



The Role of Extension Programs in Developing the Fisheries Sector, White Nile State, Sudan

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Abstract

The importance of this study is to identify the role of some extension training programs and methods used in extension training program for fishermen to enable them to develop their occupation, in addition to determine the social characteristics of fishermen. This survey was conducted in the White Nile State in period from 2019 - 2020. The study samples are 180 samples, divided to four stations. The study used questionnaires, field surveys, and as primary sources of data collection. SPSS used in analysis beside the Excel. The results of the results found majority age range is 30 – 60 years their rate is 73.9%, while married dominantly among responders and the family number from 5 to 10 individuals in high percentage 43.3%. Fishing occupation dominant between responders it represents 90.0%. The extension programs provide to fishermen are manufacturing net and repair, fishing methods, Manufacturing equipment of preservation, beside other programs, and have different responses. The training contributed an increase in revenue through marketing. The study recommended that the training programs which provide have simple benefits in livelihood of fishermen communities to increase those benefits from training programs must make a good planning to determine fishermen needs, and how can implement them.

المستخلص

الأهمية من هذه الدراسة هي التركيز على دور بعض البرامج الإرشادية والأساليب المستخدمة في برنامج الإرشاد للصيادين لتمكينهم من تطوير مهنتهم، بالإضافة إلى تحديد الخصائص الاجتماعية للصيادين. أجري هذا المسح في ولاية النيل الأبيض الفترة من 2019 إلى 2020. عينات الدراسة هي 180 عينة، مقسمة إلى أربع محطات. استخدمت الدراسة الاستبيانات والدراسات الاستقصائية الميدانية، ومصدر رئيسي لجمع البيانات. SPSS أستخدم في التحليل. نتائج الدراسات وجدت أن الفئة العمرية للأغلبية تتراوح بين 30 و 60 سنة ومعدلها 73.9 في المائة، في حين أن المتزوجين يشكلون الاغلبية بين المستجيبين وعدد الأسر يتراوح بين 5 و 10 أفراد بنسبة عالية 43.3 في المائة. يعتبر الصيد المهنة المهيمن بين المستجيبين أنه يمثل 90.0%. برامج الإرشاد التي تقدم للصيادين هي تصنيع الشبكة وإصلاحها ، وأساليب الصيد ، ، وتصنيع معدات الحفظ ، إلى جانب برامج أخرى ، ولها استجابات مختلفة. ساهم التدريب في زيادة الإيرادات من خلال التسويق. وأوصت الدراسة بأن برامج التدريب التي توفر فوائد بسيطة في سبل عيش لمجتمعات الصيادين لزيادة تلك الفوائد من برامج التدريب يجب أن يتم التخطيط الجيد لتحديد احتياجات الصيادين، وكيف يمكن تنفيذها.

Key word: Fisheries - Training - Programs - Communication - Extension methods

Introduction

The natural of fisheries in Sudan seem into two main sectors; the inland fisheries (freshwater fisheries) and the marine fisheries (Ahmed and et al, 2015). Ahmed, 2009 explain that, the inland fisheries are mainly on the River Nile and its tributaries, contributing over 90% of the estimated production potential of the country. In 2017 Hassan clarify the fishermen considered as



one of the most neglected and poorest groups within society, having traditional inherited fishing methods that are not adapted to modern fishing, gears, handling, and preservation. The same conditions are dominant in Sudan, there is a huge gap between the world's modern fisheries and the traditional fisheries in the country. The fisheries sector plays an important role in many developing countries, contributing towards food security, generation of employment and procurement of foreign exchange. (Mohammed,2012). In 2019 Jumaa defines the basic concept of extension is that it is education; we can define education as shaping of behavior of the individual for adequate adjustment in the society, change in behavior means, change in knowledge and understanding, skill and attitude. Extension education is an applied science consisting of content derived from researchers, accumulated field experiences and relevant principles drawn. Mohammed, 2018 explains the objective of extension service is to help target group holding to gain new information and develop new abilities as well as to apply directly on the farm the latest scientific knowledge. Extension works in development of new guidelines for small and medium projects and agriculture holding. Investment, business plans, plan for development of regions for agriculture production and processing, and addition activities. Jumaa, 2019 reported that extension methods play significant role in achieving extension program goals. Khalid, 2012. said the training is a planned activity designed to attend changes in the individual and community in terms of information, experience, skill, and trends which make this individual or that group fit to do their duties efficiently with high productivity. She also defines the training methods as knowledge transfer of skill from extension agency to target group via various way and means, or it the set of procedures and steps and tasks that must carried out by agency to target group to achieve the objectives of training programs.



Material and methods

Sampling: Primary data was collected by using questionnaires, which design for fishermen, besides observations observed during the survey period. The surveys period 2019 - 2020. The samples of the study are 180 individuals divided to four stations (**Aljbleen, Kosite, Aldwam, and Almoner**) according to Administration of fisheries and Aquatic organisms (report, 2018). The researcher takes 40 sample from four fisheries stations, she used simple random sampling to collect data.

The questionnaires included question about the social- characteristics condition of fishermen using the survey indicators like age distribution marital status, educational status, family number, main occupation. Beside information about support and training. A detailed analysis is made on these parameters

Analysis: The approach to analyzed collected data is the analytical descriptive approach. Statistical Package of Social Science (SPSS) and Excel. Frequencies and percentage tables, in addition to cross tabulation different issues used, to achieve the objectives of the study.

Results and discussion

In this part display the results of data collection which used in survey. The figures below display some the social characteristics of responders.

Age per years

Knowledge of age distribution is important in estimating potentiality of the human resources. In the present study, age classified into three groups as (Less 30 years, 30 -60 years, and more than 60 years). The investigation showed majority of the fishing professional belongs to group 30-60 year represent 73.9% flow by less than 30 their rate 23.9% and 2.2% age more than 60 years.

Age per years

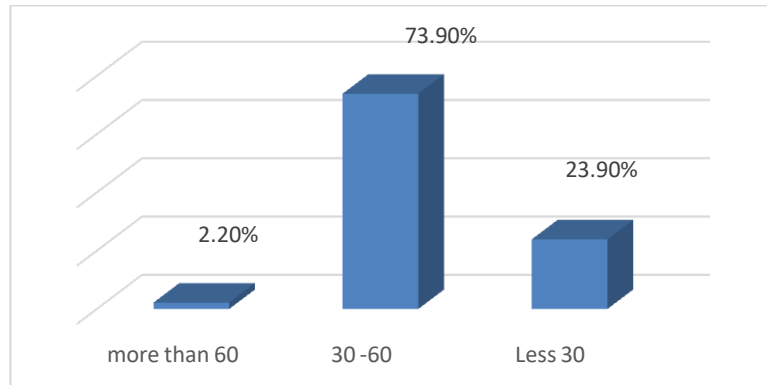
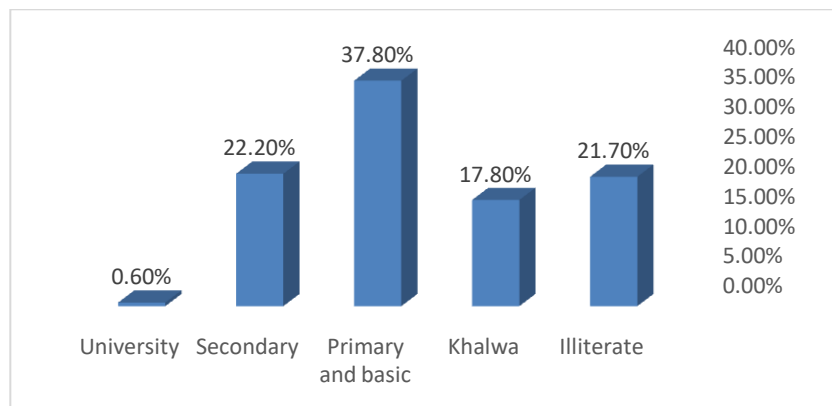


Figure (1) explain the age of responders

Educational level: - the figure (1) shows education level in the study area, a majority of responders are attended primary and basic school represent 37.8%, fallow by 22.2% for secondary school, illiteracy among the responders is about 21.7%, while *khalwa* there rate 17.8%, and the rest they attended university.

Education level



The figure (2) explains the educational level of responders

Family number

The number of family individuals has great importance from an economic point of view since it indicates if income from this occupation supports a big or small family. Therefore, figure (1) explains that the family number 5 - 10 individuals present 43.4%, those who have family size in the range of less than individuals

are 36.1% and 18.3% of responders have family size in the range 10 and more individuals, the rest have no family.

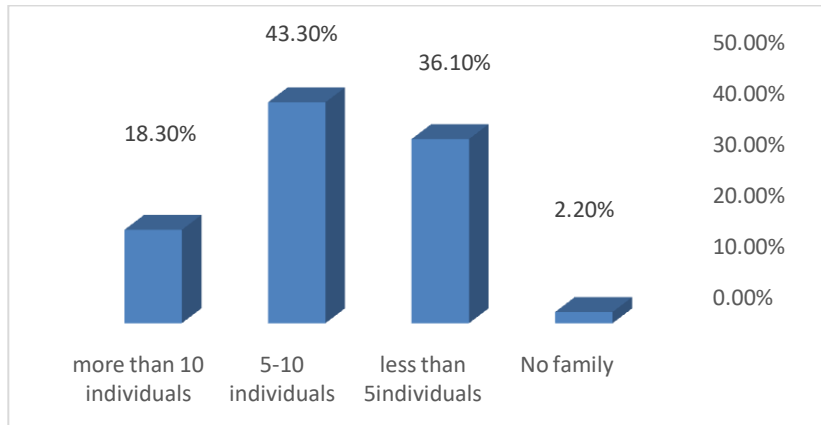


Figure (3) explain the family number of responders

Married status

The figure shows that minority of responders are unmarried

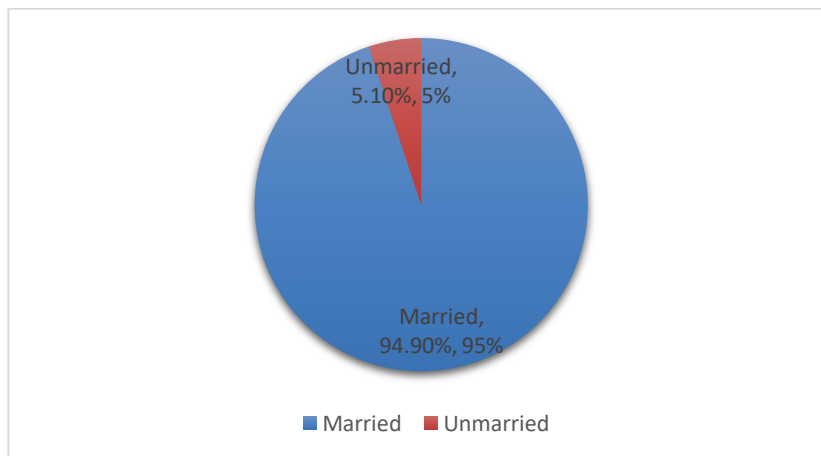


Figure (4) explain the married status of responders

Main occupation

figure (1) shows that the rate of responders who practices fishing as main occupation is 90.0%, follow by 6.1. % Is a farmer While 2.2% traders and the rest of them are workers 1.7%.

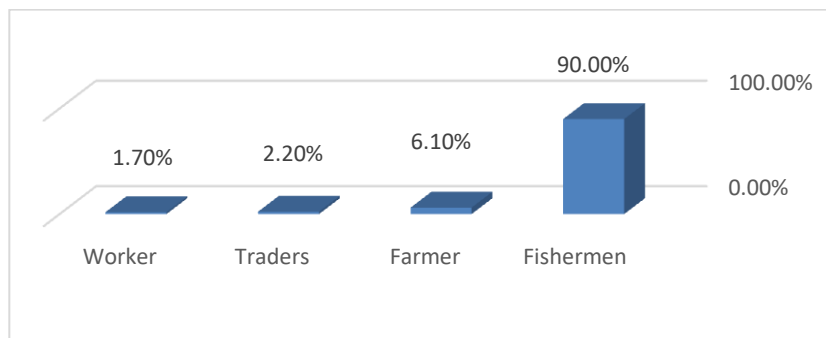


Figure (5) explain the main occupation of responders

Support information

Table (1) support information

		N=180	%
do you take any support type?	No	137	76.1%
	Yes	43	23.9%
Statement		N=43	%
who provide support	government	2	1.1%
	organization	24	13.3%
	Government and organization	17	9.4%
kind of support	financial support and training	17	9.4%
	training	26	14.4%
financial support	Don't receive financial support	171	95.0%
	Receive financial support	9	5.0%
Support	Gears and filament	16	8.9%
	Boats	14	7.8%
	Devices to determine position	0	0.0%
	Refrigerators	1	0.6%
	maintenances equipment	0	0.0%

multiple response analysis



The table (1) shows that responders don't take any type of support their rate 76.1%, while 23.9% take support, that means the chances don't disturb equal between responders. By referring to the table above illustrated that the majority of support came from organizations, their rate is 13.3%, and however, both organizations and government support 9.4% and support came through government 1.1%. The support which provides to responders present in finance support and training or just training. The support which provides to responders present in different ways, financial support and training they rate 9.4% and who take just training support their rate 14.4%, gears and filament, boats and refrigerators. Present different ration display respectively 5.0% of financial support, 8.9% to gears and filament and 7.8% of boats, while 0.6% taker refrigerators.

The fishermen take several types of training to improve their silk in fishing and develop themselves, it's represented in net manufacturing and repair, fish processing, improve food culture, boats manufacturing and maintenance, manufacturing equipment of preservation, cooling and package, fish handling, fish marketing and capacity building They were different response trend the programs ranged from weak, middle, good and v- good response as display in table (2).

Training programs

Table (2) Training programs

statement	response	N=43	%
manufacturing net and repair	weak	0	0.0%
	middle	1	0.6%
	good	16	8.9%
	v-good	20	11.1%
Methods of fishing	Weak	0	0.0%
	middle	5	2.8%
	good	14	7.8%
	v-good	16	8.9%
Boats manufacturing and maintenance	weak	2	1.1%
	middle	4	2.2%
	good	9	5.0%
	v-good	1	0.6%
Manufacturing equipment of preservation, cooling and package	weak	0	0.0%
	middle	1	0.6%
	good	9	5.0%
	v-good	2	1.1%
Fish handling	Weak	0	0.0%
	middle	1	0.6%
	good	11	6.1%
	v-good	10	5.6%
Fish marketing	weak	0	0.0%
	middle	2	1.1%
	good	9	5.0%
	v-good	6	3.3%
Capacity building	middle	1	0.6%
	good	1	0.6%
	v-good	3	1.7%
Improve food culture	weak	0	0.0%
	middle	0	0.0%
	good	0	0.0%
	v-good	5	2.8%
Fish processing	weak	0	0.0%
	middle	0	0.0%
	good	2	1.1%
	v-good	15	8.3%

Survey 2020: multiple response analysis

Extension methods to deliver information

Table (3) Extension methods

Method	Depend on it	N=43	%
Lectures	sometime	13	7.2%
	often	19	10.6%
Practical training	sometime	21	11.7%
	often	7	3.9%
publication	sometime	12	6.7%
	Often	2	1.1%
Field visit	sometime	2	1.1%
	Often	8	4.4%
Interview	sometime	2	1.1%
	Often	3	1.7%
Radio	sometime	5	2.8%

Survey 2020: multiple response analysis

The table (3) found the responders receive information through various methods. 10.6% of them often depending on Lectures and 7.2% sometime depending on it. While 11.7 % sometime depending on practical training 3.9% often depending on it, 6.7% sometime depending on publication to get information 1.1% often depending on it, on other hand field visit sometime done by 1.1% whilst interview method of get information need responder meet official that make it cost and need effort to meet 1.7% often do it, 1.1% sometime do it Radio is available method and don't need effort information received to responders in their place, although that a few of responders depending on it 2.5% sometime depending on it. Above table explain the stated

of Khalid, 2012 who said the training methods as knowledge transfer of skill from extension agency to target group via various way and means,

Training contribution

Table (4) Training contribution

Statement	N	%	
Training contribution	Training contributed to an increase in revenue through good marketing.	42	23.3%
	Training decrease fish loss	40	22.2%
	Increase in fishing equipment	37	20.6%
	Decrease time and effort and increase production good marketing	36	20.0%

Survey 2020: multiple response analysis

By refer to the table (3) noted that, the training has significant effect in responders who take training, 23.3% of responders said that training contributed to an increase in revenue through good marketing, while 22.2% belief that training contributed to some extend to decrease fish loss. By good knowing, 20.6% said training increase in fishing equipment, and decrease time and effort and increase production the ratio 20.0%, this results approval with Mohammed, 2018 who explains the objective of extension service is to help target group holding to gain new information and develop new abilities as well as to apply directly on the farm the latest scientific knowledge.

Conclusion

The main occupation of responders is fishing By refer to table (1) we found the percent of responders who take kind of support are little 23. % From the total percent, the majority support comes from organization the main extension methods used to deliver information is practical training. The raise awareness



of fishermen communities toward fisheries to protected it is the first step of develop.

Recommendation

- The study recommended that to develop fishing occupation through using modern extension methods.
- The training programs which provide have simple benefits in livelihood of fishermen communities to increase those benefits from training programs must make a good planning to determine fishermen needs, and how can implement them.
- Evaluation of training programs to determine the benefits gains from training. Any program that provides must pay attention to teaching fishermen how to protect fisheries by introducing new technology in fishing and teaching them new occupations in order to reduce fishing effort, and help fishermen in the rainy seasons because fishing become more difficult.

References

- Ahmed Fawzi, Haroon Elgaili Idres Yagoubi and Safa fadlalseed, (2015).** Socio economic Characteristics of Fishermen in Jabel Awlia and Elmawrada at Khartoum State, Sudan. In Scienceline Publication. Online Journal of Animal and Feed Research. 5 (14): 95-100.
- Ahmed Somia (2009).** The Economic of Fish Production &Marketing in White Nile State – Sudan, Msc. University of Khartoum, Sudan. P 5, 9
- Hassan Sana, (2017).** Problems and Constraints of Fish Production and Marketing Case Study Sawakin area - Red Sea State- Sudan. M.sc in Fish Science and Technology. Sudan University of Science and Technology. Sudan. P 12
- Jumaa Rugaia, (2019).** Effect of Extension Campaigns on Livestock Producers Knowledge and Skills (Case Study South Kordofan State). A thesis Submitted for Partial Fulfillment of the Requirement for Master Degree in Animal production in the Tropics. Sudan University of Science and Technology College of Graduate Studies. P 5,6



- Khalid Omeima, (2012).** Training and Impact on Rural Leaders Capacity Building (A case Study of Community Development Fund Project – Kassala State. PhD. College of Graduate Studies, Department of Agricultural Extension and Rural Development, Sudan University of science &Technology. P 7, 15
- Mohammed Ismail , (2018).** An Assessment of Role of Agriculture Extension on Land Tenure Conflicts Management in El Salam Locality – Western Kordofan State – Sudan. Ph.D. Degree in Agriculture extension and Rural Development. Sudan university of science and technology. P 93



Triazole Compounds for the Treatment of Cancer

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Abstract

Malignant tumors are another name for cancerous tumors. Benign tumors do not migrate or invade surrounding tissues. When benign tumors are surgically removed, they seldom reoccur, although melanoma sometimes does. Malignant tumors, on the other hand, may sometimes grow to be enormous. Many, even benign brain tumors, may be fatal or produce severe symptoms. The kind of therapy selected depends on the kind of cancer and its stage of progression. The kind of therapy selected depends on the kind of cancer and its stage of progression. Although cancer therapy is the most prevalent, it has adverse effects ranging from mild, such as weight loss, to severe, such as nausea. Triazoles and triazole-containing substances have several important uses in the dyeing and pigment, corrosion preventive, and polymer markets. Such chemicals have several biological properties, including antibacterial, antioxidant, antiviral, anti-HIV, antitubercular, and anticancer properties. Evaluating contemporary sustainable methods to triazole-forming biologically active molecules, particularly anticancer drugs, would be crucial for both the pharmaceutical sector and the worldwide scientific society. The current study presents an overview of the anticancer potential of various triazole derivatives.

Keywords: Chemotherapy; Apoptosis; triazole compounds, Intrinsic Pathway

Introduction

Cancer is a group of illnesses that spread to different organs and tissues (Alam *et al.*, 2021). Following cardiovascular illnesses as the second biggest reason of death worldwide, cancer is a significant medical consequence. Regarding the influence of illness consequences on the long-term well-being of

people, cancer prevention and therapy has received much focus. Cancers were identified as a Worldwide Burden of Illness and are projected to become one of leading reasons of mortality in the next generations (Prachayasittikul *et al.*, 2015). Consequently, the quest for new anticancer drugs has risen to the forefront of medication research and discovery (Cui *et al.*, 2020).

There are a variety of successful anticancer therapies, but their adverse effects, including resistance to anticancer drugs, lack of distinction among malignant and noncancerous cells, the limitations of radiotherapy, and the need for surgical intervention, necessitate the development of substitute therapies with various mechanisms (Schirmacher, 2019; Lind, 2020). Because of the inability of presently existing anti-cancer medications to distinguish between normal and malignant cells, there are a number of side effects associated with their toxicity to normal cells as well (Stewart *et al.*, 2016). Due to ineffective chemotherapy caused by drug resistance and the inability of some medications to discriminate normal cells from malignant cells, a systematic approach to the development of innovative chemotherapeutics with superior efficiency, reduced toxicity, and enhanced specificity is essential (Mashayekh and Shiri, 2019).

Heterocycles have performed a crucial role in the development of anticancer medications. Triazole compounds are heterocyclic molecules having the chemical formula $C_2H_3N_3$ having a five-membered carbon- and nitrogen-containing ring (Sonawane *et al.*, 2017). The relative positions of the nitrogens differ between 1,2,3-Triazoles and 1,2,4-Triazoles, the two isomers (Figure 1). All of them include 2 tautomers with varying nitrogen-hydrogen bonds. Commonly, cycloaddition procedures are used to create 1,2,3-triazoles (Souza and Miranda, 2019). Amongst the methods is the use of [organic azides and

alkynes]. The Huisgen azide-alkyne 1,3-dipolar cycloaddition is a process that can be carried out despite the absence of reagents at the correct temperature. However, this approach produces two distinct analogs: 1,4-disubstituted 1,2,3-triazole and 1,5-disubstituted 1,2,3-triazole. Utilizing metal catalysts favorably produces a certain enantiomer (V Kouznetsov *et al.*, 2016; Jalani *et al.*, 2017). When 1,4-disubstituted 1,2,3-triazoles are synthesized in the presence of copper(I) catalysts, the resulting method is referred to as copper-catalyzed azide-alkylation (Di Girolamo *et al.*, 2022).

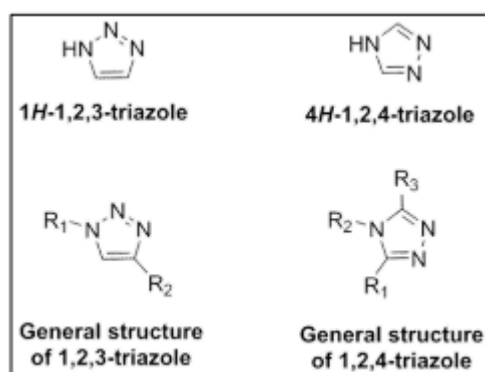


Figure 1. 1H-1,2,3-triazole and 1H-1,2,4-triazole chemical structures, as well as their generalized frameworks.

Anticancer Activity of Triazole Derivatives

Triazoles' isometric qualities enable for anticancer, anti-inflammatory, antiviral, and other medicinal actions. Owing to its characteristics, it's used in medical chemistry. 1,2,3-triazole and its analogues were used with click chemistry to decrease harmful waste/byproducts (Figure 2) (Hou *et al.*, 2017; El Azab *et al.*, 2021). Despite scientific improvements, cancer is still the second-leading reason for mortality worldwide. Various tumors respond variably to anticancer medicines. Anticancer medications can't distinguish among normal cells and those accountable for human tumor cell growth (Ciliberto, 2022).

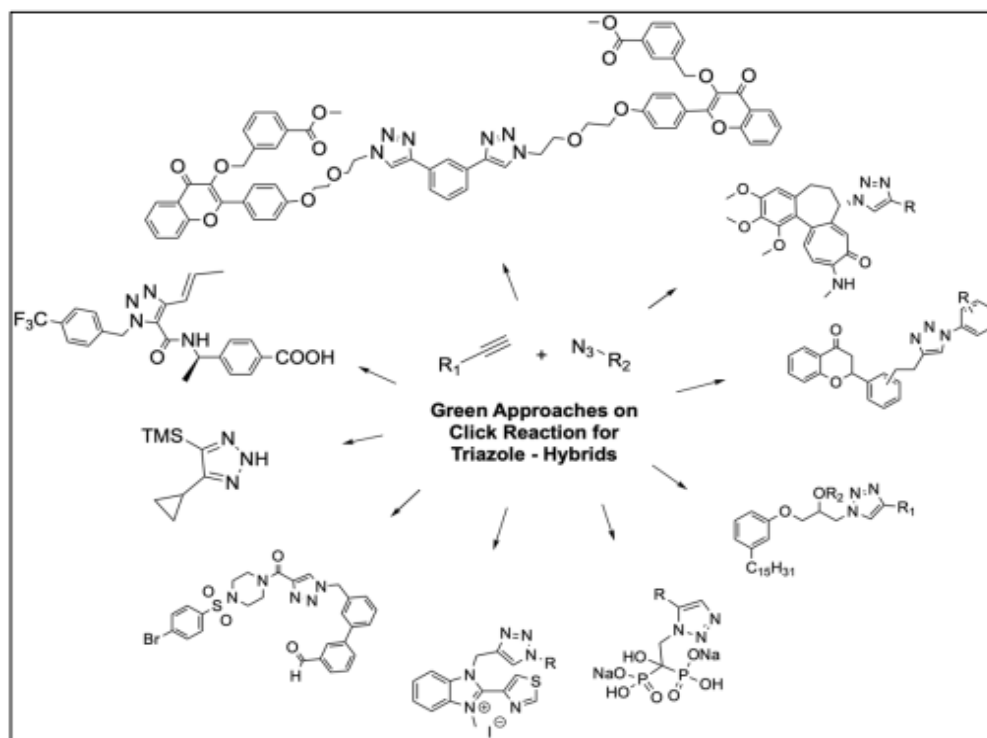


Figure 2. Click chemistry of triazole hybrids (Ciliberto, 2022).

Numerous types of bacteria, marine organisms, and fungus possess compounds with a triazole ring (Neves *et al.*, 2021). The medicinal potential of vicinal diaryl triazoles is the subject of intensive investigation (Davies, 2020). Additional biological and chemical actions include anticancer, cyclooxygenase (COX) suppressive, anti-inflammatory, antibacterial, anti-obesity, and antiviral (Yadav *et al.*, 2018). Deferasirox, a commonly available iron chelator, incorporates vicinal diaryl triazole. Vicinal diaryl triazoles and tetrazoles were found as prospective components for the manufacture of novel pharmaceuticals with applications as cyclooxygenase-2 (COX-2) inhibitors, tubulin

polymerization inhibitors, and Cannabinoid receptor type 1 (CB1) receptor antagonists (Karypidou, 2019).

The Anticancer Potential of 1,2,3-triazoles

Many of 1,2,3-triazole's derivatives are now employed in clinics or preclinical studies to treat cancer. Hybrid chemicals have a crucial impact in cancer prevention and management, and mixing the 1,2,3-triazole molecule with various additional anticancer chemicals might be a helpful therapeutic method for cancer treatment, especially for drug-resistant cancer (Bozorov *et al.*, 2019). The anticancer activities of 1,2,3-triazoles have been established through a number of ways. Thymidylate synthase (TS), aromatase, carbonic anhydrases (CAs), vascular endothelial growth factor receptor (VEGFR), tryptophan, epidermal growth factor receptor (EGFR), and 2,3-dioxygenase (TDO) all have a part in the progression of this fatal disease (Figure 2) (Alam, 2022). Carbonic anhydrases (CAs), thymidylate synthase (TS), aromatase, tryptophan 2,3-dioxygenase (TDO), vascular endothelial growth factor receptor (VEGFR), and epidermal growth factor receptor (EGFR) are inhibited to produce anticancer effects. Novel paeonol Schiff baselinked 1,2,3-triazoles, (E)2[1([1(2-fluorophenyl)1H1,2,3-triazol-4-yl]methylimino)ethyl], (E)2[1([1(2-fluorophenyl)1H1,2,3-triazol-4-yl]methylimino)5-methoxyphenol] and (E)2[1([1(3-chlorophenyl)1H1,2,3-triazol-4-yl]methylimino)5-methoxyphenol] exhibited cytotoxicity with IC₅₀ values of 45.1 μM and 78.9 μM, respectively, against the highly proliferative, metastatic, and invasive human lung cancer A549 cells, in contrast to paeonol (IC₅₀ value of 883.0 μM). A botulin-linked 1,2,3-triazole, 3,28-O-di[2,4-(hydroxymethyl)1H1,2,3-triazol-1-yl]acetyl]betulin, demonstrated superior cytotoxicity with 20 percent and 70 percent

antiproliferation action in MCF7 human breast cancer cell line (Luminal A” subtype of noninvasive cell line and is highly responsive to chemotherapy) and HCT116 (the invasive human colorectal carcinoma cell line), respectively, compared to betulin, which demonstrated 30percent (MCF7) and 90percent (HCT116) antiproliferation action after 48 h of incubation (Sanphanya *et al.*, 2013; Banerji *et al.*, 2018; Jiang *et al.*, 2020).

The Anticancer Potential of 1,2,4-triazoles

Numerous anticancer drugs, such as ribavirin, triadimefon, tebuconazole, and fluconazole, include a 1,2,4-triazole moiety, highlighting their prospective for the development of novel anticancer treatments (Abdelrahman and Ali, 2016). Owing to their many biochemical effects, especially as anticancer drugs, 1,2,4-triazoles are one of the most significant nitrogen-containing scaffolds in biomedical sciences. By means of hydrophobic contacts, hydrogen bonding, van der Waals forces, and dipole–dipole interactions, this class of chemicals may establish a variety of non-covalent interactions with physiologically active molecules. Several 1,2,4-triazole-incorporating compounds, notably the 3-nitro-1,2,4-triazole derivative of [18F]FMISO ([18F]3-NTR, 1), are being evaluated for the treatment of cancer-related hypoxia in clinical studies (Nagarsenkar *et al.*, 2016).

Triazole molecules containing at least one 1,2,4-triazole ring were discovered to possess some of the most potent antifungal properties. Fluconazole, (2-(2,4-difluorophenyl)-1,3-di(1H-1,2,4-triazol-1-yl)propan-2-ol) and itraconazole, (4-(4-(4-((2S,4R)-2-((1H-1,2,4-triazol-1-yl)methyl)-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl)methoxy)phenyl)piperazin-1-yl)-phenyl)-1-((S)-sec-butyl)-1H-1,2,4-triazol-5(4H)-one) were amongst the first to be synthesized, although

they were not without flaws. Efinaconazole, voriconazole, albaconazole, posaconazole, isavuconazole, and ravuconazole are second-generation triazoles that are copies of fluconazole or itraconazole and are intended to substitute for the deficiencies of their parent treatments (Moghimi *et al.*, 2022).

Anticancer Properties of Triazole Substances

Triazole compounds show anticancer action by damaging DNA, generating Double Strand Breaks (DSB), and then inducing cell cycle arrest and death (Mingard *et al.*, 2020). There were a few papers in grasping how they start their anti-tumor effect, so 2 distinct routes were investigated, and one of the methods detailed the induction of apoptosis (Ghanaat *et al.*, 2021).

Programmed cell death

Chemotherapy aims to destroy tumor cells and inhibit tumor development. Also, they should contain autophagy, necrosis, and mitotic catastrophe (no apoptotic death) (Vitale *et al.*, 2011). Autophagy, apoptosis, entosis, and necrosis are four types of cell death. Apoptosis, or type I cell death, is produced by the caspase proteolytic cascade. Autophagy, sometimes called type II cell death, eliminates faulty proteins and organelles by forming an autophagosome. Necrosis includes pyroptosis and necroptosis. Entosis is a kind IV cell death that requires engulfing cells. Parthanatos, neurotic cell death, ferroptosis, lysosomal dependent cell death, alkalosis, and oxeiptosis are others (Adjemian *et al.*, 2020). Das *et al.* (2019) found NMK-T-057 (-1,2,4-triazole) linked to -secretase. NMK-T-057 inhibits breast cancer cell proliferation, engraftment, and migration in MDA-MB-231, MDA-MB-468, MCF-7 (estrogen receptor (ER)/progesterone receptor (PR)–positive cell line), and 4T1 (triple-

negative cells) with low deleterious effects on noncancerous cells (MCF-10A and peripheral blood mononuclear cells). NMK-T-057 activated apoptosis and suppressed epithelial-mesenchymal transition (EMT) and stemness in BC cells. EMT is linked to cancer progression and is involved in metastasis, treatment resistance, embryonic development, and the inflammatory process. Cells lose epithelial proteins and gain mesenchymal proteins during EMT. This transformation enables cancer cells to penetrate through the basement membrane and boost their invasive capabilities. Cancer cells that undergo EMT not only lose their epithelial phenotype cell-cell adhesion and display enhanced motility and invasion, but they also develop increased resistance to chemotherapy treatments. Furthermore, activation of EMT results in the formation of cancer cells with stem cell-like properties (Sung *et al.*, 2016). NMK-T-057 cause's autophagy-mediated cell death by inhibiting γ -secretase-mediated Notch signaling. Extensive experimental evidence links autophagy activation with Notch signaling suppression, with Notch-1 down-regulation inducing autophagy-mediated cancer cell death. Autophagy causes necroptosis, sometimes called autosis (Das *et al.*, 2019).

Extrinsic cell death

Apoptotic signalling via the extrinsic route starts with the binding of tumour necrosis factor (TNF), to the extracellular portion of trans membrane receptors [fatality domains, such as the kind 1 TNF receptor] (TNFR1). When a ligand binds to a mortality receptor, it binds via its intracellular mortality motif to a matching protein pattern in connector proteins. Coupling of TNF and TNFR1 activates the NF κ B system, which promotes cell survival or apoptosis depending on the cell type and biological conditions (Nirmala and Lopus, 2020).

Triazols and extrinsic apoptosis

Apoptotic signalling via the extrinsic route starts with the binding of tumour necrosis factor (TNF), to the extracellular portion of trans membrane receptors [fatality domains, such as the kind 1 TNF receptor] (TNFR1). When a ligand binds to a mortality receptor, it binds via its intracellular mortality motif to a matching protein pattern in connector proteins. Coupling of TNF and TNFR1 activates the NF_κB system, which promotes cell survival or apoptosis depending on the cell type and biological conditions. implicated in the extrinsic apoptotic process. As a result, it promotes apoptosis in human leukaemia HL-60 cells via both intrinsic and extrinsic routes (Rajalekshmi Devi, 2016).

Moreover, Kulabaş *et al.* (2016) stated that compound 19, novel 2-(4H-1,2,4-triazole-3-ylthio) acetamide derivative, activated the extrinsic route greatly by generating caspase-8 activity at just 50 μM ($p < 0.01$). Caspase-8 is a key apoptosis protein which is triggered in both the death receptor and the mitochondrial route. Caspase-8 sequentially activates Bid, a pro-apoptotic Bcl-2 family member. As well they demonstrated that it enhanced MMP degradation, decreased the production of anti-apoptotic Bcl-2 protein, and activated caspases-8 and -3 in these cells, suggesting the participation of both intrinsic and extrinsic apoptotic mechanisms in apoptosis.

Milošev *et al.* (2017) indicated that treatment of chronic myelogenous leukaemia K562 cells with compound 5b, a 1,2,4-triazole-3-thione containing adamantane moiety, increased the rates of pro-apoptotic protein Bax, caspase-8, and the primary activator caspase-3, indicating that this triazole activated both intrinsic and extrinsic apoptotic pathways. One of the acknowledged techniques in current apoptosis-based anticancer treatment is the identification of tiny

compounds which trigger pro-apoptotic Bcl-2 proteins⁴² such as Bax. Similarly, 5b, a known activator of apoptosis, may have anticancer activity as a small-molecule Bax agonist.

Intrinsic apoptosis

Regarding pathological conditions, the BH3-only polypeptides (BAK and BAX) stimulate and incorporate into the external mitochondrial membrane, causing the discharge of cytochrome c as well as other mitochondrial proteins such as endonuclease G and apoptosis-inducing factor (AIF), the serine protease, as well as Smac/DIABLO (2nd mitochondria-derived inducer of caspase/direct IAP-binding protein with limited PI). Following that, in the cytosol, cytochrome c binds with apoptotic protease-activating factor 1 (Apaf1) to create the apoptosome. The apoptosome, a multi-protein substrate consisting of a seven-spoke ring-shaped structure, triggers starter caspase (typically caspase-9) that triggers executing caspase-3 and commences a caspase cascade, finally leading to cell destruction. Apparently, AIF as well as endonuclease G can induce caspase-independent apoptosis by causing chromatin constriction and nuclear DNA breakage. AIF has appeared as a crucial protein for cell viability in addition to its crucial involvement in caspase-independent apoptosis. Omi/HtrA2 and Smac/DIABLO, two major mitochondrial proteins secreted in the cytoplasm, providing an extra pathway for caspase stimulation. Omi/HtrA2 and Smac/DIABLO link with IAP proteins and increase caspase engagement by antagonising them.

Triazols and intrinsic apoptosis

Several scholars have discussed that triazoles analogues trigger intrinsic apoptosis. For, instance, Ahmed *et al.* (2018) synthesized and characterized a number of new 1, 2, 4-triazole/chalcone combinations using various spectroscopic approaches. The synthesized compounds shown impressive cytotoxic action against several cancer cell types. Substances 24, 25, 27, 41, and 47 demonstrated the maximum cytotoxicity towards human lung adenocarcinoma A549 cells, with IC_{50} varying from 4.4 to 16.04 μ M, contrasted to cisplatin, which had an IC_{50} of 15.3 μ M. Flow cytometric examination of the studied substances revealed a dose-dependent surge in the prevalence of apoptotic cells. Subsequent pharmacological research revealed that 1, 2, 4-triazole-chalcone combinations triggered caspase-3 dependent apoptosis through both extrinsic and intrinsic pathways through elevated levels of proapoptotic protein Bax, mitochondrial cytochrome c leakage, and stimulation of caspase-3/8/9 proteins.

López-Soto *et al.* (2017) developed and synthesized a series of 30 new uracil derivatives as TS antagonists using a molecular assembling concept relied on the structures and pharmacological characteristics of 2 categories of TS inhibitors. The MTT test was used to assess the anticancer potential of these drugs towards OVCAR-3, A549, HepG2, and SGC-7901 cancer cell lines. The majority of them had outstanding activity against all of the cell lines examined. Moreover, the findings of the hTS experiment revealed that these substances had the unique capacity to block hTS properties *in vitro*. Interestingly, substance 13j had the most powerful effect towards A549 cells ($IC_{50} = 1.18 \mu$ M) and the most pronounced enzyme inhibition ($IC_{50} = 0.13 \mu$ M), outperforming pemetrexed



(PTX, $IC_{50} = 3.29 \mu\text{M}$ and $IC_{50} = 2.04 \mu\text{M}$). Flow cytometric studies revealed that the chemical 13j inhibited A549 cell growth by stopping the cell cycle in the G1/S phase and then inducing cell death. Additional western blot research revealed that substance 13j might block cell growth by down-regulating the cycle barrier proteins cyclin D1 and cyclin E, and subsequently trigger intrinsic apoptosis by triggering caspase-3 and decreasing the bcl-2/bax proportion. In 2020, Arulnathan *et al.* (2020) examined the mechanism of cell death induced by N-(4-chlorophenyl)-2-(4-(3,4,5-trimethoxybenzyloxy)benzoyl)-hydrazinecarbothioamide, a triazole analogue, now known as compound P7a, in the MCF-7 breast cancer cell line.

Conclusion

Triazole is a crucial heterocycle in medicinal chemistry, and its derivatives have shown significant anticancer activity. The compounds shown anticancer potential by inhibiting enzymes such as EGFR, VEGFR, and PARP that contribute to the advancement of this fatal illness. In this study, we offered an overview of many topics related to computational-method-aided novel drug development processes in general, and anti-cancer therapeutic discovery in particular. We examined research on anticancer drug designs using computational approaches to evaluate some of the most prominent instances and clarify essential concepts. This review, which included publications published between 2013 and 2022, underlined current breakthroughs in triazole-containing hybrids with anticancer potential.

References

- Abdelrahman, M.H. and Ali, M.M.,(2016).** Synthesis of Novel Pyrazole Derivatives Bearing 1, 2, 4-Triazole Moiety as Potential Anticancer Agents. *Bulletin of Pharmaceutical Sciences. Assiut*, 39(1), pp.53-71.
- Adjemian, S., Oltean, T., Martens, S., Wiernicki, B., Goossens, V., Vanden Berghe, T., Cappe, B., Ladik, M., Riquet, F.B., Heyndrickx, L. and Bridelance, J., (2020).** Ionizing radiation results in a mixture of cellular outcomes including mitotic catastrophe, senescence, methuosis, and iron-dependent cell death. *Cell death & disease*, 11(11), pp.1-15.
- Ahmed, F.F., Abd El-Hafeez, A.A., Abbas, S.H., Abdelhamid, D. and Abdel-Aziz, M., (2018).** New 1, 2, 4-triazole-Chalcone hybrids induce Caspase-3 dependent apoptosis in A549 human lung adenocarcinoma cells. *European journal of medicinal chemistry*, 151, pp.705-722.
- Alam, M.M., Nazreen, S., Almalki, A.S., Elhenawy, A.A., Alsenani, N.I., Elbehairi, S.E.I., Malebari, A.M., Alfaifi, M.Y., Alsharif, M.A. and Alfaifi, S.Y., (2021).** Naproxen based 1, 3, 4-oxadiazole derivatives as EGFR inhibitors: Design, synthesis, anticancer, and computational studies. *Pharmaceuticals*, 14(9), p.870.
- Alam, M.M., (2022).** 1, 2, 3- Triazole hybrids as anticancer agents: A review. *Archiv der Pharmazie*, 355(1), p.2100158.
- Arulnathan, S.B., Leong, K.H., Ariffin, A., Kareem, H.S. and Cheah, K.K., (2020).** Activation of Intrinsic Apoptosis and G1 Cell Cycle Arrest by a Triazole Precursor, N-(4-chlorophenyl)-2-(4-(3, 4, 5-trimethoxybenzyloxy) benzoyl)-hydrazinecarbothioamide in Breast Cancer Cell Line. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, 20(9), pp.1072-1086.
- Banerji, B., Chandrasekhar, K., Sreenath, K., Roy, S., Nag, S. and Saha, K.D., (2018).** Synthesis of triazole-substituted quinazoline hybrids for anticancer activity and a lead compound as the EGFR blocker and ROS inducer agent. *ACS omega*, 3(11), pp.16134-16142.
- Bozorov, K., Zhao, J. and Aisa, H.A., (2019).** 1, 2, 3-Triazole-containing hybrids as leads in medicinal chemistry: A recent overview. *Bioorganic & medicinal chemistry*, 27(16), pp.3511-3531.
- Ciliberto, V.C., (2022).** Green Approach Toward Triazole Forming Reactions For Developing Anticancer Drugs.
- Cui, W., Aouidate, A., Wang, S., Yu, Q., Li, Y., & Yuan, S. (2020).** Discovering anti-cancer drugs via computational methods. *Frontiers in pharmacology*, 11, 733.



- Das, A., Narayanam, M.K., Paul, S., Mukhnerjee, P., Ghosh, S., Dastidar, D.G., Chakrabarty, S., Ganguli, A., Basu, B., Pal, M. and Chatterji, U., (2019). A novel triazole, NMK-T-057, induces autophagic cell death in breast cancer cells by inhibiting γ -secretase-mediated activation of Notch signaling. *Journal of Biological Chemistry*, 294(17), pp.6733-6750.
- Davies, S., (2020). Tetrazine-Triggered Bioorthogonal Decaging Reactions for Prodrug Activation (Doctoral dissertation, University of Cambridge).
- Di Girolamo, A., Monti, F., Mazzanti, A., Matteucci, E., Armaroli, N., Sambri, L. and Baschieri, A., (2022). 4-Phenyl-1, 2, 3-triazoles as Versatile Ligands for Cationic Cyclometalated Iridium (III) Complexes. *Inorganic Chemistry*.
- El Azab, I.H., El-Sheshtawy, H.S., Bakr, R.B. and Elkanzi, N.A., (2021). New 1, 2, 3-triazole-containing hybrids as antitumor candidates: Design, click reaction synthesis, DFT calculations, and molecular docking study. *Molecules*, 26(3), p.708.
- Ghanaat, J., Khalilzadeh, M.A. and Zareyee, D., (2021). Molecular docking studies, biological evaluation and synthesis of novel 3-mercapto-1, 2, 4-triazole derivatives. *Molecular diversity*, 25(1), pp.223-232.
- Hou, W., Luo, Z., Zhang, G., Cao, D., Li, D., Ruan, H., Ruan, B.H., Su, L. and Xu, H., (2017). Click chemistry-based synthesis and anticancer activity evaluation of novel C-14 1, 2, 3-triazole dehydroabiatic acid hybrids. *European Journal of Medicinal Chemistry*, 138, pp.1042-1052.
- Jalani, H. B., Karagöz, A. Ç., & Tsogoeva, S. B. (2017). Synthesis of substituted 1, 2, 3-triazoles via metal-free click cycloaddition reactions and alternative cyclization methods. *Synthesis*, 49(01), 29-41.
- Jiang, Y., Li, Y., Yang, T., Shi, X., Suo, H., Zhang, W., Xu, G. and Li, W., (2020). Design, synthesis, and antitumor activity research of novel paeonol Schiff base derivatives containing a 1, 2, 3-triazole moiety. *Journal of the Chinese Chemical Society*, 67(1), pp.165-171.
- Karypidou, K., (2019). Synthesis of novel compounds with potential antiandrogen activity.
- Kulabaş, N., Tatar, E., Özakpınar, Ö.B., Özsavcı, D., Pannecouque, C., De Clercq, E. and Küçükgül, İ., (2016). Synthesis and antiproliferative evaluation of novel 2-(4H-1, 2, 4-triazole-3-ylthio) acetamide derivatives as inducers of apoptosis in cancer cells. *European Journal of Medicinal Chemistry*, 121, pp.58-70.
- Lind, M.J., (2020). Principles of systemic anticancer therapy. *Medicine*, 48(2), pp.90-96.
- López-Soto, A., Gonzalez, S., Smyth, M.J. and Galluzzi, L., (2017). Control of metastasis by NK cells. *Cancer cell*, 32(2), pp.135-154.
- Mashayekh, K. and Shiri, P., (2019). An overview of recent advances in the applications of click chemistry in the synthesis of bioconjugates with anticancer activities. *ChemistrySelect*, 4(46), pp.13459-13478.



- Milošev, M.Z., Jakovljević, K., Joksović, M.D., Stanojković, T., Matić, I.Z., Perović, M., Tešić, V., Kanazir, S., Mladenović, M., Rodić, M.V. and Leovac, V.M., (2017). Mannich bases of 1, 2, 4- triazole- 3- thione containing adamantane moiety: Synthesis, preliminary anticancer evaluation, and molecular modeling studies. *Chemical Biology & Drug Design*, 89(6), pp.943-952.
- Mingard, C., Wu, J., McKeague, M. and Sturla, S.J., (2020). Next-generation DNA damage sequencing. *Chemical Society Reviews*, 49(20), pp.7354-7377.
- Moghimi, S., Shafiei, M. and Foroumadi, A., (2022). Drug design strategies for the treatment azole-resistant candidiasis. *Expert Opinion on Drug Discovery*, 17(8), pp.879-895.
- Nagarsenkar, A., Prajapati, S.K., Guggilapu, S.D., Birineni, S., Kotapalli, S.S., Ummanni, R. and Babu, B.N., (2016). Investigation of triazole-linked indole and oxindole glycoconjugates as potential anticancer agents: novel Akt/PKB signaling pathway inhibitors. *MedChemComm*, 7(4), pp.646-653.
- Neves, A.R., Pereira, D., Gonçalves, C., Cardoso, J., Pinto, E., Vasconcelos, V., Pinto, M., Sousa, E., Almeida, J.R., Cidade, H. and Correia-da-Silva, M., (2021). Natural benzo/acetophenones as leads for new synthetic acetophenone hybrids containing a 1, 2, 3-triazole ring as potential antifouling agents. *Marine Drugs*, 19(12), p.682.
- Nirmala, J.G. and Lopus, M., (2020). Cell death mechanisms in eukaryotes. *Cell biology and toxicology*, 36(2), pp.145-164.
- Prachayasittikul, V., Pingaew, R., Anuwongcharoen, N., Worachartcheewan, A., Nantasenamat, C., Prachayasittikul, S., Ruchirawat, S. and Prachayasittikul, V., (2015). Discovery of novel 1, 2, 3-triazole derivatives as anticancer agents using QSAR and in silico structural modification. *Springerplus*, 4(1), pp.1-22.
- Rajalekshmi Devi, S., (2016). Development of Novel anti-estrogens for endocrine resistant Breast Cancer (Doctoral dissertation, Virginia Tech).
- Sanphanya, K., Wattanapitayakul, S.K., Phowichit, S., Fokin, V.V. and Vajragupta, O., (2013). Novel VEGFR-2 kinase inhibitors identified by the back-to-front approach. *Bioorganic & medicinal chemistry letters*, 23(10), pp.2962-2967.
- Schirmmacher, V., (2019). From chemotherapy to biological therapy: A review of novel concepts to reduce the side effects of systemic cancer treatment. *International journal of oncology*, 54(2), pp.407-419.
- Sonawane, M.D., Narwate, B.M. and Darandale, A.S., (2017). Review On Chemistry And Pharmacological Significance Of Triazole Derivatives. *International Journal of Pharmaceutics and Drug Analysis*, pp.161-176.
- Souza, R.O. and Miranda, L.S., (2019). Strategies towards the synthesis of N 2-substituted 1, 2, 3-triazoles. *Anais da Academia Brasileira de Ciências*, 91.



- Stewart, B.W., Bray, F., Forman, D., Ohgaki, H., Straif, K., Ullrich, A. and Wild, C.P., (2016).** Cancer prevention as part of precision medicine: 'plenty to be done'. *Carcinogenesis*, 37(1), pp.2-9.
- Sung, W.J., Kim, H. and Park, K.K., (2016).** The biological role of epithelial-mesenchymal transition in lung cancer. *Oncology reports*, 36(3), pp.1199-1206.
- Vitale, I., Galluzzi, L., Castedo, M. and Kroemer, G., (2011).** Mitotic catastrophe: a mechanism for avoiding genomic instability. *Nature reviews Molecular cell biology*, 12(6), pp.385-392.
- V Kouznetsov, V., Y Vargas-Mendez, L. and I Zubkov, F., (2016).** Recent Advances in the Synthesis of Bioactive Quinoline-Based 1, 2, 3-Triazoles via Cu-Catalyzed Huisgen 1, 3-Dipolar Cycloaddition ("Click Reaction"). *Mini-Reviews in Organic Chemistry*, 13(6), pp.488-503.
- Yadav, M.R., Murumkar, P.R. and Ghuge, R.B. (2018).** Vicinal Diaryl Substituted Heterocycles: A Gold Mine for the Discovery of Novel Therapeutic Agents. Elsevier.



Left Ventricular Myosin Heavy Chains Ratio after Administration of L-Name in Combination with Dexamethasone

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Abstract

The main goal of the study is to investigate the effect of Co-administration of Dexamethasone and N^G-Nitro- L-Arginine Methyl Ester (L-NAME) on the left ventricular myosin heavy chains (LVMHCs) redistribution and compare it with the cardiac MHCs redistribution caused by L-NAME treatment. 44 white adult Swiss mice CDI (weight range between 18g & 33g, and their ages range between 8-10 weeks) were used in this study. Animals were distributed randomly into four groups in a group of eleven as follows: Control group left without treatment during the whole period of the study, group I were given L-NAME 600mg/L in the drinking water and injected subcutaneously with dexamethasone phosphate 1mg/kg/day for 22 days, group II were given 600mg/L of L-NAME in the drinking water for 22 days, group III were injected subcutaneously with dexamethasone phosphate 1mg/kg/day for 22days. Myosin was extracted by minicing the left ventricular muscle in Laemmli buffer. Cardiac MHCs were separated with SDS-PAGE procedures. we found that; L-NAME treatment in the group II shifts α : β ratio towards β LVMHCs compared to control with P values 0.045 . This transition occurs without significant increase in HW/BW ratio (P values 0.171). Interestingly, dexamethasone treatment shifts α : β ratio towards β LVMHCs compared to control (P value 0.025) with a significant increase in the HW/BW ratio (P value 0.000). Remarkably, when

dexamethasone was injected in combination with L-NAME caused a significant increase in HW/BW ratio (P value 0.001) without a significant change in alpha or beta LVMHCs compared to control (P value 0.216). L-NAME treatment changed the LVMHCs from alpha to beta, and this changing occurs before left ventricular hypertrophy. While dexamethasone treatment changed the α : β ratio towards β LVMHCs with an increase in the HW/BW ratio. Finally when dexamethasone administrated in combination with L-NAME reverse the effect of L-NAME on the LVMHCs with an increase in the HW/BW ratio.

Key words: Myosin heavy chains, myosin isozymes, alpha myosin heavy chain, beta myosin heavy chain, Dexamethasone, and L-NAME

Introduction

Myosin is a mechanoenzyme molecule which converts the chemical energy stored as adenosin triphosphate (ATP) into mechanical energy (muscle contraction). The force and motion generated by the molecules of myosin occurs via cyclic interactions with actin molecules (Nikitina et al, 2008). There are 15 classes of myosin showed by phylogenetic analysis; class II myosin known as conventional myosin while the rest of myosin classes known as unconventional myosin (Sellers et al, 2000). The myosin present in the heart is the myosin class II which is also expressed in skeletal muscle, smooth muscle (Nikitina, et al 2008) and non muscular tissues (Sellers et al, 2000, Muhanad, et al 2019).

The five distinct components of the cardiac myosin molecules could be classified according to their electrophoretic mobility into A1 and A2 in the atria and V1, V2, and V3 in the ventricles (Hoh et al, 1978). These components have different calcium – activated myosin ATPase activity (Hoh et al, 1978). When the activity of the Calcium – activated myosin ATPase was analyzed; revealed that A1, A2 & V1 isozymes have about the same activity while V3 has the lowest activity and V2 has intermediate activity (Hoh et al, 1978). The V1($\alpha\alpha$) which has a much higher Calcium ATPase activity resembles



fast- twitch skeletal muscle myosin while V3 $\beta\beta$) behaves like slow – twitch skeletal muscle myosin (Pope et al, 1980).

The physiological factors affecting cardiac MHC isoforms redistribution are age and routine physical exercise. The effect of age on cardiac MHC isoforms redistribution is not associated with heart hypertrophy (Lompre et al, 1981). This redistribution depends on species; in mice and rats the amount of alpha MHC isoform increases with age, while the amount of beta MHC isoform decreases with age (Lompre et al, 1981). Similarly the distribution of MHC due to hypertrophy depends on type of exercise at least in rat. Hypertrophy after swimming exercise shows that MHC is shifted toward alpha while hypertrophy after running exercise is not associated with changes in the cardiac MHCs distribution ((Lompre et al 1981, Rupp et al 1989, and Rupp et al 1981).

Nitric oxide contributes to the pathogenicity of septic shock (Kiehl et al, 1997 and Petros et al, 1991); human septic shock is often characterized by considerable systemic vasodilation with low vascular resistance and hypotension refractory to treatment with vasopressors (Avontuur et al, 1998). Inhibition of NO production by competitive enzyme inhibitors like NG- Nitro-L-arginine methyl ester (L-NAME) can be used therapeutically to reverse hypotension in patient with severe septic shock (Kiehl et al, 1997 and Petros et al, 1991). Moreover, Dexamethasone is well known as anti-inflammatory drug (Sharon et al, 2009), so it can be used in the case of the septic shock. Furthermore, it has been documented that L-NAME & dexamethasone combination could be used clinically in the treatment of cases of pancreatitis (Sugiyama et al, 2005). Since a combination of L-NAME & Dexamethasone can be used in the clinical practice (human septic shock & pancreatitis), it is important to know the effect of the co-administration of these drugs on the left ventricular myosin heavy chains ration, and if there is a change should be investigated is it associated with cardiac hypertrophy or not?

Methods

This experimental study was approved by the Scientific Research Committee at Gezira university. The ethical approval was obtained from the medical faculty research committee and the Animal Care and Use Committee (ACUC) at Gezira University.

Animals grouping and treatment

Forty four white adult male and female Swiss mice CD1 of either sex were obtained from the animal house of the faculty of pharmacy university of Khartoum, weight ranged between 18g up to 33g and their ages ranged between eight to ten weeks, were used in this study. Animals were kept in different cages at room temperature and 12 hours light and dark cycles. Each cage lined with sawdust which was replaced every four to seven days to keep them fit and clean. Also, they had free access to food and water ad libitum. The mice were distributed randomly into four groups see table 1.:

Table 1: Study group, number, and treatment

Study group	No, of mice	treatment
Control	11	--
I	11	600 mg /L of L-NAME (Gokcimen et al 2007) in the drinking water and injected subcutaneously with dexamethasone phosphate 1mg/kg/day (Muangmingsuk et al 2000) for twenty two days
II	11	600 mg /L of L-NAME (Gokcimen et al 2007) in the drinking water for twenty two days.
III	11	subcutaneously with the dexamethasone phosphate 1mg/kg/day (Muangmingsuk et al 2000) for twenty two days

Extraction of the heart and MHCs preparation

Animals were sacrificed at the end of the experiment; body weight was recorded, the heart was removed, and flushed. The extra wetness was removed from the heart by blotting it with filter paper and weighted. Part of the left ventricular wall was excised and



chopped by scissor and sharp blade into fine pieces until the heart muscle was converted to paste like substrate (the process of chopping was performed on ice). The chopped heart muscle was mixed with Laemmli sample buffer (Laemmli UK 1970).

Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis (SDS-PAGE)

A non gradient large gel electrophoresis (VS20 maxi cleaver scientific) with parallel glass slabs 20*20 cm and 1 mm spacer were used . A very porous polyacrylamide resolving gel (3.8%) was used to obtain maximum separation of the two cardiac MHCs. The procedure belong to the Bio-rad protocol, which mentioned in the booklet supplied by the company itself.

The suitable voltage was continuous 120V per slab. After staining and destaining procedures, the gel was scanned with a computer scanner (Canon pixma MP 280) & Syngene documentation system with X ray light box instead of its ultraviolet box. The protein quantification analyzed with Total Lab Quantity computer program.

Identification of the myosin heavy chain bands

We believed that these bands separated on SDS-PAGEs which their migrations are similar to MHCs in the smooth muscle tissue because of their high molecular weight (around 200 KD) and they showed darkest coloration (highest concentration) among all protein bands in the specimen taken from heart muscle (figure 1). Also they have mobility on the SDS PAGE faster than standard protein with accuracy more than 95% (250 KD) and the high molecular weight protein filament of the chicken gizzard 250 KD (Wang K et al 1975). Moreover their mobility on SDS PAGE corresponds to the mobility of MHCs of the smooth muscle (figure 1).

Statistical analysis

The statistical analysis was done using the independent two samples T test of the Minitab computer program for analysis in the health science. The values were considered statistically significant when the P value was less than or equal to 0.05. The data were presented as mean \pm standard error of the mean (SE).

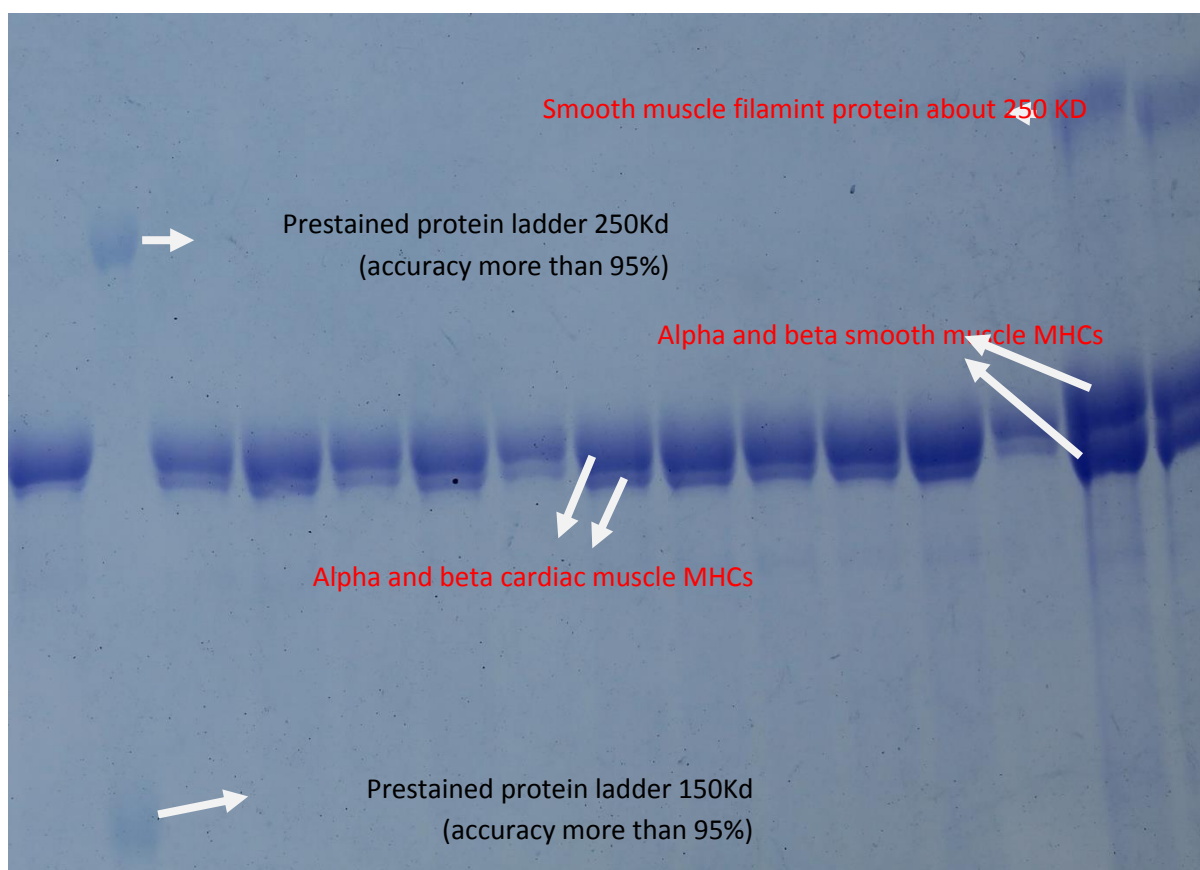


Figure 1: Identification of the MHCS band

RESULTS

Thirty four of forty four mice completed the experiments. The missed mice were dead, and they were from all of the study groups except the control group.

Observations during the experiment

All of the mice treated with L-NAME were fit during the whole period of the experiment, except for one mouse died (group II)

Five mice died from group I which was treated with a combination of L-NAME & dexamethasone phosphate. Worth-mentioning that; all of the dead animals were males, while the females looked fit during the whole period of the study. The last mouse died during the preparation of the killing material and instruments, but its data had been collected. Interestingly, each mouse in this group showed a blue tail & shape deformity two days before death.

Four mice died from group III which was injected with dexamethasone phosphate. The death in this group was two from each gender. The cause of death of the two females was an attack from a different animal. Four of the mice (one male & three females) that completed the study showed lower limb edema.

Degree of the heart hypertrophy

The degree of the heart hypertrophy was assessed by calculating the heart weight / body weight ratio (HW/BW ratio) and compared with the control group.

L-NAME treated group (group II) did not show significant change in the degree of heart hypertrophy. The HW/BW ratio was higher in group II (0.4400 ± 0.0075) compared to control group (0.4309 ± 0.0058) with P value 0.171 (Table 2 and fig2).

Dexamethasone treated group (Group III) showed a highly significant degree of heart hypertrophy. HW/BW ratio was higher in group III (0.4843 ± 0.011) compared to control group (0.4309 ± 0.0058) with P value 0.000. Even when dexamethasone was injected in combination with L-NAME (group I) also produced a highly significant increase in the

degree of heart hypertrophy. The HW/BW ratio was elevated in group I (0.4914 ± 0.02) compared to control group (0.4309 ± 0.0058) with a P value 0.001 (table 2 and fig 2).

Table 2:- Heart weight/ Body weight ratio for each study group. *= P ≤ 0.02**

Experimental group	Number	mean ± SE	P value
Control	11	0.4309 ± 0.0058	-----
I	7	0.4914 ± 0.02	0.001 ***
II	10	0.44 ± 0.0075	0.171
III	7	0.4843 ± 0.011	0.000 ***

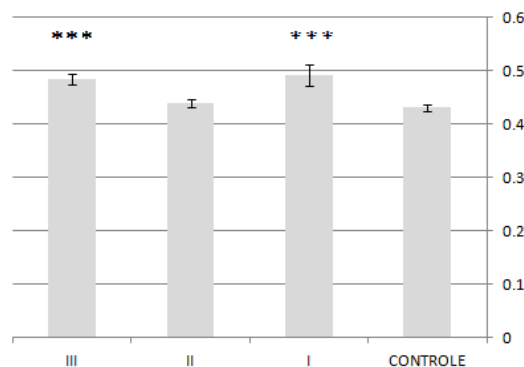


Figure 2: Difference in the HW/BW ratio for each study group. *= P ≤ 0.02**

As noted in table 3 and figure 3 , there was no significant change in the Heart weight / Body weight ratio between: group I VS group III with P values 0.761.

Table 3: Comparison between the study groups I & III on the degree of the heart hypertrophy.

<i>Experimental group</i>	<i>Mean ± SE</i>	<i>P value</i>
I	0.4914 ± 0.02	0.761
III	0.4843 ± 0.011	

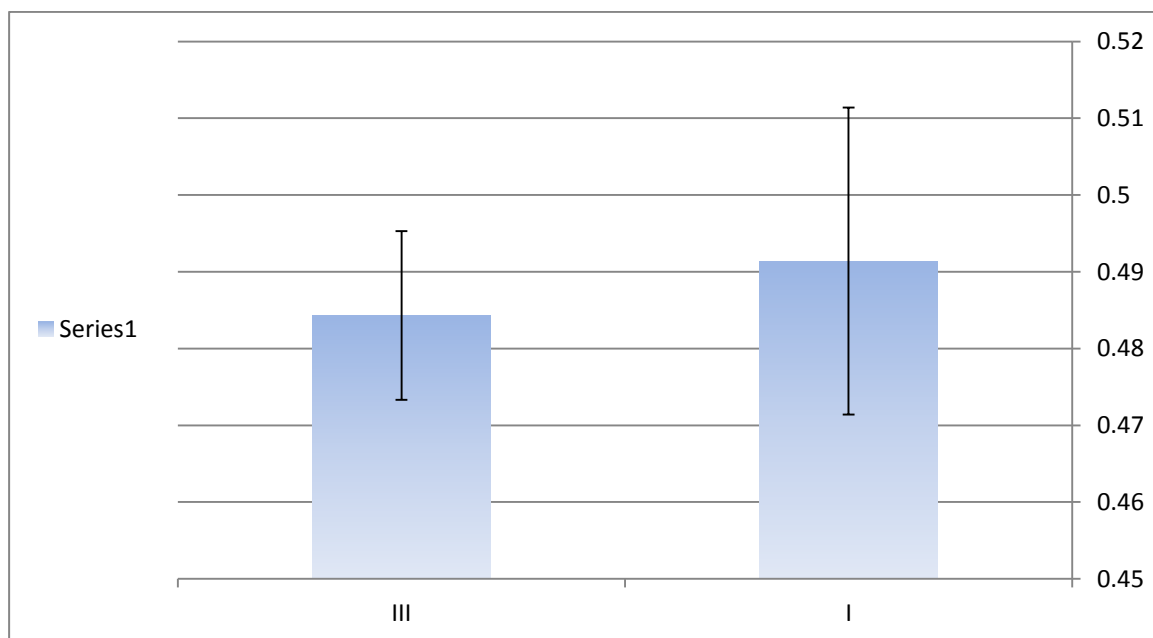


Figure3: Comparison between group I VS group III on the degree of the heart hypertrophy.

Myosin Heavy Chains redistribution after treatment with different drugs

The effect of L-NAME, Dexamethsone, and a combination of L-NAME & Dexamethasone on the redistribution of the Left Ventricular Myosin Heavy Chains (LVMHCs) has been detected.



The MHCs has been classified according to their electrophoretic migration into slow migrating MHC & fast migrating MHC which correspond to Alpha & Beta MHCs respectively. The L-NAME treated mice (group II) showed a lower percentage of the slow migrating LVMHCs compared to control group. The slow migrating LVMHCs bands had means of 72 ± 0.96 in group II compared to 74.92 ± 1.3 in the control group with P value 0.045. Therefore, L-NAME treated mice (group II) showed a higher percentage of the fast migrating LVMHCs compared to the control group. The fast migrating LVMHCs bands have means of 28 ± 0.96 in group II compared to 25.08 ± 1.3 in the control group with P value 0.045 (table 4 and fig 4).

The Dexamethasone treated mice (Group III) showed a lower percentage of the slow migrating LVMHCs compared to control group. The slow migrating LVMHCs bands had means of 70.56 ± 1.6 in group III compared to 74.92 ± 1.3 in the control group with P value 0.025. Also group III produced a higher percentage of fast migrating LVMHCs compared to control group. The bands of the fast migrating LVMHCs have means of 29.44 ± 1.6 in group III compared to 25.08 ± 1.3 control group with P value 0.025 (table 4& fig 4).

Remarkably, when the dexamethasone was injected in combination with L-NAME, it reversed the effect of L-NAME in the redistribution of the left ventricular myosin heavy chains. There was no change in the slow migrating LVMHCs bands which has a mean of 73.54 ± 0.68 in group I compared to 74.92 ± 1.3 in the control group with P value 0.216. Similarly the fast migrating LVMHCs bands had a mean of 26.46 ± 0.68 compared to 25.08 ± 1.3 of the control with a P value 0.216 (table 4& fig 4).

Table 4: Slow migrating LVMHCs percentage in different study groups. *= $p \leq 0.05$, and ** = $P \leq 0.030$

Experimental group	number	Mean \pm SE	P value
Control	11	74.92 \pm 1.3	-----
I	7	73.54 \pm 0.68	0.216
II	10	72 \pm 0.96	0.045 *
III	7	70.56 \pm 1.6	0.025 **

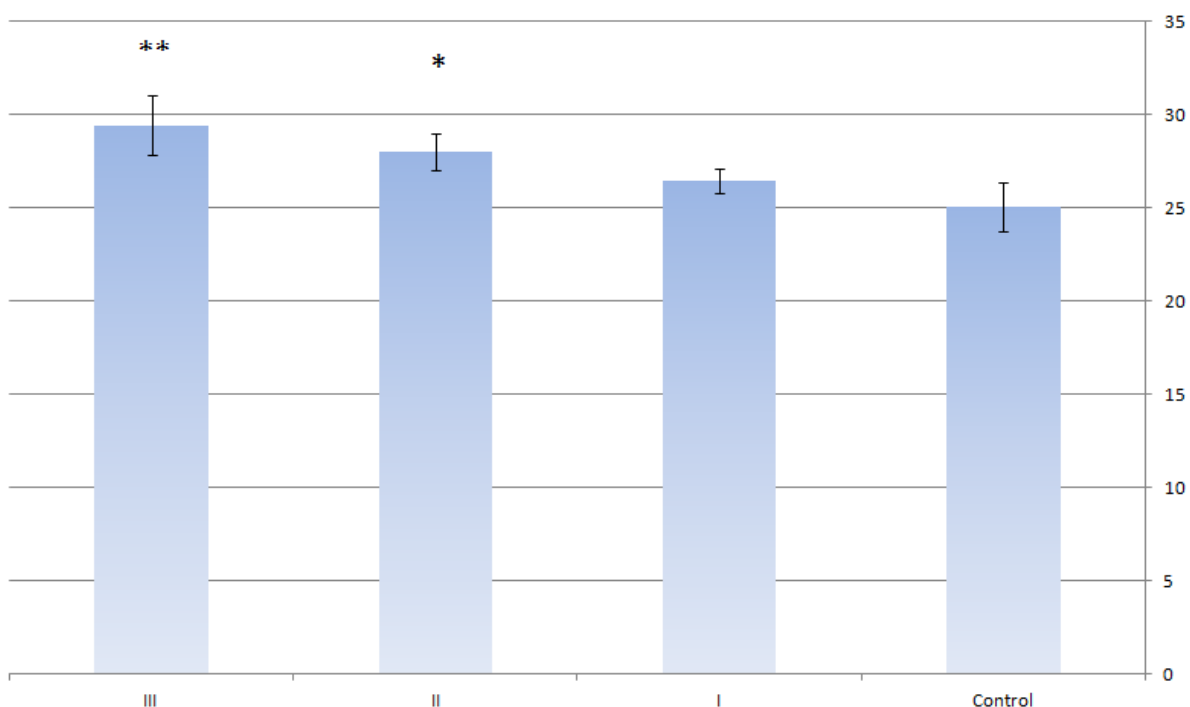


Figure4: Fast migrating LVMHCs percentage in different study groups. *= $p \leq 0.05$, and ** = $P \leq 0.030$.

As noted in table 5 and figure 5 there was no significant change in the slow migrating LVMHCs between; group I VS II with P values 0.126. Similarly, there were no

significant change in the fast migrating LVMHCs between group I VS group II with P values 0.126

Table 5: Comparison between the experimental groups on the percentage of the slow migrating LVMHCs.

<i>Experimental group</i>	<i>Mean ± SE</i>	<i>P value</i>
I	73.54 ± 0.68	0.126
II	72 ± 0.96	

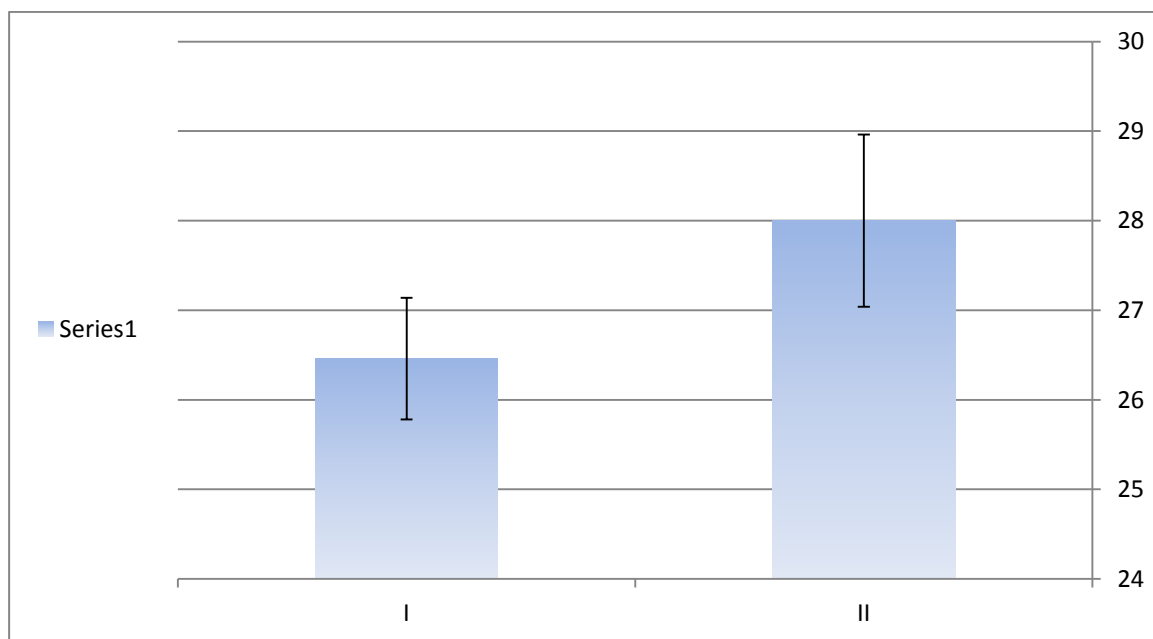


Figure 5: Comparison between group M VS Group D on the percentage of the fast migrating LVMHCs.



Discussion

This project expresses the effect of co-administration of L-NAME & dexamethasone on the left ventricular MHCs, and whether or not their effect are associated with a change in the degree of heart hypertrophy. It is important to see this effect since L-NAME & dexamethasone could be used clinically in the treatment of cases with septic shock or pancreatitis. Sugiyama et al, 2005 showed that; both dexamethasone and L-NAME suppress the severity of pancreatitis induced by Caerulein administration, and the effect of L-NAME compared with dexamethasone is more potent against mild pancreatitis but less so against severe pancreatitis. This phenomenon could be due to the effect of L-NAME on the peripheral resistance, and the anti-inflammatory effect of the dexamethasone. Taking into account that cardiac muscle contraction requires interaction between myosin and actin molecules, and the myosin molecule consists of two heavy chains and four light chains (McNally et al 1989). Worth-mentioning that; the mechanical properties of the cardiac muscle depends on types of MHCs (Hoh et al 1978).

Treatment with L-NAME did not cause a significant change in the HW/BW ratio with a P values 0.171 in group II L-NAME treated mice (see Table 2). Some studies disagreed with our result like, Hropot et al 1994 who showed that treatment with L-NAME causes hypertension, cardiac hypertrophy and renal insufficiency. This fact could be due to a short duration of our experiment.

Dexamethasone treated group (group III) showed a highly significant degree of heart hypertrophy. HW/BW ratio was higher in group III compared to control group with a P value 0.000 (fig 2). Even when dexamethasone injected in combination with L-NAME also produced a highly significant increase in the HW/BW ratio compared to control group with a P value 0.001(Fig 2, Table 2). This results is in agreement with La Mear NS et al 1997 who demonstrated that; treatment with dexamethasone increases the HW/BW



ratio with the elevation of the total protein content, actin content, and total protein to total DNA ratio.

As showed in table 3 there was no significant difference in the HW/BW ratio between mice treated with a combination of dexamethasone & L-NAME (group I) vs mice treated with dexamethasone (group III) with P values 0.761 . This phenomenon might be because of the short duration of our experiment that did not allow L-NAME to cause its effect on the HW/BW ratio.

Since both L-NAME & dexamethasone can be used in the treatment of pancreatitis, we want to investigate the effect of their combination on the left ventricular myosin heavy chain. Does dexamethasone counteract the effect of L-NAME on the MHCs redistribution? It was found that L-NAME treated mice (group II) show a lower percentage of the left ventricular α MHC compared to control with P values 0.045, and a higher percentage of the left ventricular β MHC compared to control with P values 0.045 (table 4 and fig 4). Thus L-NAME treatment caused a shifting in the MHCs ratio toward β MHC. This is in agreement with Zhang Y. et al 2003, and Zhao Y. et al 2006 who reported cardiac MHCs shift toward β MHC at the nuclear level in L-NAME treated animals, also they showed this transition occurs even in the absence of left ventricular hypertrophy. Furthermore, the group of mice treated with dexamethasone (group III) showed a lower percentage of the left ventricular α MHC, and a higher percentage of the left ventricular β MHC compared to control with P value 0.025 (table 4 & fig 4). This observation is in disagreement with Muangmingsuk et al (2000) who reported that; treatment with dexamethasone caused heart hypertrophy with the shifting of the myosin heavy chain ratio towards alpha. This difference between the result in our hand and Muangmingsuk S et al 2000 could be due to species differences used in the experiment, in which our experiment used Swiss mice CD1, while Muangmingsuk S et al 2000 used rats in their experiment. Remarkably, when dexamethasone injected in combination with



L-NAME reverse the effect of L-NAME in the redistribution of the left ventricular myosin heavy chains. There was no significant change in the alpha or beta left ventricular myosin heavy chain when the animal treated by Dexamethasone in combination with L-NAME with a P value 0.216 (Table 4, and Figure 4).

In the present study treatment with L-NAME or dexamethasone caused changes in the MHCs redistribution toward fast migrating MHC (corresponding to β MHC), and this effect seems to be the same as most of the pathological factors effect in animal. Rupp 1981 showed in the case of cardiac hypertrophy due to hypertension because of renal causes (Goldblatt II) there was a shift of the ventricular myosin isozyme toward native V3 ($\beta\beta$) myosin isoform. This is reinforced by the observation of Yazaki et al 1989 where they demonstrated the left ventricular hypertrophy was evident from 3 days after pressure loading and the isozymic transition of myosin heavy chain from the slow migrating alpha MHC isoform to the fast migrating beta MHC isoform was detected within 24 hours after aortic constriction. Thus pressure overload causing MHC shift toward fast migrating beta MHC isoform. Does shifting of MHCs toward beta is mechanically beneficial to heart under this condition? Some investigators found that heart muscle containing large proportion of beta MHC became more economical (Narolska et al 2005). As noted in table 5, fig 5 there was no significant change in the alpha or beta left ventricular myosin heavy chains between group I VS group II with P values 0.126.

Interestingly, all of the mice that died during the experiment were males, while the females looked fit during the whole period of the experiment. We suggest the presence of male sex hormone interacting with the administrated drugs. Furthermore, each mouse died in the group I (treated with a combination of dexamethasone and L-NAME) showed a blue tail & shape deformity for two days before death. This phenomenon could be due to the potent immunosuppressant properties and the high rate of gluconeogenesis of



dexamethasone. Remarkably, four mice from group III (dexamethasone treated mice) showed lower limb edema. we suggested that, the left ventricular hypertrophy caused by dexamethasone lead to right heart failure which in turn lead to lower limb edema.

In light of our findings concerning the administration of L-NAME and dexamethasone on the redistribution of the LVMHCs, we suggested that; usage of these drugs on humans could not affect the LVMHCs ratio because human ventricles contain predominantly β MHCs (Reiser PJ et al 2001).

Conclusion

L-NAME treatment changed the LVMHCs from alpha to beta, and this change occurs before left ventricular hypertrophy. While dexamethasone treatment changed the α : β ratio towards β LVMHCs with an increase in the HW/BW ratio. Finally when dexamethasone was administrated in combination with L-NAME, dexamethasone counteract the effect of L-NAME on the LVMHCs with an increase in the HW/BW ratio was noted.

References

- Avontuur JA, Buijk SL, Bruining HA. (1998). Distribution and metabolism of N(G)-nitro-L-arginine methyl ester in patients with septic shock. *Eur J Clin Pharmacol*; 54:627-31.
- Avontuur JA, Tutein Nolthenius RP, van Bodegom JW, Bruining HA. (1998). Prolonged inhibition of nitric oxide synthesis in severe septic shock: a clinical study. *Crit Care Med.*, 660-7.
- Gokcimen A, Kocak A, Kilbas S, Bayram D, Kilbas A, Cim A, Kockar C, Kutluhan S. (2007). Effect of lisinopril on rat liver tissues in L-NAME induced hypertension model. *Mol Cell Biochem*; 296:159-64.
- Hoh JF, McGrath PA, Hale PT. (1978). Electrophoretic analysis of multiple forms of rat cardiac myosin: effects of hypophysectomy and thyroxine replacement. *J Mol Cell Cardiol.*;10:1053-76.

- Hropot M, Grötsch H, Klaus E, Langer KH, Linz W, Wiemer G, Schölkens BA. (1994).** Ramipril prevents the detrimental sequels of chronic NO synthase inhibition in rats: hypertension, cardiac hypertrophy and renal insufficiency. *Naunyn Schmiedebergs Arch Pharmacol.*;350:646-52.
- Kiehl MG, Ostermann H, Meyer J, Kienast J. (1997).** Nitric oxide synthase inhibition by L-NAME in leukocytopenic patients with severe septic shock. *Intensive Care Med.*;23:561-6.
- La Mear NS(1), MacGilvray SS, Myers TF. (1997).** Dexamethasone-induced myocardial hypertrophy in neonatal rats. *Biol Neonate.*;72(3):175-80.
- Laemmlli UK. (1970).** Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.*;227:680-5.
- Lompre AM, Mercadier JJ, Wisnewsky C, Bouveret P, Pantaloni C, D'Albis A, Schwartz K. (1981).** Species- and age-dependent changes in the relative amounts of cardiac myosin isoenzymes in mammals. *Dev Biol.*;84:286-90.
- McNally EM, Kraft R, Bravo-Zehnder M, Taylor DA, Leinwand LA. (1989).** Full-length rat alpha and beta cardiac myosin heavy chain sequences. Comparisons suggest a molecular basis for functional differences. *J Mol Biol.*;210:665-71.
- Muangmingsuk S, Ingram P, Gupta MP, Arcilla RA, Gupta M. (2000).** Dexamethasone induced cardiac hypertrophy in newborn rats is accompanied by changes in myosin heavy chain phenotype and gene transcription. *Mol Cell Biochem.*;209:165-73.
- Muhanad S.A, Mukhallad AM, Abdelwhab HM, Mansour AB, Mazin SA, (2019).** Cardiac Myosin Heavy Chains. *Cardiology and Angiology: An International Journal*, 8(1), 1-7. <https://doi.org/10.9734/CA/2019/46305>
- Narolska NA, Eiras S, van Loon RB, Boontje NM, Zaremba R, Spiegelen Berg SR, Stoker W, Huybregts MA, Visser FC, van der Velden J, Stienen GJ. (2005).** Myosin heavy chain composition and the economy of contraction in healthy and diseased human myocardium. *J Muscle Res Cell Motil.*;26:39-48.
- Narolska NA, van Loon RB, Boontje NM, Zaremba R, Penas SE, Russell J, Spiegelenberg SR, Huybregts MA, Visser FC, de Jong JW, van der Velden J, Stienen GJ. (2005).** Myocardial contraction is 5-fold more economical in ventricular than in atrial human tissue. *Cardiovasc Res.*;65:221-9.
- Nikitina LV, Kopylova GV, Shchepkin DV, Katsnel'son LB. (2008).** Assessment of the mechanical activity of cardiac isomyosins V1 and V3 by an in vitro motility assay with regulated thin filaments. *Biofizika.*;53:956-61.
- Petros A, Bennett D, Vallance P. (1991).** Effect of nitric oxide synthase inhibitors on hypotension in patients with septic shock. *Lancet.*;338:1557-8.
- Pope B, Hoh JF, Weeds A. (1980).** The ATPase activities of rat cardiac myosin isoenzymes. *FEBS Lett.*;118:205-8.
- Reiser PJ, Portman MA, Ning XH, Schomisch Moravec C. (2001).** Human cardiac myosin heavy chain isoforms in fetal and failing adult atria and ventricles. *Am J Physiol Heart Circ Physiol.*;280(4):H1814-20.



- Rupp H. (1981).** The adaptive changes in the isoenzyme pattern of myosin from hypertrophied rat myocardium as a result of pressure overload and physical training. *Basic Res Cardiol.*;76:79-88.
- Rupp H. (1989).** Differential effect of physical exercise routines on ventricular myosin and peripheral catecholamine stores in normotensive and spontaneously hypertensive rats. *Circ Res.*;65:370-7.
- Sellers JR. (2000).** Myosins: a diverse superfamily. *Biochim Biophys Acta.*;1496:3-22.
- Sharon L.H. Ong, YI Zhang and Judith A. (2009).** Whitworth, Mechanism of dexamethasone induced hypertension current hypertension reviews, (5): 61-74.
- Sugiyama Y, Kato S, Abe M, Mitsufuji S, Takeuchi K. (2005).** Different effects of dexamethasone and the nitric oxide synthase inhibitor L-NAME on caerulein-induced rat acute pancreatitis, depending on the severity. *Inflammopharmacology.*;13:291-301.
- Wang K, Ash JF, Singer SJ. (1975).** Filamin, a new high-molecular-weight protein found in smooth muscle and non-muscle cells. *Proc Natl Acad Sci U S A.*;72:4483-6.
- Yazaki Y, Tsuchimochi H, Kurabayashi M, Komuro I. (1989).** Molecular adaptation to pressure overload in human and rat hearts. *J Mol Cell Cardiol. Suppl 5*:91-101
- Zhang Y, Carreras D, de Bold AJ. (2003).** Discoordinate re-expression of cardiac fetal genes in N(omega)-nitro-L-arginine methyl ester (L-NAME) hypertension. *Cardiovasc Res.*;57:158-67.
- Zhao Y, Bell D, Smith LR, Zhao L, Devine AB, McHenry EM, Nicholls DP, McDermott BJ. (2006).** Differential expression of components of the cardiomyocyte adrenomedullin/intermedin receptor system following blood pressure reduction in nitric oxide-deficient hypertension. *J Pharmacol Exp Ther.*;316:1269-81.



Prevalence of Resistant *H. pylori* to Clarithromycin and Metronidazole in Central Hospitals, Gezira State, Sudan

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Abstract

Infections with H. pylori affect more than 50% of the world's population. The estimated lifetime risk of gastric ulcer is 20% and the estimated lifetime risk of stomach cancer is 1-2%. Clarithromycin- and metronidazole-resistant H. pylori strains pose a global public health problem. These strains usually carry mutations within the 23S rRNA gene associated with clarithromycin and metronidazole resistance. The aim of this study was to detect mutations associated with H. pylori and clarithromycin and metronidazole resistance in Sudanese patients from Gezira state. A cross-sectional study was conducted at central hospitals (Rufaa, Hassaheisa, and Wadmedani) in Gazira state, Sudan, with the aim of estimating the prevalence of H. pylori infection in randomly selected individuals. For this purpose, 334 faecal samples were collected and tested. Another goal was to detect the presence of H. pylori DNA. While concurrently detecting mutations associated with resistance to clarithromycin and metronidazole. Gastric biopsy was taken from each enrolled patient by endoscopy. Molecular detection of resistance genes to metronidazole (rdx gene) and clarithromycin was done by TaqMan real-time PCR due to this 80 biopsy samples were taken and tested. Three hundred and thirty four patients, 197 (58.9%) males and 137 (41.01%) females participated. The prevalence of H. pylori infection was 73.7% (246/334). Eighty patients, 45 male (56.25%) and 35 female (43.75%) involved. Of the 80 samples, H. pylori was detected in 56 (~70%) samples by 16's RNA. Allele-specific PCR detected the A2142G variant in 32/56 (~57.14%) of the samples and the A2143G mutation in 25/56 (~44.64%). Variant detected by allele-specific PCR Mtz(rdxAS131) in 34/56 (~60.71%) sample, while Mtz (rdxAS59) mutation was found in 26/56 (~44.42%) Eradication of H. pylori has become difficult due to global increasing of its antimicrobial resistance. This study revealed a high frequency (32% and 25%) of

mutations associated with clarithromycin resistance (A2142G and A2143G) respectively and metronidazole (34% and 26%) of mutations associated with metronidazole resistance (*rdxAS131* and *rdxAS59*) respectively. This information should be taken into consideration to avoid eradication therapy failure. Continuous surveillance for detection of this resistance should be done to help physicians in selection of optimum eradication therapy.

Keywords: Helicobacter Pylori, Clarithromycin, Metronidazole, Resistance, PCR, Prevalence , Molecular testing

Introduction

H. pylori is a Gram-negative, spiral-shaped, microaerophilic bacterium. It is one of the most common bacterial infections worldwide and is believed to be the cause of chronic active gastritis (Nashaat *et al.*, 2010). *H. pylori* infection is considered one of the most common chronic bacterial infections in humans, affecting more than half of the world's population. Overall prevalence is high in developing countries. Although this is a global problem, prevalence varies by country (Naous *et al.*, 2007). *H. pylori* infection is acquired in early childhood and becomes a chronic infection if left untreated (Alvarado-Esquivel, 2013). The majority of infected individuals remain asymptomatic, and usually only a few develop symptoms in adulthood (Wasiam *et al.*, 2007). *H. pylori* causes upper gastrointestinal diseases such as gastritis, peptic ulcer disease, and also increases the risk of stomach cancer (Naous *et al.*, 2007; Wasiam *et al.*, 2007). *H. pylori* is most commonly found in the gastrointestinal tract and is associated with chronic gastritis, gastroduodenal ulcers, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma (MALT). *H. pylori* are transmitted by the following routes: person-to-person, oral-oral, or fecal-oral, and vertical transmission of *H. pylori* through contaminated water consumption and breastfeeding can also occur. Most people infected with *H. pylori* remain asymptomatic (Alvarado-Esquivel, 2013). Because *H. pylori* prevalence is closely related to socioeconomic conditions, infection is more common in developing countries than in developed countries (Wasiam *et al.*, 2007). *H. pylori* prevalence is associated with low socioeconomic status, low household income, educational attainment, living in overcrowded housing, contaminated

drinking water sources, lifestyle habits, smoking, and the risk of alcohol consumption for *H. pylori*. Differences in infection rates between populations suggest that parameters such as age, cultural background, genetic predisposition, socioeconomic status, and environmental factors all play a role in organism acquisition and transmission. . Improving living conditions, including adequate sanitation, drinking water and improved basic hygiene, balanced diet, and avoidance of overcrowding are key to *H. pylori* infection (Cardaropoli *et al.*, 2014). Introduction of a molecular approach to *H. pylori* antibiotics Resistance testing was facilitated by the fact that the mechanism of action of clarithromycin is well understood and is due to several point mutations in the peptidyltransferase region of domain V of the 23S rRNA gene. *H. pylori* have two copies of this gene, and the mechanism of clarithromycin resistance appears to impede macrolide action by reducing macrolide ribosomal binding and inhibiting protein biosynthesis. . Primary resistance to metronidazole in *H. pylori* is very high in many countries, with reported rates ranging from 10% to 70% (Canton *et al.*, 2001). There are conflicting views about the clinical significance of Metronidazole resistance in eradication; nevertheless, there is growing evidence that resistance significantly reduces the efficacy of metronidazole-containing regimens. Increased rates of resistance have been attributed to repeated administration of metronidazole in regimens that only partially inhibit metronidazole in the treatment of non-*H. Pylori* infections, leading to the selection of resistance to *H. pylori* (Buckley *et al.*, 1997; Houben *et al.*, 1999). The main objective is To investigate the prevalence of *H. pylori* infection and genetic factors associated with its resistance to treatment in central Hospitals, Gezira state, Sudan.

Materials and Method

Study Design

This cross-sectional hospital-based study carried out between December 2019 up to June 2022 among patients attending the outpatient clinic in central hospitals in Gazira State Sudan.



Study Area and Population

The study was conducted in Central hospitals (Rufaa, Hasaheisa and Wadmedani Hospitals) Gezira State Sudan which is the biggest state in the middle of Sudan. With population of 2,796,329 referenced by Wikipedia 2018 with an area of about 10.387 square miles specking the localities. Patients with age less than 20 years were excluded. Likewise, the patients with history of long-term drugs known to cause gastritis such as NSAIDs, steroids, anticoagulants, and lesions suggestive of malignancy on endoscopy were excluded. All patients presented to the outpatients with/without GIT symptoms were included in this study.

Data Collection

Stool sample was collected from 334 patients in wildly mouth containers and data were collected by a structural interview questionnaire of all subjects. For molecular study tissue biopsy were collected from 80 patients.

Preparation of Genomic DNA from Gastric Biopsy Samples

Vivisection samples were taken from the gastric antrum. Genomic DNA was uprooted from gastric vivisection samples using WizPrep gDNA MiniKit (cell/ towel) from WIZBIOSOLUTIONS, Korea) , following manufacturer guidelines. Briefly, a gastric vivisection sample (10 mg) was placed in an eppendorf tube with 50 μ L absolute alcohol.

Each sample was taken and centrifugated for 5 min to precipitate towel and proteins. Supernatant discarded and 200 μ l of GT1 added and homogenized the sample towel by vortexing. 200 μ l of GT2 were added to the sample and 20microlitter of proteinase K prepared well by vortexing kept for night in 56 °C. Absolute alcohol 200 μ l were added and mixed well by vortexing.

Sample transferred to spin column and centrifugated for 2 min at 14000 rpm. Filtrate was kept with deposit produced from first centrifugation. 500 μ l of W1 added to the spin column centrifugated for 1 min at 13000 rpm. Filtrate discarded and spin column reconnected. 700 μ l W2 added to

the spin column centrifugated for 1 min at 13000 rpm. Spin column centrifugated for 2 min at 13000 rpm. Spin column and new 1.5 ml tube were connected. 50- 100 µl of Elution Buffer added and incubated at room temperature for 1 min and also centrifugated for 1 min at 13000 rpm. Spin column discarded and eluted purified DNA used for coming step. Eluted DNA stored at -20 °C.

Detection of *H. Pylori* 16S rRNA Gene

Two manual sets were used for the discovery of the bacteria, targeting 16S rRNA (532bp), uprooted DNA was used to amplify the universal 16S rRNA gene using the following manuals (manuals F5' AGAGTTTGATCCTGGCTCAG-3') (R5'CTACGGCTACCTTGTTACGA 3'). PCR modification was carried out with the HOT FIREP PCR mix master Mix (SOLIS BIODYNE). The temperature cycle for the PCR was Carried out using protocol for modification of 16S rRNA as follows original activation at 95 °C for 3 twinkles, followed by 35 cycles at 95 °C for 20s, 55 °C for 30s, and 72 °C for 45s, and a final extension at 72 °C for 2 twinkles. To descry the DNA, 10 µl of each PCR products was loaded onto 1.5 agarose gels stained with 20 µl ethidium platitude (10 mg/ ml) and subordinated to electrophoresis in 1x Tris EDTA Buffer (TEB buffer) (89 mM of Tris base, 89 mM Boric acid, and 2 mM EDTA dissolved in 1 waste H₂O) for 20 min at 120 V and 50 mama. The gel was imaged under UV light illumination. A 100 bp DNA graduation (aps labs Company, Maharashtra, India) was used in each gel as a molecular size standard. The amplified product for the specific rrs gene is 522 bp.

Detection of 23S rRNA Gene Conferring Clarithromycin Resistance

Allele-specific PCR was used for the discovery of A2142G and A2143G point mutations using four manuals called FP- 1, RP- 1, RP2142G, and FP2143G (Table 1). When the strain is wild type (wt), neither RP2142G nor FP2143G anneals with the template and polymerase chain response (PCR) modification proceeds between FP- 1 and RP- 1, performing in a 320 bp amplicon. In the case of the presence of A2142G mutation, the PCR modification primarily takes place between FP1 and RP2142G, which



results in an amplicon of 238 bp. Also, in the case of the A2143G mutation, the PCR modification goes between FP2143G and RP- 1, performing in an amplicon of 118 bp.

Discovery of 23S rRNA Gene Conferring Clarithromycin Resistance

The real- time (Applied Biosystems, USA) response admixture Per sample comported of 15.75 µl distilled water, 2.5 µl PCR buffer(10x), 0.5 µl dNTPs(10mM), 1µl each of PF(10 µM), PR(10 µM), A2142G RP(10 µM) and A2143G FP(10 µM) and 0.25 µl Taq polymerase(5U/ µl). The PCR response used was an original step of 2 min at 95 °C, followed by denaturation for 30 sec at 95 °C, annealing for 1 min at 56 °C, and manual extension for 45 sec at 72 °C. After the 40th cycle, the final extension step was dragged for 2min to complete the conflation of beachfront.

Detection of Metronidazole Resistant Genes

Multiplex PCR assay was performed to descry Metronidazole resistance genes (rdxAS59-F and rdxAS131F) using specific manuals.

PCR modification involved original denaturation at 950C for 5 min, followed by 40 cycles of denaturation at 950C for 30s, annealing at 540C for 1 min, extension at 720C for 1 min and a final at 720C for 5 min. Allele-specific PCR was used for the discovery of rdxAS59- F and rdxAS131- F point mutations using four manuals called rdx- R, rdx- F, rdxAS59- F, and rdxAS131- F(Table 1). When the strain is wild type(wt), rdxAS59- F, nor rdxAS131- F anneals with the template and polymerase chain response(PCR) modification proceeds between FP- 1 and RP- 1, performing in a 886bp amplicon. In the case of the presence of rdxAS59-F mutation, the PCR modification primarily takes place betweenFP1and rdxAS59- F, which results in an amplicon of 681 bp. also, in the case of the mutation, the PCR modification goes between rdxAS131- F and RP- 1, performing in an amplicon of 463 bp

Table (1): Primers sequences used for detection of *H.pylori*, metronidazole and clarithromycin resistant genes

Primer name	Sequence
Hp23s-FP-3	CTCCATAAGAGCCAAAGCCCTTAC
Hp23s-RP-2	GAAGGTTAAGAGGATGCGTCAGTC
HP23s2143G	CCGCGGCAAGACAGAGA
HP23s2142G	AGTAAAGGTCCACGGGGTATTCC
rdx-R	GCAGGAGCATCAGATAGTTCT
rdx-F	GGGATTTTATTGTATGCTACAA
rdxAS59-F	GCATTTTGTGATGGTACTG
rdxAS131-F	TCAACCACAGCATGCAAAG

Data Analysis

Statistical Package for Social Sciences program (SPSS Inc., Chicago, IL, USA) version 20 was used for data entry and for analysis of the patients demographic characteristics; Laboratory findings of *H. pylori* infection and demographic data were analyzed by simple descriptive statistics. *p* value less than 0.05 were statistically significant.

Ethical Approval

The ethical approval was obtained from the ethical committee faculty of medicine, Al-Butana University.

Results

Eradication of *H. pylori* has come difficult due to global adding of its antimicrobial resistance. Nonstop surveillance for discovery of this resistance should be done to help croakers in selection of optimum eradication remedy (24), (25). This study showed the frequency of *H. pylori* infection to be 73.7, as a high frequency. In the current study more males 131(53.3)



were positive for *H. pylori* infection compared to ladies 115 (46.7) this was statistically significant ($p = 0.000$). This study illustrated that there were 151(61.4) of *H. pylori* cases included in this study from pastoral areas, while 95 (38.6) of them were from civic areas. Regarding the age distribution it was set up that, utmost of the positive *H. pylori* subjects 97(39.4) were ranged in age group of 20- 30 times while 63(25.6) of them were ranged between 31- 40 times, 61 (24.8) of them were ranged further than 50 times and 25 (10.2) of them were ranged between 41- 50 times, it was statistically significant ($p = 0.000$). The standard of education is a strong index of socioeconomic class [30]. In our study there was association between *H. pylori* infection and the position of education and it was statistically significant($p = 0.000$). There was no association between *H.pylori* frequency with employment status and occupancy (civic vs pastoral). In the present study, there were high *H. Pylori* infection frequency in individualities who participated a bed with their family members, 201(81.7) compared to those who didn't 45(18.3) ($p = 0.000$).*H. pylori* infection was high with the use of a hole latrine 176 (71.5) of *H. pylori* cases and the association of *H. pylori* infection with the type of restroom was insignificant ($p=0.533$). In term of party occupation, it was set up that 69 (28) of *H.pylori* cases included in this study were workers, while 16(6.5) of them were employers, while 43(17.5) of them were Dealers, while 96(39) of them were House women and while 22 (8.9) of them were scholars a statistically significant difference was set up between *H. pylori* and occupation of actors ($p=0.018$). Unexpectedly, this study had detected antithetical results about the relation between *H. pylori* and smoking. We set up no statistically significant difference between smokers, non-smokers in the positivity rate of *H. pylori* ($P = 0.896$). Interestingly, in the present study, we set up that non-drinkers displayed a significantly advanced rate of *H. pylori* infection compared with alkie, demonstrating a negative association, which is analogous to preliminarily reported studies.

In the current study, *H. pylori* infection was detected in 56(70) of gastric necropsies of enrolled cases. Concerning symptoms and endoscopic

finding in *H. pylori* infected cases, severe verbose gastritis with duodenitis 131(53.6), moderate gastritis 52 (21.4), severe gastritis 44 (17.9), gastritis 8 (3.6) GERD with gastro duodenitis 4(1.8), and verbose nodular hyperplasia 4(1.8) were the most frequent and they were significantly associated with discovery of *H. pylori* infection as shown in figure 1.

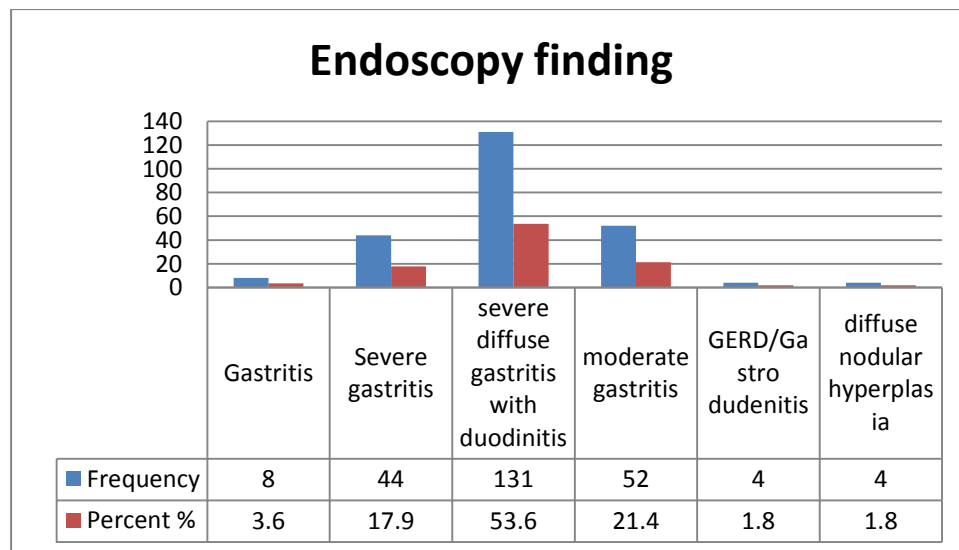


Figure (1): Endoscopy finding

In the current study, mutations in the rdxAS59 gene were detected in 26 (46.4) *H. pylori*-infected cases, of which 12 were male (46.2) and 14 were female (53.8). Mutations in the rdxAS131 gene were also detected in 34 (60.7) *H. pylori*-infected cases, including 21 males (61.8) and 13 females (38.2), as shown in the figure 2.

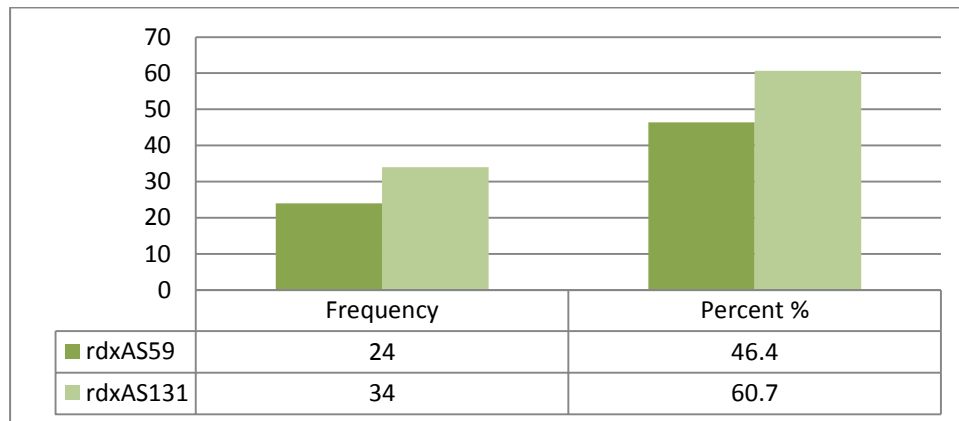


Figure (2): Detection of rdxAs59 and rdxAs131 points mutations by allele-specific PCR

In this study, the A2143G and A2142G mutations were identified in *H. pylori* clarithromycin-resistant strains based on the PCR-RFLP assay. The A2142G mutation was detected in 32 individuals (57.1), 22 of whom were male (68.8) and 10 were female (31.2). As shown in Figure 3, the A2143G mutation was detected in 25 individuals (44.6), 13 of whom were major (52) and 12 were female (48)

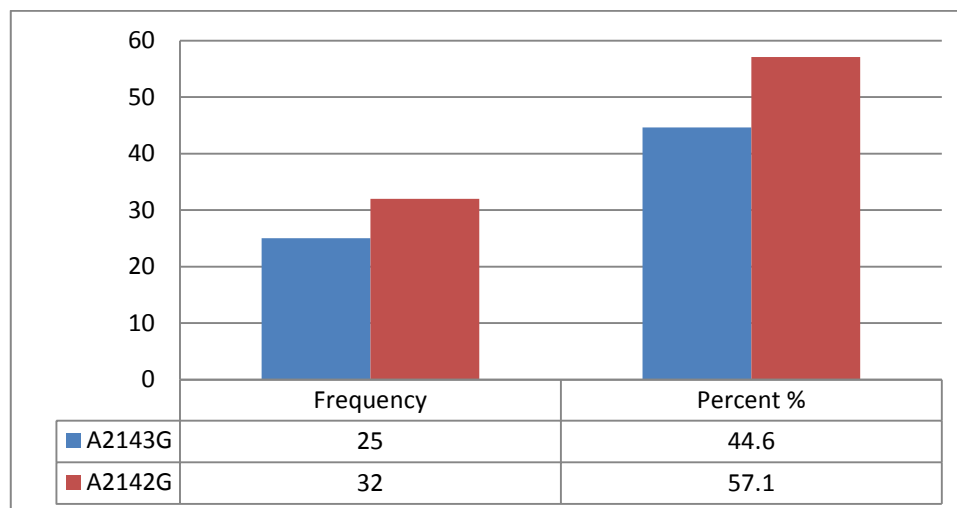


Figure (3): Detection of A2142G and A2143G points mutations by allele-specific PCR

Discussion

This study showed that the incidence of *H. pylori* infection was 73.7. It is more frequent and more advanced than the study demonstrated by Azim *et al.* (1994). In a recent study in central Sudan, a frequency of 48 was reported by Abdalsadeg *et al.* (2012). In the current study, more men (n=13153.3) than women (n=11546.7) were positive for *H. pylori* infection, which was statistically significant (p=0.000). Reported by Moayyedi *et al.* (2002) and Ndip *et al.* (2004) showed a higher incidence in males than in females. 97 (39.4) *H. pylori* patients were graded in the 20-30 age group, 63 (25.6) were graded 31-40, 61 (24.8) were graded ≥ 50 and 25 (10.2) were graded ≥ 50 . Divided. They had 41-50 fold statistical significance (p=0.000). The findings of this study are similar to studies in South Africa (Dube *et al.*, 2009) and Pakistan (Rasheed *et al.*, 2012). Education level is a strong indicator of socioeconomic class (Nishizawa and Suzuki, 2014). In our study, an association between *H. pylori* infection and educational status was statistically significant (p = 0.000). Rashid *et al.* (2012) reported no association was found between *H. pylori* infection and educational level. This is consistent with his EUROGAST study group (1993), Torres *et al.*(1998) and Graham *et al.* (1991) .There was no association between *H.pylori* frequency with employment status and occupancy(civic vs pastoral). Whilst the spread of The *H. Pylori* organism has been shown to be associated to water (16), our study didn't find any association between *H pylori* infection and the source of drinking water (p = 0.376), a analogous finding in the study by Rasheed *et al.* (2012) further than half of actors drinking valve water were set up positive to *H. Pylori* (n = 211;85.8). In the present study, there was a high *H. pylori* infection frequency in individualities who participated a bed with their family members,81.7 compared to those who didn't18.3 (p = 0.000). This is in keeping with studies done by Moayyedi *et al.* (2002) and Rothenbacher *et al.* (1998). Interestingly the association between *H. pylori* infection and those who participated a bed with their parents wasn't statistically significant, (p = 0.998), this was analogous to findings in the study by Moayyedi *et al.*(2002) (p = 0.65). *H. pylori* infection was high with the use of a hole potty71.5 of *H.*



pylori cases and the association of *H. pylori* infection with the type restroom wasn't statistically significant($p = 0.533$). In term of party occupation, a statistically significant difference was set up between *H. Pylori* and occupation of actors($p=0.018$).

Among the considered occupations ,house women showed the loftiest frequence to *H. pylori*($n = 96$; 39) followed by workers 69 (Agudo *et al.*, 2011), dealers 43 (17.5), scholars 22 (8.9) and employer 16 (6.5). In term of gastrointestinal characteristics,

utmost of the actors have mentioned suffering from gastrointestinal disturbances. Interestingly, a statistically significant difference wasn't set up between the *H. pylori* infection and the gastrointestinal disturbances. Actors suffering from PUD were set up largely current to *H. Pylori* 224 (91.1) followed by IBS 143 (58.1), epigastric pain 224 (91.1), heart burn 84 (34.1), and dyspepsia 207 (84.1).

Unexpectedly, this study had detected antithetical results about the relation between *H. pylori* and smoking. We set up no statistically significant difference between smokers, non-smokers in the positivity rate of *H. Pylori* ($P= 0.896$) and analogous results have been reported by Khalifa *et al.* Among dyspepsia Egyptian cases (Khalifa *et al.*, 2014). Severalcross sectional studies have delved the relationship between alcohol consumption and *H. pylori* infection. Some studies reported a significantly inverse association with *H. pylori* infection (21.22), while others set up no significant association. Some results indicate that consumption of moderate quantities of alcohol in the form of wine, beer and spirits may cover against *H. pylori* infection. Three mechanisms may explain the negative relationship. originally, alcohol may ply an anti-bactericidal effect against new infection. Secondly, alcohol may be bactericidal against being *H. pylori* infection (Ogihara *et al.*, 2000), and eventually, some alcoholic potables are known to stimulate gastric acid stashing, which may annihilate *H. pylori* by lowering the pH in the stomach(Bujanda, 2000).

Interestingly, the present study found that non-drinkers had significantly higher *H. pylori* infection rates compared to drinkers, a negative association

similar to previously reported studies. Drug-resistant bacteria are increasing around the world, making it difficult to eradicate *H. pylori*. Continuous surveillance to detect this resistance should be performed so that physicians can select the most appropriate eradication therapy (Shetty *et al.*, 2019), (Hu *et al.*, 2020). In the current study, *H. pylori* infection was detected in gastric biopsies (n = 56; 70%) of the included patients. Symptoms and endoscopic findings in *H. pylori*-infected patients included severe diffuse gastritis with duodenitis 131 (53.6%), moderate gastritis 52 (21.4%), severe gastritis 44 (17.9%), gastritis 8 (3.6%), GERD with gastroduodenitis 4 (1.8%) and diffuse nodular hyperplasia 8 (1.8%) were the most common and were significantly associated with evidence of *H. pylori* infection. This was consistent with Ayana and her colleagues who reported that 61.1% of dyspepsia patients had gastritis on endoscopy (Ayna *et al.*, 2014). In the current study, mutations in the rdxAS59 gene were detected in *H. pylori*-infected patients (n=26; 46.4%), of whom 12 were male (46.2%) and 14 were female (53.8%). rdxAS131 gene mutations were also detected in *H. pylori*-infected patients (n=34; 60.7%), of which 21 were male (61.8%) and 13 were female (38.2%). This is similar to Diab and his colleagues who found rdx A deletion mutations in 25% of *H. pylori* examined [27]. In this study, A2143G and A2142G mutations were found in *H. pylori* clarithromycin-resistant strains based on PCR-RFLP assays. The A2142G mutation was detected in (n=32; 57.1%), of whom 22 were male (68.8%) and 10 were female (31.2%). The A2143G mutation was detected in (n=25; 44.6%), of which 13 were male (52%) and 12 were female (48%). This finding is consistent with the results of Agdo *et al.* [28] From Spain, Droud *et al.* (Doroud *et al.*, 2005) From Iran, from Nishizawa and Suzuki (2014).

Conclusion

We concluded that high prevalence *H. pylori* resistance to metronidazole and clarithromycin may be associated with the prevalence used of clarithromycin for respiratory infection and metronidazole for parasitic infection.

Reference

- Abdalsadeg NA, Adam AA, Abdul-Aziz H, Omer WH, Osman HA, Bolad AK. (2012). Comparison of different diagnostic methods of *Helicobacter pylori* infection in Sudanese patients. *Al Neelain Med. J.* 2(4): 27-34.
- Agudo S, Perez-Perez G, Alarcon T, Lopez-Brea M. (2011). Rapid detection of clarithromycin resistant *Helicobacter pylori* strains in Spanish patients by polymerase chain reaction-restriction fragment length polymorphism. *Rev Esp Quimioter*; 24(1):32–6.
- Ayana SM, Swai B, Maro VP and Kibiki GS. (2014). Upper gastrointestinal endoscopic findings and prevalence of *Helicobacter pylori* infection among adult patients with dyspepsia in northern Tanzania. *Tanzan J. Health Res.*; 16(1):16-22
- Azim Mirghani YA, Ahmed S, Ahmed M, Ismail MO, Fedail SS, Kamel M, et al. (1994). Detection of *Helicobacter pylori* in endoscopic biopsies in Sudan. *Trop Doct.*; 24(4): 161-163.
- Buckley MJM, Xia HX, Hyde DM, et al. (1997). Metronidazole resistance reduces efficacy of triple therapy and leads to secondary clarithromycin resistance. *Dig. Dis. Sci.*;42:2111–15.
- Bujanda L. (2000). The effects of alcohol consumption upon the gastrointestinal tract, *Am. J. Gastroenterol*, V. 95: 3374-82.
- Dube, C. Nkosi, T.C. Clarke, A.M. Mkwetshana, N. Green, E. Ndip, R.N. (2009). *H. Pylori* in an asymptomatic population of Eastern Cape Province, South Africa: public health implication, *Rev. Environ. Health*; 24 (3): 249–255.
- Canton R, Martin de Argila C, de Rafael L, et al. (2001). Antimicrobial resistance in *Helicobacter pylori*. *Rev. Med. Microbiol*;12:47–61.
- Cardaropoli S, Rolfo A, Tudors T. (2014). *Helicobacter pylori* and pregnancy-related disorders. *World J. of Gastroenterol*; 20(3): 654-664.
- Cosme Alvarado-Esquivel. (2013). Seroepidemiology of *Helicobacter Pylori* Infection in Pregnant Women in Rural Durango, Mexico. *Inter. J. of Biomed. Sci.*; 9: 4-8.
- Rothenbacher, D.; Bode, G.; Peschke, F.; Berg, G.; Alder, G. and Brenner, H. (1998). Active infection with *Helicobacter pylori* in an asymptomatic population of middle aged to elderly people, *Epidemiol. Infect.* 120: 297–303.
- Graham, D.Y.; Malaty, H.M.; Evans, D.G.; Evans, D.J.; Klein, P.D. and Adam, E. (1991). Epidemiology of *Helicobacter pylori* in an asymptomatic population in the United States, *Gastroenterology* 100 (1991) 1494–1501.



- Diab, M.; El-Shenawy, A.; El-Ghannam, M.; Salem, D.; Abdelnasser, M.; Shaheen, M.; Abdel-Hady, M.; El Sherbini, E. and Saberet, M. (2018). Detection of antimicrobial resistance genes of *helicobacter pylori* strains to clarithromycin, metronidazole, amoxicillin and tetracycline among Egyptian patients. *Egypt. J. of Med. Human Genetics* 19:417–423.
- Doroud, D. Mohajerani, N. Massarrat, S. Mohammadi, M. (2005). Are molecular methods appropriate substitutions for traditional antimicrobial assays in detecting *Helicobacter pylori* antibiotic resistance in Iranian clinical trials? The 4th National Biotechnology Congress Islamic Republic of Iran. Kerman.
- Ehab H. Nashaat, MD, Ghada M. Mansour, MD (2010). *Helicobacter pylori* and Hyperemesis Gravidarum. Department of Obstetrics and Gynecology, J. of Nature and Science; 8(8): 22-26.
- EUROGAST (1993). Epidemiology of, and risk factors for, *Helicobacter pylori* infection among 3194 asymptomatic subjects in 17 populations. The EUROGAST Study Group, *Gut* 34 1672–1676.
- Rasheed, F. Ahmad, F.T. and Bilal, R. (2012). Prevalence and risk factors of *Helicobacter pylori* infection among Pakistani population, *Pak. J. Med. Sci.* 28(4):661–665.
- Houben; M.H.M., Van DeBeek; D., Hensen; E.F., et al. (1999). A systematic review of *Helicobacter pylori* eradication therapy—the impact of antimicrobial resistance on eradication rates. *Aliment Pharm. Ther.*;13:1047–55.
- Hu Y., Zhu Y. and Lu N.H. (2020). Recent progress in *Helicobacter pylori* treatment. *Chin. Med. J.* 5; 133(3):335-343.
- Torres, J.; Leal-Herrera, Y.; Perez-Perez, G.; Gomez, A.; Camorlinga-Ponce, M.; Cedillo-Rivera, R.; Tapia-Conyer, R. and Muñoz, O. (1998). A community-based seroepidemiologic study of *Helicobacter pylori* infection in Mexico, *J. Infect. Dis.* 178 (4):1089–1094.
- Khalifa, M., Khodiar, S. and Abd Almaksoud, A. (2014) Cigarette Smoking Status and *Helicobacter pylori* Infection in Non-Ulcer Dyspepsia Patients. *Egyptian J. of Chest Diseases and Tuberculosis*, 63, 695-699.
- Kuepper-Nybelen J., Thefeld W., Rothenbacher D., et al. (2005). Patterns of alcohol consumption and *Helicobacter pylori* infection: results of a population-based study from Germany among 6545 adults, *Aliment Pharma. Ther.*, V. 21 (pg. 57-64).
- Naous A, Al-Tannir M, Naja Z, Ziade F, El-Rajab M. (2007). Fecoprevalence and determinants of *Helicobacter pylori* infection among asymptomatic children in Lebanon. *Lebanon Journal of Medicine*; 55 (3).



- Tanih, N.F. Ndip, R.N. (2013).** South African perspective on *Helicobacter pylori*: prevalence, epidemiology and antimicrobial chemotherapy, *Afr. J. Microbio. Res.* 7(21):2430–2437.
- Nishizawa T, Suzuki H. (2014).** Mechanisms of *Helicobacter pylori* antibiotic resistance and molecular testing. *Front Mol. Biosci*;1:19. LImm
- Ogihara A Kikuchi S Hasegawa A, et al. (2000).** Relationship between *Helicobacter pylori* infection and smoking and drinking habits, *J. Gastroenterol Hepatol.*, V. 15; 271-6.
- Moayyedi, P. Axon, A.T.R. Feltbower, R. Duffet, S. Crocombe, W. Braunholtz, D. Richards, I.D.G. Dowell, A.C. and Forman, D. (2002).** Relation of adult lifestyle and socioeconomic factors to the prevalence of *Helicobacter pylori* infection, *Int. J. Epidemiol.* 31: 624–631.
- Ndip, R. N. Malange, A.E. Akoachere, J.F.T. MacKay, W.G. Titanji, V.P.K. and Weaver, L.T. (2004).** *Helicobacter pylori* antigens in the faeces of asymptomatic children in the Buea and Limbe health districts of Cameroon: a pilot study, *Trop. Med. Int. Health*, 9: 1036–1040.
- Shetty V., Lamichhane B., Tay C.Y., Pai G.C., Lingadakai R., Balaraju G., Shetty S., Ballal M. and Chua E.G. (2019).** High primary resistance to metronidazole and levofloxacin, and a moderate resistance to clarithromycin in *Helicobacter pylori* isolated from Karnataka patients. *Gut Pathog.* 13;11: 21, 8.
- Wasiam D., Benjamin C.Y., and Wong, M.D. (2007).** American College of Gastroenterology Guideline on the Management of *Helicobacter pylori* Infection. *American J. of Gastroenterology*;102.

Evaluation of the Activity of *Senna alaxandrina* Plant Extracts Against *Fasciola gigantica* worms in Kassala Town Cattle

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Abstract

In this study 4370 animal were examined (1935 cows, 1912 sheep, 441 goats and 82 camels, for their gastrointestinal *Fasciola gigantica* infection. The results showed that 0.87% cases were affected by *Fasciola gigantica*. Ratio of cows that were affected by *Fasciola gigantica* was 1.6 %, sheep 0.15% goats 0.68% and the camels were not affected. The ratio of cows infected by *Fasciola gigantica* because they depended on different species of herbs for nutrition. This is where the intermediate host is found, the metacercarie beside Shrubs as well as existing of water lakes where the cows drink. The study showed that prevalence of *F. gigantica* in male cattle was higher than in female. *Fasciola gigantica* disease in high proportion (1.75) was recorded In July. But in August, September and October *Fasciola gigantica* disease proportion was recorded 0.83%, 0.28% and 0.79% Consequently. That referred to raining season in autumn which cause momentum of grasses, hence lead to increase of metacercarie. Infection in November, December, January and February (Winter season) decreased to 1.06%, 1.08%, 0.85% and 0.54. In Summer season the *Fasciola gigantica* infection proportion declined to zero in March up to 0.84% in April and 1.08%, 0.88% in May and June respectively. Ethanol & petroleum ether extracts of *Senna alaxandrina* active against *Fasciola gigantica*. While *Senna alaxandrina* petroleum ether extract activity were the highest 100%, 100%, 100% respectively.

Key words: *Fasciola gigantica*, *Senna alaxandrina*, Extracted petroleum Ether Ethanol.

Introduction

Fascioliosis is a food-borne trematode infection caused by the liver flukes *Fasciola hepatica* and *Fasciola gigantica*. This infection is a zoonotic disease and is associated with a wide range of severe clinical signs in animals, mainly ruminants, but also humans (Verónica *et al*, 2020). In general, cattle are infected by trematode when they swallow metacercariae

attached to the grass and water (Muhammad *et al*, 2020). Moreover, the density of metacercariae is influenced by the population size of snails *Bulinus* spp. and *Planorbis* spp. as the intermediate hosts. The prevalence of paramphistomiasis and fasciolosis related to several factors, among them the grazing system (Muhammad *et al*, 2020). Fascioliasis is a neglected tropical disease (NTD) that mostly affects the farm animals and human populations of developing and under-developed countries. The World Health Organization (WHO) estimated that at least 2.4 million people are infected in >70 countries worldwide, with several million at risk. Human cases occurred occasionally, but are now increasingly reported from Europe, America, Africa, South and South East Asia (Parismita *et al*, 2019). Fascioliasis is prevalent in >150 countries worldwide, posing a serious threat to both human health and livestock that attributes to mortality, reduction in milk and meat production, secondary bacterial infections, and expensive anthelmintic treatment (Garcia and Modi,2008 ; Schmidt and Roberts ,2005).This disease is principally treated with a single WHO-approved drug, Triclabendazole (TCZ), which is active against both immature and adult parasites (Parismita *et al.*, 2019).The increasing incidence of animal as well as human fascioliasis, along with the emergence of TCZ-resistant parasite populations makes vaccination an alternative strategy for controlling the disease (Parismita *et al*, 2019).*F. gigantica* is restricted to tropical areas (Ashrafi *et al.*, 2014).In Sub-Saharan Africa, the most widespread species is *F. gigantica*, although *F. hepatica* was reported in Eastern and Southern Africa, including Zambia, Kenya, Zimbabwe, Tanzania, Ethiopia, and South Africa (Verónica *et al*, 2020). This fascioliasis is highly endemic in the Sudan specially in the white Nile area , also the fascioliasis is reported in many areas of the country e.g. the State of Darfour , Khartoum , Blue Nile , Kassala , as well as Northern (Almahy, 2013). The objective of this study to investigate the prevalence of *F.gigantica* affect cattle at Kassala Town. To evaluate the biological activities extracts of *Senna alaxdrina* against adult worm of *F.gigantica* in vitro.



Plant material

Study area

The plant material proposed will be collected from bank of the Delta Elgash at Kassala city. The selected plant species collected between February 2017 and April 2017. The plants material used for study were barks and fruits of *Senna alexandrina*. The plant will be identified and authenticated by the taxonomists of Medicinal and Aromatic Plant and Traditional Medicine Research Institute, National Centre for Research (MAPTMRI). All plant parts will be air dried, coarsely powdered and then used for extracts preparation.

Materials and Methods

Preparation of Crude extracts

Extraction was carried out according to the method described in (Harbone, 1984). To prepare extracts for screening of anti-fasciola activity, briefly ; parks and fruits were separated from the other parts of the plant .

The coarsely powdered plants material (\cong 30 gram) will be exhaustively extracted for 20 hours with chloroform in Soxhlet apparatus. The chloroform extracts of each plant will be filtered and evaporated under reduced pressure and the extracted plant materials will be air dried, repacked in the Soxhlet exhaustively extracted with methanol. The methanolic extracts will be filtered and evaporated under reduced pressure. Extract will be redissolved or suspended in methanol and the final volume will be adjusted to give the appropriate concentration and will be kept in refrigerator till the time of their use.

Simultaneously, water extracts will be prepared by adding 100 ml water to sample of 20 gm from coarsely powdered plants materials followed by occasional shaking for 2 hours .The aqueous extract will then filtered and the remaining macerated material will be washed with small volume of boiled water and added to the filtrate which will be then used immediately.

Medium Preparation

A sample of 20 gm of RPMI powder was exactly weighed in a 2 liters beaker and 4 gm of NaHCO₃ and 2 liters of distilled water were added, mixed well and shaken: 5 pellets of NaOH were also added followed by addition of 20 ml of gentamicin. The solution was stirred for five minutes using a magnetic stirrer. The medium was prepared and immediately sterilized by filtration just before the test.

Anti-fasciola Bioassay

The infected cattle livers were obtained from El Gandahar Slaughter House at Omdurman and transported immediately to the parasitological laboratory, MAPTMRI. National Centre for Research. The adult worms were collected, then we prepare Media RPMI -1640 in concentration as 125PAM , 250PPM ,500 PMM in the room temperature(25c). After that we took certain amount about 130 *Fasciola gigantica* as samples from the infection cows, and be grown in poetry dishes that contained the nutrition mediator, two *Fasciola gigantica* a per each poetry dishes in addition to six fasciola worms in three control dishes.

Statistical analysis

Analysis of variance (ANOVA) will be conducted to test the effect of the different concentrations of the plant extracts on both snails. Date analysis will be carried out using probit regression analysis as recommended by the expert of committee of world health organization (1979). Probit regression analysis (SPSS/inc) will be carried out for all plants determine the lethal dose that gives 50% mortality (LD50) and 95% mortality (LD95) as well their confidence limits.

Results

Two extractions from each of the following *Senna alexandrina*. Ethanol 96% & petroleum ether (40C – 60C) .A Medium RPMI -1640 in concentrations as 125pmm , 250pmm,500 pmm in the room temperature 25C. Time / hours.

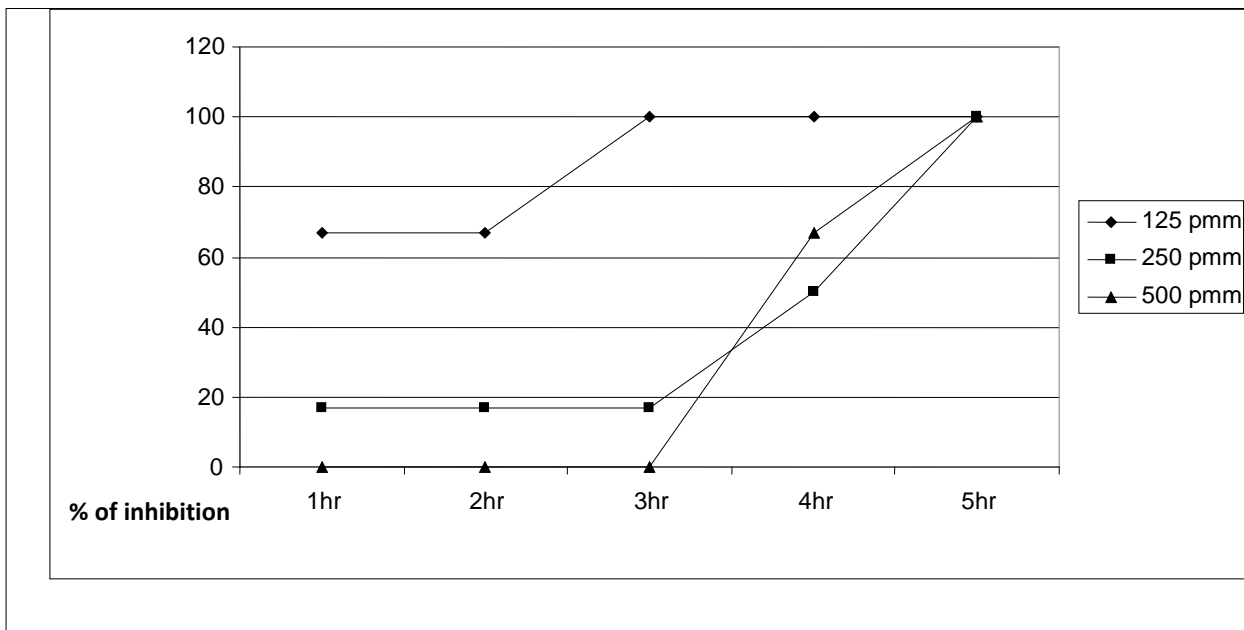


Fig.(1): The activity of *Senna alexandrina* petroleum ether

extract against *F.gigantica*.

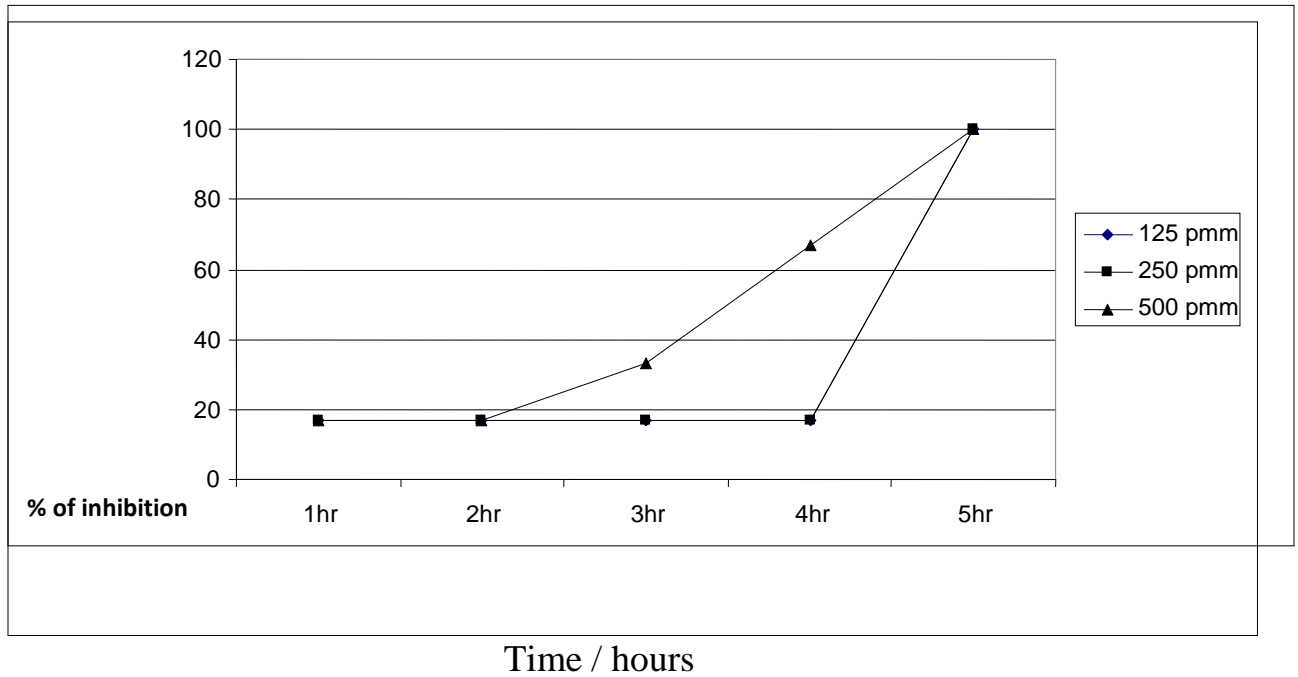


Fig.(2): The activity of *Senna alexandrina* ethanol extract against *F.gigantica*.

Table (1-A): Prevalence of *F.gigantica* affect cattle at Kassala Town

Area	Type of animal	No.of Sample	No.of positive	%
Kassala	Cows	1935	32	1.6
	Sheep	1912	3	0.15
	Goats	441	3	0.68
	Camels	82	0	0
Total		4370	38	0.87

Table (1-B): Prevalence of *F.gigantica* affect cattle at Kassala Town

Sex	Total	positive	%
Male	2174	22	0.01
Female	2196	16	0.73
Total	4370	38	0.87

Table (2): Monthly total infection among examined animals

Month	Total	positive	%
Jan	350	3	0.85
Feb	370	2	0.54
March	386	0	0
April	358	3	0.84
May	368	4	1.08
Jun	340	3	0.88
Jul	342	6	1.75
Aug	359	3	0.83
Sept	369	3	0.28
Oct	379	3	0.79
Nov	378	4	1.06
Dec	371	4	1.08
Total	4370	38	0.87

Discussion

This study was carried at Kassala Town to evaluate live stock gastrointestinal *Fasciola gigantica* infection during the period from April 2017 till March 2018 according to data collection form.

The study covered 4370 samples from the animal (1935 cows , 1912 sheep , 441 goats and 82 camels. The results showed that 0. 87% cases were affected by *Fasciola gigantica*. Ratio of cows that was infected by *F. gigantica* was 1.6 % , sheep 0.15% , but goats 0.68% and there was no camel affected. The study showed the prevalence of *F. gigantica* in male cattle was higher than in females (Table 1-B). The females are rarely used in plowing in the paddy field, especially in gravid condition, and therefore they have less contact with metacercariae, a source of infection (Muhammad *et al.*,2020).

F. gigantica disease high proportion (1.75) was recorded in July. But in August, September and October *F. gigantica* disease proportion was recorded 0.83% , 0.28% and 0 .79% consequently. That referred to Autumn season raining which causes momentum of grasses , hence lead to increase of metacercaria and water in lagoons (Burton, 2005) . Infection in November , December , January and February (Winter season) decreased to 1.06% , 1.08% , 0.85% and 0.54 consequently for few grasses and little lakes water in Summer season the *F. gigantica* infection proportion decline to zero in March up to 0.84% in April and 1.08% , 0.88% in May and June consequently. They are infection in Summer season was fewer than in Autumn and Winter season. For cattle and cow grazing in farms, as for few land grasses (Almahy, 2013).

Senna alexandrina is an evergreen shrub in all the seasons of the year which is 60-80 cm tall, glabrous to sub glabrous(Săvulescu *et al.*, 2018). The majors effects of sennosides, ow the main constituents of senna are sennosides with having defensive, antioxidant, natural defensive strengthening, anti-inflammatory properties and having many other medicinal properties which are also beneficial for health (Farid *et al.*, 2020). *Senna alexandrina* were extracted and screened for their

fasciolicidal activity *in vitro*. This practical processes confirmed that *Senna Alaxandrina* petroleum ether 0% , 25% , 75% the first hour.

While *Senna alexandrina* petroleum ether efficiency was the highest after twenty four hours 100% , 100% , 100% respectively. After the six hours *Senna alexandrina* extracts petroleum ether and ethanol registered the highest efficiency 50%, 75%, 100 % and 50%, 50%, 75% respectively because plant poisonous fresh fruits and leaves (Braun *et al.*, 1991) .It contains very active chemical component known as sennosides that act on the lining of the bowel causing a emetic effect Constipation or hard stools is one of the generating factors for causing and worsening hemorrhoids (Syed and Gulistan, 2020).

Conclusion

The study showed the prevalence of *F. gigantica* in male cattle was higher than in females. That means the infection in Summer season was less than in Autumn and Winter season. After the six hours *Senna alexandrina* extracts petroleum ether and ethanol registered the highest efficiency. We can use *Senna alexandrina* extracts anti worms because plant poisonous fresh fruits and leaves.

Recommendations

- Continuous diagnosis for cattle for animals against Fascioliasis to insure absence of disease.
- Controlling borders must be done to prevent cattle entering to reduce diseases which might come from neigh developing countries.

References

- Almahy, M. E. (2013). Helminthes Infection among Animals in Kassala Town and Antifasciola Activity of Five Sudanese Medicinal Plants. M.Sc. Thesis, Sudan Academy of Sciences.p.1
- Ashrafi, K., Bargues, M.D., O'Neill, S., Mas-Coma, S. (2014). Fascioliasis: a worldwide parasitic disease of importance in travel medicine. Travel Med Infect Dis. 12:636-49.



- Braun, M. Burgstaller, H, Hamdoum A.M & Walter, H. (1991).** Common weeds of Central Sudan GTZ. Eschborn, Germany.
- Burton, J.B. Clint, E.C. and Thomas, N.O (2005).** Liver flukes. *Human Parasitology*, 3: 206- 207.
- Farid, A., Kamel, D., Abdelwahab Montaser, S., Mohamed Ahmed, M., El Amir, M., and El Amir, A. (2020).** Synergetic role of senna and fennel extracts as antioxidant, anti-inflammatory and anti-mutagenic agents in irradiated human blood lymphocyte cultures. *Journal of Radiation Research and Applied Sciences*, 13(1), 191-199.
- Garcia, H.H., Modi, M.(2008).** Helminthic parasites and seizures, *Epilepsia* 49, 25–32.
- Harbone , JB., (1984).** Phytochemical methods. 2nd ed. New York, Champan&Hall, 4:4-7.
- Muhammad, H., Rizka, A., Henni, V., Amiruddin, A., and Farida A. (2020).** Occurrence of *Fasciola gigantica* and *Paramphistomum* spp Infection in Aceh Cattle. *E3S Web of Conferences* 151, 01025.
- Parismita, K., Denzelle,L.L., Aditya, K.P., Harish, S., and Timir, T. (2019).** Development of multi-epitope driven subunit vaccine against *Fasciola gigantica* using immunoinformatics approach. *International J. of Biological Macromolecules*.
- Săvulescu, E., Georgescu, M. I., Popa, V., and Luchian, V. (2018).** Morphological and Anatomical Properties of the Senna Alexandrina Mill.(*Cassia Angustifolia* Vahl.). Paper presented at the “Agriculture for Life, Life for Agriculture” Conference Proceedings.
- Schmidt, G.D., Roberts, L.S. (2005).** *Foundations of Parasitology*, 7th Ed. McGraw-Hill Publishing Company, New York, New York.
- Syed, R. A., and Gulistan, R. (2020).** Medicinal Significance of Alexandrian Senna. *J. of natural sciences*.
- Verónica, C.G., Joshua, L., Javier, G., Elora, V.G., Olorugum, J. A., Ngozi ,P. C.,and María, M.V.(2020).** *Fasciola hepatica* and *Fasciola gigantica* coexistence in domestic ruminants in Nigeria: application of a PCR-based tool. *Tropical Animal Health and Production*.



Clinical and Laboratory Diagnosis of Mange Infestation among One-humped Camels (*Camelus Dromedarius*) at Tamboul Livestock Market, Sudan.

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Abstract

This study was conducted from Tamboul livestock market, East of Gezira state and about (150) km south of Khartoum. Three hundred suspected camels of different ages, sex that came from different parts of Sudan to Tamboul livestock market were studied in different seasons to determine the incidence rate of mange mite's infestation and predisposing factors association with mange in camels during period from 2015 to 2016. The incidence of 24.7% mange mite's infestation was recorded during this study; visually the main characterization of lesion was loss of hair, irritation, corrugation, crust conformation and skin thickening, Some cases infested by sarcoptic mange revealed barrier with accumulation of white necrotic materials, Lesions were noticed in all parts of the body and mostly in the head, neck, thorax and gluteal region. Microscopically sarcoptic mange and psoroptic mange was detected; also nymph, larvae and eggs were noticed. according to season the result was revealed significant ($p < 0.05$) higher incidence rate in winter (51.4%) followed by autumn (24.3%) and summer (24.3%) respectively, while recorded no significant variation in effect of mange on gender and age groups. also the mange infestation registered significant increase of MCHC, hemoglobin and eosinophil. The study concluded that the parasitological technique using 10% KOH is rapid, economic and reliable technique to discover mange mite's infestation among camel's herds and strategic application of anti-parasite drugs against mange disease during cold and wet season was strongly recommended.

Keywords: Dromedary camel, Mange, Infestation, Tamboul livestock market, Sudan.

Introduction

Mange is an important zoonotic parasite affecting camel production and risk to human health (Ahmed *et al.*, 2020) and second most common parasitic disease in camels (Mochabo *et al.*, 2005). Mange is wide spread in Butana area (mid –eastern Sudan) and prevalence of 55.12% mange infestation was detected; Sarcoptic mange is a chronic and highly contagious disease, which spreads rapidly and affects both sexes and all ages of camels (Nayel and Abu-Samra, 1986). Sarcoptic mange in camels caused by *Sarcoptes scabiei var cameli* was considered to be one of the most serious, contagious, zoonotic disease affecting both dromedary, Arabian and Bactrian camels (Singh and Veer, 2005). The economic impact of camel's production is hampered by highly contagious and zoonotic diseases (Lamuka *et al.*, 2017). Poor body condition and poor nutritional status also made camels more prone to having sarcoptic mange (Jain *et al.*, 2005). The mange disease caused by one or a combination of several species of mite includes *Sarcoptes*, *Psoroptus*, *Chorioptes* and *Demodex* and some species are more widely distributed globally than others (Muller, 1989). The main clinical signs observed included intensive itching, hairless, thickened and gray in color and high incidence of mange lesion were recorded on the neck (60.5%) and the lowest incidence were on the tail(5.2%) while No lesion observed on the hump (Ramahi and khalaf, 2009). Most studies revealed that prevalence of mange in the camel population of different countries ranges from 3.5% to 83% (Agab and Abbas, 1999) and (Megersa, 2010). The camel has received very little attention as compared to other species of domesticated animals, For this reason, camels are affected by several diseases including

mange mites (Jarso *et al.*, 2018), stress due to other disease, overcrowding and poor management are the important predisposing factor addition that the mites can survive outside the body for about 1-2 week and they propagate in low temperature and high humidity (Sahani *et al.*, 2003). One of the main strategies for disease control is early detection of the parasite combined with prevention/control of the major risk factors associated with the infection (Ahmed *et al.*, 2020).

Materials and methods

Study area

The present investigation was conducted from Tamboul livestock market, it is one of the biggest markets of camels founded in Sudan, Tamboul District is located at latitude 14° 52' N and longitude 33° 31' E, it lay east to Gezira state and about 150 km to the south of Khartoum; the capital of the Sudan.

Study design

Observational and analytical study type aimed to determine the prevalence of mange infestation among one hump camels in study area within examined the suspected camels by 10% KOH direct examination and also to assess risk factors associated with infestation.

Study population and samples size

Three hundred suspected camels (300) of different age, sex, locations and different season were provided to Tamboul livestock market or Tamboul abattoir by different breeds and ecotypes were examined. Sample size required was calculated based on the formula $N = Z^2pq/d^2$ (Thrusfield, 2007) and prevalence of ringworm probability was

taken according to (Nayel and Abu-Samra, 1986) that revealed 55.12 % of camels in Sudan were infested with Mange.

Sample Collection

Skin scrapings from the lesion for Three hundred suspected camels with clear skin lesions were collected with a blunt scalpel in sterile petri-dishes. All specimens were labeled for age, sex and date of collection and then transferred to the laboratory for the diagnosis of Mange mites using direct examination technique.

Direct 10%KOH Examination technique

The collected skin scrapings were transferred to test tubes and mixed with a small amount of 10% potassium hydroxide solution and left to stand for 0.5–1 h until the skin particles have partly disintegrated. The tubes were centrifuged at 3000 revolutions per minute. The supernatant fluid was discarded, and a drop of sediment was investigated under a microscope for the detection of the various stages of sarcoptic mites and their eggs. (Köhler-Rollefson *et al.*, 2001).

Clinical investigations

Three hundred camels with different age, sex and location in different season (winter, summer and autumn) were examined visually for mange and clinical sings with lesions characterization for all suspected camels were detected. Also the collected data was labeled for age, sex and date of collection.

Blood samples

Venous blood samples were obtained in three seasons; during winter, summer and autumn (100 in each season). Blood samples were collected 300 from Jugular vein of suspected camels by Using Vacationer

EDTA tube, vacuoner needle, syringe, needle, needle holder, and disinfectant for blood parameters. At the same time, 3 ml Blood without anticoagulant used to obtain serum for biochemical tests, the blood was centrifuged at 3000 rpm for 15 minutes and the serum were kept in Eppendorf tubes then stored at -20°C until used (Kemal, 2014).

Hematological and Biochemical parameters

Hematological parameters were measured according to (Kemal, 2014), and total protein and albumin was determined according to manufacturer's instructions while globulin was measured according to formula **Globulin g/dl** = (Total protein _Albumin).

Statistical Analysis

Data collected from this study were compiled using an appropriate statistical package SPSS version 16. Results were summarized as means \pm standard deviation (S.D) and Levels of Significance was taken at ($P \leq 0.05$).

Results

From examined Three hundred (300) skin scrape conducted from suspected camels provided to Tamboul livestock market 74 (24.7%) out of 300 suspected camels were infested with mange mites.

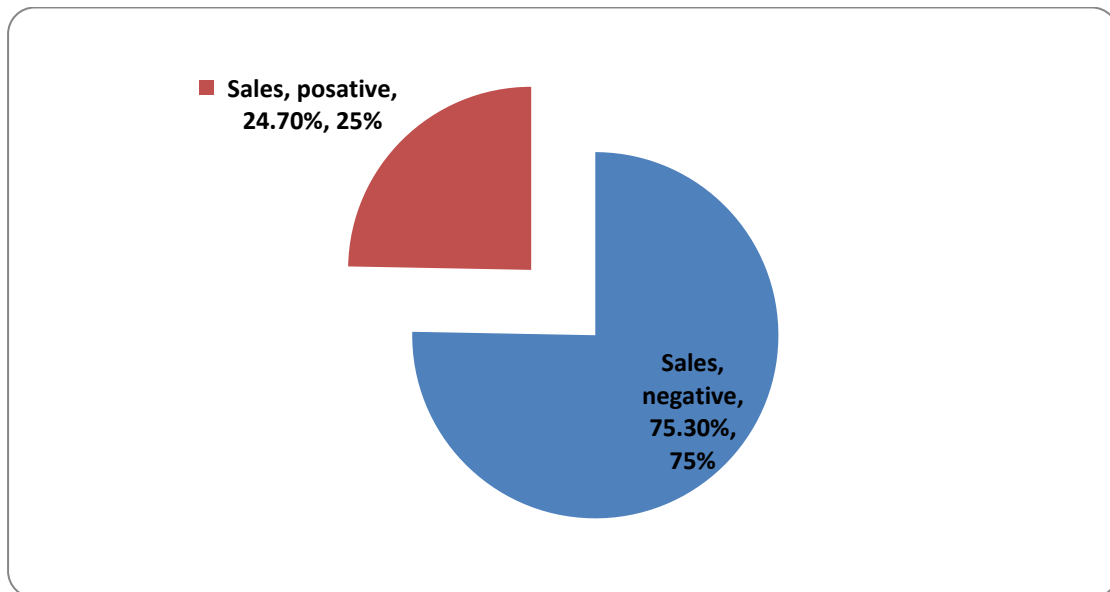


Fig. (1): Incidence rate of mange infestation among suspected dromedary camels.

The clinical observations recovered from infested camels with parasitic mange (*psoroptic mange* and *sarcoptic mange*) were characterized by loss of hair, irritation, corrugation, crust conformation and skin thickening. Some cases infested by sarcoptic mange revealed barrier with accumulation of white necrotic materials. Lesions were noticed in all parts of the body and mostly in the head, neck, thorax and gluteal region Figure (2) and (3).



Fig. (2): Camel infested by *sarcoptic mange* revealed alopecia, thickening, corrugation and crust.



Fig. (3): Camel infested by *psoroptic mange* revealed corrugation and irregular alopecia.

The present study was revealed two types of mange mites, *psoroptic mange* (fig4) and *sarcoptic mange* (fig5) and also nymph (fig6), larvae and eggs (fig7) were discovered.



Fig.(4): *Sarcoptic mange* under microscope showed by the 10× lens.



Fig. (5): *Psoroptic mite* under microscope showed by the 10× lens

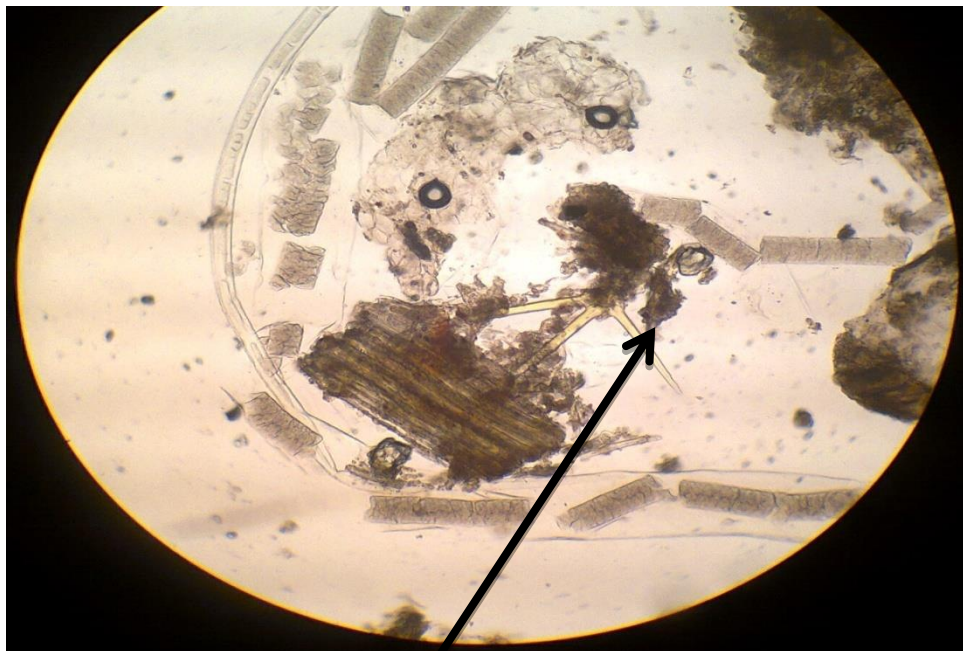


Fig.(6): Photomicrograph of Nymph of mite prepared by 10% KOH.

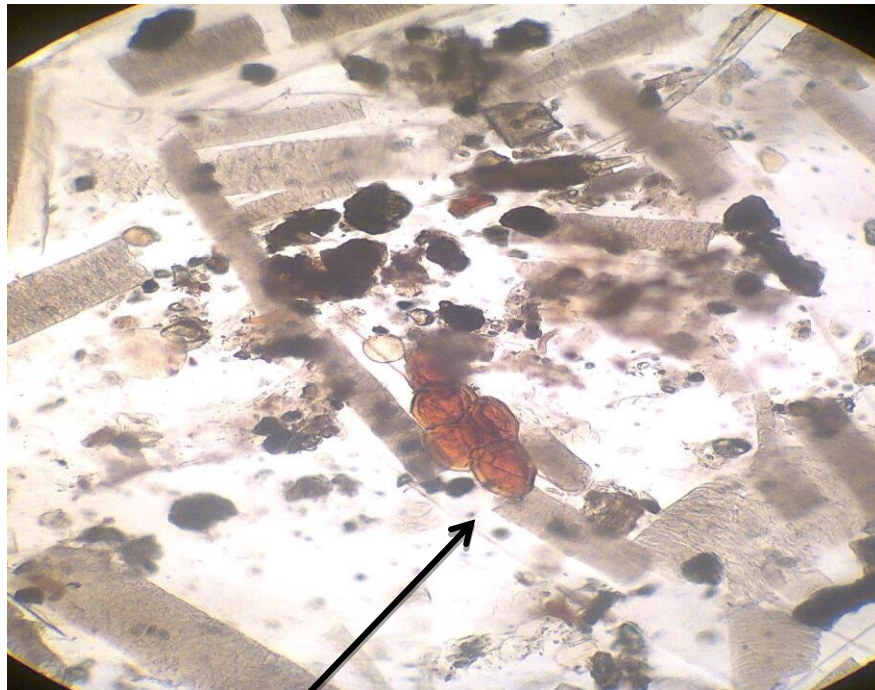


Fig. (7): Photomicrograph showing egg of mange prepared by 10% KOH.

According to season the result was revealed significant ($p < 0.05$) higher incidence rate in winter (51.4%) followed by autumn (24.3%) and summer (24.3%) respectively show in table fig (8).

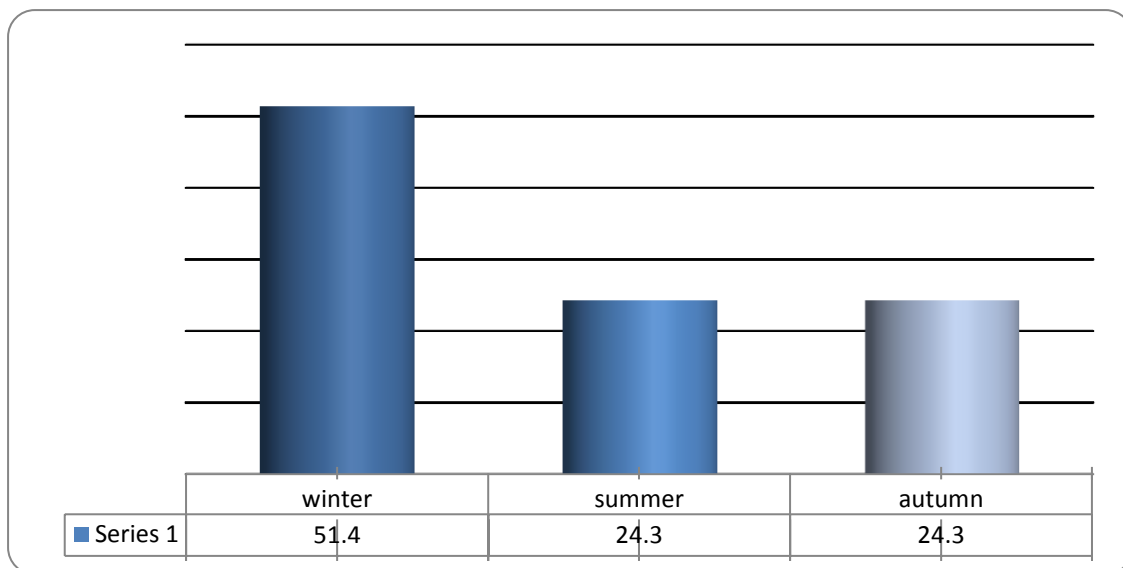


Fig. (8): Prevalence of mange infestation among camels at Tamboul livestock market according to season

In effect of age on mange disease, the examined camels were divided into four age-groups, (1-5) years, (6-10), (11-15) and (16-20) years old. High incidence rate was detected in age group (6-10) 64.8% followed by (11-15) 23% years and (1-5) 12.2% years while no cases recorded in age (16-20) years, statistically no significant variation in effect of age on incidence of mange mites infestation.

According to gender the Results showed no significant effect on mange disease (P value > 0.01). Also the mange infestation registered significant increase ($p < 0.05$) of MCHC, hemoglobin and eosinophil and recorded (52.5 ± 5.7), (14 ± 2) and (7.9 ± 4.9) respectively in animal infested with mange, when compared to mean values in healthy control animals. no significant variation in effect of mange mites infestation on others hematological and biochemical parameters.

Discussion

The incidence of 24.7% mange mite's infestation was recorded during this study which was less than prevalence of 31.6% recorded in Sudan by Nayel and Abu-Samra (1986) and 55.12% discovered by Agab and Abbas (1999) in eastern Sudan while similar to infestation rate 25.9% detected by Abebe (2001) how recorded 27.80% camels from eastern Ethiopia were infested with mange and Ramahi and khalaf (2009), This variation in the prevalence of camel mange mite especially in Sudan might be attributed to the wide use of anti-parasite (Ivermectin) among camels herds man.

The characterization of mange mite's infestation was loss of hair, irritation, corrugation, crust and skin thickening. Some cases infested by sarcoptic mange revealed barrier with accumulation of white necrotic

materials. Lesions were noticed in all parts of the body and mostly in the head, neck, thorax and gluteal region the result was agree with other result showed by Ramahi and khalaf (2000); AL-Ani (2004); singh and Momin (2008) and Hussain *et al* (2012).

Chi square revealed significant ($p < 0.05$) higher incidence rate in winter (51.4%) followed by autumn (24.3%) and summer (24.3%) respectively, this is same result discovered by Nayel and Abu-Samra, (1986) ; Mahran and Saleh (2004); Ashraf (2014) and Zahid *et al* (2015) showed that prevalence of ring worm was high during winter and the rainy season. According the gender and age groups the present study registered no significant variation ($P > 0.05$) in the prevalence of *mange mite's* infestation among camels, this result is similar to result obtained by Ramahi and khalaf (2009); Megersa *et al* (2012) and Zahid *et al* (2015)

Significant Increased MCHC values in positive cases in this study was agreed with result described by Manisha *et al* (2005) and Parmar *et al* (2005), while significantly increased of Hemoglobin concentration in camels infested with mange was not supported by Mal *et al* (2002) and Mal *et al* (2006), This different result may be the non-infested camels were infected with other causative agent lead to anemia particularly ectoparasite namely ticks, lice were observed. This may explained the role of this ectoparasite in anemia induction. Increased mean values ($p < 0.00$) for eosinophil in animal infected with mange was recorded (7.9 ± 4.9) when compared to healthy control camels (2.9 ± 3), this result was same to result recorded by Momenah (2014).

The study concluded that the mange mites is wide spread in Sudan, an incidence rate of 24.7% mange infestation was detected with two species; *psoroptic mange* and *sarcoptic mange*, mixed skin infection by one or more causative agent like dermatophytes spp and *Dermatophilus* spp was recorded. Parasitological technique using 10% KOH is rapid, economic and reliable technique to discover mange mite's infestation among camel's herds and strategic application of anti-parasite drugs against mange disease during cold and wet season was strongly recommended.

References

- Abebe, F. (2001).** Prevalence and intensity of ectoparasite infestation in Issa camels, Eastern Ethiopia. DVM thesis, FVM, AAU, Debre Zeit, Ethiopia, 6-26.
- Agab, H., & Abbas, B. (1999).** Epidemiological studies on camel diseases in the eastern Sudan. *J. of World Animal Review*, 92: 42-51. ISSN: 1014-6954.
- Ahmed, M. A., Elmahallawy, E. K., Gareh, A., Abdelbaset, A. E., El-Gohary, F. A., Elhawary, N. M., ... & Abushahba, M. F. (2020).** Epidemiological and Histopathological Investigation of Sarcoptic Mange in Camels in Egypt. *Animals*, 10(9), 1485.
- Al-Ani, F. K. (2004).** Camel Management and Diseases. Animal/ Diseases/ Camel/ Infection Diseases. 1st Ed. AL-Sharq Printing Press, Jordan.
- Ashraf, S.H (2014).** Prevalence of Common Diseases in Camels of Cholistan Desert, Pakistan, *J. of Infection and Molecular Biology*, 2 (4): 49-52. <http://dx.doi.org/10.14737/jimb.2307-5465/2.4.49.52>.
- Hussain, M. H, Habasha, F. G and Faraj, M. K. (2012).** Demodectic mange in Iraqi camels Qadisiya *J. of Vet.Med.Sci.* Vol. /11 No./1.
- Jain, G. K., Singh, A. P., Tanwer, J., Marwaha, S., & Chahar, A. (2005).** Epidemiological studies on sarcoptic mange in camels (*Camelus dromedarius*) in Bikaner district (West Rajasthan). *J. of Parasitic Diseases*, 29(1), 67-70.
- Jarso, D., Birhanu, S. and Wubishet, Z. (2018).** Review on Epidemiology of Camel Mange Mites, *Biomed J. Sci & Tech Res*, ISSN: 2574-1241 pp: 6313-6316.
- Kelly, W.R, (1984).** Veterinary clinical diagnosis. 3^{ed} Ed., London.

- Kemal, J. (2014).** Laboratory manual and review on clinical pathology. Foster City, USA. OMICS group ebooks. ISO 690.
- Köhler-Rollefson, I., Mundy, P., & Mathias, E. (2001).** A field manual of camel diseases: traditional and modern health care for the dromedary. ITDG publishing.
- Lamuka, P. O., Njeruh, F. M., Gitao, G. C., & Abey, K. A. (2017).** Camel health management and pastoralists' knowledge and information on zoonosis and food safety risks in Isiolo County, Kenya. *Pastoralism*, 7(1), 20.
- Mahrán, O. M., & Saleh, M. A. (2004).** Prevalence of ectoparasite and their effect on some biochemical indices in camels (*Camelus dromedarius*) at Shalatin City. *Assiut Vet. Med. J*, 50, 164-187.
- Mal, G., Kumar, R., Sena, D. S., & Sahani, M. S. (2002).** Hematological and mineral values in mange affected and healthy camels. *Indian veterinary journal*, 79(10), 1026-1027.
- Mal, G., Sena, D. S., & Sahani, M. S. (2006).** Haemato-biochemical changes in camels infested with mange during winter and summer season. *J. of Camel Practice and Research*, 13(2), 173-174.
- Manisha, M., Hemant, D., Sharma, G. D., & Sandeep, K. (2005).** A study of haemato-biochemical changes in camels affected with cutaneous ectoparasitoses in Rajasthan. *Veterinary Practitioner*, 6(2), 131-132.
- Megersa B. (2010).** An epidemiological study on major camel diseases in the Borana lowland, Southern Ethiopia. Oslo, Norway: DCG Report No. 58: 67-98.
- Megersa, B., Damena, A., Bekele, J., Adane, B., & Sheferaw, D. (2012).** Ticks and mange mites infesting camels of Boran pastoral areas and the associated risk factors, southern Ethiopia. *Journal of Veterinary Medicine and Animal Health*, 4(5), 71-77.
- Mochabo, K. O. M., Kitale, P. M., Gathura, P. B., Ogara, W. O., Catley, A., Eregae, E. M., & Kaitho, T. D. (2005).** Community perceptions of important camel diseases in Lapur Division of Turkana District, Kenya. *Tropical animal health and production*, 37(3), 187-204.
- Momenah, M. A. (2014).** Some blood parameters of one humped she camels (*Camelus dromedaries*) in response to parasitic infection. *Life Science Journal*, 11(5), 118-123.
- Muller GH, Kirk RW, Scott DW (1989).** Small animal dermatology, 4th Ed. WB Saunders Company, Philadelphia, Pennsylvania 1,007.
- Nayel, N.M and Abu-Samra, M.T (1986).** Sarcoptic mange in one hump camels (*camelus dromedarius*), Sudan *J. Arid Envir.*, 10: 199-211.



- Parmar, A. J., Singh, V. E. E. R., & Sengar, Y. S. (2005).** Epidemiological Studies on Sarcoptic mange in camel (*Camelus dromedarius*) in Banaskantha district (North Gujarat). *J. of Parasitic Diseases*, 29(1), 67-70.
- Ramahi H.M. and Khalaf A.M. (2009).** A study on the prevalence of mange among Arabian camels in Najaf province\ Iraq, *J. of Natural and Applied Science*, ISSN: 2073-0764. pp: 109-114.
- Sahani, M.S. (2003).** Sarcoptic mange (scabies) in camel's preventives measure and treatment, national research center on camels, Bikaner-334001(Rajasthan).
- Singh, A. and Veer, M. (2005).** Parasitic zoonosis.1st Ed. Poimer publication, Jaipur, India.
- Singh, V., & Momin, R. R. (2008).** Common parasitic diseases of camel. *Veterinary World*, 1(10), 317.
- Thrusfield, M. (2007).** Sample size determination. *Veterinary Epidemiology*, 3, 185-189.
- Zahid, M., Maqbool, A., Anjum, S., Ashraf, K., Khan, M., & Ahmad, N. (2015).** Prevalence of sarcoptic mange in camels in Punjab, Pakistan. *J. of Animal and Plant Science*, 25, 1259-1263.



Seroprevalence of *Toxoplasma gondii* Infection in Chickens (*Gallus domesticus*), Pigeons (*Columba livia*) and Turkeys (*Meleagris gallopavo*) in Dongola, Northern Sudan

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Abstract

This study aimed to investigate the seroprevalence and possible risk factors associated with toxoplasmosis in poultry in Northern State. A Toxoplasma gondii seroprevalence survey during October 2016 –June 2017, was conducted in chickens, pigeons and turkeys in Dongola, Northern, Sudan. The sera of 1490 (612 indigenous chickens, 415 exotic chickens, 351 pigeons and 112 turkeys) were examined using the modified agglutination test (MAT). The obtained results revealed that antibodies against Toxoplasma gondii were detected in a rate of 18.1%, 11.1%, 14.9% and 12.5% in indigenous chickens, exotic chickens, pigeons and turkeys respectively. A very high prevalence of the parasite was found in chickens raised in free-range backyards. The soil contamination might be an effective indicator for Toxoplasma gondii infection. Regarding sex, no significant difference between males (14.0%) and females (15.5%) was found ($P > 0.05$). This study showed that prevalence of Toxoplasma gondii infection in poultry is high; represent a potential source of toxoplasmosis for humans in particular in the study area. We concluded that, the results of this study confirm the presence of Toxoplasma gondii in chickens, pigeons and turkeys in Dongola locality, Northern Sudan

Keywords: Toxoplasma gondii, chickens, pigeons, Turkeys

Introduction

Toxoplasma gondii is a ubiquitous apicomplexan parasite which causes toxoplasmosis in a wide range of animals, man and birds (Radosttits *et al.*,



1994). *Toxoplasma* infection has been also reported in a number of marine mammal species indicating that the organism has entered the marine environment (David *et al.*, 2017). Feline species especially cat are definitive hosts and play important role in the epidemiology of the disease (Dubey and Beattie 1988). The main sources of infection are meat, other foods, water and environment (Schlundt *et al.*, 2007). The transmission of the disease occurs mainly through consumption of food or water contaminated with sporulated oocysts shed by cats (Dubey, 2007). The oocysts in the environment may cause direct infection through ingestion of soil by different herbivorous and hence may lead to formation of tissue cysts in farm animals (Aretas *et al.*, 2017). The disease is a serious risk for seronegative pregnant women and immunocompromised persons (Pappas *et al.*, 2009). Clinical signs, when present, were not specific; therefore serological tests become important in the diagnosis of the disease (Switaj *et al.*, 2005). Bioassay in mice or cats has been used as highly sensitive and specific test to isolate viable *Toxoplasma gondii*. Most acquired toxoplasmosis in poultry, include, weight loss, in appetite, shrunken comb, drop in egg production, whitish diarrhea, in coordination, trembling, opisthotonos, torticollis and blindness (Opsteagh *et al.*, 2010). All chickens infected before eight weeks of age, developed clinical signs. Chicken has been suggested as more efficient intermediate host for *T. gondii* and



possibly plays more important rule in the epidemiology of the parasite than rodents, because they were clinically resistant to *T. gondii* and life longer than rodents (Dubey, 2007). Compared to other continents, few studies have been conducted on toxoplasmosis in Africa. Studies available on the seroprevalence of toxoplasmosis in African countries were still fragmented except Ethiopia (Ayinmode *et al.*, 2012). Information on the incidence of toxoplasmosis in Sudan and the risk of infection associated with food sources was limited.

Materials and methods

Study area

This study was carried out between October 2016 – June 2017 and performed on chickens, pigeons and turkeys of Dongola locality, in the North of the Sudan, in altitude of 25 15.19 : 32 18 47.7 M, 16 35 8.35 :22 105 N longitude. It is desert climate. The temperature is 41 C, average humidity is 25% and annual average of rain fall is 25 mm.

Poultry investigated

Indigenous chickens: A total of 612 household free range indigenous chickens which more than one year old (410 female -202 male) were selected randomly as study samples. The chickens were kept free ranging on ground



cereal grains might be given in the morning and the birds will scratch for food in the surrounding during the rest of the day.

Exotic chickens: A total of 415 exotic chickens which were commercially raised (512 female – 103 male). The chickens were kept as farmed chicken on concentrate floors.

Pigeons: A total of 351 domestic Pigeons which were more than one year old (218 female – 133 male) were selected randomly as study samples.

Turkey: A total of 112 household free range turkeys which were more than one year old (95 female – 17 male) were selected randomly as study samples.

Samples collection

Five ml of blood were collected individually from examined chickens, pigeons and turkeys. After clotting at room temperature blood was centrifuged at 3000 rpm for 15 minutes and collected sera were stored at -20°C until assayed for antibodies for toxoplasmosis.

Samples investigation

Commercial serum agglutination kits (Toxo-HAIFUMOUZE) for toxoplasma antibodies detection were used. Diluted two fold blood serum



samples starting at 1:40 to 1:2560 were assayed for *T. gondii* antibodies with modified agglutination test (MAT) conducted as the test was performed according to the manufacture's described by Debye and Dismounts (Debey. J.P 198) instructions. MAT titers of 1:80 or higher were considered positive for *T. gondii* antibodies.

Statistical analysis

The obtained data were computed and analyzed using chi square test in SPSS version 5. The differences were considered statically significant when $P < 0.05$. Data presented as percentage.

Results and discussion

The MAT test revealed the presence of antibodies to *Toxoplasma gondii* in all types of poultry examined in study area. Positive results obtained in the sera of poultry examined were shown in (Tables 1, 2).

Table (1): Seroprevalence of *T.gondii* in different poultry by MAT

Type of poultry	Number collected	Number tested	Seropositivity (%)	Number of poultry with MAT titer		
				1:80	1:160	1:320
1-Indigenous chickens	216	600	109 (18.1%)	49	32	28
2-Exotic chickens	415	411	46(11.1%)	26	14	6
3- Pigeons	351	342	51 (14.9%)	32	19	-
4- Turkeys	112	112	14(12.5%)	7	8	2
Total	1490	1465	220 (15%)	114	70	36

Table (2): Seroprevalence of *T. gondii* in different poultry by MAT according to sex

Type of poultry	Number Tested		Number positive (%)	
	male	female	male	female
Endogenous	202	398	36 (17.8%)	73 (18.3%)
1-Chicken				
2- Ex. Chicken	101	310	11 (10.8%)	35 (11.2%)
3- Pigeons	124	218	14(11.2%)	37 (16.9%)
4- Turkey	91	21	12 (11.5%)	2 (9.5%)
Total	518	947	73 (4.0%)	147 ((15.5%)



Chickens were considered one of the most important hosts in epidemiology of *Toxoplasma gondii* infection because they are an efficient source of infection for cats that excrete the environmentally resistant oocysts and because humans may become infected with this parasite after eating undercooked infected chicken meat (Jones and Dubey, 2012). There were few reports of *T. gondii* in animals in Sudan (Shadia *et al.*, 2013), but many studies were revealed that toxoplasmosis was quiet prevalent among Sudanese citizen (Mohamed *et al.*, 2017). The first report of toxoplasmosis in chickens from Sudan was mentioned by (Mohamed *et al.*, 2017) where *T. gondii* antibodies were detected in free range chickens in river Nile, Khartoum and Sennar States. The present study was conducted to investigate the prevalence of toxoplasmosis in chickens, pigeons and turkeys in Dongola locality. The results showed high prevalence of *T. gondii* antibodies in free range indigenous chickens (18.1%) when compared with farmed exotic chickens (11.1%), pigeons were (14.9%) and turkey were (12.5%) in the study area. The seroprveillance rate (18.1%) for *T.gondii* in free range indigenous chickens in this study is lower than that found in Nigeria (40%) reported by Ayinmode *et al.*, (2014) in Ethiopia (38.4%) reported by Tiham *et al .*, (2013) and in Egypt (20.0%) reported by Shawky *et al .*, (2013). Our study showed that there was high prevalence of the disease in free range chickens (18.1%) than farmed



exotic chickens (11.1%). This finding is similar to the reports of some previous studies such as, Zhu *et al.*, (2008) Aboelhadid *et al.*, ((2013) and Horold Salant, (2016) and might be explained that an efficient bio security conditions prevent a widespread oocyte contamination of the environment because of fecal contamination of the soil and ground water either by domestic or feral cats . With regard to the sero prevalence of toxoplasmosis in pigeons (14.9%), the result are in agreement with Kirkpatric *et al.*, (1990), Mushi *et al.*, (2001).Tsai *et al.*, (2006) and Waop *et al.*, (2008). Pigeons are mostly flying free bird and may be a resvior in the spread of toxoplasmosis and source of infection for predators and humans, hence, may play a role in epidemiology of toxoplasmosis. Regarding turkey, the seroprevalence of toxoplasmosis was (12.5%), which is lower than in Iran (64%) recorded by Ghorbani *et al.*, (1990), in Iraq (76.6%) recorded by Butty (2009) and in Egypt (29.4%) recorded by Harfoush *et al.*, (2010). Few studies were carried out about turkey because of limitation in study numbers and sample size. In our study we assessed for the first time, the prevalence of *T.gondii* infection in Turkey in Sudan. Our study showed that there was no significant difference ($P>0.05$) in the seroprevalence of *Toxoplasma gondii* infection between females and males (14.0%). Suggesting that gender does not influence exposure to *T.gondii* infection. This finding is in accordance with reports recodes by Gondofli *et al.*,

(1993), Uneke *et al.*, (2007) and Aboehadid *et al.*, (2013). In conclusion, the results of this study confirm the presence of *Toxoplasma gondii* antibodies in chickens pigeons and turkeys in Dongola locality, Northern Sudan. Thus further studies will be needed to clarify the impact of toxoplasmosis on the animal industry and transmission of the disease to humans in Sudan.

Recommendations

We recommended optimal surveillance and monitoring methods in animal populations and humans are needed. Furthermore, post-harvest aspects such as *Toxoplasma* monitoring and sampling strategies in meat, milk and process products are recommended.

Conclusion

In conclusion, the results of this study confirm the presence of *Toxoplasma gondii* antibodies in chickens, pigeons and turkeys in Dongola locality, northern Sudan. Thus further studies are needed to clarify the impact of toxoplasmosis in animal industry and transmission of the disease to humans in Sudan.

References

- Aboehadid S.M.; Abdel-Ghany A.E.; Ibrahim M.A. and Mahran H.A. (2013). Seroprevalence of *Toxoplasma gondii* infection in chickens and humans in BeniSuef, Egypt. *Glob. Vet.* 11. 139-144.
- Aretas B.N.; Yao A.; Philippe S.; Camus A.; Eric Y.; Yaovi G.; Marc N.; Issaka Y. and Souaibou F. (2017). *Toxoplasma gondii* infection in meat animals in Africa: Systemic review and meta-analysis of sero-epidemiological studies. *Veterinary World*, EISSN: 2231- 0916 : 194-199.

- Ashraf M.B.; Salem M. and El Newishy A.M. (2012). Zoonotic Chickens Toxoplasmosis in Some Egyptian Governorates 15(17) 821-826.
- Ayinmode A.B. and J.P. Dubey (2012). *Toxoplasma gondii* infection: Mini-review and seroprevalence Study in Oyo State, Nigeria. *African J.of Biomedical Research*. 15: 145-148.
- Butty E.T. (2009). Diagnostic Study of *Toxoplasma gondii* in turkey (*Meleagris allopavo*) in some Regions in Ninevah governate in Iraqu. *Iraqi J. of Vet.Sci*.Vol.23:57-62.
- Candolfi E.; Berg M. and Kien T. (1993). Prevalence of Toxoplasmosis in Point –Noire in Congo. *Bulletin De La Societe De Pathologie Exotique et De Ses Filiales* 86: 358-362.
- David J. Blyde (2016). Toxoplasmosis: An Emerging Disease of Marine Mammals in Australia. 47th. Annual IAAAM meeting and conference May 21-26 Virginia Beach.
- Dubey J.P. (2007). The History and Life cycle of *Toxoplasma gondii* In:Weiss LM.Kim K.editors pp.1-17.
- Dubey J.P. and Beattie C.P.(1988). Toxoplasmosis of Animals and Man Boca Raton, FL:CRC Press ;pp.1-220.
- Ghorbani M.; Gharavi M.J. and Kahnamoui A. (1990). Serological and Parasitological investigation on Toxoplasma infection in domestic fowls in Iran *J. Publ.Health*, 19 -9-17.
- Harfoush M. and Tahoou A.E. (2010). Seroprevalence of *Toxoplasma gondii* in duck, free-range chickens, turkeys and rabbits in Kafr –El-Sheikh Governate, Egypt *J of Egyptian Society of Parasitology*.40:295-302.
- Harold Slant (2016). Seroprevalence of *Toxoplasma gondii* infection in poultry kept under different housing conditions in Israel. Msc. University of Pretoria Faculty of Veterinary Medicine.
- Jones J.L. and Dubey J.P. (2012). Food borne toxoplasmosis *Clin.Infect.Dis*.55 (6) :845-851
- Kirkpatrick C.E.; Colvin B.A. and Dubey J.P. (1990). *Toxoplasma gondii* antibodies in Common barn-Owls (*Tyto alba*) and Pigeons (*Columba livia*) in NewJersy. *Vet.Parasitol* 36: 177-180.
- Mohamed O.H.; Shima H.A.; Abdel Rahim M.E. (2017). Seroprevalence of Toxoplasma *gondii* in Chickens (*Galus domesticus*) in Sudan. *Int.J.Infect*.4(3) 34-41.
- Mucker E.M.; J.P. Dubey; M.J. Lavallo and JG.Humpherys (2006). Seroprevalence of antibodies to *Toxoplasma gondii* in Pennsylvania bobcat. *J. of Wilde Life Diseases*, 42: 188-191.
- Mushi E.Z.; Binta M.G.; Chabo R.G.; Ndebele R. and Panzirah R. (2001). Seroprevalence of *Toxoplasma gondii* and *Chlamidia psittacii* in domestic pigeons (*Columba livia*) at Sebele, Gaborone, Botswana. *J.VetRes*.68:159-161.

- Opsteegh M.; Langelear M.; Sprong H.; Den Hartog L.; De Croeye S.; Bokken G.; Ajenberg D.; Kijlstra A.; and Van der Giessin (2010).** Direct detection and genotyping of *Toxoplasma gondii* in meat samples using magnetic capture and PCR. *Int.J Food Microbiol.* 139:193-201.
- Papps G.; Roussos N.; and Falagas M.E. (2009).** Toxoplasmosis Snapshots: Global Status of *Toxoplasma gondii* Seroprevalence and implications for Pregnancy and Congenital toxoplasmosis. *Int. J. of Parasitology.*39 :1385-1394.
- Radostits O.M.; Blood D.C. and C.C..Gay (1994).** Text Book of the diseases of cattle, sheep, pigs, goats and horses. 8th Ed. W.B Saunders, London UK 1201-1206.
- Schlundt J.; Toyofuku H.; Jansen J. and Herbst S.A. (2004).** Emerging food borne diseases *Revue Scientifique et Technique OIE* 33:513-533.
- Shadia M.A.; Abdalla M.I.; Nabaa K.A.; Rabab H.M.; Ahmed A.I. and Tamador E.A. (2013).** The First Report on Seroprevalence of *Toxoplasma gondii* in Working Horses and Donkeys in the Sudan. *J of Life Sciences* Vol.7.No.12:1284-1287.
- Switaj K.; Master A.; Skrzypczak M.; and Zaborowski P. (2005).** Recent trends in molecular diagnostics for *Toxoplasma gondii* infections. *Clinical Microbiology and Infection* 3: 170-176.
- Tilahun N.T.; L.R. Ferreira; S.Choudhary; S.Oliveira; SK. Verma ;OCH Kowk; B.Molla ; WJA. Saville; G .Medhin; T.Kassa;H. Aleme;WA.Gebreys; C.Su; JP.Dubey (2013).** Prevalence of *Toxoplasma gondii* from free-range Chickens (*Gallus domesticus*) from Addis Ababa, Ethiopia. *J.Parasitol.* 99(4) 740-741.
- Unkke C.J.; Duhlińska D.D.; Ngwa B. A. and Njoku M.O. (2007).** Seroprevalence of *Toxoplasma gondii* on Kwal, Nigeria. *Afr. J. of Med. Sciences.* 36: 109-113.
- Waop P.H.; Vilares A.; Rebelo E.; Gomes S. and Angelo H. (2008).** Epidemiological and genetic characterization of *T.gondii* in urban pigeons from area of Lisbon (Portugal).*Vet.Parasitol* 157: 306-309.
- Zhu J.; Yin J.; Xiao Y.; Jiang N.; Andle J.; Lind J. and Chen Q. (2008).** Sero-epidemiological survey of *Toxoplasma gondii* in free –range and caged and chickens in northeast China. *Veterinary Parasitology.*158.360-363.

Design a Security Framework for Portable Media Devices inside Organization that Contains Sensitive Data

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Abstract

Portable media device spread few years ago. They are cheap and light weight what make them accessible for the staff. They also participate in increasing companies' productivity moreover they are suitable for all situation and time, though they have many disadvantages if the users do not follow the right principles when dealing with them in a correct way that might lead to counter-productive. This paper aimed to study the types of removable device and the problems that the users might face at the abuse of these devices and the internal threats caused by the users. The paper methodology contains a questionnaire which check whether there are systems to control the work using portable devices, also to find if there are problems while use these devices, and design security framework and how to control and apply them in organization like have sensitive data. At the end of this paper there is a summary and recommendations for further work in this field.

Keywords: SFPMD, USB, CD, DVD.

Introduction

OVERVIEW

Many companies live in fear of the day when someone steals their sensitive corporate data. That's why companies deploy firewalls, manage wireless connectivity, control network-access privileges, and install antivirus

software. But these solutions focus on the front door and can leave the back door wide open to a new kind of security breach. Like Trojan horses, USB and other removable storage devices such as flash drives and USB memory sticks, MP3 players, CDs and DVDs enter the corporate environment and attach to computing assets without arousing suspicion or triggering security alerts. Characterized by ever-increasing storage capacity, these devices can be used to surreptitiously download larger and larger amounts of sensitive corporate data. (Graham Sharpless, 2004).

PROBLEM

There was really problem when employee used portable media devices in the organizations, the organizations don't aware for that, this research focus is exclusively Insider Threat Management - understanding & addressing risks caused by employees and other trusted entities, Solutions & services for managing employee behaviors for mobile devices usage, including insider threat assessments, mechanism for encrypted mobility devices data, social policy for employee to reduce the risk of using mobile devices.

OBJECTIVE

To study the situation how to deal with portable media devices in Organization contains sensitive data and to design a security framework for portable media devices usage.

IMPORTANCE

The importance of this research comes from the importance and higher security nature of the datum in the organizations, these data exist in the user's computer machines, so it may be affected with the abuse of these machines and accommodated data through use of portable media devices.

Methodology

A social study will be adopted followed by practice study for security policy. The following steps are used to implement the methodology:

1. Using policy to control access to USB ports.
2. Enforce USB security without overburdening the IT staff.
3. Strict the types of removable media devices are allowed to connect with company computers and network.
4. Prove that data leakage has not occurred.
5. Provides recommendations for protecting mobile data.

BOUNDARIES

The boundaries of this research are just for controlling the use of portable media devices in organization systems, and find a mechanism, then prove it to restrict using these devices. The controlling will be for the following portable media devices in organization systems:

- Laptops
- USB flash drives.
- USB memory sticks.
- MP3 players.
- CDs and DVDs.

The space boundary of the research Organization contains sensitive data.

Portable Media Devices

Introduction

An improved portable media device and methods for operating a media device are disclosed. According to one aspect, the portable media device can also function as a solid-state drive for data storage. The form factor of the

portable media device can be hand-held or smaller, such that it is highly portable. The portable media device can use one or more status indicators. The portable media device can also include a peripheral bus connector, a rechargeable battery, and one or more input devices. According to another aspect, the portable media device has the capability to store media device status information in persistent memory before powering down. Thereafter, when the portable media device is again powered up, the stored media player status information can be retrieved and utilized. According to still another aspect, the portable media device can form and/or traverse a media asset playlist in an efficient manner.

As portable media players have become more popular, there has been an increased demand for improved media player design. Thus, there remains a need for media players having improved user controls, small sizes, and longer battery life.(Steve Jobs, Anthony M. Fadell, Jonathan P, 2009,).

Insider Threats While Use PMD in Organization's

In the previous part, the appearance and characteristics of portable devices was mention, their types and characteristics such as USP flash drive, memory sticks, PDA's, MP3 players, Hard disk drives, CD's / DVD's, Laptop computers.

In this section, the problems and internal threats associated with the use of these devices by employees within the organizations and the risks that follow the use of the devices concerned. will discussed,

Organization's Protection

Statistics demonstrate that 98% of all crimes committed against companies in the U.K. had an insider connection.

Data theft, legal liabilities, productivity losses and corporate network security breaches are all dangers that corporations have to face if malicious insiders or careless employees misuse portable storage devices at their workplace.

Efforts to estimate how often companies face attacks from within are difficult to make. It has been suggested that insider attacks are under-reported to law enforcement and prosecutors.

The reasons for such under-reporting include an insufficient level of damage to warrant prosecution, a lack of evidence or insufficient information to prosecute, and concerns about negative publicity. (Keeney & Michelle , 2005,pp 12)

Insider Threats

Employees pose the greatest threat to the inadvertent or deliberate disclosure of personally identifiable information, trade secrets, intellectual property, and sensitive or confidential information. Additionally, not only can portable media devices used on the organization's network easily and rapidly download massive amounts of data, they can also introduce viruses or malicious code.

Moreover, due to their trusted nature, these devices can bypass intrusion detection systems (IDSs) and antivirus protection safeguards.

PDA's, iPods, smart phones, and USB flash drives are all items that fit in the pocket and as a result, they're easily forgotten in taxis, subways, restaurants, or airports.

But the consequences of losing a laptop or a large storage device, such as a USB flash drive holding sensitive data about an organization's business, could lead to financial ruin and might destroy its reputation. Despite the devastating consequences of this high risk, a recent Pointset study indicated

that 99 percent of removable media devices don't have encryption capabilities. (FAITH M.HEIKKILA AUGUST 2007) .

Data theft

The actual act of stealing corporate data by insiders is quite simple in itself and today software that is easily available for download automates the whole process. Insider's only need to plug in the portable storage device on a corporate workstation and all data, including sensitive data, is automatically copied, without any additional user intervention. This automated process, commonly known as 'pod slurping', is able to copy whole databases and other confidential records to a portable storage device in a matter of a few minutes

Legal liabilities

When confidential information is 'lost' or illicit/objectionable data is introduced on the corporate network through portable storage devices, corporations might become legally liable for any information that is stolen or illicitly.

Measuring the use of mobile devices in enterprises

Introduction

The researcher visited many organizations to make interviews and observation with some organization officers to check the notification of using portable media devices in their work and identify the problems that they might face, to do this, researcher designed a questionnaire to collect data from different private and government organizations.

This questionnaire consists of 30 questions, and takes about 15 minutes to fill out. Following the differentiation and analysis of this questionnaire, 150 samples were distributed to public, private and government organizations, which collected only 93 samples.

After analysis the questionnaire data, the **result** is to design a security framework that includes a set of policies for employees to control and handle mobile devices. So as to prevent the internal threats in the organizations by employees.

Based on the three randomly questions as a sample of the questionnaire, as shown in the Figures (1), (2), (3), the use of portable devices in a widespread in the organizations, and this is a big problem, especially Organizations dealing with sensitive data

Q1. Is it permitted to use portable media devices and all benefits of internet inside the organization?

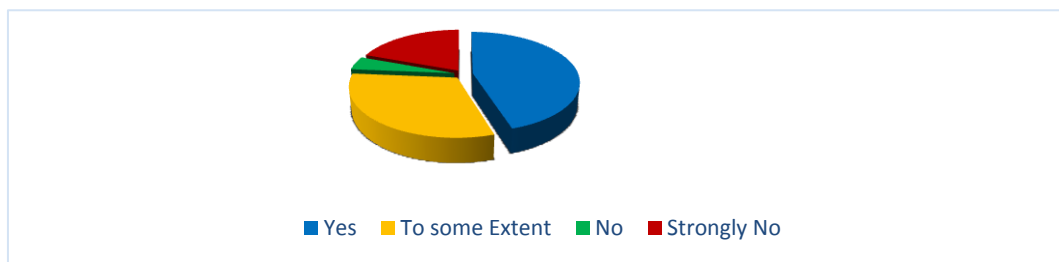


Figure (1):Use portable media device inside the organization

Q2. Do you expect any damage after using these devices and internet inside the organization?

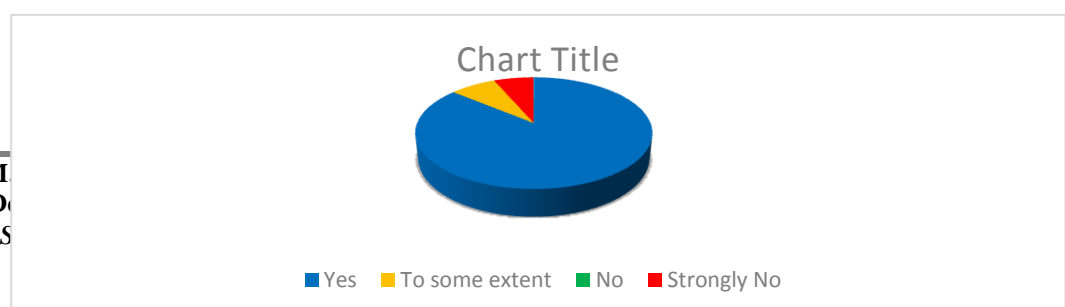


Figure (2): expect damage after using Portable media devices

Q3. Backup Information is available to recover lost data from portable media devices?

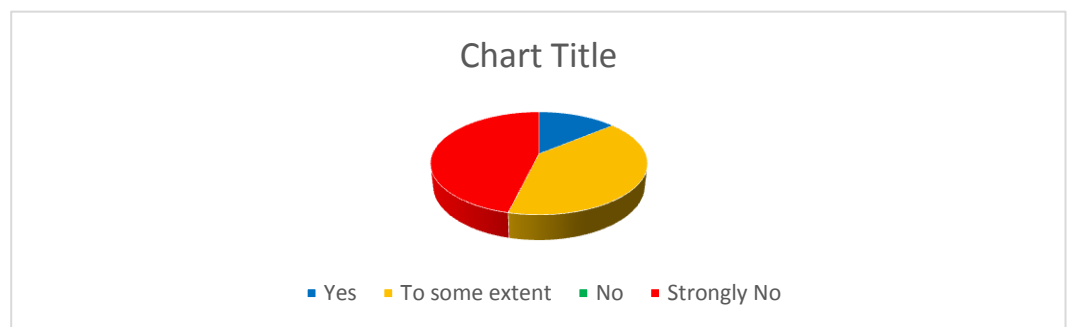


Figure (3): Backup Information to recover lost data from portable media devices



Figure (4): A proposed Security framework for portable media devices

A proposed Security Framework for Portable Media Devices

A framework Figure (4), shows that there is a set of policies for controlling mobile devices and includes the following policies:

Physical policy, general policy for all mobile devices, policy for memory control, MP3, MP4, PDA and smartphone control policy, HD control policy,

CD / DVD policy, LAPTOP control policy, network and Internet control policy.

All these policies control the use of mobile devices within the institution. There must be training courses and awareness of staff, to identify the seriousness of the use of these devices at random may lead to security problems for the institution

Introduction

In this section, the stages of the proposed framework will be explained along with an explanation of the detailed framework of each stage. The framework includes a set of policies aimed at handling and controlling the use of mobile devices and Internet services. The proposed framework should apply to all organizations.

General Policy for all Portable Media devices

- Mobile devices must comply with the Network Connection Policy including (if supported) the installation of a recognized and updated anti-virus package and any relevant operating systems patches.
- Passwords must not be stored within email clients, browsers and login scripts on a mobile device – please see the (Password Policy for guidance on password creation and usage).
- Mobile devices that contain or access sensitive information, or have been used to access sensitive information in the past, must be processed to ensure all data is permanently removed in a manner that prevents recovery before their disposal or transfer to another user. Deleting files
- and/or reformatting a device are insufficient to prevent recovery of data.
- In the event that a mobile device is lost or stolen, the Head of the IT Department must be notified. The police should also be notified and a crime number obtained for insurance purposes. And notify the Legal Compliance

team and IT Security team who will conduct a risk assessment and notify the Information Commissioners Office if required.

- Appropriate backup of mobile devices must be undertaken.

Physical Security(policy) – all mobile devices

- Mobile devices should not be left unattended and, where possible, must be physically locked away or secured.
- Mobile devices should be transported as hand luggage whenever possible.
- Mobile devices should be marked using a scribing tool, Smart Water or security label and indicate a method of return in case the device is lost. Their serial numbers must be recorded by the owner. Owners must be aware that even if a lost device is recovered, the data on it may have been copied in the meantime.

PDA's, USB Key/Flash drive, Memory sticks, Hard disk, MP3 players, Writable CD/DVD (Policy)

There are a number of recommendations and software and hardware solutions to ensure the secure use of USB flash drives.

- Implement a risk assessment methodology to ensure the correct controls to minimize risks throughout the lifecycle of the devices. A risk assessment will allow for understanding in detail the risks related to the use of devices and costs providing the basis to develop a strategy for closing these gaps.
- Implement security policies/guidelines around the use of these devices and storing of corporate data on to a personal mobility device.
- Implement security policies before any data/security breach happens. Develop a company security policy which has every employee signing an agreement for not connecting their personal devices drive to the network and transport data. Eventually allow the use of corporate devices, specifying employee responsibilities and rules for safe use and blocking devices that

have no valid business use. Thus, define what types of hardware are allowed to access the network.

- Corporate policies should be comprehensive but not so restrictive as to impede employee productivity. This is why many large organizations choose to monitor and log access to sensitive files rather than block them outright. These rules will vary depending on the roles and responsibilities of each employee.
- Introduce a procedure to assess the loss and/or damage of a corporate asset, such as mobility devices. Use forms to collect and analyse information from personnel involved as appropriate.
- Implement a centralized endpoint security policy through a dedicated solution. Deploying and managing portable storage devices across an organization can be complex and expensive. A centralized management enables organizations to overcome these challenges by:
 - Managing and eventually blocking ports, encryption and identity management software will not make devices 100 % secure. Monitor every port on every workstation and lock out unauthorized devices. Furthermore, it is also possible to audit port and record what devices are in use or be set to allow only specific devices, such as encrypted drive issued to particular employees.
 - Looking for a system that allows tracking offline usage of mobility devices and comparing mobile data files against the originals to determine if they have been opened, altered or copied to another device.
 - Recovering user passwords centrally, using a challenge response mechanism.
 - Managing the corporate device drives centrally.
 - Demonstrating compliance with security standards.

- Protecting assets and brand by demonstrating that devices were encrypted at the time of loss or stolen with an extensive auditing.
- **Audit and enforce policies:** once policies are in place ensure that they are followed, the audits can range from the physical inspection of employee workstations (e.g. monitor the use of devices in the organization eventually limiting the use of devices to company authorized devices) to virtual audits using network-based applications that follow data as it moves through an organization. Simply establishing corporate policies without any means of enforcing the rules or detecting violations is useless.
- **Asset management:** assess/identify all hardware and portable devices used to access the network. Eventually use software to identify every device that has ever been connected to the network. You will need this information to define your policies around the type of devices that can be used in the organization, the employees who will allow using them and the type of protection required.
- **Assess companies:** readiness for cases of loss of data if USB flash drives are lost or stolen.
- **Limit access:** limit the access to certain types and amount of sensitive data to certain employees. In a more complex organization, establish data usage rules specifying the personnel who can be authorized to get access to sensitive data files, what kind of data files can be portable and how they should be treated. Look for software that can automate this process by scanning files on network drives and client machines, checking for key words.
- **Attach USB drives** to key chains/lanyards to avoid loss of media: the reduced size of USB flash drives makes these devices easier to lose or be stolen and higher storage capacity increases the potential amount of data at risk for unauthorized access.

- **Invite users** to put the USB flash drive in read-only mode to avoid virus transmission: some USB flash drives include a physical switch to put the drive in a read-only mode to avoid the host computer from writing or modifying the data on the drive.
- Scan devices after copying files from an untrusted machine to avoid virus transmission.
- **Require users to authenticate:** prevent unauthorized access to data with a mechanism that requires users to authenticate using a password and/or fingerprint. Set a maximum number of password or biometric authentication retries to counter attacks.
- **Use of encryption:** performed either by software or hardware means, whereby data is altered in order to make it inaccessible without proper key to decrypt the data. In this way data will be useless without the required key and will remain always secure wherever it travels. A solution is to require sensitive data to be stored only on encrypted storage devices such as enterprises-grade mobility devices with mandatory password protection for all files. Another solution, which is widely acknowledged as one of the best, is to use hardware-based encrypted devices which perform the encryption on-board inside the devices. The major advantage of hardware-based encryption keys is that they never leave the devices are not susceptible to any outside attacks and virtually don't cause performance loss. Finally, encryption is a powerful security technology but is a tool which can be used. It should be used when data moves and access controls rights are not specific enough. Evaluate third-party data encryption tools with appropriate defences for all high-risk systems, those that contain sensitive data or are likely to be stolen and use for corporate espionage.
- **Protect an infrastructure** from malicious codes: use of antivirus protection to:

- **Stop viruses:** block, clean and remove viruses and Trojans from mobility devices.
- **Protect PCs:** prevent the devices from acting as a carrier of viruses that can be transmitted when you plug into a PC.
- **Backup information:** be able to recover data residing on devices drives.
- **Train the workforce:** train employees on policies around technology usage to make them aware of the risk involved with storing and transporting corporate data on portable media devices, explain how to avoid data leaks and remind them to report those that happen. Keep them informed of possible changes in policies and ensure they follow guidelines in their daily work. User education, awareness and acceptance are critical for the success of any security policy or implemented technical solution.
- **Run a survey:** to check if users are familiar with their organization's policies regarding portable media devices usage.
- **Start at the top:** start with senior management and personnel who travel with sensitive data before moving on to the rest of the organization. The best defence against data leaks is an educated workforce.
- **All PDA, USB Key/Flash drive,** memory sticks, External Hard disks devices should be password protected.
- **Any documents carried** on a mobility device should be password protected or use fingerprint recognition for security purpose.

Laptops (Policy)

Introduction

Laptop security policy is a document that states in writing the rules and practices to be conformed to at all times by the employees of an organization in order to ensure the safety of laptops issued to them and the data stored in the machines. Laptop security policy is ideally framed by IT professionals of the

organization and signed by all employees. If the security policy has not been implemented or communicated effectively across the organization and signed by all employees, it might leave the company vulnerable to laptop and data theft. A security policy is a formal document to be read by employees so that security practices can be standardized and agreed upon by them.

Rules and responsibilities

- Laptops and home personal computers should not be used for business activities without appropriate security measures, including up to date security “patches” and virus protection.
- Where sensitive information is held on laptops or mobile storage devices, data encryption must be applied to that information or to the entire device.
- Strong passwords must be used (see Passwords).
- All laptop computers must have a properly configured firewall, up to date antivirus software and patched software.
- When using a laptop, do not process personal or sensitive data in public places e.g. on public transport.
- Disable infrared ports, wireless cards and remove PCMCIA cards when not in use.
- The physical security of your laptop is your personal responsibility so please take all reasonable precautions. Be sensible and stay alert to the risks.
- Lock the laptop away out of sight when you are not using it, preferably in a strong cupboard, filing cabinet or safe. This applies at home, in the office or in a hotel. Never leave a laptop visibly unattended in cars.
- Carry and store the laptop in a padded laptop computer bag or strong briefcase to reduce the chance of accidental damage. Don't drop it or knock it about! An ordinary-looking briefcase is also less likely to attract thieves than an obvious laptop bag.

- Keep a note of the make, model, serial number and the Georgia Legal Services Program asset label of your laptop but do not keep this information with the laptop. If it is lost or stolen, notify the Police immediately and inform the IT administrator as soon as practicable (within hours, not days, please).
- Corporate laptops are provided for official use by authorized employees. Do not loan your laptop or allow it to be used by others such as family and friends.
- Avoid leaving your laptop unattended and logged-on. Always shut down, log off or activate a password-protected screensaver before walking away from the machine.

Passwords (Policy)

Introduction

Passwords are a define in maintaining proper portable media devices security. Passwords are particularly prone to attack and if broken provide the easiest path into a portable media device. Good passwords are hard to ‘crack’ but bad ones offer little or no protection.

- **Passwords should be constructed as follows:**

- ✓ At least 8 characters in length.
- ✓ Must include 3 of the following uppercase, lowercase, numeric, no alphabetic e.g. %, #
- ✓ Must not contain all or part of a user id
- ✓ Must be different to the last 24 used
- ✓ Do not use a password that is easily identified to yourself (name, car registration, telephone number)
- ✓ Do not use valid English words which can be easily identified.
- ✓ Keep your password confidential and do not share it.

Practical Tips to Prevent Theft

- Ensure employees report cases of lost or stolen portable devices to the IT department.
- Conduct a damage assessment for every mobility device that goes missing.
- Establish how and where they went missing.
- Review your policies/guidelines to ensure major sources of loss are covered.
- Highlight potential risks associated with the innocent use of mobility devices by employees and for other less legitimate purposes such as smuggling information out of the company.
- Take special measures for business units/departments which are handling sensitive data.
- Monitor and report incidents on a regular basis.
- Train and send out reminders to employees.
- Benchmark your performance against other similar enterprises.
- Collect feedback to further fine-tune the enforced solutions and policies for maximum accuracy and understand the patterns that increase the risk of data loss.

Benefits

An overview of the many benefits linked to a secure use of portable media devices will help and lead the enterprises to better decide about this matter. The following benefits were identified.

- Enhance and boost employee productivity through mobility and remote connectivity.
- Flexible and secure solutions will:
 - Protect corporate assets.
 - Reduce total cost of ownership.
 - Prove that devices were encrypted when stolen or lost.

- Defend enterprises from data leakage.
- Enforce mandatory company-wide security policies.
- Sanities any PC, anywhere. Allow connection to PC by authorized devices.
- Extend security policy beyond the perimeter:
 - Track all activity on mobility devices.
- Comply with the three pillars or classifications of information, security, confidentiality, availability and integrity, and security standards.

Conclusions

In today's organizations, sensitive data is stored and accessed on a variety of portable media devices; the storage capacity, size, low price and plug-and-play functionality are some of the reasons why their use has increased enormously. Portable media devices are often handling corporate information, such as financial information, forms, employee documents and customer data. These portable media devices remain largely unprotected and uncontrolled by IT departments, leaving business susceptible to consequences which may be devastating such as lost reputation, jobs and profits. Loss of company information is the result of employee ignorance about the risks associated with the use of portable media devices or their willingness to skirt policies in order to work more productively. Thus, most of the actions are not intentional or malicious but accidental and unintended. Although there is increasing awareness of the risks and costs related to the insecure usage of portable media devices, there is still a significant amount of work to do. It is therefore crucial that IT asset managers prepare themselves and their organization to regulate, manage and audit the use of portable media devices as ensuring the ability to secure information on the network and the opportunity to manage data which enter and leave the company environment is key for any organization

regardless of its size and maturity. With the increased number of portable devices used in business, with employees traveling and taking work home, a secure use of portable media devices and awareness of the related risks should be an integral part of the organization overall security strategy.

The benefits of this framework include, protect corporate assets, reduce total cost of ownership, prove that devices were encrypted when stolen or lost, enhance and boost employee productivity through mobility and remote connectivity, defend enterprises from data leakage, enforce mandatory company-wide security policies, sanitizes any PC, anywhere. Allow connection to PC by authorized devices, track all activity on mobility devices and comply with the three pillars or classifications of information security, confidentiality, availability and integrity, and security standards. This security frame work needs to be audited from time to time so that any developments in technology or changes in the nation's data security legislation can be incorporated. organization also need to put in place powerful mechanisms to ensure the implementation and applying of this security framework.

References:

- Andrew Charlesworth , 2009 , "The ascent of smartphone", Institution of Engineering and Technology, IEEE Engineering & Technology.**
- Carlton S. Rebeske, 2001 , " LAPTOP COMPUTER", Patent N0 US 6,295,038 B1.**
- Cheng Yu Huang, 2005, "Portable hard disk drive", Patent US 6891721 B2.**
- D.J. Parker, 1999 , "Defining DVD", IEEE Multimedia.**
- Daryl E. Anderson, Makarand P. Gore, Paul J. Mcclellan , 2003 , "Integrated cd/dvd recording and labeling", WO 2003032299 A2, Hewlett-Packard Company.**
- David L. MOSS & Erica J. scholder , 1997 , "LAPTOP TABLE FOR PORTABLE COMPUTERS", Patent 5,623,869, Dell Computer orporatlon, Apr. 29, 1997.**



- Der-sheng Huang , 2006 , "Memory card shaped card reader" ,Patent US 7104809 B1,DatafabSystemInc.
- Faith M. Heikkila, 2007 , "Encryption: Security Considerations for Portable Media Devices", IEEE Computer Society, IEEE Security & Privacy.
- GFI white paper " The threat posed by portable storage devices " ,
<https://www.gfi.com/whitepapers/combating-corporate-data-theft.pdf>
Published on 18 Oct. 2006, Last Updated on 19 July 2013.
- Graham Sharpless, 2004 , "An Introduction to DVD Formats", Deluxe Global Media Services Ltd.
- Jeff Grady, 2004 , "FM TRANSMITTER AND POWER SUPPLY/CHARGING ASSEMBLY FOR MP3 PLAYER", Pub. No US 2004/0058649 A1.
- Keeney, Michelle , Kowalski, Eileen ,Cappelli, Dawn , Moore, Andrew ,Shimeall, Timothy , Rogers, Stephanie," Insider Threat Study: Computer System Sabotage in Critical Infrastructure Sectors", NATIONAL THREAT ASSESSMENT CTR WASHINGTON DC, <http://www.dtic.mil/get-tr-doc/pdf?AD=ADA636653> , May 2005.
- KheinSengPua & Chee Kong Awyong, 2002, "UNIVERSAL SERIAL BUS FLASH MEMORY STORAGE DEVICE", No.: US 2002/0147882 A1.
- LasseSiitonen & RistoRonkka, 2000 , "Personal digital assistant with real time search capability", US6049796 A , Nokia Mobile Phones Limited.
- Mark Evan Cohen, 2004, "Thermal management of a laptop computer ",US 6760649 B2, International Business Machines Corporation.
- Sohail Khan & Mohammad Nauman & Shahrulniza Musa, 2012, "How secure is your smartphone: An analysis of smartphone security mechanisms " , IEEE Computer Society, Cyber Security, Cyber Warfare and Digital Forensic (CyberSec), International Conference on Kuala Lumpur, Malaysia.
- Steve Jobs, Anthony M. Fadell, Jonathan P. Ive, 2009, "Highly portable media device".



Toxicity of *Nerium oleander* Extractions on Nematode (*Pratylenchussudanensis*) on Bt Cotton, Gezira State, Sudan

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Abstract

*Plant parasitic nematodes are known as animals invertebrates, widespread, cylindrical, worm-shaped, which infect all crops causing direct damage by absorbing food from plant cells, leading to heavy losses in terms of production quantity, and indirect damage through direct infection with fungi and bacteria, in addition to transfer of pathogenic viruses from one plant to another. Cotton is one of the most important crops in the world due to its great economic importance. An ethanolic and aqueous extract of the *Nerium oleander* plant was prepared in the laboratory of the Plant Pathology Center. The effect of these extracts was studied on cotton cultivar *Gossypium hirsutum* (Bt) artificially infected with *Pratylenchussudanensis* through selecting treated cotton seeds and planted under greenhouse conditions. Then treated with both ethanolic and aqueous extracts after two weeks of planting by injecting (3 cc) of the extract per pot. All pots were injected with an aqueous suspension containing 40 nematodes from *P. sudanensis* worms, then the nematodes were extracted from soil and roots of laboratory plants two months after injection of the extract, the results showed that the effect of these treatments was significant, leading to a high increase in plant growth and a decrease in the compared to the control plants in the soil and roots tissue. The average nematode population in the soil was 10.7 and in the roots 1.7 for the plants treated with the ethanolic extract, while the aqueous extract was 16.3 in the soil and on the roots 2, compared to the control was 19.5 in the soil*



and on the roots 5 , and it was noted that the increase in the growth of plants treated with the ethanolic extract of *N. oleander* was greater than the treatment with the aqueous extract.

INTRODUCTION

Cotton (*G. hirsutum*) is known globally as a leading fiber and oil cash crop which is commercially grown in many countries in the Tropic and Subtropics regions. Cotton has been growing in Sudan since early 19th century. Until recent time, Sudan is considered the world second largest producer of long-staple cotton. The crop is grown in both irrigated and rain-fed sectors. The irrigated sector is symbolizes 90% of the total cultivated cotton area in Sudan (Salim, 2007). The main important cotton production areas in Sudan include Gezira, Suki, Rahad, New Halfa, White Nile area and Nuba Mountains. There are many commercial varieties cultivated such as Genetically Modified Cotton (Bt), Barakat 90 and Abdin. The crop participated in financial development of local communities in the region where cotton is cultivated.

Cotton crop in the Sudan is known to be affected by many pests and diseases, the insect pests are African boll worm (*Heliothis armigera*), white flies (*Bemisia tabaci*), Jassids (*Empoasca lybica*), Thrips (*Caliothrips sudanensis*), Termites (*Microtermes thoracalis*), Pink bollworm (*Platyedra gossypiella*), Sudan bollworm (*Diparopsis watersi*), Aphids (*Aphis gossypii*) (Hargreaves, 1948). Numbers of diseases includes pre-emergent such as damping off, Rhizoctonia and post-emergent such as root rot (*Fusarium*), Charcoal rot (*Macrophomina*), Alternaria leaf spots (*Alternaria spp*), leaf curl virus, the most important disease is black arm or Bacterial Blight of cotton (*Xanthomonas axonopodis pv. malvacearum*) In the Sudan, the association of plant-parasitic nematodes with the cotton crop, as plant parasites or from the soil around the crop root system, a preliminary survey conducted by Decker *et*

al. (1981) showed the presence of *Tylenchorhynchuscylindricus*, *Ditylenchusmyceliobagas*, *Telotylenchussp*, *Tylenchussp*, *Aphelenchoidessp* and *Aphelenchusavenaewere* found in association with roots of cotton, cv (.Barakat and Acala). Yassin (1973) reported that at least two species were isolated from within and round the roots of cotton with stunted growth areas,e.g., the Gezira, Khashm, Elgirba and Guneid, these are *pratylenchussudanensis*sn.sp. Loof and Yassin and *P.delattreiluc*. The commonest is, however ,*P. sudanensis*. Other plant parasitic Nematodes associated with cotton crops in the world .e.g. *Meloidogyne incognita*, *Rotylenchulusreniformis*, *Hoplolaimusspp*. *Tylenchorhynchusspp*., *Helicotylenchusspp*, *Aphelenchoides*, *Longidorusspp*., *Xiphinemaspp*. Kinloch and Sprenkel (1994), Sasser (1972), Smart and Perry (1969). The nematode which chosen for this study is *Pratylenchussudanensis* which was recorded for the first time on cotton in the Sudan . In this study, field and laboratory work is conducted to achieve the following objectives:

Bioassay of *N. oleander*extracts on *P. sudanensis* infesting cotton Bt cultivar to evaluate and compare the effect of these extract on the plant and nematode population development

Materials and Methods

Collection of plant sample

The fully mature leaves of *Narium oleander* were collected from Wad-Medani the leave were thoroughly washed and shade dried for 10 days .

Site of the experiments

The experiments worked up into laboratory of Plant Pathology Centre ,Faculty of Agricultural Sciences, University of Gezira .

Preparation of Nerium powder



The collected samples of the leaves were dried at room temperature, and powdered using an electric blender. The powdered leaves were kept in glass bottles for further use.

Extractions

All solvents and chemicals used should be of analytical grade. Two solvent, ethanol and water, were used for the extraction. Two hundred grams of powdered plant part was placed in a conical flask (1litre). The flask was shaken for 2 hours and then kept overnight in a refrigerator. After filtration under vacuum through Hyflosupercel (Prolabo, France). The filtrate and the percolate were combined and concentrated using a rotary evaporator (water-bath temperature, 40°C). The residue was finally dissolved in ethanol (25 ml) from which 5ml was used for the crude bioassay. This procedure was repeated for ethanol and water using the same plant debris.

Effect of *N. oleander* extracts on *P. sudanensis* on cotton *G. hirsutum* (Bt)

This experiment was conducted to determine and compare the effect of *Nerium oleander strmonium* extracts (ethanolic and aqueous) on *P.sudanensis* infecting cotton. Selective methods were used to evaluate the effects of these treatments on the nematode and the test plants rate of growth.

Procedure

120 cotton seedlings were planted in 40 droplet plastic. It contains 9 kg of silt and sand at a ratio of 2:1. In each plastic pot, 3 well-grown seedling. I left for two months under the conditions of the plastic house. Plant extracts were prepared from *Narium Oleander* as mentioned previously and 50 ml of extract was taken for every 15 pots, by drawing 3cc and injecting them in to each bucket separately. Ulceration nematode inoculations are added after their extraction, as previously mentioned on treated and un treated cotton plants, two

weeks after adding the plant extracts. By adding it in the middle of the planted seedlings with 40 *Pratylenchus* in a water suspension that has been modified to be poured into a small hole at a depth of 5 cm below the surface of the pot soil, using a pipette with a wide opening. The treated and un treated plants were then left for two months after inoculation. Immediately after that evaluations for the effect of the nematode on the test plants development as well as the nematode population in plant tissue and pots soil ,compared with control were determined as previously described pages(18 -21) . The experiment was made in a randomized complete block design with 5 replicates.

Results are shown in Tables 3-5, Appendix Table 2 and Figures.1-3.

Statistical analyses

Data were transformed $\sqrt{x + 0.5}$ and subjected to analyses of variance (ANOVA) Procedure ($P \leq 0.05$). Means were separated by using Duncen's Mtugll Ranye Test (DMRT). Statistical analysis was done by using the software statistics 8.

Results

Effect of *N. oleander* extract on *Pr. sudanensis* feeding on cotton cultivar *G. hirsutum* (Bt) as measured by plant height and root lengths

From the results in table (1), it can be seen that is *Narium oleander* extract compounds produced similar highly significant ($p < 0.05$) effects on *P. sudanensis* by increasing the plant height and root lengths .of the test cultivar compared with control.

The mean average of plant height of cotton plant treated by Neriumethanolic extract was 8.4 regarding to aqueous extract it was 8.2 in comparison with the control which was 5.9 The mean average of

root length of cotton plant treated by Neriumethanolic extract was 5.7 regarding to aqueous extract it was 5.4 in comparison with the control which was 4.5

Table (1): Effect of *N. oleander* extracts on plant height and root lengths of cotton cultivar *G. hirsutum*(Bt) inoculated with *P. sudanensis*

Cultivar cotton	Treatment	Plant height (cm)	Root length (cm)
BT	Control	5.9	4.5
	Ethanollic extract	8.4	5.7
	Aqueous extract	8.2	5.4
	Grand Mean	7.5	5.2
	SE	0.3	0.2
	CV	7.5	6.6

Effect of *N. oleander* extract on *P. sudanensis* feeding on cotton cultivar *G. hirsutum*(Bt) as measured by number of branches and prunus

The results in table 2 show that the *Nearium oleander* extract gave highly significant effects on the nematode ($P < 0.05$), as reflected by the increase of the cotton cultivar *G. hirsutum*(Bt). Number of branches and number of prunus, compared with control.

The mean average of Number of Branches of cotton plant treated by Neriumethanolic extract was 6.5 regarding to aqueous extract it was 3.5 in

comparison with the control which was 3.0 The mean average of Number of prunus of cotton plant treated by Neariumethanolic extract was 2.3 regarding to aqueous extract it was 2.2 in comparison with the control which was 1.1 .

Table (2): Effect of *Narium oleander* extract on number of branches and prunus of cotton cultivar *Gossypiumhirsutum*(Bt) inoculated with *Pratylenchussudanensis* (Mean of 5 reps)

Cultivar	Treatment	Number of Branches	Number of prunus
BT	Control	3.0	1.1
	Ethanollic extract	6.5	2.3
	Aqueous extract	3.5	2.2
	Grand Mean	4.3	1.9
	SE	1.4	0.2
	CV	58.4	20.5

Effects of *N.oleander* extract on *P. sudanensis* feeding on cotton cultivar *G. hirsutum*(Bt) as measured by Leaf and root fresh and dry weights

From the result as presented in table 3 ,it can be seen that these treatments produce highly significant effects ($P<0.05$) against *Pratylenchussudanensis* as demonstrated by the increase of Leaf and root fresh and dry weights of the cultivars, compared with the control. Again these results illustrate that nariumethanolic extract is shown to be slightly more effective in increasing the test plants parts than the aqueous extract The mean average of leaf (fresh and dry) weight of cotton plant treated by Nariumethanolic extract was

8.8, 6.5 regarding to aqueous extract it was 7.8 , 6.5 in comparison with the control which was 6.4, 5.1 The mean average of root (fresh & dry) weight of cotton plant treated by Neriummethanolic extract was 2.8, 2.2 regarding to aqueous extract it was 2.0, 1.5 in comparison with the control which was 1.0, 0.6.

Table (3): Effect of *N. oleander* extract on leaf and root fresh and dry weights of cotton cultivar *G. hirsutum*(Bt) inoculated with *P.sudanensis*(Mean of 4 reps)

Cultivar cotton	Treatment	Leaf fresh weight	Leaf dry weight	Root fresh weight	Root dry weight
BT	Control	6.4	5.1	1.0	0.6
	Ethanollic extract	8.8	6.5	2.8	2.2
	Aqueous extract	7.8	6.5	2.0	1.5
	Grand mean	7.7	6.0	1.9	1,4
	SE	1.4	0.8	0.9	0.3
	CV	38.9	28.3	72.4	36.2

Effect of *N. oleander* extract of *Pratylenchussudanensis* feeding on cotton cultivar as measured by nematode population

Nematode reproduction test results illustrated in Table 4 indicated that the alcoholic extract of the *Narium oleander* plant gave the highest effect on reducing the growth rate of nematode *Pratylenchussudanensis* obtained from roots and soil . These results also, show that Aqueous extract was slightly less effective in reducing the nematode population.

Table (4): Effect of *N. oleander* extracts on soil and roots population of *P.sudanensis* infesting in cotton cultivar.

Cultivar	Treatments	Soil population	Roots population
Cotton	Control	19.5	5
	Alcoholic extract	10.7	1.7
	Aqueous extract	16.3	2
	Grandmean	15.5	2.9

Discussion

In this work it has been found to be greater effects were produced by *N. oleander* extracts (Alcoholic & Aqueous) in increasing cotton plant rate of growth and reducing of *P. sudanensis* nematode populations obtained from roots and soil of cotton cultivars, and this coincides with what was mentioned by (Hug *et al.*, 1999). Hussein and Gorski (2011) that the *N. oleander* plant contained anti-bacterial substances. The above results also agree with the findings of Jude (2013) where the great effectiveness of ethanolic *Nerium oleander* extract against bacteria and fungi was proved. In another study by Shirzadian *et al.* (2009) they reported that plant extracts derived by ethanol had more antifungal activity against some plant pathogenic fungi including *F. solani* and *F. oxysporum* than some extracts performed by water. Although some researchers who only used the aqueous extracts in their studies (Bhardwaj, 2012). The present study revealed that leaves of *N. Oleander* possessed antifungal activity. Similarly Yadav *et al.*, (2013) reported alkaloids,



flavonoids, Carbohydrates, glycosides and tannins in different Parts of *N. Oleander*. In another study by Bakr *et al.*, (2015) they reported that significantly effect of all tested *N. Oleander* extracts on egg hatching and larvae mortality under laboratory conditions . A great reduction in number of galls; eggmasses ;femaies/ root system of tomato plants and number of second stage juveniles,(J2S)/250gsoil was recorded compared to the treated plants with nematode alone .Tomato plant growth parameters and oxidative enzymes were markedly enhanced compared to the non-treated control Bakr *et al.*,(2015) .Similar results were reported by Zasadaet *el.*,(2002) and Elbadriet *al.*, (2008) mentioned that oleander petroleum ether extract at the high concentration 3% was the most effective in larvae mortality could be attributed to the predominant presence of acidic compounds and their derivatives and amino acids and their derivatives.Our results were in agreement with that reported by Tiyagi et al., (2012)who mentioned that *N. indicum* significantly reduced the number of root galls caused by *M. incognita* in treated plants and that of Zasadaet *el.*,(2002) who reported that *N.Oleander* reduced the root galling caused by *M. incognita* on tomato plants. Current results in agreement with those by Zasadaet *el.*,(2002) who reported that the water extract of *N.Oleander* reduced *M.javanica* egg hatching.

References

- Bakr, R.A.; M.E. Mahdy; E.M.Mousa and M.A.Salem .(2015) . Egyption Journal Crop Protection,Vol .,(10) ,No. (1):1-13.
- Decker, E.M., El-Amin, A.M. and Yassin, Y.A.B. Zeidan. (1981) Plant parasitic nematodes in the Toker Delta, Sudan, Beitrage trop. Land wirtsch.Veterinatmea. 3,353-357.



- Elbadri , G.A.A.; Dong Woon Lee ,Jung Chan Park , Hwang Bin Yu and Ho Yul Choo (2008) . Evaluation of Various plant extracts for their nematicidal efficacies against juveniles of *Meloidogyne incognita* .Journal of Asia-Pacific Entomology ,11:99-102.
- Hargreaves, H.A. (1948) .list of Recorded Cotton Insects of the World .London :Common.Ent,50pp.
- Hug, M.M., Jabbar, A., Rashid, M.A. and Hasan, C.M. (1999).A novel antibacterial and cardiac steroid from the roots of *Nerium oleander*.*Fitoterapia* 70(1): 5–9.
- Hussain, M.A. and Gorski, M.S. (2011).Antimicrobial Activity of *Nerium oleander* Linn. *Asian J. Pl.Sci.*, 3 (2) : 177 – 180
- Jude, C.A. (2013) Extraction, Characterization and industrial applications of Tobacco Seed Oil (*Nicotianatabacum*). *Chemistry and Materials Research* 3(2): 19–21.
- Kinloch, R.A. and Sprengel, R. K. (1994). Plant- Parasitic nematodes associated with cotton inflorida .*suppl Jour .Nematology* 26:749 -752.
- Salim,H.A. (2007). Combining ability for yield and its components in half diallelcrosses of local and introduce cultivars of upland cotton (*Gossypiumhirsutum* L.) .M.Sc. Thesis 2007.Pp46.
- Sasser, J.N. (1972).Nematode diseases of cotton. Pp. 187-214, In J.M.Webster (ed).*Economic Nematology* .Academic Press. New York .
- Shirzadian, S.; Afshari-Azad, H. and Khalghani, J. (2009).Introductory study of antifungal activities of bryophyte extracts-*ApplEntomolphytopathol* ,77:1-22.
- Smart, G.C.Jr. and Perry, J.G. (1969).*Tropical Nematology*. University of Florida Press ,Gainesville ,Florida , USA.
- Yadav, C.H.S.; Bharadwaj,D.N.S;Yedukondalu, M.; Methushala, C.H. and Kumar, A.R. (2013). Phytochemical evaluation of *Nyctanthesarbortristis*, *Nerium oleander* and *catharathnusroseus* .*Ind .J.Respharm Biotechnol*, 1 (3) : 333-338.s



- Yassin, A.M. (1973). A root lesion nematode parasitic to cotton in the Gezira 1973 :Emp.cott . Gr. Rev. 50 :161-168
- Zasada, I. A., H. ferris and L. Zheng (2002).Plant Sources of Chinese Herbal Remedies:LaboratoryEfficacy,Suppression of *Meloidogyne javanica* in Soil, and Phytotoxicity Assays.Journal of Nematology, 34(2):124-129 .

Using Floyd Algorithm to find the Shortest Path for Marketing the Gum Arabic in Sudan

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Abstract

In this paper Floyd's algorithm was utilize to find out the shortest path for marketing cities of Gum Arabic between fourteen major marketing cities in Sudan. After completing the algorithm we found that the route with smallest distance is (Nyala, AlObied, Omdurman, Atbara, Port Sudan) with length 1650 Km

المستخلص

في هذا البحث استخدمت خوارزمية فلويد لاكتشاف أقصر طريق لتصدير الصمغ العربي من مدن الانتاج الى بورتسودان . كان الاختيار بين أربع عشرة مدينة رئيسية في السودان كانت خيار ليمر بها الطريق. بعد الانتهاء من الخوارزمية وجدنا أن أصغر مسار هو (نياالا ، الابيض ، أم درمان ، عطبرة ، بورتسودان) بطول 1650 كم.

Key words: Graph theory, shortest path problem, Floyd's algorithm

Introduction

In the eighteenth century, seven bridges connected four regions in the former city of Kongsberg (now Kaliningrad) when the people went on strolls through town they wondered if there was a way to travel across all seven bridges and return to the starting point without crossing each bride twice. This problem was solved by Leonard Euler in 1736, which as a solution consisted of representing the problem by a graph, with the four regions represented by four vertices and the seven bridges by seven edges as follows.(Trudeau 2013, Marcus 2020)

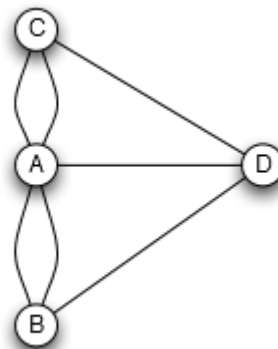


Fig (1) [regions represented]

Graph theory is a branch of mathematics that concerns a network of points connected by lines. The concept has expanded and is used in many applications such as chemical bonding, genetics, and computer science. The most visible application of basic graph theory is vacation planning. MapQuest, a popular site used for finding information like driving directions and quickest routes, relies heavily on graph theory concepts in order to create the routes. Graph theory is now a major tool in mathematical research, electrical engineering, computer programming and net

The second issue relating to paths in digraphs is finding the best path between two vertices. The simplest form of the problem is to compute the path between two vertices that uses the fewest edges. We generalize somewhat and allow the edge of the digraph to be assigned weights that have nonnegative values. We call such a digraph a weighted digraph. The weight of the path is then the sum of the weights of the edges in the path. The shortest path between two vertices u and v is called the distance from u to v . If there is no path from u to v , the distance is said to be infinity (∞). There are many algorithms for finding shortest paths in digraphs. The one we present here was developed by Floyd's.

In this paper we discuss the Floyd's algorithm and application it for find the shortest path for marketing the gum Arabic in Sudan

The algorithm works by updating two matrices, namely D_k and Q_k , n times for a n - node network. The matrix D_k , in any iteration k , gives the value of the shortest distance (time) between all pairs of nodes (i, j) as obtained till the

K^{th} iteration. The matrix Q_k has q_{ij}^k as its elements. The value of q_{ij}^k gives the immediate predecessor node from node i to node j on the shortest path as determined by the k^{th} iteration. D_0 and Q_0 give the starting matrices D_n and Q_n give the final matrices for an n -node system. The first task is to determine D_0 and Q_0 . D_0 is taken up first. The elements d_{ij} of matrix D_0 are defined as follows: If a link (branch) exists between nodes i and j the length of the shortest path between these nodes equals length $l(i, j)$ of branch (i, j) which connects them. Should there be several branches between nodes i and node j , the length of the shortest path d_{ij}^0 must equal the length of the shortest branch, i.e.: (Pešić, Šelmić et al. 2020)

$$d_{ij}^0 = \min[l_1(i, j), l_2(i, j), \dots, l_m(i, j)]$$

Where m is the number of branches between node i and node j .

It is clear that $d_{ij}^0 = 0$ when $i = j$. In the case when there is no direct link between node i and node j , we have no information at the beginning concerning the length of the shortest path between these two nodes so we treat them as though they were infinitely far from each other, that is,

$$d_{ij}^0 = \infty$$

Elements d_{0j}^0 of the predecessor matrix Q_0 are defined as follows:

If a link (branch) exists between nodes i and j the length of the shortest path between these nodes equals length $l(i, j)$ of branch (i, j) which connects them. Should there be several branches between nodes i and node j , the length of the shortest path d_{ij}^0 must equal the length of the shortest branch, i.e.:

$$d_{ij}^0 = \min[l_1(i, j), l_2(i, j), \dots, l_m(i, j)]$$

First, we assume that $q_{0j}^0 = i$, for $i = j$, i.e. that for every pair of nodes (i, j) for $i = j$, the immediate predecessor of node j on the shortest path leading from node i node j is actually node i . After defining D_0 and Q_0 the following steps are used repeatedly to determine D_n and Q_n . (Shukla 2013)

Step (1): Let $k = 1$

Step (2): We calculate elements d_{ij}^k of the shortest path length matrix found after the k -th passage through algorithm D_k using the following equation:

$$d_{ij}^k = \min[d_{ij}^{k-1} + d_{ik}^{k-1} + \dots + d_{kj}^{k-1}]$$

Step (3): Elements d_{ij}^k of predecessor matrix Q_k found after the k -th passage through the algorithm are calculated as follows:

$$d_{ij}^k = \begin{cases} q_{kj}^{k-1}, & \text{for } d_{ij}^k \neq d_{ij}^{k-1} \\ d_{ij}^k, & \text{otherwise} \end{cases}$$

Step (4): If $k = n$, the algorithm is finished. If $k < n$, increase k by 1, i.e. $K = k + 1$ and return to step 2.

Let us now look at the algorithm in a little more detail. In step (2), each time we go through the algorithms we are checking as to whether a shorter path exists between nodes i and j other than the path we already know about which was established during one of the earlier passages through the algorithm. If we establish that $d_{ij}^k \neq d_{ij}^{k-1}$, i.e. if we establish during the k -th passage through the algorithm that the length of the shortest path d_{ij}^k between nodes i and j is less than the length of the shortest path d_{ij}^{k-1} known previous to the k -th passage, we have to change the immediate predecessor node to node j . Since the length of the new shortest path is:

$$d_{ij}^k = d_{ij}^{k-1} + d_{kj}^{k-1}$$

it is clear that in this case node k is the new immediate predecessor node to j , and therefore:

$$q_{ij}^k = q_{kj}^{k-1}$$

This is actually done in the third algorithmic step. It is also clear that the immediate predecessor node to node j does not change if, at the end of step 2, we have established that no other new, shorter path exists. This means that:

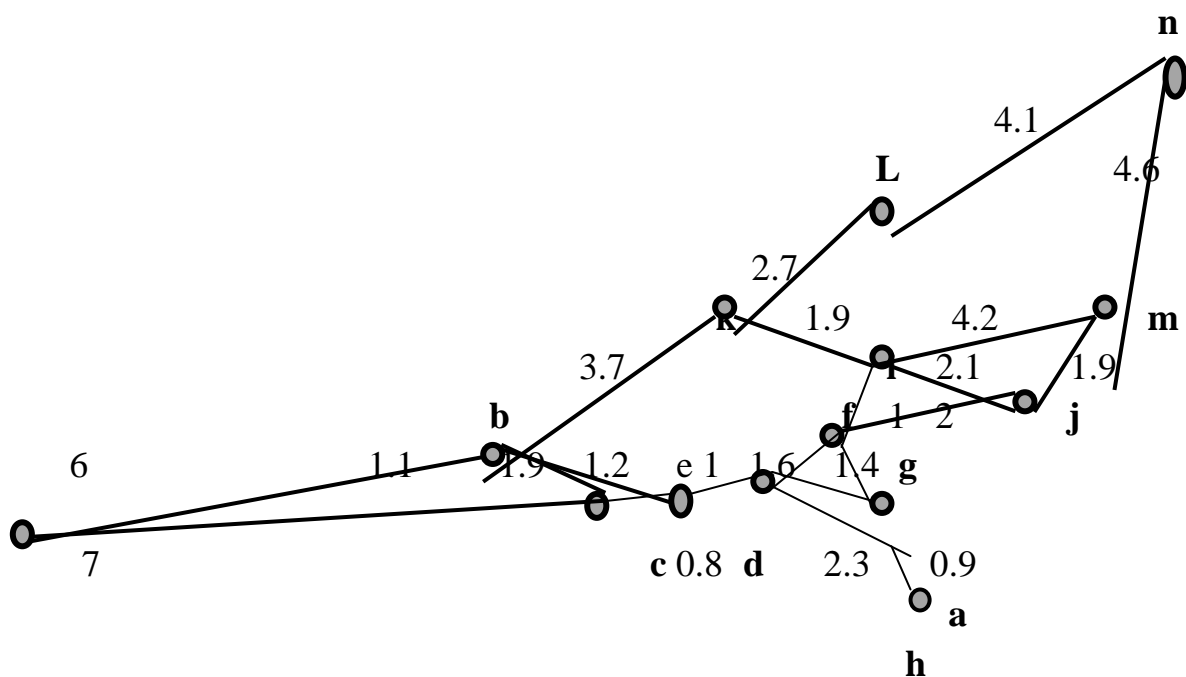
$$q_{ij}^k = q_{kj}^{k-1} \text{ for } d_{ij}^k = q_{ij}^{k-1}$$

When we go through the algorithm n times (n is the number of nodes in the transportation network), elements d_{ij}^n of final matrix D_n will constitute the shortest path going from node i to node j .

The shortest path for marketing the gum Arabic in Sudan

Shortest path problem is a project using the application of graph theory for shortest path problem. The question is how efficiently graph theory can be used in route planning for a marketing of gum Arabic. The question is essentially two fold; what is route with the shortest distance between Nyala and Port Sudan and length of this route.

In the following graph **a, b, c, d, e, f, g, h, i, j, k, L, m, and n** represents the marketing cities .i.e. **a** → Nyala, **b** → Elobeid, **c** → Ummruwaba, →**d** Tendelti, **e** → Rabk ,**f**→ Sinner, **g** →Wadennial, →**h** Eldamazeen, **i** → wad madni, **j** → Elgadarif, **k**→ Omdurman, **L**→Atbara, **m**→ cassala **n**→Bortsudana, and the distance between cities represents by 1cm≡100Km



Represents the marketing cities

Fig (4.1)

using Floyd’s algorithm to finding the shortest path between Nyala and Port Sudan starting by the matrices D_0 and Q_0

The matrices D_0 and Q_0 give the initial representations of the graph. D_0 is symmetrical.

Starting matrix D_0 is as follows:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	6	7	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞
2	6	0	1.1	1.9	∞	∞	∞	∞	∞	∞	3.7	∞	∞	∞
3	7	1.1	0	0.8	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞
4	∞	1.9	0.8	0	1.2	∞	∞	∞	∞	∞	∞	∞	∞	∞
5	∞	∞	∞	1.2	0	1	1.6	2.3	∞	∞	∞	∞	∞	∞
6	∞	∞	∞	∞	1	0	1.4	∞	1	2	∞	∞	∞	∞
7	∞	∞	∞	∞	1.6	1.4	0	0.9	∞	∞	∞	∞	∞	∞
8	∞	∞	∞	∞	2.3	∞	0.9	0	∞	∞	∞	∞	∞	∞
9	∞	∞	∞	∞	∞	1	∞	∞	0	2.1	1.9	∞	4.2	∞
10	∞	∞	∞	∞	∞	2	∞	∞	2.1	0	∞	∞	1.9	∞
11	∞	3.7	∞	∞	∞	∞	∞	∞	1.9	∞	0	2.7	∞	∞
12	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	2.7	0	∞	4.1
13	∞	∞	∞	∞	∞	∞	∞	∞	4.2	1.9	∞	∞	0	4.6
14	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	4.1	4.6	0

Starting matrix Q_0 is as follows:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	–	1	1	1	1	1	1	1	1	1	1	1	1	1
2	2	–	2	2	2	2	2	2	2	2	2	2	2	2
3	3	3	–	3	3	3	3	3	3	3	3	3	3	3
4	4	4	4	–	4	4	4	4	4	4	4	4	4	4
5	5	5	5	5	–	5	5	5	5	5	5	5	5	5
6	6	6	6	6	6	–	6	6	6	6	6	6	6	6
7	7	7	7	7	7	7	–	7	7	7	7	7	7	7
8	8	8	8	8	8	8	8	–	8	8	8	8	8	8
9	9	9	9	9	9	9	9	9	–	9	9	9	9	9
10	10	10	10	10	10	10	∞	10	10	–	10	10	10	10
11	11	11	11	11	11	11	11	11	11	11	–	11	11	11
12	12	12	12	12	12	12	12	12	12	12	12	–	12	12
13	13	13	13	13	13	13	13	13	13	13	13	13	–	13
14	14	14	14	14	14	14	4	14	14	14	14	14	14	–

Starting matrix D_1 is as follows:

We now go to the first algorithm step. Let $k = 1$. As an illustration of step 2 we calculate the elements of the first row of matrix D_1

$$d_{12}^1 = \min\{d_{12}^0, d_{11}^0 + d_{12}^0\} = \min\{6.0 + 6\} = 6$$

$$d_{13}^1 = \min\{d_{13}^0, d_{11}^0 + d_{13}^0\} = \min\{7.0 + 7\} = 7$$

$$\begin{aligned}
 d_{14}^1 &= \min\{d_{14}^0, d_{11}^0 + d_{14}^0\} = \min\{\infty.0 + \infty\} = \infty \\
 d_{15}^1 &= \min\{d_{15}^0, d_{11}^0 + d_{15}^0\} = \min\{\infty.0 + \infty\} = \infty \\
 d_{16}^1 &= \min\{d_{16}^0, d_{11}^0 + d_{16}^0\} = \min\{\infty.0 + \infty\} = \infty \\
 d_{17}^1 &= \min\{d_{17}^0, d_{11}^0 + d_{17}^0\} = \min\{\infty.0 + \infty\} = \infty \\
 d_{18}^1 &= \min\{d_{18}^0, d_{11}^0 + d_{18}^0\} = \min\{\infty.0 + \infty\} = \infty \\
 d_{19}^1 &= \min\{d_{19}^0, d_{11}^0 + d_{19}^0\} = \min\{\infty.0 + \infty\} = \infty \\
 d_{110}^1 &= \min\{d_{110}^0, d_{110}^0 + d_{14}^0\} = \min\{\infty.0 + \infty\} = \infty \\
 d_{111}^1 &= \min\{d_{111}^0, d_{11}^0 + d_{111}^0\} = \min\{\infty.0 + \infty\} = \infty \\
 d_{112}^1 &= \min\{d_{112}^0, d_{11}^0 + d_{112}^0\} = \min\{\infty.0 + \infty\} = \infty \\
 d_{114}^1 &= \min\{d_{114}^0, d_{11}^0 + d_{114}^0\} = \min\{\infty.0 + \infty\} = \infty
 \end{aligned}$$

D_1 is as matrix follows

and Q_1 is as follows:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	–	1	1	1	1	1	1	1	1	1	1	1	1	1
2	2	–	2	2	2	2	2	2	2	2	2	2	2	2
3	3	3	–	3	3	3	3	3	3	3	3	3	3	3
4	4	4	4	–	4	4	4	4	4	4	4	4	4	4
5	5	5	5	5	–	5	5	5	5	5	5	5	5	5
6	6	6	6	6	6	–	6	6	6	6	6	6	6	6
7	7	7	7	7	7	7	–	7	7	7	7	7	7	7
8	8	8	8	8	8	8	8	–	8	8	8	8	8	8
9	9	9	9	9	9	9	9	9	–	9	9	9	9	9
10	10	10	10	10	10	10	∞	10	10	–	10	10	10	10
11	11	11	11	11	11	11	11	11	11	11	–	11	11	11
12	12	12	12	12	12	12	12	12	12	12	12	–	12	12
13	13	13	13	13	13	13	13	13	13	13	13	13	–	13
14	14	14	14	14	14	14	4	14	14	14	14	14	14	–

Step (2) set $k = 2$ then $D_2 =$

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	6	7	(7.9)	∞	∞	∞	∞	∞	∞	(9.7)	∞	∞	∞
2	6	0	1.1	1.9	∞	∞	∞	∞	∞	∞	3.7	∞	∞	∞
3	7	1.1	0	0.8	∞	∞	∞	∞	∞	∞	(4.8)	∞	∞	∞
4	(7.9)	1.9	0.8	0	1.2	∞	∞	∞	∞	∞	(5.6)	∞	∞	∞
5	∞	∞	∞	1.2	0	1	1.6	2.3	∞	∞	∞	∞	∞	∞
6	∞	∞	∞	∞	1	0	1.4	∞	1	2	∞	∞	∞	∞
7	∞	∞	∞	∞	1.6	1.4	0	0.9	∞	∞	∞	∞	∞	∞
8	∞	∞	∞	∞	2.3	∞	0.9	0	∞	∞	∞	∞	∞	∞
9	∞	∞	∞	∞	∞	1	∞	∞	0	2.1	1.9	∞	4.2	∞
10	∞	∞	∞	∞	∞	2	∞	∞	2.1	0	∞	∞	1.9	∞
11	(9.7)	3.7	(4.8)	(5.6)	∞	∞	∞	∞	1.9	∞	0	2.7	∞	∞
12	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	2.7	0	∞	4.1
13	∞	∞	∞	∞	∞	∞	∞	∞	4.2	1.9	∞	∞	0	4.6
14	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	4.1	4.6	0

Q_2 is as follows:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	-	1	1	2	1	1	1	1	1	1	2	1	1	1
2	2	-	2	2	2	2	2	2	2	2	2	2	2	2
3	3	3	-	3	3	3	3	3	3	3	2	3	3	3
4	2	4	4	-	4	4	4	4	4	4	2	4	4	4
5	5	5	5	5	-	5	5	5	5	5	5	5	5	5
6	6	6	6	6	6	-	6	6	6	6	6	6	6	6
7	7	7	7	7	7	7	-	7	7	7	7	7	7	7
8	8	8	8	8	8	8	8	-	8	8	8	8	8	8
9	9	9	9	9	9	9	9	9	-	9	9	9	9	9
10	10	10	10	10	10	10	∞	10	10	-	10	10	10	10
11	2	11	2	2	11	11	11	11	11	11	-	11	11	11
12	12	12	12	12	12	12	12	12	12	12	12	-	12	12
13	13	13	13	13	13	13	13	13	13	13	13	13	-	13
14	14	14	14	14	14	14	4	14	14	14	14	14	14	-

Step (3) set $k = 3$ then $D_3 =$

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	6	7	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞
2	6	0	1.1	1.9	∞	∞	∞	∞	∞	∞	3.7	∞	∞	∞
3	7	1.1	0	0.8	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞
4	∞	1.9	0.8	0	1.2	∞	∞	∞	∞	∞	∞	∞	∞	∞
5	∞	∞	∞	1.2	0	1	1.6	2.3	∞	∞	∞	∞	∞	∞
6	∞	∞	∞	∞	1	0	1.4	∞	1	2	∞	∞	∞	∞
7	∞	∞	∞	∞	1.6	1.4	0	0.9	∞	∞	∞	∞	∞	∞
8	∞	∞	∞	∞	2.3	∞	0.9	0	∞	∞	∞	∞	∞	∞
9	∞	∞	∞	∞	∞	1	∞	∞	0	2.1	1.9	∞	4.2	∞
10	∞	∞	∞	∞	∞	2	∞	∞	2.1	0	∞	∞	1.9	∞
11	∞	3.7	∞	∞	∞	∞	∞	∞	1.9	∞	0	2.7	∞	∞
12	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	2.7	0	∞	4.1
13	∞	∞	∞	∞	∞	∞	∞	∞	4.2	1.9	∞	∞	0	4.6
14	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	4.1	4.6	0

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	6	7	(7.8)	∞	∞	∞	∞	∞	∞	9.7	∞	∞	∞
2	6	0	1.1	1.9	∞	∞	∞	∞	∞	∞	3.7	∞	∞	∞
3	7	1.1	0	0.8	∞	∞	∞	∞	∞	∞	4.8	∞	∞	∞
4	(7.8)	1.9	0.8	0	1.2	∞	∞	∞	∞	∞	5.6	∞	∞	∞
5	∞	∞	∞	1.2	0	1	1.6	2.3	∞	∞	∞	∞	∞	∞
6	∞	∞	∞	∞	1	0	1.4	∞	1	2	∞	∞	∞	∞
7	∞	∞	∞	∞	1.6	1.4	0	0.9	∞	∞	∞	∞	∞	∞
8	∞	∞	∞	∞	2.3	∞	0.9	0	∞	∞	∞	∞	∞	∞
9	∞	∞	∞	∞	∞	1	∞	∞	0	2.1	1.9	∞	4.2	∞
10	∞	∞	∞	∞	∞	2	∞	∞	2.1	0	∞	∞	1.9	∞
11	9.7	3.7	4.8	5.6	∞	∞	∞	∞	1.9	∞	0	2.7	∞	∞
12	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	2.7	0	∞	4.1
13	∞	∞	∞	∞	∞	∞	∞	∞	4.2	1.9	∞	∞	0	4.6
14	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	4.1	4.6	0

Q_3 is as follows:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	–	1	1	3	1	1	1	1	1	1	2	1	1	1
2	2	–	2	2	2	2	2	2	2	2	2	2	2	2
3	3	3	–	3	3	3	3	3	3	3	2	3	3	3
4	3	4	4	–	4	4	4	4	4	4	2	4	4	4
5	5	5	5	5	–	5	5	5	5	5	5	5	5	5
6	6	6	6	6	6	–	6	6	6	6	6	6	6	6
7	7	7	7	7	7	7	–	7	7	7	7	7	7	7
8	8	8	8	8	8	8	8	–	8	8	8	8	8	8
9	9	9	9	9	9	9	9	9	–	9	9	9	9	9
10	10	10	10	10	10	10	∞	10	10	–	10	10	10	10
11	2	11	2	2	11	11	11	11	11	11	–	11	11	11
12	12	12	12	12	12	12	12	12	12	12	12	–	12	12
13	13	13	13	13	13	13	13	13	13	13	13	13	–	13
14	14	14	14	14	14	14	4	14	14	14	14	14	14	–

Step (4) set $k = 4$ then $D_4 =$

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	6	7	7.8	(9)	∞	∞	∞	∞	∞	9.7	∞	∞	∞
2	6	0	1.1	1.9	(3.1)	∞	∞	∞	∞	∞	3.7	∞	∞	∞
3	7	1.1	0	0.8	(2)	∞	∞	∞	∞	∞	4.8	∞	∞	∞
4	7.8	1.9	0.8	0	1.2	∞	∞	∞	∞	∞	5.6	∞	∞	∞
5	(9)	(3.1)	(2)	1.2	0	1	1.6	2.3	∞	∞	(6.8)	∞	∞	∞
6	∞	∞	∞	∞	1	0	1.4	∞	1	2	∞	∞	∞	∞
7	∞	∞	∞	∞	1.6	1.4	0	0.9	∞	∞	∞	∞	∞	∞
8	∞	∞	∞	∞	2.3	∞	0.9	0	∞	∞	∞	∞	∞	∞
9	∞	∞	∞	∞	∞	1	∞	∞	0	2.1	1.9	∞	4.2	∞
10	∞	∞	∞	∞	∞	2	∞	∞	2.1	0	∞	∞	1.9	∞
11	9.7	3.7	4.8	5.6	(6.8)	∞	∞	∞	1.9	∞	0	2.7	∞	∞
12	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	2.7	0	∞	4.1
13	∞	∞	∞	∞	∞	∞	∞	∞	4.2	1.9	∞	∞	0	4.6
14	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	4.1	4.6	0

Q_4 is as follows:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	-	1	1	3	4	1	1	1	1	1	2	1	1	1
2	2	-	2	2	4	2	2	2	2	2	2	2	2	2
3	3	3	-	3	4	3	3	3	3	3	2	3	3	3
4	3	4	4	-	4	4	4	4	4	4	2	4	4	4
5	4	4	4	4	5	-	5	5	5	5	4	5	5	5
6	6	6	6	6	6	6	-	6	6	6	6	6	6	6
7	7	7	7	7	7	7	7	-	7	7	7	7	7	7
8	8	8	8	8	8	8	8	8	-	8	8	8	8	8
9	9	9	9	9	9	9	9	9	9	-	9	9	9	9
10	10	10	10	10	10	10	∞	10	10	-	10	10	10	10
11	2	11	2	2	4	11	11	11	11	11	-	11	11	11
12	12	12	12	12	12	12	12	12	12	12	12	-	12	12
13	13	13	13	13	13	13	13	13	13	13	13	13	-	13
14	14	14	14	14	14	14	4	14	14	14	14	14	14	-

Step (5) set $k = 5$ then $D_5 =$

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	6	7	7.8	9	(10)	(10.6)	(11.3)	∞	∞	9.7	∞	∞	∞
2	6	0	1.1	1.9	3.1	(4.1)	(4.7)	(5.4)	∞	∞	3.7	∞	∞	∞
3	7	1.1	0	0.8	2	(3)	(3.6)	(4.3)	∞	∞	4.8	∞	∞	∞
4	7.8	1.9	0.8	0	1.2	(2.2)	(2.8)	(3.5)	∞	∞	5.6	∞	∞	∞
5	9	3.1	2	1.2	0	1	1.6	2.3	∞	∞	6.8	∞	∞	∞
6	(10)	(4.1)	(3)	(2.2)	1	0	1.4	∞	1	2	(7.8)	∞	∞	∞
7	(10.6)	(4.7)	(3.6)	(2.8)	1.6	1.4	0	0.9	∞	∞	(8.4)	∞	∞	∞
8	(11.3)	(5.4)	(4.3)	(3.5)	2.3	∞	0.9	0	∞	∞	(9.1)	∞	∞	∞
9	∞	∞	∞	∞	∞	1	∞	∞	0	2.1	1.9	∞	4.2	∞
10	∞	∞	∞	∞	∞	2	∞	∞	2.1	0	∞	∞	1.9	∞
11	9.7	3.7	4.8	5.6	6.8	(7.8)	(8.4)	(9.1)	1.9	∞	0	2.7	∞	∞
12	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	2.7	0	∞	4.1
13	∞	∞	∞	∞	∞	∞	∞	∞	4.2	1.9	∞	∞	0	4.6
14	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	4.1	4.6	0

Q_5 is as follows:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	—	1	1	3	4	5	5	5	1	1	2	1	1	1
2	2	—	2	2	4	5	5	5	2	2	2	2	2	2
3	3	3	—	3	4	5	5	5	3	3	2	3	3	3
4	3	4	4	—	4	5	5	5	4	4	2	4	4	4
5	4	4	4	4	—	5	5	5	5	5	4	5	5	5
6	5	5	5	5	6	6	—	6	6	6	5	6	6	6
7	5	5	5	7	7	7	7	—	7	7	5	7	7	7
8	5	5	5	8	8	8	8	8	—	8	5	8	8	8
9	9	9	9	9	9	9	9	9	9	—	9	9	9	9
10	10	10	10	10	10	10	∞	10	10	—	10	10	10	10
11	2	11	2	2	4	5	5	5	11	11	—	11	11	11
12	12	12	12	12	12	12	12	12	12	12	12	—	12	12
13	13	13	13	13	13	13	13	13	13	13	13	13	—	13
14	14	14	14	14	14	14	4	14	14	14	14	14	14	—

Step (6) set $k = 6$ then $D_6 =$

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
1	0	6	7	7.8	9	10	10.6	11.3	(11)	(12)	9.7	∞	∞	∞	
2	6	0	1.1	1.9	3.1	4.1	4.7	5.4	(5.1)	(6.1)	3.7	∞	∞	∞	
3	7	1.1	0	0.8	2	(3)	3.6	4.3	(4)	(5)	4.8	∞	∞	∞	
4	7.8	1.9	0.8	0	1.2	2.2	2.8	3.5	(3.2)	(4.2)	5.6	∞	∞	∞	
5	9	3.1	2	1.2	0	1	1.6	2.3	(2)	(3)	6.8	∞	∞	∞	
6	10	4.1	3	2.2	1	0	1.4	∞	1	(2)	7.8	∞	∞	∞	
7	10.6	4.7	3.6	2.8	1.6	1.4	0	0.9	(2.4)	(3.4)	8.4	∞	∞	∞	
8	11.3	5.4	4.3	3.5	2.3	∞	0.9	0	(4.3)	(5.3)	9.1	∞	∞	∞	
9	(11)	(5.1)	(4)	3.3	(2)	1	(2.4)	(4.3)	0	2.1	1.9	∞	4.2	∞	
10	(12)	(6.1)	(5)	(4.2)	(3)	2	(3.4)	(5.3)	2.1	0	(9.8)	∞	1.9	∞	
11	9.7	3.7	4.8	5.6	6.8	7.8	8.4	9.1	1.9	(9.8)	0	2.7	∞	∞	
12	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	2.7	0	∞	
13	∞	∞	∞	∞	∞	∞	∞	∞	4.2	1.9	∞	∞	∞	0	
14	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	4.1	4.6	0

Q_6 is as follows:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	—	1	1	3	4	5	5	5	6	6	2	1	1	1
2	2	—	2	2	4	5	5	5	6	6	2	2	2	2
3	3	3	—	3	4	5	5	5	6	6	2	3	3	3
4	3	4	4	—	4	4	4	4	6	6	2	4	4	4
5	4	4	4	4	5	—	5	5	6	6	4	5	5	5
6	5	5	5	5	6	6	—	6	6	6	5	6	6	6
7	5	5	5	5	7	7	7	—	7	6	6	5	7	7
8	5	5	5	5	8	8	8	8	—	6	6	5	8	8
9	6	6	6	6	6	9	6	6	—	6	9	9	9	9
10	6	6	6	6	6	10	6	6	6	—	6	10	10	10
11	2	11	2	2	4	5	5	5	11	6	—	11	11	11
12	12	12	12	12	12	12	12	12	12	12	12	—	12	12
13	13	13	13	13	13	13	13	13	13	13	13	13	—	13
14	14	14	14	14	14	14	4	14	14	14	14	14	14	—

Step (7) set $k = 7$ then D_7

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	6	7	7.8	9	(10)	(10.6)	11.3	(11)	(12)	9.7	∞	∞	∞
2	6	0	1.1	1.9	3.1	(4.1)	(4.7)	5.4	(5.1)	(6.1)	3.7	∞	∞	∞
3	7	1.1	0	0.8	2	(3)	3.6	4.3	(4)	(5)	4.8	∞	∞	∞
4	7.8	1.9	0.8	0	1.2	(2.2)	2.8	(3.5)	(3.3)	(4.2)	5.6	∞	∞	∞
5	9	3.1	2	1.2	0	1	1.6	2.3	(2)	(3)	6.8	∞	∞	∞
6	(10)	(4.1)	(3)	(2.2)	1	0	1.4	∞	1	(2)	(7.8)	∞	∞	∞
7	(10.6)	(4.7)	(3.6)	(2.8)	1.6	1.4	0	0.9	∞	(3.4)	(8.4)	∞	∞	∞
8	(11.3)	(5.4)	(4.3)	(3.5)	2.3	∞	0.9	0	∞	(5.3)	(9.1)	∞	∞	∞
9	(11)	(5.1)	(4)	(3.3)	(2)	1	(2.4)	(4.3)	0	2.1	1.9	∞	4.2	∞
10	(12)	(6.1)	(5)	(4.2)	(3)	2	(3.4)	(5.3)	2.1	0	∞	∞	1.9	∞
11	9.7	3.7	4.8	5.6	6.8	(7.8)	(8.4)	(9.1)	1.9	∞	0	2.7	∞	∞
12	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	2.7	0	∞	4.1
13	∞	∞	∞	∞	∞	∞	∞	∞	4.2	1.9	∞	∞	0	4.6
14	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	4.1	4.6	0

Q_7 is as follows:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	—	1	1	3	4	5	5	5	1	6	6	1	1	1
2	2	—	2	2	4	5	5	5	2	6	6	2	2	2
3	3	3	—	3	4	5	5	5	3	6	6	3	3	3
4	3	4	4	—	4	4	4	4	4	6	6	4	4	4
5	4	4	4	4	5	—	5	5	5	6	6	5	5	5
6	5	5	5	5	6	6	—	6	6	6	6	5	6	6
7	5	5	5	5	7	7	7	—	7	7	6	7	7	7
8	5	5	5	5	8	8	8	8	—	8	6	8	8	8
9	6	6	6	6	6	9	6	6	—	9	9	9	9	9
10	6	6	6	6	6	10	6	6	10	—	10	10	10	10
11	2	11	2	2	4	5	5	5	11	11	—	11	11	11
12	12	12	12	12	12	12	12	12	12	12	12	—	12	12
13	13	13	13	13	13	13	13	13	13	13	13	13	—	13
14	14	14	14	14	14	14	4	14	14	14	14	14	14	—

Step (8) set $k = 8$ then $D_8 =$

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	6	7	7.8	9	10	10.6	11.3	11	12	9.7	∞	∞	∞
2	6	0	1.1	1.9	3.1	4.1	4.7	5.4	5.1	6.1	3.7	∞	∞	∞
3	7	1.1	0	0.8	2	3	3.6	4.3	4	5	4.8	∞	∞	∞
4	7.8	1.9	0.8	0	1.2	2.2	2.8	3.5	3.3	4.2	5.6	∞	∞	∞
5	9	3.1	2	1.2	0	1	1.6	2.3	2	3	6.8	∞	∞	∞
6	10	4.1	3	2.2	1	0	1.4	∞	1	2	7.8	∞	∞	∞
7	10.6	4.7	3.6	2.8	1.6	1.4	0	0.9	∞	3.4	8.4	∞	∞	∞
8	11.3	5.4	4.3	3.5	2.3	∞	0.9	0	∞	5.3	9.1	∞	∞	∞
9	11	5.1	4	3.3	2	1	2.4	4.3	0	2.1	1.9	∞	4.2	∞
10	12	6.1	5	4.2	3	2	3.4	5.3	2.1	0	∞	∞	1.9	∞
11	9.7	3.7	4.8	5.6	6.8	7.8	8.4	9.1	1.9	∞	0	2.7	∞	∞
12	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	2.7	0	∞	4.1
13	∞	∞	∞	∞	∞	∞	∞	∞	4.2	1.9	∞	∞	0	4.6
14	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	4.1	4.6	0

Q_8 is as follows:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	-	1	1	3	4	5	5	5	1	6	6	1	1	1
2	2	-	2	2	4	5	5	5	2	6	6	2	2	2
3	3	3	-	3	4	5	5	5	3	6	6	3	3	3
4	3	4	4	-	4	4	4	4	4	6	6	4	4	4
5	4	4	4	5	-	5	5	5	5	6	6	5	5	5
6	5	5	5	6	6	-	6	6	6	6	5	6	6	6
7	5	5	5	7	7	7	-	7	7	6	6	7	7	7
8	5	5	5	8	8	8	8	-	8	6	6	8	8	8
9	6	6	6	6	6	9	6	6	-	9	9	9	9	9
10	6	6	6	6	6	10	6	6	10	-	10	10	10	10
11	2	11	2	2	4	5	5	5	11	11	-	11	11	11
12	12	12	12	12	12	12	12	12	12	12	12	-	12	12
13	13	13	13	13	13	13	13	13	13	13	13	13	-	13
14	14	14	14	14	14	14	4	14	14	14	14	14	14	-

Step (9) set $k = 9$ then $D_9 =$



	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	6	7	7.8	9	10	10.6	11.3	11	12	9.7	∞	(15.2)	∞
2	6	0	1.1	1.9	3.1	4.1	4.7	5.4	5.1	6.1	3.7	∞	(9.3)	∞
3	7	1.1	0	0.8	2	3	3.6	4.3	4	5	4.8	∞	(8.2)	∞
4	7.8	1.9	0.8	0	1.2	2.2	2.8	3.5	3.3	4.2	(5.1)	∞	(7.5)	∞
5	9	3.1	2	1.2	0	1	1.6	2.3	2	3	(3.9)	∞	(6.2)	∞
6	10	4.1	3	2.2	1	0	1.4	∞	1	2	(2.9)	∞	(5.2)	∞
7	10.6	4.7	3.6	2.8	1.6	1.4	0	0.9	∞	3.4	(4.3)	∞	(6.6)	∞
8	11.3	5.4	4.3	3.5	2.3	∞	0.9	0	∞	5.3	(5.2)	∞	(8.5)	∞
9	11	5.1	4	3.3	2	1	2.4	4.3	0	2.1	1.9	∞	4.2	∞
10	12	6.1	5	4.2	3	2	3.4	5.3	2.1	0	(4)	∞	1.9	∞
11	9.7	3.7	4.8	5.6	6.8	7.8	8.4	9.1	1.9	∞	0	2.7	(6.1)	∞
12	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	2.7	0	∞	4.1
13	(15.2)	(9.3)	(8.2)	(7.5)	(6.2)	(5.2)	(6.6)	(8.5)	4.2	1.9	(6.1)	∞	0	4.6
14	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	4.1	4.6	0

Q_9 is as follows:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	—	1	1	3	4	5	5	5	6	6	2	1	9	1
2	2	—	2	2	4	5	5	5	6	6	2	2	9	2
3	3	3	—	3	4	5	5	5	6	6	2	3	9	3
4	3	4	4	—	4	4	4	4	6	6	9	4	9	4
5	4	4	4	5	—	5	5	5	6	6	9	5	9	5
6	5	5	5	6	6	—	6	6	6	6	9	6	9	6
7	5	5	5	7	7	7	—	7	6	6	9	7	9	7
8	5	5	5	8	8	8	8	—	6	6	9	8	9	8
9	6	6	6	6	6	9	6	6	—	9	9	9	9	9
10	6	6	6	6	6	10	6	6	10	—	9	10	10	10
11	2	11	2	9	9	9	9	9	11	9	—	11	9	11
12	12	12	12	12	12	12	12	12	12	12	12	—	12	12
13	9	9	9	9	9	9	9	9	13	13	9	13	—	13
14	14	14	14	14	14	14	4	14	14	14	14	14	14	—

Step (10) set $k = 10$ then $D_{10} =$

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	6	7	7.8	9	10	10.6	11.3	11	12	9.7	∞	(13.9)	∞
2	6	0	1.1	1.9	3.1	4.1	4.7	5.4	5.1	6.1	3.7	∞	(8)	∞
3	7	1.1	0	0.8	2	3	3.6	4.3	4	5	4.8	∞	(6.9)	∞
4	7.8	1.9	0.8	0	1.2	2.2	2.8	3.5	3.3	4.2	5.6	∞	(6.1)	∞
5	9	3.1	2	1.2	0	1	1.6	2.3	2	3	6.8	∞	(4.9)	∞
6	10	4.1	3	2.2	1	0	1.4	∞	1	2	7.8	∞	(3.9)	∞
7	10.6	4.7	3.6	2.8	1.6	1.4	0	0.9	∞	3.4	8.4	∞	(5.3)	∞
8	11.3	5.4	4.3	3.5	2.3	∞	0.9	0	∞	5.3	9.1	∞	(7.2)	∞
9	11	5.1	4	3.3	2	1	2.4	4.3	0	2.1	1.9	∞	(4)	∞
10	12	6.1	5	4.2	3	2	3.4	5.3	2.1	0	∞	∞	1.9	∞
11	9.7	3.7	4.8	5.6	6.8	7.8	8.4	9.1	1.9	∞	0	2.7	6.1	∞
12	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	2.7	0	∞	4.1
13	(13.9)	(8)	(6.9)	(6.1)	(4.9)	(3.9)	(5.3)	(7.2)	(4)	1.9	6.1	∞	0	4.6
14	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	4.1	4.6	0

Q_{10} is as follows:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	—	1	1	3	4	5	5	5	1	6	6	1	10	1
2	2	—	2	2	4	5	5	5	2	6	6	2	10	2
3	3	3	—	3	4	5	5	5	3	6	6	3	10	3
4	3	4	4	—	4	4	4	4	4	6	6	4	10	4
5	4	4	4	5	—	5	5	5	5	6	6	5	10	5
6	5	5	5	6	6	—	6	6	6	6	5	6	10	6
7	5	5	5	7	7	7	—	7	7	6	6	7	10	7
8	5	5	5	8	8	8	8	—	8	6	6	8	10	8
9	6	6	6	6	6	9	6	6	—	9	9	9	10	9
10	6	6	6	6	6	10	6	6	10	—	10	10	10	10
11	2	11	2	2	4	5	5	5	11	11	—	11	10	11
12	12	12	12	12	12	12	12	12	12	12	12	—	12	12
13	10	10	10	10	10	10	10	10	10	13	10	13	—	13
14	14	14	14	14	14	14	4	14	14	14	14	14	14	—

Step (11) set $k = 11$ then $D_{11} =$

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	6	7	7.8	9	10	10.6	11.3	11	12	9.7	(12.4)	13.9	∞
2	6	0	1.1	1.9	3.1	4.1	4.7	5.4	5.1	6.1	3.7	(6.4)	8	∞
3	7	1.1	0	0.8	2	3	3.6	4.3	4	5	4.8	(7.5)	6.9	∞
4	7.8	1.9	0.8	0	1.2	2.2	2.8	3.5	3.3	4.2	5.6	(8.3)	6.1	∞
5	9	3.1	2	1.2	0	1	1.6	2.3	2	3	6.8	(6.6)	4.9	∞
6	10	4.1	3	2.2	1	0	1.4	∞	1	2	7.8	(10.5)	3.9	∞
7	10.6	4.7	3.6	2.8	1.6	1.4	0	0.9	∞	3.4	8.4	(11.1)	5.3	∞
8	11.3	5.4	4.3	3.5	2.3	∞	0.9	0	∞	5.3	9.1	(11.8)	7.2	∞
9	11	5.1	4	3.3	2	1	2.4	4.3	0	2.1	1.9	(4.6)	4	∞
10	12	6.1	5	4.2	3	2	3.4	5.3	2.1	0	∞	(12.5)	1.9	∞
11	9.7	3.7	4.8	5.6	6.8	7.8	8.4	9.1	1.9	∞	0	2.7	6.1	∞
12	(12.4)	(6.4)	(7.5)	(8.3)	(6.6)	(10.5)	(11.1)	(11.8)	(4.6)	(12.5)	2.7	0	(8.6)	4.1
13	13.9	8	8.2	6.1	4.9	3.9	5.3	7.2	(4)	1.9	6.1	(8.6)	0	4.6
14	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	4.1	4.6	0

Q_{11} is as follow

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	6	7	7.8	9	10	10.6	11.3	11	12	9.7	12.4	13.9	(16.5)
2	6	0	1.1	1.9	3.1	4.1	4.7	5.4	5.1	6.1	3.7	6.4	8	(10.5)
3	7	1.1	0	0.8	2	3	3.6	4.3	4	5	4.8	7.5	8.2	(11.5)
4	7.8	1.9	0.8	0	1.2	2.2	2.8	3.5	3.3	4.2	5.6	8.3	6.1	(12)
5	9	3.1	2	1.2	0	1	1.6	2.3	2	3	6.8	6.6	4.9	(10.7)
6	10	4.1	3	2.2	1	0	1.4	∞	1	2	7.8	10.5	3.9	(9.7)
7	10.6	4.7	3.6	2.8	1.6	1.4	0	0.9	∞	3.4	8.4	11.1	5.3	(11.1)
8	11.3	5.4	4.3	3.5	2.3	∞	0.9	0	∞	5.3	9.1	11.8	7.2	(12)
9	11	5.1	4	3.3	2	1	2.4	4.3	0	2.1	1.9	4.6	4	(8.7)
10	12	6.1	5	4.2	3	2	3.4	5.3	2.1	0	∞	12.5	1.9	(10.8)
11	9.7	3.7	4.8	5.6	6.8	7.8	8.4	9.1	1.9	∞	0	2.7	6.1	(6.8)
12	12.4	6.4	7.5	8.3	6.6	10.5	11.1	11.8	4.6	12.5	2.7	0	∞	4.1
13	13.9	8	8.2	6.1	4.9	3.9	5.3	7.2	4	1.9	6.1	∞	0	4.6
14	(16.5)	(10.5)	(11.5)	(12)	(10.7)	(9.7)	(11.1)	(12)	(8.7)	(10.8)	(6.8)	4.1	4.6	0

Q_{12} is as follows:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	–	1	1	3	4	5	5	5	1	6	6	11	10	12
2	2	–	2	2	4	5	5	5	2	6	6	11	10	12
3	3	3	–	3	4	5	5	5	3	6	6	11	9	12
4	3	4	4	–	4	4	4	4	4	6	6	11	10	12
5	4	4	4	5	–	5	5	5	5	6	6	11	10	12
6	5	5	5	6	6	–	6	6	6	6	5	11	10	12
7	5	5	5	7	7	7	–	7	7	6	6	11	10	12
8	5	5	5	8	8	8	8	–	8	6	6	11	10	12
9	6	6	6	6	6	9	6	6	–	9	9	11	10	12
10	6	6	6	6	6	10	6	6	10	–	10	11	10	12
11	2	11	2	2	4	5	5	5	11	11	–	11	9	12
12	11	11	11	11	11	11	11	11	11	11	12	–	12	12
13	10	10	10	10	10	10	10	10	13	13	9	13	–	13
14	12	12	12	12	12	12	12	12	12	12	12	14	14	–

Step (13) set $k = 13$ then $D_{13} =$

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	6	7	7.8	9	10	10.6	11.3	11	12	9.7	12.4	13.9	16.5
2	6	0	1.1	1.9	3.1	4.1	4.7	5.4	5.1	6.1	3.7	6.4	8	10.5
3	7	1.1	0	0.8	2	3	3.6	4.3	4	5	4.8	7.5	8.2	(11.5)
4	7.8	1.9	0.8	0	1.2	2.2	2.8	3.5	3.3	4.2	5.6	8.3	6.1	(10.7)
5	9	3.1	2	1.2	0	1	1.6	2.3	2	3	6.8	6.6	4.9	(9.5)
6	10	4.1	3	2.2	1	0	1.4	∞	1	2	7.8	10.5	3.9	(8.5)
7	10.6	4.7	3.6	2.8	1.6	1.4	0	0.9	∞	3.4	8.4	11.1	5.3	(9.9)
8	11.3	5.4	4.3	3.5	2.3	∞	0.9	0	∞	5.3	9.1	11.8	7.2	(11.8)
9	11	5.1	4	3.3	2	1	2.4	4.3	0	2.1	1.9	4.6	4	(8.6)
10	12	6.1	5	4.2	3	2	3.4	5.3	2.1	0	∞	12.5	1.9	(6.5)
11	9.7	3.7	4.8	5.6	6.8	7.8	8.4	9.1	1.9	∞	0	2.7	6.1	6.8
12	12.4	6.4	7.5	8.3	6.6	10.5	11.1	11.8	4.6	12.5	2.7	0	∞	4.1
13	13.9	8	8.2	6.1	4.9	3.9	5.3	7.2	4	1.9	6.1	∞	0	4.6
14	16.5	10.5	(10.7)	(9.5)	(8.5)	(9.9)	(15.2)	(11.8)	(8.6)	(6.5)	6.8	4.1	4.6	0

Q_{13} is as follows:

Step (14) set $k = 14$ then $D_{14} =$

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	6	7	7.8	9	10	10.6	11.3	11	12	9.7	12.4	13.9	16.5
2	6	0	1.1	1.9	3.1	4.1	4.7	5.4	5.1	6.1	3.7	6.4	8	10.5
3	7	1.1	0	0.8	2	3	3.6	4.3	4	5	4.8	7.5	6.9	11.5
4	7.8	1.9	0.8	0	1.2	2.2	2.8	3.5	3.2	4.2	5.1	7.9	6.1	10.7
5	9	3.1	2	1.2	0	1	1.6	2.3	2	3	3.9	6.6	4.9	9.5
6	10	4.1	3	2.2	1	0	1.4	2.3	1	2	2.9	5.6	3.9	8.5
7	10.6	4.7	3.6	2.8	1.6	1.4	0	0.9	2.4	3.4	4.3	7	5.3	9.9
8	11.3	5.4	4.3	3.5	2.3	2.3	0.9	0	3.3	4.3	5.2	7.9	7.2	11.8
9	11	5.1	4	3.2	2	1	2.4	3.3	0	2.1	1.9	4.6	4	8.6
10	12	6.1	5	4.2	3	2	3.4	4.3	2.1	0	4	6.7	1.9	6.5
11	9.7	3.7	4.8	5.1	3.9	2.9	4.3	5.2	1.9	4	0	2.7	5.9	6.8
12	12.4	6.4	7.5	7.9	6.6	5.6	7	7.9	4.6	6.7	2.7	0	8.6	4.1
13	13.9	8	6.9	6.1	4.9	3.9	5.3	7.2	4	1.9	5.9	8.6	0	4.6
14	16.5	10.5	11.5	10.7	9.5	8.5	9.9	11.8	8.6	6.5	6.8	4.1	4.6	0

Q₁₄ is as follows:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	-	1	1	3	4	5	5	5	1	6	6	11	10	12
2	2	-	2	2	4	5	5	5	2	6	6	11	10	12
3	3	3	-	3	4	5	5	5	3	6	6	11	9	12
4	3	4	4	-	4	4	4	4	4	6	6	11	10	12
5	4	4	4	5	-	5	5	5	5	6	6	11	10	12
6	5	5	5	6	6	-	6	6	6	6	5	11	10	12
7	5	5	5	7	7	7	-	7	7	6	6	11	10	12
8	5	5	5	8	8	8	8	-	8	6	6	11	10	12
9	6	6	6	6	6	9	6	6	-	9	9	11	10	12
10	6	6	6	6	6	10	6	6	10	-	10	11	10	12
11	2	11	2	2	4	5	5	5	11	11	-	11	9	12
12	11	11	11	11	11	11	11	11	11	11	12	-	12	12
13	10	10	10	10	10	10	10	10	13	13	9	13	-	13
14	12	12	12	12	12	12	12	12	12	12	12	14	14	-

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	-	1	1	3	4	5	5	5	1	6	6	11	10	12
2	2	-	2	2	4	5	5	5	2	6	6	11	10	12
3	3	3	-	3	4	5	5	5	3	6	6	11	9	13
4	3	4	4	-	4	4	4	4	4	6	6	11	10	13
5	4	4	4	5	-	5	5	5	5	6	6	11	10	13
6	5	5	5	6	6	-	6	6	6	6	5	11	10	13
7	5	5	5	7	7	7	-	7	7	6	6	11	10	13
8	5	5	5	8	8	8	8	-	8	6	6	11	10	13
9	6	6	6	6	6	9	6	6	-	9	9	11	10	13
10	6	6	6	6	6	10	6	6	10	-	10	11	10	13
11	2	11	2	2	4	5	5	5	11	11	-	11	9	12
12	11	11	11	11	11	11	11	11	11	11	12	-	12	12
13	10	10	10	10	10	10	10	10	13	13	9	13	-	13
14	12	13	13	13	13	13	13	13	13	13	12	14	14	-

The final matrices D_{14} and Q_{14} contain all the information needed to determine the shortest route between any two nodes in the graph. From node 1 to node 14 is $d_{1,14} = 16.5cm$, since $1\text{ cm} \equiv 100\text{km} \rightarrow d_{1,14} = 1650\text{ km}$ is the shortest distance between Nyala and Port Sudan is 1650 km. to determine the associated route, recall that a linked through at least one other intermediate node, because $q = 12 \neq 14$, the route is initially given as $1 \rightarrow 12 \rightarrow 14$, Now because $q_{1,12} = 11 \neq 12$ the segment (1,12) is not a direct link, and $1 \rightarrow 12$ is replaced with $1 \rightarrow 11 \rightarrow 12$ and $q_{1,11} = 2 \neq 11$ the segment (1,11) is not a direct link, and $1 \rightarrow 11$ is replaced with $1 \rightarrow 2 \rightarrow 11 \rightarrow$

$12 \rightarrow 14$ defines the shortest route.(i.e) the shortest route is (Nyala, AIObied, Omdurman, Atbara, and Port Sudan

Reference

Marcus, D. A. (2020). Graph theory, American Mathematical Soc.

Pešić, D., et al. (2020). "Finding optimal route by two-criterion Fuzzy Floyd's algorithm—case study Serbia." Operational Research 20(1): 119-138.

Shukla, K. T. (2013). "Fuzzy Floyd's algorithm to find shortest route between nodes under uncertain environment." Int J Math Comput Appl Res 3(5): 34-54.

Trudeau, R. J. (2013). Introduction to graph theory, Courier Corporation.