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FACULTY OF MEDICAL LABORATORY SCIENCES

SMLJ

وَلَقَدْ آتَيْنَا دَاوُودَ وَسُلَيْمَانَ عِلْمًا

وَقَالَا الْحَمْدُ لِلَّهِ الَّذِي

فَضَّلَنَا عَلَىٰ كَثِيرٍ مِّنْ عِبَادِهِ الْمُؤْمِنِينَ

النمل15

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Medical Laboratory sciences are fast-growing fields, and it is a challenge to keep our journal up to date. The clinical laboratory has a major role in modern medicine, and as new information and concepts emerge and our understanding evolves, the material we teach must also change. Each article in this journal has been revised to encompass this new information.

Each of the authors is an acknowledged trusted in the paper he or she is writing about. The authors, editors and I have tried to eliminate errors that appeared in this issue without interfering with the objective or the meaning of the article. We are grateful to our colleagues, editors and authors for help in identifying mistakes and inconsistencies, and we ask for their continued aid in improving future issues.

Health professionals, health administrators, health policy-makers and nongovernmental organizations, among others, can and should use the scientific method to guide their work for improving the health of individuals and communities. It is hopefully that we gave them useful information through this journal.

I would like to take this opportunity to thank Prof. MB Saad, and Dr. Kamal Abdelsalam for their effort in establishing the journal. Special thanks also go to authors for their interest and encouragement. Once again, welcome to the journal and I hope you find the papers both interesting and thought provoking.

Sincerely,

Dr. Mohammed Abdel Hamid K.Elsid

Editor-in-Chief

Preface from the Editors

We are proud and happy to bring to you this new issue of **Sudan Medical Laboratory Journal (SMLJ)** assembled under our joint editorship.

Laboratory medicine might well claim to be the most popular and the most glamorous of biological sciences today. Accompanied with the fast growing of science and technology, the communication and information exchanges among the worldwide scientists are getting more and more important.

Numerous scientists, researchers, professors, and related experts working in the universities, institutes, hospitals, and pharmaceutical enterprises have been engaged in the research and development from different aspects, such as functional mechanism, toxicology, pharmacology, clinical evaluation, quality control, etc.

So SML journal is initiated to have a very broad scope to reflect medical laboratory scientific researches and other sciences in various aspects of medicine as well as regional and international relevant research. It supports insiders to share their insights on theory, application, and achievements.

In addition, it provides information about the recent tests for anyone who is concerned about human health. We sincerely expect that the increasing number of people in the world would benefit from this journal.

The journal is your friend, my friend, and our friend to connect us together. The journal is your home, my home, and our home to let us share human's intelligence in this field and to develop further.

Dr. Kamal A Abdelsalam
Director Editor
Dr. Mohammed B Saad
Advisor editor

Preface from the College

Welcome to the new issue of **Sudan Medical Laboratory Journal** (SMLJ) the official journal of the **Faculty of Medical Laboratory Sciences** in **Omdurman Islamic University**.

It has been said that over 80% of all medical decisions encompass clinical laboratory data in the decision process! While we know of no evidence to support this statement it is probably not far off the mark. Laboratory medicine plays an integrated role in the diagnosis, prognosis, treatment, and long-term management of disease. Proper selection and interpretation of laboratory tests is critical for quality patient care. In the last few decades, there has been an information explosion in the field of laboratory medicine, making it difficult for health care professionals to remain fluent in all aspects of laboratory testing. Sudan Medical Laboratory journal (SMLJ) aimed to provide a comprehensive overview of modern laboratory medicine in a "real-life" case-based or research-based format.

We hope SMLJ will be an excellent resource for medical technologists, laboratorians, physicians, residents, nurses, physician's assistants, medical students, educators, and many other allied health workers.

Dr. Isam AM Sadig
Vice Dean

Aims and scopes

Sudan Medical Laboratory Journal (SMLJ) is issued by Omdurman Islamic University, College of Medical Laboratory Sciences. This is a peer-reviewed journal published yearly. Its main objective is to reflect medical laboratory scientific research in various aspects of medicine as well as regional and international relevant research. Basic scientific research clinical practice, experiences that help in patient management are also welcome. Review articles, original articles, case reports are welcome. Local research in sciences education and history of laboratory and medicine will be considered for publication.

Manuscripts must be solely submitted to this journal. All authors must sign approving the submitted version. Any conflict of interest must be stated clearly. Ethical clearance must be presented in relevant submission.

Manuscript submission: only electronic version sent to this e mail addressed to the Editor-in-Chief will be considered smljournal@gmail.com.

Directions to contributors

Sudan Medical Laboratory Journal (SMLJ) publishes works, general and clinical articles, reviews, abstracts, society news and matters pertaining to human health and human sciences.

Papers are considered for publication provided that they have not been published before or will not be sent for publication in any other journal.

Articles for publication always should be prepared in a 1.5cm-spaced typewritten on size A4 paper and with 3cm margin on all sides. A covering letter signed by all authors must be forwarded and submitted in 3 paper copies and should be accompanied with a computer CD copy.

Names of authors should be followed by their official address. Names should be written as follow: last name + initials i.e. Cornel, C. E[1]. Ford, E. J. H[2]. Evans, J[3].

All papers should be accepted for publication after being subjected to minor reviewing by an assessor if necessary. No reasons will be given in case of rejecting the article.

One journal issue will be sent free of charge irrespective of the number of authors. Order for extra issues will be considered. Article should be subdivided into **abstract** (not more than 300 words), **introduction** (short concise overview of the current state with background information), **methods** (a clear description of the methodology used), **patient selection** (Inclusion and exclusion criteria), **results** (in form of text, tables and figures, avoid repetition of data), **discussion** (discussing the results obtained) and **references** (Vancouver style).

References should be written as follows: name of author(s) + (year) + title + journal + pages. For example:

One author: Tamir, M. J. (2000). The role of socioeconomic status in plasma concentration. Sud. Med. J. 15-20.

Two authors: Tamir, M. J. and Sami, R. T. (2000). The role of socioeconomic status in plasma concentration. Sud. Med. J. 15-20.

Three authors: Tamir, M. J. Sami, R. T. and Ahmed, S. F. (2000). The role of socioeconomic status in plasma concentration. Sud. Med. J. 15-20.

For books: name of author(s) + (year) + title + edition + publisher + pages.

Manuscripts should be sent to this address:

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Message from the Editor-in-Chief

Dr. Mohammed Abdel Hamid K.Elsid

Editor-in-Chief

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Dear readers,

Our newly issued journal, "Sudan Medical laboratory Journal" (SMLJ), has reached its 2nd issue with a satisfying content. The first issue took a lot of attention and it was appreciated by the scientists all over the world. As an editorial team, we are happy to acknowledge receipt of many valuable submissions in various aspects of medical and educational sciences. We sincerely hope to see this effort continues and remain.

As the scientific quality of the papers published in SMLJ is quite important and well known, and we require our authors and readers to put emphasis on the good quality of the scientific data for submission.

Considering the increasing importance of medical laboratory sciences as well as all other medical sciences, we are looking forward to receiving contributions from the researchers in Sudan and other parts of the globe as well.

Hope to meet you in the 3rd issue of SMLJ.

Sudan Medical Laboratory Journal, Volume 1, Issue 2, June-December, 2011

Original Article

Sudan Medical Laboratory Journal

Correlation between urea level and HbA1c level in type 2 diabetic patients

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Abstract

The objective of this study was to determine relation between HbA1c and urea level in type 2 diabetic patients. This study was carried between March2009 - June2010. A total of 150 diabetic patients were included in this study, ages between (40-70 years old) chosen among male and female attending several diabetic clinics in the private sector in Khartoum state were included. Fifty healthy subjects were included as a control.

Fasting blood samples were collected for estimations of blood glucose, HbA1c, and blood urea. Urea concentration was measured by Berthelot reaction and HbA1c was measured by glycohemoglobin spectrophotometry method. By SPSS (Statistical Package for Social Sciences) and student t-test statistical analysis was determined in which statistical significance was measured as p-value less than 0.05.

HbA1c, and urea levels increased significantly ($P<0.01$) in patients with diabetes compared to the control group. Regression and correlation analysis showed a significant positive correlation between HbA1c and serum urea levels. Insignificant changes between men and women were found.

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Introduction:

Diabetes mellitus is a major health problem worldwide. Diabetes is one of the leading causes of morbidity and mortality throughout the world. Diabetes mellitus is a major health problem worldwide. About 2.2% to 3% of the world's population suffers from type 2 diabetes mellitus [(1)]. Diabetes mellitus (DM) is a chronic metabolic disorder that can lead to severe cardiovascular, renal, neurological and retinal complications [(2, 3)]. It is a serious debilitating and deadly disease that has now reached epidemic proportion and the prevalence rates are expected to go even higher in the future. Diabetic patients may reach End Stage Renal Disease (ESRD) if diabetes mellitus is not adequately controlled. In most countries diabetic nephropathy has become the single most frequent cause of ESRD [(4, 5)].

The Glycosylated hemoglobin (HbA1c) is widely accepted and used as the most reliable test for assessment of chronic glycaemia [(6)]. The HbA1c reflects the overall blood glucose levels over a period of 2-3 months and the major use of the

HbA1c assay is to assess changes in metabolic control that follow an alteration in treatment [7].

The nephropathy is common in diabetic patients and usually associated with vascular complications. The long-term complications of diabetes have major consequences for individual and health care providers. The blood glucose was considered as a prime test for optimizing treatment of diabetes mellitus. But the HbA1c determination is the new better method to monitor the long term glucose control irrespective of glucose measurement for patient management. It would prevent or delay the further diabetic complications. Diabetic patients with oral hypoglycemic therapy should go for HbA1c test as recommended by the American diabetes association [8].

Therefore, in the present investigation, the effect of the HbA1c in diabetes associated nephropathy is proposed to explore in Sudanese population.

Materials and methods:

Study area: The study was conducted in Khartoum state**,** Sudan

Study size: A total of 90 diabetic patients, ages (40-70) years old comprising males and females attending several diabetic clinics in the private sector in Khartoum state were chosen in the study. Informed consent was obtained from each patient. Fifty healthy subjects with no known history of hyperglycemia and renal disease were included as a control group.

Study Population: The study was conducted on the type II diabetic patients who have DM for 5 years and more, based on WHO criteria. [9].

Patients with type 1 diabetes mellitus, gestational diabetes and any known mental illness, macro vascular disease prior to diagnosis of type II diabetes, or the patients who refused to participate in the study were excluded. Subjects with hypertension, renal failure, cardiac problems, malaria, and/or obese were also excluded.

Samples collection: Overnight fasting blood samples were collected from the antecubical vein, by venipuncture without venous stasis, in lithium-heparin coated tube after no medications were taken for the previous 12h or longer for estimations of blood glucose, HbA1c, and serum urea.

A previously structured questionnaire was used to record the demographic features of all subjects. Age, sex and duration of DM affect.

Methods: HbA1c was estimated by glycohemoglobin spectrophotometry (from Analar Diagnosis Kits, Spain). Serum urea was estimated by the Berthelot reaction [10].

Statistical analysis: Statistical analysis was performed using statistical package for social sciences (SPSS). Statistical significance and difference from control and test values were evaluated by Student's t-test. Correlation coefficient and regression analysis were used to describe the effects of elevated serum HbA1c on urea.

Results:

The results showed that there were significant variations between diabetic patients and the control group (p value<0.05) in both results of

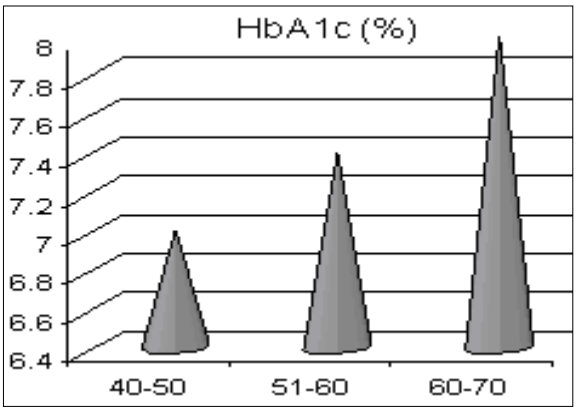
urea and Hb A1c. Also there was a significant correlation between urea and HbA1c

Table (1): results between DM group and control group:

Type	HbA1c (%)	Urea (mg/dl)
Control	**6.73**	**29.55**
Diabetes Mellitus	**7.45**	**47.9**
P value	**<0.05**	**<0.05**

In this study the highest level of Hb A1c (8.6%) was reported among the 60 - 70 age groups, while the lowest level (6.9%) was reported among the 40-50 age group (figure 1). This difference was found to be statistically significant (P value=0.04).

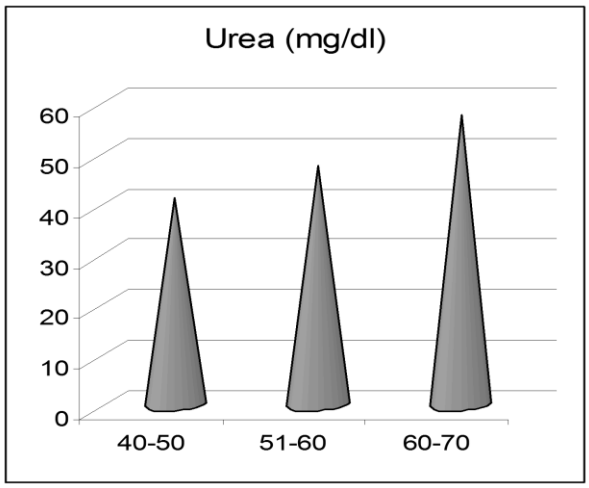
Figure (1): Results of HbA1c in DM group according to age:



* Significant changes between (60-70years) and (40-50years) age groups

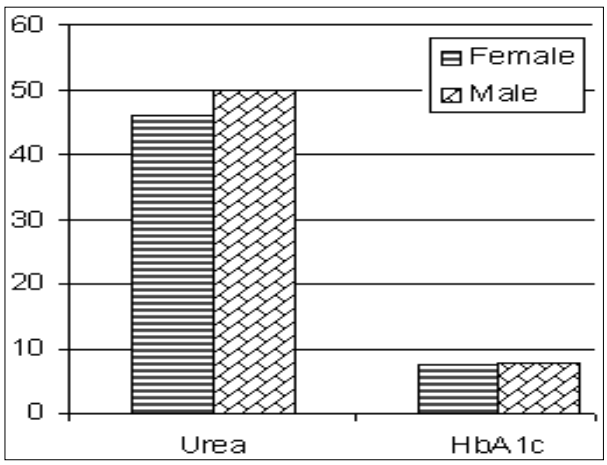
In this study the highest level of urea (56 mg/dl) was reported among the 60 - 70 age groups, while the lowest level (38 mg/dl) was reported among the 40-50 age group (figure 2). This difference was found to be statistically significant (P value<0.05).

Figure (2): Results of urea in DM group according to age



The result revealed that the highest levels of urea and HbA1c (50 mg/dl, 8.7% respectively) were reported among male diabetic patient compared to female (45mg/dl urea and 8.5% HbA1c) (figure 3). This difference was found to be statistically insignificant (P value=0.5).

Figure (3): Results of urea and HbA1c in DM subjects according to sex



Discussion:

In the present investigation, diabetes mellitus type II associated nephropathy showed significant elevated levels of HbA1c in blood. The present study (table 1), HbA1c and urea levels were increased significantly (P<0.05) in DM compared to the control group. This was in agreement with Murugan [8] who reported that there was a strong relationship between fasting blood sugar level, postprandial blood sugar level and HbA1c level in diabetic patients. Also table 1 showed that there was a significant correlation between urea and

HbA1c. Similarly, it has been reported that the blood glucose and HbA1c levels considerably increase in diabetic patients [(11)]. The findings of the current study are also in agreement with Bernadette *et al* [(12)] who investigated the elevated levels of HbA1c in diabetes. Previous studies [(8, 13, and 14)] also reported that urea level is elevated in DM patients.

The age had a significant increasing affect ($P<0.05$) on the levels of HbA1c and urea (diabetic patients) in the elderly group (60-70years) compared to the youngest study group (40-50years) (figure 2 and figure 3). Many studies proved that the urea levels are increased by age [(15)] which in line with our findings; but we disagree with Wiener *et al* [(16)] who reported that HbA1c raises but insignificantly affected by age.

In this study, the levels of urea and HbA1c was not affected significantly ($p>0.05$) by sex (figure 3). These findings were in agreement with Yang et al [(17)] and Waleed et al [(18)].

Acknowledgment:

We gratefully acknowledge the help and support from laboratories of Khartoum North Teaching Hospital for providing facility to work for this project.

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Original Article Sudan Medical Laboratory Journal

Relationship between follicular fluid selenium of Sudanese women with polycystic ovary syndrome

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Abstract:

The aim of this study was to investigate the concentration of selenium in the follicular fluid of Sudanese women patients with polycystic ovary syndrome. A case control study of 56 Sudanese women patients with polycystic ovary syndrome was compared with 55 healthy subjects as a control group; all mean age was 33 year. A follicular fluid sample was taken and selenium levels were analyzed using flameless atomic absorption. The mean (±SD) follicular fluid selenium level in control case was 61.41±29.14µg/L while among polycystic ovary syndrome patients, the follicular fluid selenium was 83.1±32.72µg/L. There was statistically significant ($P<0.05$) difference in follicular fluid selenium level between women patients and normal subjects.

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Introduction:

As reported by Cheung [(1)], polycystic ovary syndrome (PCOS), firstly described in 1935, by Dr. Stein and Dr. Leventhal. It is also known as functional ovarian hyperandrogenism, ovarian dysmetabolic syndrome and hyperandrogenic chronic anovulation disease. It is characterized by amenorrhea, hirsutism and obesity, in association with enlarged polycystic ovaries.

PCOS diagnosis and differential diagnosis remains confused to many clinicians. This is in part due to the lack of a specific diagnostic test for the disorder. In other part, the clinical history and a few laboratory tests are not enough to make the diagnosis and exclude other entities that may present in such the same way [(2)].

Selenium is the trace element essential for the activity of glutathione peroxidase (GSH-Px). Glutathione peroxidase protects against the damaging effect of peroxide, which is produced when fats are oxidized. The role of the selenium as an antioxidant is closely related to other micronutrients, vitamin E and vitamin C [(3)].

The role of selenium in fertility was greatly increased, and it was found that in vitro fertilization of the egg cell depends on the activity of the selenium containing enzyme GSH-Px in the follicular fluid. It was also found that the selenium level in the follicular fluid was lower in women with unexplained infertility [(4)].

Although selenium is an essential trace element, it is toxic if taken in excess. Exceeding the tolerable upper intake level of 400 micrograms per day can lead to selenosis. Symptoms of selenosis include a garlic odour on the breath, gastrointestinal tract disorders, hair loss, sloughing of nails, fatigue, irritability and neurological damage. Extreme cases of selenosis can result in cirrhosis of the liver, pulmonary oedema and death [(5)].

Materials and methods:

This case-control study included 56 women Sudanese patients attending the Reproductive Health Care Centre (RHCC), Sudan Assisted Reproduction Centre (SARC) and Asia Hospital. 55 subjects were used as baseline control. Both of study and control groups were with age between 24-43 years. Baseline value were formulated by considering those patients (age-matched) who presented with PCOS symptoms but in whom after investigation, no PCOS disease could be elicited (n=56). Patients were subsequently advised to have vaginal ultragraphy for PCOS confirmation patients with evidence of PCOS at the time of presentation (n=55). The women enrolled in this study were from different Sudanese tribes.

All patients underwent in vitro fertilization process. They were examined by the obstetricians, especially abdominal and vaginal ultrasound using four dimensions Doppler machine. PCOS morphology was defined as at least one ovary with 10 or more follicles of 2-10 mm in a single plane or an ovarian volume greater then 10 ml in the absence of a dominant follicle greater than 10 mm, a corpus luteum, or a cyst.

Student's t-test was used to calculate a pooled estimate of the variance of the data $P<0.05$ was considered significant.

Results:

The mean (±SD) follicular fluid selenium in the normal case was 61.41±19µg/L. Among the PCOS patients, the follicular fluid selenium level was 83.11±32.7µg/L. There were significant statistical differences ($P<0.05$) in the mean follicular fluid selenium levels between patients and control group (table.1).

Table (1): Comparison of the follicular fluid selenium between study group and control group:

Parameter	**Patients** means ±SD n= 56	**Control** means ±SD n= 55	**P value**
Follicular fluid selenium	83.11 ± 32.72	61.41 ± 29.14	<0.05

Table(2): The residents in the whole study group:

Parameter	PCOS N=55	Controls N=56
Khartoum state N (percentage)	47 (87%)	44 (80%)
Gazira state N (percentage)	3 (5.6%)	6 (10.9%)
Others N (percentage)	4 (7.4%)	5 (9.1%)

The participants in this study were from different states in Sudan. The higher numbers of participants were from Khartoum state (87% and 80% from both groups of PCOS and control respectively), and from Gazira state (5.6% and 10.9% from PCOS and control respectively), while other states were the least number inparticipations (7.4% and 9.1% from PCOS and control respectively) (table 2).

58.7% of women included in this study were housewives, and 41.3% were employees. 69.1% of PCOS patients were house-wives, while 30.9% were employees increased incidence of polycystic ovary disease among the housewives.

Table (3): The occupations of PCOS patients and the controls

Parameters	PCOS N=55	Controls N=56	Total N=111
House wives N (percentage)	38 (69.1%)	27 (48.2%)	65 (58.7%)
Employees N (percentage)	17 (30.9%)	29 (51.1%)	46 (41.3%)

Discussion:

All women included in the study were in the reproductive age (between 24 and 43 years). Concerning the residents, the study covered 10 Sudanese states (Khartoum, Gezira, Kassala, White Nile, Northern State, Korodfan, Darfur, Gadarif, Southern State and Sennar). The result of the study revealed that 83.5% of the study populations were living in the Khartoum State, while 8.3% were from Gezira State, the rest 8.2% distributed among the other 8 states.

Concerning the occupational conditions, 58.7% of women included in this study were housewives, and 30.9% were employees. 69.1% of PCOS patients were house-wives, while 51.8% were employees. Increased incidence of polycystic ovary disease was among the housewives.

The mean level of follicular fluid selenium in patients with PCOS was significantly higher ($P<0.05$). In general, this elevated follicular selenium level, suggests one of the following: the selenium level in the Sudanese food may be higher than recommended dietary allowance (RDA), which is 55-70µg/day [(6)] as long as the level of selenium is geographical region dependent, and the availability of selenium in soil varies widely and is reflected in the selenium content of vegetation grown there [(7)]. The second suggestion for the increased level of follicular selenium in the PCOS patients is that the most available data was not from human follicular fluid.

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Original Article

Sudan Medical Laboratory Journal

Low birth weight and risk factors among neonates delivered in Khartoum Teaching Hospital in January – February 2007

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Abstract

The aim of this study was to investigate prevalence and possible risk factors for LBW in Khartoum Teaching Hospital, Sudan.

Fifty pregnant women, 14-45 years old, were selected randomly from those admitted to the labor ward of Khartoum Teaching Hospital during the period from January to February 2007. A structured questionnaire was administered to each woman to gather social and medical information. Neonates were weighed immediately using a standardized weight scale to the nearest 50 g. LBW was considered when birth weight was less than 2500g.

The percentage of LBW among neonates of the study group was 12%, normal birth weight was 78%, whereas high birth weight was 10%. Cigarette passive smoking was found to be significantly related to LBW ($p<0.05\%$). Other risk factors like, age, education, occupation, parity, past history of twins and anemia had no effect on LBW.

The proportion of LBW among neonates delivered in Khartoum Teaching Hospital was found to be 12%. The relation of passive smoking to low birth weight was found to be highly significant.

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Introduction:

Low birth weight (LBW) is defined by the WHO as the weight of an infant at birth of less than 2.5 kg, irrespective of gestational age. Although about one half of all LBW infants in industrialized countries are born preterm (<37 wk gestation), most LBW infants in the developing countries are born at term and are affected by intrauterine growth restriction that may begin early in pregnancy [(1)]. LBW is a key determinant of infant survival, health and development [(2)]. It is found to be one of the major causes of high mortality and morbidity rates [(3)]. The Birth weight is governed by two major processes: duration of gestation and intrauterine growth rate. LBW is thus caused by either a short gestation period or retarded intrauterine growth or a combination of both. Prematurity is usually defined as a gestational age of less than 37 weeks whereas intrauterine growth retardation (IUGR) has generally no standard definition [(4)]. Low birth weight infants are at greater risk of having disability and diseases such as cerebral palsy, visual problems, learning disabilities and respiratory problems [(2, 4)]. Premature

infants, especially those weighing less than 1500g (also known as very low birth weight infants) have a far greater risk of developing hyaline membrane disease, aponea, intrauterine haemorrhage, sepsis, retrolental fibroplasia, and other conditions related to physiological immaturity. IUGR infants are far more likely to exhibit growth deficiencies, which appear to be permanent [(4)]. Many factors are thought to be associated with higher incidence of LBW. These include female neonates, maternal age (<20 or >35 years), primiparity and preterm live births [(5)]. On the other hand, pregnancy maternal body mass index, unbooked status, preeclampsia and bad obstetric history are significant maternal factors resulting in LBW babies [(2)]. Additional factors are related to socioeconomic status, calorie intake, urinary tract infection, quality of antenatal care [(6)], ethnicity, marital status, birth interval and educational level [(2)]. Fetal factors include genetic and/or chromosomal aberrations [(2)]. In addition to all these factors, a close association is found between LBW and medical conditions during pregnancy such as hypertension, diabetes, malnutrition, bleeding, anemia, infections, placental or fetal anomalies and multiple pregnancies [(7)].

A multi-factorial inter-relationship exists between environmental factors and the growth rate of the fetus [(8)]. One of these factors is the tobacco smoke, the factor most often cited [(9)]. Both active and passive smoking were found to be highly associated with low birth weight and premature births [(10)]. Smoking causes fetal hypoxia which arises as a consequence of increased carboxyhemoglobin levels, attenuated blood oxygen unloading and vasoconistriction of maternal blood supply to the placenta [(11, 12)]. Fetal oxygen deprivation results in growth retardation and weight reduction in the new born [(12)].

Materials and methods:

Study design: This is a descriptive, cross sectional hospital based study.

Study population and study area: The population studied composed of 50 pregnant women selected randomly from those admitted to Khartoum Teaching Hospital (KTH) for delivery, during the period from 1st January to 2nd February2007. Their ages ranged between 14 and 45 years old and they all live in Khartoum State.

Methods of data collection:

Questionnaire: The selected ladies responded to the interviewer who administered a questionnaire requiring information about maternal age, educational level, occupation, parity clinical profile, date of the last menstrual period and outcome of previous pregnancy (delivery and miscarriage). The date of the last menstrual period was used to determine gestational age. They were asked about active smoking and whether or not they were exposed for at least two hours per day during pregnancy to passive smoking, either at home or at work. The modes of delivery, past history of twins and presence or absence of anemia were recorded.

Measurement of neonatal body weight: Immediately after birth, the weight of each newborn was taken using standardized weight scale to the nearest 50g. LBW was considered when the birth weight was less than 2500 g.

Ethical consideration: Approval was taken from the consultants and doctors supervising the labor

ward in the hospital. Informed consents were taken from the pregnant women before entry into the study.

Statistical analysis: The collected data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 14. The chi square test was used to test distribution of categorical variables and student's *t* test was used for continuous variables. Statistical significance was accepted when P value is less than 0.05.

Results:

Age distribution of the mothers is shown in figure 1. It was found that more than half (52%) of all mothers in the study group were within the age group from 25-34 years.

Figure (1): Age distribution of pregnant women in the study group

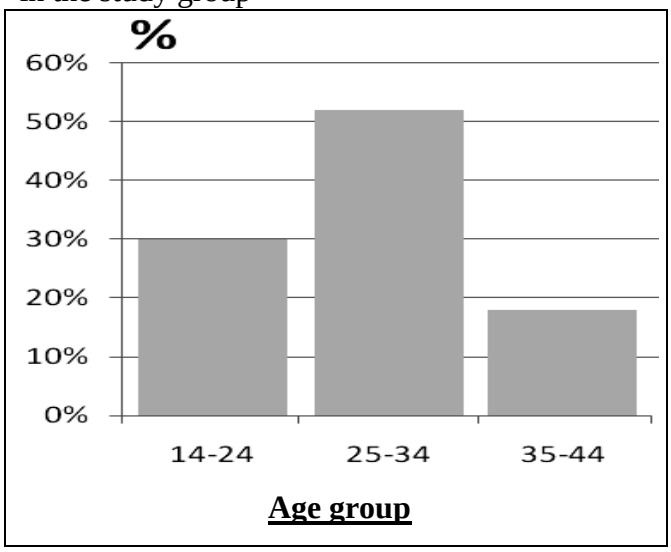


Table 1 showed that the majority of the mothers (86%) were house wives.

Table 1: Occupation of mothers in the study group:

Occupation	Frequency	Percent
House wife	34	86 %
Employee	3	6 %
Worker	4	8%
Total	50	100%

P=0.4388 not significant

Educational attainment is described in **table 2.** It shows that 32% of the mothers attained primary school education, 34% attended higher secondary school education and 30% attended university education.

Table 2: Educational attainment among the mothers:

Education	Frequency	Percent
Not Educated	2	4 %
Primary Education	16	32 %
Secondary Education	17	34%
University Level	15	30 %
Total	50	100%

P=0.5161not significant

Table 3 describes the distribution of birth weight in relation to different age groups. Neonates with low birth weight were (12%), normal birth weight(78%) and high birth weight (10%). The relation between birth weight and different age groups was statistically insignificant.

Table 3: Distribution of birth weight in relation to age of pregnant women

Total	High birth weight	Low birth weight	Normal birth weight	Age group
14 - 24	26 %	30 %	4 %	0
25 - 34	34 %	46 %	4 %	8 %
35 - 45	18 %	24 %	2 %	4 %
Total	78 %	100%	10 %	12 %

Not significant (p>0.05)

Modes of deliveries are shown in table 4. About 60% of all deliveries were cesarean sections. The difference of the modes of delivery in the study group was found to be statistically insignificant (P=0.9080).

Table 4: Modes of delivery in the study group

Percent	Frequency	Mode of delivery
40 %	20	Vaginal
60 %	30	Cesarean Section
100%	50	Total

P=0.9080 not significant

The percentage of mothers exposed to cigarette smoke during pregnancy was about 54%. The relation between passive smoking and LBW was found to be statistically significant (P<0.05). (Table 5)

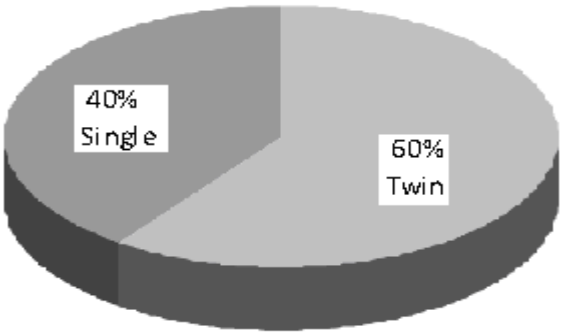
Table 5: Passive smoking in relation to low birth weight in the study group

Percent	Frequency	Passive Smoking
54 %	27	Yes
46 %	23	No
100%	50	Total

P=0.0085 highly significant

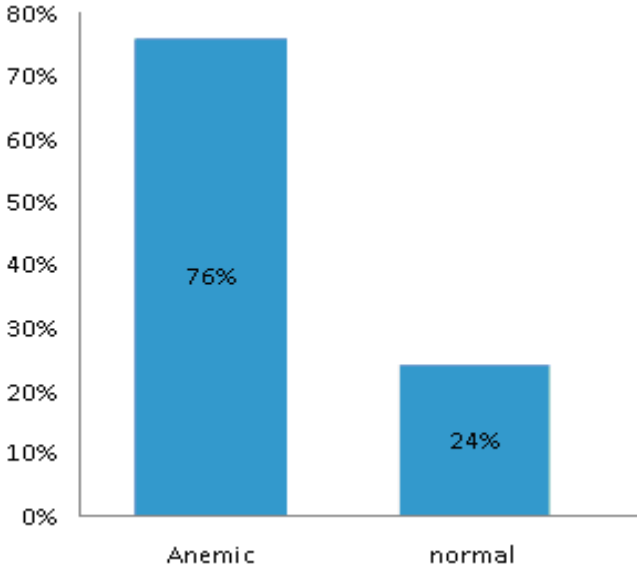
The percentage of mothers with past history of twins was 60% (figure 2).

Figure 2: Past history of pregnancy with twins in the study group:



Anemia was found in 76% of pregnant ladies (figure 3).

Figure 3: Percentage of anemia among pregnant ladies in the study group



Discussion:

Birth weight is an important indicator for future growth and development of children, hence periodical studies had been conducted to detect the prevalence of LBW. The documented

prevalence of LBW in Sudan (UNCEF-1992) was 15%. More recently, a prevalence of 12.6% was found in central Sudan [13] and 15.3% in eastern Sudan [14]. Although the sample size in this study was small, the estimated prevalence in Khartoum teaching hospital was very similar to the previous studies conducted in other parts of the Sudan.

Complete duration of pregnancy (full term) was about 88% of all pregnancies and incomplete (preterm) was about 12% with a ratio of (7:3). This finding is quite different from findings in central Sudan where the ratio was (2:9) in the community and only (1.3) in the hospitals [15]. The higher ratio of term to preterm deliveries in Khartoum may reflect better antenatal follow up in Khartoum compared to other parts in the country. In this study, no significant relation was found between mother age and LBW. This finding is consistent with other findings in Sudan which found insignificant association between the young age of mothers and LBW[13]**.** However, international studies confirmed presence of strong association between LBW and this factor [5]. In addition to maternal age, other factors like educational attainment, history of anemia, past history of twins and medical insurance were found to have insignificant correlation with low birth weight. These findings are quite different from previous findings in other parts of the Sudan which confirmed a close association between LBW and many other factors like low socio-economic status, lack of antenatal care, short birth intervals, poor obstetric history, complications of pregnancy and malaria infection [13-16]**.** It is worth noting that the majority of mothers in this study were educated. University graduates were about 30%.

The percent of active smoking was zero and this is a good finding which is different from level of active smoking in the developed countries. Most probably because in Sudan, smoking practice is religiously and socially unacceptable, especially among females. On the other hand, positive history of passive smoking was found in 54%. This is a serious finding because it indicates lack of knowledge about the negative effects of passive smoking on growth and development of the fetus during intra-uterine life. A highly significant relation was found between passive smoking and LBW in this study ($P<0.05$). Similar results were found in other countries [12]. In addition to the LBW, neonates exposed to passive smoking during pregnancy may develop other complications like asthma and reduced lung function [17].

Acknowledgements:

We are very grateful to all the women who participated in this study. Also, we deeply appreciate the help and cooperation of Khartoum Teaching Hospital staff, especially doctors of the obstetric unit and all who helped us in carrying out this work.

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Original Article

Sudan Medical Laboratory Journal

Prevalence of obesity and overweight among the female students in basic school levels in Omdurman- Sudan

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Abstract:

This study attempts to define obesity and overweight in Sudanese basic academic schoolgirl levels in Omdurman – Sudan.

The participants were 80 female students, age 5-13 years from grade 1st; 4th; 5th; and 7th during the academic year 2006-2007. Data was collected by direct interview with school girls and a consent form was given to their parents to allow their daughters to participate in the study. Body weight in kilograms and height in meters were taken to determine body mass index (BMI). Also, percentile of obesity was determined using a suitable growth reference for age and sex. Data was analyzed using statistical package for social science (SPSS) and presented in the form of tables.

Results revealed that the percentage of students at risk of obesity and overweight were particularly high (37.5%). Also, this percentage was close to the percentage of normal weight (55%). The prevalence of underweight was 7.5%. Also, the results showed that the prevalence of obesity and overweight was found significantly changed ($p<0.01$) in female children according to age. The percentage of obesity and overweight among school girls were 26.32%, 39.47% and 43.48% in age groups of 5-7 years, 8-10 years, and 11-13 years respectively.

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Introduction:

The prevalence of childhood obesity has been increasing at unsettling rates across the globe [1]. In addition to striking the developed world, this pattern has also been noted in developing countries undergoing rapid epidemiological transitions, including those in East Africa [2]. In Sudan, a study of children in secondary school in the capital Khartoum found that rates of overweight and obesity were 28.5% and 5.6% respectively [3]. Rates of obesity for younger schoolchildren in East Africa remain unclear, though obesity at younger ages may carry greater importance because younger children possess improved potential for early intervention [4]. The global prevalence of obesity in children aged 5-17 years is approximately 10%, but this is unequally distributed, with the prevalence ranging from less than 2% in sub-Saharan Africa to over 30% in the Americas [5]. The prevalence of childhood obesity is high in the Middle Eastern, Central and Eastern European countries [6]. Iran has been reported to be one of the seven countries with the highest prevalence of

childhood obesity [7]. In Saudi Arabia, one in every six children aged 6-18 years old is obese [8]. Childhood obesity and adolescent prevalence rate have steadily increased in industrial countries in the last 20 years [9]. Obesity means deposition of excess fat in the body [10].

Obesity in children -mean BMI- for age as at or above the 95th percentile and overweight at or above 85th percentile [4,11]. There are serious problems that can come from being obese as a child, including high cholesterol, liver problems, hip and other bones problems, early puberty, sleep-apnea, reproductive problems and some types of cancer. Overweight children may also be prone to low self-esteem that stems from being teased, bullied, or rejected by peers [12].

Materials and methods:

Study area: This study was carried out in Amena Bent Wahab School which is one of the primary basic school level for girls in Omdurman – Khartoum state – Sudan.

Study subjects and study design: The study was conducted on 80 girls. Their ages ranged between 5-13 years. The participants were sampled out of four classes (grades), first, fourth, fifth, and seventh classes. The age, weight, height and number of participants from each class were recorded. Consent forms were given to parents to allow their daughters to participate in the study. Meeting between head master and students was arranged to discuss aims and methods of research.

Data collection and calculations:

Measurement of weight and height: Normal balance was used to measure child's weight in kilograms and meters were used to measure height. Height was measured in light clothes .The weighing scale was calibrated daily before the first measurement was taken. Height and weight were measured with the child's shoes removed.

Calculation of body mass index: Body mass index was computed using the standard formula BMI= weight in kilograms/height square in meters. Measurement of body mass index is calculated as reported by Dehghan et al [4].

Calculation of obesity percentile: From the calculated value of BMI, the child's BMI percentile for age and sex were calculated on growth curves published and distributed by the National Center for Chronic Disease Prevention and Health Promotion in America for female ages from 2-20 years. School girls were classified as obese or overweight, normal and under weight based on their body mass index. European researchers classified overweight as at or above 85th percentile and obesity as at or above 95th percentile of BMI [4].

Statistical analysis: A completely randomized design was selected for this study. The data had been presented in tables. The data were analyzed using the statistical program for Social Sciences (SPSS). Statistical significance was set at $p<0.05$ and $p<0.01$. The correlation was used to identify the relation between age with obesity and overweight.

Results:

General information about the studied population**,** were shown in table 1. The mean and standard deviation for age, height, weight and number of participants in each class were given.

Table 1: The Studied population:

Class	Age	Height	Weight	Number
First	6 ± 0.46	1.11 ± 00.06	19.8 ± 4.13	19
Fourth	8.73 ± 0.69	1.32 ± 0.08	32.36 ± 24.70	22
Fifth	10.2 ± 0.51	1.4 ± 0.08	37.8 ± 9.73	20
Seventh	12.11 ± 0.45	1.49 ± 0.05	43.84 ± 12.32	19

Categories of obesity and corresponding percentile were shown in table 2.

Table 2: Categories and percentile of obesity:

Case	Percentile
Obese	More than 95th
Overweight	More than 85th
Normal	More than 5th
Underweight	Less than 5th

Prevalence of obesity, overweight, normal and underweight among studied population and frequencies were presented in table 3. The percentage of students at risk of obesity and overweight was particularly high (37.50%). Also this percentage was close to the percentage of normal weight (55%).

Table 3: Distribution of weight status among studied population:

Study Population	Frequency	Percentage	P value
Obese	15	18.75%	<0.05
Overweight	15	18.75%	
Normal	44	55%	
Underweight	6	7.5%	
Total	80	100%	

Table 4 showed the prevalence and distribution of weight status in each class. The summation of frequency of obesity and overweight in each class was very close to the normal frequency of weight in each studied class.

Table 4: Distribution of weight status in each class:

Class	Obese	Overweight	Normal	Underweight	Total	P value
First	3	2	13	1	19	<0.05
Fourth	6	3	11	2	22	
Fifth	3	6	11	-	20	
Seventh	3	4	9	3	19	

Prevalence of obesity and overweight in studied population according to age were presented in table 5. The percentage of obesity and overweight among school girls were 26.32%, 39.47%, and 34.48% for in age groups of 5-7 years, 8-10 years, and from 11-13 years respectively.

Table 5: Distribution of obesity and overweight according to age:

Age (years)	Number	Frequency	Percentage	P value
5-7	19	5	26.32 %	<0.05
8-10	38	15	39.47 %	
11-13	23	10	43.48 %	
Total	80	30	100 %	

Table 6 showed the distribution and prevalence of obesity and overweight in each class. Percentage of obesity and overweight were 30% in the fourth and fifth classes. While it was 23.33% and 16.67% in the seventh and the first classes respectively.

Table 6: Distribution of obesity in each class:

Class	Frequency	Percentage	P value
First	5	16.67 %	<0.05
Fourth	9	30.00 %	
Fifth	9	30.00 %	
Seventh	7	23.33 %	
Total	30	100 %	

Table 7: Correlation between BMI and age:

Factor	Age
BMI/Pearson Correlation	0.546**

**The correlation is significant at the 0.01 level (2-tailed).

Discussion:

Our study revealed that the combined prevalence of obesity and overweight is 37.50% (18.75% in each case) among the included subjects .The prevalence of obesity and overweight among female children is increasing and is comparable to that found in the developing countries[2,3].

The present study showed that the prevalence of obesity and overweight in female students of basic school level were significantly increasing with age. This is similar to the result obtained by Osama and Enayat [3] and also agreed with Bose et al [5] in their study on obese children.They found that there was a significant increase in the rate of overweight with increasing age.

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Original Article Sudan Medical Laboratory Journal

Gastrointestinal parasites among inmates in Omdurman prison

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Abstract:

The results showed that 49% of inmates were harboring gastrointestinal parasites. The parasites detected were as follows: *Giardia lamblia* (16.3%), *Entamoeba histolitica* (17.4%), *Entamoeba coli* (5.3%), *Ascaris lumbricoides* (5.3%), *Schistosoma mansoni* (1%) and *Taenia spp* (11.7%). In males, the highest rate (16.6%) was detected among the 31 to over 40years age group, while in females it was high among the 21-30 years old group (20.6%).The highest rate (17.7%) was detected among those from southern Sudan and the lowest (9.3%) was observed among those from Somalia and Ethiopia. The highest detection rate (78.2%) was reported with the formal ether technique, while the lowest (14.9) was reported with the sodium chloride flotation technique.

Introduction:

The three factors that separate the underdeveloped world from developing world are access to dirty water, bad sanitation and bad nutrient. Gastrointestinal parasites (GIT) are associated with these factors [(1)].

Intestinal parasites are parasites that populate the gastrointestinal tract. The term is not merely a collective term [(2)], but it can include a group of diverse parasites that vary greatly in many aspects e.g. biology, pathology, epidemiology. Taxonomically, the intestinal parasites are composed of two major subgroups: Protozoa: It includes *Giardia lambilia*, *Entamoeba histolytica* ,*Balantidum coli* and *Cryptosporidium parvum*. Helminthes: The intestinal helminthes are represented by both flat worms and nematodes [(3)]. Intestinal nematodes constitute by far the most common parasitic infection in human [(4)]. It includes *Ascaris lumbericoides, Enterobious vermicularis, Strongyloides stercoralis* and *Trichuris trichiura*. In the other branch, it includes flat worms (Trematodes and Cestodes). They include *Hymenolepis nana*, *Taenia species* and *Fasiola hepatica*. They parasitize human, small intestine and are highly adapted to cause a minimum harm. The frequency of the infection is a general indication of local level of development of hygiene and sanitation.

Material and Methods:

Study area: The study was conducted in Omdurman prison which is located 4 kilometers far away from Khartoum center.

Study Population: The study was conducted on the inmates (male and females) of Omdurman prison.

Sample size: A total of 300 inmates (150 males and 150 females) were examined for the presence of gastrointestinal parasites. The inmates were categorized according to different age groups and different state of origins as follows:

Group 1: age between 10-20 years.

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Group 2: age between 20-30 years

Group 3: age over 40 years

Sampling collection: Each selected inmate was provided with a labeled container which is transparent, clean and with wide mouth for faecal sample collection. From each selected inmate, the labeled container was checked to ensure that the number of the container corresponds to the serial number on the individuals request form.

All those steps were done in order to ensure the quality control measures.

Methodology: The following techniques were used for detection of different parasitic infections: 1.Direct smear examination 2.Formal ether concentration technique 3.Saturated sodium chloride floatation technique 4.Saturated sugar floatation technique.

Results:

Out of the 300 inmates (150 males and 150 females) examined for the presence of gastrointestinal parasites, 147 were found to harbor parasites in their gastrointestinal tract. This constituted an overall prevalence rate of 49% (table 1).

Out of 150 male inmates, 73 were positive for parasites infection which constituted a 48.7% prevalence rate. In females, the prevalence rate was found to be 49.3% (table 2). This difference was found to be statistically insignificant (p value=0.91).

The result showed that the highest prevalence rate of gastrointestinal parasites (18%) was found among the 21-30 age group, while the lowest prevalence rate (1.3%) was reported among the 10-20 age group (table 3). This difference was found to be statistically insignificant (p value=0.20).

In males the highest prevalence rate (16.6%) was reported among the 31- 40 age groups, while the lowest prevalence rate (0%) was reported among the 10-20 age group (table 4). This difference was found to be statistically insignificant (P value=0.44).

In females, the highest prevalence rate (20.6%) was reported among the (21-30) age group, while the lowest prevalence rate (2.6%) was reported among the 10-20 age group (table 5). This difference was found to be statistically insignificant (P value=0.48).

The result revealed that the highest prevalence rate (17.7%) was reported among inmates from the southern region, while the lowest rate 9.3% was reported among others who represent those from Somalia and Ethiopia (table 6). This difference was found to be statistically insignificant (P value=0.41).

The parasites encountered during the study were *Entamoeba histolytica* with a prevalence rate of 14.7%,*Ascarislumbricoides* (5.3%), *Hymenolepis nana* (5.3%), *Entamoeba coli* (2.7%), *Giardia lambilia* (16.3%), *Taenia spp* (11.7%) and *Shistosoma mansoni* (10%) (table7). This difference was found to be statistically significant (P value= 0.04).

The results demonstrated that the highest detection rate (78.2%) was reported for the ether technique while the lowest detection rate (14.9%) was reported for saturated sodium chloride technique (table 8). This difference was found to be statistically significant (P value=0.01).

Table 1: The overall prevalence rate of gastrointestinal parasites among inmates in Omdurman prison

Number examined	Number positive	Prevalence
300	147	49%

Table 2: The prevalence of gastrointestinal parasites among inmates in Omdurman prison according to gender

Sex	Number examined	Number positive	prevalence	P-value
Males	150	73	48.7	
Females	150	74	49.3	0.91
Total	300	147	49	

Table 3: The prevalence of gastrointestinal parasites among inmates in Omdurman prison according to age groups

Age group	Number examined	Number positive	Prevalence	P-value
10 - 20	11	4	1.3%	
21- 30	94	54	18%	
31 - 40	103	49	16.3%	0.20
Over 40	92	40	13.3%	
Total	300	147	49%	

Table 4: The prevalence of gastrointestinal parasites among inmates in Omdurman prison according to age groups in males

Age group	Number examined	Number positive	Prevalence	P-value
10 - 20	1	0	0%	
21- 30	40	23	15.3%	
31 - 40	52	25	16.6%	0.44
Over 40	57	25	16.6	
Total	150	73	48.7%	

Table 5: The prevalence of gastrointestinal parasites among inmates in Omdurman prison according to age groups in females

Age group	Number examined	Number positive	Prevalence	P-value
10 - 20	10	4	2.6%	
21- 30	54	31	20.6%	
31 - 40	51	24	16%	0.48
Over 40	35	15	10%	
Total	150	49.3	4903%	

Table 6: The prevalence of gastrointestinal parasites among inmates in Omdurman prison according to states of origin

States of origin	Number examined	Number positive	Prevalence	P-value
South	108	53	17.7%	
West	81	36	12%	
North	64	30	10%	0.41
Others	47	28	9.3	
Total	300	147	49%	

Table 7: The prevalence of different gastrointestinal parasites encountered among inmates in Omdurman prison

Parasite	Number examined	Number positive	Prevalence	P-value
Entamoeba histolytica	300	44	14.7%	
Ascaris lumbricoides	300	16	5.3%	
Hymenolepis nana	300	16	5.3%	
Entramoeba coli	300	8	2.7%	0.04
Giardia lamblia	300	49	16.3%	
Taenia spp.	300	35	11.7%	
Schistosoma mansoni	300	3	1%	

Table 8: Detection rates of different techniques used for the diagnosis of gastrointestinal among inmates in Omdurman prison

Technique used	Number positive	Number detected	Detection rate	P-value
Wet smear	147	66	44.9%	
Ether technique	147	115	78.2%	0.01
Sodium chloride	147	22	14.9%	
Sugar technique	147	36	24.4%	

Discussion:

From the results, it is obvious that the overall prevalence rate of gastrointestinal parasites among inmates in Omdurman prison is extremely high (49%). This rate was found to be higher than the rate reported by Awole *et al* [(5)] in Ethiopia (34.4%). However, our rate was found to be lower than the rate reported by Develoux *et al* [(6)] in Juba (66 %).

The highest prevalence rate was reported among the age groups 21 – 30 and 31 – 40 years old. This was also true for both age groups in males and females. This finding was in agreement with Eman [(7)] who reported higher rates among the 11 -20 and 21 – 40 age groups in Elrenk district.

From the findings, parasites were mostly encountered in those who came from the southern region and were least encountered in those who came from Somalia and Ethiopia. Our finding for those who came from the south was in line with the finding reported by other workers who conducted similar studies in the south as Marnell *et al* [(1)] and Homeida [(8)] reported a 66% prevalence rate in Juba. In Somalia and Ethiopia, higher rates were reported (15.1% and 34.4% respectively) [(5, 9)], while the rate reported in our study was 9.3% for both.

The highest prevalence (16.3%) was reported for *G. lambilia* followed by exceptionally higher rate for *E. histolytica* and *Taenia spp* with small rates for the others. Similar prevalence rate of *G. lambilia* (16.3%) was reported by Develoix *et al* [(6)] in Niger. This rate was greater than the rate reported by Obiamiwe and Nmorise [(10)] in Nigeria (1.4 %).

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Our rate withy *E. histolytica* was higher than the rate reported by Obimiwe and Nmorise [10] in Niger (3.9%) and lower than the rate reported by Devaloux *et al* [6] in Niger also (29.3%). In this study, *Taenia spp* were found in 11.7% of the population examined. This rate was greater than the reported by Prescika [11] (3.2%). For *Ascaris* and *Shistosoma*, the rate was low (5.3% and 1% respectively). These low rates might be attributed to the different origins of the inmates. *Ascaris* was prevalent in those individuals most probably coming from the south and *S. mansoni* is endemic in certain irrigated areas of Sudan. Higher rates of those two parasites are expected in individuals who are residing in endemic areas. Other parasites reported in this study have no limited area i.e. common in all parts of the Sudan.

As far as the detection rates for the 4 techniques used, it was obvious that the highest detection rate (78.2%) was reported for the ether technique and the lowest rate (14.0%) was reported for sodium chloride technique, while the wet smear and sugar technique showed rates of 44.9% and 24.4% respectively.

Our result for the ether technique was in agreement with Eissa [12] who reported 90% detection rate. However, the detection rate reported in our study was greater than the detection rate reported by Eman [7] (44%). The study revealed that the detection rate for the wet preparation was almost similar to the detection rate reported by Eman [7] (41.4%). Sodium chloride technique was very less efficient in our study compared with Eissa [12] who reported detection rate of 47% while ours was 14.9%.

The detection rate for the sugar floatation technique (24.4%) was lower than the detection rate obtained by Duria [13] (58.6%).

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Original Article

Sudan Medical Laboratory Journal

Schistosoma mansoni infection and its association with hepatitis B virus in Keryab Village

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Abstract

The study was conducted in Keryab village to investigate the association, if any, between *S.mansoni* infection and hepatitis B surface antigen. The study revealed that the overall infection rate was 15% (45 out of 300 stool samples examined). The prevalence in females was higher than that of males (17.8% and 13.4respectively).

The highest infection rate (17.5%) was reported among the 11-15 years age group while the infection was less (14%) among the 16-20 years age group, and lowest infection (11.1%) was reported among the 5-10 years age group. On the other hand, 20 blood samples out of 300 were positive for hepatitis B surface antigen which constitutes 6.7% prevalence rate.

The highest rate of hepatitis B surface antigen (10%) was reported among the 16-20 years age group while the infection was less (6.25%) among the 11-15 years age group.

Out of 45 positive *S. mansoni* cases, 11 were found positive with hepatitis B surface antigen which constitutes 24.44% of the positive cases. On the other hand, 9 positive hepatitis B surface antigens were found among the negative *S. mansoni* cases which constitute 3.52% of the negative cases.

Introduction:

Schistosomiasis is considered to be the most important helminthic disease of man since it infects about 200 million individuals in about 74 countries in the world. Subjects are affected in different manners and most infected individuals are asymptomatic carriers of the parasite. The most serious form is the hepatosplenic one, whose major complication is upper digestive hemorrhage secondary to portal hypertension [(1)].

In pure and uncomplicated cases, hepatocellular function is usually preserved up to the more advanced phases of the disease. Two aspects motivated the study of the role of viral hepatitis in shistosomiasis. The first was the observation that in case of schistosomiasis that progressed to the decompensated form, unusual cirrhotic changes could be observed in liver paraenchyma. The second was the fact that patients with schistosomiasis show altered behavior when in contact with different pathogens, with possible modifications occurring in the course of the disease in the associated presence of enterobacteria, other helminthes and different protozoa [(1, 2, 3 & 4)]. Pioneering studies by Daneshmend *et al* [(5)] demonstrated a significantly higher incidence of B virus surface antigen (HBsAg) in individuals with hepatosplenic schistosomiasis (HSS) and suggested that the antigenemia of patients tended to persist. Anatomopathological studies demonstrated the role

of active chronic hepatitis as a decomposing factor in schistosomiasis [2, 6].

Materials and methods:

The study was conducted in Keryab village, sharg Al Nil which is located 30 kilometers far from Khartoum centre.

Study population: The study was conducted on 300 individuals selected randomly. Search for Hbs antigens was done for both positive and negative S. mansoni cases. The population was divided according to gender and age groups (5- 10, 11- 15 and 16-20 years).

Sample collection: Stool samples were collected in sterile containers. Blood samples were collected in filter papers.

Stool examination: Faecal samples collected were examined by the locally modified Kato method described by Teesdate and Amin [7].

Blood examination:

Specimen processing: About 300 µl of whole blood was obtained from each suspected patient in filter papers (whatman) type and allowed to dry in room temperature and stored in closed container to avoid contamination and touch of each paper.

Preparation of eluate: 24 hrs before testing, the spot of filter paper was soaked by 300 µl of PBS. The tube was tightly sealed and kept at room temperature over night to be tested the next day by ELISA test (DIA. PRO, Diagnostic Bioprobes Srl. Italy).

Test procedures:

1. The required numbers of strips was placed in the plastic holder and washed once to hydrate the well. The wells for controls, calibrator and sample were carefully identified.
2. Well Al was left empty for blanking purposes.
3. 150 µl of the negative control were pipetted in triplicate, 150 µl of the calibrator in duplicate and 150 µl of the positive control was all pipetted into the assigned wells. This was followed by addition of 150 µl of the eluate of each sample.
4. Presence of samples in wells was checked by naked eye (there is a marked color difference between empty and full wells) or by reading at 450/620nm (samples show OD values higher than 0.100).
5. 100 µl of diluted enzymatic conjugate were dispensed in all wells, except for Al, used for blanking operations.
6. Addition of the conjugate was followed, checking that the color of the samples has changed from yellowish to red and then the micro plate was incubated for 120 min at 37ºC.
7. When the first incubation is over; the micro wells were washed very well.
8. 200 µl chromogen/substrate were pipetted into all the wells, including Al.
9. The micro plate was incubated and protected from light at 18- 24 ºC for 30 min. wells were dispensed with the positive control, the calibrator and the positive samples will be turned from clear to blue.
10. 100 µl Sulphuric acid were added into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 8. Addition of the acid solution turned the positive control, the calibrator and positive samples from blue to yellow.

11. The color intensity of the solution in each well was measured using a 540 nm filter (reading) and if possible a 620- 630 nm filters, blanking the instrument on Al.

Statistical analysis: Statistical analyses were performed using statistical package for social sciences (SPSS). Proportions were compared using Chi Square test. Significance was determined at the 0.05 probability level in all analysis.

Results:

Out of the 300 faecal samples examined, 45 were found positive for *S.mansoni*. This constitutes a 15% prevalence rate (table 1).

Out of the 193 male examined, 26 were found positive for *S.mansoni* which constitutes 13.4% prevalence rate, while out of the 107 females examined, 19 were found positive for *S.mansoni* which constitutes 17.8% prevalence rate (table 2). This difference was found to be statically insignificant (P value=0.268).

The results showed that, the highest prevalence rate of S. mansoni (17.5%) was observed among the (11-15 years) age group while the lowest prevalence rate (11.1%) was detected among the (5-10 years) age group (table 3). This difference was found to be statistically insignificant (P value=0.268).

The detection of antibodies against Hepatitis-B surface Ag (HBs Ag) among the school children in Kerayab village revealed prevalence of 6.7% (table 4). Among those who were infected with *S.mansoni*, the detection rate was 24.44% while among those who were negative for *S.mansoni*, the detection rate was 3.52% (table 5). This difference was found to be statically significant (P value=0.01).

The result showed that the highest prevalence rate of Hepatitis-B surface Ag (10%) was observed among the 16-20 years age group while the lowest prevalence rate (5.5%) was detected among the 5-10 years age group (table 6). This difference was found to be statistically insignificant (P value= 0.663).

Out of the 193 males examined, 16 were found positive for Hepatitis-B surface Ag (HBs Ag) which constitutes 8.3% prevalence rate, while out of the 107 females examined, 4 were found positive for Hepatitis-B surface Ag (HBs Ag) which constitutes 3.7% prevalence rate (table 7). This difference was found to be statistically insignificant (P value= 0.168).

Table 1: Overall prevalence rate of S. mansoni among school children in Keryab village:

Number examined	Number Positive	Prevalence
300	45	15%

Table 2: The prevalence rate of S. mansoni among school children in Keryab village according to gender:

Gender	Number examined	Number positive	Prevalence	P value
Male	193	26	13.4%	0.268
Female	107	19	17.8%	

Table 3: The prevalence rate of S.mansoni among school children in Keryab village according to age group**:**

Age group	Number examined	Number positive	Prevalence	P value
5 – 10	90	10	11.1%	0.268
11 – 15	160	28	17.5%	
16 - 20	50	7	14%	

Table 4: Seropositivity of hepatitis B surface antigen among school children in Keryab village:

Number examined	Number Positive	Prevalence
300	20	6.7%

Table 5: Seropositivity of hepatitis B surface antigen among Schistosoma mansoni positive and negative cases in Keryab village*:*

	Number tested	Number positive	Prevalence	P value
+ve	45	11	24.44%	0.01
-ve	255	9	3.52%	

Table 6: The prevalence rate of hepatitis B surface antigen among school children in Keryab village according to age group:

Age group	Number examined	Number positive	Prevalence	P value
5 – 10	90	5	5.5%	0.663
11 – 15	160	10	6.25%	
16 - 20	50	5	10%	

Table 7: The prevalence rate of hepatitis B surface antigen among school children in Keryab village according to gender:

Sex	Number examined	Number positive	Prevalence	P value
Male	193	16	8.3%	0.168
Female	107	4	3.7%	

Discussion:

The result obtained reveled that the overall prevalence rate of *S.mansoni* in Kerayab village was found to be 15%. This rate was lesser than the rate reported by Tagwa [(8)] who reported 40% prevalence rate. This marked reduction in the prevalence rate of *S.mansoni* in the area might be attributed to control measures launched in the area before the start of our study.

In this investigation, there was no correlation between age and the occurrence of *S.mansoni* infection among the studied population. This finding is in contrast with the findings of Nasr [(9)] and Butterworth *et al* [(10)] who attributed the correlation to age dependent acquisition of immunity to super- infection. The prevalence and intensity of infection was higher in females (17.8%) than in males (14.3%). In spite of exposure rate and water contact which is usually higher in males by virtue of their activities. Although this is an agriculture scheme, farming is normally practiced by male and female particularly the young and middle aged ones. The land is irrigated by canals which harbor infected snails. These same canals act as swimming pools for children and youngest boys and girls. Since this is one of the main items for recreation, it is customary to find them daily swimming from early to late afternoon or washing clothes. The result obtained showed that HBs Ag was high among bilharzial patient than in non-bilharzial individuals. Similar results were reported from Egypt [(11, 12, 13, & 14)], Brazil [(15)], Kuwait [(16)] and Sudan [(5)]. Such results can be regarded as evidence for the existence of an association between HBV and *S.mansoni* infection.

In contrast, some investigators [(17, 18, 19, 20, & 21)], showed that there is no link between the two diseases. Some of them referred the association found in the former studies to selection bias *i.e* those studies showed positive correlation in selected hospitalized patients with *S.mansoni* infection. As is generally accepted that *S.mansoni* infection alone does not lead to cirrhosis or chronic active hepatitis [(18 & 22)], interaction between HBV and *S.mansoni* infection was suggested to cause a serious form [(14)].

The result obtained showed that the bilharzial patients with or without oraganomegally had comparable seropositivity for HBsAg. This indicates that they are similary susceptible to HBV infection. Similar finding were reported by Khalil *et al* [(13)] and Ghaffar *et al* [(14)]. In contrast, Bassily *et al* [(11)] and Al Nakib *et al* [(16)] reported that HBV infection was higher in hepatosplenic than in intestinal schistosomiasis. However, the orgaomegally found in these subjects might not be due to only Schistosoma infection, because the study area is endemic for other diseases such as malaria.

Tosswil and Rideiy [(23)] suggested the presence of shared antigens between *S.mansoni* and HBV infection, however, the introduction of highly specific methods for detection of HBsAg made this possibility rather remote. The initial defense mechanisms against viral infection are the innate immunity defense such as interferon-y (INF-y), Natural Killer (NK) and macrophages. INF-y inhibits viral replication and enhances adaptive immune response by stimulating and increasing the expression of major histocompatibility complex (MHC) class 1 class 11 and it is also potential activator of macrophages and NK cells. Since HBV is an intracellular organism, MHC1 is the most important mechanism for eliminating the virus infected cells through CD-8 T cells [(14)].

The overall prevalence rate of the present study was 24.4%. This rate was found to be higher than the rate reported by Eltoum *et al* [(20)], Hyams *et al* [(18)], and Taha [(24)] who reported 18.5%, 10%, and 9% rate respectively. However, our rate was less than the rate reported by Nasr [(9)] who reported 30% rate in Sudan.

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Original Article

Sudan Medical Laboratory Journal

Study of hypoglycemic and anti-diabetic effect of grape fruit ethanolic extract on hyperglycemic glucose induced rats.

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Abstract

This experiment was conducted to study the hypoglycemic and anti-diabetic effect of grape fruit ethanolic extract (outer external layer) in hyperglycemic rats induced by injection of glucose at the rate of 2g/kg body weight, after fasting 18 hours.

According to our experiment rats weighing 80-120g were used for this study. Rats were divided into 5 groups (n=6). Group I was injected 10 mg/kg body weight of glibenclamide (Daonil), this is the standard group. Group II left as control and treated with 10 mg/kg distilled water, group III was treated with 200 mg/kg body weight and group IV was treated with 400 mg/kg of ethanolic grape fruit extract. The last group (group V) was treated with 800 mg/kg of the extract. The rats in the standard group have low concentration of blood glucose after 2 hrs, 4 hrs (86.3 – 76.8 mg/dl respectively). Group III (200 mg/kg extract) have blood glucose level of 121.8 and 124.2 mg/dl respectively. Group IV (400 mg/kg) have blood glucose 147.2 and 135.7 mg/dl respectively, while the rats in last group (800 mg/kg) have the lowest level of blood glucose 90.8 and 103 mg/dl respectively which was the best group, and the blood glucose was nearly similar to that of the standard.

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Introduction:

Diabetes mellitus (D.M) is a major metabolic disorder, characterized by chronic glycaemia as relative or absolute lack of insulin or the action of insulin leading to structural changes in a range of cells specially those of the vascular system, nerves and the organs system [(1)].

Type I diabetes mellitus (IDDM – insulin dependant D.M or juvenile diabetes) is characterized by beta cells destruction caused by an autoimmino-process, usually leading to absolute insulin deficiency, over 95% of persons with type I develop the disease at the age of 25 with an equal incidence in both sexes. Type II diabetes mellitus (NIDDM, non-insulin dependent D.M or adult onset) is characterized by insulin resistance in peripheral tissue and insulin secretary defect of beta cell. NIDDM is usually

associated with family history of adults, elder age, obesity and lack of exercise, and it is more common in women [(2)].

The outer layer of grape fruit contains emulin extract which lowers the carbohydrate, and reduces the glucose manufactured by the liver. It also lowers the high level of glucose in liver so it acts like insulin. It reduces the glucose level in the blood by about 27 %.

Naringin yellow flavoniod compound is found in the external layer of grape fruit. It has been identified as anti diabetic and hypoglycemic, in addition to hypolipidemic effect. Hesperedin is a flavonoid found in grapefruit, and is used as traditional remedy in some local tribes. The mechanism where by naringin and hesperdin have hypoglycemic and hypolipidemic action in type II diabetes have rarely been investigated [(3)].

Ali and Elkader [(4)] found that 80 mg/kg of naringin as citrus flavonoid have shown a great effect as hypoglycemic and anti-oxidant effect. Naringin inhibited glucose uptake in the in the intestine and also have inhibitory action towards glucose uptake in renal brush border membrane vesicle [(5)]. Effect of naringin (30mg/kg) with 50 mg/kg vitamin C was similar to the effect excreted by injection of 6 units/kg insulin [(6)].

Materials and methods:

Method of ethanol extraction: 50 grams of dried sample (outer layer of the fruit) was extracted with 80% ethanol using shaker apparatus for about 48hours. Solvent was evaporated under reduced pressure using rotary evaporator apparatus. The extract was placed in open Petri-dishes till complete dryness (yield about 15.6%).

Glucose-loaded model rats: Rats of either sex weighing 80-120 g were used. The animals were fasted for 18hours prior to the experiment and then allotted to five groups of 6 rats each. After administration of the extract, the five groups were injected intraperitonially with 50% glucose solution at a dose of 2g/kg body weigh. The glucose level was monitored at 0 hr, 2hrs and 4hrs intervals [(7)].

Group I: Each rat was given 10 ml/kg body weight of glucose, then blood glucose was measured for each rat at 2hr and 4hr. This was considered as the control group.

Group II: Each rat was injected intraperitonially with 50% glucose solution at a dose of 2 g/kg body weight, then each rat was injected with 10mg/kg weight with glibenclamide. The glucose level was monitored at 0 hr, 2hr, and 4hr. This was considered as the standard group.

Group III: Each rat first injected with 50% glucose to induce diabetes, and then each rat was given the extract (0.2g/kg body weight) orally. The blood glucose was monitored at intervals of 2hr, and 4hrs.

Group IV: Each rat was first injected with 50% glucose to induce diabetes, then each rat was given an extract (0.4 g/kg body weight). The blood glucose was monitored at 2hrs, and 4hrs intervals.

Group V: Each rat injected with 50% glucose, and then each rat was given orally (0.8 g/kg body weight). The blood glucose was monitored at 2hrs, and 4hrs intervals.

The blood was taken by using capillary tube where the eye was pressed to outside by pressing its head down to allow the flow of blood to the capillary tube.

Glucose was estimated by glucose oxidase enzymatic method [8]

Results:

In the standard and 800mg/kg treatment groups, there was no much difference between these two groups and others where the glucose level was 86.3 and 103.0 respectively (table 1).

Table 1: Effect of control, standard and different concentrations of grape fruit extract on blood glucose level at 2hr, 4hr intervals

Grape fruit Extract	Concentration of glucose after 2hr	Concentration of glucose after 4hr
control	132.7	127.8
standard	86.3	76.8
200 mg/kg	121.8	124.2
400mg/kg	147.2	135.7
800mg/kg	103	90.8
least significant difference (L.S.D)	20.3	40

If we compare concentration of glucose regarding time (2hrs & 4hrs) for all treatments (table 2), from the concentration of glucose after 4hrs is less significant (111.1 mg/dL) if we compared with 2hrs time (118.2 mg/dL). There was no significant difference of glucose at 2hrs, and 4hrs in groups of (standard, 200 mg/kg, 400 mg/kg), but in group of (800 mg/kg) and especially after 4hrs this treatment record a significant differences (90.8 mg/dL) compared to (103.0 mg/dL) result obtained after 2hrs.

Table 2: Comparison of blood glucose level after 2hr, 4hr for all treatment, standard and (200 mg/kg, 400mg/kg, 800mg/kg)

Glucose level (mg/dL)	After 2hrs	After 4hrs	Significant difference
for all treatments	118.2	111.1	**
standard	86.3	76.8	ns
200 mg/kg	121.8	124.2	ns
400 mg/kg	147.2	135.7	ns
800 mg/kg	103.0	90.8	**

ns (no significant difference)
** There is significant difference at 1%

Discussion:

In the standard and 800mg/kg treatment groups, there was no much difference between these two groups and others where the glucose level was 86.3 and 103.0 respectively (table1). The other group recorded glucose concentration -after 2hrs- 132.7 mg/dL in control, 121.8 mg/dL in 200mg/kg, and 147.2 mg/dL in 400mg/kg. 200mg/kg record the least average (taken control and 400 mg/kg) we compare the concentration of these treatment. The decrease of level of glucose in group (800mg/kg treatment) we compare it to

the other treatments (control, 200 mg/kg, 400 mg/kg) it was 22.2%, 15.4% and 30% respectively. This proved that the treatment 800mg/kg after 4hr is the best treatment when compared to others.

If we compare concentration of glucose regarding time (2hrs & 4hrs) for all treatments (table 2), from the concentration of glucose after 4hrs is less significant (111.1 mg/dL) if we compared with 2hrs time (118.2 mg/dL).

As in table (2), there was no significant difference of glucose at 2hrs, and 4hrs in groups of (standard, 200 mg/kg, 400 mg/kg), but in group of (800 mg/kg) and especially after 4hrs this treatment record a significant differences (90.8 mg/dL) compared to (103.0 mg/dL) result obtained after 2hrs. The rate of decrease of glucose concentration was 11.9% which was similar to the rate in treatments 200 mg/kg, 400 mg/kg and 800 mg/kg for the results after 4hrs when compared to the results after 2hrs.

The ethanolic extracts of grape fruit include naringin and hesperedin. Naringin exert a variety of pharmacological effects such as anti oxidant activity, blood lowering, anti cancer activity, and inhibition of selected drug metabolizing cytochrome P450, including CYP 3A4 and CYPIA2 which may result in drug interaction in vivo. Ingestion of naringin may increase or decrease in circulating drug level.

Naringin is absent from juice [9], grape fruit juice did not produce interaction [10].

Naringin inhibited in vitro felodipine and nifedipine metabolism that was much less potent than its glycone naringenin [11, 12].

Now we are about to find the effect of grape fruit extract flavonoid in reducing the glucose level, but more research work must be carried not to find the effect of naringin, hesperedin, Emulin (separately or mixed) of pure forms of these components to produce anti diabetic and hypoglycemic, and hypolipimic effect. Also we need to know the different effect of naringin and it's a glycone naringenin.

Hesperidin and naringin both significantly increased the glucose kinase RNA level, while naringin lowered the RNA expression of phospho-enolpyruvate carboxkinase and glucose-6-phosphate in the liver [3]. Hesperidin and naringin effectively lowered the plasma free fatty acids and plasma and hepatic triglyceride levels and simultaneously reduced the hepatic fatty acid oxidation. There was suppression of glucose-6-phosphate dehydrogenase and increasing in the plasma and hepatic cholesterol levels, this due to decrease in hydroxy-3-methylglutaryl. Coenzyme (HMG. GA) reductase and acyl CoA, cholesterol acyl transferase (ACAT) activities and increase fecal cholesterol [3].

Exogenous administration of individual gradual dose dependant of naringin to hyperglycemic rats causes a dose dependant decrease of the glucose level and an increase concentration of insulin, decrease of the H_2O_2 as well as an increase of the antioxidant enzymes (catalyse CAT, super-oxide

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dismutase (SOD), glutathione peroxidase (GPX), and paraoxonase (PON)).

The greatest effect of naringin was observed at 80 mg/kg body weight [(4)].

Naringenin a flavonoid present in citrus fruits significantly inhibited glucose uptake in the intestine also has an inhibitory effect towards glucose uptake in renal brush-border membrane vesicles [(5)].

A dose of 30 mg/kg naringin and 50 mg/kg of vitamin C. was similar to the effect exerted by insulin 6units/kg [(6)].

Naringin have anti oxidant activity, blood lipid lowering, anti-cancer activity, inhibition of selected drug-metabolizing cytochrome P450 enzymes including CYP 3A4 and CYP IAZ which may result in drug interaction in vivo, so this all effect the intestinal absorption of certain drugs leading to an increase or decrease in circulating drug level. Naringin inhibited in vitro felodipine and nifedipine metabolism, but this was much less in naringenin [(13)].

It seem that naringin have good effect in lowering the glucose and also reducing the level of lipids; but the issue in inhibition of absorption of certain drugs this will face the commercial pharmaceutical application of naringin as anti diabetic reagent; researcher may think about the effect of naringin (the aglycone of narigin), also the mixed effect of naringin and hesperidin, or the effect of emulin which reduce the level of blood glucose by 27%.

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Original Article

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Evaluation of various techniques used for the diagnosis of *Schistosomiasis*

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Abstract:

The main objective of the study was to determine the rates of prevalence for both species of *Schistosoma* amongst the population with special emphasis on school children. Moreover, the study aimed at the determination of sensitivity of various techniques for the diagnosis of each of the two *Schistosoma* species.

1000 specimens were, in total, collected; 500 samples of urine and 500 samples of stool from the population in the area around Sinnar city. The surrounding environment and social behavior of the communities were taken into consideration, especially the habits that aid the spread of infection. For stool detection, direct faecal smear, formal ether centrifugation technique, formal detergent quantitative technique, ordinary kato technique, and modified kato-katz technique were used. For urine detection, sedimentation technique by centrifugation, sedimentation technique by gravity, and filtration technique were used.

The overall rate of prevalence of *S. mansoni* was 24.8%, while *S. haematobium* was 43.0%. Amongst the diagnostic methods for the examination of the samples, formal ether technique proved to be the most sensitive (24.8%) for detecting and determining the mean egg count of *S. mansoni*, while filtration technique proved to be the most sensitive (43.8%) for detecting and determining the mean egg count for *S. haematobium*. Other methods showed relatively less sensitivity and quantitative efficiency

Introduction:

Schistosomiasis is considered to be one of the major public health problems in Sudan, and also is considered to be the most important tropical disease next to malaria. Christopherson **(1)** in 1918 held the view that schistosomiasis was introduced into the Sudan by the Egyptians. Human schistosomiasis or bilharziasis is caused by three main species; *Schistosoma mansoni*, *Schistosoma haematobium and Schistosoma japonicum*, which infect 10% of the world population. *S.haematobium* causes urinary schistosomiasis, *S. mansoni* and *S.japonicum* cause hepatosplenic and intestinal schistosomiasis. The estimated global total annual loss from reduced productivity amounted to 642 million US dollars. This sum did not include the cost of public health programs, medical care, or compensation for illness **(2)**.

The simplistic equation: man + snail + water = schistosomiasis, is valid because of high reproductive potentiality of life cycle **(3)**.

The peak age of infection is in the first two decades of life. In the population, the prevalence at this age approaches 100% **(4)**. Schistosomiasis causes chronic morbidity, with mortality estimated at 2-10%. It is predominantly a rural disease, commonly infecting farmers, and is

acquired through contact with contaminated water [5]. Children in particular, with their high infection level indiscriminate habits of excretion, and predilection for playing in water, are very important in the transmission of the disease [6]. The distribution of *S. haematobium* has been reviewed in a World Health Organization questionnaire. It is endemic in all countries in Africa, except Rwanda and Burundi, and occurs in islands off the African mainland including Zanzibar and Madagascar [7]. The parasite is often endemic in only a part of a country where it occurs - reflecting the distribution and population dynamics of the snail host and variation in mass contamination of, and contact with, the water bodies where these snails are present [8]. Nevertheless, in a few studies, surprisingly low mean egg counts have been found, in association with significant levels of prevalence, perhaps, due to technical factors such as the time of specimen collection [9].

A considerable number of studies have been carried on *S. haematobium* infection in the Sudan. In 1933 [10], urinary schistosomiasis was reported in Kordofan and in Darfour regions.

Over the years, repeated surveys showed the gradual establishment of the disease in the Gezira. Stephenson [11] reported a prevalence rate of 21% among adults and 45% among children for *S. haematobium*, and suspected a fairly high non-documented prevalence for *Schistosoma mansoni*. Omer, *et al* [12] reported an over all prevalence of *S. mansoni* infection, on Elgalgala village in northern Gezira of 50% and a peak of 80% in the age group 10 – 20 years. Only 0.2% of cases were suffering from *S. haematobium*. Amin and Fenwick [13] reported a prevalence of 70 – 86% for *S. mansoni* and only 1.13% for *S. haematobium*. They noticed the ever increasing prevalence of *S. mansoni* and the dwindling incidence of urinary schistosomiasis. This is in spite of the widespread distribution of both types of snails.

This study aimed to compare the efficiency of various methods used for the diagnosis of *S.mansoni* and *S.haematobium*; and also to determine the prevalence of *S.mansoni* and *S. haematobium* in Sinnar Province.

Materials and methods:

Study area: The crescent around Sinnar town that lies about 280 km south from the capital, Khartoum was chosen as the survey area. The samples were collected from sporadically scattered places in the vicinity of Sinnar town, Wad Hashim village, Al-muraffaa Umda, Hillat Albeer, Sinnar Junction, and Mahala village

Collection of specimens: Samples of urine and stool were collected from 1000 randomly selected individuals of various sexes and mainly school children of 5-16 years of age (500 faecal and 500 urine samples). The persons from whom samples were collected were also requested to respond to a questionnaire comprising beside the personal particulars (name, age and occupation etc), the environmental factors and surroundings that could most probably lead to infection.

Collection of faeces samples: Faecal specimens were collected in clean, dry wide-mouthed plastic containers with tightly fitted lids.

Collection of urine samples: The urine samples were collected between 10a.m-2p.m. 30 ml volume of urine was taken. When it was not

possible to examine the fresh specimen within the first hour of collection, it was preserved with formalin (1ml per 100ml of urine) to avoid hatching of eggs.

Methods:

Stool samples:

- **Direct faecal smear**: using standard method published by Engels et al [14].
- **Formal ether centrifugation technique:** using method published by Katz et al [15].
- **Formal detergent quantitative technique:** using the method published by Borel et al [16].
- **Ordinary Kato technique:** using the method published by Ebrahim et al [17].
- **Modified Kato-Katz technique:** using standard method published by Erko et al [18].

Urine samples:

- **Sedimentation technique by centrifugation:** using method published by Fisher et al [20].
- **Sedimentation technique by gravity:** using standard method published by Gutierrez [21].
- **Filtration technique:** using standard method published by Kean et al [22].

Results:

S. mansoni

The examined fecal samples in this study were 500 samples. 24.8% of them were positive for *S.mansoni* (table 1).

Table (1): The overall prevalence rate of S. mansoni in the surveyed individuals:

Number of samples	Number of positive samples	Percentage
500	124	24.8%

The positive samples for *S.mansoni* in males' faecal samples showed insignificant less prevalence percentage (23.9%) than females (25.3%) in this study (table 2).

Table (2): Prevalence of S. mansoni according to sex of school children:

Sex of children	Examined	Positive	Percentage	P value
Boys	394	94	23.9%	>0.05
Girls	87	22	25.3%	

Out of 124 positive stool samples, the highest sensitive technique used for detection of *S. mansoni* was formal ether (100% sensitive) which was increased significantly compared to other techniques. The mean number of egg count obtained by this technique has also showed the highest number. While the lowest sensitivity was shown by the wet preparation (24.2%) which decreased significantly compared to other techniques (table 3 and figure 1).

Table (3): The number found positive by the respective techniques and their sensitivity:

Techniques	Wet preparation	formal ether	formal detergent	modified kato	ordinary kato	P value
Positive	30	124	110	103	100	0.08
Relative sensitivity	24.2%	100%	88.7%	83.1%	80.6%	

Insignificant changes

Figure (1): Mean number of egg count obtained by different techniques applied for stool examination:

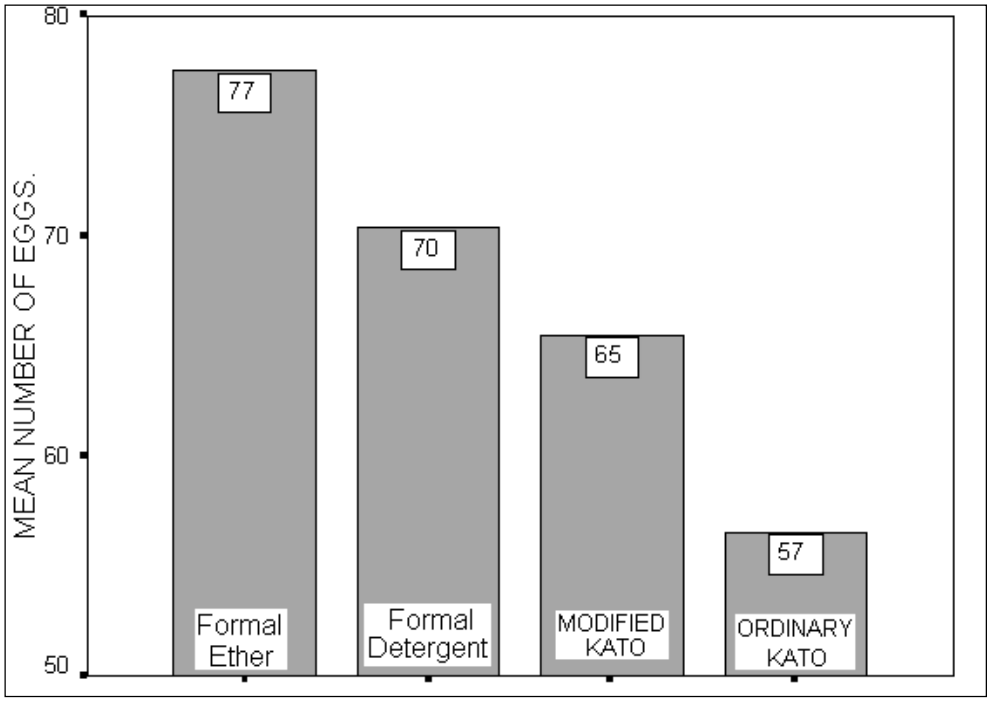

Figure (1): Mean number of egg count obtained by different techniques applied for stool examination:

***S. haematobium*:**

The examined fecal samples in this study were 500 samples. 43% of them were positive for *S.haematobium* (table 4).

Table (4): The overall prevalence rate of *S. haematobium* in the surveyed population:

Number of samples	Number of positive	Percentage
500	216	43%

The positive samples for *S.haematobium* in males' faecal samples showed insignificant increase prevalence percentage (45.9%) than females (34.6%) in this study (table 5).

Table (5): Prevalence of S. haematobium according to sex of school children:

Sex of children	No examined	No positive	Percentage	P value
Boys	399	183	45.9%	>0.05
Girls	78	27	34.6%	

With no significant variations, out of 216 positive urine samples (table 6), the highest sensitive technique used for detection of *S. haemato*bium was the filtration technique (100% sensitive), while the lowest sensitivity was shown by the sedimentation with gravity technique (97.2% sensitive).

Table (6): The number found positive by each technique and their sensitivity:

Technique	Filtration	Sedimentation with centrifugation	Sedimentation with gravity	P vale
Positive	216	213	210	>0.05
Relative sensitivity	100%	98.6%	97.2%	

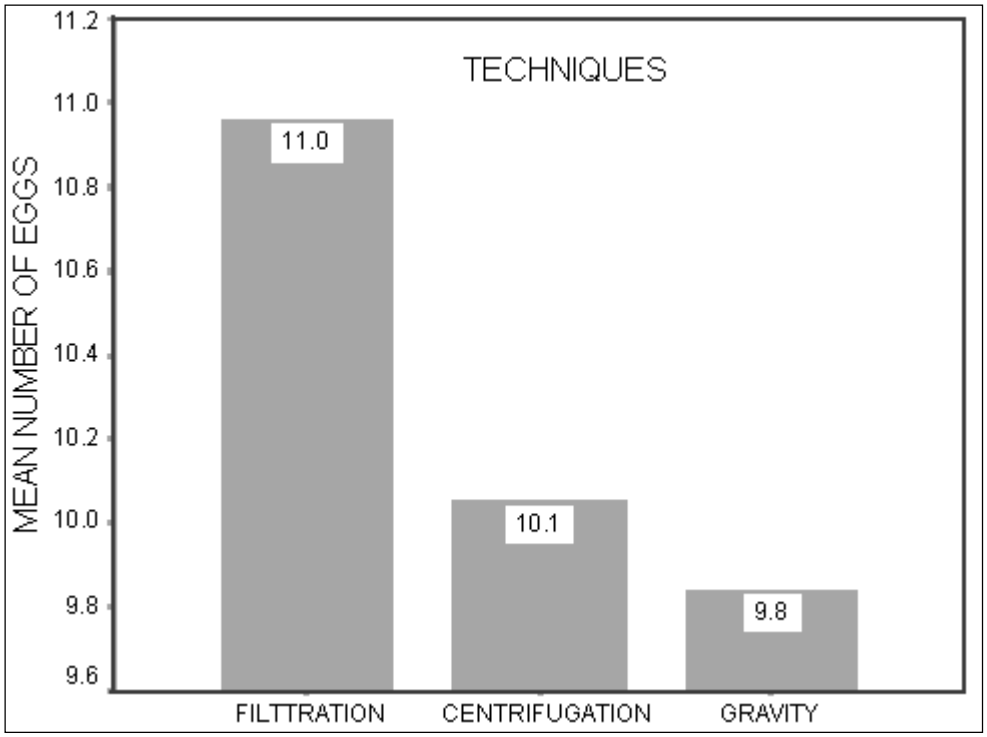

Figure (2): Mean number of eggs count obtained by different techniques applied for urine examination:

Discussion:

The results from the present study show that, from 500 collected samples for each of *S. mansoni* and *S. haematobium* detection, the overall prevalence was 24.8 % and 43% respectively. This reveals that several factors could be held responsible for the transmission of the disease. Among these factors: the social behavior, non-hygienic community practices, improper disposal of human waste and lack of health care.

Stephenson [(11)] had reported a very high prevalence of *S. mansoni* (35 %) and *S. haematobium* (40%) from the northern province. This finding was in agreement with the findings obtained in this study.

This study concentrated particularly upon school children in the age range of 5 to 16 years. The rate of positive cases resulting from the present study, for *S. mansoni* among boys and girls was 23.8% and 25.3% respectively. The prevalence rate of *S. haematobium* for boys and girls was 45.9% and 34% respectively. This difference between infection in boys and girls is, perhaps, attributed to the more frequent visits paid by boys to the contaminated water supply sources.

El Alamy and Cline [(7)], accounted for the epidemiology of *S. haematobium* that it differs from that of other species in respect to relationship between age and the prevalence and intensity of infection. *S. haematobium* infection characteristically has peak intensity and prevalence of infection in the 5 to 16 years old age group, after which there is a fall to much lower levels of intensity and prevalence in middle age.

For the detection of *S. mansoni*, the present results proved that formal ether yielded the highest relative sensitivity value of 100% in the examined samples, whereas other methods

showed less sensitivity. For instance, formal detergent method could recognize 88.7% of the examined samples. The wet preparation technique proved the least reliable in this respect. It showed only 24.2% of the positive samples. Yet, though the wet preparation technique is not considered a quantitative method, for egg count, it is distinguished by being a quick and simple method and requires small amount of sample. It is not efficient in detecting light and chronic infections. Ordinary Kato and modified Kato Katz gave considerable and reproducible results, 80.6% and 83.1%, respectively. The mean egg count showed varied results among these methods considerably. The mean egg count with the Formal ether was 77, formal detergent 70, modified Kato Katz 65, whereas with the ordinary Kato was only 57. This denotes that great quantity of sample contributes to the reliability of detection, since the quantity taken for formal ether detection weighs up to 1g of stool sample. Formal detergent detection requires 0.33g of stool sample. Meanwhile, for modified Kato Katz 25mg and ordinary Kato, only 50 mg of stool sample is prescribed. Engels et al **(14)**, stated that modified Kato Katz gave better results than the wet preparation method for detection of helminthes, and that it is better than ordinary Kato. Ebrahim et al **(17)** made a comparison between formal ether technique and modified Kato Katz. He found that the overall sensitivity of a single modified Kato Katz smear was 70.8%, whilst the sensitivity of the formal ether sedimentation technique was 83.3%. He concluded that formal ether method gave better results than modified Kato Katz in slight infections. The above findings correspond with the results of the present study. In a comparison made by Borel et al **(16)**, they concluded that the principal advantage of formal detergent method over modified Kato Katz technique was its better sensitivity to detect light infections resulting from larger amounts of stool processed.

In the present study, the techniques used for the detection of *S. haematobium* in urine samples showed highly sensitivity without significance between them ($p>0.05$). Out of 216 positive urine samples for *S. haematobium,* the sensitivity of filtration, sedimentation by centrifugation and sedimentation by gravity techniques were 100%, 98.6% and 97.2% respectively. This shows, indisputably, the higher relative sensitivity of the filtration technique. As for the mean egg count is concerned, almost similar results were procured, since a mean egg count 11.0 resulted from the filtration method, 10.1 resulted from sedimentation by centrifugation and 9.8 resulted from. In South Africa, Schutte et al **(24)** reported that the filtration technique was the mostsensitive in detecting ova in urine. Furthermore, there was little agreement between the mean egg count obtained using the filtration technique and those obtained with the other two techniques.

Richards et al **(25)** reported that sedimentation by centrifugation gave statistically higher values than all other techniques, including filtration, as for the number of eggs recovered in the preserved urine. This is mainly attributed to the fact that the preserved urine, unlike fresh urine, frequently caused obstruction of the polycarbonate and Whatmain No. 1 filters.

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Original Article

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Serological and molecular diagnosis of toxoplasmosis in pregnant women in Omdurman, Khartoum state

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Abstract:

This study was conducted to determine the sero-diagnosis and risk factors of *toxoplasmaosis* among pregnant women in Omdurman, Khartoume state, during the period of June 2006 to July 2008. The study included 455 pregnant women between 25 – 35 years of ages.

The blood samples were collected and examined by direct latex agglutination, ELISA IgG and IgM and ten by PCR test. All serological investigations were carried out in the center for research and laboratory sciences, Faculty of Medicine, Ain Shams University, Egypt. Data were collected from the pregnant women by interview using modified questionnaire.

The results showed that the prevalence of *Toxoplasma gondii* was 17.8% by direct latex agglutination, 38.9% by ELISA IgG (indicating chronic infections) and 12.9% by IgMindicating acute infections. The results of PCR test showed 16.7%. Although of questionable accuracy, the results of the present study revealed a relatively high sero-prevalence of acute infection in the pregnant women, with *Toxoplasma*.

Introduction:

Frankel et al [(1)] reported that *Toxoplasma gondii* was first discovered by Nicolle and Manceaux in (1908), Hutchison in (1965) isolated *Toxoplasma* oocysts from cats.

Toxoplasma gondii is a worldwide polyxenous intracellular coccidian parasite in which cats are known to be the definitive hosts and probably all warm blooded animals and man are intermediate hosts. Human toxoplasmosis may occur as an acquired infection or as a congenital infection in infants of infected mothers. Intra-uterine infection with *Toxoplasma gondii* can cause severe and often fatal cerebral damage to the fetus. *Toxoplasma gondii* is serving as source of infection to humans and animals and usually leads to economic losses due to miscarriage. Although, occasionally, *Toxoplasma* can be isolated in smears of lymph nodes, bone marrow and other tissues, diagnosis of toxoplasmosis in man and also in animals relies entirely on detection of circulating antibodies by serological tests.

The risk of infection with *Toxoplasma gondii* can be reduced by avoiding ingestion of contaminated food and by avoiding contact with infected and / or stray cats.

Toxoplasmosis in Sudan:

The first report of human toxoplasmosis dates back to 1966 when Carter and Fleck [(2)] studied four types of population from different parts of Sudan, using the Dye test. They reported a seroprevalence rate of 22% in Kordofan and Darfur and southern provinces, whereas in the

northern provinces, the seroprevalence rate was as high as 70%. The difference in the prevalence rates is thought to be due to racial habits which may affect the transmission of toxoplasmosis [(2)].

An overall prevalence rate of 41.7% was reported by Abd Elhameed [(3)] among the residents of Gezira province aged 10 years and above using IgG ELISA, ISAGA and latex agglutination test. In the study made by Carter and Fleck [(2)] among the residents of Khartoum and Gezira, excluding children under 10 years, the overall *Toxoplasma gondii* seroprevalence rate was reported to be as high as 72.8%. The prevalence rate reported by Abd Elhameed [(3)] among females aged 20-49 years was significantly higher than that of males of the same age; a percentage of 44.8 and 39.1, respectively. Exposure of females to *Toxoplasma gondii* cysts in uncooked meat during food preparation, and consumption of raw liver, and Mararra (raw viscera of herbivores) are suggested to be the attributable factors for the high prevalence rate of *Toxoplasma gondii* infection among females.

Another *Toxoplasma gondii* seroepidemiological study among pregnant women was made by Elnahas [(4)]. He detected an obvious increase in IgG seroprevalence status with low levels of education and socioeconomic status. The socio cultural habit of eating raw liver and marrara was found to be an important risk factor for acquiring *Toxoplasma gondii* infection. As cats are not popular pets among Sudanese populations, the study has shown no significant correlation between cat contact and transmission of *Toxoplasma gondii* infection. *Toxoplasma gondii* IgG seroprevalence was found to be not significantly correlated with women with history of miscarriage, preterm labor, low birth weight and congenital anomalies. He concluded that, serologically, toxoplasmosis is not uncommon among Sudanese pregnant women; and recommended screening and follow up programmes as well as health education.

Materials and methods:

Study area: Samples collection was carried out during the period June 2006-July 2007 at different obstetric and gynecology department in different hospitals in Khartoum state. A total of 455 out patients, were screened. Of this number, 230 were checked at Omdurman center which in located in Wad Nubawi, Omdurman and 120 were examined at the friendships hospital and 105 at Omdurman Saudi hospital.

Study population: The target group comprised women presented to the hospital with history of miscarriage and pregnant women at various stages of pregnancy who were attending the antennal clinic. Total of 455 samples were examined for presence of anti-*Toxoplasma* antibodies using direct latex agglutination test and 180 samples were examined by ELISA IgG, IgM and PCR test.

Ethical clearance: Ethical approval of the study taken from the Research Committees, Omdurman Islamic University.

Samples collection and preservation:

* Blood samples were collected from both aborted and normally delivered women using direct latex agglutination to screen *Toxoplasma*.

* 5ml of venous blood was collected from all the study and control individuals. Samples were

allowed to clot at room temperature and centrifuged of 3000 rpm to separate the sera. The sera were kept frozen at -20C° for ELISA test.

* Blood samples were also collected from aborted women for molecular studies.

All the tubes were labeled with the name of the patient and the date of collection.

All tests including ELISA test, DNA extraction, and genetic analysis were performed at the serology and molecular biology laboratory/ medical research center –faculty of medicine –El Neelin university, and medical research center – faculty of medicine- Ain Shams University. Cairo-Egypt.

Methodology: Three techniques were used in this study; these are:

1- Screening assays which was carried firstly as a direct latex agglutination test for detection of *antitoxoplasma* antibodies. The commercial kits produced by Spin-react, (Girona) Spain were used.

2- Enzyme linked Immuno Sorbent Assay (ELISA) was used for detection of IgG and IgM.

3- Polymerase chain reaction (PCR) was used for detection of affecting of toxoplasmosis among pregnant women. The primers used were B1F1 (5'GGAACTGCATCCGTTCAT GAG 3') B1R1 (5'TCTTTAAAGCGTTCGTGGTC3')(TIB-MOLBIOL -Berlin). The primers correspond to the *Toxoplasma gondii*. All PCRs were performed in Gene Amp ® PCR System 9700.

Statistical analysis: SPSS program was used to analyze the demographic and clinical data.

Results

Total population: The results showed that out of 455 women screened by latex agglutination test 72 (17.8%) were found to be sero-positive (table1).

Table 1: The prevalence rate of anti – Toxoplasma antibodies, in all study groups as examined by latex agglutination test:

Group tested	No. examined	No.+ ve	Percentage
Study	405	72	17.8%
Control	50	8	16.0%
Total	455	80	16.9%

Seropositivity in age groups: The percentage of seropositive cases detected by ELISA test ranged between 22 – 27% in women when ages were 15 to 35 years ,the percentage of seropositive cases in 35- 39 years women reached 15.4% .The rate in over 40 years was low as it did not exceed 10.0% (table 2).

Table 2: Percentage of seropositivity (S.P) and sero prevalence (S.R) rate by age group:

Age group in years	No .study groups	S.P (+ve)	S.R
15-25	43	9	21.1%
25-35	168	41	22.9%
35-39	13	2	15.4%
>40	10	1	10.0%
Total	230	53	26.1%

S.P=Seropositivity
S.R=Seroprevalence rate

Association between sero – prevalence status and obstetrical history:

Out of 104 recently aborted women, 22 (21.2%) showed positive anti-*Toxoplasma* antibodies by ELISA test. Out of 50 controls, the sero-positivity was 8 (16%) (Table3.12-Fig3.17). There was no significant difference between them (x 2= 9.43, P. value 0.0021)

Table 3: Comparison between prevalence of anti- *Toxoplasma* antibodies in women with history of miscarrage and control examined by ELISA test:

Group tested	**No. examined**	**No. +ve**	**Percentage**
miscarriage	104	22	21.2%
controls	50	8	16.0%
Total	154	30	19.5%

Association between Toxoplasmosis and pregnancy:

The prevalence rate of anti- *Toxaplama* antibodies was determined by ELISA test in pregnant women who had no previous history of miscarriage. Out of 51 pregnant women 24 (47.1%) were positive .In controls (non-pregnant women), 8(16%) out of 50 were found positive. There was no significant difference between the two groups (x = 2.25, p value 1.339) (table 4).

Table 4: The rate of anti- *Toxaplama* antibodies in pregnant women and controls obtained by ELISA test:

Group tested	**No. examined**	**No.+ve**	**Percentage**
Pregnant women	51	24	47.1%
Controls	50	8	16.0%
Total	101	32	31.7%

Previous history of congenital toxoplasmosis:

Women whose babies were born with congenital malformations in the study groups had sero-prevalence rate of 16 (32%) compared to others with no past history of malformation 3(6%) There is significant difference between them (x = 5.98, P. value = 0.1226) (table 5)**.**

Table 5: The rate of Anti-Toxoplasma antibodies in pervious history of congenital toxoplasmosis examined by ELISA test:

Previous history of congenital toxoplasmosis	No. examined	No.+ve	Percentage
Yes	50	16	32.0 %
No	50	3	6.0 %
total	100	19	19.0 %

Blood transfusion:

Table 6 shows the rate in women who were given blood transfusion. There was no significant difference between the study and control groups in relation to blood transfusion (x = 1.46, P. value = 0.69).

Table 6: Women with previous history of blood transfusion:

Blood transfusion	No. examined	No. +ve	Percentage
Yes	7	1	14.3%
controls	50	6	12.03%
total	57	7	12.3%

Direct latex agglutination test in pregnant women:

When pregnant women examined for anti-*Toxoplasma* antibodies by latex agglutination test, The results showed antibodies titers between 1/8 iu/l to 1/32 iu/l. The number of women who were positive at this range amount to 12 (16%) out of 51 examined (table 7). (X 2:3.533, P. Value: 0.171).

Table 7: Titer of anti-*Toxoplasma* antibodies by latex agglutination test in pregnant women:

Titers	No. examined	No. +ve	Percentage
1\8	4	1	10.0 %
1\16	12	3	44.0 %
1\32	19	8	11.0 %
1\64	16	0	0.0 %
Total	51	12	16.0 %

Table 8: Seropositive of anti-*Toxoplasma* antibodies by latex agglutination test in pregnant women and control:

Group test	No. examined	No. +ve	Percentage
Pregnant women	76	12	15.8%
Control	40	8	20.0 %
Total	116	20	17.3%

Antibody titers by latex agglutination test in aborted women:

Out of 104 examined women 18(17.3%) were with history of miscarriage. The antibodies titers ranged between 1/8 to 1/64. In non–aborted controls, the percentage was 8% showing no significant difference between titers in aborted and non-aborted women (X 2: 6.62, P. Value: 0.0850) (table 9).

Table 9: Titer of anti- *Toxaplama* antibodies examined by latex agglutination test in women with history of miscarriage:

Titers	No. examined	No. +ve	Percentage
1\8	22	8	44.0 %
1\16	37	6	77.0 %
1\32	29	3	3.8 %
1\64	16	1	1.3 %
total	104	18	17.3 %

IgG level determined by ELISA:

The results showed that out of 180 women tested by ELISA, 70 (39.0%) were sero-positive .As for controls, out of 50 women, 16(32%) were found positive (table 10). There is no significant difference in sero-positivity rate between the two groups (X 2: 0.793, P. value: 0.373).

Table 10: IgG level by ELISA among study and control groups:

Group	No. examined	No. +ve	Percentage
Study group	180	70	39.0%
Controls	50	16	32.0%
Total	230	86	37.4%

IgM antibodies by ELISA in examined women:

Out of 70 examined women, 9 (12.9%) were found positive compared to 4(10.0%) out of 40 controls (table 11). There was no significant difference between the two groups (X 2: 0.190, P. value: 0.656).

Table 11: Sero- positive status and ELISA IgM among study and control groups:

Group	No. examined	No. +ve	Percentage
Study group	70	9	12.9%
Controls	40	4	10.0 %
Total	110	13	11.8 %

Polymerase chain reaction (PCR):

Application of PCR test in the study group revealed 30 (16.7%) positive cases, while 50 (18%) women were positive in the control group (table 12). No significant difference was found between the examined and controls (x2= .049.P.value = 0.825).

Table 12: Positive cases by PCR among examined women.

Group	No. examined	No. +ve	Percentage
Study group	180	30	16.7%
Controls	50	9	18.0%
Total	230	39	16.7%

Association between direct latex agglutination and PCR test in the study groups:

In study group (table 13), out of 26 women positive examined by direct latex agglutination test 21 (80.8%) were positive whereas (19.2) were negative by PCR test .Out 18(100%) were negative by latex agglutination test were negative by PCR the difference statistically was highly significant (X 2: 3.905 P. value: 0.049).

Table 13: Association between direct latex agglutination and PCR test in study

Test	**No. examined**	**No. +ve**	**Percentage**
DLA	26	21	80.8%
PCR	18	18	100.0%

DLA=Direct latex agglutination

Association between ELISA IgG in aborted and PCR test in study group:

59.1% (13 out of 22 aborted women positive by ELISA IgM) were PCR positive whereas 9 (41%) were negative by PCR test all the 82 aborted women negative by ELISA. IgG test were negative by PCR test statistically there was highly difference significant between ELISA and PCR test (X 2: 36.723, P. Value: 0.001) (table 14).

Table 14: Association between ELISA IgG in women with history of miscarrage and PCR test in study group :

	ELISA I gG Ser (+Ve)		ELISA I gG Ser(-Ve)	
	N0	%	N0	%
positive	13	59.1%	82	100.0%
negative	9	41.0%	0	0.0%
Total	22	100.0%	82	100.0%

Association between ELISA IgG test and PCR test examined in pregnant women:

Table 15 shows the association between ELISA IgG as examined in pregnant women and PCR test, 18(64.3%) were positive, whereas 10 (35.7%) were negative by PCR test .All the 37 women negative by PCR test, were negative by ELISA test. Statistically there was significant difference between ELISA and PCR test. (X 2 : 7.12,P. Value: 0.0676).

Table 15: Association between ELISA IgG test and PCR test as examined in pregnant women:

	ELISA I gG. Ser +Ve		ELISA I Gg. Ser –Ve	
	N0	%	N0	%
positive	18	64.3%	37	100.0%
negative	10	35.7%	0	0.0%
Total	28	100.0%	37	100.0%

Association between ELISA IgM and PCR test in study group:

In study group (Table 16), 18 out of the 61 women negative by ELISA IgM test were positive by PCR test (29.5), and 70.5% were negative (43 out of 61) .All the 9 positive women by ELISA IgM were positive by PCR test. Statistically there was significant different between them. (X 2: 16.448-P. Value: 0.001).

Table 16: Association between ELISA IgM and PCR test in study group:

	I gM sero- positive		I gM sero- negative	
	N0	%	N0	%
positive	9	100.0 %	18	29.5%
negative	0	0.0 %	43	70.5%
Total	9	100.0 %	61	100.0%

Association between ELISA and PCR tests:

When the rate of anti-*Toxoplasma* antibodies by IgG were tested in all study groups, 38.9% were positive for IgG by ELISA test, and 16.6% were positive by PCR test (table 17). There was statistically significant difference between ELISA and PCR (X 2: 22.15, P. value: 0.001).

Table 17: Comparison of the prevalence rate of anti-Toxoplasma antibodies examined by ELISA and PCR .Tests in all study groups.

Group tested	**No. examined**	**No. + ve**	**Percentage**
ELISA	180	70	38.9%
PCR	180	30	16.6%
Total	360	100	27.8%

Discussion:

The proportion of women at risk of acquiring the infection during pregnancy in many countries, including Sudan, is not well known. Primary infection with *Toxoplasma* during pregnancy may lead to severe complications, if not fatal infection of the foetus [(5)].Therefore, emphasis is placed on preventive measures and early diagnosis of the infection to prevent these severe complications. The ideal situation for the diagnosis of *Toxoplasma gondii* infection in pregnancy of having antibody –negative serum sample collected at the very begging of pregnancy or preferably before conception is usually not possible [(6)]. In Sudan, as in some other countries such as United States, testing for antibodies to *Toxoplasma* in pregnancy is performed in only suspected cases.

In the present study, although the prevalence rate was higher by latex agglutination test and ELISA in the aborted women compared to normally pregnant women, there was no significant difference between the two groups, who also found that women with history of miscarriage was not correlated to *Toxoplasma gondii* seroprevelance in Sudan. Elnhas [(4)] found in a study carried out in Khartoum, Sudan prevalence rates of 35.4% and 34% among women with past history of miscarriage and others with no past history, with no significant difference between them. Compared to some countries, in Saudi Arabia, the prevalence of *Toxoplasma gondii* antibodies was not found to be associated with women with history of miscarriage [(7)]. El.Ridi et al [(8)] reported that in Egypt there was no correlation between *Toxoplasma gondii* seropositivity rate and women with history of miscarriage. The present finding was also in accordance with those of Abdel-Hafez [(9)] and Qublan et al [(10)] who found no correlation between *Toxoplasma gondii* sero-prevalence and women with history of miscarriage in Amman-Jordan. The present results were also similar to

those of Ashfunnessa [11] who reported that *Toxoplasma gondii* seropositivity was not correlated to recurrent fatal loss among pregnant women from Bangladesh.

Despite the variations in percentages of infections examined by the foemtioned authors which may be attributed to some epidemiological factors, sample sizes and different techniques, our results point out to the fact that the diseases in aimilocaly common in susceptible individuals including pregnant ladies and that pregnancy does not initiate nor trigger infections.

The results of the present study agree with those Elnahas [4] who found that *Toxoplasma gondii* sero-prevalence gradually increased by age but he did find not statistically significant correlation between the under 30 versus the over 30 years old.

In contrast, Sahawi et al [12] showed that there was correlation between seropositivity in pregnancy.

The role of toxoplasmosis in women with history of miscarriage is still unsettled [13]. There are conflicting data concerning its relationship to habitual or sporadic women with history of miscarriage. Women with previous history of miscarriage in the study group showed *Toxoplasma gondii* sero- prevalence rate of 21.2% compared to 27.4% among others with no past history of miscarriage with no significant difference between them (P value=0.21).

In the control group, 17% were the sero-prevalence rates found in women with previous history of miscarriage. Statistically, the difference between them was also not significant.

Several studies found no association between *Toxoplasma gondii* sero-prevalence and history of miscarriage [10, 12, and 13]. Similarly result of Al Hindy [14] and Elnahas [4] in Khartoum showed no strong association between toxoplasmosis and women with history of miscarriage. In contrast, a study involving 5000 patients, made by Kimble et al [15], concluded that toxoplasmosis may be the cause for women with history of miscarriage and will vary in cultural habits and geographical areas. Their results suggested but did not prove a causative relationship with infection.

The highest *Toxoplasma gondii* sero- prevalence rate in the study group was shown by women past history of having babies with congenital malformations than others with no past history of malformations (30% and 6%) respectively. Statistically, the difference between them was insignificant (P=0.1226). Significant correlation between *Toxoplasma gondii* antibodies sero-prevalence and past history of congenital malformations was observed in the control group as well. Similar findings were reported by Maha [16] who found significant correlation between *Toxoplasma gondii* antibodies and past history of congenital malformations. These results agreed with those of Elfakahani et al [17] who found 60% among mothers who delivered congenitally abnormal babies using PCR test. Many studies showed that congenital disease can be caused by acquired as well as congenital *toxoplasmosis* [18, 19].

The present results showed that 14.3% of women were positive for the blood transfusion compared to 12% of the control with no significant difference between them.

It is well know that *Toxoplasma gondii* may cause serious toxoplasmosis with clinical manifestations when the host in immune - compromised. The infection can be transmitted through blood and some cases of post – transfusion. Similar study in china showed that 4.86% should be expected in the population of blood donors [(20)].

Application of PCR test on all study groups, showed similar positivists in pregnant women and control groups. No statistical significant difference was found between the groups examined.

Compared with direct latex agglutination test in the study group, percentage of positive women was 80.7%, whereas, 5% (19.23%) were negative by PCR test and out of 43 negative by direct latex agglutination, 34.8% were positive by PCR test and 65.2% were negative by PCR test. Statistically, the difference was highly significant (p value= 0.049).

When immunoglobulis were titrated by ELISA, the results revealed that the level of IgG in the study and controls groups was similar and there were no statistical differences between them. Similarly, it also indicated that the level of IgM was similar in both groups. Since there is no increase in antibody titers, it seems reasonable to conclude that there appears to be no recent infections among the studied pregnant women or that the infections may be sub patient to an extent that antibody level is maintained in low titers.

When these IgM results were related to the PCR findings it was found that all the samples (100%) positive by IgM in the study group were positive by PCR (9 out of 9), compared with none in the control group. Previous studies confirmed that the PCR could actually detect *Toxoplasma gondii* in blood samples of women before or during pregnancy [(21)]. This may be due to the fact that *Toxoplasma* DNA may not be cleared soon from the blood of patients with acute *Toxoplasmic*. Based on this, the presence of *Toxoplasma* DNA in the maternal blood most probably indicated a recent infection or an indicator of apparent parasitaemia, which likely to be clinically significant. Other explanation was that, serological test may have limitation; they may fail to detected specific *Toxoplasma* IgG or IgM antibodies during the active please of *Toxoplasma gondii* infection [(22)].

When IgG was negative, the IgM could be positive become IgM antibodies appear earlier than IgG and they stay for a short time [(23)]. Comparing the IgM results with others should be taken with caution because generally; positive IgM is either an indication of recent infection or might be a false positive result [(24, 25)]. This might also be due to generation of false positive PCR results which commonly occur during DNA processing and PCR reaction. The laboratory contamination of the samples can not be completely present even when different stages of the PCR reaction procedure are carried out in separate rooms and the carry over contamination is controlled [(26)].

However, a negative PCR result dose not exclude recent infections because the sensitivity of the PCR, in which a single trophozoite can be detected in clinical sample has potential problems for some types of specimen and become the exact kinetics of parasitaemia in infected people are not well known .The short duration of parasitaemia, or if the numbers of the trophozites circulating in peripheral blood are low, could lead to a sampling error that will produce false negative result in such cases. The sensitivity of the PCR was found to be significantly higher for maternal infections that infections that occurred between 17 and 21 weeks gestation ($p<0.02$) when the amniotic fluid was tested [(27)]. If the some applies to blood samples, further studies are needed.

A pervious study of serial blood samples from acutely infected pregnant women indicated that in the presence of *toxoplasmosis* specific IgG and IgM antibodies, and additional presence of high direct latex agglutination test titer were insufficient criteria for identifying *Toxoplasma* infection in early pregnancy because some acute infections will not be detected [(28)].

Conversely, some women will be falsely identified was being infected [(25)], and undergo unnecessary diagnostic amniocentesis and anti-parasitic treatment [(6)].

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Original Article

Sudan Medical Laboratory Journal

Anti bacterial activity of *Psidium guajava* and *carthamus tinctoruis* against respiratory tract infection

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Abstract:

The aim of this study was to enrich the information about the antimicrobial activity of some medicinal plants of the Sudan. Also, to verify the claimed activity of certain Sudanese medicinal plants used in traditional medicine as antimicrobial agents, to subject these plants for further antimicrobial studies against clinical isolates, to determine their minimum inhibitory concentration (MICs) and to compare their activity with the commonly used antibacterial agents in the Sudan. The chloroform, methanol and aqueous extract of *Psidium guajava* leaves (family: myrtacae) and *Carthaumus tinctorius* seeds (family: Asterceae) were screened for their antimicrobial activity against four standard bacteria: two gram positive (*Staphylococcus aureus* and *Streptococcus pyogenes*) and two gram negative *(Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) using the cup plate agar diffusion method. All six extracts were active against one or more of the four bacteria. Four of these extracts inhibited the growth of all microorganisms. *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were the most sensitive organisms to all extracts, while *Streptococcus pyogene*s showed less susceptibility. The antibacterial activity of five reference drugs was determined against the tested four bacteria and their activity was compared to the activity of plants extracts.

Introduction:

Antibacterial activity is a substance that kills or inhibits the growth of bacteria. Some plants have been investigated scientifically for antimicrobial activity and as a large numbers of plant products have been shown to inhibit the growth of pathogenic microorganisms. Recently, works in medical field returned back to nature particularly in the use of medical plant to treat human ailments [(1)].

Some plants are known as medicinal because they contain active substances that cause certain reactions that cure diseases of the human [(2)]. Many countries use plants in treatment of diseases as in China [(3)], South America [(4)] and Iran [(5)]. In African and other developing countries, traditional medicines from plants continue to form the basis of rural medicinal care because traditional medicines are easily available and cheap [(1)]. The number of medicinal plants around the world range between 250,000 to 300,000 and more than two third of this plant growing in developing countries [(6)]. Almagaboul *et al* [(7)] investigated 40 extracts belonging to 18 families from Sudan for antibacterial activity against one or more microorganisms. Ahmed *et al* [(8)] tested antibacterial activity of *Julifterinean* (alkaloid isolated from *Prosopis juliflora)* invitro against six Gram negative and ten Gram positive bacteria at comparable concentration of penicillin, streptomycin, erythromycin, sulphamethoxazole, ampicillin and tetracycline. Abdelrahim, *et al* [(9)] and

Verma, *et al* [10] also reported a microbial effect of *guava* sprout extract upon many bacterial species.

Different extracts from traditional plants have been tested. Many report showed the effectiveness of traditional herbs against microorganisms [11].

The chemotherapeutic index compares the maximum dose that can be tolerated by the host without causing death, with minimum dose that cures the particular infection [12].

Antimicrobial activity is measured in vitro [13] in order to determine:

a. The potency of an antimicrobial agent in solution.
b. The sensitivity of given microorganism to known concentration of the drug.

Determination of these quantities may be undertaken by one method (dilution method) [14, 15, 16].

The aim of agar dilution method is to determine the lowest concentration of assayed antimicrobial agent (minimum inhibitory concentration (MIC)) that, under defined test condition, inhibits the visible growth of the bacterium being investigated. MIC values are used to determine susceptibilities of bacteria to drugs and also to evaluate the activity of new antimicrobial agent.

In this method, the medium is inoculated with test organism and the samples to be tested are mixed. The zones of inhibition results are dependent upon both the diffusibility of the agent in the medium and the degree of susceptibility of the organism. The speed of growth and the size of inoculums can influence to a marked degree the size of inhibitory zones.

The common bacteria causing respiratory infections are *staphylococcus aureus, streptococcus pyogenes, klebsiella pneumoniae* and *pseudomonas aeruginosa* [17].

Materials and methods:

Chemicals and regents:

- Alcohol, hydrogen peroxide, crystal violet, distill water, immersion oil, lugol's iodine, methanol, methyl red, para- dimethyl-aminobenzaldehyde (Kovac's reagent), petroleum ether, suffranine.

Culture media: Blood base agar, DNase media, kliger iron agar, Koser's citrate, MacConkey agar, mulltor hintton agar, nutrient agar, nutrient broth, urea agar.

Medicinal plants extracts:

- *Carthamus tinctoius*
- *Psidium guajava.*

Bacterial Microorganism:

- *Klebsiella pneumonia* ATCC53657 (Gram –ve bacteria).
- *Pseduomonas aeruginosa* ATCC27853 (Gram –ve bacteria).
- *Staphylococcus aureus* ATCC25923 (Gram+ ve bacteria).
- *Streptococcus pyogenes* ATCC26722 (Gram+ ve bacteria).
- American type culture collection (ATCC) Rockville, Maryland,USA.

Chemotherapeutic agents: amoclan, ampcillin, ciprofloxacin, erythromycin, gentamycin.

Methods:

Plants material:

The two plants used in this study were collected from Gazira state (*psidium guajava* from Al.

Kasamber, *Carthamus tinctoirus* from Al-Sharafa Elbaher).

Hundred grams of each plant sample were powdered by grinder and extracted as described below and then subjected to antimicrobial activity screening.

Preparation of crude extract: [19]

Each of the coarsely powdered plant material (100g) was extracted for 4 hours with petroleum ether in soxhlet apparatus. The petroleum ether extract was filtered and evaporated under reduced pressure using Rota- vapor. The extracted plant material was then air- dried, repacked in the soxhlet and exhaustively extracted with methanol. The methodic extract was filtered and evaporated under reduced pressure again using Rota- vapor. Each residue was weighted and the yield percentage was determined. The petroleum ether residue (1.6g) was dissolved or suspended in petroleum (16ml) to a final volume (16ml) conc. (10mg/ml). The methanol residue (1.6g) was dissolved in methanol (16ml) conc. (10mg/ml), and kept in refrigerator until used.

Aqueous extract for each dried ground plant (10g) was prepared by infusion using boiled distilled water. It was allowed to soak for 2 hours, then, it was filtered, and 1 ml taken from the residue was then dried and weighted and the yield percent age was obtained. The final volume of the residue was used immediately.

Preparation of bacterial suspensions:

The test organisms were aseptically inoculated in broth media and incubated at 37ºC for 24 hours. From this media, 1ml was taken and distributed on nutrient agar slopes, and incubated again at 37ºC for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8- 10^9 colony forming units per ml (C.F.U/ml). The suspension was stored in the refrigerator at 4ºC.

In vitro testing for antibacterial Activity:

The cup- plate agar diffusion method [16] was adopted to assess the antibacterial activity of the prepared extracts. One ml of the standardized bacterial stock suspension 10^8- 10^9 (C. F. U/ml) was thoroughly mixed with 100ml of Mullor hintton agar which was maintained at 45, 20 ml aliquots of the inoculated Mullor hintton agar. They were distributed into sterile plates.

The agar was left to set and in each of these plates, 4 cups (10 mm in diameter) was cut using a sterile cork borer (No.4) and agar discs were removed. Alternate cups were filled with 0.1 ml samples of each of the extracts using automatic microlitre pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for 18 hours.

Two replicates were carried out for each extract against each of the test organisms. After incubation, the diameters of the result growth inhibition zones were measured, averaged and the mean values were tabulated.

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Results:

Screening for antimicrobial activity of *Psidium guajava* leaves and *Carthamus tinctoirus* seeds:

In the preliminary screening for antibacterial activity of two Sudanese medicinal plants, belonging to two families, the total number of extracts examined against the tested organism (standard) was six; four of these extracts exhibited inhibitory activity against one or more of four tested bacteria. The two other extracts were devoid of any activity.

Four extracts exhibited inhibitory effects against both gram positive organisms (*S. aureus and S. pyogenes*) and gram negative organisms (*P. aeruginosa and K. pneumoniae*), but *S. aureus* was the most sensitive organism being inhibited by four extracts, also *Pseudomonas aeruginosa* was more susceptible whereby *Streptococcus pyogenes* and *Klebsiella pneumoniae*) were less susceptible (table 1). One of two extracts of petroleum ether showed no activity against all tested organisms. Whereas, all methanol extracts exhibited inhibitory effect against all tested organisms. Meanwhile, one out of two aqueous extracts examined for antibacterial activity was inhibitory to all tested organisms. It is evident that methanolic extract exhibited the highest level of inhibitory effect against the bacteria tested, it could be due to the presence of polar compounds, two methanolic extract exhibited inhibitory effect against the four tested bacteria (table 1).

The *Psidium guajava* leaves extract provided high activity against *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *S. aureus*. Whereby, this extracts showed moderate activity against *S. pyogenes* (table 2). Figure 1 shows activity of petroleum ether extract of *Psidium guajava* leaves extracts against standard organisms. In contrast, *Carthamus tinctorius* seeds extracts provided no activity against all standard organisms used in this study (table 3). Whereas, reference antibacterial drugs used against standard organisms provided various results. Ciprofloxacin produced activity against all standard organisms, whilst ampicillin provided no activity against any one of all standard organisms (table 4).

Figure 1: activity of petroleum ether extract of *psidium guajava* leaves extracts against standard organisms:

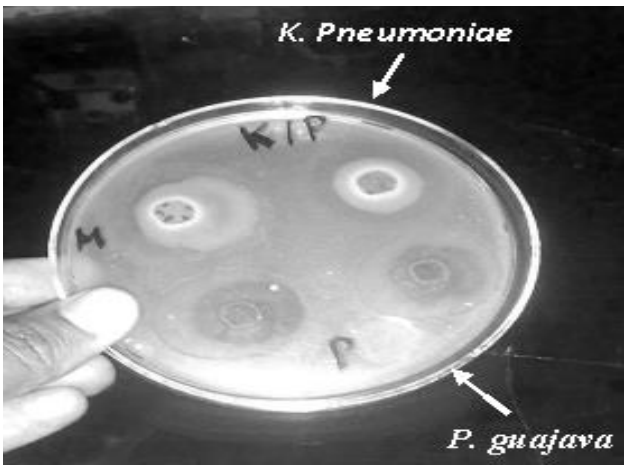

Table 1: Antibacterial activity of two plants extracts against the standard organisms:

Family/ Botanical/ vernacular names	Part used	Yield %	Solvent	Test organisms used MDIZ mm			
				S.aureus	***S.pyogenes***	***K.pneumoiae***	***P.aeruginosa***
Myrtaceae	Ieaves	2.099	$CHCl_3$	22	14	22.5	22.5
Psidium guajava		15.258	MeOH	25.5	14.5	18.5	22.5
(guava)		0.05	H_2O	12.5	14.5	18.5	20
Asteraceae	Seeds	7.273	$CH\ Cl_3$	-	-	-	-
Carthamustinctorius		21.578	MeOh	16	11.5	14.5	16.5
Osfer		0.035	H_2O	-	-	-	-

$CHCl_3$ = chloroform
MeOH = Methanol
H_2O = water
MDIZ = Mean diameter of growth inhibition zone in (mm) average of (2) replicates.
MDIZ > 18 Sensitive, 14- 18 Moderate, 14 resistant, (-) No activity

Table 2: The activity of *Psidium guajava* leaves extract against standard organisms:

Standard organism	Solvent	No. of standard organism		
		Sensitive	**Intermediate**	**Resistant**
S .aureus	$CHCl_3$	1	0	0
	MeOH	1	0	0
	H_2O	0	0	1
S. Pyogenes	$CHCl_3$	0	1	0
	MeOH	0	1	0
	H_2O	0	1	0
K. pneumeniae	$CHCl_3$	1	0	0
	MeOH	1	0	0
	H_2O	1	0	0
P. aurginosa	$CHCl_3$	1	0	0
	MeOH	1	0	0
	H_2O	1	0	0

Sensitive organism exhibit inhibition zone > 18 mm
Concentration used = 10 mg/ml at 0.1 ml/cup
MeOH = Methanol
H_2O = water
$CHCl_3$ = chloroform

Table 3: The activity of *Carthamus tinctorius* seeds extract against standard organisms:

Standard organism	Solvent	No. of standard organism		
		Sensitive	**Intermediate**	**Resistant**
S. aureus	$CHCl_3$	0	0	1
	MeOH	0	1	0
	H_2O	0	0	1
S. Pyogenes	$CHCl_3$	0	0	1
	MeOH	0	0	1
	H_2O	0	0	1
K. pneumeniae	$CHCl_3$	0	0	1
	MeOH	0	1	0
	H_2O	0	0	1
P. aurginosa	$CHCl_3$	0	0	1
	MeOH	0	1	0
	H_2O	0	0	1

Sensitive organism exhibit inhibition zone > 18 mm
Concentration used = 10 mg/ml at 0.1 ml/cup

MeOH = Methanol
H_2O = water
$CHCl_3$ = chloroform

Table 4: Antibacterial activity of reference drugs against standard organisms

Drugs	**Concentration used mg/ ml**	**Standard organisms used MDIZ mm**			
		S.aureus	***S.pyogenes***	***K.pneumoniae***	***P.aeruginosa***
Ampicillin	40	-	-	-	-
	20	-	-	-	-
	10	-	-	-	-
	5	-	-	-	-
Ciprofloxacin	40	24	35	25	34
	20	21	35	25	32
	10	20	30	24	26
	5	19	30	20	20
Erythromycin	40	-	11	-	-
	20	-	11	-	-
	10	-	11	-	-
	5	-	11	-	-
Amoclan	40	13	11	14	15
	20	-	-	13	14
	10	-	-	12	-
	5	-	-	11	-
Gentamycin	40	15	25	17	26
	20	12	23	15	23
	10	-	15	12	12
	5	-	15	11	-

Discussion:

In this study, the petroleum ether leaves extract of *Psidium guajava* gave high activity against *Staphylococcus aureus, Klebsiella pneumonia* and *Pseudomonas aeruginosa*, and Moderate activity against *Streptococcus pyogenes*. This study agreed with Abdelraheem *et al* [(9)] and Almagboul *et al* [(5)].

The methanolic leaves extract of *Psidium guajava* gave high activity against *Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, and moderate activity against *Streptococcus pyogenes*. This result was in line with Abdelraheem *et al* [(9)], Almagboul *et al* [(5)] and Verma *et al* [(10)].

The aqueous leaves extract of *Psidium guajava* exhibited high activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa,* and moderate activity against *Streptococcus pyogenes* and low activity against *Staphylococcus aureus*. This finding agreed with the finding of Abdelraheem *et al* [(9)], Almagboul *et al* [(5)] and Holets *et al* [(4)].

The petroleum ether seeds extract of *Carthamus tinctorius* exhibited no activity against all four organisms. Meanwhile, the methanolic seeds extract of *Carthamus tinctorius* exhibited moderate activity against *staphylococcus aureus, klebsiella pneuomoniae* and *pseudomonas aeruginosa,* and low activity against *streptococcus pyogenes.*

The aqueous seeds extract of *Carthamus tinctorius* showed no activity against all standard organisms.

The petroleum ether extract of *Psidium guajava* leaves inhibited *staphylococcu saureus* higher than 20 mg/ml of ciprofloxacin, and inhibited *Klebsiella pneumoniae* higher than 5 mg/ml of ciprofloxacin and *Pseudomonas aeruginosa* inhibited higher than 5mg/ml of ciprofloxacin. In contrast, it inhibited *Streptococcus pyogenes* lower than 5 mg/ml of ciprofloxacin.

The petroleum ether extract of *Psidium gujava* leaves inhibited all four standard organisms higher than 40 mg/ml of amoclan. Moreover, this extract of *Psidium guajava* leaves inhibited *Staphylococcus aureus* higher than 40mg/ml of gentamycin, adding to that it inhibited *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* higher than 10mg/ml of gentamycin, whereby, it inhibited *Streptococcus pyogenes* lower than 5ml/ml of gentamycin.

On the other side, methanolic extract of *Psidium guajava* leaves inhibited *Staphylococcus aureus* higher than 40mg/ml of ciprofoloxacin, amoclan and gentamycin, inhibited *Streptococcus pyogenes* higher than 40 mg/ml of amoclan and lower than 5 mg/ml of gentamycin and ciprofloxacin. Also, this extract inhibited *Klebsiella pneumoniae* higher than 40 mg/ml of amoclan and gentamycin, and lower than 5 mg/ml of ciprofloxacin. Moreover, it inhibited *Pseudomonas acerugionsa* higher than 40 mg/ml of amoclan, higher than 10mg/ml of gentamycin, and lower than 5 mg/ml of ciprofloxacin.

The aqueous extract of *Psidium guajava* leaves inhibited *Staphylococcus aureus* higher than 10 mg/ml of gentamycin and amoclan, and lower than 5 mg/ml of ciprofloxacin, inhibited *Streptococcus pyogenes* higher than 10 mg/ml of amoclan, and lower than 10 mg/ml of gentamycin and ciprofloxacin and inhibited *Klebsiella pneumoniae* higher than 10 mg/ml of gentamycin and amoclan, and lower than 5mg/ml of ciprofloxacin. This extract inhibited *Pseudomonas aerugionsa* equal to 5 mg/ml of ciprofloxacin, and higher than 10 mg/ml of amoclan and gentamycin.

The petroleum ether extract of *Carthamus tinctorius* seeds inhibited all tested standard organisms lower than 5 mg/ml of ciprofloxacin, amoclan and gentamycin. Whereas, methanolic extract of *Carthamus tinctorius* seeds inhibited *Staphylococcus aureus* higher than 40 mg/ml of amoclan and gentomycin, and lower than 5 mg/ml of Ciprofloxacin, inhibited *Streptococcus pyogenes* higher than 40 mg/ml of amoclan, lower than 10 mg/ml of gentamycin and lower than 5 mg/ml of ciprofloxacin, inhibited *Klebsiella pneumoniae* higher than 40 mg/ml of amoclan, and gentamycin, and lower than 5 mg/ml of ciprofloxacin, inhibited *Pseudomonas aeruginosa* higher than 40mg/ml of amoclan, higher than 10 mg/ml of gentamycin and lower than 5 mg/ml of ciprofloxacin.

The aqueous extract of *Carthamus tinctoruis* seeds inhibited all tested standard organisms lower than 5mg/ml of ciprofloxacin, amoclan and gentamycin.

In this study, the ampicillin and erythramycin had no activity against four standard organisms.

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Original Article

Sudan Medical Laboratory Journal

Potential of faba bean and tomato extracts as microbiological culture media

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Abstract:

This study was undertaken to investigate and assess the potentiality of some plant materials (tomato and faba bean) as sources of nutrient for cultivation of bacteria. The pH values of these plant extracts were determined and adjusted to 7.4. Tomato and faba bean (dry and soaked) were used as culture media for *Kiebsiella sp*. and *Staphylococcus aureus*.

Viable counts of *Klebsielia sp*. on different plant extracts were similar to that on the nutrient agar (control). Culture medium prepared from tomato extract supported growth of *Staphylococcus sp*. where the faba bean medium was unable to support the growth of this organism.

Introduction:

Generally microorganisms need various nutrients to maintain growth, multiplication and reproduction. The most important ingredients of solid media include water; in fact approximately 80% of the living cells of bacteria are water (1).

Plant media used as modified culture media for the microorganism growth:

Davis (2) compared the morphology of lactobacillus by growing on oxid tomato juice agar and other media. Atles (3) used tomato at meal past agar for cultivation of flexi bacteria species.

Another study by Mohammed (4) investigated degradation of microbes of ten fungal and 70 different bacterial isolates using Gum Arabic.

This study refine the ability of bacteria to grow on Gum Arabic but grow the lead to the drop of pH values due to production of organic acid while the media became alkaline in case of the fungal isolate with the production of ethanol.

Osman (5) using twenty plants extracts as culture media for bacteria and the fungi. The results indicated that the viable count of some bacteria of some plant extracts were larger or similar when they are compared with those produced on the control nutrient agar and blood agar. The results of the survival period of both staphylococcus sp and *Kiebsielia sp* extended for several weeks at 37°C and for long time at 4°C.

Plant materials used as microbial culture media in this study:

Tomato (*Lycopersicones culentuin mill*): is one of most important vegetable in Sudan the dry seeds contain (in 100 g of food product) 4.0 g crude fibers, 55g carbohydrate, 0.6 g fat, 24.0 g crude protein, 60mg calcium, 32 mg phosphorous, 3.0 g ash, and 7 mg Fe^{++} (6).

Faba bean (*Vicia faba*): one of the most main source of protein in diets for Sudanese and most of third world nation, it is rich in calcium, phosphorus, zinc and lysine (7) and it is deficient in sulphur containing amino acid. The dry seed was found to contain about 28% protein, 3% fat,

2% glucose, 48% carbohydrates, 3% minerals salts [6].

The objective of this study was development and evaluation of culture media derived from plant products (tomato and faba bean) because the culture media solid as dehydrated preparation, have become very expensive in the local market and in the most instances are not available.

Materials and Methods:

Collection of samples:

Tomatoes and faba beans purchased from local market of Khartoum State, so as to be used as culture media for bacterial growth.

Bacterial growth on the different extracts:

Klebsiella sp and *Staphylococcus aureus* strains were obtained from Faculty of Basic Medical Science, Omdurman Islamic University.

Procedure for plant materials extracts [8]:

Specific amount of each plant sample was weighted. The samples were mixed with distilled water in 500 ml conical flask. Then the mixtures were boiled in an autoclave at 121°C for 15 minutes. The plant extract were removed from the autoclave and allowed to cool, then filtered through sterile gauze. Then the volume was completed to one liter by adding distilled water. These extracts were stored at 4°C. 20 g of purified agar (agarose) were added to one liter of the above extract.

The pH of these extracts was adjusted to 7.4 by pH meter using alkaline solution (NaOH) and acidic solution (HCl). Then after autoclaving, the extracts were cooled to 45°C and distributed into disposable Petri dishes (about 17 ml for each).

Preparation of nutrient agar media [8]:

Twenty grams of agar were added to 1000 ml nutrient broth components, and then allowed to dissolve in a water bath. The pH was adjusted using pH meter to 7.4. Then autoclaving at 121°C for 15 minutes allowed to col and distributed into disposable Petri dishes in about 17 ml for each.

Preparation of inoculums: A loopful of over night pure culture of each strain was taken and dipped into a pure sterile nutrient broth 5 ml, incubated at 37°C for 24 hours, smeared and stained before use.

Viable count on different plant extracts:

Serial dilution, of *Klebsiella sp*. and *Staphylococcus aureus* were made, according to surface viable counts method the oven-dried nutrient agar and plant extracts agar Petri dishes ,were divided into two half, on each half one drop (0.02 ml) from the diluted culture was spread. Then the plates were incubated aerobically at 37°C for 24 hours. The duplicate plates were counted according to the average method [9].

Results:

Growth of *Staphylococcus aureus*:

The tomato extract media supported the *Staphylococcus aureus* growth but the media prepared from faba bean (A and B) failed in supporting the *Staphylococcus aureus* growth as in table 1.

Growth of *Klebsiella sp.*:

Referring to table 2 and figure 2 the growth of *Klebsiella* sp. on tomato and faba beans (A and B) was similar to that on the nutrient agar (control).

The colony size of *Klebsiella sp*. on the plant media were smaller than that on nutrient agar, but

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the colony size of *Staphylococcus aureus* on the plant media were very small than that on nutrient agar

Table (1): The Growth Rate *of Staphylococcus aureus* on different plant media (log_{10} of cfu/ml) and nutrient agar**:**

Media Source	Viable Count	Colony	
		Size (mm)	Morphology
Tomato	11.71180723	(0.4 - 0.6)	Circular smooth raised glistening golden yellow
Nutrient Agar (control)	11.72427587	(2 — 3)	Circular smooth flat opaque gray white
Faba bean(A)*	No growth	No growth	-
Faba bean(B)**	No growth	No growth	-

* dry
** soaked

Table (2): The Growth Rate of *Kiebsiella sp* on different plant media (log_{10} of cfu/ml) and nutrient agar:

Media Source	Viable Count	Colony	
		Size (mm)	Morphology
Tomato	11.05115252	(3-6)	Circular large raised entire glistening mucoid gra
Nutrient Agar (control)	11.00	(1—3)	Circular large flat very mucoid light pink
Faba bean(A)*	10.82930377	(1—4)	Circular large raised mucoid light pink
Faba bean(B)**	11.09691001	(3 —7)	Circular very large raised mucoid

* dry
** soaked

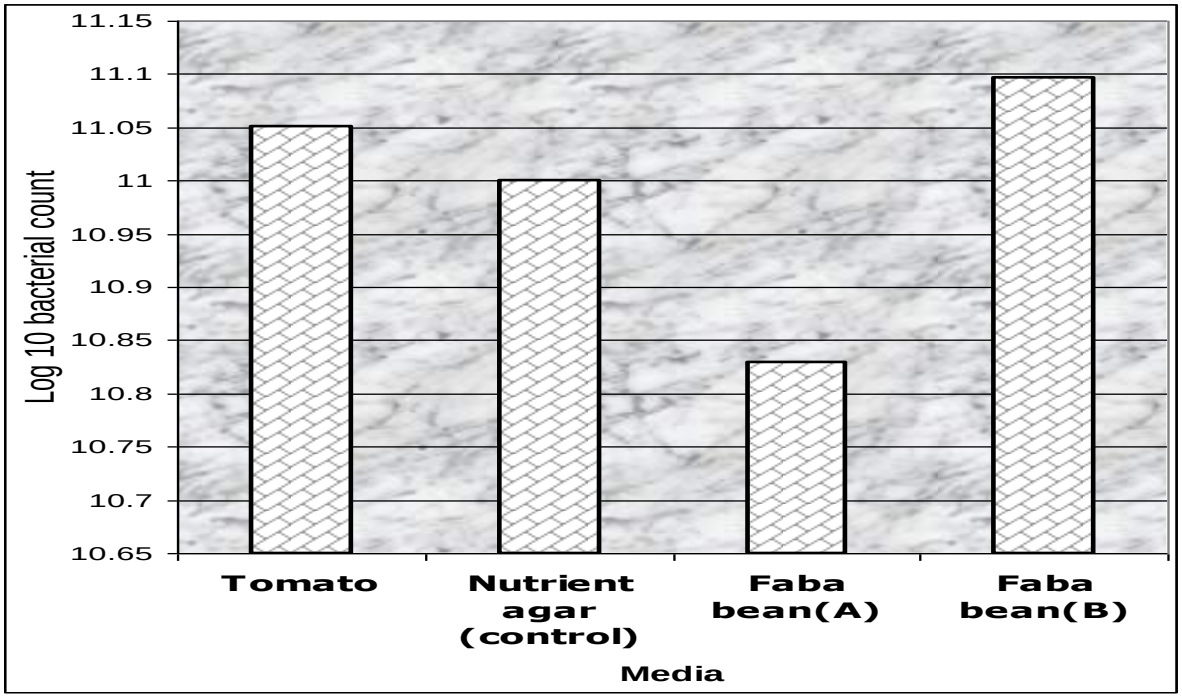

Fig. (1): The growth rate of *Klebsiella sp* on different plant extract and control

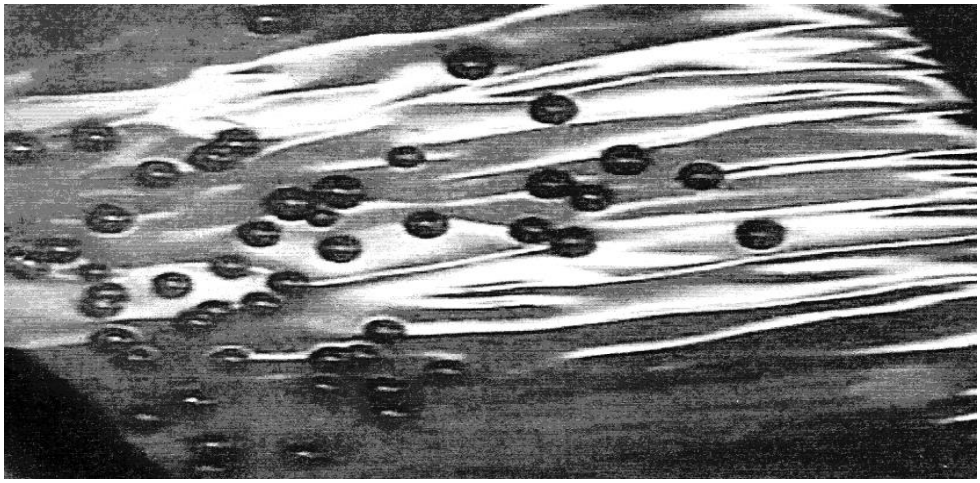

Fig.2: Colonies of *Klebsiella sp* on faba bean (A)

Discussion:

The search for bacterial culture media derived from plant materials as a source of nutrients has been investigated in this work.

The pH values of the culture media prepared from plant extracts, for bacterial growth were adjusted to the requirements of the bacteria under cultivation, for obtaining maximum growth.

Compared to nutrient agar (control), the tomato and faba bean extracts were able to support the growth of both Gram (-ve) bacteria *Kiebsiella sp.* and *Staphylococcus aureus* Gram (+ve) bacteria and the viable count, of these bacteria were similar to that on Nutrient Agar (control) this 'because these plant extracts, are rich in proteins, fats, carbohydrates and essential elements which help the bacteria to grow.

Staphylococcus aureus failed to grow on faba bean (dry and soaked) although faba beans have a high content of protein carbohydrates and fats hut this variation in nutrients concentration, may lead to inhibit the *Staphylococcus aureus* growth, and might be due to the absence of active transport across the cell wall. These findings were in agreement with the observations of Osman [(5)].

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Short Communication **Sudan Medical Laboratory Journal**

Effect of hypertension and smoking on lipid profile levels

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Abstract

The study aimed to establish effect of cigarette smoking and hypertension on lipid profile. 50 hypertensive (HTN) nonsmoker, 50 smoker non-hypertensive persons, 50 non hypertensive nonsmoker persons (control), age between (25-45 yrs) and all without history of hyperlipidaemia were included in the study

Lipid profile on the serum was performed with an autoanalyzer using standard methods. A significant increase in lipid profile was reported in hypertensive and smoker patients compared to control while HDL-C showed significant decrease. The triglyceride and low density lipoprotein (LDL-C) increased significantly in smokers than hypertensive patients, while the total cholesterol and HDL-C are insignificantly changed when comparing smokers with hypertensive patients.

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Introduction:

Hypertension and cigarette smoking induces a wide variety of physiological responses, some of which appear likely to be involved in accelerating atherosclerosis. Hypertension and cigarette smoking are firmly established as risk factors for coronary heart disease.

Smoking alters the lipid profile adversely causing dyslipidemia and the changes become more marked with the number of cigarettes [(1)].

Though the exact mechanism by which smoking induces changes in lipid profile is unclear, it is presumed that: **a-** Nicotine stimulates sympathetic adrenal system leading to increased secretion of catecholamines resulting in increased lipolysis and increased concentration of plasma free fattyacids, which further results in increased synthesis of hepatic triglycerides, along with VLDL-cholesterol in the blood stream [(2)]. **b-** Smoking results in fall in estrogen levels, which further leads to decrease in HDL-cholesterol levels [(3)]. **c-** Presence of hyperinsulinaemia in smokers leads to increased total cholesterol, LDL-cholesterol, VLDL-cholesterol and triglyceride levels due to decreased activity of lipoprotein lipase [(4, 5)].

Blood pressure is another important risk predictor for vascular disease, particularly coronary artery disease [(6)]. Hypertension is one of the major risk factors for coronary artery disease and stroke, and with smoke it is an independent risk factor for atherosclerosis [(7)].

It is a well known fact that hypertension is associated with abnormal changes in lipid profile

(dyslipidemia) which is a cause for atherosclerosis [8].

So the role of plasma lipid and lipoprotein in development of coronary heart disease (CHD) has been studied [9].

Materials and methods:

Study design: Across- sectional hospital based study.

Study area: The study was carried out in Wad Madani teaching hospital and Khartoum teaching hospital, between January 2009 and May 2010.

Study population: This study included 150 participants divided into three groups. The first group comprised 50 hypertensive patients (HTN) non smokers. The second group comprised 50 smokers but non-hypertensive persons. The third group comprised 50 healthy, non hypertensive and non smoker persons (control). Ages of all groups were between 25-45 year olds, and all with no history of cardiac problems

In all patients, a clinical check was done, including the body mass index and the blood pressure. Hypertensive patients who were smoking were excluded from this study as well as diabetic, obese, or any subject with history of hyperlipidaemia.

Ethical consideration: This study was approved by the ethical committee of Khartoum Teaching Hospital. Informed consent was obtained from each participant.

Sampling: Venipuncture was performed after 5 minutes in the sitting position, using the tourniquet as briefly as possible without venous stasis. 5ml of overnight fasting blood collected from the antecubital vein in serum separator tube. Serum was separated after 20 minutes and analyzed immediately after separation.

Methods: The concentration of total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were measured using direct kit methods [10] (from Bio-Diagnostics Kits, Spain)

Statistical analyses of data: Statistical analysis was performed using SPSS (Statistical Package for Social Sciences). Differences in mean values between groups were evaluated by a one-way analysis of variance (ANOVA) and Student's *t*-test. Two-tailed *P*-values were used and statistical significance was considered at $P<0.05$.

Results:

The results showed that there were significant increase in smokers and hypertensive patients compared to control group. The levels of serum TG and LDL-C in smokers was increased significantly compared to the control group as well as HTN group (table1).

Table 1: Cholesterol, TG, HDL and LDL levels (mg/dl) in serum of the study groups compared to the control groups

Lipid profile	Control	Smoke	HTN
TCL	121.7	225.20*	215.50*
TGL	122.5	218.00*	203.00**
HDL	48.8	34.95*	37.00*
LDL	79.3	147.35*	125.40**

* Significant from control group ($p<0.05$).

** Significant from control and smoke group ($p<0.05$).

Discussion:

Hyperlipoproteinemia is regarded as one of the most important risk factors for the development

of artheriosclerotic diseases especially when confounded with other risk factors **(11).**

In the present study, our results showed that hypertension and smoking cigarettes have significant effect on lipid levels. In both groups of smoking and hypertensive patients, we found that there were significant high levels (P<0.05) of triglycerides, LDL-Cholesterol and cholesterol, and significant decreased levels of HDL-cholesterol (P<0.05) compared to the control group. These results were consistent with the results of Christos et al **(12)**.

Although, smoking is known to affect serum lipid levels, especially decreasing the HDL-cholesterol **(3)**, in this study it did not appear to have any significant impact on lipid profile compared with hypertensive patients.

When comparing the effect of hypertension with smoking on lipid profile, we found that there are significant increase (P<0.05) in triglyceride and LDL-cholesterol in smoking more than in hypertension, which agrees with Bo-qing Zhu **(13)**; while the levels of total cholesterol and HDL-cholesterol were changed insignificantly (P<0.05) in smokers comparing to hypertensive patients.

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Short Communication **Sudan Medical Laboratory Journal**

Cholesterol level in serum of adult men and women diabetic Sudanese patients (Type II)

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Abstract

The aim of the study was to compare the glucose and total cholesterol in fasting serum between males and females type II diabetic Sudanese patients. The average fasting blood glucose and serum total cholesterol for men was 166.5mg/dl and 136.63mg/dl respectively while for women was 174.5mg/dl and 166.13mg/dl respectively. The control was 89.1mg/dl and 141mg/dl respectively. The cholesterol and glucose results of females were significantly higher than those of males. Statistically, there was positive correlation between fasting blood glucose and cholesterol in women group.

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Introduction:

Diabetes is a condition where the blood sugar level is higher than normal. There are two main types of diabetes.

1. Type 1 diabetes or insulin – dependent diabetes. It is usually seen in young people.
2. Type 2 diabetes – usually non insulin-dependent. It tends to affect adults over 40 and overweight people [(1)].

There are many factors which lead to increase the level of glucose in the blood; the increase in weight especially in females, type of diet, the infection of pancreas, and also increased of level of glucose during pregnancy [(2)].

The major lipids in the body are fatty acids, triglycerides, cholesterol and low density lipoprotein (LDL) [(3)]. The cholesterol present in the living cells of the animals, especially in nerve-cells, but not present in plant cells. Human body can synthesize cholesterol as 1-5 g/day, while the cholesterol taken from food as 0.3g/day[(4)].

Cholesterol concentration in the blood depends on age, sex and type of food ingested. The cholesterol increases with the increasing of age, where maximum levels reach in 50-60 years [(5)]. An increased level of cholesterol above 300 mg/dl leads to heart attack [(6)].

Materials and methods:

Study area: The study was conducted in Khartoum state.

Study population: The study was conducted on Sudanese patients with type 2 diabetes mellitus. A total of 30 patients (15 males and 15 females) and 30 healthy controls (15 males and 15 females) were included in this study.

Sampling collection: After overnight fasting, was separated and examined for glucose and total cholesterol.

Methods: Glucose was measured by glucose oxidase method [(7)] and cholesterol is measured by cholesterol esterase/oxidase enzymatic method [(8)].

Results and discussion:

The control group showed glucose and cholesterol mean values of 89.1mg/dl & 141mg/dl respectively, while in diabetic patients the levels increased significantly ($p<0.05$) to reach 171.67 mg/dl for glucose and 151.43 mg/dl for cholesterol (table 1)

Table 1: Descriptive data showed the level of F.B.G and cholesterol in normal and DM patient of the studied sample.

Variables	Control	Patients	p value
Glucose	89.1	171.67	<0.01
Cholesterol	141	151.43	<0.05

The diabetic females showed higher significant value ($p<0.05$) compared to diabetic males. The breakdown of glucose to ATP in females to produce more energy is less, so there is excess of glucose in their blood [(4)]. Diabetic women in U.S.A are 82% and men 17.5% [(5)].

The high level of glucose in females may be due to two reasons, either higher uptake of sugars in their meals, or there is low physical activities compared to males.

For all studied patients, statistical analysis revealed that there was insignificant correlation between fasting blood glucose and cholesterol levels in patients, as well as insignificant correlation in male patients; but there was a significant ($P < 0.05$) correlation in female D.M patients (table 2)

Table 2: Descriptive data showed the level of F.B.G and cholesterol in male and female and correlation between glucose and cholesterol levels in DM patient.

Variables	Male	Female	P value
Glucose	166	174.5	<0.05
Cholesterol	136.73	166.13	<0.05
Correlation probability	0.101	0.048*	

* Significant correlation.

Previous research of Omar and Amira [(9)] showed that there is positive correlation between the level of triglyceride, low density lipoprotein and cholesterol in D.M patients. D.M patients have to avoid foods which have high level of cholesterol.

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