International Journal of TROPICAL DISEASE & Health

26(2): 1-12, 2017; Article no.IJTDH.36282
ISSN: 2278–1005, NLM ID: 101632866

Evaluating Toxicity Profile of Garlic (*Allium sativum*) on the Liver, Kidney and Heart Using Wistar Rat Model

A. A. Fowotade[1], A. Fowotade[2*], B. U. Enaibe[3] and G. O. Avwioro[4]

[1]Department of Morbid Anatomy, University of Ilorin Teaching Hospital, Ilorin, Nigeria.
[2]Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria.
[3]Department of Anatomy, University of Ilorin, Nigeria.
[4]Department of Medical Laboratory Sciences, Delta State University, Abraka, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author AAF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BUE and GOA managed the analyses of the study. Author AF managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJTDH/2017/36282
Editor(s):
(1) Zhiheng Zhou, Thyroid Cancer Research Laboratory, Massachusetts General Hospital, Harvard medical school, Boston, USA.
Reviewers:
(1) Juan Carlos Troiano, University of Buenos Aires, Argentina.
(2) Dinithi Peiris, University of Sri Jayewardenepura, Sri Lanka.
Complete Peer review History: http://www.sciencedomain.org/review-history/21238

Original Research Article

Received 22nd August 2017
Accepted 20th September 2017
Published 6th October 2017

ABSTRACT

Background: The medicinal values of *Allium sativum* has been extensively described in a number of studies. Additionally, it has shown antimicrobial activity against a wide spectrum of microorganism; bacteria, fungi and parasites. The widespread benefit of garlic for its medicinal properties has resulted in its increased usage thus justifying the need to study the potential toxicity of garlic extracts on vital organs of the body. The present study aims to evaluate the toxicity profile of garlic extract in Wistar rats.
Materials and Methods: Thirty five male rats of Wistar strains were randomly grouped into seven (A-G) of five animals each. Animals in the control group received 1ml of physiological saline intraperitoneally for 38 days. Group A (5 mice) were given 250 mg/kg of garlic extract daily. Group B (5 mice) received 300 mg/kg of garlic extract daily, Group C (5 mice) received 350 mg/kg of garlic

**Corresponding author: Email: temilabike@gmail.com;*

extract daily, Group D (5 mice) received 400 mg/kg of garlic extract daily. Group E (5 mice) received 450 mg/kg of garlic extract daily. Group F (5 mice) received 500 mg/kg of garlic extract daily. All administration were done intraperitoneally for 38 days following which blood specimens were collected for biochemical analysis and the animals sacrificed for histological analysis.

Results: The result of the study showed a dose dependent increase in levels of liver enzymes 9AST, ALT and ALP) as well as an increase in serum creatinine levels. Additional findings included dose dependent histologic alterations in hepatic, renal and cardiac functions compared with the control group 6.

Conclusion: This study therefore highlights the safety of garlic at low levels and the potential toxicity of high dose garlic extract to the liver, heart and kidney.

Keywords: Toxixicty; Allium sativum; wistar rats.

1. INTRODUCTION

Medicinal plants are plants which, in one or more of its organs contain substances that can be used for therapeutic purposes or as a precursor for the synthesis of useful drugs [1]. The medicinal value of plants has been known from ancient times and their uses have been scientifically proven by research. Prior to the advent of orthodox medicine, several ailments were treated with tea made from the bark of a Willow tree [2]. Further research has shown that Willow's bark contains salicylic acid; the active ingredient in aspirin [2]. It has also been established that the plants which naturally synthesize and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatile oils, minerals and vitamins possess medicinal properties [1].

Allium sativum, commonly known as garlic, is one of the species in the onion family, *Alliaceae*. Garlic is a small perennial herb cultivated throughout West Africa, West Indies and Brazil. There are about 400 species. Garlic is among the oldest of all cultivated plants and is popularly ingested for both culinary and medicinal purposes [3]. Garlic is a plant of economic importance and is produced in high demand in the Northern regions of Nigeria where it is consumed both in raw and cooked form [1]. The use of Garlic for medicinal purposes varies from usage in therapy for; abdominal discomfort, diarrhea, otitis media and respiratory tract infections in Nigeria [4] to treatment of common colds, hay fever and asthma in Europe and India. In Russia, Garlic is commonly used both as topical and systemic antimicrobial agent [5].

In 1858, Louis Pasteur observed garlic's antibacterial activity, and it was used during World Wars I and II as antiseptic on gunshot wounds to prevent gangrene [6]. More recently, a clinical trial showed that mouthwash containing 2.5% fresh garlic was associated with good antimicrobial activity [7]. However, a significant number of participants reported an unpleasant taste and halitosis [7]. Garlic is used to reduce abnormal cholesterol and blood pressure levels [8-9]. The most striking reported effect is its antispasmodic effect in infantile abdominal colic [10]. Previous investigational studies in humans, have suggested possible cardiovascular benefits of garlic [11]. A Czech study has also demonstrated that garlic supplementation reduces accumulation of cholesterol on the vascular walls of animals [11].

Lack of standardization is a major drawback to the use of herbal derivatives in developing nations. This is because of the potential hepatotoxic and cardiotoxic effects of such extracts. Despite the numerous beneficial effects of *Allium sativum*, its deleterious effect on sperm functions has also been documented [12].

The widespread benefit of garlic for its medicinal properties and the dearth of information on its potential toxicity justify the need for detailed study in order to investigate its potential toxicity on vital organs of the body. Hence this study was designed to assess the dose dependent effects of *Allium sativum* on the liver, kidney and heart of Wistar rats.

2. MATERIALS AND METHODS

2.1 Animal and Experimental Design

The study was carried out at the University of Ilorin, Ilorin, Nigeria. A total of thirty five (35) adult albino Wistar rats weighing approximately 122 grams (age ranges between 9-12 weeks) were used for the experiment. The rats were obtained from the animal house of the Department of Anatomy, College of Health

Sciences; University of Ilorin and housed in a well-ventilated, hygienic environment and fed with standard pelleted food and clean water. Animal handling was consistent with the ethical guidelines approved by the University of Ilorin Ethics Committee for scientific and medical research. They were maintained and habituated in wooden cages of the animal house of the University. The floors of the cages were filled with saw dusts while the cleaning was done on daily basis. Habituation conditions were 25 – 32°C and relative humidity 50 ± 15% with 12 hours light and dark cycle. The Wistar rats were randomly grouped into seven (A-G) of five animals each.

Group A (n=5): received 250 mg/kg body weight of garlic extract daily.

Group B (n=5): received 300 mg/kg body weight of garlic extract daily.

Group C (n=5): received 350 mg/kg body weight of garlic extract daily.

Group D (n=5): received 400 mg/kg body weight of garlic extract daily.

Group E (n=5): received 450 mg/kg body weight of garlic extract daily.

Group F (n=5): received 500 mg/kg body weight of garlic extract daily.

Group G (n=5): Control group received 1ml of physiological saline.

All administration was done intraperitoneally for 38 days following which blood specimens were collected for biochemical analysis. The animals were anaesthetized with chloroform and dissected. The organs (liver, kidney and heart) were removed at necropsy and fixed in 10% formol saline.

2.2 Plant Collection and Identification

Local cultivars of garlic (*A. sativum*.) were purchased from a market in the city of Ilorin, Nigeria during the dry season (February). The Department of Plant Biology, University of Ilorin identified, authenticated and confirmed them.

2.3 Plant Extract Preparation

The garlic cloves were peeled and cut into small pieces and cold extraction was carried out at room temperature (18-22°C) as described by [13]. Briefly, the whitish inner bulbs were ground into a fine paste using a mechanical grinder. 500 g of the paste were put in a standard volumetric flask and covered with about 1000 ml of 70% ethanol stoppered with cotton wool and allowed to stand in the dark at room temperature for 48 hours for complete extraction. The ethanolic extract was filtered off into pre-weighed evaporating dishes, while the residue in the flask was washed with a further 1000 ml of 80% ethanol and added to the extracts in the evaporating dishes. The filtrates were evaporated into a syrupy residue using a rotary extractor at 40°C. The dishes were then weighed again on a triple beam balance and the weight of extract was calculated as follows:

> Weight of extract = weight of evaporating dish after evaporation – weight of evaporating dish before addition of extract.
>
> e.g. weight of extract = (71 – 51) g = 20 g

The extracts were pooled together into an air-tight container and stored at -4°C until required for use.

2.4 Extract Administration

A portion of the extract was weighed and dissolved in normal saline solution. Fresh preparations were made on each day of the experiment. The resulting solutions were injected intraperitoneally into the mice [13]. The administered volume was determined by the procedure described by [14] as follows:

> 500 mg of garlic extract is contained in 1 mL of saline.

Hence,

$$\text{Administered volume} = \frac{\text{Effective dose (E.D.)}}{\text{Stock solution}}$$

Effective dose = Dose assigned to each group

Stock solution = 500 mg/mL of normal saline e.g.

For Group A,

$$\text{Administered volume} = \frac{250}{500} = 0.5 \text{ mL of freshly prepared extract solution.}$$

2.5 Collection of Samples

After the specified duration (38 days), the blood samples were collected by cardiac puncture

under anesthesia. About 3 ml of blood was obtained from each rat into EDTA specimen bottle for the evaluation of haematological parameters and into sterile bottles for biochemical assays. The blood samples in the sterile bottles (plain bottles) were allowed to clot and retract at room temperature. Sera in EDTA bottles were separated into plastic vials and stored in the freezer at -30 degrees centigrade until required for use.

2.6 Haematological Parameters

The blood samples were analyzed for packed cell volume (PCV) using a Microhaematocrit reader according to [15] and white blood cells (WBC) count was also estimated using the improved Neubauer counting chamber as described by [15].

2.7 Biochemical Parameters

Assays for liver function tests such as; serum alanine amino transferase (ALT) and aspartate amino transferase (AST) were performed using Randox kits (Randox Laboratories, UK) and based on the method described by [16]. Serum alkaline phosphatase (ALP) was determined by the method described in [17]. Kidney function tests such as; calcium and phosphate ions were estimated using Agappe kits based on the methods described in [18-19] respectively. Meanwhile, urea and creatinine were estimated using Fortress kits (Fortress Diagnostics, UK) while serum creatinine kinase-MB (CK-MB) level was determined by using Agappe kit [20]. Lipid peroxidation was assessed by measuring plasma malondialdehyde (MDA) level using method described by [21]. Total Cholesterol level was measured using commercial cholesterol reagent kit by Cell Biolabs Inc, San Diego, USA which is based on the Cholesterol Oxidase-Peroxidase colourimetric method described by [22]. Serum triglyceride level was measured using commercial triglyceride reagent kit made by Randox Laboratories (Antrim, UK) and estimated values were read as described by [23]. Serum High density Lipoprotein-cholesterol (HDL-C) was performed using the commercial HDL-precipitant kit made by Quimica Clinical Amplicada S. (QCA.) and measured as described by [24]. Serum Low density cholesterol (LDL-C) was measured using the LDL Cholesterol assay kit (Cell Biolabs, Inc, San Diego, USA) and assayed as described by [24]. All tests were carried out according to manufacturer's instruction.

2.8 Histopathological Parameters

All organs were examined for gross lesions. Tissues of interest were taken at necropsy were fixed in 10% formol saline (1 part of tissue to 10 parts of fixative) and then labeled. The tissues were dehydrated through ascending grades of alcohol, cleared in xylene, wax impregnated and finally embedded in paraffin wax. They were sectioned at 5 µm on a Rotary microtome and stained by Mayer's haematoxylin and Eosin (H & E) method for microscopic assessment. Any evidence of histopathological changes was observed using Brightfield Leitz microscope.

2.9 Statistical Analysis

The data were analyzed with Statistical Package for Social Sciences (SPSS 17.0 for Windows). Analysis of variance (ANOVA) was employed for analyzing all the data. A 5% significance level ($P<0.05$) and two-tailed tests were used for all hypothesis tests.

3. RESULTS

The results revealed significant ($P<0.05$) weight gain in the Wistar rats with increasing doses of *Allium sativum* administration (Table 1). As shown in Table 2, there was no significant increase ($P>0.05$) in packed cell volume and white blood count when compared with the control.

To assess the dose dependent effects of *Allium sativum* administration on hepatic biochemical function, we assayed for serum levels of liver enzymes and found that there was significant decrease ($P<0.05$) in serum Alkaline phosphatase (ALP), Alanine amino transferase (ALT) and Aspartate amino transferase (AST) levels when compared with the control (Table 3). There was a significant drop in the serum AST level ($P<0.05$) following the administration of 300 mg/kg after which there was a progressive rise in the serum level up until a dose of 400 mg/kg was administered. The AST levels then stabilized at a level of 140 IU/L. For serum ALT level, there was a significant decrease in the serum ALT level ($P<0.05$) after the second dose of 300 mg/kg was administered. This was followed by a progressive rise in the serum AST level until levels stabilized following the administration of 450 mg/kg.

There was a significant decrease in the serum levels of calcium and phosphate ions ($P<0.05$) as

shown in Table 4, when compared with the control. Contrastingly, there was a significant increase in the serum creatinine level ($P<0.05$) while the decrease observed in serum urea level was not statistically significant ($P>0.05$). There was a significant decrease in the serum levels of MDA and CK-MB ($P<0.05$) when compared with the control (Fig. 1A and B).

The results of the H & E reactions revealed that administration of ethanolic extract of garlic above 350 mg/kg body weight/day, resulted in varying degrees of histological alterations in liver architecture including; pyknotic nuclei, cellular hypertrophy and dilatations of sinusoids and central vein of the liver in the treatment groups (Groups D, E and F) when compared to the control groups as well as Groups A, B and C which received low doses of garlic extract. Similarly, high doses of ethanolic garlic extract was associated with histologic changes in the heart including; irregular arrangement of muscle fibres, wider interfibre spaces and flattened nuclei of the muscle fibres. The significant histologic findings in the kidney at high doses of garlic extract include; pronounced degeneration of the tubular epithelial cells lining of the Bowman's capsule and enlargement of the Bowman's space (Fig. 3).

4. DISCUSSION

Natural remedies have been investigated over several decades for a wide variety of ailments. Garlic has particularly attracted special attention for its widespread potential medicinal benefits [25-26]. Nonetheless, there has been little scientific support for its therapeutic and pharmacological properties as well as its potential toxic effects. The increasing usage of medicinal plants in alternative medicine, justifies the need for toxicological assessment. The present study was undertaken to evaluate the biochemical toxicity profile of *Allium sativum* at varied doses.

Our findings revealed significant final body weight gain in the treated animals (Table 1). The weights increased progressively with increasing doses of *Allium sativum*. This finding is in keeping with the observations of [27], which showed a reversal of radiation-induced weight loss in experimental rats, thus showing that garlic was able to restore the weight loss caused by radiation.

Dose dependent treatment with ethanolic extract of garlic resulted in an increase in packed cell volume and white blood cell count, however the increase was not statistically significant (Table 2). This finding is at variance with a previous report which showed a significant rise in total white cell count and packed cell volume in adult Wistar rats fed with *Allium sativum* for 38 days [28]. The difference in our observation might be due to the variation in the number of days of treatment, which is longer in our own study.

Liver enzymes such as AST, ALT and ALP are marker enzymes for liver function and integrity [29]. These enzymes are usually elevated in acute hepatotoxicity or mild hepatocellular injury, but tend to decrease with prolonged intoxication due to liver damage [30]. When the liver cell membrane is damaged, varieties of enzymes normally located in the cytosol are released into the blood stream [29]. The results from the serum biochemical analysis shown in Table 1 reveal a significant decrease in the levels of serum AST, ALT and ALP) when compared with the control after the administration of garlic extract at different concentrations for 38 days. These findings agree with previous observations that fresh garlic protects the liver against induced toxicity [13,31].

Urea and creatinine are waste products of protein metabolism that need to be excreted by the kidney, therefore a marked increase in serum urea and creatinine levels confirm the possibility of functional damage to the kidney [32]. Urea levels can be increased as a consequence of factors such as dehydration, use of anti-diuretic drugs and diet. Creatinine is however more specific to the kidney, since kidney damage is the only significant factor that causes elevated serum creatinine levels [32]. In this study, intraperitoneal administration of ethanolic garlic extract at different concentrations for each group resulted in lower levels of serum urea and creatinine levels when compared with the control. However, the effects became more pronounced and sustained with higher doses (>350mg/kg body weight/day) of the ethanolic extract (Table 4). This study confirms earlier report of possible functional damage to the kidney [32].

Additionally we observed that intraperitoneal administration of ethanolic extract of garlic lowers the serum MDA level when compared with the control. This lowering effect becomes more stable with high doses of garlic extract (see points E and F in Fig. 1). The significantly lower concentration of serum MDA suggests that the intraperitoneal administration of the garlic extract

increase the antioxidant potential and antioxidant enzyme activities as a protective mechanism against oxidative stress [33]. This finding supports the hypothesis that plant products are effective chemo-preventive agents [33].

Interestingly, there was a reduction in the levels of serum Creatine kinase following administration of low doses (250-350 mg/kg) of *Allium sativum.* Increasing doses (>350 mg/kg body weight/day) of *Allium sativum* extract resulted in a progressive rise in the serum creatine kinase-MB levels when compared with the control (Fig. 1B). These results are in keeping with the previous studies on the cardiovascular effects of *Allium sativum* [34-35]. The cardioprotective nature of *Allilum sativum* could be due to the presence of cardiac glycosides in garlic [36]. Cardiac glycosides are useful in the treatment of congestive heart failure and cardiac arrhythmias and are found as secondary metabolites in several plants and some animals [36].

Earlier studies have highlighted the hypolipidemic effects of garlic [37-39]. The present study agrees with this report In that intraperitoneal administration of varying concentrations of the ethanolic extract of garlic, caused a significant reduction in serum total cholesterol (TC), triglycerides (TG) and low density lipoprotein-cholesterol (LDL-C) levels after 38 days of garlic extract administration when compared with the control. Interestingly, the serum high density lipoprotein-cholesterol (HDL-C) was significantly increased following prolonged during administration of *Allium sativum* (Fig. 2A and Table 5). These effects are more pronounced with high dose of ethanolic garlic extract (450 or 500 mg/kg body weight/day). In the same respect, Chi [40] as well as Olaniyan [28] suggested that the significant reduction in serum lipids by the garlic extracts may be due to the inhibition of 3-hydroxyl-3-methyl-glutaryl-CoA (HMG-CoA) reductase; which is a rate limiting enzyme in cholesterol biosynthesis. It has also been shown to depress the hepatic activities of other lipogenic, cholestrogenic enzymes such as malic enzymes, fatty acid synthase, and glucose -6- phosphate dehydrogenase. Thus the triglyceride lowering effect of garlic may be due to the inhibition of fatty acid biosynthesis [41].

The histological findings in the liver are in accordance with previous report by Alnaqeeb [42]. The cellular hypertrophy observed in the current study may be caused by the cytotoxic effects of high doses of the ethanolic extract of garlic. High doses of ethanolic extract of garlic which ranged from 400 – 500 mg/kg body weight/day was associated with some histopathological changes in the architecture of the heart as observed with Groups D, E and F. These histopathological changes include irregular arrangement of muscle fibres, wider interfibre spaces and flattened nuclei of the muscle fibres. Similar to our observation with the liver histology, the control sections of the heart as well as the low doses of Groups A, B and C appeared morphologically normal. Our findings are in agreement with the observation of Rahman [43]. The significant histological findings in the kidney were found in the high dose groups D, E and F. These changes include; pronounced degeneration of the tubular epithelial cells lining of the Bowman's capsule, tubular dilatation and enlargement of the Bowman's space. These observed histopathological changes are in accordance with Barnejee [44].

Interestingly, the observed histological changes in the liver, heart and kidney following garlic administration at different doses for each group further support the biochemical findings shown in Tables 3 and 4.

Table 1. Dose-dependent effect of ethanolic extract of *Allium sativum* on the body weight of Wistar rats

Group	*Initial body weight (g)	*Final body weight (g)	Body weight gain (g/d)
Control (1 ml of saline)	122 ± 1.5	232 ± 2.2	1.83 ± 0.7
Group A (250 mg/kg)	122 ± 1.5	236 ± 1.8	1.90 ± 0.3
Group B (300 mg/kg)	122 ± 1.2	238 ± 2.5	1.93 ± 1.3
Group C (350 mg/kg)	122 ± 1.8	240 ± 2.2	1.97 ± 0.4
Group D (400 mg/kg)	124 ± 2.2	248 ± 2.5	2.06 ± 0.3
Group E (450 mg/kg)	125 ± 1.5	261 ± 2.2	2.27 ± 0.7
Group F (500 mg/kg)	130 ± 1.5	275 ± 2.5	2.42 ± 1.3

**Values were expressed as Mean ± SEM; n=5 for each treatment group.*
Body weight gain (g/d) = [Final body weight (g) - Initial body weight (g)] ÷ 60

Table 2. Dose-dependent effect of ethanolic extract of *Allivum sativum* on some haematological parameters of Wistar rats

Group	Parameters	
	*PCV (%)	*WBC (10^3/mm^3)
Control (1 ml of saline)	30.4 ± 0.4	3.6 ± 0.2
Group A (250 mg/kg)	31.5 ± 0.5	3.8 ± 0.2
Group B (300 mg/kg)	31.2 ± 0.4	3.7 ± 0.5
Group C (350 mg/kg)	31.5 ± 0.5	3.8 ± 0.4
Group D (400 mg/kg)	31.4 ± 0.5	3.8 ± 0.4
Group E (450 mg/kg)	31.8 ± 0.4	3.9 ± 0.5
Group F (500 mg/kg)	32.2 ± 0.5	4.2 ± 0.2

**Values were expressed as Mean ± SEM for five animals in each group after treatment with the ethanolic extract of A. sativum for 38 days. There was no significant increase (P>0.05) in packed cell volume and white blood count when compared with the control*

Table 3. Dose-dependent effect of ethanolic extract of *Allium sativum* on serum levels of liver enzymes in Wistar rats

Group	Parameters (IU/L)		
	*ALT	*ALT	*AST
Control (1 ml of saline)	279 ± 2	36 ± 2	185 ± 2
Group A (250 mg/kg)	200 ± 2	19 ± 2	142 ± 2
Group B (300 mg/kg)	168 ± 2	11 ± 2	115 ± 1
Group C (350 mg/kg)	131 ± 2	20 ± 2	133 ± 1
Group D (400 mg/kg)	169 ± 2	20 ± 2	181 ± 1
Group E (450 mg/kg)	172 ± 2	18 ± 1	142 ± 2
Group F (500 mg/kg)	191 ± 4	18 ± 2	142 ± 2

**Values were expressed as Mean ± SEM for five animals in each group after treatment with the ethanolic extract of A. sativum for 38 days. There was a significant decrease (P<0.05) in Alkaline phosphatase (ALP), Alanine amino transferase (ALT) and Aspartate amino transferase (AST) when compared with the control*

Table 4. Dose-dependent effect of ethanolic extract of *Allium sativum* on the renal function of Wistar rats

Group	Parameters (mmol/L)			
	*Calcium	*Phosphate	*Urea	*Creatinine
Control (1 ml of saline)	2.66 ± 0.02	1.84 ± 0.01	7.5 ± 0.20	46 ± 1.00
Group A (250 mg/kg)	2.38 ± 0.01	1.56 ± 0.01	3.90 ± 0.00	60 ± 1.00
Group B (300 mg/kg)	2.33 ± 0.01	1.64 ± 0.02	5.6 ± 0.10	68 ± 1.00
Group C (350 mg/kg)	2.40 ± 0.02	1.66 ± 0.01	5.7 ± 0.10	61 ± 1.00
Group D (400 mg/kg)	2.43 ± 0.03	1.66 ± 0.01	6.8 ± 0.10	61 ± 0.00
Group E (450 mg/kg)	2.46 ± 0.01	1.73 ± 0.03	6.8 ± 0.10	65 ± 2.00
Group F (500 mg/kg)	2.46 ± 0.02	1.73 ± 0.01	6.9 ± 0.00	65 ± 1.00

**Values were expressed as Mean ± SEM for five animals in each group after treatment with the ethanolic extract of A. sativum for 38 days. There was a significant decrease in the serum levels of calcium and phosphate ions (P<0.05) as shown above when compared with the control. However, the decrease in the serum level of urea was not significant (P>0.05) while there was a marked significant increase (P<0.05) in the serum creatinine level*

Table 5. Dose-dependent effect of ethanolic extract of *Allium sativum* on serum Lipid levels of Wistar rats

Group	Parameters (mmol/L)			
	*TC	*TG	*LDL-C	*HDL-C
Control (1 ml of saline)	2.93 ± 0.01	1.87 ± 0.01	1.95 ± 0.01	2.40 ± 0.01
Group A (250 mg/kg)	1.43 ± 0.01	0.93 ± 0.01	0.66 ± 0.02	2.42 ± 0.01
Group B (300 mg/kg)	1.82 ± 0.01	1.10 ± 0.02	0.75 ± 0.01	2.48 ± 0.02
Group C (350 mg/kg)	2.55 ± 0.01	1.37 ± 0.02	0.93 ± 0.02	2.53 ± 0.02
Group D (400 mg/kg)	2.47 ± 0.02	1.45 ± 0.02	1.00 ± 0.02	2.60 ± 0.01
Group E (450 mg/kg)	2.25 ± 0.02	1.44 ± 0.01	1.06 ± 0.02	3.05 ± 0.01
Group F (500 mg/kg)	2.25 ± 0.01	1.44 ± 0.01	1.04 ± 0.02	3.05 ± 0.01

**Values were expressed as Mean ± SEM for five animals in each group after treatment with the ethanolic extract of A. sativum for 38 days. There was a significant decrease in the serum levels of TC, TG and LDL-C (P<0.05) when compared with the control. Meanwhile, there was a significant increase in the serum level of HDL-C as shown in the table above when compared with the control*

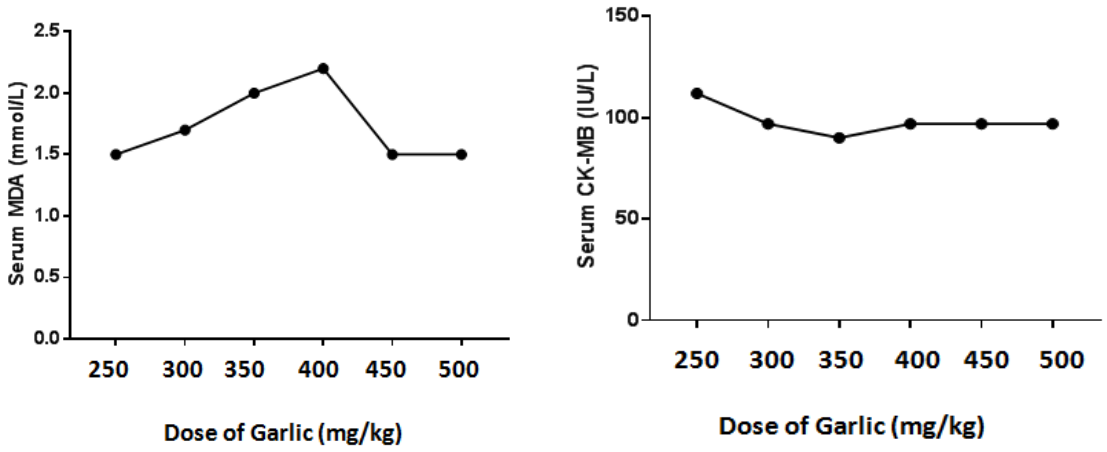

Fig. 1. Dose-dependent effect of ethanolic extract of *Allium sativum* on serum MDA and Creatine kinase-MB (CK-MB) activities in Wistar rats

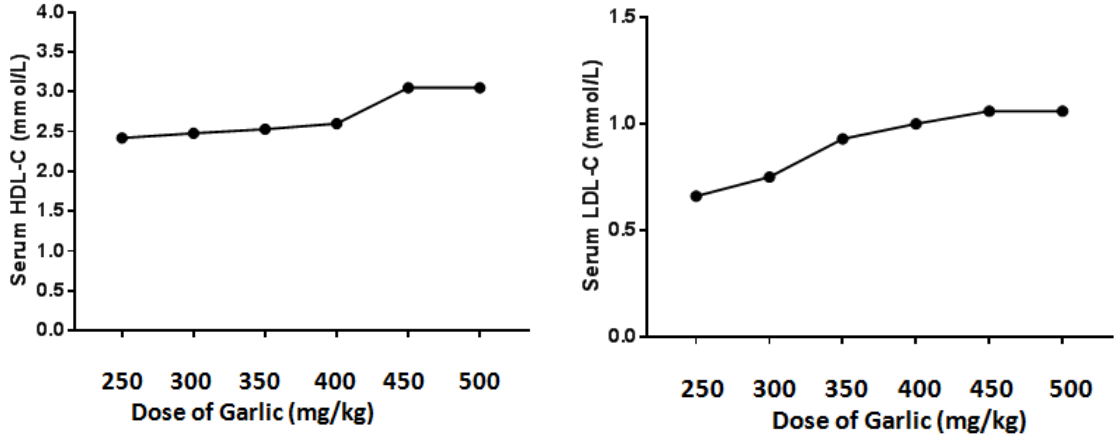

Fig. 2. Dose-dependent effect of administration of *Allium sativum* on serum lipid profile of Wistar rats

The Fig. 1 illustrates the pattern of change in (A) serum Malondialdehyde (MDA) levels and (B) serum levels of Creatine kinase-MB in rats following administration of varied doses of extracts of *Allium sativum*. There was a progressive increase in levels of MDA following increased doses and duration of administration of Allium sativum (A-D) after which the levels of MDA dropped and stabilized at 1.5 mmol/L at concentrations E and F. *Allium sativum* administration resulted in a depletion of CK-MB levels with increasing doses and duration (A-C). Increased doses subsequently resulted in a slight increase (D) followed by a plateau at 97 IU/L shown at points D-F. The observed changes were statistically significant ($P<0.05$) when compared with the control.

This figure illustrates the effect of chronic administration of *Allium sativum* on (A) serum High density lipoprotein-cholesterol (HDL-C) and (B) serum Low density lipoprotein – cholesterol (LDL-C). There was a significant increase in the serum HDL-C level ($P<0.05$) after few days of the extract administration when compared with the control (2.40 mmol/L ± 0.01). The serum (HDL-C) level became stable at 3.05 mmol/L (E and F). Fig. 1B summarizes the Low density lipoprotein-cholesterol (LDL-C) profile and shows a significant decrease in the serum LDL-C level ($P<0.05$) after few days of the extract administration (A and B) when compared with the control (1.95 mmol/L).

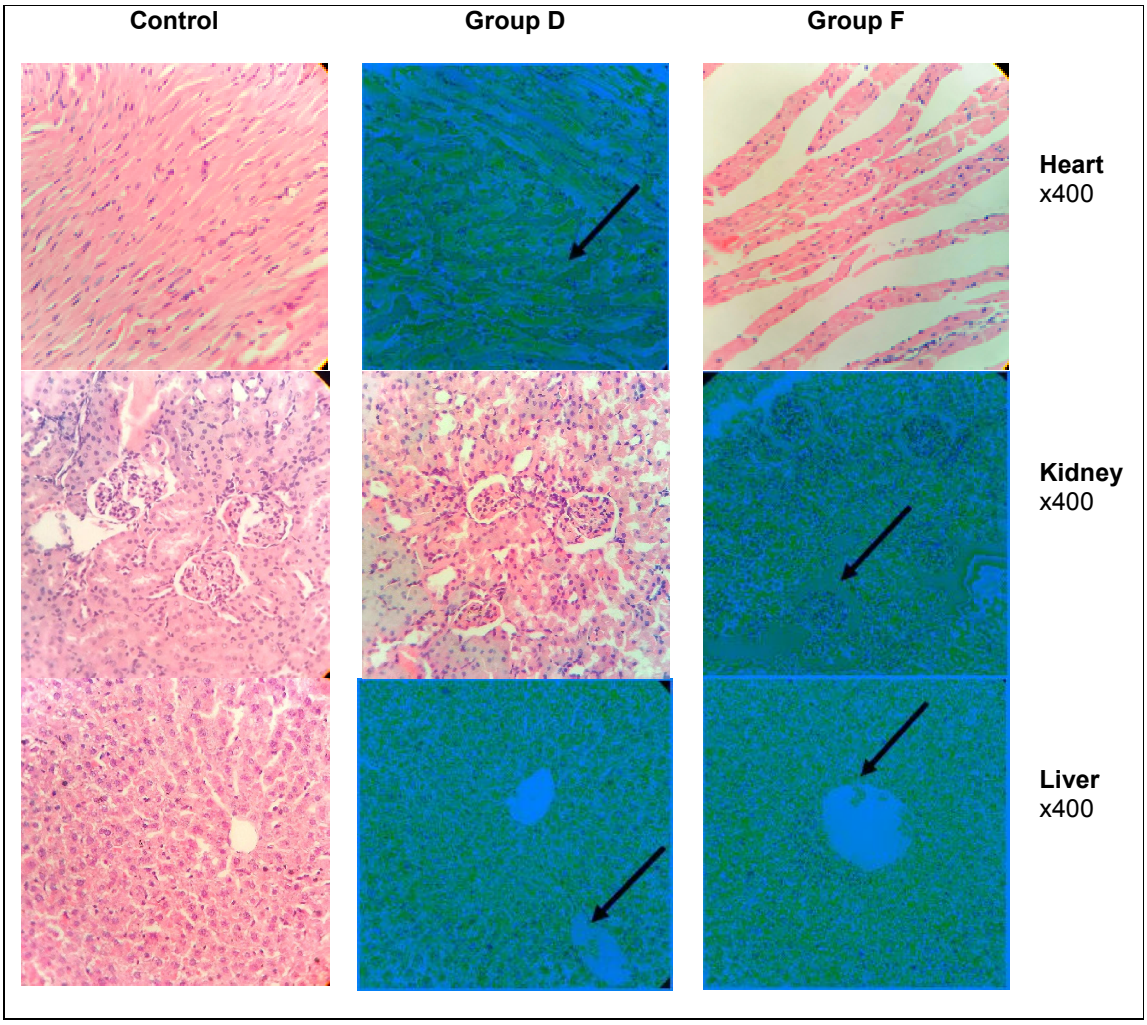

Fig. 3. Dose-dependent effect of administration of *Allium sativum* on histological profile of Wistar rats

Longitudinal sections of the heart, kidney and liver following 38 days treatment with D (400 mg/kg) and F (500 mg/kg) of *Allium sativum* extract as compared with the control (1 mL of physiological saline). Sections show a preserved architecture of all three organs for the control group. Groups D and F show varied histological changes from widening of the cardiac interfibre spaces, nuclei flattening and irregularity of cardiac muscles (arrow). Sections of the kidney show widening of the Bowman's space (arrow) and loss of flattened squamous tubular epithelial cells lining the Bowman's space of the kidney. Sections from the liver show dilated sinusoids, cellular hypertrophy with mild congestion of central vein of the liver (arrow). Magnification was set x400.

5. CONCLUSION

There has recently been an increased interest in research on medicinal plants with the view to providing alternatives to or supplementing imported (generic) drugs there by preserving foreign exchange reserves. The toxic side effects of these medicinal plants are limitation to their potential usefulness. The current study has shown that low doses of garlic extract (250 – 350 mg/kg body weight/day) has no deleterious effects on the organs of the Wistar rats while high doses (>400 mg/kg body weight/day) pose severe threat to selected body organs. This study therefore, highlights the potential ability of garlic extract to induce morphological changes in the liver, kidney and heart of humans consuming high doses of garlic for medicinal purposes.

6. RECOMMENDATIONS

It is therefore recommended that garlic consumption should be restricted to a safe daily margin of 250-350 mg/kg as suggested from this study in other to prevent the possible dose dependent toxic effects of garlic on the liver, kidney and heart.

Further studies are needed to investigate the exact mechanism(s) of action of the garlic extract which is not well understood presently.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sofowora A. Medicinal plants and traditional medicine in Africa. Chichester: John Wiley & Sons Ltd. New York. 1982;9-12, 142-146.
2. Dalziel JM. Useful plants of West Tropical Africa; Crown agents for the colonies. River: Pearson Education Print. 1956;179-183.
3. Gernot katzer Spice pages: Garlic (*Allium sativum* L.); 2005. Available:http://www.uni-graz.at/katzer/engl/Alli_sat.html (Retrieved 28-08-2007)
4. Jaber MA, Al-Mossawi A. Susceptibility of some multiple resistant bacteria to garlic extracts. Afr. J. Biotechnol. 2007;6(6):771-776.
5. Timbo BB, Ross MP, McCarthy PV, Lin CT. Dietary supplements in a survey of Prevalence of use and reports of adverse events. Am. Diet Assoc. 2006;106(12): 1966-1974.
6. Tattleman E. Health effects of garlic. American Family Physicians. 2005;72: 102-106.
7. Groppo F, Ramaccido J, Motta R, Ferraresi P, Sartoratto A. Antimicrobial Activity of Garlic against oral streptococci. Int Journal Dental Hyg. 2007;5:109-115.
8. Angelo A. Izzo. Efficacy and safety of *Allium sativum* (garlic). Focus on Alternative and Complementary Therapies. 2007;12:162-166.
9. Nwokocha CR, Ozolua RI, Owu DU, Nwokocha MN, Ufearo CS, Iwuala MOE, et al. Antihypertensive properties of *Allium sativum* on normotensive and two kidney one clip hypertensive rats. Niger J Physiol Sci. 2011;26(2):213-218.
10. Dutta AC. Botany for degree students. 5th Edition, Sylvia, NC. Herald Publishing Co. 1980;25:767-769, 824-827.
11. Sonova M, Sova P. Pharmaceutical importance of *Allium sativum* L. hypolipemic effects *in-vitro* and *in-vivo*. Ceska Slov Farm. 2004;5(3):117-23.
12. Morakinyo AO, Oloyo AK, Raji Y, Adegoke OA. Effects of aqueous extract of garlic (*Allium sativum*) on testicular functions in the rat. Nigeria J Health and Biomedical Sci. 2008;7(2):26-30.
13. Ezeala CC, Nweke IN, Unekwe PC, El-Safty IA, Nwaegerue EJ. Fresh garlic extract protects the liver against acetaminophen-induced toxicity. The Journal Nutrition and Wellness. 2009;7(1): 162-6.
14. Bernas GC, Gonzales RE, Solevilla RC, Ysrael MC. Pharmacology-toxicology. In: Guevara BQ, editor. A guide book to plant screening: Phytochemical and biological. Santo Tomas Philippines: Research Centre for Natural Sciences, university of Santo Tomas. 2004;103-132.
15. Baker FJ, Silverton RE, Kilshaw D. The full blood count. Introduction to Medical Laboratory Technology; 6th Edition. Butterworth and Co. (Publishers) Limited. 1985;323-327.
16. Reitman S, Frankel S. A colourimetric method for determination of serum. Am J Clin Pathol. 1957;28:56.
17. Klein B, Read PA, Babson AL. Raoid method for the quantitative determination of serum alkaline phosphatase. Clin Chem. 1960;6:269-275.

18. Connerty HV, Brigss AR. Determination of serum calcium by means of orthocresolphthalein complexone. Am J Clin Pathol. 1966;45:3-1.
19. Tietz W. Clinical guide to laboratory tests. 2nd Edition; W. B. Saunders Co. Philadelphia U.S.A. 1990;554–556.
20. Di. Witt, C. Trendelenburg, J. CK-NAC Clin Chemie, Clin. Bioch. 1982;20:235.
21. Stocks J, Domandy TL. The antioxidation of human red cell lipid induced by hydrogen peroxide. Br. J. Haematol. 1971; 20:95-111
22. Abel LL, Levy BB, Brodie BB. A simplified method for the estimation of total cholesterol in serum and determination of its specificity. J. Biol. Chem. 1952;195: 357-366.
23. Jacobs NJ, Vandemark PJ. The purification and properties of the α-glycerophosphate-oxidizing enzyme of Streptococcus feacalis 10C1. Arch Biochem. Biophys. 1960;88:250-255.
24. Asman G, Herbert PN, Fredrickson DS. Isolation and characterization of an abnormal HDL-cholesterol in Tangier disease. J. Clin. Invest. 1977;60:242-252.
25. Hammami I, Nahdi A, Mauduit C. The inhibitory effects onadultmale reproductive functions of crude garlic (*Allium sativum*) feeding. Asian J Androl. 2013;10(4):593-601.
26. EL-Demerdash FM. Dietary garlic and onions reduced the incidence of the rieogenic diet-induced cholesterol gallstones in experiment mice. Brit J Nutr. 2008;5:1-9.
27. Behnaz H. Effect of garlic (*Allium sativum*) hydroalcoholic extraction on estrogen, progesterone and testosterone levels in rats exposed to cell phone radiation. Zahedan J Res Med Sci. 2014;16:1.
28. Olaniyan OT, Meraiyebu AB, Arogbonlo A, Dare JB, Shekins O, Shafe MO. Effects of aqueous extract of garlic (*Allium sativum*) on blood parameters in adult wistar rats (*Rattus norvegicus*). Int J Pharm Sci Inv. 2013;2(3):42-45.
29. Rao J. Sublethal effects of an organophosphorus insecticide on biochemical parameters of tilapia *Oreochromis mossambicus*. Comparative Biochemistry and Physiology Part C. 2006; 143:492–498.
30. Arti S, Veena S, Leena K. Amelioration of lead-induce hepatotoxicity by *Allium sativum* extracts in swiss albino mice. Libyan J Med. 2010;5. Incl Supplements.
31. Sabah GE, Ahmed MA, Hafez A, Sara ME. Effect of garlic consumption on blood lipids and antioxidant/oxidant parameters in rat males exposed to chlorpyrifos. Slovak J. Anim. Sci.; 2009.
32. Garba SH, Adelaiye LYAB, Msheila. Histopathology and biochemical changes in the Rats kidney following exposure to a pyrethroid based mosquito coil. J Appl Sci. Res. 2007;3(12):1788-1792.
33. Arivazhaghan S, Balascnthil S, Nagiri S. Chemopreventive potential of garlic and neem leaf extracts during gastric carcinogenesis induced by N-methyl-N-nitro-N-nitrosoguanidine. Med. Sci. Res. 1999;27:209-212.
34. Rahman K, Lowe GM. Garlic and cardiovascular disease: A critical review. J. Nutr. 2006;136:S736-40.
35. Khalid S. Al-Numair. Hypocholesteremic and antioxidant effects of garlic *Allium sativum* extract in rats fed high cholesterol diet. Pak J Nutr. 2009;8(2):161-166.
36. Mikail HG. Phytochemical screening, elemental analysis and acute toxicity of aqueous extract of *Allium sativum* L. bulbs in experimental rabbits. J Med Plants Res. 2010;4(4):322-326.
37. Bordia AK, Sodhya SK, Rathore AS, Bhu N. Essential oil of garlic on blood lipids and fibrinolytic activity in patients of coronary heart disease. J. Asso. Physician Ind. 1975;26:327-331.
38. Banerjee SK, Maulik M, Mancahando SC, Dinda AK, Gupta SK, Maulik SK. Dose-dependent induction of endogenous antioxidants in rat heart by chronic administration of garlic. Life Sci. 2002;70: 1509-1518.
39. Thomson M, Al-Qattan KK, Bordia T, Ali M. Including garlic in the diet may help lower blood glucose, cholesterol and triglycerides. J. Nutr. 2006;136:800S-802S.
40. Chi MS, Koh ET, Stewart TJ. Effect of garlic on lipid metabolism in rats fed cholesterol or lad. J Nutr. 1982;122:241-248.
41. Yu-Yan Y, Liu L. Cholesterol lowering effect of garlic extracts and organ sulfur

compounds. Human and animal studies. J Nutr. 2001;131:9895-9935.

42. Alnaqeeb MA, Thomson M, Bordia T, Ali M. Histopathological effect of garlic on liver and lungs of rats. Toxicol Lett. 1996;85(3): 157-64.
43. Rahman K. Effects of garlic on platelet biochemistry and physiology. Mol. Nutr. Food Res. 2007;51(11): 1335-44.
44. Banerjee SK, Maulik M, Manchanda SC, Dinda AK, Das TK, Maulik SK. Garlic induced alteration in rat liver and kidney morphology and associated changes in endogenous antioxidant status. Food Chem Toxicol. 2001;39:793-7.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21238