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journal homepage: www.jadweb.orgOriginal article <http://dx.doi.org/10.1016/j.joad.2016.05.002>Antimicrobial evaluation, acute and sub-acute toxicity studies of *Allium sativum*Bashir Lawal^{1*}, Oluwatosin Kudirat Shittu¹, Florence Inje Oibiokpa¹, Hadiza Mohammed¹, Sheriff Itopa Umar¹, Garba Muhammed Haruna²¹Department of Biochemistry, Tropical Disease Research Unit, Federal University of Technology, P.M.B. 65, Minna, Nigeria²Federal College of Wild Life Management, New Bussa, Niger State, Nigeria

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ABSTRACT

Objective: To evaluate the antimicrobial and toxicological effects of aqueous garlic (*Allium sativum*) bulbs extract in wister rat using biochemical and hematological parameters.

Methods: A total of 20 wister rats were assigned into four (A–D) groups of five animals each. Group A served as the control and was administered 1 mL of distilled water. Groups B–C were given 300, 600 and 1200 mg/kg body weight of garlic (*Allium sativum*) bulbs extract for 5 weeks.

Results: Garlic bulbs extract produced significant inhibitory activities against all bacteria tested at concentrations of 120 and 160 mg/mL. However, at concentration of 80 mg/mL, the extract had no inhibitory activities against *Klebsiella pneumoniae* and *Salmonella typhi*. The minimal inhibitory concentration and minimal bactericidal concentration ranged between 80–120 mg/mL and 120–160 mg/mL respectively. Toxicological study revealed that the extract did not cause any significant ($P > 0.05$) alteration to serum aspartate transaminase, alkaline phosphatase activities, total bilirubins, Na, K, creatinine, red blood cell, hematocrit, hemoglobin, mean corpuscular hemoglobin concentration, granulocyte and organs-body weight ratio. However, serum alanine transaminase activities, total proteins, direct bilirubins, Cl^- concentrations and body weight gain were significantly ($P < 0.05$) lowered while the concentrations of urea, albumin, white blood cell, mean corpuscular hemoglobin and mean corpuscular volume count were raised significantly ($P < 0.05$) in rats dosed with 600 and 1200 mg/kg of the extract. However, at a dose of 300 mg/kg only, the concentrations of Cl^- , urea and albumin were mildly altered.

Conclusions: The extract caused selective changes in some biochemical parameters of organ function; however, since only mild alteration was observed at a dose of 300 mg/kg, the garlic bulb may be considered to be relatively safe and could be explored as an oral remedy at this dose.

1. Introduction

Microbial infectious diseases have always been considered as a global leading cause of morbidity and mortality in humans.

The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics have raised concern on untreatable bacterial infections and call for urgent search for antimicrobial agents from natural products.

Nature has bestowed to humanity the gift of immense therapeutic knowledge with wide varieties of medicinal plant^[1]. Effort has been made towards validating the therapeutic claimed of African indigenous natural products^[2,3]. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment^[4]. More than 80% populations of the third world countries depend majorly on natural products to meet up their daily health care needs. In supports of these practices, World Health Organization has

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All experimental procedures involving animals were conducted in accordance to Canadian Council on Animal Care Guidelines and Protocol Review and approved by the Federal university of Technology, Minna Committee on Ethics for Medical and Scientific Research.

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recommended the use of these natural products but emphasized that safety/toxicity should always be considered when using the natural products for health care needs^[5].

Garlic [*Allium sativum* Linn. (*A. sativum*)], family Alliaceae is a perennial flowering plant that grows up to 30–60 cm tall. It has a characteristic pungent smell with a large variety of flavors and textures when it is raw or cooked^[6]. Garlic is one of the most important *Allium* species consumed world wide and has generated a lot of interest for decades as a medicinal remedy for many diseases^[7,8]. It's traditionally used as antiseptic, expectorant, antihypertensive, stimulant, carminative, aphrodisiac, diaphoretic, anthelmintic, diuretic, antiscorbutic and for the treatments of viral infections. Biological activities including antioxidant, insecticidal, antinociceptive, antitrypanosomal and antimicrobial activities^[8], have been documented^[9–12].

A non-protein amino acid (alliin) and an enzyme (alliinase) are the most active ingredients contained in garlic bulb. When fresh *A. sativum* is chewed or grounded, these compounds (alliin and alliinase) interacted to form allicin, which is the main biologically active component in garlic that is responsible for garlic's strong smell^[9]. This compound could also be responsible for the therapeutic and toxic virtues of the bulb.

Isaac *et al.* had reported the absence of visible toxic effects following 14 days administration of an aqueous extract of garlic in rats^[13]. Literature survey revealed the scanty information on toxic effect of garlic in the same manner as it is claimed to be used in Nigerian folklore medicine for the management of several diseases. In this study, we have evaluated the antimicrobial and toxic effects of garlic using the biochemical and hematological studies.

2. Materials and methods

2.1. Collection and preparation of garlic extract

The garlic (*A. sativum*) bulbs were obtained from Bosso Market, Minna, Nigeria in July, 2015. The garlic extract was prepared as described by Gupta *et al.* with slight modification^[8]. The outer covering of the bulbs was peeled off and the cloves were separated, sliced and grounded repeatedly with mortar and pestle. About 50 g of the grounded garlic bulbs were further homogenized with 250 mL of distilled water. The froth was allowed to settle down before filtering through Whatman filter paper No. 1.

2.2. Experimental animals

A total of 32 apparently healthy wister rats (*Rattus norvegicus*) with average weight of 120.54 ± 5.67 were procured from the Small Animal Breeding Unit of Biochemistry Department, Federal University of Technology Minna. The rats were housed in clean plastic cages and maintained under standard laboratory conditions [temperature: (22 ± 3) °C; photoperiod: 12 h natural light and 12 h dark and humidity: 40%–45%]^[14]. The rats were maintained on standard animal feeds (Bendel Feeds and Flour Mills, Edo State, Nigeria) and tap water *ad libitum*. The principles governing the use of laboratory animals as laid out by the Federal University of Technology, Minna Committee

on Ethics for Medical and Scientific Research and also existing internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review were duly observed^[15].

2.3. Sources of microorganisms

Pure isolates of *Klebsiella pneumoniae* (*K. pneumoniae*), *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E. coli*) and *Salmonella typhi* (*S. typhi*) were procured from Microbiology Unit, Faculty of Life Sciences Federal University of Technology, Minna, Nigeria. Biochemical test and Gram staining test were used to confirm the identity of organism.

2.4. Assay for antibacterial activity

Antibacterial activity of garlic bulb was carried out using agar-well diffusion method as described by Gaherwal *et al.* and ciprofloxacin (40 µg/mL) as standard drug^[16]. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by tube dilution method for each of the test organism in triplicates.

2.5. Assay kit

The biochemical assay kits including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total protein, albumin and electrolytes were obtained of Randox Laboratories Ltd, United Kingdom. While urea and creatinine assay kits were produced (Quinica Clinica Aplicada S.A., Spain).

2.6. Determination of LD₅₀

Four groups of three rats each were used. The rats were given garlic (*A. sativum*) bulbs extract through oral route at doses of 100, 1000, 2500 and 5000 mg/kg body weight in each group. The extract was administered once and the rats were observed for death and sign of toxicity within 24 h.

2.7. Experimental design

A total of 20 adult wister rats were randomly assigned into four (A–D) groups of five animals each. Group A served as the control rats and was administered with 1 mL of distilled water. Groups B–C were treated like those of the control except they received the same volumes containing 300, 600 and 1200 mg/kg body weight of garlic (*A. sativum*) bulbs extract. All treatments were administered orally on daily basis for 5 weeks.

2.8. Collection of blood, serum and organs

The blood, serum and organs were collected as described previously^[17]. At the end of the five weeks treatment, the animals were denied their feeds but still had water *ad libitum* for 24 h before they were kindly sacrificed under ether anesthesia. The whole blood for hematological analyses

was collected in sample bottles containing ethylene diamine tetraacetic acid. Another whole blood was collected in a clean and free centrifuge tube containing ethylene diamine tetraacetic acid which was allowed to stand for 10 min at room temperature before had been centrifuged at 1000 r/min for 15 min to get the serum which was used for biochemical analyses. The animals were thereafter quickly dissected and the organs (liver, kidneys spleen and heart) were removed, cleaned and weighed.

2.9. Determination of hematological and biochemical parameters

The hematological components including hemoglobin, hematocrit, red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), granulocyte count and lymphocytes were determined using the automated hematologic analyzer (Sysmex, KX-21, Japan) as described by Dacie and Lewis^[18].

The activities of serum ALP, AST and ALT were determined using standard procedures^[19,20]. The levels of serum total protein, albumin, bilirubin, serum urea and creatinine were determined using standard procedures^[21–23]. Na⁺, K⁺ and Cl⁻ concentrations were determined by flame photometry^[19].

2.10. Determination of body weight and relative organ weight

The body weights of rats were determined before and after the experiment and the weight gains were computed. Relative organ weights were computed by expressing the absolute weight of the organs to the body weight of animals as described below.

Weight gain = Final weight of rat (g) – Initial weight of rat (g)

Relative organ weight = organ weight (g)/body weight (g) × 100

2.11. Statistical analysis

Data were analyzed using SPSS version 16 and presented as means ± SEM. Comparisons between different groups were done using ANOVA and Duncan's multiple range test. Values of $P < 0.05$ were considered as statistically significant as described by Yalta^[24].

3. Results

3.1. Antimicrobial study

Garlic bulbs extract produced a significant inhibitory activities against all the microorganism tested at the concentrations of 120 and 160 mg/mL. However, at concentration of 80 mg/mL, the extract showed no inhibitory activities against *K. pneumoniae* and *S. typhi*. The MIC of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus* and *S. typhi* was 120, 120, 120, 80 and 120 mg/mL respectively and MBC of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus* and *S. typhi* was 120,

160, 120, 120 and 160 mg/mL respectively. Antimicrobial activities of garlic (*A. sativum*) bulbs extract were showed in Table 1.

Table 1

Antimicrobial activities [zone of inhibition (mm)] of garlic (*A. sativum*) bulbs extract against some pathogenic microorganisms (mg/mL).

Pathogenic microorganisms	Extract concentrations			Ciprofloxacin
	80	120	160	
<i>E. coli</i>	6.60 ± 0.42	12.35 ± 0.15	23.76 ± 0.56	26.00 ± 1.09
<i>K. pneumoniae</i>	–	4.56 ± 0.20	17.05 ± 0.10	26.00 ± 0.20
<i>P. aeruginosa</i>	5.00 ± 0.56	13.45 ± 1.23	26.34 ± 0.32	28.00 ± 0.34
<i>S. aureus</i>	7.68 ± 0.42	9.56 ± 0.56	16.95 ± 0.65	24.00 ± 0.30
<i>S. typhi</i>	–	6.78 ± 0.50	18.67 ± 1.35	26.00 ± 0.56

3.2. Acute toxicity

In the acute toxicity studies, no death was recorded during the treatment period at all doses of the garlic bulb extract administered. The animals were apparently healthy with no sign of toxicity up to the dose of 2500 mg/kg. However, at 5000 mg/kg, animals were weak and had intense ethrema tachy-cardia and disorientation but no death was recorded. Thus, LD₅₀ was more than 5000 mg/kg.

3.3. Hematological parameters

Administration of aqueous garlic bulb extract at doses of 300, 600 and 1200 mg/kg body weight for 5 weeks caused significant increase ($P < 0.05$) in WBC and decrease in lymphocyte (Figure 1) but had no effects on granulocyte neutrophile. However, among the erythrocytic indices, MCH and MCV were significantly ($P < 0.05$) raised by the extract while RBC, hematocrit, hemoglobin and MCHC were compared well with the control rats at all doses of the garlic tested (Figure 2).

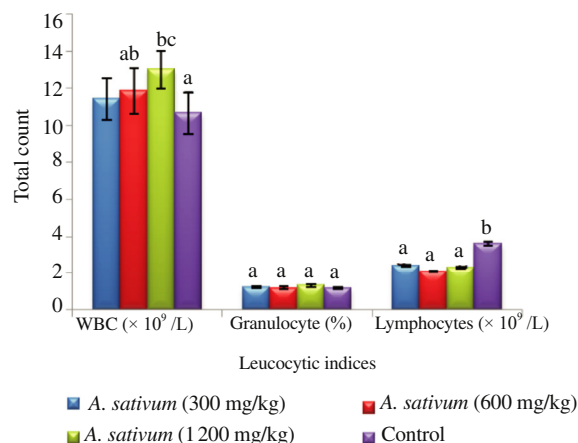


Figure 1. Effect of garlic extract on leucocytic indices in rats. Values were expressed as mean ± SEM of 5 rats. Bars with different superscripts were significantly different ($P < 0.05$).

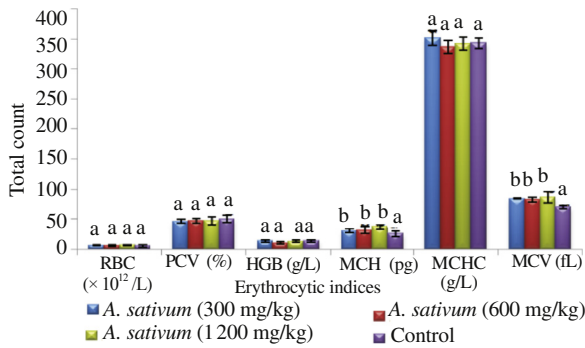


Figure 2. Effect of garlic bulb extract on erythrocytic indices in rats. PVC: Packed cell volume; HGB: Hemoglobin. Values were expressed as mean \pm SEM of 5 rats. Bars with different superscripts were significantly different ($P < 0.05$).

3.4. Biochemical parameters

Serum ALT activities, total proteins, direct bilirubins and Cl^- concentrations were significantly ($P < 0.05$) lowered while the concentrations of urea and albumin were raised in rats dosed with 600 and 1200 mg/kg for 5 weeks when compared with the control rats. At dose of 300 mg/kg only, the concentrations of Cl^- , urea and albumin were altered. However, serum AST and ALP activities, total bilirubins, Na, K and creatinine concentrations following 5 weeks administrations of garlic (*A. sativum*) bulbs extract at all doses (300, 600 and 1200 mg/kg) were not significantly ($P > 0.05$) different from the control rats (Figures 3–5).

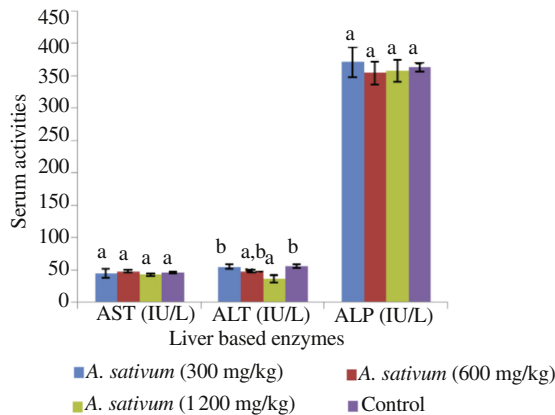


Figure 3. Effect of garlic extract on serum activities of some liver based enzymes in rats. Values were expressed as mean \pm SEM of 5 rats. Bars with different superscripts were significantly different ($P < 0.05$).

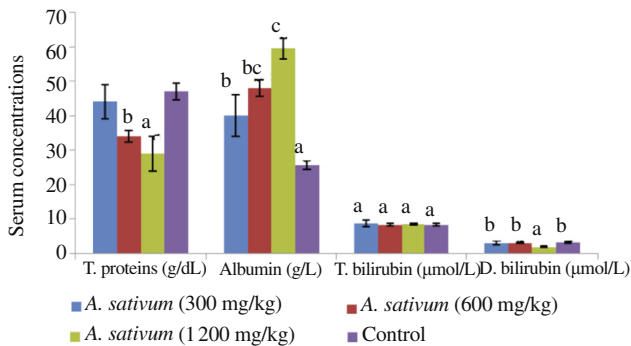


Figure 4. Effect of garlic extract on some biochemical parameters in rats. T. proteins: Total proteins; T. bilirubin: Total bilirubin; D. bilirubin: Direct bilirubin. Values were expressed as mean \pm SEM of 5 rats. Bars with different superscripts were significantly different ($P < 0.05$).

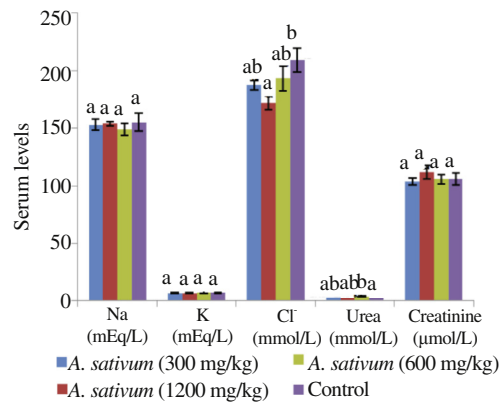


Figure 5. Effect of garlic extract on serum electrolyte, urea and creatinine in rats. Values were expressed as mean \pm SEM of 5 rats. Bars with different superscripts were significantly different ($P < 0.05$).

3.5. Body weight and relative organ weight

Body weight gain in groups of rats administered aqueous extract of *A. sativum* for 5 weeks were lower when compared with the control rats (Table 2). However, the relative organ/body weight ratios indicated that the liver, spleen, kidney, and heart body weight ratios of the rats were not significantly ($P > 0.05$) different from those of the control rats (Table 3).

Table 2

Effect of aqueous extract of *A. sativum* on body weight gain of albino rats.

Groups	Initial weight (g)	Final weight (g)	Body weight gain (g)
<i>A. sativum</i> (300 mg/kg)	153.46 \pm 5.98	176.11 \pm 8.82	22.65
<i>A. sativum</i> (600 mg/kg)	144.07 \pm 4.77	166.05 \pm 12.01	21.98
<i>A. sativum</i> (1200 mg/kg)	133.43 \pm 6.00	153.41 \pm 8.82	19.98
Control rats	144.09 \pm 3.86	171.14 \pm 12.01	27.05

Values were expressed as mean \pm SEM of 5 determinations.

Table 3

Effect of aqueous extract of *A. sativum* on relative organ weight of albino rats.

Parameters	<i>A. sativum</i> (300 mg/kg)	<i>A. sativum</i> (600 mg/kg)	<i>A. sativum</i> (1200 mg/kg)	Control rats
Kidney	0.0053 \pm 0.000	0.0060 \pm 0.0010	0.0051 \pm 0.0000	0.0055 \pm 0.0010
Liver	0.0430 \pm 0.000	0.0440 \pm 0.0050	0.0490 \pm 0.0010	0.0430 \pm 0.0020
Heart	0.0050 \pm 0.001	0.0056 \pm 0.0000	0.0061 \pm 0.0010	0.0055 \pm 0.0010
Spleen	0.0043 \pm 0.002	0.0042 \pm 0.0000	0.0040 \pm 0.0000	0.0049 \pm 0.0000

Values were expressed as mean \pm SEM of 5 determinations.

4. Discussion

Plants have been well documented for their medicinal uses for thousands of years and traditional medicines are still a major part of habitual treatments of different maladies in different parts of the world^[25]. The specific molecular principles of medicinal plants in their natural states possess a variety of influences on

human physiological and biochemical systems as raised concern over their safety.

The antibacterial activity of garlic bulb extract was investigated against five bacteria (*S. aureus*, *S. typhi*, *E. coli*, *P. aeruginosa* and *K. pneumoniae*). Increase in the concentration of the extract results in corresponding increase in the zones of inhibitions. This linear relationship between extract concentrations and zones of inhibition could be that the extract was able to diffuse into the inoculated nutrient agar. The extract was found highly active at concentration of 160 mg/mL. The antimicrobial activities demonstrated by this plant extract could be linked to its phytochemical constituents especially tannins which has been reported to exert antimicrobial activities^[12].

Evaluations of hematological parameters (erythrocytes, leucocytes and thrombocytes) provide valuable information on the adverse effects of foreign components on the blood and also explain blood-related functions of chemical compounds^[26]. It has been established that oral ingestion of medicinal plants or drugs can alter the normal values of hematological indices^[27,28].

In the present study, administration of aqueous garlic bulb extract at various doses of 300, 600 and 1200 mg/kg for 5 weeks can cause significant increase ($P < 0.05$) in WBC, decrease in lymphocyte but had no effects on granulocyte neutrophile count (Figure 1). WBC and its differentials are known for their defensive role against foreign body and infectious agents through the production, transportation and distribution of antibodies in immune response^[26]. The significant increased WBC counts following 5 weeks oral administration of garlic (*A. sativum*) bulbs extract reflect leucopoietin-release and possible immunomodulatory effects of the garlic extract which augmented the production of WBC in order to overcome the stress induced by the extract^[15]. This will increase the animal's capability of generating antibodies in the process of phagocytosis which have high degree of resistance to diseases and enhance adaptability to local environmental and disease prevalent conditions.

Among the erythrocytic indices evaluated in this study, MCH and MCV were significantly raised by the extract while RBC, hematocrit, hemoglobin and MCHC were compared well with the control rats at all doses of the garlic tested (Figure 2). This is an indication that garlic extract did not cause destruction of existing RBC as well as did not inhibit or stimulate the erythropoietin release in the kidney, which is the humoral regulator of RBC production^[29]. Hemoglobin and RBC are very essential in transferring respiratory gases^[28]. This finding also indicates that the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues have not been compromised by 5 weeks administrations of garlic extract.

Evaluation of serum biochemical indices in animals has become the most valuable tools for assessing the integrity and functionality of organs as well as risk assessment, pathological condition and general health status of the body^[30–35]. AST and ALT are biomarkers of hepatic integrity and to a certain level, can be used to assess the extent of hepatocellular damage^[31], the ALT activities, however, give more valuable information relevant to the integrity of the hepatocyte than AST^[32]. Consequently, in the present work, serum ALT activity was significantly lowered in rats dosed with 600 and 1200 mg/kg for 5 weeks when compared with the control rats. The constituents of the garlic must have inhibited the enzyme activity or diminution of important molecules needed for the optimum activities of the enzyme^[33]. Such decreased ALT

activities will, however, adversely affect the metabolism of amino acid and carbohydrate with consequent diminishing effect on ATP generation^[35]. It appears that the extract might have selectively affected the transaminases since AST activities in the serum of the animals were not altered. This may not be unconnected to the earlier mentioned, selective toxicity of natural products especially the plants extracts^[30,36].

Alkaline phosphatases are often used to assess the integrity of plasma membrane and endoplasmic reticulum^[37]. The observed non-significant difference from the control values in the activities of ALP after 5 weeks of garlic extract administration suggested that the integrity and functionality of endoplasmic reticulum and plasma membrane have not been comprised^[36]. It also indicates that the extract did not inhibit or activate the activities of the enzyme molecule *in situ*^[38].

The levels of albumin, bilirubins, total protein, electrolytes creatinine and urea play important roles in determining the synthetic and excretory roles of the kidney and liver^[39]. The observed decrease in the total proteins and direct bilirubins content in rats dosed with 600 and 1200 mg/kg garlic suggests that the extract might have interfered with the equilibrium in the rate of synthesis or destruction of total protein and direct bilirubins from the system of the animals. Such decrease could, however, lead to hydration which is detrimental to cellular homeostasis. This will negatively affect the metabolic activities of the liver and consequently the health of the animals^[30,33].

The kidneys regulate the excretion of urea and reabsorption of electrolytes into the blood. When there is a compromise of normal glomerular function, substances normally cleared by the kidneys such as urea and creatinine accumulate in the biological fluid. The significant increase in serum urea following the administration of the garlic may be due to the increased protein catabolism. The insignificant effects in the level of total bilirubins, Na, K and creatinine concentrations following 5 weeks administrations of garlic (*A. sativum*) bulbs extract at all doses tested (300, 600 and 1200 mg/kg) suggest that normal functioning of liver and kidney tubules as regard to these electrolytes was preserved^[39].

Organ body weight ratios are normally investigated to determine whether the size of the organ has changed related the weight of the whole animal. The absence of an effect on the computed organs/body weight ratios suggests that the extract did not cause any form of swelling, atrophy and hypertrophy on the organs^[30].

In conclusion, the extract can cause selective changes in some biochemical parameters of organ function. Therefore, since only mild alteration was observed at dose of 300 mg/kg, the garlic bulb may be considered to be relatively safe and could be explored as oral remedy at this dose.

Conflict of interest statement

The authors report no conflict of interest.

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