CAFOBIOS
Cameroon Forum for Biological Sciences

Cameroon Journal of Experimental Biology 2005 Vol. 01, N°01, 39-45.

CAJEB

Original Article

Pharmacology

Toxicological evaluation of the aqueous extract of *Allium sativum* bulbs on laboratory mice and rats

Donatien GATSING[1*], Roseline ALIYU[2], Jules R. KUIATE[1], Ibrahim H. GARBA[3], Kiri H. JARYUM[2], Nestor TEDONGMO[1], Félicité M. TCHOUANGUEP[1] and Godwin I. ADOGA[2]

1. Department of Biochemistry, Faculty of Science, University of Dschang, P.O. Box 67 Dschang, Cameroon.
2. Department of Biochemistry, Faculty of Medical Sciences, University of Jos, P.M.B. 2084 Jos, Nigeria
3. Chemistry Programme, School of Science, Abubakar Tafawa Balewa University, Bauchi, Nigeria.

*Corresponding Author, E-mail: gatsingd@yahoo.com

ABSTRACT

The possible toxicological risks of *Allium sativum* aqueous extract upon consumption were assessed in mice and rats using acute and sub-chronic treatments. 36 male Swiss albino mice were used, and the various doses administered were 0, 2, 4, 8, 16 and 32 g/kg body weight. Mice were observed for behavioural changes and for mortality. For sub-chronic study, 50 Wistar albino rats were used, and the various doses administered were 0, 75, 300, 1200 and 4800 mg/kg body weight. After four weeks of administration of the extract to rats, the serum total protein and total protein titre of organs were determined by the biuret method, whereas changes in serum transaminases were determined by the kinetic method. Haematocrit values were also determined. Results obtained showed that mice administered the extract exhibited a reduced reaction to noise, an increased social interaction, a reduced reaction to pinch and losses in body weight. The exact value of lethal dose 50 (LD_{50}) of the extract was not determined; however it is greater than 32 g/kg. Rats administered the extract also showed increased liver and spleen to body weight ratios, increases in serum total protein concentration and decreases in total protein titre of the liver and lungs. Also, increases in transaminase activities were observed in treated male and female rats, whereas marked decreases in haematocrit values were observed in the same animals. These data suggest that this extract may have a depressant effect on the central nervous system, a sedative effect, and may induce a decrease in plasma prostaglandin levels. Also, this extract, at high doses, may induce injury to liver, spleen and lungs, loss of appetite, and anaemic conditions.

Keywords: *Allium sativum*, toxicity, liver, spleen, anaemia.

RESUME

Les risques toxicologiques pouvant être liés à la consommation de l'extrait aqueux d'*Allium sativum* ont été évalués chez les souris et les rats par les tests de toxicité aiguë et sub-chronique. 36 souris albinos mâles ont été utilisés et les doses administrées ont été de 0, 2, 4, 8, 16 et 32 g/kg de poids corporel. Les changements de comportement, et la mortalité, ont été évalués chez les souris après administration de l'extrait. Pour l'évaluation de la toxicité sub-chronique, 50 rats albinos ont été utilisés, et les doses administrées ont été de 0, 75, 300, 1200 et 4800 mg/kg de poids corporel. Après quatre semaines d'administration de l'extrait aux rats, la méthode de biuret a été utilisée pour déterminer les concentrations des protéines totales sériques et des protéines totales dans les organes. Les variations de concentration des transaminases sériques ont été évaluées par la méthode cinétique. Les valeurs d'hématocrite ont aussi été déterminées. Les résultats obtenus montrent une baisse de la réaction au bruit et une augmentation de l'interaction sociale, associée à une réduction de la réaction au pincement et des pertes de poids chez les souris. La valeur exacte de la dose létale 50 (DL_{50}) de cet extrait n'a pas été déterminée; cependant elle est supérieure à 32 g/kg. Les rats ayant reçu l'extrait ont montré une augmentation des ratios foie et rate au poids corporel, une augmentation de la concentration en protéines sériques totales et une réduction de la concentration en protéines totales du foie et des poumons. Des augmentations ont été également observées dans les activités des transaminases sériques, tandis que les valeurs d'hématocrite ont été très faibles chez les rats mâles et femelles ayant reçu l'extrait. Ces résultats suggèrent que cet extrait aurait un effet dépressif sur le système nerveux central, un effet sédatif, et induirait une réduction de la concentration des prostaglandines. A des doses élevées, cet extrait de plante induirait des lésions dans le foie, la rate et les poumons, une perte d'appétit et l'anémie.

Mots clés : *Allium sativum*, toxicité, foie, rate, anémie

INTRODUCTION

Allium sativum Linn (Liliaceae) is a herbaceous plant. It is a bulb consisting of a number of bulblets or "cloves" rested on a common bulb base. *Allium sativum* (garlic) has been found to possess anticarcinogenic property [1]. *A. sativum* oil has been reported to be effective antioxidants against the oxidative damage caused by nicotine [2]. The effects of *A. sativum* on levels of serum lipids and on atherosclerosis have been investigated extensively [3]. *A. sativum* oil has been used in the partial treatment of alloxan-induced and streptozotocin-induced diabetes mellitus in rats [4,5]. Garlic has also been studied for its hypotensive and hypocholesterolaemic activities [6]. The bulb extract of *A. sativum* has also been reported to possess antimicrobial activity against some bacteria showing resistance to certain antibiotics. Greater anti-

candidal activity was shown by *A. sativum* than by nystatin [7]. Also, *A. sativum* bulb extract has been reported by Gatsing *et al.* [8] to possess antisalmonellal properties and to contain organosulfur compounds (allicin derivatives), flavonoids, cardiac glycosides, polyphenols and steroids.

The purpose of the present study was to investigate the possible side effects upon short term and long-term consumption of *A. sativum* extracts in mice and rats, using acute and sub-chronic toxicity techniques.

MATERIALS AND METHODS

Experimental animals

In this study, 36 male Swiss albino mice (11 – 12 weeks old) weighing 24 – 29 g, and 50 Wistar albino rats (25 males and 25 females, 11 – 12 weeks old) weighing 120 – 130 g, were used. These animals were bred in the animal house of the University of Dschang, Cameroon.

Plant material and preparation of extract

The bulbs of *Allium sativum* (garlic) were purchased from Dschang main market. The cloves of *A. sativum* were extracted using warm water technique, as described by Gatsing *et al.* [8]. The cloves were thoroughly ground and water was added (100 ml per 100 g of cloves); then the suspension was warmed at 45 °C for 15 minutes, with constant stirring. The mixture was filtered, using Wattman paper No 1, while still warm, and the filtrate was concentrated in a drying oven at 45 °C. The yield was about 4 g of yellowish extract per 100 g of cloves.

Animal treatment

For acute toxicity studies, 36 male Swiss albino mice were divided into 6 groups of 6 mice each. All animals were subjected to 15 hours fast prior to administration of the plant extract. Animals in groups 2, 3, 4, 5 and 6 were treated with graded doses of the plant extract, that is, 2, 4, 8, 16 and 32 g/kg body weight, respectively, while animals in group 1 served as the control group (i.e. 0 g/kg) and received distilled water (1 ml per 30 g of body weight). The animals in all the groups were observed during the first 3 hours after a single oral administration of the extract, for behavioural changes: communication, locomotion, aggressiveness, the state of excrement, the state of the tail, reaction to noise, reaction to pinch. When the mice are gathered together, it is an indicator of communication (i.e. normal social interaction); they are said to be in activity (i.e. locomotion) when they are roaming in the cage; they are said to be aggressive when to any attempt to touch them they react by biting; normal reaction to noise is when the mice are unsettled on hearing a noise; the cries of mice when pinched on their tail is an indicator of normal reaction to pinch; the tail is normal when it is flexible (i.e. not rigid); a rigid tail is a sign of anger. After the first 3 hours of observation, all the animals had free access to food and water. The deaths were counted within the first 48 hours; the surviving animals were further observed for one week, after which their weights were recorded.

For sub-chronic toxicity studies, 50 Wistar albino rats (25 males and 25 females) were used. The rats in each sex group were divided into 5 subgroups of 5 animals each. Rats in subgroup 1 (controls) were given distilled water (1.5 ml per 200 g of body weight), while rats in subgroups 2, 3, 4 and 5 were given 75, 300, 1200 and 4800 mg/kg doses of the crude extract, respectively. These doses were calculated from the therapeutic dose (i.e. 300 mg/kg) derived from the minimum bactericidal concentration (4 mg/ml) of *A. sativum* bulb extract, as reported by Gatsing *et al.* [8]. The administration of the various doses, and distilled water, were done by gastric gavage once in two days, for four consecutive weeks. All the animals had free access to food and water. At the end of each week the food and water intakes were evaluated and the animals were weighed.

Collection of blood and isolation of organs during sub-chronic toxicity study

At the end of the treatment period, the rats were anaesthetized using chloroform vapour prior to dissection. Blood was collected by cardiac puncture into heparinised tubes for haematocrit and into non-heparinised tubes for serum transaminases (ALT and AST) and total protein. During the dissection the organs, namely liver, heart, kidneys, lungs and spleen were excised, weighed (using an electronic balance, Mettler PE 160) and stored at –30 °C for protein titre determination.

Preparation of serum sample

The blood was allowed to clot by standing at room temperature for one hour and then refrigerated for another one hour. The resultant clear part was centrifuged at 3000 x g for 10 min, then the serum (supernatant) was isolated and stored at –30 °C until required for analysis.

Preparation of tissue homogenates

The homogenate of each organ was prepared in 0.9% NaCl solution at the concentration of 15% (i.e. 15g of organ in 100 ml of solution).

Some indices of tissue damage

Possible damage to the liver, kidneys, heart, lungs, spleen and red blood cells of mammals as a result of repeated administration of the aqueous extract of *A. sativum* was studied using some biochemical indices of tissue damage. Total protein titres of the above-mentioned organs and serum were determined by the biuret method, as described by Gornall *et al* [9]; serum transaminase (ALT and AST) activities were determined by the kinetic method, using the commercial kit of Human Gesellschaft für Biochemica und Diagnostica mbH, Germany; haematocrit values were determined using the packed cell volume to

whole blood volume ratio method, as described by OMS [10].

Statistical analysis

Statistical analyses were performed with the aid of SPSS for Windows software programme (Release 10.0). Group comparisons were done using the Student's t-test and One-way ANOVA. A p value of < 0.05 was considered statistically significant.

RESULTS

Acute toxicity

The behavioural changes observed during acute treatment are summarised in Table 1. The mice were observed for social interaction (communication), activity (locomotion), aggressiveness, reaction to noise, reaction to pinch, state of the tail, state of the excrement, and for mortality (within 48 hours). After the administration of the various doses of the crude *A. sativum* extract, the mice in groups 2 (2 g/kg), 3 (4 g/kg) and 4 (8 g/kg) gathered together (i.e. normal social interaction, reduced activity) whereas mice in group 1 (control, i.e. 0 g/kg) were still roaming in the cage. The social interaction increased in groups 5 (16 g/kg) and 6 (32 g/kg) (no activity). Generally, mice receiving the extract were not aggressive. The reaction to noise was reduced in groups 4 and 5, and profoundly reduced in group 6. Also, reaction to pinch was reduced in groups 2, 3 and 4, and profoundly reduced in groups 5 and 6. The mice in all the groups had normal tail (flexible) and granular excrement. No mortality was observed within 48 hours after administration of the extract. The lethal dose 50 (LD_{50}) of this extract was greater than 32 g/kg, since up to this dose no death was recorded.

The weight variations (Δw) of the surviving mice recorded one week after the administration of plant extract are presented in Table 2. From this Table, it can be observed that the Δw carries a negative sign in all the treated groups, suggesting that the animals in these groups lost weight in the process, as compared to their initial weight and to that of the control group. The Δw in groups 2 and 3 was significantly ($p < 0.05$) decreased as compared to control values. Also, statistically significant ($p < 0.05$) decrease was seen in the Δw of groups 4 and 5. There was also a significant ($p < 0.01$) decrease in the Δw of group 6, as compared to control.

Table 1 : Behaviour of mice during the first 3 hours of observation in acute toxicity studies with *A sativum* extract.

Parameters	Doses (g/kg) and Behaviour of Animals					
	0	2	4	8	16	32
Social interaction	-	+	+	+	++	++
Activity	+	-	-	-	--	--
Aggressiveness	+	+	+	+	+	+
Reaction to noise	+	+	+	-	-	--
Reaction to pinch	+	-	-	-	--	--
State of the tail	+	+	+	+	+	+
State of the excrement	g	g	g	g	g	g
Mortality (within 48 hours)	NM	NM	NM	NM	NM	NM

Key: + = normal; ++ = increased; - = reduced; -- = profoundly reduced; g = granular; NM = no mortality

Table 2 : Weight variation of mice as affected by doses of *A. sativum* extract during acute toxicity.

Dose (g/kg)	Weight Variation (g)
0	0.93 ± 0.25
2	-1.84 ± 1.49^{c}
4	-1.58 ± 1.07^{c}
8	-2.58 ± 0.96^{c}
16	-2.26 ± 0.66^{c}
32	-2.92 ± 0.82^{b}

All the tabulated values are Mean $\pm$ SD of six determinations.
Key: b: $p < 0.01$ vs control; c: $p < 0.05$ vs control.

Sub-chronic toxicity

The quantities of food and water taken by rats (male and female) during the four weeks of sub-chronic toxicity studies are presented in Tables 3 and 4, respectively. In the male and female groups treated with *A. sativum* aqueous extract, the food and water intakes generally decreased with the increase in the dose of the extract, throughout the four weeks of study as compared to control (group administered distilled water alone).

As can be seen from Table 5, total weight gain generally decreased in both male and female groups

treated with this extract during the four weeks of study, as compared to controls.

The results of the effects of *A. sativum* aqueous extract on organ to body weight ratios of both male and female rats are summarised in Table 6. The organs studied were heart, liver, lungs, kidneys and spleen. For the male rats treated with this extract, the liver and spleen to body weight ratios increased. The liver to body weight ratio was significantly ($p < 0.05$ and $p < 0.01$) increased in male groups treated with 1200 mg/kg and 4800 mg/kg, respectively. Also, the spleen to body weight ratio was significantly ($p < 0.05$ and $p < 0.001$) increased in male groups treated with 1200 mg/kg and 4800 mg/kg, respectively, as compared to control. In the female groups treated with this extract, the same pattern of increase in liver and spleen to body weight ratios as that of male rats was seen in rats receiving 1200 mg/kg and 4800 mg/kg, as compared to control.

Table 3: Food intake as affected by doses of *A. sativum* extract during four weeks of administration to rats.

Sex of Animal	Dose (mg/kg)	Time and Food Intake (g)			
		Week 1	Week 2	Week 3	Week 4
Males	0	165.31 ± 7.23	149.55 ± 8.44	100.47 ± 6.21	103.95 ± 11.22
	75	160.13 ± 4.45	139.61 ± 5.61	84.62 ± 4.11^c	93.88 ± 7.34
	300	151.05 ± 2.12^c	136.08 ± 2.80^c	84.07 ± 13.31	89.07 ± 4.56
	1200	122.15 ± 4.55^a	117.11 ± 4.00^b	79.12 ± 11.71^c	79.76 ± 5.08^c
	4800	105.06 ± 5.14^a	103.66 ± 6.44^a	70.43 ± 8.86^b	71.69 ± 5.49^b
Females	0	145.36 ± 5.56	140.67 ± 6.17	100.13 ± 4.40	100.77 ± 6.10
	75	142.05 ± 2.33	140.00 ± 3.13	94.68 ± 7.31	96.69 ± 3.78
	300	131.35 ± 4.36^c	128.11 ± 3.08^c	85.51 ± 5.22^c	86.06 ± 4.54^c
	1200	112.65 ± 7.47^b	107.12 ± 4.64^b	83.26 ± 5.53^c	83.47 ± 2.56^c
	4800	102.13 ± 4.00^a	102.05 ± 5.00^a	76.38 ± 6.65^c	77.21 ± 2.94^b

Tabulated values are Mean ± SD of five determinations.

Key: a: $p < 0.001$ vs control; b: $p < 0.01$ vs control; c: $p < 0.05$ vs control.

Table 4: Water intake as affected by doses of *A. sativum* extract during four weeks of administration to rats.

Sex of Animal	Dose (mg/kg)	Time and Water Intake (ml)			
		Week 1	Week 2	Week 3	Week 4
Males	0	116.54 ± 4.31	109.94 ± 8.62	111.56 ± 5.09	112.65 ± 6.11
	75	105.45 ± 5.42^c	100.37 ± 6.48	101.38 ± 6.69	103.41 ± 5.03
	300	102.95 ± 9.40	95.45 ± 4.35^c	96.65 ± 7.08^c	96.85 ± 4.03^c
	1200	90.32 ± 6.05^b	88.71 ± 9.21^c	86.44 ± 6.95^c	87.76 ± 3.15^b
	4800	82.23 ± 4.00^a	81.38 ± 8.45^c	75.03 ± 2.16^a	79.89 ± 6.39^b
Females	0	113.03 ± 8.36	105.03 ± 3.41	114.04 ± 10.31	112.65 ± 9.25
	75	103.35 ± 6.04	96.05 ± 3.04^c	95.56 ± 9.90	104.36 ± 5.66
	300	99.14 ± 2.33^c	90.57 ± 4.65^c	88.45 ± 5.40^c	92.81 ± 6.12^c
	1200	90.44 ± 3.16^c	90.04 ± 4.30^c	85.55 ± 3.11^b	89.69 ± 4.09^c
	4800	84.51 ± 4.56^b	85.65 ± 5.15^c	81.05 ± 4.73^b	86.12 ± 4.13^c

Tabulated values are Mean ± SD of five determinations.

Key: a: $p < 0.001$ vs control; b: $p < 0.01$ vs control; c: $p < 0.05$ vs control.

The results of the effects of *A. sativum* aqueous extract on the total protein titre of organs, for both male and female rats, are presented in Table 7. For the male rats treated with the extract at 4800 mg/kg significant ($p < 0.05$) decreases were observed in the total protein titre of the liver and lungs, as compared to control. The total protein titre of the other organs (i.e. heart, kidneys and spleen) of the treated rats generally increased, as compared to control. For the female rats treated with the same extract, significant decreases were observed in the total protein titres of the liver ($p < 0.001$) and lungs ($p < 0.05$), in the groups receiving 1200 mg/kg and 4800 mg/kg, as compared to control.

The results of the effects of *A. sativum* extract on the haematocrit values, serum total proteins and serum

transaminases (AST, ALT), are summarised in Table 8. For both male and female rats, significant ($p < 0.01$ and $p < 0.001$) decreases in haematocrit values were observed in the groups treated with 1200 mg/kg and 4800 mg/kg, respectively, as compared to control. Serum total protein concentration significantly increased in male ($p < 0.05$ and $p < 0.001$) and female ($p < 0.01$ and $p < 0.001$) rats receiving 1200 mg/kg and 4800 mg/kg, as compared to control. The serum activity of ALT showed significant ($p < 0.01$) increases in both male and female rats treated with the extract at 4800 mg/kg, as compared to control. Also, the serum activity of AST showed significant ($p < 0.01$ and $p < 0.05$) increases in male and female rats, respectively, receiving 4800 mg/kg, as compared to the control values.

Table 5: Total weight gain as affected by doses of *A. sativum* extract during four weeks of administration to rats.

Sex of Animal	Dose (mg/kg)	Time and Total Weight Gain (g)			
		Week 1	Week 2	Week 3	Week 4
Males	0	37.31 ± 4.45	60.13 ± 5.45	71.90 ± 12.05	75.47 ± 9.55
	75	32.45 ± 3.94	52.65 ± 5.67	61.85 ± 6.06	66.21 ± 7.61
	300	26.38 ± 2.57[c]	43.35 ± 4.41[c]	50.35 ± 4.31[c]	53.41 ± 14.68
	1200	26.01 ± 2.61[c]	45.41 ± 3.45[c]	50.01 ± 5.41[c]	52.85 ± 3.41[b]
	4800	22.51 ± 3.15[b]	43.02 ± 3.55[b]	47.21 ± 3.15[b]	51.76 ± 5.81[b]
Females	0	29.35 ± 3.16	47.91 ± 6.00	59.46 ± 3.19	67.35 ± 6.33
	75	28.07 ± 3.55	42.36 ± 2.41	54.31 ± 3.22	60.85 ± 4.28
	300	25.65 ± 4.66	38.08 ± 4.54	48.55 ± 8.91	53.25 ± 7.91
	1200	20.08 ± 2.31[c]	31.11 ± 2.53[b]	41.06 ± 5.31[b]	44.09 ± 3.25[a]
	4800	16.69 ± 4.41[c]	24.19 ± 5.02[a]	32.85 ± 4.43[a]	35.12 ± 4.66[a]

Tabulated values are Mean ± SD of five determinations.
Key: a: $p < 0.001$ vs control; b: $p < 0.01$ vs control; c: $p < 0.05$ vs control.

Table 6: Organ to body weight ratios as affected by doses of *A. sativum* extract after four weeks of administration to rats.

Sex of Animal	Dose (mg/kg)	Organ to Body Weight Ratios (g/100g)				
		Heart	Liver	Lungs	Kidneys	Spleen
Males	0	0.317 ±0.027	3.311 ±0.251	0.622 ±0.041	0.596 ±0.014	0.184 ±0.021
	75	0.316 ±0.031	3.214 ±0.305	0.624 ±0.040	0.578 ±0.030	0.187 ±0.022
	300	0.317 ±0.020	3.415 ±0.423	0.636 ±0.035	0.555 ±0.035	0.190 ±0.035
	1200	0.313 ±0.018	3.847 ±0.200[c]	0.611 ±0.038	0.564 ±0.031	0.245 ±0.021[c]
	4800	0.312 ±0.034	3.985 ±0.173[b]	0.657 ±0.052	0.590 ±0.017	0.268 ±0.026[a]
Females	0	0.363 ±0.044	3.050 ±0.241	0.643 ±0.052	0.601 ±0.025	0.196 ±0.020
	75	0.363 ±0.038	3.072 ±0.252	0.643 ±0.049	0.581 ±0.021	0.202 ±0.031
	300	0.362 ±0.040	3.216 ±0.205	0.645 ±0.041	0.587 ±0.032	0.235 ±0.030
	1200	0.356 ±0.024	3.665 ±0.121[b]	0.664 ±0.082	0.605 ±0.041	0.250 ±0.014[c]
	4800	0.349 ±0.058	3.787 ±0.232[b]	0.656 ±0.064	0.595 ±0.035	0.261 ±0.017[b]

Tabulated values are Mean ± SD of five determinations.
Key: a: $p < 0.001$ vs control; b: $p < 0.01$ vs control; c: $p < 0.05$ vs control.

DISCUSSION

Generally, acute toxicity studies did not reveal any grossly negative behavioural changes such as excitement, restlessness, respiratory distress, convulsions or coma in the mice administered the various doses of *A. sativum* extract. Moreover, no mortality was recorded with this extract. However, a reduced reaction to noise was observed, suggesting that the extract may have a depressant effect on the central nervous system. The increased social interaction observed at doses 16 g/kg and 32 g/kg suggest that this extract may have a sedative or tranquillising effect. The administration of this extract to mice caused a reduced reaction to pinch. This decreased sensitiveness may be due to decrease in prostaglandin levels, since prostaglandins have been reported to function in regulating the perception of pain [11]. Prostaglandins are not stored, and their release is dependent on biosynthesis [12]. Evidently, various medications that prevent the perception of pain inhibit the conversion of arachidonic acid by inhibiting the release of prostaglandin synthetase or by interfering in some other way with the synthesis of prostaglandins [12].

It was also observed during our study that *A. sativum* extract caused significant loss in body weight to mice

administered the various doses (2, 4, 8, 16 and 32 g/kg). This reduction in weight may be due to less food and water intake, which may be secondary to a feeling of fullness and loss of appetite after administration of the extract [13]. It may also be due to the triglyceride-lowering effect of garlic extract [14]. In spite of the above side effects, the very high values of the LD_{50} (i.e. more than 100 times the therapeutic dose) make the extract of *A. sativum* practically non-toxic.

Table 7: Total protein titre of organs as affected by doses of *A. sativum* extract after four weeks of administration to rats

Sex of Animal	Dose (mg/kg)	Total Protein Titre of Organ (mg/g)				
		Heart	Liver	Lungs	Kidneys	Spleen
Males	0	70.15 ±11.41	135.85 ±3.10	124.61 ±4.16	122.15 ±6.35	97.51 ±6.41
	75	84.45 ±4.13	136.86 ±4.11	127.91 ±4.00	126.13 ±4.65	109.31 ±7.85
	300	97.37 ±5.65[c]	137.95 ±2.61	134.46 ±7.31	153.65 ±12.65[c]	110.48 ±10.86
	1200	102.61 ±7.61[c]	130.05 ±4.54	117.07 ±5.13	143.35 ±11.31[c]	145.61 ±10.35[a]
	4800	99.74 ±7.84[c]	124.13 ±2.71[c]	110.12 ±3.61[c]	148.45 ±9.05[c]	154.30 ±8.52[a]
Females	0	68.35 ±5.63	214.97 ±4.32	129.48 ±6.63	156.43 ±2.36	160.41 ±10.01
	75	77.41 ±4.67	221.33 ±7.41	129.51 ±4.05	172.39 ±4.90[c]	157.35 ±8.31
	300	89.36 ±7.31[c]	217.56 ±5.85	133.87 ±3.85	176.45 ±6.81[c]	178.31 ±5.03[c]
	1200	89.65 ±5.61[c]	188.15 ±3.35[b]	120.04 ±2.00[c]	176.88 ±4.87[c]	179.25 ±4.98[c]
	4800	97.88 ±3.45[b]	173.22 ±4.04[a]	115.13 ±3.19[c]	185.77 ±5.95[b]	175.35 ±2.06[c]

Tabulated values are Mean ± SD of five determinations.
Key: a: $p < 0.001$ vs control; b: $p < 0.01$ vs control; c: $p < 0.05$ vs control.

Table 8: Effects of doses of *A. sativum* extract on some biochemical parameters after four weeks of administration to rats.

Sex of Animal	Dose (mg/kg)	Biochemical Parameters			
		Serum total protein (g/dl)	Serum ALT (U/L)	Serum AST (U/L)	Haematocrit (%)
Males	0	4.03 ± 1.10	25.61 ± 2.32	24.89 ± 1.46	48.67 ± 1.21
	75	4.41 ± 0.81	26.63 ± 1.72	23.61 ± 1.67	48.71 ± 1.0
	300	4.92 ± 0.50	24.85 ± 2.01	23.37 ± 2.60	46.03 ± 1.91
	1200	6.47 ± 0.76 [c]	25.87 ± 0.84	25.01 ± 3.20	37.00 ± 1.53 [b]
	4800	8.85 ± 1.31 [a]	35.46 ± 3.21 [b]	29.86 ± 1.4 [b]	32.00 ± 0.40 [a]
Females	0	3.92 ± 0.63	18.02 ± 1.47	15.75 ± 2.15	45.32 ± 2.24
	75	3.93 ± 0.74	18.33 ± 3.07	15.75 ± 1.08	46.22 ± 1.16
	300	5.04 ± 0.91	17.71 ± 1.62	17.07 ± 1.50	44.17 ± 0.75
	1200	6.83 ± 0.35 [b]	20.55 ± 3.13	16.34 ± 2.02	36.06 ± 1.0 [b]
	4800	8.41 ± 0.68 [a]	27.53 ± 2.12 [b]	19.76 ± 0.71 [c]	31.32 ± 0.43 [a]

Tabulated values are Mean ± SD of five determinations.
Key: a: $p < 0.001$ vs control; b: $p < 0.01$ vs control; c: $p < 0.05$ vs control.

In the sub-chronic toxicity studies, significant decreases in total weight gain were observed in rats administered the extract of *A. sativum*, as compared to controls. This reduction in total weight gain may be due to less food and water intake after administration of *A. sativum* extract, as also reported by Joseph *et al* [13]. The reduction in total weight gain may also be due to the anti-lipidaemic effect of *A. sativum* extract [15]. The rats administered this extract at the doses of 1200 and 4800 mg/kg exhibited increases in serum total protein concentration on the one hand, and decreases in total protein titre of the liver and lungs on the other hand. These data suggest liver and lungs injury. In fact it has been reported that enhancement in the level of serum proteins is an indication of tissue injury and that significant decrease in protein contents of the liver is a reflection of hepatic toxicity [16].

The enzymes AST and ALT are concerned with amino acid metabolism. Large amounts of AST are present in the liver, kidneys, cardiac muscle and skeletal muscle. Small amounts of the enzyme are present in the brain, pancreas and lungs. ALT is found principally in the liver [17]. The serum or plasma levels of both AST and ALT rise whenever there is liver cell damage. The higher the activities of both enzymes the greater the degree of liver damage [17]. In the present study, significant increases in ALT and AST activities were observed in male and female rats receiving *A. sativum* extract at 4800 mg/kg. The increase in the serum activities of these enzymes (ALT and AST) indicates that this extract, at high doses, may have significant cytotoxic effect on the liver. The extract could

affect the permeability of the cell membrane causing the membrane to become leaky. This would then induce the release of these enzymes from the cell into the blood thereby causing the subsequent plasma or serum elevation of the enzymes [16].

Anaemia is the condition of having too few red blood cells or too little haemoglobin to transport oxygen to tissues. At the biochemical level, anaemia is identified and classified by measurements of several parameters, most importantly haemoglobin (the oxygen-carrying protein in the blood) and haematocrit (the percent of red blood cells per standardised volume of blood). Anaemia is present when these numbers fall below the cut-offs [18]. Normal values for haematocrit range from 35% to 50% in human [19]. In the present study, significant decreases in haematocrit values were observed in male and female rats treated with *A. sativum* extract at 4800 mg/kg, as compared to both the controls and the normal range. These abnormal values (32.00 ± 0.40% for males, and 31.32 ± 0.43% for females) may be indicative of anaemic conditions. The presence of steroid saponins in the crude extract of *A. sativum* [20], in addition to low food and water intake, may account for these signs of anaemia. Saponins possess haemolytic activity which varies from strong to weak depending on the type of substituents on the flavonoid ring.

In the light of the foregoing, it appears that *A. sativum* extract, at high doses, may induce injury to liver, spleen and lungs, loss of appetite leading to weight loss, and anaemic conditions.

ACKNOWLEDGEMENTS: We wish to express our gratitude to Dr Telesphore B. Nguelefack (Department of Animal Biology, FS, University of Dschang, Cameroon) and Mr Minkame Nicephor (Zootechny Laboratory, FASA, University of Dschang, Cameroon), for their co-operation.

REFERENCES

1. Balasenthil S., Arivazhagan S., Ramachandran C.R. and Nagini S. 1999. Effects of garlic on 7, 12-dimenthylbenzazanthracene-induced hamster buccal pouch carcinogenesis. *Cancer Detection and Prevention* **23** (6): 534-538.
2. Helen A., Rajasree C.R., Krishnakumar K., Augusti K.T. and Vijayammal P.L. 1999. Antioxidant role of oils isolated from garlic (Allium sativum Linn) and onion (Allium cepa Linn) on nicotine-induced lipid peroxidation. *Veterinary and Human Toxicology* **41** (5): 316 - 319.
3. Abramovitz D., Gavri S., Harats D., Levkovitz H., Mirelman D., Miron T., Eilat-Adar S., Rabinkov A., Wilchek M., Eldar M, and Vered Z. 1999. Allicin-induced decrease in formation of fatty streaks (atherosclerosis) in mice fed with a cholesterol-rich diet. *Coronary Artery Disease* **10** (7): 515-519.
4. Adoga G.I. 1990. Effects of garlic oil on some plasma enzymes in streptozotocin-induced diabetic rats. *Medical Science Research* **18**: p. 523.
5. Adoga G.I. and Ohaeri 1991. Effect of garlic oil on prothrombin, thrombin and partial thromboplastin times in streptozotocin-induced diabetic rats. *Medical Science Research* **19**: 407 - 408.
6. Miller L.G. 1998. Herbal medicinals. *Archives of Internal Medicine* **158**: 2200-2211.
7. Arora D.S. and Kaur J. 1999. Antimicrobial activity of spices. *International Journal of Antimicrobial Agents* **12** (3): 257-262.
8. Gatsing D., Aliyu R., Meli W.B., Adoga G.I. and Tchouanguep M.F. 2003. Phytochemical profile and antisalmonellal properties of *Allium sativum* bulb extract. *West African Journal of Biological Sciences* **14**: 29 – 36.
9. Gornall A.G., Bardawill C.J., and Maxima D. 1949. Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry* **177**: 751-766.
10. OMS. 1982. *Manuel des Techniques de Base pour le Laboratoire Médical*. Genève: Organisation Mondiale de la Santé. 487 p.
11. Lehninger L.A. 1982. *Principles of Biochemistry*. New York: Worth Publishers, Inc. 1011p.
12. Eisenhauer L., Nichols W.L., Spencer T.R. and Bergan W.F. 1998. *Clinical Pharmacology and Nursing Management*. Philadelphia, New York. Lippincott: 779-809.
13. Joseph P.K., Rao K.R. and Sundaresh C.S. 1989. Toxic effects of garlic extract and garlic oil in rats. *Indian Journal of Experimental Biology* **27**: 977-979.
14. Kyo E., Uda N., Kasuga S., Itakura Y. and Sumiyoshi H. 1999. Garlic as an immuno-stimulant. In: H. Wagner (ed). *Immuno-modulatory Agents from Plants*. Basel, Boston, Berlin: Birkhäuser Verlag Basel: 273-288.
15. Arora R.C. and Arora S. 1981. Comparative effect of clofibrate, garlic and onion on alimentary hyperlipidemia. *Atherosclerosis* **39** (4): 447-452.
16. Emerson F.S., Shadara A.C. and Devi P.U. 1993. Toxic effects of crude extract of *Plumbago rosea* (Rokta chitraka). *Journal of Ethnopharmacology* **38**: 79-84.
17. Cheesbrough M. 1991. *Medical Laboratory Manual for Tropical Countries*. 2nd Ed. ELBS. **1**: 605 p.
18. MIP. 2002. *The Role of Vitamins in the Prevention and Control of Anemia*. Basel: Roche Vitamins Ltd. 22 p.
19. Alexander R.R. and Griffiths J.M. 1993. *Basic Biochemical Methods*. 2nd Ed. WILEY-LISS, Inc. 353 p.
20. Hiromichi M. 2001. Saponins in garlic as modifiers of the risk of cardiovascular disease. *Journal of Nutrition* **131** (35): 10005-10055.