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TOXICOLOGICAL STUDIES OF THE AQUEOUS LEAF EXTRACT OF *CASSIA PETERSIANA* BOLLE (CAESALPINIACEAE), AN ANTITYPHOID MEDICINAL PLANT

Donatien Gatsing [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, PMB 2084 Jos, Nigeria

***Corresponding author e-mail:** gatsingd@yahoo.com

ABSTRACT

Typhoid fever continues to be a serious health problem in developing countries. In our search for therapeutic agents from natural sources with potential for the treatment of typhoid fevers, an anti-typhoid aqueous extract was obtained from the leaves of *Cassia petersiana* and tested for possible toxicity risks. Toxicological study was done in mice and rats using both acute and sub-chronic techniques. Results obtained showed that, at high doses, the extract may have a depressant effect on the central nervous system, and may induce hypersensitiveness. The LD_{50} (lethal dose 50) values of the extract were 20 g/kg and 22 g/kg, for male and female mice, respectively. Rats administered *C. petersiana* extract showed significantly increased kidneys and liver to body weight ratios, increased serum total protein, and a decrease in total protein titre of the liver at high doses. Also, significant increases in serum transaminase (ALT and AST) activities were observed at high doses. Significant decreases in serum total protein and urinary protein were seen in male and female rats administered the extract at 75 mg/kg. However, urinary protein showed a significant increase in male rats treated at 4800 mg/kg. Significant increases in haematocrit values were observed in male and female rats receiving the dose 300 mg/kg. These data suggest that this extract, at moderate doses, may be used without any toxicity risk, and besides may have strengthening effects on the liver and kidneys. However, at high doses, it may create injury to the liver and kidneys.

Keywords: Strengthening effects, liver, kidneys, sedative, hypersensitiveness.

INTRODUCTION

Cassia petersiana Bolle is a tree belonging to Caesalpiniaceae family; it grows on sandy soils, and has pinnate green leaves and yellow flowers (Gatsing *et al.*, 2007). The plant roots have for long been used in folk medicine for the treatment of cough, syphilis, stomach ache and helminthic infections (Djemgou *et al.*, 2007). Aqueous and organic fractions from *C. petersiana* have been found to show *in vitro* antimalarial activity against the multi-drug-resistant strain of *Plasmodium falciparum* (Connelly *et al.*, 1996). Antitumor and immunostimulatory activities have been observed with chromones and other constituents isolated from the leaves of *C. petersiana* (Djemgou *et al.*, 2006). In some parts of Africa (e.g. Nigeria, Cameroon), decoctions of the leaves of *C. petersiana* are used for the treatment of typhoid fevers. Also, *C. petersiana* aqueous leaf extract has been reported by Gatsing and Adoga (2007) to show antibacterial activity against *Salmonella typhi*, *Salmonella paratyphi* A and *Salmonella paratyphi* B, the causative agents of typhoid, paratyphoid A and paratyphoid B fevers, respectively. Besides, phytochemical screening has revealed the presence of classes of compounds such as polyphenols, saponins, tannins, anthraquinones, anthocyanins, steroids and phlobatannins in this leaf extract of *C. petersiana*.

Despite the various biological activities shown, no work has been done on the toxicological effects of the leaf extract of *Cassia petersiana*. The present study was undertaken to investigate the possible side effect upon short-term and long-term consumption of *C. petersiana* aqueous leaf extract in mice and rats, using acute and sub-chronic toxicity techniques.

MATERIALS AND METHODS

Experimental animals: In this study, 36 male and 36 female Swiss albino mice (11–12 weeks old) weighing 23–30 g, and 50 Wistar albino rats (25 males and 25 females, 8–9 weeks old) weighing 118–125 g, were used. These animals were bred in the animal house of the University of Dschang, Cameroon.

Plant sample and preparation of extract: The leaves of *Cassia petersiana* were collected from the garden of the University of Jos main campus, Jos, Nigeria, in April 2004. The plant sample was identified by Mr. Kareem I.A, a plant taxonomist at the Federal College of Forestry, Jos, Nigeria, and was further authenticated by Dr. Onana at the Cameroon National Herbarium, Yaoundé, where a voucher specimen (N° 6494/SFR/Cam) is deposited. The leaves of *C. Petersiana* were air-dried, pulverized, and the powder sample (500 g) was soaked in 2.5 l of water, boiled for 15 minutes, and filtered. The filtrate was concentrated in a drying oven at 45 °C, and the yield was 8.35%.

Animal treatment: For acute toxicity studies, 36 male and 36 female Swiss albino mice were divided into 6 groups of 6 mice each, in each sex. All animals were subjected to 15 hours fast prior to administration of the plant extract. In each sex, 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, reactivity, 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 reactive 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. The LD_{50} values were determined by calculation, using the formula described by Behrens and Karber (1983), as also reported by Bidie *et al.* (2010):

$$LD_{50} = LD_{100} - \frac{\sum (z\ x\ d)}{n},$$

where, DL_{50} is the dose that kills 50% of animals in a group; DL_{100} is the dose that kills 100% of animals in a group; z is the half-sum of animals died in two groups corresponding to two consecutive doses; d is the difference between the doses of the two consecutive groups; n is the number of mice in each group.

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. 75 mg/kg) derived from the minimum inhibitory concentration (1.0 mg/ml) of *C. Petersiana* leaf extract, as reported by Gatsing *et al.* (2007b). The administration of the various doses, and distilled water, were done by gastric intubation 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 subjected to 15 hours fast after which the urine was collected, and they were anaesthetized using ether vapour, quickly brought out of jar and dissected. Sample collection was done between 8-10 am. Blood was collected by cardiac puncture into heparinised tubes for haematocrit and into non-heparinised tubes for serum transaminases [ALT (alanine aminotransferase) and AST (aspartate aminotransferase)] 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. 15 g of organ in 100 ml of solution) (Gatsing *et al.*, 2005).

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.* (1949); urinary protein concentration (proteinuria) was determined by the protein dye-binding micro-assay technique of Bradford (1976); 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 (1982).

Statistical analysis: The data were expressed as mean ± SD and analyzed by One-way ANOVA, followed by Waller Duncan test to determine significant differences between groups at $p < 0.05$.

Ethics: This work was carried out with respect for the welfare of animals, as recommended by WHO (1992).

RESULTS

In general, acute toxicity study did not show any negative behavioural changes at low doses of extract. Also, the values of LD_{50} obtained for both male and female mice make the *C. petersiana* aqueous leaf extract practically non toxic. Moreover, it appears from Sub-chronic toxicity study that, at moderate doses, this extract is not toxic, and besides may strengthen the liver and kidneys. However, at high doses, the extract may damage the liver and kidneys.

Acute toxicity: The behavioural changes observed during acute treatment are summarised in Table 1. the mice were observed for locomotion (activity), reaction to noise, reaction to pinch, state of the tail, state of the excrement, and for mortality (within 48 hours). For both male and female animals, after the administration of the various doses of the crude *C. petersiana* extract, the mice in groups 4 (8 g/kg), 5 (16 g/kg) and 6 (32 g/kg) gathered together (i.e. reduced activity). Reaction to noise was normal (i.e. the noise scattered the mice) in groups 2 (2 g/kg), 3 (4 g/kg) and 4 (8 g/kg), while it was reduced in group 5 (16 g/kg) and profoundly reduced in group 6 (32 g/kg), as compared to that in group 1 (control). Reaction to pinch was increased in group 4 and profoundly increased in groups 5 and 6 (i.e. on a pinch the mice cried out). The state of the tail was normal. The excrement was liquid in groups 2 and 3, and less granular in group 4, as compared to that in groups 1, 5 and 6 which was granular and solid. For males, two and six mice died in groups 5 and 6, respectively, whereas for females, one mouse and six mice died in groups 5 and 6, respectively, within 48 hours after administration of extract. Therefore, the LD_{50} (dose of the extract killing 50% of mice) values of the extract were 20 g/kg and 22 g/kg, for male and female mice, respectively.

The weight variation (Δw) of the surviving male and female mice recorded one week after the administration of *C. petersiana* extract are presented in Table 2. From this table, it can be seen that for the groups receiving the extract, statistically significant ($p < 0.05$) decreases in Δw were observed in groups 4 (2.60 ± 0.12 and 2.32 ± 0.20 g for males and females respectively) and 5 (2.38 ± 0.34 and 2.15 ± 0.16 g for males and females respectively) as compared to groups 1 (control) (3.06 ± 0.14 and 2.84 ± 0.13 g for males and females respectively), 2 and 3.

Sub-chronic toxicity: The quantities of food and water taken by rats during the four weeks of sub-chronic toxicity studies are presented in Tables 3 and 4, respectively. In both male and female groups treated with *C. petersiana* aqueous leaf extract there was a general increase in food intake throughout the period of study, as compared to control values (e.g. 181.39 ± 8.80 g for male receiving 4800 mg/kg in week 1, and 152.00 ± 4.99 g for the control) . For the male rats treated with the extract, water intake showed significant ($p < 0.05$) increases in weeks 1 (104.98 ± 8.04 ml), 2 (127.88 ± 2.89 ml) and 3 (138.94 ± 2.29 ml), respectively, for the group receiving the dose of 4800 mg/kg, as compared to control values (88.88 ± 6.91, 104.56 ± 9.29 and 85.36 ± 8.18 ml in weeks 1, 2 and 3, respectively). For the female rats treated with the extract, water intake was

found to be significantly ($p < 0.05$) increased in weeks 1 (114.94 ± 4.05 ml), 2 (128.80 ± 4.93 ml), 3 (113.50 ± 7.31 ml) and 4 (97.48 ± 4.90 ml), respectively, for the group receiving 75 mg/kg, as compared to control (90.74 ± 5.16, 110.90 ± 9.08, 90.54 ± 5.12 and 80.02 ± 9.00 ml in weeks 1, 2, 3 and 4, respectively). Total weight gain generally increased during the four weeks of study, for both male (e.g. 39.02 ± 6.18 and 87.43 ± 6.96 g in weeks 1 and 4 respectively, for rats receiving 4800 mg/kg, against 26.72 ± 2.56 and 64.94 ± 9.25 g respectively, for control) and female (e.g. 45.12 ± 5.55 and 60.72 ± 6.89 g in weeks 2 and 3 respectively, for rats receiving 4800 mg/kg, against 35.14 ± 2.33 and 42.62 ± 2.34 g respectively, for control) rats treated with the extract (Table 5).

The results of the effects of *C. petersiana* aqueous leaf 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 the extract the kidneys to body weight ratio was significantly ($p < 0.05$) increased in the group receiving 4800 mg/kg (0.631 ±0.016 g/100g), as compared to control (0.580 ±0.029 g/100g). For the female rats treated with the extract the liver to body weight ratio was significantly ($p < 0.05$) increased in the group treated with 1200 mg/kg (3.590 ±0.346 g/100g), as compared to control (2.857 ±0.157 g/100g).

The results of the effects of the extract on the total protein titre of organs, for both male and female rats, are presented in Table 7. In the male groups treated with *C. petersiana* aqueous leaf extract at the various doses (75, 300, 1200 and 4800 mg/kg) the total protein titre generally and significantly ($p < 0.05$) increased in all the organs studied and with all the doses, as compared to controls. However, the increase was not dose dependent, and the highest increase was seen at 300 mg/kg for the heart (103.84 ±4.38 mg/g), liver (234.23 ±10.33 mg/g) and spleen (165.77 ±18.19 mg/g), 1200 mg/kg for the kidneys (192.10 ±6.85 mg/g), and 4800 mg/kg for the lungs (183.26 ±3.55 mg/g). For the female rats, significant ($p < 0.05$) decreases were observed in the total protein titre of the liver in the groups treated with 1200 mg/kg (168.93 ±6.73 mg/g) and 4800 mg/kg (216.96 ±4.79 mg/g), as compared to control (234.86 ±3.72 mg/g). For the spleen, the general decrease in total protein titre noticed was not statistically significant ($p > 0.05$). for the other organs (heart, lungs and kidneys) the total protein titres rather increased, as compared to control values.

The results of the effects of *C. petersiana* aqueous leaf extract on the haematocrit values, serum total proteins, serum transaminases (AST, ALT) and urinary proteins are summarised in Table 8. For both male and female rats treated with the extract, significant ($p < 0.05$) increases in haematocrit values were seen in the groups receiving 300 mg/kg (52.65 ± 0.63 and 51.93 ± 1.15% for males and females, respectively), as compared to control (48.19 ± 1.68 and 46.62 ± 1.75% for males and females, respectively). For the male rats treated with the extract at the various doses (75, 300, 1200 and 4800 mg/kg), no statistically significant ($p > 0.05$) change was observed in the serum total protein concentration, as compared to control. However, for the female rats treated with the same extract serum total protein concentration showed a significant ($p < 0.05$) decrease in the group receiving 75 mg/kg (2.49 ± 0.15 g/dl), whereas significant ($p < 0.05$) increases were observed in the groups receiving 1200 mg/kg (4.94 ± 0.28 g/dl) and 4800 mg/kg (5.83 ± 0.31 g/dl), as compared to control (3.33 ± 0.32 g/dl).

For the female rats treated with the extract, the serum activity of ALT was significantly ($p < 0.05$) increased in the groups receiving 1200 mg/kg (23.92 ± 1.84 U/L) and 4800 mg/kg (34.65 ± 0.81 U/L), as compared to control (17.85 ± 2.11 U/L). This extract had no significant ($p > 0.05$) effect on the serum activity of ALT in male rats. For the rats treated with the extract, the serum activity of AST was significantly ($p < 0.05$) increased in female rats receiving 4800 mg/kg (21.42 ± 1.61 U/L), as compared to control (16.94 ± 1.73 U/L). The increases in AST activity observed in male rats treated with the same extract were not statistically significant ($p > 0.05$), as compared to the control values.

For the male rats treated with the extract urinary protein concentration showed a significant ($p < 0.05$) decrease in the group receiving 75 mg/kg (0.040 ± 0.002 g/dl), whereas in the group treated with 4800 mg/kg urinary protein concentration rather significantly ($p < 0.05$) increased (0.155 ± 0.006 g/dl), as compared to control values (0.064 ± 0.005 g/dl). For the female rats treated with the same extract, significant ($p < 0.05$) decreases in urinary proteins were observed in all the treated groups, as compared to control.

DISCUSSION

Several plant products of diverse nature and biological activities are used as medicine and in

many instances scientific information on the possible side effects upon consumption of such products may not be available. It is therefore important that the effects of these plant products are investigated to avoid immediate or long term toxic effects. It is in this line that the toxicity of *C. petersiana* aqueous leaf extract was evaluated.

Acute toxicity: In general, acute toxicity study did not reveal any negative behavioural changes at low doses (≤ 4 g/kg) of extract as compared to the control in both sexes. However, reduced activity (i.e. reduced locomotion) and reaction to noise were observed as from the doses of 8 g/kg and 16 g/kg respectively, as compared to control, suggesting that the aqueous leaf extract of *C. petersiana* may have a depressant or sedative effect on the central nervous system (Gatsing *et al.*, 2005) at high doses. This extract may acts as myorelaxant or tranquilliser on the nervous centres or on the motor fibres (Schmitt, 1973).

The increased reaction to pinch observed as from the dose 8 g/kg may be due to anaphylactoid reactions, secondary to the release of algogenic substances (e.g. prostaglandins, histamine). The extract may enhance the production of these algogenic substances. In effect, as stated by Okoye (1992), vasoactive amine mediators of anaphylactoid reactions are released from mast cells and basophils, or, are induced in tissues, and their release is triggered by exposure to a foreign agent. Histamine seems to be the most important of the mediators. Many plant extracts contain chemical agents that can induce release of these vasoactive chemical mediators from mast cells and basophils, and *C. petersiana* extract may be one of them.

In spite of the above side effects, the very high values of the LD_{50} (i.e. 20 g/kg and 22 g/kg, for male and female mice, respectively) make this extract practically non toxic (Gome *et al.*, 2011).

Sub-chronic toxicity: In this study, rats administered the extract of *C. petersiana* showed significant increase in total weight gain, and this seemed to be normal since significant increases were also observed in food and water intake with the same animals, suggesting that the extract may stimulate the appetite of these rats. Male rats administered *C. petersiana* extract (4800 mg/kg) showed significantly increased kidneys to body weight ratio, whereas female rats administered the same extract (1200 mg/kg) showed significantly increased liver to body weight ratio, suggesting the hypertrophy of these organs (kidneys and liver).

The decreased serum total protein seen in female rats treated with *C. petersiana* extract (75 mg/kg) may suggest a protective effect of this extract (at that dose) on the cell membrane and organs (e.g. the liver), since necrosis or membrane disruption would release enzymes and some other proteins into the circulation (Emerson *et al.*, 1993). The presence of polyphenols (Gatsing and Adoga, 2007) further gives credence to the hepatostrengthening effect of *C. petersiana* extract. In fact, natural flavonoids and polyphenolic compounds have been reported to exhibit protective and strengthening activities on liver cells (Adzet *et al.*, 1987; Akamatsu *et al.*, 2004; Oh *et al.*, 2004). Enhancement in serum total protein is an indication of tissue injury while a significant decrease in total protein contents of the liver is a reflection of hepatic toxicity (Aliyu *et al.*, 2007a). Significant increases in serum total protein were seen in female rats administered *C. petersiana* extract at the doses of 1200 mg/kg and 4800 mg/kg, as compared to controls. This seems to be corroborated by the significant decrease in total protein titre of the liver in female rats treated with *C. petersiana* extract (1200 mg/kg and 4800 mg/kg) and the conclusion that *C. petersiana* extract, at high doses, may have significant cytotoxic effect on the liver.

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 (Aliyu *et al.*, 2007a). Small amounts of the enzyme are present in the brain, pancreas and lungs (Cheesbrough, 1991a). ALT is found principally in the liver (in the cytosol of hepatocytes) and is considered a more sensitive marker of hepatotoxicity than AST (Azza *et al.*, 2012). The serum or plasma levels of both AST and ALT become raised whenever there is liver cell damage. The higher the activities of both enzymes the greater the degree of liver damage (Aliyu *et al.*, 2007b). In this study, significant increases in ALT and AST activities were observed in female rats receiving *C. petersiana* extract at 1200 and 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. In fact, 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 serum elevation of the enzymes (Gatsing *et al.*, 2005; Padmaja, 2011; Sourabie *et al.*, 2012).

The urine tests with the greatest medical usefulness for the diagnosis or screening of specific pathologies

of the kidneys are protein and sodium analyses (Ricos *et al.*, 1994). It is clear that two factors are important in excluding proteins at the glomerular level. The first or these is the molecular size, the other one being the molecular charge. Almost all physiological proteins are, at the pH of plasma, negatively charged: that is, they behave as anions (Baron *et al.*, 1989). Proteinuria (i.e. presence of protein in the urine in more than the usual trace amounts (Ganong, 1991); presence of more than 150 mg/24 h of protein in the urine (Cumming *et al.*, 1995) may occur through either of these two mechanisms: loss of the normal anionic charge on glycoproteins and proteoglycans forming the glomerular filtration barrier (glomerular basement membrane) (Groop *et al.*, 1990) or a structural alteration causing an increase in pore size (Power and Simpson, 1988). Heparan sulfate is the predominant glycosaminoglycan (GAG) proteoglycan in the glomerular basement membrane (GBM). It confers a negative charge on the GBM, and its loss has been related to the presence of protein (albumin) in the urine (Reddi and Jyothirmayi, 1993).

In this study, significant decreases in urinary protein were seen in female rats administered *C. petersiana* extract at various doses (75, 300, 1200, 4800 mg/kg), as compared to controls. Also, a significant decrease in urinary protein was observed in male rats treated with *C. petersiana* extract at 75 mg/kg. However, urinary protein showed a significant increase in male rats treated with *C. petersiana* extract at 4800 mg/kg, compared to controls. These results suggest that *C. petersiana* extract, at moderate doses, may enhance the biosynthesis of the glomerular basement membrane, and that at high doses this extract may create injury to the kidneys.

Significant increases in haematocrit values (52.65 ± 0.63% and 51.93 ± 1.15%) were seen in male and female rats respectively, treated with *C. petersiana* extract at 300 mg/kg, as compared to both the controls and the normal range [i.e. 35% to 50% (Alexander and Griffiths, 1993)], suggesting that the extract at that dose may cause dehydration (i.e. reduction in plasma volume).

CONCLUSION

In the light of the foregoing, it appears that *C. petersiana* aqueous leaf extract, at moderate doses, may not be toxic, and besides may stimulate the appetite leading to weight gain and may have strengthening effects on the liver and kidneys. However, at high doses, this extract may have a depressant or sedative effect on the central nervous system, may induce hypersensitiveness, and may create injury to the liver and kidneys.

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Declaration of interest: The authors report no declarations of interest.

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

Sex of Animal	Parameters	Doses (g/kg) and Behaviour of Animals					
		0	**2**	**4**	**8**	**16**	**32**
Males	Locomotion	+	+	+	-	-	-
	Reaction to noise	+	+	+	+	-	- -
	Reaction to pinch	+	+	+	+ +	+++	+++
	State of the tail	+	+	+	+	+	+
	State of the excrement	g	L	L	g-	g	g
	Mortality (within 48 hours)	NM	NM	NM	NM	2	6
Females	Locomotion	+	+	+	-	-	-
	Reaction to noise	+	+	+	+	-	- -
	Reaction to pinch	+	+	+	+ +	+++	+++
	State of the tail	+	+	+	+	+	+
	State of the excrement	g	L	L	g-	g	g
	Mortality (within 48 hours)	NM	NM	NM	NM	1	6

Key: + = normal; + + = increased; +++ = profoundly increased; - = reduced;
- - = profoundly reduced; L = liquid; g = granular; g- = less granular; NM = no mortality.

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

Dose (g/kg)	Weight variation of males (g)	Weight variation of females (g)
0	3.06 ± 0.14^{a}	2.84 ± 0.13^{a}
2	3.15 ± 0.76^{a}	2.88 ± 0.14^{a}
4	4.13 ± 0.99^{a}	3.51 ± 0.62^{a}
8	2.60 ± 0.12^{b}	2.32 ± 0.20^{b}
16	$2.38 \pm 0.34^{\#\,b}$	$2.15 \pm 0.16^{*\,b}$
32	-	-

Tabulated values are Mean ± SD of six determinations, except # (four determinations) and * (five determinations).
- : No surviving animal.
a, b: values on the same column with different letters are significantly different at $P < 0.05$.

Table 3: Food intake as affected by doses of *C. petersiana* 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	152.00 ± 4.99^{a}	138.91 ± 2.73^{d}	91.24 ± 10.39^{b}	124.62 ± 8.03^{e}
	75	189.37 ± 8.46^{b}	160.25 ± 8.24^{c}	118.85 ± 5.05^{c}	136.73 ± 9.25^{e}
	300	162.46 ± 7.05^{a}	158.16 ± 8.04^{c}	134.25 ± 4.60^{a}	136.36 ± 7.61^{e}
	1200	174.28 ± 8.36^{b}	149.37 ± 4.55^{c}	153.56 ± 4.00^{d}	118.89 ± 7.80^{e}
	4800	181.39 ± 8.80^{b}	182.44 ± 5.86^{e}	164.10 ± 14.49^{d}	172.84 ± 13.56^{a}
Females	0	133.90 ± 5.08^{c}	125.49 ± 5.96^{a}	80.96 ± 15.27^{b}	98.05 ± 5.31^{b}
	75	150.02 ± 6.50^{a}	145.83 ± 8.61^{d}	115.48 ± 5.36^{c}	118.29 ± 3.40^{d}
	300	143.28 ± 4.66^{c}	139.92 ± 4.28^{d}	118.87 ± 6.24^{c}	95.62 ± 8.69^{b}
	1200	151.39 ± 6.55^{a}	145.81 ± 3.67^{d}	130.15 ± 5.15^{a}	115.32 ± 7.83^{d}
	4800	162.52 ± 8.87^{a}	164.53 ± 11.20^{b}	130.85 ± 7.95^{a}	137.25 ± 10.58^{e}

Tabulated values are Mean ± SD of five determinations.
a, b, c, d, e: values on the same column with different letters are significantly different at $P < 0.05$.

Table 4: Water intake as affected by doses of *C. petersiana* 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	88.88 ± 6.91^{a}	104.56 ± 9.29^{b}	85.36 ± 8.18^{a}	83.26 ± 8.67^{a}
	75	101.70 ± 7.24^{a}	115.80 ± 4.09^{b}	99.14 ± 7.42^{a}	96.38 ± 5.61^{a}
	300	94.46 ± 9.41^{a}	114.74 ± 9.00^{b}	98.84 ± 9.71^{a}	96.68 ± 6.26^{a}
	1200	89.10 ± 4.99^{a}	106.02 ± 7.39^{b}	83.54 ± 5.14^{a}	90.80 ± 4.00^{a}
	4800	104.98 ± 8.04^{b}	127.88 ± 2.89^{c}	138.94 ± 2.29^{d}	93.80 ± 2.58^{a}
Females	0	90.74 ± 5.16^{a}	110.90 ± 9.08^{b}	90.54 ± 5.12^{a}	80.02 ± 9.00^{a}
	75	114.94 ± 4.05^{b}	128.80 ± 4.93^{c}	113.50 ± 7.31^{b}	97.48 ± 4.90^{e}
	300	99.54 ± 4.56^{a}	119.58 ± 2.63^{b}	101.18 ± 11.84^{a}	91.30 ± 6.20^{a}
	1200	95.92 ± 5.48^{a}	111.42 ± 7.59^{b}	94.58 ± 6.47^{a}	85.14 ± 2.78^{a}
	4800	96.00 ± 4.44^{a}	109.24 ± 10.03^{b}	92.30 ± 6.21^{a}	89.85 ± 6.61^{a}

Tabulated values are Mean ± SD of five determinations.
a, b, c, d, e: values on the same column with different letters are significantly different at $P < 0.05$.

Table 5: Total weight gain as affected by doses of *C. petersiana* 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	26.72 ± 2.56[a]	49.46 ± 3.03[c]	53.72 ± 6.59[c]	64.94 ± 9.25[e]
	75	34.04 ± 7.49[a]	50.96 ± 9.97[c]	68.78 ± 17.13[c]	82.45 ± 25.75[e]
	300	28.92 ± 3.56[a]	50.14 ± 6.77[c]	58.38 ± 9.30[c]	70.80 ± 9.61[e]
	1200	35.62 ± 3.65[b]	56.24 ± 3.04[d]	64.32 ± 8.34[c]	80.74 ± 3.93[d]
	4800	39.02 ± 6.18[b]	65.32 ± 4.05[e]	70.10 ± 4.34[e]	87.43 ± 6.96[d]
Females	0	24.54 ± 2.02[a]	35.14 ± 2.33[b]	42.62 ± 2.34[c]	44.70 ± 5.21[c]
	75	29.00 ± 9.79[a]	42.76 ± 9.08[b]	59.60 ± 15.29[c]	65.72 ± 17.91[c]
	300	26.08 ± 5.23[a]	39.96 ± 5.23[b]	52.82 ± 7.24[d]	62.46 ± 11.70[e]
	1200	26.38 ± 4.67[a]	48.64 ± 7.05[d]	61.62 ± 6.92[e]	72.66 ± 6.92[e]
	4800	28.62 ± 3.26[a]	45.12 ± 5.55[d]	60.72 ± 6.89[e]	66.83 ± 3.08[e]

Tabulated values are Mean ± SD of five determinations.
a, b, c, d, e: values on the same column with different letters are significantly different at $P < 0.05$.

Table 6: Organ to body weight ratios as affected by doses of *C. petersiana* 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	0.326 ±0.035[a]	3.471 ±0.506[b]	0.618 ±0.053[d]	0.580 ±0.029[d]	0.191 ±0.025[a]
	75	0.326 ±0.028[a]	3.561 ±0.238[b]	0.608 ±0.034[d]	0.576 ±0.032[d]	0.237 ±0.049[a]
	300	0.364 ±0.019[a]	3.593 ±0.270[b]	0.612 ±0.075[d]	0.545 ±0.026[d]	0.194 ±0.040[a]
	1200	0.326 ±0.011[a]	3.345 ±0.363[b]	0.666 ±0.050[d]	0.568 ±0.041[d]	0.203 ±0.042[a]
	4800	0.334 ±0.028[a]	2.849 ±0.359[b]	0.616 ±0.012[d]	0.631 ±0.016[e]	0.196 ±0.008[a]
Females	0	0.370 ±0.035[a]	2.857 ±0.157[b]	0.695 ±0.091[d]	0.623 ±0.012[d]	0.220 ±0.009[a]
	75	0.349 ±0.027[a]	3.102 ±0.301[b]	0.672 ±0.020[d]	0.594 ±0.035[d]	0.228 ±0.041[a]
	300	0.348 ±0.023[a]	3.081 ±0.285[b]	0.642 ±0.056[d]	0.588 ±0.030[d]	0.208 ±0.019[a]
	1200	0.349 ±0.056[a]	3.590 ±0.346[c]	0.642 ±0.049[d]	0.618 ±0.046[d]	0.249 ±0.031[a]
	4800	0.357 ±0.051[a]	3.047 ±0.276[b]	0.642 ±0.148[d]	0.576 ±0.042[d]	0.253 ±0.058[a]

Tabulated values are Mean ± SD of five determinations.
a, b, c, d, e: values on the same column with different letters are significantly different at $P < 0.05$.

Table 7: Total protein titre of organs as affected by doses of *C. petersiana* 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	65.09 ±7.27[b]	129.75 ±6.11c	133.54 ±7.35[c]	111.21 ±8.31[a]	108.48 ±4.94[a]
	75	85.83 ±1.36[a]	129.81 ±3.48c	134.01 ±5.60[c]	141.92 ±1.80[c]	131.65 ±3.75[c]
	300	103.84 ±4.38[a]	234.23 ±10.33[d]	160.08 ±12.24[e]	187.05 ±4.05[b]	165.77 ±18.19[e]
	1200	83.20 ±8.84[a]	164.93 ±12.56[e]	168.93 ±10.52[ea]	192.10 ±6.85[b]	131.86 ±7.31[c]
	4800	98.74 ±4.82[a]	200.63 ±4.34[a]	183.26 ±3.55[a]	146.39 ±3.10[c]	149.56 ±4.47[c]
Females	0	77.94 ±12.96[a]	234.86 ±3.72[d]	136.28 ±4.63[c]	169.56 ±7.71[e]	168.51 ±17.29[e]
	75	90.15 ±11.53[a]	235.91 ±5.87[d]	144.50 ±12.67[c]	171.04 ±4.25[e]	159.03 ±8.62[e]
	300	88.26 ±7.46[a]	240.76 ±3.54[d]	135.02 ±11.86[c]	189.82 ±10.04[b]	161.56 ±10.91[e]
	1200	88.04 ±15.05[a]	168.93 ±6.73[e]	139.23 ±3.75[c]	172.09 ±4.97[e]	153.77 ±7.14[e]
	4800	114.01 ±7.99[c]	216.96 ±4.79[a]	152.87 ±11.45[e]	167.20 ±6.85[e]	166.41 ±12.31[e]

Tabulated values are Mean ± SD of five determinations.
a, b, c, d, e: values on the same column with different letters are significantly different at $P < 0.05$.

Table 8: Effects of doses of *C. petersiana* 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)	Urinary protein (g/dl)	Haematocrit (%)
Males	0	3.52 ± 0.51^{a}	28.93 ± 1.96^{c}	24.65 ± 0.85^{c}	0.064 ± 0.005^{d}	48.19 ± 1.68^{b}
	75	3.41 ± 0.60^{a}	26.90 ± 2.25^{c}	24.21 ± 1.20^{c}	0.040 ± 0.002^{b}	47.58 ± 2.00^{b}
	300	3.11 ± 0.67^{a}	26.54 ± 3.03^{c}	26.22 ± 3.06^{c}	0.063 ± 0.004^{d}	52.65 ± 0.63^{e}
	1200	3.72 ± 0.26^{a}	29.07 ± 1.08^{c}	25.36 ± 2.30^{c}	0.069 ± 0.004^{d}	47.62 ± 1.85^{b}
	4800	4.13 ± 0.20^{a}	28.94 ± 2.10^{c}	25.57 ± 0.88^{c}	0.155 ± 0.006^{e}	48.67 ± 1.13^{b}
Females	0	3.33 ± 0.32^{a}	17.85 ± 2.11^{d}	16.94 ± 1.73^{d}	0.081 ± 0.003c	46.62 ± 1.75^{b}
	75	2.49 ± 0.15^{b}	16.41 ± 1.03^{d}	16.91 ± 1.02^{d}	0.045 ± 0.004^{b}	49.04 ± 1.72^{b}
	300	3.30 ± 0.17^{a}	17.35 ± 0.79^{d}	17.25 ± 1.10^{d}	0.021 ± 0.003^{a}	51.93 ± 1.15^{e}
	1200	4.94 ± 0.28^{c}	23.92 ± 1.84^{b}	17.92 ± 1.90^{d}	0.028 ± 0.004^{a}	49.23 ± 2.33^{b}
	4800	5.83 ± 0.31^{e}	34.65 ± 0.81^{a}	21.42 ± 1.61^{b}	0.067 ± 0.004^{d}	48.67 ± 1.55^{b}

Tabulated values are Mean ± SD of five determinations.
a, b, c, d, e: values on the same column with different letters are significantly different at $P < 0.05$.
ALT: alanine aminotransferase; AST: aspartate aminotransferase.

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