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Acute and sub-chronic toxicity profile of methanol leaf extract of *Gouania longipetala* in rats

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A B S T R A C T

Ethnopharmacological relevance: *Gouania longipetala* leaves are commonly used in folkloric medicine in Africa and other parts of the world for treatment of edema, febrifuges, veneral diseases, lumbago, heart diseases, diabetes mellitus malaria, *etc.* This study therefore evaluated safety profile of the methanol leaf extract of the plant using acute and sub-chronic studies in rat model.

Materials and methods: Acute toxicity test of the plant lasted for 48 h with oral administration of graded doses (100–4000 mg/kg) of *Gouania longipetala* extract (GLE) in rats. The rats were observed for signs of toxicity and death. The sub-chronic toxicity was evaluated by administration of different doses (2.5, 5 and 10 mg/kg) of GLE daily in feed for 90 days. On days, 30, 60 and 90, blood samples collected from the retro-orbital plexus of the eye of the rats were used for evaluation of serum biochemistry, hematology, lipid peroxidation and *in vivo* antioxidant activities. Histopathological evaluations of the kidney, liver, lungs and heart were also done.

Results: The acute toxicity test revealed no observable signs of toxicity or morbidity. Sub-chronic study showed that GLE significantly ($p < 0.05$) increased relative liver weight on day 90 at 10 mg/kg. There were no significant variations in the hematological parameters of both GLE treated and untreated rats. The extract significantly ($p < 0.05$) reduced total cholesterol, triglycerides, very low density lipoproteins and increased high density lipoproteins which was more prominent on day 90 at the dose of 10 mg/kg. The extract significantly ($p < 0.05$) increased liver enzyme markers at the doses used. GLE also significantly ($p < 0.05$) increased serum urea at the dose of 10 mg/kg on day 90. The extract caused dose-dependent and significant ($p < 0.05$) increase in superoxide dismutase and decrease in malondialdehyde. Histopathological studies revealed degenerative changes in the kidney and liver.

Conclusion: The results of the study suggest that *Gouania longipetala* is well tolerated in short term therapies, but may have long term toxic effects on the kidney and liver.

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1. Introduction

Plant derived products have been used for medicinal purposes for centuries and presently it is estimated that about 80% of the world's population are dependent on medicinal plants or plant-derived products for their health needs (Shri, 2003). Herbal preparations and natural remedies are commonly employed in developing countries for the treatment of various diseases. This practice is an alternative way to compensate for some perceived deficiencies in orthodox pharmacotherapy (Zhu et al., 2002).

Also there is the worldwide green revolution which is reflected in the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs (Williamson et al., 1996) and medicinal plants have been documented to have advantage in toxicity considerations based on their long term use and one might expect bioactive compounds obtained from such plants to have low animal and human toxicity (Fabricant and Farnsworth, 2001).

It is in the light of the above that World Health Organization (WHO) has recognized the role of traditional alternative/complementary medicine and has encouraged member nations to develop national policies appropriate for their situations taking cognizance of the fact that the use of medicinal plants is the most common form of traditional medication worldwide (WHO, 2005). However, patients are often unaware of important similarities and differences between medicinal herbs and approved medications; some mistakenly think of herbs as natural alternatives to chemicals, failing to recognize that herbs are composed of bioactive chemicals some of which may be toxic and a number of studies

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have reported the toxic effects of herbal medicines (Jaouad et al., 2004; Taziebou et al., 2008).

The use of traditional and herbal medicine is widely practiced in Nigeria and *Gouania longipetala* is one of such plants used in Nigerian folkloric medicine.

Gouania longipetala (Hemsl.) belongs to the family Rhamnaceae. It is a scandent shrub or liana mainly present in closed forests, forest margins and in jungle regrowths. (Burkill, 1985). Its salient characteristics are the watch-spring tendrils, spike-like thyrsus, a more or less lobed disc, inferior ovary and longitudinally 3-wined septicidal fruits (Buerki et al., 2011).

Gouania longipetala has been used in ethnomedicine for the treatment of different ailments. The leaves are used for the treatment of swellings, edema venomous stings, gout, febrifuges. It is also used as genital stimulants, laxatives and for treatment of veneral diseases. Also the stem and leaves are used to treat abdominal pain and stomach troubles. The leaf sap is used for eye treatments, as pain killers and for treating heart diseases. Also the plant is used for treating lumbago, rickets and wounds. (Burkill, 1985; Abbiw, 1990). The stem of the plant has been shown to possess antibacterial and anti-inflammatory activities (Ekuadzi et al., 2012). In Orba village, in Udenu local Government Area of Enugu State, South Eastern Nigeria (where the plant is known as “Asha”), the leaves of *Gouania longipetala* are used to treat diabetes mellitus. Also the leaves and sap are used for the treatment of malaria (Focho et al., 2009). The preparations are administered in most disease conditions over a long period of time without proper dosage monitoring and consideration of toxic effects that might result from prolonged usage. This study was therefore undertaken to determine the acute and sub-chronic toxic effects of methanol leaf extract of *Gouania longipetala* using rat model.

2. Materials and methods

2.1. Plant collection and identification

Fresh leaves of *Gouania longipetala* were collected from its natural habitat at Orba- Nsukka, Enugu state, Nigeria in the month of July, 2011and identified by a taxonomist, Mr. A.O. Ozioko of the Bioresources Development and Conservation Program (BDCP), Aku Road, Nsukka, Enugu State where a voucher specimen number VPP/2011/68 was deposited in their herbarium.

2.2. Preparation of plant material

The plant was extracted using cold maceration method. The leaves were washed, cut into small pieces, dried at room temperature and pulverized into coarse powder of about 1 mm in diameter. 500 g of the plant material was macerated in 80% methanol for 48 h with intermittent shaking at 2 h interval. The extract was then filtered using Whatman no 1 filter papers and concentrated *in vacou* using rotary evaporator at 40 °C and 210 mbar and a vacuum lyophilizer. The percent yield was calculated and the extract was stored in a refrigerator at 4 °C as *Gouania longipetala* extract (GLE) until time of use (Mansi and Lahham, 2008).

2.3. Experimental animals

Mature Wistar albino rats, bred in the laboratory animal unit of the Faculty of Veterinary Medicine, University of Nigeria Nsukka, were used for the experiments. They were housed in an environment of normal ambient temperature and the lighting period was 12 h daily and relative humidity of 40–60%. The weight of the rats varied between 120 and 175 g. The rats were kept in stainless steel cages, supplied with clean drinking water and fed *ad libitum* with standard rat commercial pelleted feed (Vital feed®, Nigeria). Ethical conditions governing the conduct of experiments with life animals were strictly observed as stipulated by Ward and Elsea (1997) and all animal experiments were conducted in compliance with NIH Guide for Care and Use of Laboratory Animals (pub. No. 85-23, Revised 1985). The experimental protocol was approved by the institution′s ethical committee for the use of laboratory animals.

2.4. Acute toxicity study

The method of Lorke (1983) was adopted for this study. Mature albino rats (35) of both sexes were randomly grouped into seven groups (1–7) of five rats per group. Groups 1–6 were given GLE at 100, 500, 1000, 2000, 3000 and 4000 mg/kg respectively, by oral gavage. Group 7 rats received distilled water (10 ml/kg). The rats were allowed free access to feed and drinking water and were observed for 48 h for signs of toxicity and death.

2.5. Sub-chronic toxicity study

The sub-chronic toxicity study lasted for 90 days (OECD, 1998)

2.5.1. Preparation of experimental feed containing different concentrations of GLE

Gouania longipetala at different doses (2.5, 5 and 10 mg/kg was incorporated in feed (Grower mash, Vital feed®, Jos, Nigeria).

The extract of each dose was first dissolved in 20 ml of water and then uniformly made up to 2.5 l/3 kg feed. The feed and water- containing extract were mixed thoroughly and the mixture pelleted. The pelletized feed was dried for 3 days under mild sunlight. The feed was then stored in a dry environment with intermittent drying to prevent fungal growth until time of use.

2.5.2. Experimental procedure

Fifty six (56) Wistar albino rats of both sexes were randomly grouped into 4 (1–4) groups of 14 rats per group. Males and females were separated in different cages to avoid breeding. The rats were then treated as follows:

Group 1: feed without extract (control).
Group 2: 2.5 mg/kg extract in feed.
Group 3: 5.0 mg/kg extract in feed.
Group 4: 10 mg/kg extract in feed.

The rats were fed in accordance with the normal feed consumption rates of rats at 10 g feed/100 g body weight/day (Hafez, 1970), while clean drinking water was provided *ad libitum*. The rats were routinely weighed on a weekly basis to determine the quantity of feed to be taken for the week. Blood samples were collected from four (4) rats in each group through the retro-orbital plexus of the eye of the rats on days 30, 60 and 90 for hematological and biochemical analyses after which the rats were sacrificed by cervical dislocation under light ether anesthesia and organs (liver, lungs, kidney and heart) collected for histopathological examination.

2.5.3. Determination of parameters

2.5.3.1. Body weights and relative organ weights (ROW). The changes in body weights were recorded and the organs (liver, kidney, lungs and heart) were weighed using sensitive weighing balance to calculate the ROW for different groups on days 30, 60 and 90.

Table 1
Mean body weights of rats given GLE in feed for 90 days.

Group	Treatment	Mean body weights of rats (g)			
		Day 1	Day 30	Day 60	Day 90
1	Control (without extract)	108.1 ± 2.71	133.4 ± 2.30^a	148.2 ± 1.33^a	192.3 ± 3.04^b
2	GLE(2.5 mg/kg in feed)	102.2 ± 1.93	142.2 ± 2.44^a	173.4 ± 2.1^b	202.5 ± 2.12^c
3	GLE (5.0 mg/kg in feed)	105.9 ± 2.06	148.6 ± 1.99^a	180.1 ± 3.04^b	211.3 ± 2.77^c
4	GLE (10 mg/kg in feed)	99.5 ± 1.48	140.5 ± 3.06^a	189.2 ± 1.68^b	233.6 ± 1.48^c

[a] $p < 0.05$ when compared to day 1.
[b] $p < 0.001$ when compared to day 1.
[c] $p < 0.0001$ when compared to day 1.

2.5.3.2. Hematology. Blood samples from 4 rats in each group were collected on days 30, 60 and 90 for analysis using Hematological auto-analyzer(Mindray BC-2800 Auto Hematological Analyzer, England). The hematological parameters analyzed included: Packed cell volume (PCV), Red Blood Cell (RBC) count, White Blood Cell (WBC) count and Hemoglobin (Hb) concentration. Others were; mean corpuscular volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC).

2.5.3.3. Lipid profile assay. Total cholesterol was evaluated using enzymatic colorimetric chod-pad test method described by Allain et al. (1974) with Quimica Applicada test kit; Triglycerides was also determined spectrophotometrically using the method of Tietz (1990); High density lipoproteins (HDL) was evaluated by the method of Grove (1979) as described in Quimica Clinica Applicada test kit; low density lipoprotein (LDL) was determined as the difference between total cholesterol and cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulfate (PVS) in the presence of polyethylene-glycol monomethyl ether (Bergmenyer, 1985) and Very low density cholesterol(VLDL) was calculated according to the method of Wilson et al. (1981) as $VLDL = 0.2 \times TG$ (where TG is triglycerides).

2.5.3.4. Liver markers enzymes. Aspartate aminotransferase (AST) was evaluated using the method of Reitman and Frankel (1957) as described by Randox laboratories, United Kingdom using Randox kits; Alanine aminotransferase (ALT) was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenyl hydrazine using the method of Reitman and Frankel (1957) as described in Randox kits; alkaline phosphatase (ALP) was assayed based on the methods of Kind and King (1972); total protein in serum was assayed using direct biuret method (Gornall et al., 1948) while total and conjugated bilirubin were determined according to the method of Jendrasik and Grof (1938).

2.5.3.5. Effect of Gouania longipetala on kidney function tests

2.5.3.5.1. Estimation of serum urea. This was done following the method of Bauer et al. (1982). Urea in serum was hydrolyzed to ammonia in the presence of urease. The ammonia was then measured photometrically by Berthelot's reaction.

2.5.3.5.2. Estimation of serum creatinine. The method of Cockcroft and Gault (1976) was employed for the estimation of serum creatinine in which at alkaline pH values; creatinine reacts with picric acid to produce a colored compound creatinine alkaline picrate which is photometrically read at 546 nm.

2.5.3.6. Lipid peroxidation/in vivo antioxidant activities of GLE

2.5.3.6.1. Malondialdehyde (MDA). Lipid peroxidation was determined spectrophotometrically by measuring the level of lipid peroxidation product, malondialdehyde (MDA) as described by Draper and Hadley (1990) where MDA reacts with thiobarbituric acid (TBA) to form a red or pink colored complex which absorbs maximally in acid solution at 532 nm.

2.5.3.6.2. Estimation of superoxide dismutase (SOD). Adrenaline (10 mg) was dissolved in 17 ml of distilled water to make adrenaline solution. Serum (0.1 ml) was added to 0.9 ml of phosphate buffer (pH 7.8). 0.2 ml of the extract was taken in triplicate and 2.5 ml of buffer added inside a cuvette and 0.3 ml of adrenaline solution added, mixed well and absorbance was read at 450 nm at 30 s interval for 5 times (Xin et al., 1991).

2.5.3.6.3. Determination of catalase. This assay was done according to the method of Aebi (1983).

This was done using the principle that the ultraviolet absorption of hydrogen peroxide can be easily measured at 240 nm and that as hydrogen peroxide decomposes with catalase, the absorption decreases with time, hence, catalase activity can be measured.

2.5.3.7. Histopathology. On days 30, 60 and 90, four rats each from the treatment groups and the control group were sacrificed by cervical dislocation and liver, kidney, heart and lungs were collected for histopathological studies. Tissue samples collected were fixed in 10% formal saline for 24 h. They were washed in ascending grades of ethanol, cleared with xylene, embedded in paraffin wax, sectioned with a microtome and stained with haematoxylin and eosin (H and E) and mounted on Canada balsam (Bancroft and Stevens, 1977). All the sections were examined under a light microscope under different ($\times 100$ and $\times 400$) magnifications. Photomicrographs of lesions were taken with an Olympus photo microscope for observations and documentation of histopathological lesions.

2.6. Data analysis

The results were presented as mean ± SEM and analyzed using one way analysis of variance (ANOVA). The differences between the means were tested using post hoc LSD and values of $p < 0.05$ were considered statistically significant. SPSS for windows version 17 was used for the statistical analysis

3. Results

3.1. Acute toxicity study

After 48 h, no death or signs of toxicity was observed in the rats treated with different doses (100, 500, 1000, 2000, 3000 and 4000 mg/kg) of GLE.

3.2. Clinical observation

Throughout the duration of the experiment, the rats did not show any observable signs of toxicity or morbidity as they looked bright, were feeding well and their feces looked normal. Also no mortality was recorded.

3.3. Body weights

Table 1 shows the changes in the body weights of rats fed different doses (2.5, 5.0 and 10 mg/kg) of *Gouania longipetala* extract in feed. Within each group there was a significant ($p<0.05$) increase in the body weights from day 1 to day 90 including the control group but it was more pronounced in the extract treated rats. Also there was a highly significant ($p<0.01$) and dose dependent increase in the body weights of the rats when compared to negative control group.

3.4. Relative organ weights (ROW)

There was no significant ($p>0.05$) difference in the ROW of liver, lungs and heart of the treated group of rats when compared to the control ROW (3.70 ± 0.29, 6.0 ± 0.72 and 3.8 ± 0.20 ($\times10^{-3}$ g) for liver lungs and heart, respectively) throughout the duration of the study. However, the kidney showed significant ($p<0.\ 02$) increase in weight in rats treated with 10 mg/kg of GLE on day 90 when compared to control group of rats (Table 2).

3.5. Hematology

The result of the hematology of rats exposed to sub-chronic treatment with different doses (2.5, 5 and 10 mg/kg) of *Gouania longipetala* extract in feed showed that there was no significant ($p>0.05$) variations in the hematological indices (PCV, WBC, RBC, hemoglobin concentrations, MCV, MCH and MCHC) values of the extract treated groups of rats when compared to negative control.

3.6. Lipid profile

3.6.1. Total cholesterol

The result of the effect of sub-chronic exposure of rats to GLE on the total cholesterol of rats is presented in Table 3. The extract caused different levels of significant ($p<0.01$ and $p<0.0.005$) decrease in the total cholesterol levels of the rats which was more prominent on days 30 and 60 when compared to the control group.

Table 2
Relative kidney weights of rats given GLE extract in feed for 90 days.

Group	Treatment	Relative organ weight of kidneys $\times10^{-3}$ (g)		
		Day 28	Day 56	Day 84
1	Control (feed without extract)	2.7 ± 0.09	2.7 ± 0.24	2.8 ± 0.02
2	2.5 mg/kg extract in feed	2.7 ± 0.08	2.8 ± 0.27	2.9 ± 0.33
3	5.0 mg/kg extract in feed	2.9 ± 0.45	2.6 ± 0.27	3.1 ± 0.15[a]
4	10.0 mg/kg extract in feed	2.9 ± 0.24	2.8 ± 0.15	3.6 ± 0.24[a]

[a] $p<0.02$ when compared to control group.

Table 3
Effect of sub-chronic feeding of GLE for 90 days on the total cholesterol of rats.

Group	Treatment	Total cholesterol (mg/dl)		
		Day 30	Day 60	Day 90
1	Control	101.5 ± 2.01	91.7 ± 3.12	85.0 ± 0.21
2	GLE (2.5 mg/kg in feed)	95.3 ± 1.12[b]	88.3 ± 5.15[a]	83.6 ± 1.26
3	GLE (5 mg/kg in feed)	84.5 ± 0.82[b]	82.0 ± 4.19[b]	82 ± 0.18[a]
4	GLE (10 mg/kg in feed)	81.6 ± 2.59[b]	80.4 ± 1.12[b]	75.3 ± 2.19[a]

[a] $p<0.01$ when compared to negative control
[b] $p<0.005$ when compared to negative control.

3.6.2. Triglycerides

The result of the serum triglycerides of rats exposed to *Gouania longipetala* extract in feed is presented in Fig. 1. The extract showed a dose-dependent and significant ($p<0.01$) reduction of the serum triglycerides of the extract treated rats on days 60 and 90 when compared to the control rats. Also GLE caused marked reduction of the triglycerides of both treated and control rats on day 90.

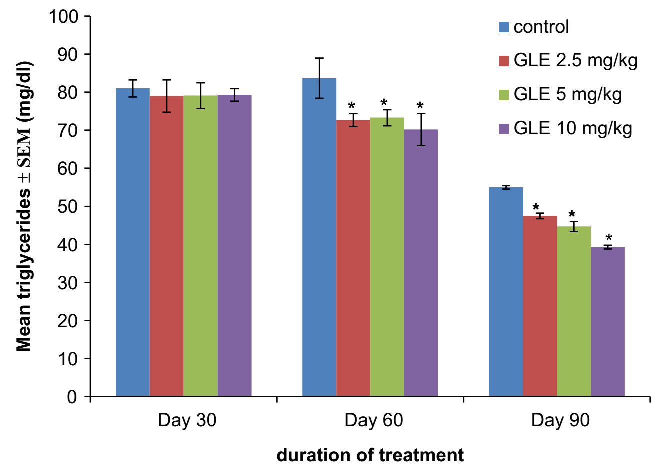

Fig. 1. Effect of sub-chronic administration of GLE on serum triglycerides of rats for 90 days. *$p<0.01$ when compared to control.

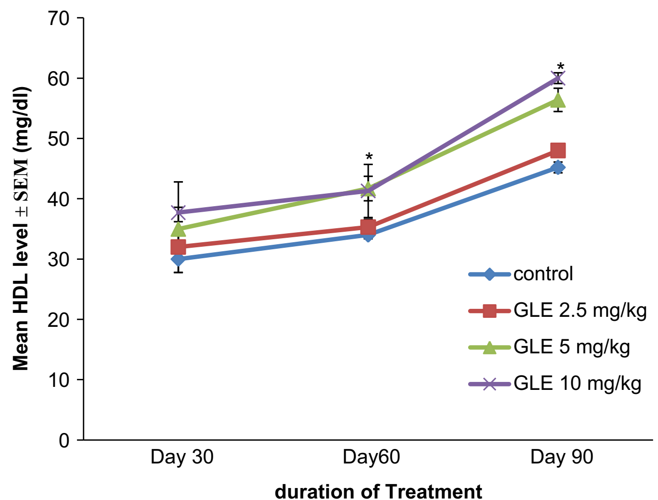

Fig. 2. High dentistry lipoproteins (HDL) of rats fed with *Gouania longipetala* extract for 90 days. *$p<0.05$ (5 and 10 mg kg GLE compared to control.

Table 4
Low density lipoproteins (LDL) of rats fed with *Gouania longipetala* extract for 90 days.

Group	Treatment	LDL(mg/dl)		
		Day 30	Day 60	Day 90
1	Control (feed without extract)	28.0 ± 0.45	29.3 ± 0.21	31.8 ± 0.85
2	GLE (2.5 mg /kg in feed)	27.0 ± 2.28	$28.5.0\pm0.73$	30.0 ± 0.36
3	GLE 5 (mg/kg in feed)	26.5 ± 2.90	27.7 ± 1.12	29.5 ± 0.81
4	GLE (10 mg/kg in feed)	25.8 ± 1.18[a]	27.5 ± 0.92[a]	24.5 ± 0.67[a]

[a] $p<0.05$ when compared to negative group.

3.6.3. *High density lipoproteins (HDL)*

The result of the HDL of rats given GLE in feed is presented in Fig. 2. The extract at the doses of 5 and 10 mg/kg caused a significant ($p < 0.01$) increase in the HDL of the treated rats on days 60 and 90 when compared to the control rats.

3.6.4. *Low density lipoproteins (LDL)*

Table 4 shows the result of the LDL of rats given GLE in feed. The result showed a dose dependent decrease in the values of LDL of the extract treated rats which was only significant ($p < 0.05$) at the dose of 10 mg/kg throughout the duration of the study.

3.6.5. *Very low density lipoproteins (VLDL)*

The result of the VLDL of the GLE treated rats and control is presented in Fig. 3. There was a significant ($p < 0.02$) reduction in the VLDL of the extract treated rats on days 60 and 90 when compared to control rats.

3.7. *Liver enzyme markers*

3.7.1. *Aspartate aminotransferase (AST)*

Fig. 4 shows the result of the AST levels of rats fed with *Gouania longipetala* extract. On days 30 and 60, there was no significant

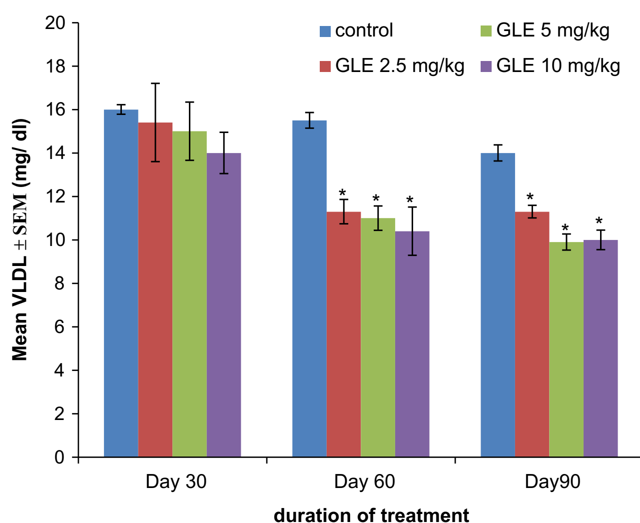

Fig. 3. Very low density cholesterol of rats fed with *Gouania longipetala* extract for 90 days. *$p < 0.02$ when compared to control.

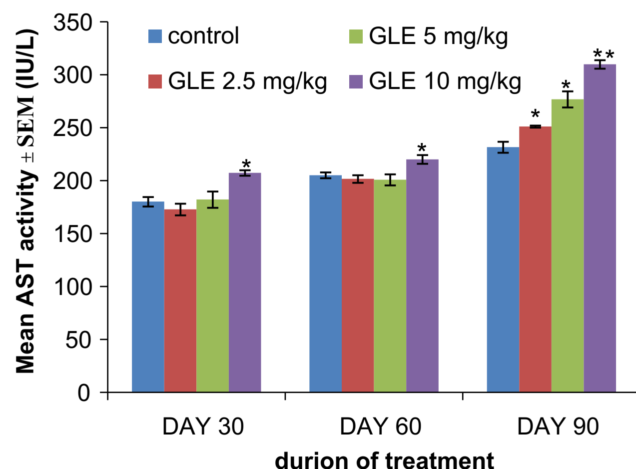

Fig. 4. Aspertate aminotransferase of rats fed with *Gouania longipetala* extract for 90 days. *$p < 0.05$, **$p < 0.001$ when compared to control.

($p > 0.05$) difference in the AST values of the treated rats at the doses of 2.5 and 5 mg/kg. However, there was a significant ($p < 0.05$) increase in AST at the dose of 10 mg/kg when compared to the control rats. On day 90 there was a significant ($p < 0.001$) increase in the values of the AST in the extract treated rats when compared to the control rats.

Table 5
Effect of oral feeding of GLE on mean alanine aminotransferase activity in rats.

Group	Treatment	ALT (IU/l)		
		Day 30	Day 60	Day 90
1	Control (feed without extract)	78.2 ± 3.12	74.8 1.68	75.0 ± 0.45
2	GLE (2.5 mg /kg in feed)	77.3 ± 2.60	76.1 ± 4.31	73.9 ± 1.12
3	GLE 5 (mg/kg in feed)	77.0 ± 1.52	76.0 ± 2.85	75.3 ± 1.72
4	GLE (10 mg/kg in feed)	71.6 ± 2.37	77.0 ± 1.59	82.3 ± 0.98[a]

[a] $p < 0.05$ when compared to control group.

3.7.2. *Alanine aminotranferase (ALT)*

The result of the ALT of rats given *Gouania longipetala* extract in feed is presented in Table 5. The result showed that there was no significant ($p > 0.05$) variations in the ALT values of the extract treated rats except on day 90 at the dose of 10 mg/kg where the extract caused a significant ($p < 0.05$) increase in ALT.

3.7.3. *Alkaline phoshatase (ALP)*

Table 6 shows the serum alkaline phosphatase of rats given different doses of *Gouania longipetala* extract in feed for 90 days. The result showed that there were various levels of significant ($p < 0.01$ and $p < 0.0001$) increase in the ALP of the extract treated rats when compared to the control rats throughout the study.

3.7.4. *Total protein*

Fig. 5 shows the result of the serum total protein of rats given *Gouania longipetala* extract in feed. There were no significant changes in the total protein values of both extract treated and control rats on days 30 and 60. However, on day 90 there was a significant ($p < 0.05$) increase in the serum total protein of rats treated with 10 mg/kg of the extract when compared to the control.

3.7.5. *Total bilirubin*

The result of the total bilirubin of both control and rats treated with *Gouania longipetala* extract in feed in presented in Table 7. The result showed that there was no significant ($p > 0.05$) difference in the total bilirubin of both control and extract treated rats throughout the duration of the experiment

3.7.6. *Albumin*

Fig. 6 shows the serum albumin of both control and GLE treated rats. There was no significant ($p > 0.05$) difference in the serum albumin levels of both the control and extract treated rats throughout the duration of the experiment. However, there was a significant ($p < 0.05$) increase in albumin level on day 90 when compared to day 30.

3.8. *Kidney function tests*

3.8.1. *Serum urea*

The result of the serum urea of rats fed with *Gouania longipetala* extract for 90 days is presented in Table 8. The result showed that there was no significant difference in the serum urea levels between the treated and control groups of rats on days 30 and 60. However, on day 90, there was a significant ($p < 0.05$)

Table 6
Effect of oral feeding of GLE on mean alkaline phosphatatse activity in rats.

Group	Treatment	ALP (IU/l)		
		Day 30	Day 60	Day 90
1	Control (feed without extract)	286.5 ± 1.53	356.3 ± 3.03	402.5 ± 0.61
2	GLE (2.5 mg/kg in feed)	326.5 ± 0.56[a]	462.0 ± 5.05[a]	478.0 ± 1.59[a]
3	GLE 5 (mg/kg in feed)	339.5 ± 3.08[a]	487.3 ± 7.53[a]	565. 7 ± 2.36[b]
4	GLE (10 mg/kg in feed)	339.0 ± 5.16[a]	591.7 ± 2.05[b]	630.7 ± 3.47[b]

[a] $p < 0.01$ when compared to control group.
[b] $p < 0.0001$ when compared to control group.

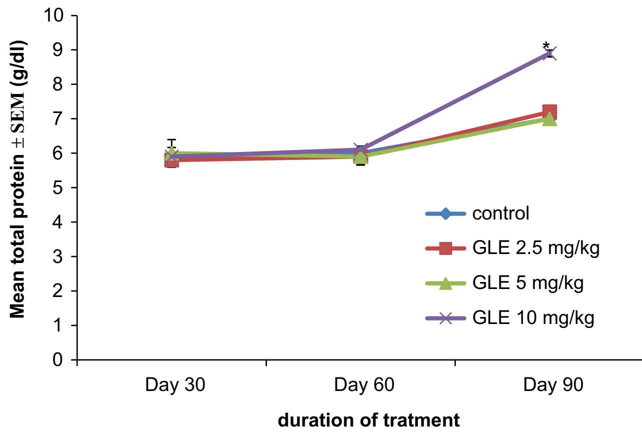

Fig. 5. Serum total protein of rats fed with *Gouania longipetala* extract for 90 days. *$p < 0.05$ when compared to control.

Table 7
Total serum bilirubin levels of rats fed with *Gouania longipetala* extract for 90 days.

Group	Treatment	Total bilirubin (mg/dl)		
		Day 30	Day 60	Day 90
1	Control (feed without extract)	0.3 ± 0.01	0.3 ± 0.02	0.5 ± 0.02
2	GLE (2.5 mg/kg in feed)	0.3 ± 0.06	0.3 ± 0.06	0.6 ± 0.06
3	GLE 5 (mg/kg in feed)	0.3 ± 0.02	0.4 ± 0.04	0.6 ± 0.06
4	GLE (10 mg/kg in feed)	0.3 ± 0.06	0.4 ± 0.04	0.6 ± 0.02

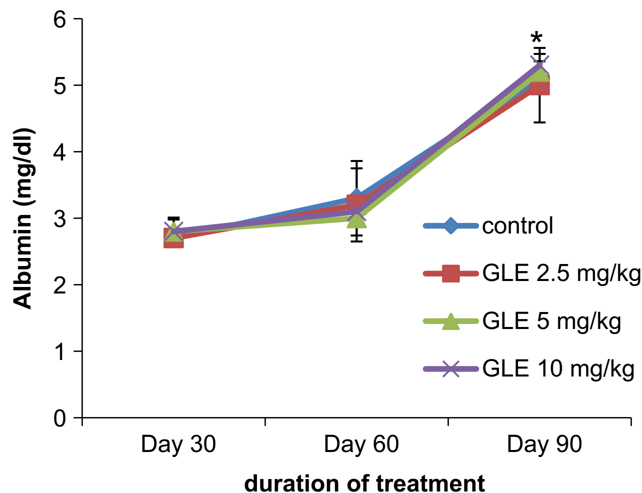

Fig. 6. Mean serum albumin levels of rats fed with GLE for 90 days. *$p < 0.05$ when day 90 is compared to day 30.

Table 8
Effect of GLE on the mean serum urea level in rats.

Group	Treatment	Urea (mg/dl)		
		Day 30	Day 60	Day 90
1	Control	36.0 ± 1.12	36.7 ± 0.92	36.2 ± 0.45
2	GLE (2.5 mg/kg in feed)	35.3 ± 3.47	36.0 ± 3.04	34.3 ± 0.95
3	GLE (5 mg/kg in feed)	35.2 ± 0.22	35.6 ± 2.56	34.7 ± 2.56
4	GLE (10 mg/kg in feed)	35.0 ± 0.37	33.0 ± 0.63	33.3 ± 1.86[a]

[a] $p < 0.05$ when compared to the control.

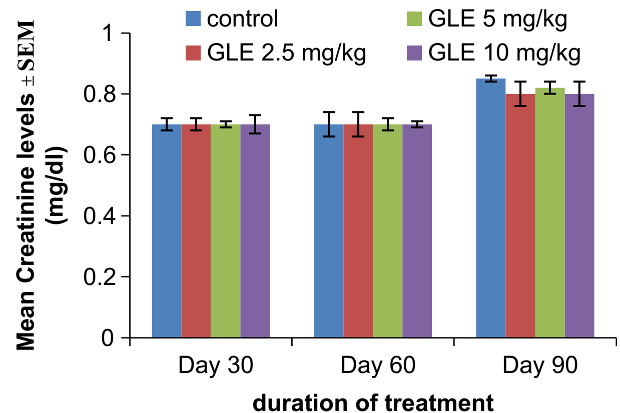

Fig. 7. Mean serum creatinine levels of rats fed with *Gouania longipetala* extract for 90 days.

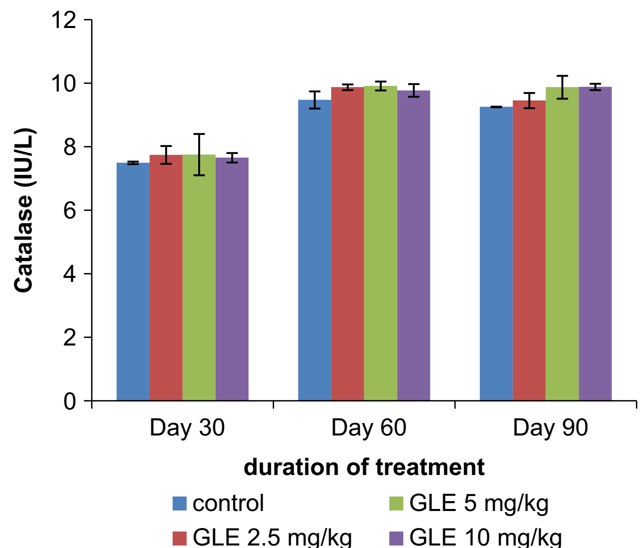

Fig. 8. Effect of oral feeding of *Gouania longipetala* extract on mean catalase activity in rats.

increase in the serum urea of the 10 mg/kg extract treated rats when compared to the control.

3.8.2. Creatinine

The serum creatinine levels of control and *Gouania longipetala* extract treated rats are presented in Fig. 7. The result showed that there was no significant difference in the creatinine levels of both treated and control rats throughout the experiment, though on day 90 there was a marginal decrease in the creatinine levels of the treated rats when compared to the control rats.

3.9. Lipid peroxidation/in vivo antioxidant activity

3.9.1. Catalase

Fig. 8 shows the result of the catalase levels of both control and *Gouania longipetala* extract treated rats. The result showed that there was no significant ($p > 0.05$) difference between the catalase levels of the treated and control rats.

3.9.2. Superoxide dismutase (SOD)

The result of superoxide dismutase activities of both *Gouania longipetala* extract treated and control rats are presented in Fig. 9. The result showed that there was no significant ($p > 0.05$) change in the SOD activities of both extract treated and control rats on day 30. On days 60 and 90, there was dose dependent and significant ($p < 0.01$) increase in the mean SOD activities of the treated rats when compared to control.

3.9.3. Malondialdehyde (MDA)

Fig. 10 shows the mean level of MDA in both control and *Gouania longipetala* extract treated rats. The result showed that there was a dose dependent and significant ($p < 0.01$) decrease in the MDA levels of the extract treated rats on day 90.

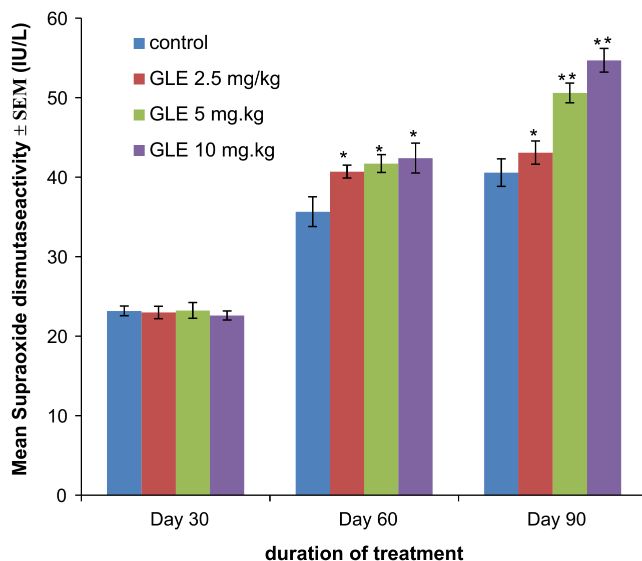

Fig. 9. Effect of 90 day treatment of GLE on superoxide dismutase activities of rats. *$p < 0.05$, **$p < 0.01$ when compared to control.

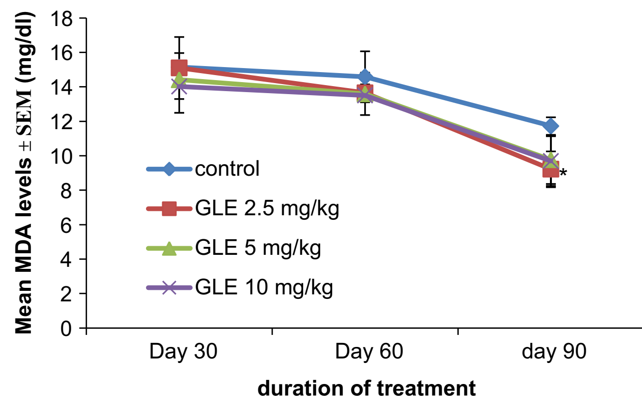

Fig. 10. Effect GLE on MDA levels of rats fed with *Gouania longipetala* extract for 90 days. *$p < 0.01$ when compared to control.

3.10. Histopathology

Histopathological studies of the organs (Heart, lungs, kidney and liver) of rats that were exposed to sub-chronic treatment with *Gouania longipetala* extract at different doses (2.5, 5 and 10 mg/kg) in feed showed that only the kidney and liver had histopathological lesions at the dose of 10 mg/kg on day 90.

The photomicrograph of sections of the kidney of rats treated with 10 mg/kg on day 90 showed pyknotic cells and desquamated tubular epithelial cells with cells found in the tubular lumen (Plate 1B) when compared to control rat kidney (Plate 1A).

Also photomicrograph of sections of the liver of rats sub-chronically exposed to GLE at the dose of 10 mg/kg on day 90 showed proliferation of bile ducts around the central vein (Plate 2B) when compared to control rat liver (Plate 2A).

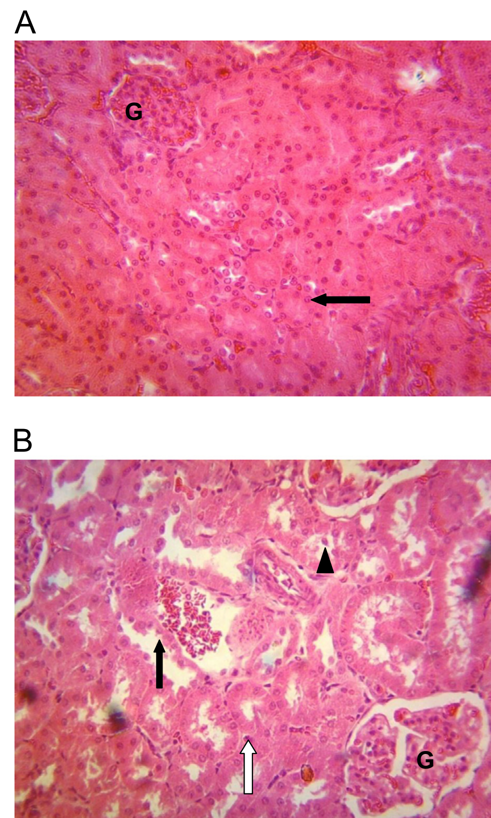

Plate 1. (A) Photomicrograph of kidney section from experimental control rats (group 1) on day 90 of sub-chronic toxicity test showing apparently normal glomerulus (G) and renal tubules with tubular epithelial cells (arrow). H and E (×400). (B) Photomicrograph of kidney section from experimental rats (group 4) after 90 days of treatment with 10 mg/kg *Gouania longipetala* extract in feed showing the glomerulus (G), sloughing off of tubular epithelial cells (black arrow), pyknotic epithelial cells (white arrow) and cells in the lumen of the renal tubule (arrow head). H and E (×400).

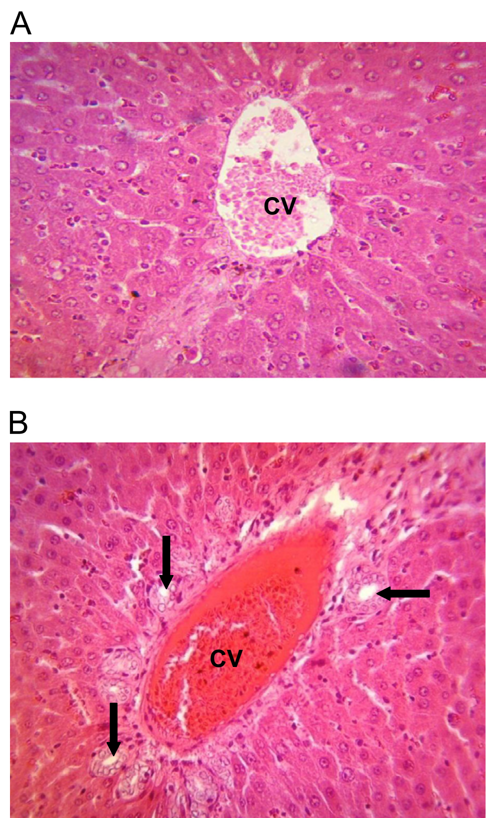

Plate 2. (A) Photomicrograph of kidney section from experimental control rats (group 1) on day 90 of sub-chronic toxicity test showing apparently normal central vein with blood cells (CV) and hepatocytes. H and E (× 400). (B) Photomicrograph of liver sections from experimental rats (group 4) after 90 days of treatment with 10 mg/kg *Gouania longipetala* extract in feed showing central vein containing blood (CV) and proliferation of bile ducts around the central vein (arrow).

4. Discussion

Generally herbal medicines are taken throughout life because of the chronic nature of the ailments they are used to treat. Little or no attention is paid to the untoward effects of these therapies. Based on this, acute toxicity test and sub-chronic toxicity study that lasted for 90 days was conducted to evaluate the safety profile of the methanol leaf extract of *Gouania longipetala* in experimental animals. Safety profile on medicinal plants is important as safety of herbal medicine use has recently been questioned due to reports of illnesses and fatalities (Park et al., 2010).

Hydromethanolic (80% methanol and 20% water) extract was used for the study since those that use the plant for traditional medicine use mostly methanol or aqueous solution and this also improves the extractive value and the storage.

General behavior and body weights are one of the critical parameters for the evaluation of first signs of toxicity (Sireeratawong et al., 2008). In acute study, there were no observable signs of morbidity or mortality throughout the duration of the experiment at all the doses used, which is an indication that the extract was well tolerated by the rats.

In the sub-acute toxicity experiment, there was gain in mean body weight of the rats in all the groups but was more prominent in the 10 mg/kg extract treated group of rats. This may be attributed to normal growth of the rats with age and also may be due to improved feed consumption by the rats.

In the mean relative organ weights, only the kidney showed significant ($p < 0.05$) increase on day 90 at the dose of 10 mg/kg of GLE in feed. This may indicate a potential toxic effect of the chronic exposure of the rats to the extract at a high dose on this organ or may be due to the kidney's attempt at constant excretion of the extracts for a long period of time.

Hematopoietic system is one of the most sensitive targets for toxic compounds and important index of physiological and pathological status. Also blood profile usually gives vital information on the response of the body to injury or stress (Mukinda and Eagles, 2010). The daily administration of GLE in feed for 90 days did not produce any significant changes in hematological parameters of the treated rats in all the doses used when compared to control group. This suggests that the extract may have no toxicological effects on the hemopoietic system.

Hyperlipidemia is well known as one of the major risk factors for atherosclerosis which leads to coronary artery disease (CAD) (Fuster et al., 2005). Researches in cardiovascular pharmacology in the past few years has been mainly focused on hypolipidemic (lipid lowering) agents including herbal drugs and diets (Tajudin and Nasirudin, 2006). Exposure of the rats to GLE in feed for 90 days caused a significant ($p < 0.05$) reduction in total cholesterol, triglycerides, LDL and VLDL and increase in HDL when compared to untreated group of rats. The results suggest that the extract may be helpful in the management of heart related diseases and also in the prevention and management of diabetes/diabetic complications. This is because, according to Moller (2001), accumulation of cholesterol and triglycerides lead to reduction in insulin mediated metabolic activity and can cause Type 2 diabetes. This observation is very important because natives who are diabetic and live on the decoction of this medicinal plant may not be at risk of diabetic complications like cardiovascular effects. However, caution should be exercised as continued intake of the plant extract may lead to abnormal low cholesterol level in users.

Liver is the major organ involved in drug biotransformation. Levels of serum liver biomarker enzymes are biochemical parameters usually performed in order to evaluate any toxic effects on the liver (Mukinda and Syce, 2007). Increases in the levels of AST, ALT and ALP in the serum are associated with liver toxicity by drugs or any other hepatotoxin (Ramaiah, 2011). However, ALT is more specific to liver and thus a better parameter for detecting liver injury as AST is also associated with diseases of other organs such as heart and muscle (Ozer et al., 2008). ALP is present mostly in cells lining the billiary duct of the liver and is used to diagnose obstruction to the billiary system. Therefore, its elevation in the blood indicates cholestatic diseases such as gallstone or tumor blocking the bile duct (Burtis and Ashwood, 2001). In this study, chronic exposure of rats to GLE at different doses (2.5, 5 and 10 mg/kg) caused a significant increase in AST and ALT only on day 90 at the dose of 10 mg/kg while there was a dose-dependent and significant ($p < 0.001$) elevation of ALP values from day 30 to day 90 when compared to negative group. This may be an indication of cholestatic disorder. Also the liver is prone to xenobiotic-induced injury because of its central role in xenobiotic metabolism (Bass and Ockner, 1996).

Bilirubin is a breakdown product of hemoglobin and is associated with hepatic diseases like jaundice and ineffective erythropoiesis and increased bilirubin levels reflect the depth of jaundice (Thapa and Walia, 2007). In this study there was no significant change in the levels of serum bilirubin and albumin of both the treated and control rats. This suggests that the extract may have no toxic effect on the erythropoietic system as reported earlier in hematological parameters.

On day 90, there was a significant ($p < 0.05$) increase in the mean total protein level of the treated rats at the dose of 10 mg/kg (Fig. 5). It can be suggested that the observed increases in total protein at that dose level on day 90 may be due to increased synthesis of globulin in the lymphoid organ and albumin with possible involvement of the liver (Donga et al., 2011).

Urea and creatinine are considered as important markers of kidney dysfunction (Mukinda and Eagles, 2010). The increase in serum urea levels observed in the high dose (10 mg/kg, GLE) treated group when compared to the untreated control may be associated with kidney dysfunction most likely by renal filtration mechanism and probably indicates that chronic exposure of GLE at the dose of 10 mg/kg for up to 90 days may interfere with the capacity of the kidney to excrete this metabolite as suggested by Crook (2006). This is may be responsible for the increase in relative organ weight of the kidney observed in this study.

The effect of GLE on lipid peroxidation in rats given the extract in feed for 90 days was evaluated using catalase and superoxide dismutase (SOD) enzymes activities and malondialdehyde (MDA) level.

Decreases in the levels of catalase and SOD activities and increases in the level of MDA signifies increased oxidative stress and reduced antioxidant activities in the system which reduces the capability of the body to get rid of free radicals (Parejo et al., 2002). In this study, GLE caused a dose-dependent decrease in the level of MDA of the extract treated rats. The extract also caused a significant increase in SOD activity on days 60 and 90 and no significant increase in the catalase activity of the extract treated group when compared to the untreated control. This suggests that GLE has beneficial effect in increasing antioxidant defense of the body and may be of value in reduction of oxidative stress and may help in prevention and management of degenerative diseases such as diabetes mellitus (Soto et al., 2003).

The biochemical signs of liver and kidney damage as a result of sub-chronic exposure of the rats to GLE for 90 days were confirmed by the histopathological findings of these organs. They were observed at the dose of 10 mg/kg on the 90th day of the study. The lesions include: pyknotic cells, sloughing off of the tubular epithelial cells and presence of cells in the lumen of kidney tubules (Plate 1B). These changes will interfere with ability of the kidney to carry out its normal excretory roles. The presence of the cells in the lumen especially can cause narrowing or blockage of the tubular lumen and this interferes with excretion of metabolites (Crook, 2006).

This may have contributed to the high levels of urea seen in the blood of rats treated with GLE at the dose of 10 mg/kg on day 90 in this study. Also the kidney is highly susceptible to toxicants because a high volume of blood flows through it and it filters large amounts of toxins which can concentrate in the kidney tubules (Emily, 2007).

Liver sections revealed proliferation or hyperplasia of the bile ducts around the central vein in rats treated with 10 mg/kg of *Gouania longipetala* extract on day 90 of the sub-chronic study (Plate 2B).

Bile duct proliferation now termed ductular reaction describes increase in the number of bile ducts structures seen in many forms of liver disease especially cholestatic injury. The role of this ductular reaction is not clear but the ducts may provide a route for the escape of bile in diseases such as primary biliary cirrhosis and primary cholangitis in which there is destruction or obstruction of bile ducts (Burt and MacSween, 1993). The proliferation of bile ducts correlates with the high level of ALP seen in the serum biochemistry in this study (Table 6), since according to Burtis and Ashwood (2001), ALP lines the cells of the bile ducts and also proliferation of bile ducts is seen in cholestatic diseases just like increased levels of ALP is associated with bile duct abnormalities.

In conclusion, *Gouania longipetala* is well tolerated in short term therapies, but caution should be exercised when using the extract for long term therapy as prolonged administration for up to 90 days may lead to some toxic effects on the liver and kidney at high doses. Therefore, for GLE to be used beneficially, treatment should be planned for a short period or very low doses should be used for a long duration of treatment.

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