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Full Length Research Paper

Evaluation of the long-term effects of *Urena lobata* root extracts on blood glucose and hepatic function of normal rabbits

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***Urena lobata* is one of the medicinal plants used to treat diabetes in Nigeria. Its hypoglycaemic and anti-diabetic activities have been demonstrated. This study was designed to evaluate the long term effects of daily oral administration of aqueous extracts of *U. lobata* roots in normal rabbits. Parameters such as body weight, blood glucose and liver function tests were monitored at specific intervals in the serum for 24 weeks, and in the tissue. *U. lobata* significantly (P<0.05) reduced the body weight and fasting blood sugar of treated rabbits. Indicators of cholestasis, alkaline phosphatase and γ-glutamyl transferase, as well as serum direct bilirubin concentration, were initially significantly (P<0.05) increased, these parameters returned to the levels of control before the 10th week of monitoring and tissue enzymes were not depleted, suggesting that the initial toxic response was not sustained. Markers of hepatocyte injury; alanine transaminase and aspartate transaminase, were initially slightly elevated but subsequently returned to control levels. *U. lobata* root had no significant effects on serum total proteins, albumin and globulins. Regulation of dose and frequency of consumption of *U. lobata* extracts may reduce its toxic side effects.**

Key words: *Urena lobata*, hypoglycaemic/anti-diabetic, liver function tests, medicinal plants, toxicity.

INTRODUCTION

Urena lobata L., Caesar weed, belongs to the family Malvaceae (ISB, 2003). Various extracts of leaves and roots are used in herbal medicine to treat such diverse ailments as colic, malaria, gonorrhea, fever, wounds, toothache and rheumatism (De Las Heras et al., 1998; Adeloye et al., 2007). The methanol extract of *U. lobata* root (Mazumder et al., 2001) and various crude extracts of the leaves and roots, as well as the solvent fractions (Adeloye et al., 2007) have been reported to show a broad spectrum of antibacterial activity. *U. lobata* has also been used in many traditional systems to treat diabetes mellitus (Mahabir and Gulliford, 1997; Lans, 2006); root extracts have been shown to have significant anti-diabetic effects in streptozotocin-induced diabetic rats (Onoagbe et al., 2010). Many Nigerians use the root and leave extracts of *U. lobata* to treat diabetes and these extracts are consumed for a long periods of time (Onoagbe et al., 2010), it is therefore necessary to evaluate the toxic effects of these extracts. This study was designed to monitor the long term effects of aqueous root extracts of *U. lobata*, orally administered daily for 24 weeks on body weight, fasting blood glucose and liver function of normal rabbits so as to ascertain its safety.

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MATERIALS AND METHODS

Chemicals and reagents

Bovin serum albumin (Sigma, London), sodium hydroxide, copper sulphate, sodium-potassium tartrate (BDH Chemical Limited, Poole, England). Glucose, Alanine transferase/Glutamate-Pyruvate Transaminase (ALT/GPT), Aspartate transferase/Glutamate-Oxaloacetae Transaminase (AST/GOT), alkaline phosphatase (ALP), gamma glutamyl transferase (γ-GT), albumin and bilirubin Randox kits product of Randox Laboratory Ltd, Ardmore, Diamond Road, Crumlin, Co. Anrtim, United Kingdom. Other analytical grade

Table 1. Summary of histological studies.

Group	Rabbit	Liver
Control	1	No visible lesions (NVL)
	2	NVL
	3	NVL
	4	NVL
	5	NVL
	6	NVL
U. lobata	1	Not severe, but generalized hepatic degeneration
	2	-
	3	-
	4	NVL
	5	Generalized hepatic degeneration
	6	NVL

chemicals were also used.

Plant materials and preparation of extract

U. lobata was obtained from open forest at Akungba-Akoko, Ondo State, Nigeria and identified by Dr A. E. Ayodele of the department of Microbiology and Botany, University of Ibadan, Ibadan, Nigeria. Herbarium specimen, with voucher number UIH 22287 was deposited at the Herbarium of the University of Ibadan, Nigeria. The aqueous plant extracts was prepared by the Onoagbe et al. (1999) method. The plant extracts were orally (by gavage) administered to the rabbits at 200 mg/kg body weight daily for 24 weeks. This dose was chosen from a pilot study of varying doses of extracts; using serum ALT and AST as indices of toxicity and fasting blood glucose as a therapeutic index. It reflects a balance between the toxic and therapeutic dose of the extract.

Animals and management

Twelve rabbits of the New Zealand strain, weighing between 800 to 1200 g, purchased from the Animal Unit of Federal University of Technology, Akure, Ondo State, were used for this research. The physical conditions of the rabbits were assessed by a veterinary doctor and they were allowed to acclimatize for three weeks before the commencement of experiments. The animals were placed on commercial feed (Ewu growers from the Bendel Feed and Flour Mill Ewu, Nigeria) and water *ad libitum*. The rabbits were treated according to the Principles of Laboratory Animal Care (NIH Publication 85-93, revised 1985). The rabbits were divided into two groups:

Group I: Normal control
Group II: Normal rabbits treated with aqueous root extracts of *U. lobata*

Blood collection

Blood was collected from the ventral vein of the rabbits' ear during the period of monitoring, at the end of the monitoring phase, the rabbits were sacrificed by stunning and while unconscious, the thoracic and abdominal regions were opened to expose the heart and other organs. Blood was collected through heart puncture, the liver was also collected. Blood samples for glucose and biochemical assays were allowed to clot and centrifuged at 1000 g for 5 min; the serum was then separated for analysis. Tissues were homogenized in ice cold normal saline (1:4 w/v), centrifuged and the supernatant stored in the freezer until analysis.

Biochemical analyses

Blood glucose was measured by the glucose oxidase method of Barham and Trinder (1972), serum and tissue ALT and AST activities were assayed by Reitman and Frankel (1957) method while serum and tissue ALP and γ-GGT activities were measured by an optimized standard method according to the recommendations of the Deutsche Gesellschaft fur Klinische Chemie (Rec. GSCC DGKC) (1972) and the Szasz (1969) methods respectively. Serum and tissue protein levels were measured by the Biuret method (Gornall et al., 1949), serum albumin levels were measured by Doumas and Biggs (1972) method while the amount of globulins was calculated as a difference between total serum proteins and serum albumin. Serum total and direct bilirubin levels were assayed by Jendrassik and Grof (1938) method. Histological investigation on the liver was done according to the method described by Lamb (1981) Table 1.

Statistical analysis

The differences between means of control and test group were analyzed by the independent samples T-test. The SPSS 11.0, SPSS Inc., Chicago, Illinois, USA, was used for this analysis. A value of $P < 0.05$ was considered as statistically significant.

RESULTS

Body weight

The body weights of rabbits were monitored weekly and after 24 weeks, the liver body weight ratio was assessed. *U. lobata* treatment significantly ($P<0.05$) lowered the body weight gain of test rabbits throughout the period of monitoring (Figure 1). Dissection of treated rabbits

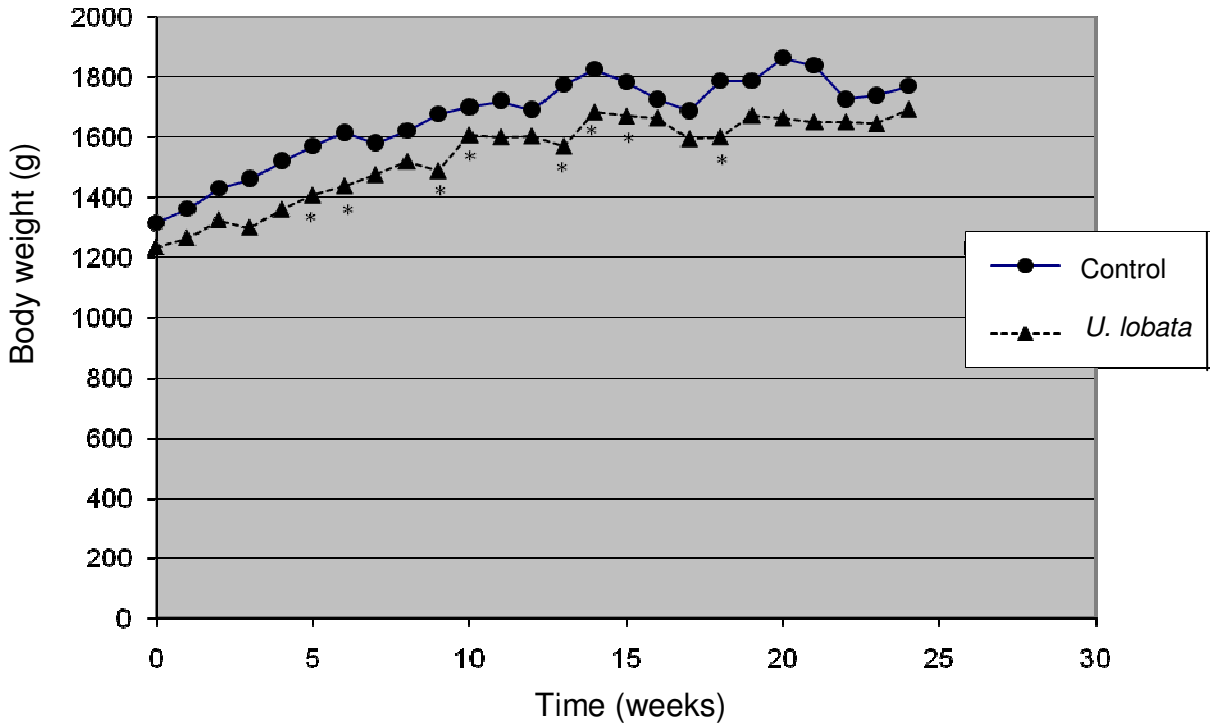

Figure 1. Time-course of body weight gain of control rabbits and rabbits orally treated daily with aqueous extracts of *U. lobata* root for 24 weeks at 200 mg/kg body weight. Data was obtained weekly and are means ± SEM of 4 to 6 determinations. Values carrying notations are statistically different from control at P<0.05.

revealed a virtual absence of subcutaneous fat. *U. lobata* administration had no significant effect on liver-body weight ratio.

Fasting blood glucose

Significant (P<0.05) decreases in fasting blood glucose levels were observed from week 4 to the end of the monitoring phase for the *U. lobata* treated rabbits (Figure 2).

Liver function tests

Increased serum ALT activities were observed from weeks 8 to12 in the *U. lobata* treated rabbits (Figure 3), also, serum AST activities were increased in weeks 2 and 18 (Figure 4). None of the increases observed in serum ALT and AST activities was more than 1.5 times the value of control. A nearly three-fold increase was observed in the serum ALP activities of the *U. lobata* treated normal rabbits at weeks 1 and 3 (Figure 5), a concomitant increase in serum GGT activities was also observed in weeks 1 and 4 (Figure 6). None of the liver enzyme activities were significantly depleted (Figure 7).

For most of the period of monitoring, the results obtained for serum total protein (Figure 8), albumin (Figure 9) and globulins (Figure 10) were similar to control. Apart from the initial slight increases (weeks 2 and 3) observed in serum total bilirubin levels (Figure 11), most values recorded were similar or lower than control, while the serum direct bilirubin were mostly slightly higher (weeks 1 to 10) than control (Figure 12).

DISCUSSION

Millions of people in developing nations, including Nigerians, have resorted to the use of medicinal plants to treat their ailments; this could be as a result of the high cost of orthodox health care or maybe as a result of the global shift towards the use of natural, rather than synthetic drugs. While the craze for natural products has its merits, care must be taken not to consume plants or plant extracts that could have deleterious effects, either on the short term or on the long term. It therefore means that these plants must be studied for their biochemical/toxicological effects.

The virtual absence of subcutaneous fat in *U. lobata* treated rabbits implies that the weight reduction was as a result of loss of fat deposits and not muscle wasting. The weight lowering effects of *U. lobata* root extracts may be related to its high fibre and plant carbohydrate content, as well as the presence of phytochemicals such as steriodal/triterpenoidal saponins (Omonkhua and

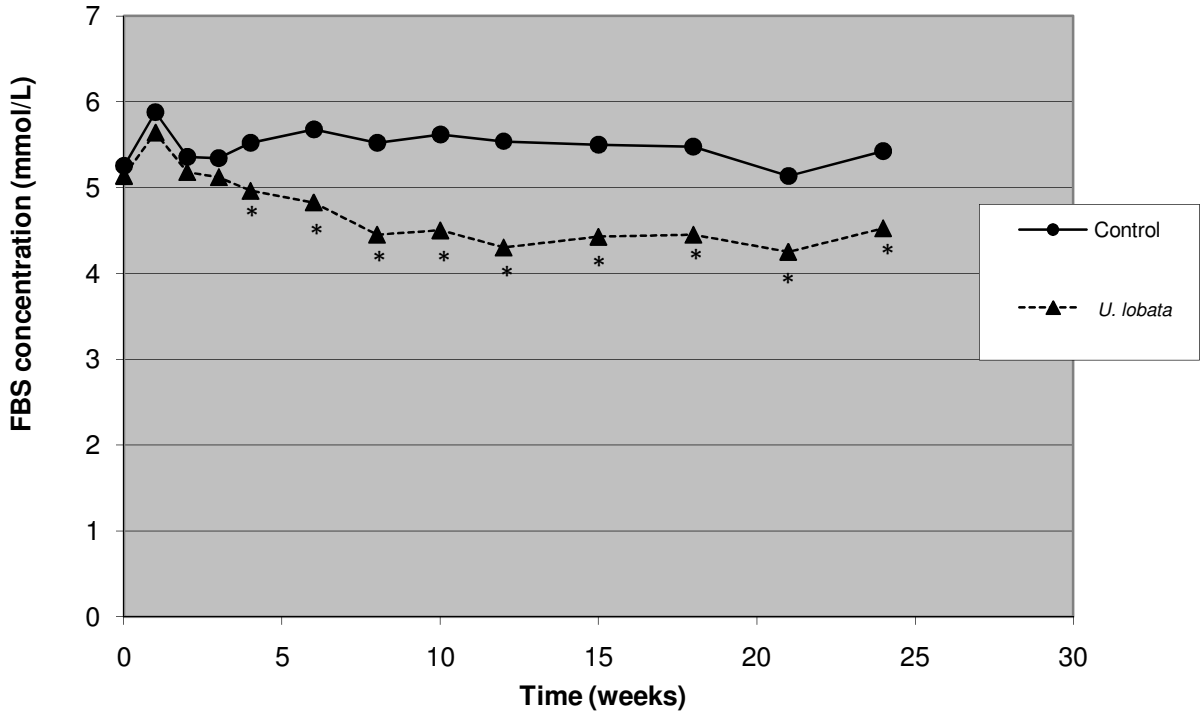

Figure 2. Effects of repeated daily oral administration of aqueous extracts of *U. lobata* root for 24 weeks at 200 mg/kg body weight on the fasting blood sugar concentration of normal rabbits. Data was obtained from serum at pre-determined intervals and are means ± SEM of 4-6 determinations. Values carrying notations are statistically different from control at P<0.05.

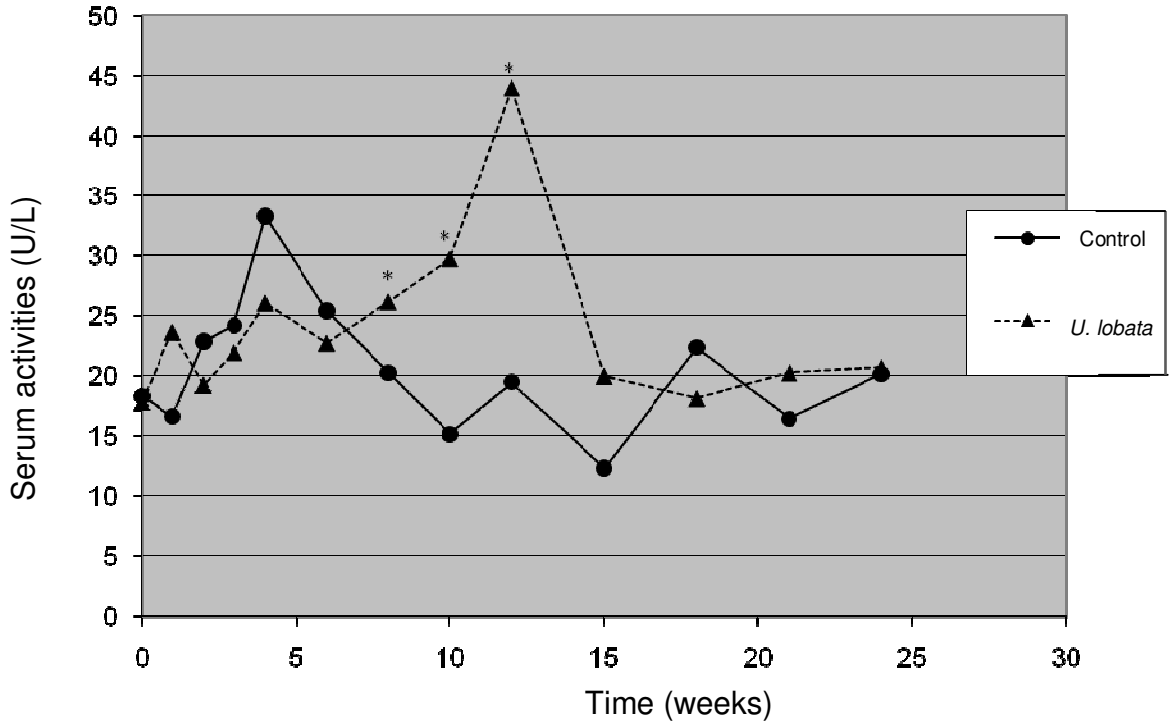

Figure 3. Effects of repeated daily oral administration of aqueous extracts of *U. lobata* root for 24 weeks at 200 mg/kg body weight on serum ALT activities of normal rabbits. Data was obtained from serum at pre-determined intervals and are means ± SEM of 4-6 determinations. Values carrying notations are statistically different at P<0.05.

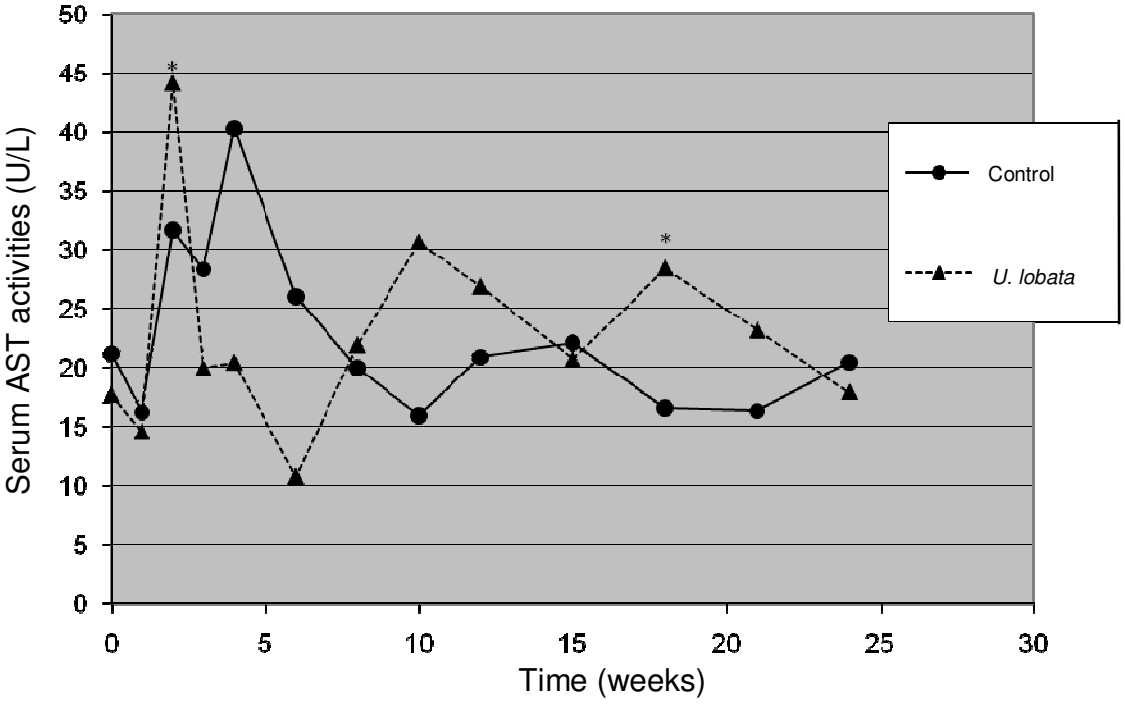

Figure 4. Effects of repeated daily oral administration of aqueous extracts of *U. lobata* root for 24 weeks at 200 mg/kg body weight on serum AST activities of normal rabbits. Data was obtained from serum at pre-determined intervals and are means ± SEM of 4-6 determinations. Values carrying notations are statistically different at $P<0.05$.

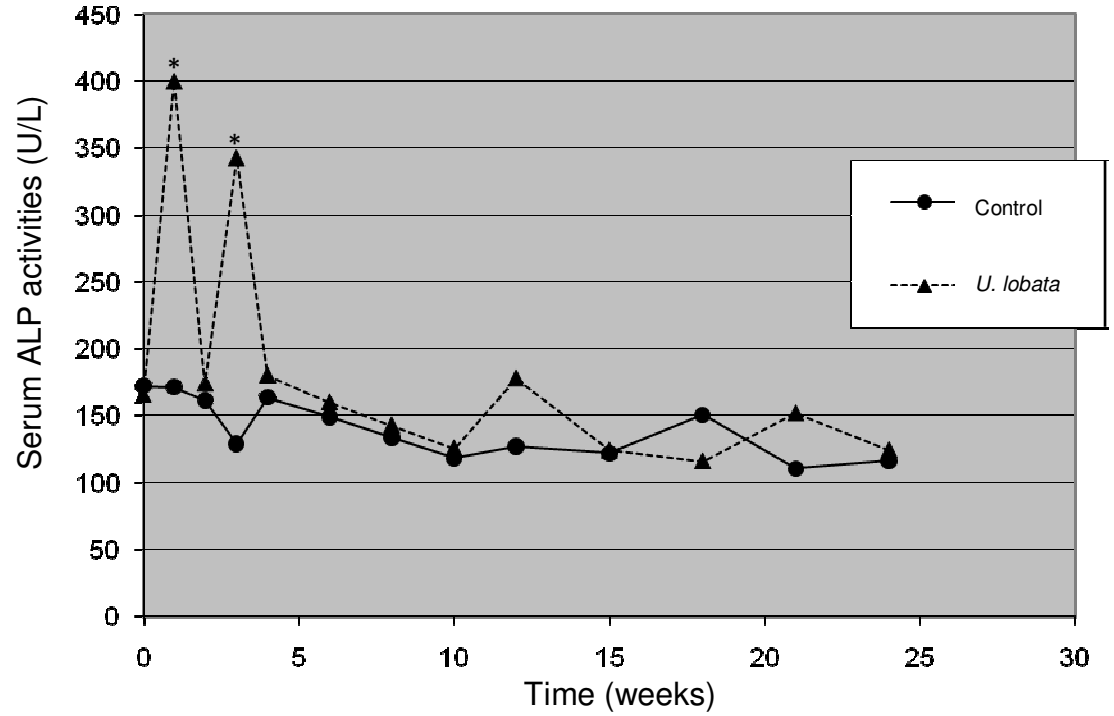

Figure 5. Effects of repeated daily oral administration of aqueous extracts of *U. lobata* root for 24 weeks at 200 mg/kg body weight on serum ALP activities of normal rabbits. Data was obtained from serum at pre-determined intervals and are means ± SEM of 4-6 determinations. Values carrying notations are statistically different at $P<0.05$.

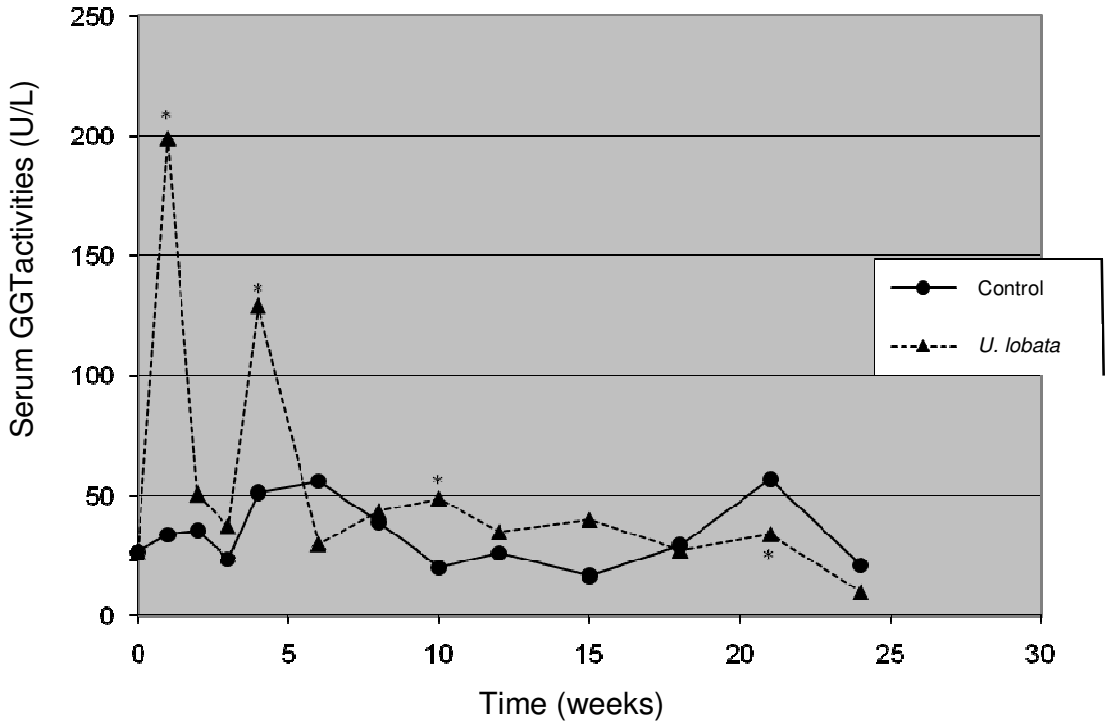

Figure 6. Effects of repeated daily oral administration of aqueous extracts of *U. lobata* root for 24 weeks at 200 mg/kg body weight on serum GGT activities of normal rabbits. Data was obtained from serum at pre-determined intervals and are means ± SEM of 4-6 determinations. Values carrying different notations are statistically different at P<0.05.

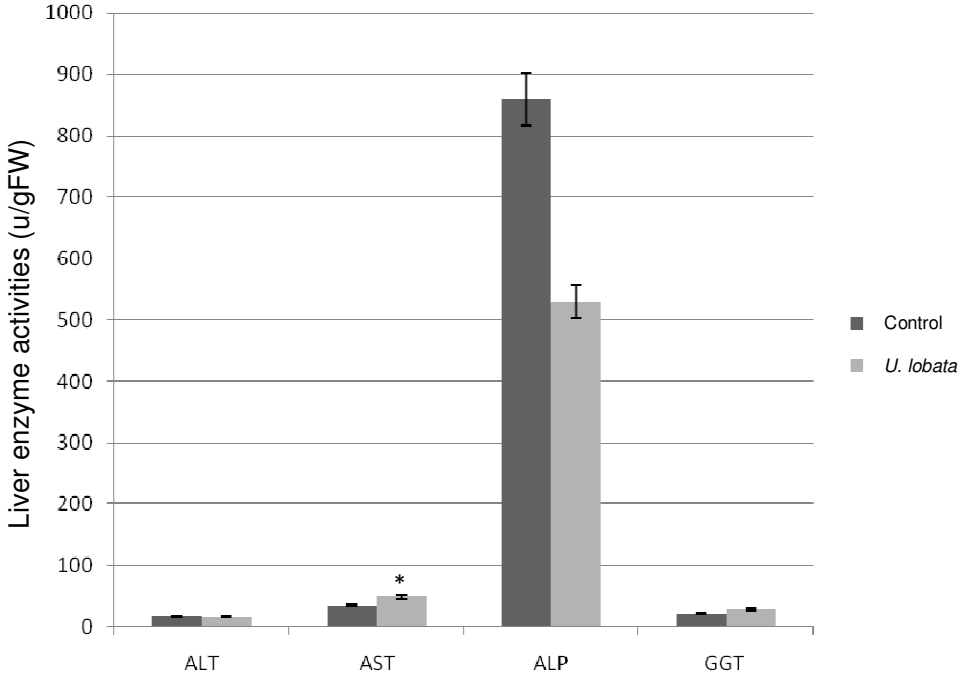

Figure 7. Effects of repeated daily oral administration of aqueous extracts of *U. lobata* root for 24 weeks at 200 mg/kg body weight on liver ALT, AST, ALP and GGT activities of normal rabbits. Data was obtained from tissue homogenates at the end of 24 week of monitoring and are means ± SEM of 4-6 determinations. Values carrying notations are statistically different from control at P<0.05.

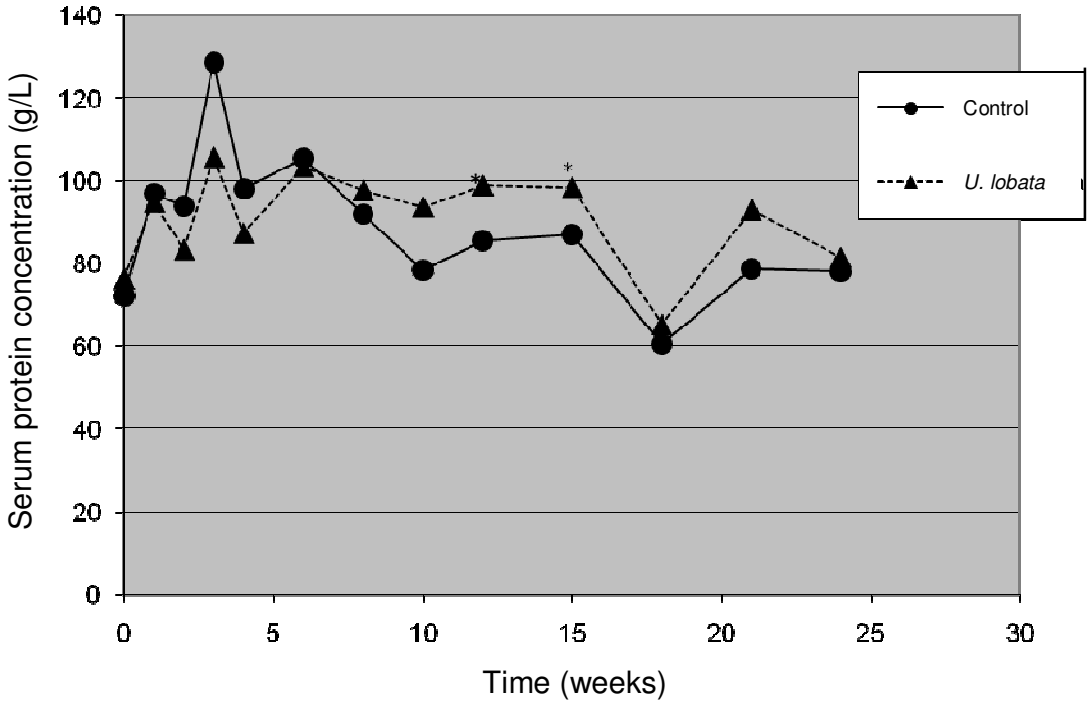

Figure 8. Effects of repeated daily oral administration of aqueous extracts of *U. lobata* root for 24 weeks at 200 mg/kg body weight on serum total protein concentration of normal rabbits. Data was obtained from serum at pre-determined intervals and are means ± SEM of 4-6 determinations. Values carrying notations are statistically different from control at P<0.05.

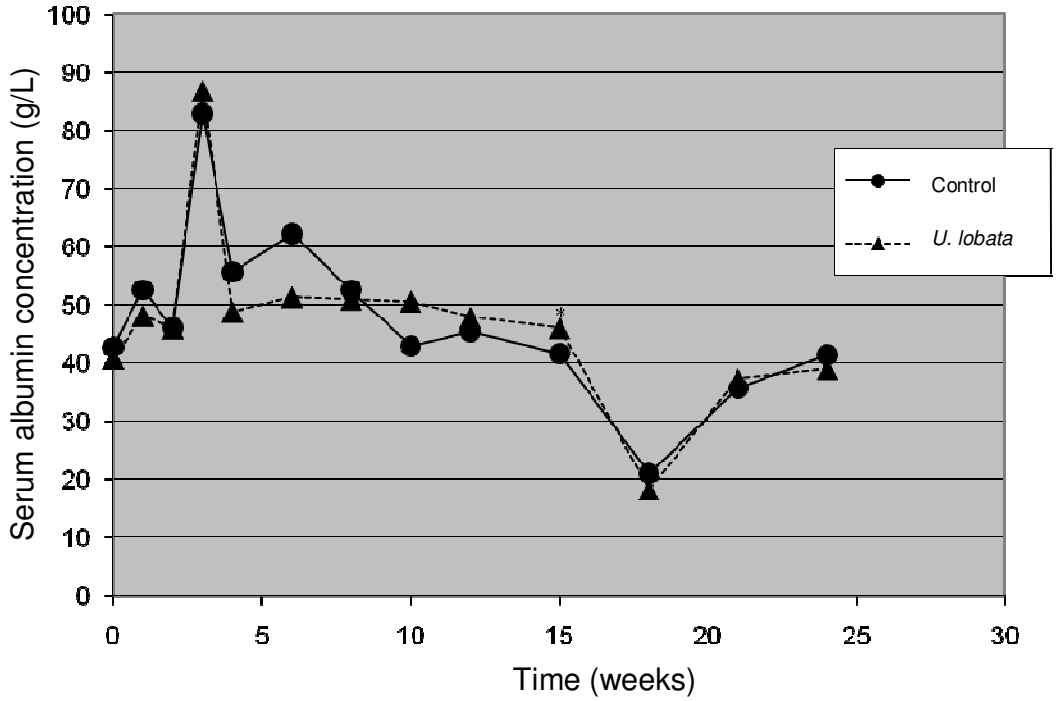

Figure 9. Effects of repeated daily oral administration of aqueous extracts of *U. lobata* root for 24 weeks at 200 mg/kg body weight on serum albumin concentration of normal rabbits. Data was obtained from serum at pre-determined intervals and are means ± SEM of 4-6 determinations. Values carrying notations are statistically different from control at P<0.05.

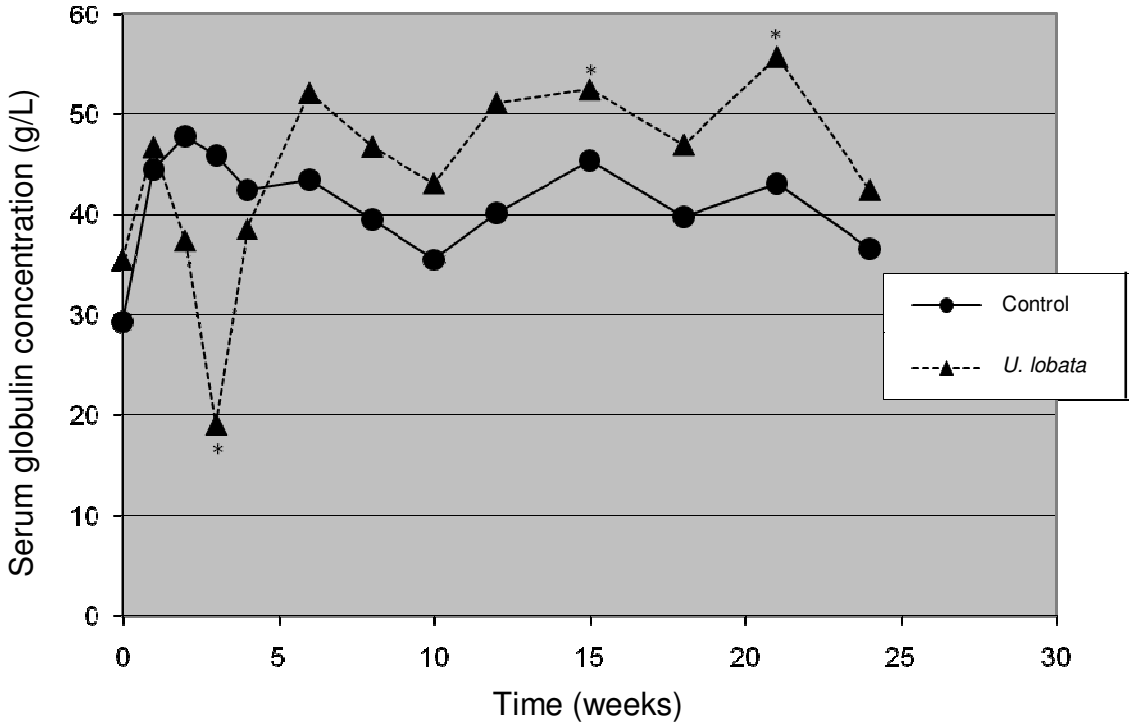

Figure **10.** Effects of repeated daily oral administration of aqueous extracts of *U. lobata* root for 24 weeks at 200 mg/kg body weight on serum globulins concentration of normal rabbits. Data was obtained from serum at pre-determined intervals and are means ± SEM of 4-6 determinations. Values carrying notations are statistically different from control at P<0.05.

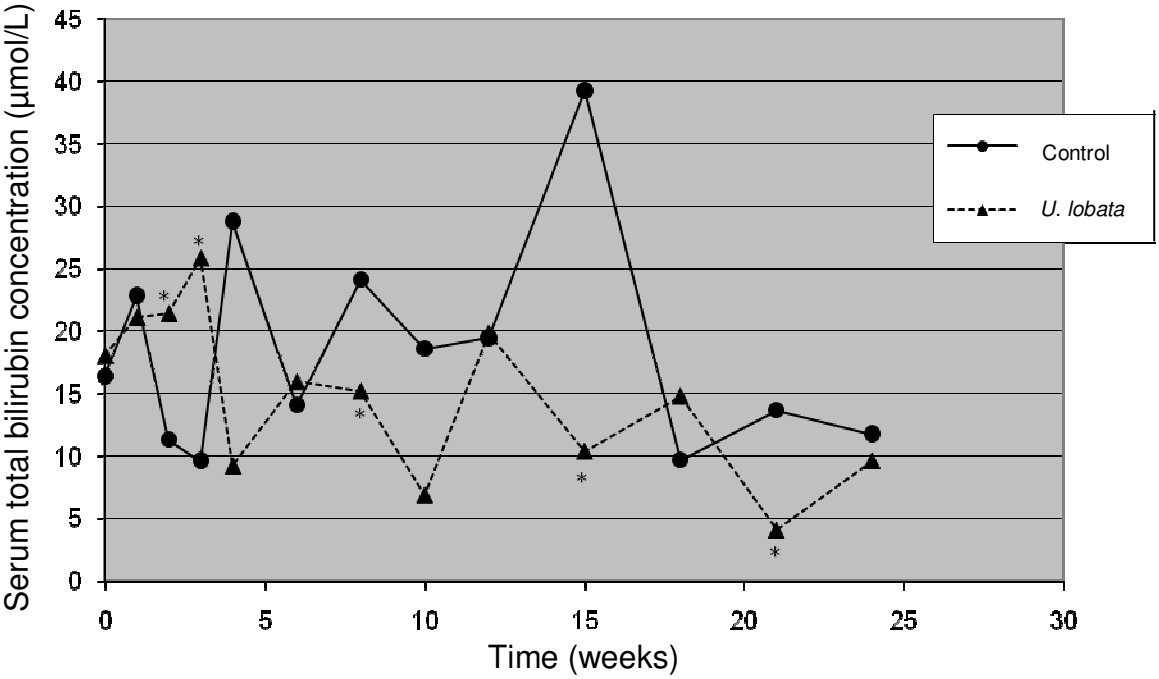

Figure 11. Effects of repeated daily oral administration of aqueous extracts of *U. lobata* root for 24 weeks at 200 mg/kg body weight on serum total bilirubin concentration of normal rabbits. Data was obtained from serum at pre-determined intervals and are means ± SEM of 4-6 determinations. Values carrying notations are statistically different from control at P<0.05.

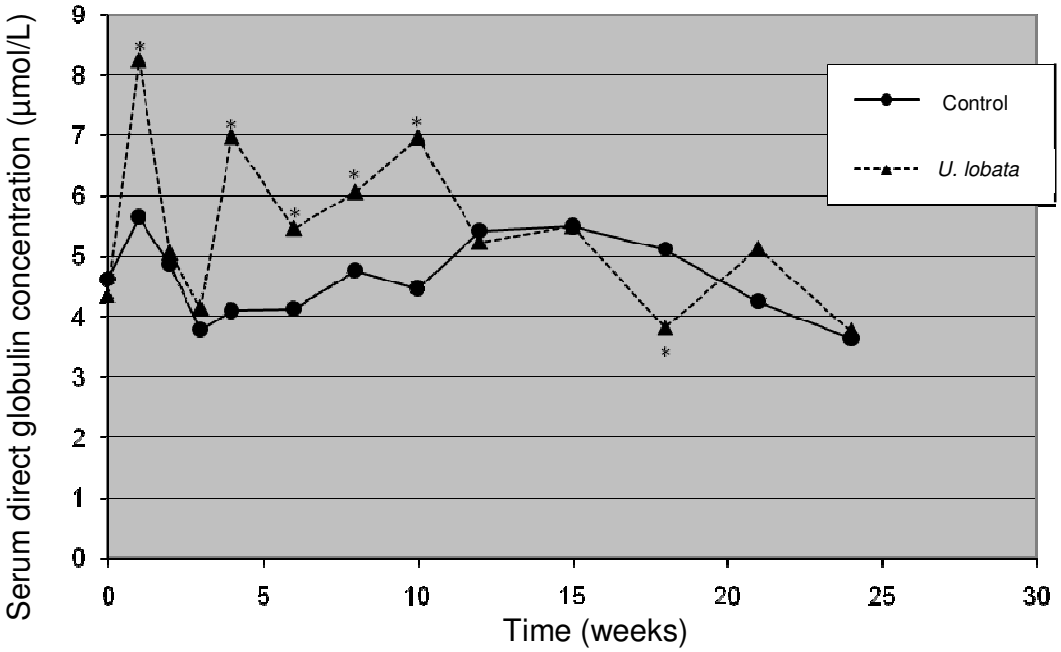

Figure 12. Effects of repeated daily oral administration of aqueous extracts of *U. lobata* root for 24 weeks at 200 mg/kg body weight on serum direct bilirubin concentration of normal rabbits. Data was obtained from serum at pre-determined intervals and are means ± SEM of 4-6 determinations. Values carrying notations are statistically different from control at $P<0.05$.

Onoagbe, 2010) and quercetin (Howarth et al., 2001). This effect may contribute to the hypoglycaemic effect of this medicinal plant by increasing insulin sensitivity (DeFronzo, 1997). Liver-body weight ratio was not altered by *U. lobata* treatment. The hypoglycaemic effect of *U. lobata* observed in this study gives scientific credence to the traditional use of this plant as an anti-diabetic remedy (Mahabir and Gulliford, 1997; Lans, 2006) and corroborates the work of Onoagbe et al. (2010). These results also show that *U. lobata* have a sustained (24 weeks) hypoglycaemic effect implying a consistent glycaemic control for diabetes. High fibre (Erdman et al., 2007) and phytochemicals such as saponins and flavonoids (quercetin) (Awika and Rooney, 2004; Murthy et al., 2009) may contribute to this hypoglycemic effect. None of the increases observed in serum ALT and AST activities was more than 1.5 times the value of control. Slight AST or ALT elevations (within 1.5 times the upper limits of normal) do not necessarily indicate liver disease (Sherman, 1991). Some histological slides of test animals showed slight to moderate hepatic degeneration. The implication of these results is that the hepatocytes membrane integrity was slightly compromised by the administration of the medicinal plant. The subsequent restoration of enzyme activities to control levels as well as the fact that liver enzyme activities were not depleted shows that hepatocytes membrane damage was not sustained. Most research on the effects of anti-diabetic medicinal plants on serum ALT and AST activities of healthy subjects report normal or lower activities (Gupta et al., 2005; Kesari et al., 2007) suggesting that these anti-diabetic plants do not exert hepatic injury. The results obtained in this study however, imply that *U. lobata* extracts administered orally at 200 mg/kg body weight for 24 weeks, elicited an initial toxic responses from the liver, which was not severe and sustained. The nearly three-fold increases observed in the serum ALP activities of the *U. lobata* treated normal rabbits at weeks 1 and 3 are indicative of bile duct obstruction, and the concomitant increase in serum GGT activities of this group further strengthens this suggestion (Johnston, 1999; Whitfield et al., 1972). Kesari et al. (2007) and Gupta et al. (2005) reported for the different anti-diabetic plants they studied, that serum ALP activities were reduced after one month of extract administration. This study however, showed that administration of *U. lobata* aqueous extracts exerted an initial toxic effect on bile production and/or flow, the subsequent reduction of serum ALP and GGT activities to control levels implies this negative effect was not prolonged. Serum albumin is frequently utilized as an index of the hepatocyte's ability to carry out synthetic function. Serum albumin does not change in mild liver injury but readily declines in the face of sub-massive liver necrosis (Johnston, 1999; Rothschild et al., 1988).

For most of the period of monitoring, the results obtained for serum total protein, albumin and globulins were similar to control suggesting that administration of

U. lobata did not diminish the protein synthetic capacity of the liver. Many different liver diseases, as well as conditions other than liver diseases (e. g. increased production by enhanced red blood cell destruction), can cause the serum bilirubin concentration to be elevated (Johnston, 1999). Apart from the initial slight increases (weeks 2 and 3) observed in serum total bilirubin levels, most values recorded were similar or lower than control, while the serum direct bilirubin were mostly slightly higher (weeks 1 to 10) than control. The implication of this is that *U. lobata* administration caused bile duct obstruction which is consistent with the increased serum ALP and GGT observed in this study. The subsequent restoration of direct bilirubin levels of treated rabbits to control values suggests that this lesion was short-lived.

Biochemical and histological evidences obtained from this study have shown that *U. lobata* administration exerted an initial toxic effect on hepatocytes and also caused bile obstruction, these events were however, not severe and sustained. It is possible that reduction in dose, frequency and duration of administration may reduce the side effects observed in this study.

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