Journal of Medicinal Plants Research Vol. 6(13), pp. 2581-2589, 9 April, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.561
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Effects of long-term oral administration of aqueous extracts of *Irvingia gabonensis* bark on blood glucose and liver profile of normal rabbits

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Accepted 30 August, 2011

***Irvingia gabonensis*, which is consumed widely in Nigeria, has been shown to possess hypoglycaemic/anti-diabetic properties. In this study, the long term effects of aqueous extracts of *I. gabonensis* bark, orally administered daily, on body weight, blood glucose and liver function of normal rabbits were monitored at pre-determined intervals for 24 weeks. Body weight and fasting blood sugar were significantly ($p<0.05$) reduced in the medicinal plant treated rabbits. Indices of hepato-cellular injury, alanine transaminase and aspartate transaminase were initially slightly elevated, while markers of cholestasis, alkaline phosphatase and γ-glutamyl transferase were initially significantly ($p<0.05$) increased. These parameters returned to the levels of control midway into the monitoring period and tissue enzymes were not depleted, suggesting that the initial toxic response was not sustained. Administration of *I. gabonensis* bark had no effect on serum total proteins, albumin and globulins, as well as serum total and direct bilirubin concentrations. The sustained anti-obesity and hypoglycaemic effects, as well as the relative low liver toxicity of *I. gabonensis* bark extracts makes it an important candidate for the treatment of diabetes.**

Key words: *Irvingia gabonensis*, hypoglycaemic/anti-diabetic, liver function, medicinal plants, toxicity; anti-obesity.

INTRODUCTION

Irvingia species, including *Irvingia gabonensis*, *Irvingia wombolu* and *Irvingia grandifolia*, are used in the treatment of ailments such as dysentery, diabetes mellitus and also used as analgesic (Oloyede, 2005). The hypoglycaemic effects of *I. grandifolia* have been reported in normal rabbits and streptozotocin-induced diabetic rats (Onoagbe et al., 1999a). The anti-diabetic effects of *I. gabonensis* seed extracts on type 2 diabetics was first reported by Adamson et al. (1990); seed extracts have also been reported to have hypoglycemic effects on humans (Ngondi et al., 2005) and on streptozotocin-induced diabetic rats (Ngondi et al., 2006). We have also observed the anti-diabetic effects of aqueous extracts of *I. gabonensis* bark on streptozotocin-induced diabetic rats. Since diabetes mellitus is a chronic disorder, and these medicinal plants are used for prolonged periods of time, it is imperative to assess the long term biochemical effects of medicinal plants so as to ascertain their safety.

Liver function tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs/substances (Harris, 2005). This study was designed to monitor the long term effects of aqueous bark extracts of *I. gabonensis*, o rally

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administered daily for 24 weeks on body weight, fasting blood glucose and liver function of normal rabbits.

MATERIALS AND METHODS

Plant materials and preparation of extract

The bark of *I. gabonensis* was obtained locally from open forest at Akungba-Akoko, Ondo State, Nigeria and identified by Dr O. A. Obembe of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko. The identity of the plant was further authenticated by Dr A. E. Ayodele of the Department of Microbiology and Botany, University of Ibadan, Ibadan, Nigeria. Herbarium specimen, with voucher number UIH 22286 was deposited at the Herbarium of the University of Ibadan, Nigeria. The aqueous plant extract was prepared by the method described by Onoagbe et al. (1999b).

Experimental animals and management

Twelve (12) 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 rabbits were treated by a veterinary doctor and 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 allowed to drink water freely. Treatment of the animals was in accordance with the Principles of Laboratory Animal Care (NIH Publication, 1985 to 1993; revised, 1985). The rabbits were divided into two groups:

Group I: Control (normal rabbits).
Group II: Normal rabbits treated with *I. gabonensis* aqueous bark extracts.

The plant extract was orally administered to the rabbits at 200 mg/kg body weight daily for 24 weeks.

Blood collection

During the monitoring phase, blood was collected from the ventral vein of the rabbits' ear, at the end of the monitoring phase, the rabbits were stunned and in this unconscious state, the thoracic and abdominal regions were opened to expose the heart and other organs. Blood was obtained through heart puncture, the liver was also collected.

Blood samples for glucose and biochemical assays were allowed to clot and centrifuge at 5,000 rpm 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

Fasting blood glucose analysis

Fasting blood glucose was measured by the glucose oxidase method of Barham and Trinder (1976) as described in the Randox Glucose kit product of Randox Laboratory Ltd, Ardmore, Diamond Road, Crumlin, Co. Anrtim, United Kingdom.

Liver function tests

Serum and tissue ALT and AST activities (Reitman and Frankel, 1957), serum and tissue ALP activities (Rec. Gscc (DGKC), 1972), serum and tissue γ-GT activities (Szasz, 1969), serum total and direct bilirubin concentrations (Jendrassik and Grof, 1938) and serum albumin concentrations (Doumas and Biggs, 1972) were assayed using Randox kits. Serum and tissue protein levels were measured by the Biuret method (Gornall et al., 1949), while the amount of globulins was calculated as a difference between total serum proteins and serum albumin. Histological investigation on the liver was done according to the method described by Lamb (1981).

Statistical analysis

The differences between means of control and test groups 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 overall effects of long-term administration of aqueous extracts of *I. gabonensis* bark on normal rabbits, was assessed by monitoring body weight for 24 weeks and organ-body weight ratio. Treatment with *I. gabonensis* aqueous extracts significantly ($p<0.05$) lowered the body weights of the animals. Upon dissection, the test animals were virtually devoid of subcutaneous fat compared to control animals (Figure 1). *I. gabonensis* had no effect on liver-body weight ratio.

Fasting blood glucose

I. gabonensis treatment caused significant ($p<0.05$) decreases in fasting blood glucose levels from week 4 to the end of the monitoring phase in treated rabbits, indicating that this plant has a sustained hypoglycaemic effect (Figure 2).

Liver function tests

Figures 3 to 8 show the effects of *I. gabonensis* bark extracts on various liver function tests.

DISCUSSION

Plants having hypoglycaemic/anti-diabetic properties abound in literature, however, searching for new anti-diabetic drugs from natural plants is still attractive because they contain substances that have alternative

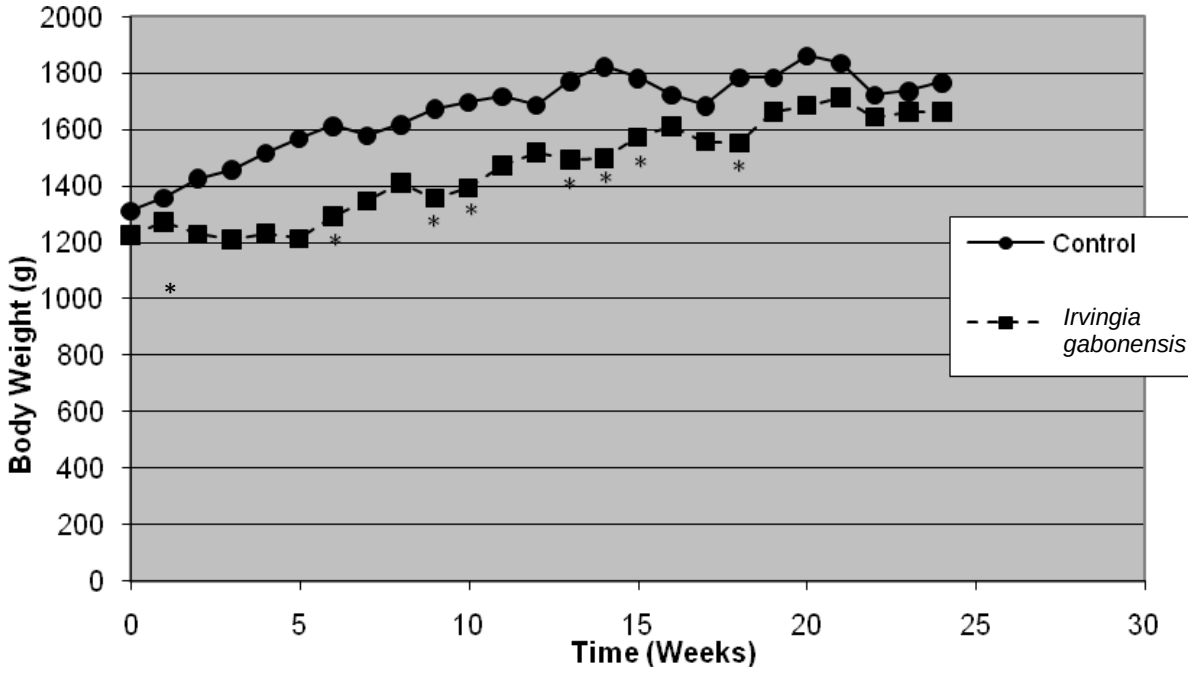

Figure 1. Time-course of body weight gain of control rabbits and rabbits orally treated daily with aqueous extracts of *I. gabonensis* bark for 24 weeks at 200 mg/kg body weight. Data was obtained weekly and are means of 4 to 6 determinations. Values carrying notations are statistically different from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

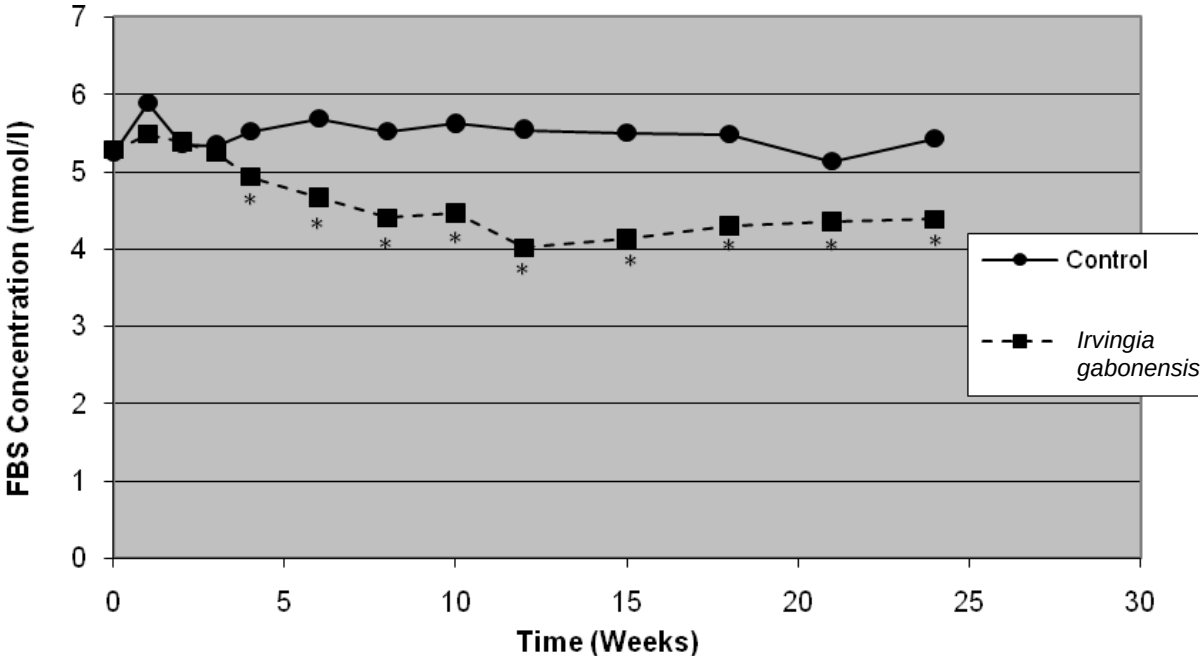

Figure 2. Effects of repeated daily oral administration of aqueous extracts of *I. gabonensis* bark for 24 weeks at 200 mg/kg body weight on the fasting blood sugar concentration of normal rabbits. Data was obtained from serum at pre-determined intervals and are means of 4 to 6 determinations. Values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

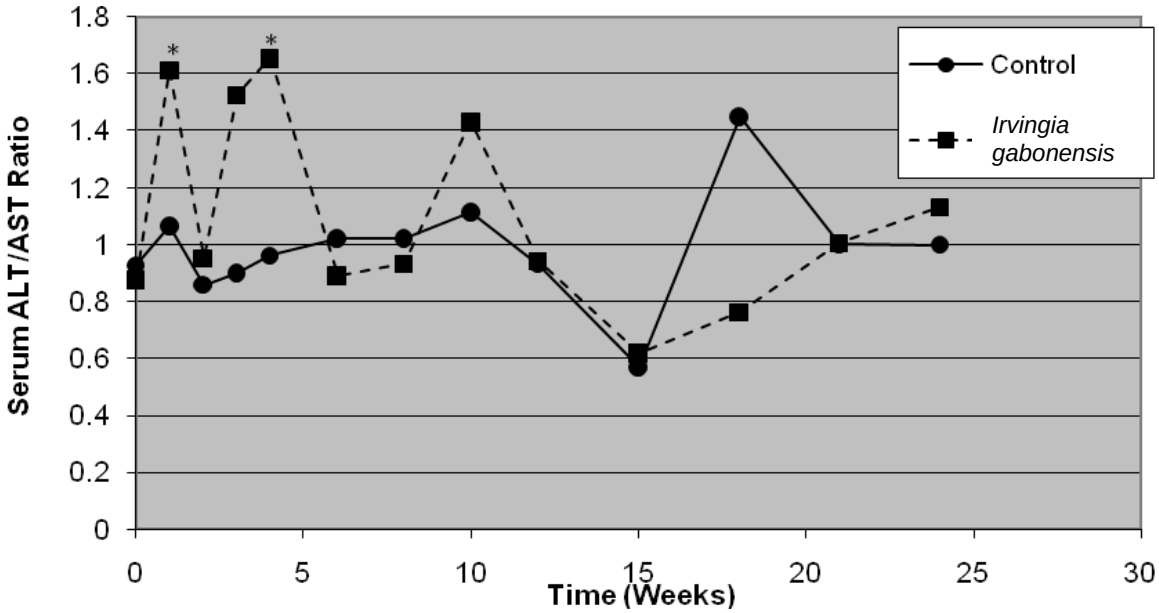

Figure 3. Effects of repeated daily oral administration of aqueous extracts of *I. gabonensis* bark for 24 weeks at 200 mg/kg body weight on serum ALT/AST ratio of normal rabbits. Data was obtained by dividing the serum ALT values by serum AST values and are means of 4 to 6 determinations. Values carrying notations are statistically different from control at $p<0.05$.

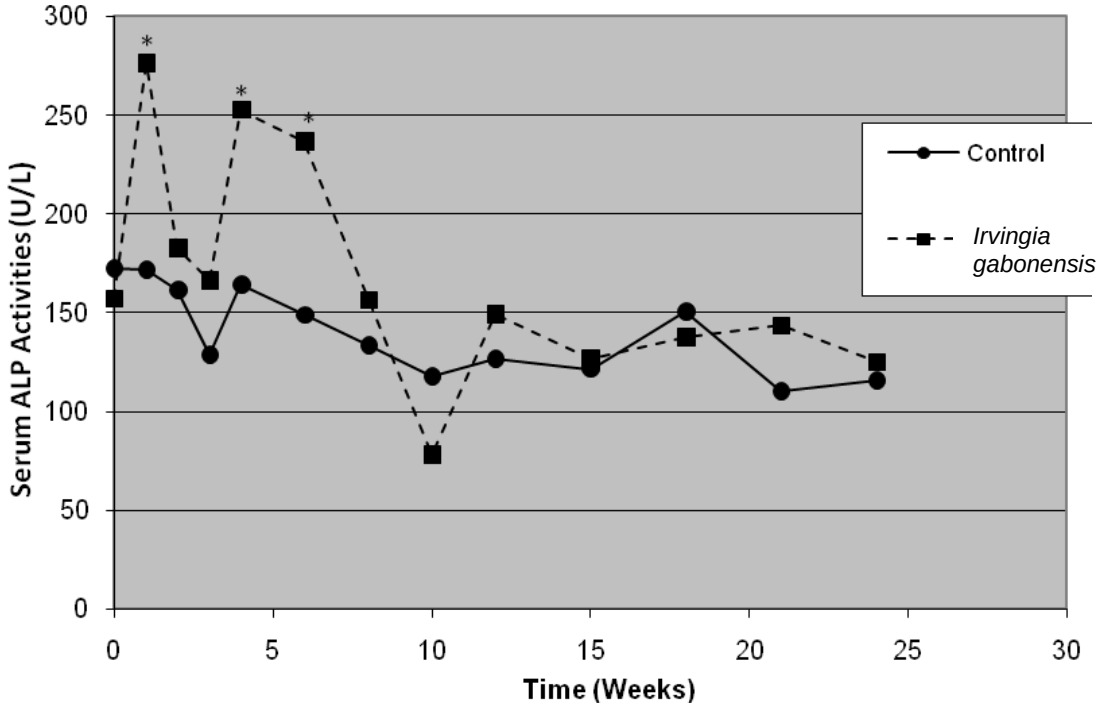

Figure 4. Effects of repeated daily oral administration of aqueous extracts of *I. gabonensis* bark for 24 weeks at 200 mg/kg body weight on serum ALP activities of normal rabbits. Data was obtained from serum at pre-determined intervals and are means of 4 to 6 determinations. Values carrying notations are statistically different from control at $p<0.05$.

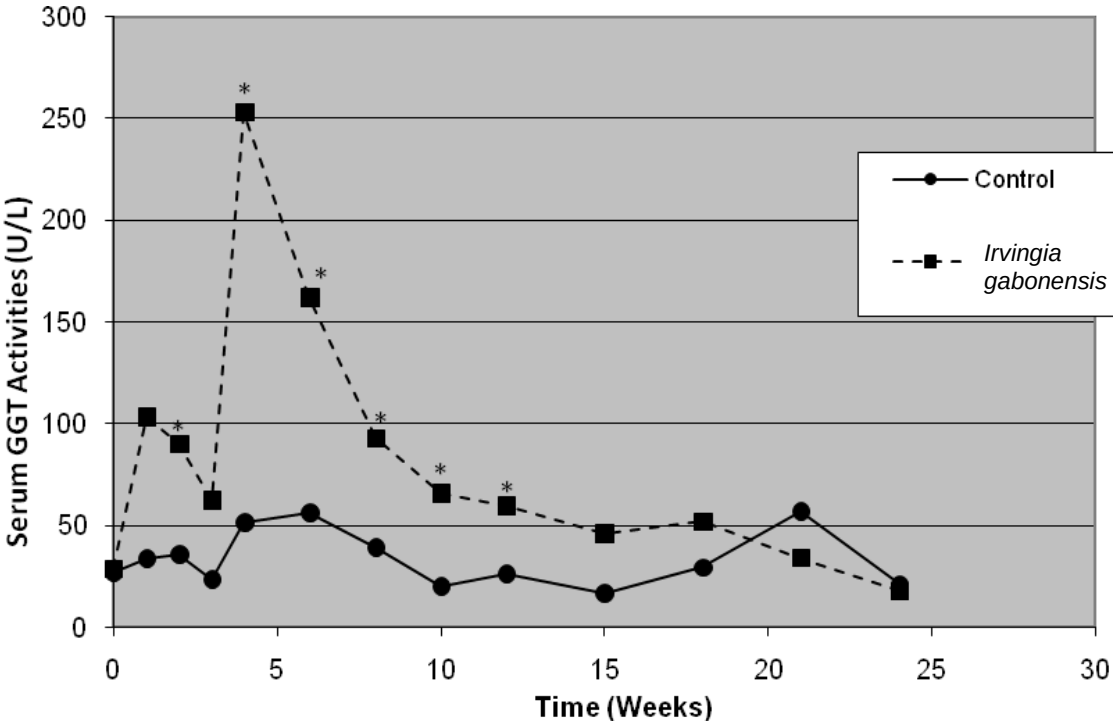

Figure 5. Effects of repeated daily oral administration of aqueous extracts of *I. gabonensis* bark for 24 weeks at 200 mg/kg body weight on serum GGT activities of normal rabbits. Data was obtained from serum at pre-determined intervals and are means of 4 to 6 determinations. Values carrying notations are statistically different from control at $p<0.05$.

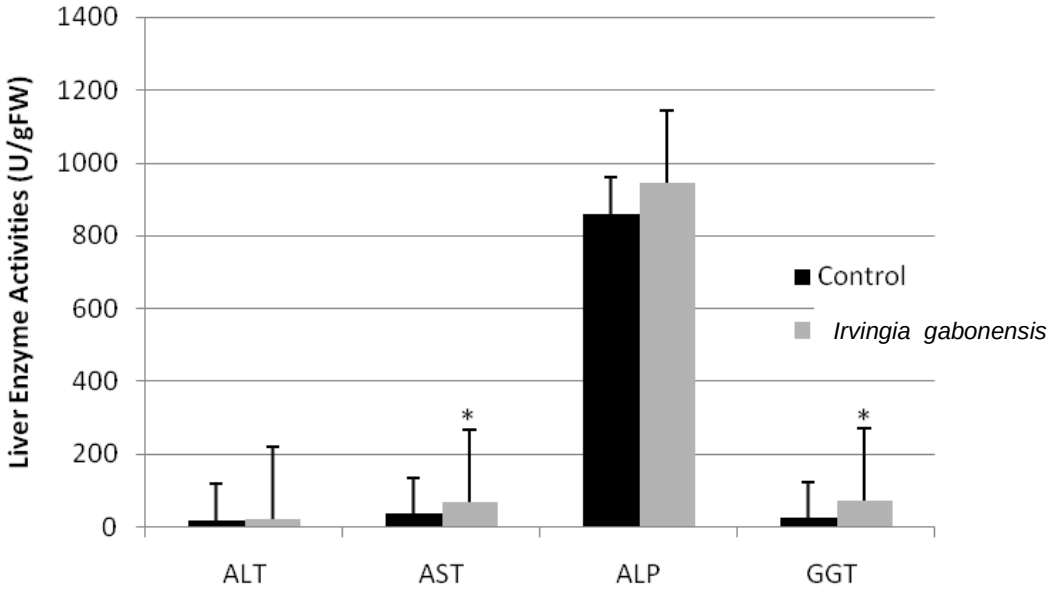

Figure 6. Effects of repeated daily oral administration of aqueous extracts of *I. gabonensis* bark for 24 weeks at 200 mg/kg body weight on liver ALT, AST, ALP and GGT activities of normal rabbits. Data was obtained from tissue homogenates at the end of 24 weeks of monitoring and are means of 4 to 6 determinations. Values carrying notations are statistically different from control at $p<0.05$.

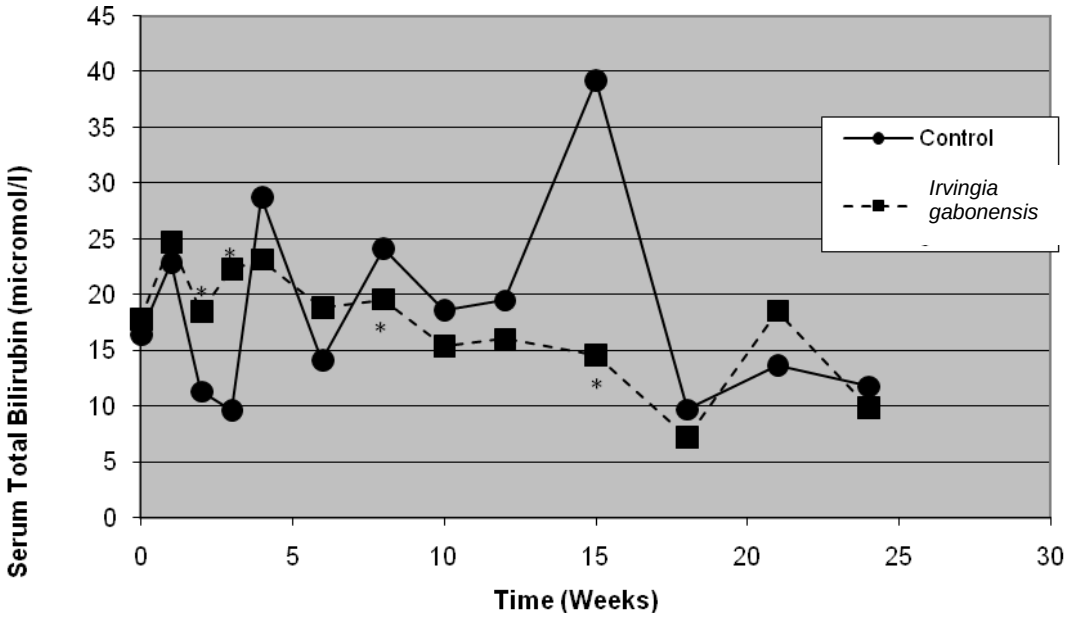

Figure 7. Effects of repeated daily oral administration of aqueous extracts of *I. gabonensis* bark for 24 weeks at 200 mg/kg body weight on serum total bilirubin concentration of normal rabbits. Data was obtained from serum at pre-determined intervals and are means of 4 to 6 determinations. Values carrying notations are statistically different from control at $p<0.05$.

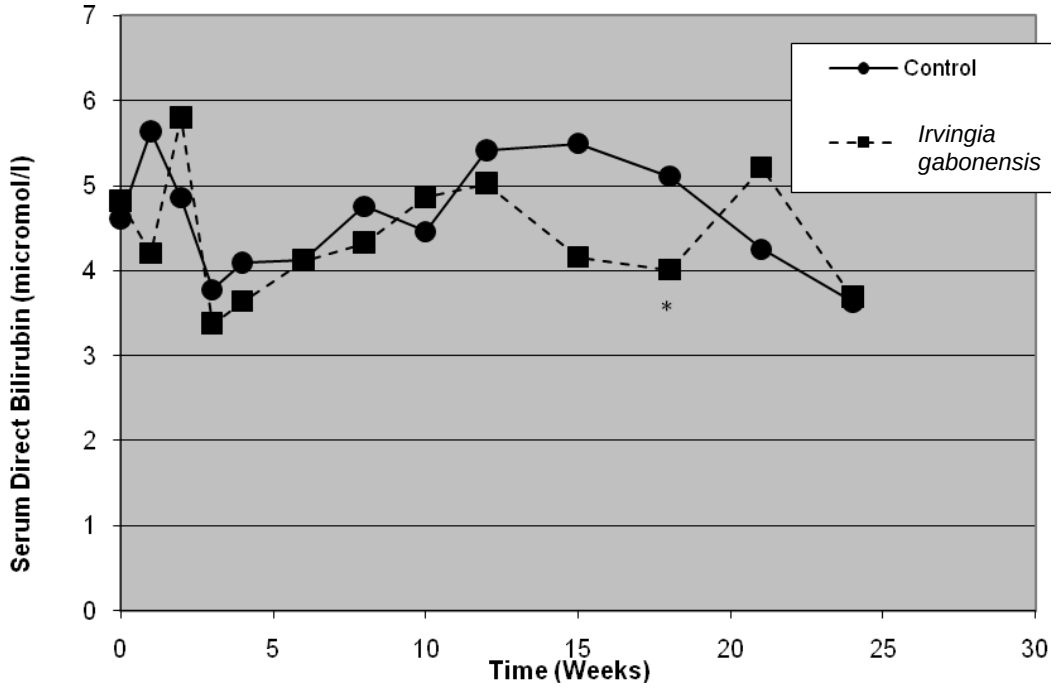

Figure 8. Effects of repeated daily oral administration of aqueous extracts of *I. gabonensis* bark for 24 weeks at 200 mg/kg body weight on serum direct bilirubin concentration of normal rabbits. Data was obtained from serum at pre-determined intervals and are means of 4 to 6 determinations. Values carrying notations are statistically different from control at $p<0.05$.

and perhaps safer effects on diabetes mellitus (Loew and Kaszkin, 2002). There are several reasons why the use of medicinal plants should be studied: Herbal remedies may have recognizable therapeutic effects (Adewole and Caxton-Martins, 2006), they may also have toxic side-effects (Mahdi et al., 2003).

The significant reductions in body weight of rabbits given *I. gabonensis* bark extracts corroborates with a number of reports on the anti-obesity effects of *I. gabonensis* (Ngondi et al., 2005; Oben et al., 2008). The significant reduction in the subcutaneous fat of the animals implies that the weight reduction was as a result of loss of fat deposits and not muscle wasting, this conclusion is supported by the findings of Oben et al. (2008) that *I. gabonensis* seed extracts inhibited adipogenesis in murine adipocytes. This weight lowering effect may contribute to the hypoglycaemic effect of this medicinal plant, since obesity is a predisposing condition in some types of diabetes (DeFronzo, 1997) and loss of weight has been shown to improve insulin sensitivity. The high fibre content and the presence of phytochemicals such as tannins and saponins (Omonkhua and Onoagbe, 2010) in *I. gabonensis* bark may be responsible for this anti-obesity effect (Kao et al., 2000; Awika and Rooney, 2004; Murthy et al., 2009).

The hypoglycaemic effect observed in this study corroborates the reports of Ngondi et al. (2005, 2006). The presence of tannins and saponins, phytochemicals with recognizable hypoglycaemic effects, as well as the presence of soluble fibre and carbohydrates in *I. gabonensis* bark, may contribute to this effect (Omonkhua and Onoagbe, 2010). Serum aminotransferases, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are sensitive tests of hepatocyte injury (Wrobleski, 1959; Ellis et al., 1978). Slight AST or ALT elevations (within 1.5 times the upper limit of normal) do not necessarily indicate liver disease (Sherman, 1991). The high ALT/AST ratio observed for the test group in weeks 1, 3 and 4 indicates a toxic response by the liver to the administered extract. However, since none of the increases observed in serum ALT activities was more than 1.5 times the value of control, severe hepatocyte damage did not occur. The results obtained for serum and tissue AST activities mirror that of ALT activities. Even though significant increases were observed in serum AST activities, none of these increases were more than 1.5 times that of control, implying that though a toxic response occurred, severe (necrotic) liver damage did not occur (Sherman, 1991). Liver ALT and AST activities were higher than control. Since tissue damage is usually indicated by increased levels of serum marker enzymes which correspond to a decrease in the tissue enzyme, the result for liver ALT and AST suggests that the damage done to the liver was not sustained to the end of the study. Cholestasis (lack of bile flow) results from the blockage of bile ducts or from a disease that impairs bile formation in the liver itself. Alkaline phosphatase (ALP) and γ-glutamyl transferase (GGT) levels typically rise to several times the normal level after several days of bile duct obstruction or intra-hepatic cholestasis. The elevations observed in the serum ALP activities of *I. gabonensis* treated normal rabbits were typically less than 1.5 times that of control. Most mild ALP elevations (less than 1.5 times normal) are resolved within six months (Johnston, 1999). From week 10 to the end of the monitoring period, the serum ALP activities of treated rabbits were similar to control. However, the increase in serum GGT activities was five times that of normal at week 4, from where it decreased gradually until it became similar to control from week 15. This high value for serum GGT is indicative of bile obstruction or impaired liver bile synthesis (Johnston, 1999). The results obtained in this study clearly show that the medicinal plant examined exerted an initial negative effect on bile production and/or flow, the fact that the serum ALP and GGT activities eventually normalized, in addition to the fact that liver ALP and GGT activities were either higher or similar to control, implies that the bile obstruction and/or impairment of liver bile synthesis, was a temporary event that eventually fizzled out.

Serum total protein reflects the synthetic function of the liver (Braunwald et al., 2001). In this study, the serum total protein levels of the medicinal plant treated normal rabbits were generally similar to control. The serum albumin levels were mostly statistically similar to control while the serum globulin levels were mostly similar or higher than control. Taken together, the implication of these results is that the medicinal plant did not diminish the protein synthetic capacity of the liver.

Apart from the initial slight increases observed in serum total bilirubin levels (Figure 7), most values recorded were slightly lower than control, while the serum direct bilirubin (Figure 8) were mostly similar to control. The implication of this is that bilirubin metabolism was not adversely altered by *I. gabonensis* administration. Gupta et al. (2005) reported that feeding normal rabbits with *Annona squamosa* pulp, an anti-diabetic plant, for one month caused a reduction in serum bilirubin; this correlates with the results obtained for serum total bilirubin in this study.

The results obtained for the liver function tests imply that *I. gabonensis* administration, under the conditions of this study, exerted an initial toxic effect on the liver of normal rabbits that was not severe and sustained. This conclusion is further strengthened by the mild hepatic degeneration observed in some of the histological slides of the liver (Table 1). Keeping in mind the profound therapeutic (anti-obesity and hypoglycaemic) effects observed in this study, it is possible that reduction in dose, frequency and duration of administration may

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
Irvingia gabonensis	1	Congested vessel, slight generalized hepatic degeneration
	2	-
	3	-
	4	NVL
	5	Slight hepatic degeneration
	6	NVL

reduce the side effects observed in this study.

ACKNOWLEDGEMENTS

We acknowledge Dr. B. O. Emikpe (DVM, MVSc {Diagnostic pathology}, Ph. D Vet Path.) of the Department of Veterinary Pathology, University of Ibadan, Nigeria, for reading and interpreting the histology slides.

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