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Effects of Long-term Administration of Aqueous Extracts of *Irvingia gabonensis* Stem Bark on α -Amylase and Lipase Activities of Rabbits

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Abstract

The increased acceptability of medicinal plants as alternatives to allopathic drugs has been greatly influenced by studies that have established their beneficial effects in the treatment of various diseases. This however, does not preclude the need to carefully assess the safety of medicinal plant preparations. *Irvingia gabonensis* has been reported to possess antidiabetic, antihyperlipidaemic, antioxidant, and anti-obesity effects. This study assessed the effect of daily oral feeding of *I. gabonensis* (at 200 mg/kg body weight) on the exocrine pancreatic function of normal rabbits. Serum and urine α -amylase, as well as serum lipase activities, were measured in rabbits at pre-determined intervals for 24 weeks; while pancreatic α -amylase and lipase activities were assessed at 24 weeks. Significant ($p < 0.05$) increases were observed in serum and urine α -amylase activities while pancreatic α -amylase activities significantly ($p < 0.05$) reduced. Also, while serum lipase significantly ($p < 0.05$) increased, pancreatic lipase activities significantly ($p < 0.05$) reduced. Phytochemicals extracted from *I. gabonensis* and other medicinal plants have been reported to exert inhibitory effects on α -amylase and lipase; which may account in part for their antidiabetic, antihyperlipidaemic, and anti-obesity effects. However, the moderate pancreatic decline observed in this study implies that the continuous administration of the extract to normal subjects should be closely monitored.

Keywords: Medicinal plants; *Irvingia gabonensis*; α -Amylase; Lipase, Long term toxicity

Introduction

Decades of studies have established the beneficial effects of medicinal plants in the management of many diseases. Many studies have identified key components of medicinal plant preparations and elucidated the mechanism by which these components exert their therapeutic effects (Ong *et al.*, 2011; Adebajo *et al.*, 2013; Casanova *et al.*, 2014). This has greatly increased the acceptability of medicinal plants as alternatives to

allopathic drugs. Besides, many allopathic drugs are derived from plant compounds (Bharel *et al.*, 1996; Schwikkard and Heerden, 2002). This however, does not preclude the need to carefully assess the safety of medicinal plant preparations. *Irvingia gabonensis*, with common names such as African mango, wild mango, Dikanut or bush mango (Ekpe *et al.*, 2007), gained some popularity after it was established that it possesses significant anti-obesity and anti-diabetic effects in animal

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and human experimental subjects (Adamson *et al.*, 1990; Ngondi *et al.*, 2005; Omonkhua and Onoagbe, 2012).

Different parts of *I. gabonensis*, such as seed and stem bark, are used in Nigeria to treat diabetes mellitus (Adamson *et al.*, 1990; Omonkhua and Onoagbe, 2012).

The treatment of chronic diseases, like diabetes, with medicinal plants normally requires long term consumption of the plant preparation. While it is instructive to assess acute and sub-chronic safety profiles of natural products, a long term toxicity assessment of plant products provides better experimental evidence of their safety.

The pancreas plays very important biological endocrine and exocrine functions. The exocrine component of the pancreas makes up 84%, while 2% constitutes the endocrine pancreas (Madole *et al.*, 2016). The endocrine pancreas produces several important hormones, including insulin, glucagon, and somatostatin; while the exocrine pancreas secretes pancreatic juice containing digestive enzymes such as α -amylase, lipase and proteases, that pass to the small intestine (Adler and Kern, 1975). These enzymes help in the further breakdown of the carbohydrates, protein, and fat in the chyme (Maton *et al.*, 1993). Pancreatic function can be assessed by evaluating the serum and urine levels of some of its exocrine secretions. Variance from the normal serum level may indicate certain disease conditions of the pancreas (Rao, 2005). Concomitant elevation of serum α -amylase and lipase is usually indicative of pancreatic injury. A number of other conditions can be attributed to the elevation of serum α -amylase alone; but when serum lipase is raised as well, the diagnosis of pancreatitis is more likely (Rao, 2005).

As part of a general toxicological evaluation of this plant, the long term (24 weeks) effect of daily oral administration of *I. gabonensis* (at 200 mg/kg body weight) on the exocrine pancreatic function of normal rabbits was assessed in this study.

Materials and methods

Chemicals and reagents

Lipase and α -amylase BioSystem kits, and BioSystems' Biochemistry control serum levels I and II, product of BioSystem S. A. Costa Brava 30, Barcelona, Spain; were purchased from Raphson Laboratories Ltd, 9 Adelabu Close, Ikeja Lagos. Other analytical grade chemicals were also used.

Preparation of Plant extract

The stem bark of *I. gabonensis* was obtained from Akungba-Akoko, Ondo State, Nigeria. The plant was authenticated in the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko and the Department of Microbiology and Botany, University of Ibadan, Ibadan, Nigeria. A specimen, with voucher number UIH 22286, was deposited at the Herbarium of the University of Ibadan, Nigeria. The aqueous plant extracts was prepared by a method described by Onoagbe *et al.* (1999).

Experimental Animals and Management

Twelve (12) rabbits of the New Zealand strain, weighing between 800-1200g, purchased from local breeders in 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 allowed free access to food and water. The study protocol was approved by the local Institutional Review Board (IRB) and treatment of

the animals was in accordance with the guideline for the Care and Use of Laboratory Animals (The National Academy of Sciences, 2011).

Page | The rabbits were divided into two groups: GROUP I: 3199 Control and GROUP II: Normal rabbits treated with *Irvingia gabonensis* aqueous extracts. The plant extracts were orally administered to the rabbits at 200 mg/kg body weight daily for 24 weeks.

Blood Collection

Blood samples were drawn from the ventral vein of the rabbits' ear before the commencement of extract administration (week 0) to serve as baseline values. Subsequently, during the monitoring phase, blood (1 ml) was collected at pre-determined intervals (week 1, 2, 3, 4, 6, 8, 10, 12, 15, 18, 21 and 24). 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 pancreas and other tissues were also collected.

Blood samples were collected in plain vacutainers, allowed to clot on ice, and centrifuged at 1,000 X g for 5 minutes. The serum was then separated for analysis. Tissues were homogenized in ice cold normal saline (1:4 w/v), centrifuged and the supernatant stored at 4 °C until analysis.

Urine collection

This was done by collecting the urine expressed by each rabbit for the period of 24 hours. To achieve this, 24 hours before the scheduled blood collection, each rabbit was transferred in a separate cage; urine was carefully

collected, centrifuged at 1,000 X g for 5 minutes and stored in the freezer till analysis.

Biochemical Analysis

Total protein

Total pancreatic protein concentration was measured by the Biuret method (Gornall *et al.*, 1949).

α -Amylase assay

Serum, urine and pancreatic α -amylase activities were measured by Lorentz (2000) method, as described in the manual of the BioSystem α -Amylase kit.

Lipase assay

Serum and pancreatic lipase activities were measured by Neumann *et al.* (1984) method, as described in the manual of the BioSystem Lipase kit.

Histological evaluation

Histological investigation on the pancreas was done according to the method described by Lamb (1981).

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

Continuous administration of *I. gabonensis* stem bark aqueous extract for 24 week at 200 mg/Kg body weight caused significant ($p < 0.05$) increases in serum α -amylase activities in weeks 3, 12 and 21 (Figure 1). Urine α -amylase increased significantly ($p < 0.05$) in weeks 1, 4 and 18 (Figure 2), while pancreatic α -amylase activities

significantly ($p < 0.05$) decreased in *I. gabonensis* treated rabbits compared to untreated controls (Figure 3).

Serum lipase activities increased significantly ($p < 0.05$) in weeks 2, 3, 12 and 24 (Figure 4), while pancreatic lipase activities were significantly ($p < 0.05$) lower in the *I. gabonensis* treated rabbits compared to control (Figure 5). The total pancreatic protein concentration significantly ($p < 0.05$) reduced (163.89 ± 57 g/l) in the medicinal plant treated rabbits compared to control (262.5 ± 51.54 g/l).

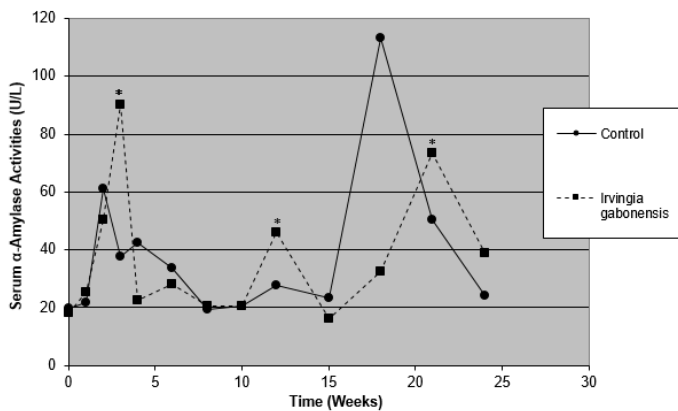


Figure 1: Effects of repeated daily oral administration of aqueous extracts of *I. gabonensis* bark for 24 weeks at 200 mg/kg body weight on serum α -amylase activities (U/L) of normal rabbits. Data was obtained from serum at pre-determined intervals and are means of 4-6 determinations. Values carrying notations are statistically different from control at $p < 0.05$.

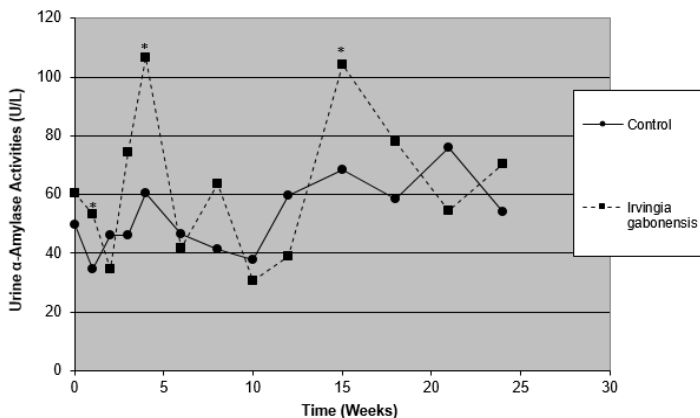


Figure 2: Effects of repeated daily oral administration of aqueous extracts of *I. gabonensis* bark for 24 weeks at 200 mg/kg body weight on urine α -amylase activities (U/L) of normal rabbits. Data was obtained from urine at pre-determined intervals and are means of 4-6 determinations. Values carrying notations are statistically different from control at $p < 0.05$.

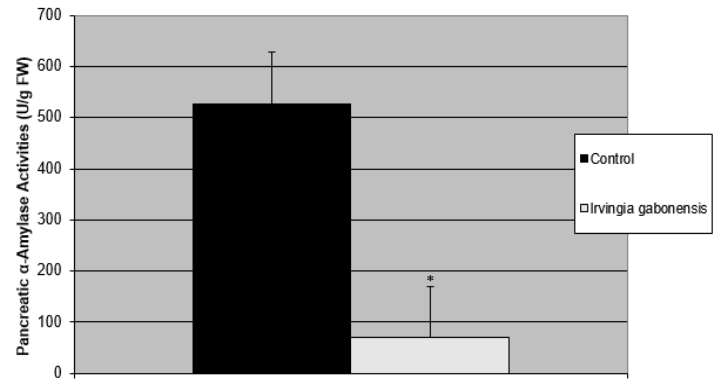


Figure 3: Effects of repeated daily oral administration of aqueous extracts of *I. gabonensis* bark for 24 weeks at 200 mg/kg body weight on pancreatic α -amylase activities (U/g FW) of normal rabbits. Data was obtained from tissue homogenates at the end of 24 week of monitoring and are means of 4-6 determinations. Values carrying notations are statistically different from control at $p < 0.05$.

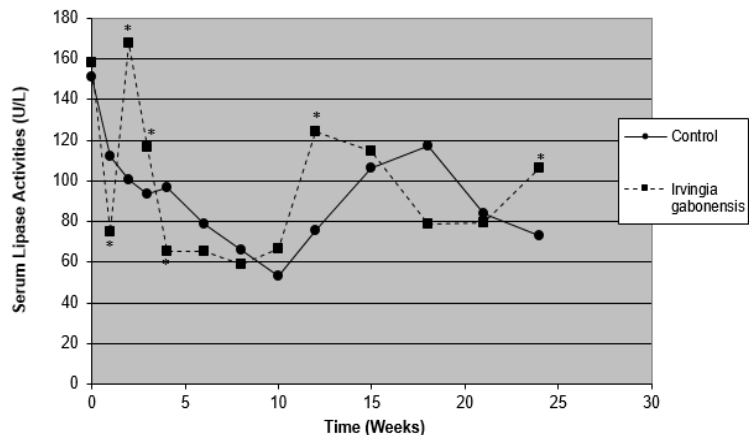


Figure 4: Effects of repeated daily oral administration of aqueous extracts of *I. gabonensis* bark for 24 weeks at 200 mg/kg body weight on serum lipase activities (U/L) of normal rabbits. Data was obtained from serum at pre-determined intervals and are means of 4-6 determinations. Values carrying notations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

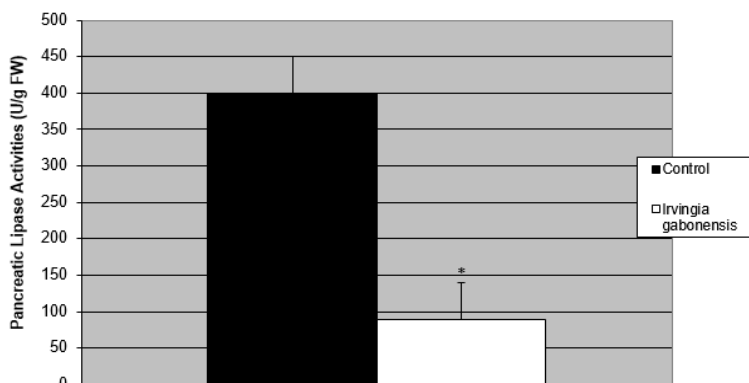


Figure 5: Effects of repeated daily oral administration of aqueous extracts of *I. gabonensis* bark for 24 weeks at 200 mg/kg body weight on pancreatic lipase activities (U/g FW) of normal rabbits. Data was obtained from tissue homogenates at the end of 24 week of monitoring and are means of 4-6 determinations. Values carrying notations are statistically different from control at $p < 0.05$.

Discussion

Normally, only low levels of α -amylase are found in the blood or urine. However, if the pancreas or salivary glands become damaged or blocked, more α -amylase is usually released into the blood and urine (Pagana and Pagana, 2002). The diagnosis of acute pancreatitis is more likely when the activity of serum amylase is three times the upper limit of normal (Matull *et al.*, 2006). In the blood, α -amylase levels rise for only a short time. In the urine, α -amylase may remain high for several days (Pagana and Pagana, 2002). High amounts of lipase may be found in the blood when the pancreas is damaged or when the tube leading from the pancreas (pancreatic duct) to the beginning of the small intestine is blocked (Keim *et al.*, 1998).

Generally, the results obtained from this study indicate increases in serum and urine α -amylase activities, and serum lipase activities while pancreatic α -amylase and lipase activities were significantly reduced. These results imply that under the conditions of this study, administration of *I. gabonensis* exerted some measure of damage to pancreatic tissues. Since pancreatic α -amylase and lipase activities were also decreased at the end of 24 weeks of monitoring, the implication is that this effect was sustained. This conclusion is supported by the presence of diffuse pancreatic necrosis in one of the pancreas histological slides of treated rabbits (result not shown), as well as the significantly ($p < 0.05$) lower pancreas total protein concentration. Many studies have shown that medicinal plants, like *I. gabonensis*, used in the treatment of diabetes and obesity possesses anti-amylase and anti-lipase activities (de Souza *et al.*, 2011; Mnafguit *et al.*, 2014; Seyedan *et al.*, 2015). Most of such studies have been conducted under *in vitro* conditions. The secretions

of the exocrine pancreas are responsible for the digestion of carbohydrates, proteins and fats. A reduction in these secretions will ultimately reduce the digestion of food substances which will eventually lead to reduction in absorption. The reduction in pancreatic amylase, which is the principle enzyme of carbohydrate degradation in the small intestine, will reduce the production and absorption of sugars. While this may provide a therapeutic strategy for the management of diseases like obesity and type 2 diabetes; the effect on normal individuals will need to be assessed further. Again, the reduction in pancreatic lipase activities observed in this study may be therapeutically significant in the management of obesity and hyperlipidaemia, where reduced intake of triglycerides is desirable. In fact, previous studies have shown that normal rabbits treated with *I. gabonensis* for 24 weeks were practically devoid of subcutaneous fat (Omonkhua and Onoagbe, 2012). Supporting the conclusion that reduced pancreatic lipase activities ultimately leads to reduction in fat deposits. It is thus possible that the *in vitro* anti-amylase and anti-lipase activities observed in many medicinal plants could be replicated *in vivo* by the ability of these plants to reduce the exocrine secretions of the pancreas.

While some desirable effects may be obtained from the inhibition of secretions from the exocrine pancreas observed in this study; the significant decrease in the activities of α -amylase and lipase observed also implies an onslaught by the components of the ingested plants on the integrity of pancreatic acinar cells. Indeed one of the histological slides of the pancreas showed diffuse pancreatic necrosis. Thus consumption of this medicinal

plant for long periods of time must be followed up with assessment of pancreatic function.

Conclusion

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Phytochemicals isolated from *I. gabonensis* and other medicinal plants have been reported to exert inhibitory effects on α -amylase and lipase; which may account in part for their antidiabetic, antihyperlipidaemic, and anti-obesity effects. However, the moderate pancreatic decline observed in this study implies that the continuous administration of the extract to normal subjects should be closely monitored.

Conflict of Interest

The authors declare no conflict of interest

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