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Ethanol Extract of *Irvingia gabonensis* (Bush Mango) Seed Improves Renal and Hepatic Functions in Wistar Rats

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Abstract: The effect of ethanol extract of *Irvingia gabonensis* seed on some renal and hepatic function biomarkers was studied in ten Wistar rats. The rats were randomly assigned into two groups of five rats each: Group 1 (Normal group) received normal rat feed and water; Group 2 (Treated group) received 250mg/kg body weight of ethanol extract of *Irvingia gabonensis* seed in addition to normal rat feed and water. Treatment lasted for twenty-one days and was done twice daily via oral gastric intubation. Blood samples were collected from each rat via cardiac puncture for analysis of hepatic and renal function biomarkers. Serum AST and total protein concentrations were significantly ($p < 0.05$) increased and ALT and ALP concentrations significantly ($p < 0.05$) decreased in the treated group compared with the normal group. Serum creatinine, Na^+ , and K^+ concentrations were not significantly different between the two groups but Ca^{2+} concentration increased significantly ($p < 0.05$) in the treated group compared with the normal group. In conclusion, *Irvingia gabonensis* seed therefore has the potential to improve liver and kidney functions in Wistar rats and may serve as a source of calcium. Increased consumption of *Irvingia gabonensis* seed is encouraged.

Keywords - Creatinine; Electrolytes; *Irvingia gabonensis*; Protein; Serum enzymes

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I. Introduction

In recent times, attention has been drawn to the use of medicinal plants in the treatment of several ailments. *Irvingia gabonensis* (IG) is one of such plants. IG belongs to the family, Irvingiaceae and it is commonly called bush mango, African mango, wild mango or Dikanut [1]. It is an edible fruit tree indigenous to Africa [2]. It consists of root, stem, leaves and fruits. The fruit consists of a fleshy part and nut. The nut itself is made up of a hard shell and kernel (seed). The various parts of the plant have been employed in traditional herbal medicine and have recorded great success. The bark is used for treating dysentery, scabies, toothache and skin diseases [3]. It is also used as anti-poison and in the treatment of hernias and yellow fever [4]. The stems of IG tree have been used as chewing sticks to help clean the teeth [5]. In Senegal, the decoction of the stem bark is used in the treatment of gonorrhoea, hepatic and gastrointestinal disorders [6]. The leaves, when combined with palm oil are used to stop haemorrhage in pregnant women [7]. The leaf extracts are known to have diuretic and hypotensive effects [8,9]. The leaf extract has also been reported to have hepatoprotective, cytoprotective and anti-ulcer effects on aspirin-induced ulcer [10]. It has also been reported to demonstrate hepatoprotective and anti-clastogenic effects on sodium arsenite-induced toxicity in Wistar rats [7]. The leaf extract also has hepatoprotective and nephronprotective effect on Cadmium chloride-induced toxicity in Wistar rats [11]. The juice has been reported to have renal and hepato-protective effects on sodium fluoride-induced toxicity in Wistar rats [12].

In addition, the nut has been employed in the management of type 2 diabetes mellitus [13]. In Nigeria, the seeds are ground and used as thickening agent in soups [14]. They can be roasted and used as flavouring agent in local salad preparation [15]. The seeds are applied to burns and also act as an astringent [16]. Aqueous extract of the seeds has been reported to have the potential to improve renal and hepatic functions [17]. From the foregoing, it has been noted that various parts of *Irvingia gabonensis* have been used extensively in the treatment of diseases traditionally with the leaves having the potential to improve renal and hepatic functions and to protect the liver and kidneys from toxic substances. However, to the best of our knowledge, no study till date has reported the effect of ethanol extract of *Irvingia gabonensis* seed on renal and hepatic functions in Wistar rats. The present study was therefore carried out to examine the effect of ethanol extract of *Irvingia gabonensis* seed on hepatic and renal functions in Wistar rats.

II. Materials and Methods

2.1 Animal Preparation

Ten Wistar rats of weight 180-200g were collected from the Animal House of Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar. The rats were handled according to the laid down principles of Helsinki [18]. They were kept under normal environmental conditions in properly ventilated wooden cages, allowed access to rat feed and water *ad libitum* and exposed to 12/12-hour light/dark cycle. The animals were allowed for seven days to explore their new environment before commencement of treatment.

2.1 Preparation of Ethanol Extract of *Irvingia gabonensis* Seed

After identification of *Irvingia gabonensis* tree by a botanist in the University of Calabar, the fresh fruits were carefully plucked from the tree in the university farm and opened to remove the seeds. The seeds were sundried and ground to powdery form using a manual hand grinder. About 100g of the powdered sample was soaked in 300mls of ethanol and covered in an air-tight container for 48 hours. The mixture was thereafter sieved to get the filtrate using a sieve cloth. The main *Irvingia gabonensis* seed extract was obtained by further filtration. This was done by pouring the filtrate into a filter cloth and finally into a filter paper. The whole extraction process was done in the Endocrinology and Medicinal Plant Research Laboratory of the Department of Biochemistry, University of Calabar.

2.3 Experimental Design and Extract Administration

The ten Wistar rats (200-250g) were randomly assigned into two groups of five rats each namely: Group 1 (Normal group) and group 2 (Treated group). The Normal group received normal rat feed and water while the Treated group received 250mg/kg body weight of *Irvingia gabonensis* seed extract in addition to normal rat feed and water. Treatment was done twice daily (8am and 3pm) via oral gastric intubation and lasted for twenty-one days. The 250mg/kg body weight of *Irvingia gabonensis* seed extract used in this study falls below the lethal dose of 2500mg/kg [19].

2.4 Collection of Blood Samples

The animals were sacrificed under chloroform anaesthesia (3.5% soaked in cotton wool) and blood collected via cardiac puncture using 5ml syringe attached to a 21SWG needle into plain capped bottles. The blood samples were allowed for 2 hours to clot. Serum was thereafter collected through centrifugation at 2500 rpm for 10mins and used for biochemical analyses.

2.5 Biochemical Analyses

2.5.1 Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST)

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were assayed according to the method described by Reitman and Frankel [20]. This method is based on the principle that the pyruvate produced by transamination reacts with 2,4-dinitrophenylhydrazine to give a high-colored hydrazone. AST catalyses the reaction between L-aspartate with α -ketoglutarate to yield glutamate and oxaloacetate. The oxaloacetate produced reacts with 2,4-dinitrophenylhydrazine to yield a high-coloured oxaloacetate hydrazone which is measured calorimetrically at 546nm. The absorbance of the hydrazone is directly proportional to the activity of AST. ALT activity was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine to produce pyruvate hydrazone. ALT activity was determined by measuring calorimetrically the concentration of pyruvate hydrazone formed at 546nm. The absorbance of the hydrazone is directly proportional to the activity of ALT.

In this procedure, two sets of test tubes were cleaned, dried and labelled B(blank) and T(test). 0.1ml of sample and distilled water were pipetted into B and T test tubes respectively. To B and T test tubes were also added 0.5ml of the buffer solution. The contents of the tubes were thoroughly mixed and incubated for 30 minutes at 37°C followed by addition of 0.5ml of 2,4-dinitrophenylhydrazine. The contents of the tubes were again well mixed and allowed to stand for exactly 20 minutes at 20-25°C followed by addition of 0.5ml of NaOH to all the tubes. At 546nm, the absorbance of the test samples was read against the reagent blank after 5 minutes.

2.5.2 Alkaline phosphatase

Alkaline phosphatase (ALP) activity was determined using commercial kit (Randox, UK) as described by King and King [21]. The activity of ALP is determined by monitoring the rate of its dephosphorylation of p-nitrophenylphosphate to p-nitrophenol, a yellow coloured compound whose concentration can be monitored calorimetrically at 405nm.

In this procedure, 1.0ml of the Randox reagent was added to 0.02ml of the plasma in a cuvette at 30°C. The contents were then mixed and the initial absorbance was taken. Readings were thereafter taken after 1, 2 and 3 minutes at 405Nm in a spectrophotometer.

2.5.3 Total Protein and Creatinine

Total protein concentration was assayed using commercial kits (Randox, UK) as described by Gornall *et al.* [22]. Creatinine concentration was determined using commercial kit (Biosystems) by the method described by Bartels and Böhmer [23].

2.5.4 Serum Electrolytes

Serum sodium concentration was determined by the modified method described by Maruna [24] and Trinder [25] and serum potassium and calcium concentrations were determined by the methods of Terri and Sesin [26] and Gindler and King [27] respectively using commercially available test kits (Teco, USA).

2.6 Statistical Analysis

Results are presented as mean \pm standard error of mean (SEM). Data were analysed using Computer software, SPSS (version 21, Microsoft Company, USA). Student's T-test was used to compare the mean difference between the two groups. $p < 0.05$ was considered statistically significant.

III. Results

3.1 Comparison of serum enzymes and total protein concentrations between the normal and treated groups

Table 1 shows serum enzymes and total protein concentrations between the normal and treated groups. Serum concentration of AST was significantly ($p < 0.05$) increased in the treated group compared with the normal group while serum ALT and ALP concentrations were significantly ($p < 0.05$) decreased in the treated group compared with normal group. Serum total protein concentration was significantly ($p < 0.05$) increased in the treated group compared with the normal group.

Table 1: Comparison of serum enzymes and total protein concentrations between the normal and treated groups

	Total protein (g/L)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Normal Group	8.70 \pm 0.09	64.53 \pm 0.90	37.75 \pm 0.85	170.55 \pm 0.58
Treated Group	9.13 \pm 0.10*	69.58 \pm 0.55*	34.08 \pm 1.01*	166.23 \pm 1.04*

Values are expressed as mean \pm SEM, n = 5

* = significantly different from normal group at $p < 0.05$

3.2 Comparison of serum creatinine and electrolytes concentrations between the normal and treated groups

Table 2 shows serum creatinine and electrolytes concentration between the normal and treated group. Serum creatine concentration was not significantly different between the normal and treated groups. There was also no significant difference in serum Na⁺ and K⁺ concentrations between the two groups. But serum Ca²⁺ concentration was significantly ($p < 0.05$) increased in the treated group compared with the normal group.

Table 2: Comparison of serum creatinine and electrolytes concentrations between the normal and treated groups

	Creatinine (mmol/L)	Na ⁺ (mmol/L)	K ⁺ (mmol/L)	Ca ²⁺ (mmol/L)
Normal Group	83.58 \pm 0.97	133.45 \pm 0.73	3.58 \pm 0.06	16.34 \pm 0.20
Treated Group	83.15 \pm 0.72	133.25 \pm 0.74	3.48 \pm 0.03	17.25 \pm 0.10*

Values are expressed as mean \pm SEM, n = 5

* = significantly different from normal group at $p < 0.05$

IV. Discussion

Irvingia gabonensis (IG) is an edible fruit tree indigenous to Africa. Various parts of the plant including the stem, leaves, nut and seeds have been used traditionally in the treatment of several health problems. This study investigated the effect of ethanol extract of *Irvingia gabonensis* seed on renal and hepatic functions by determining the serum concentrations of liver enzymes (AST, ALT and ALP), total protein, creatinine and electrolytes (Na⁺, K⁺ and Ca²⁺).

Results from the present study show that serum AST concentration increased significantly in the treated group compared with normal group but serum ALT and ALP concentrations were significantly decreased in the treated group compared with the normal group. Our result for ALT corroborates with the

findings of Gbadegesin *et al.* [7] who reported that IG (250mg/kg) leaves administered to Wistar rats significantly reduced ALT concentration. In their study, serum AST concentration was also significantly decreased but this is not the case in our study where AST increased significantly. Large amounts of ALT and AST are found in the liver and small amounts are found in the muscles, heart and kidneys. Blood level of AST, ALT and ALP is usually elevated when hepatocytes are damaged [7, 28, 29]. ALP is essential in bone mineralization and its activity is increased in bone disease and hepatobiliary disease. Increased ALP levels is an indication of bone cell damage [30] and cholestasis which probably results in progressive liver disease- biliary cirrhosis [31]. From our results, it can be said that IG seed improved hepatic function and has the potential to enhance bone mineralization since it exhibited a reducing effect on ALT and ALP concentrations. Aqueous extract of the seeds has been previously reported to have the potential to improve renal and hepatic functions [17]. Hepatoprotective effect of IG has also been previously reported [7, 10, 11]. It is unclear from this study why serum AST concentration was significantly increased in the treated group compared with the normal group. A previous study [11] showed that 200mg/kg and 400mg/kg of IG leaves administered to Wistar rats increased serum AST concentration although the increase was not significant. Serum total protein concentration was significantly increased in the treated group compared with the normal group. Ewere *et al.* [11] previously reported that ethanol extract of IG leaves significantly increased serum total protein concentration in Wistar rats. Serum proteins are produced primarily in the liver and a decrease in their concentration is indicative of liver damage [32] implying that an increase is an indication of improvement of liver function. The result from our study indicates that IG seed is able to improve protein synthesis by the liver.

Analyses of renal function biomarkers such as creatinine and electrolytes are essential in ascertaining the health of the kidneys. Results from the present study show non-significant differences in serum creatinine, Na^+ and K^+ concentrations between the normal and treated groups. Creatinine is one of the waste products in the blood undergoing continuous filtration by the kidneys and excretion into the urine. Higher than normal level of creatinine is indicative of renal function impairment. Na^+ , K^+ and Ca^{2+} are among the principal cations in the body fluid. They are involved in metabolic activities which are essential to the normal functioning of all cells. The maintenance of electrolyte balance in the body is a function of the kidneys and hormones. Estimation of the serum levels of electrolytes is an assessment of the tubular integrity of the nephron [33]. The non-significant differences in creatinine, Na^+ and K^+ concentrations observed in our study are consistent with previous finding [11] and these show that ethanol extract of IG seed exhibited nephroprotective effect. Renal protective effect of *Irvingia gabonensis* leaves and juice had been previously reported [11, 12]. This protective effect may be due to the presence of components like flavonoids, tannins and alkaloids found in IG plant [12, 34]. Serum Ca^{2+} concentration was significantly increased in the treated group compared with the normal group. This result is consistent with Emejulu *et al.* [12] who reported that IG juice significantly increased Ca^{2+} concentration. Ca^{2+} is essential for formation of new bone and repair of existing bone [35] and it contributes to blood pressure lowering effect in rats and humans [36, 37]. This result corresponds with the non-significant difference in ALP concentration between the normal and treated groups which shows the potential of IG seed to enhance bone mineralization. The increase in Ca^{2+} shows the beneficial effect of IG seed as the seed can serve as a potential source of Ca^{2+} . Ayivor *et al.* [38] had previously reported that *Irvingia gabonensis* is a rich source of Ca^{2+} .

V. Conclusion

Irvingia gabonensis seed has the potential to improve renal and hepatic functions in Wistar rats and may serve as an important source of calcium. We therefore encourage the domestication or consumption of *Irvingia gabonensis* seed.

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