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Renal and Hepato-Protective Effects of *Irvingia gabonensis* Juice on Sodium Fluoride-Induced Toxicity in Wistar Rats

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Abstract

Objective: Renal and hepato-protective effects of *Irvingia gabonensis* juice on sodium fluoride-induced toxicity was assessed in twenty-four male Wistar albino rats.

Methodology: The rats were divided into 4 groups of 6 animals each. All except normal control (NC), were intoxicated with 20 $mg.Kg^{-1}$ body weight of sodium fluoride (NaF) daily by gavage for 35 days. Sodium fluoride control group (NaFC) received only the toxicant. Test group (IG) received *I. gabonensis* juice concurrently with the toxicant, while the standard control (Q+Vit. E) received concurrently, 15 $mg.Kg^{-1}$ body weight of Quercetin+100 $mg.Kg^{-1}$ body weight of α-tocopherol throughout the 35 days. Normal control (NC) group received only standard pelletized diet and water. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, albumin, total cholesterol, serum creatinine and electrolyte levels were assessed among test, standard and control animals.

Result: *Irvingia gabonensis* significantly ($p<0.05$) reduced AST activity in the IG group (137.68 ± 12.66 U/L) compared to NaFC group (175.12 ± 10.63 U/L). This compares to the reduction in the AST activity in standard (Q +Vit. E) group (135.69 ± 10.66 U/L). ALT activity was also reduced in the IG group. Effects of *I. gabonensis* on albumin and cholesterol levels were similar to that of the standard group. Administration of *I. gabonensis* also significantly ($p<0.002$) reduced elevated creatinine and Cl^- concentrations, while significantly ($p<0.05$) elevating serum Ca^{2+} and Mg^{2+} ion levels.

Conclusion: *Irvingia gabonensis* fruit juice has some renal and hepato-protective potential which may be due to the presence of secondary plant metabolites like flavonoids, tannins and alkaloids found in the plant. The fruit is also rich in Ca^{2+} and Mg^{2+}. Increased domestication is encouraged.

Key words:

Medicinal plant; Chemical toxicity; *Irvingia gabonensis*; Organ functions

Introduction

African rain forest is filled with varieties of plants. *Irvingia* is a genus of African and Southeast Asian trees in the family Irvingiaceae. *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill is commonly known as "wild mango", "bush mango" or 'African mango' and is a commercially indigenous fruit tree of the West and Central Africa. They bear edible mango-like fruits called 'Ugiri' in Igbo land (Nigeria) and 'Dika' in Cameroon, but are especially valued for their fat- and protein-rich nuts called 'ogbono' or 'dika' nuts. The fruits are broadly ellipsoid, about 4-7 cm long, green when unripe and yellow when ripe with a fleshy mesocarp. The fruit pulp is juicy, although the taste varies between sweet and bitter, it has great commercial potentials ranging from the preparation of juices, jams and jellies to wine and soap making [1,2].

In Nigeria, *Irvingia gabonensis* is a widely domesticated and grown perennial fruit tree, enjoyed both for its succulent pulp and its malleable kernels which are domestically used as a substitute for making popular "draw soups". *Irvingia gabonensis* has been shown to possess nutritional or medicinal values [3,4].

The *I. gabonensis* trees grow to about 15-40 m in height and 1m in diameter; they may occur in gregarious clusters. Different parts of the plant are used in traditional and modern medicine for the treatment of several illnesses and in industrial processes [5]. The seeds of *I. gabonensis* have a wide variety of application including its use as a thickener in soup and stews and a source of edible oil. The bark has been widely applied in the treatment of diarrhea [6], dysentery [7], scabby skin [6] and a potent anti-inflammatory agent [7]. Leaf decoction of *I. gabonensis* and the seed extract have been reported to possess hypoglycaemic and hypolipidemic effects [8-10]. Antidiabetic effects of its bark and leaves on streptozocin-induced diabetic rats have also been reported [11].

Although its fruit is widely eaten, it has remained largely understudied. Much that is known on *I. gabonensis* is mostly on the seeds and stem bark. This lack of information on the fruit juice has contributed to its underutilization and under-exploitation. Use of some of this wild fruits can do much to combat malnutrition and sustain life. It has been established that *I. gabonensis* contains elemental micronutrients and we had observed a hypolipidemic effect of the fruit juice on sodium fluoride induced dyslipidemia in rats [12].

Toxicity of sodium fluoride has been well established. It is hepatotoxic, causing among others degenerative and inflammatory changes [13,14]. It is neurotoxic [15,16] and increases oxygen free radicals and its resultant oxidative stress [17]. More recently, low glucose utilization [18], cognitive deficits and anxiety-depression-like behaviors have been described in mice treated with NaF [19].

Increased use of sodium fluoride is witnessed worldwide, with a lot of debate going-on on its continuous usage or non-use especially in fluoridation of water. Children are most vulnerable especially through excessive consumption of toothpastes. Increased risk of fluorosis due to high water-borne fluoride concentrations is threatening many parts of the world [20] and black children are disproportionately affected [16,21] due probably to biologic susceptibility or mere greater fluoride intake [21]. With increasing rate of consumption of fluorides worldwide and particularly in our locality, the present study was therefore designed to investigate the protective effect of *Irvingia gabonensis* fruit juice on the liver and kidney of male wistar rats exposed to sodium fluoride toxicity.

Materials and Methods

Chemicals/Reagents

Sodium fluoride (Fluka-Chemie, Switzerland), Quercetin dihydrate (Sigma-Aldrich Mo USA), α-Tocopherol (Fluka-Chemie, Switzerland). ALT test kit (Randox), AST test kit (Randox), Alkaline phosphatase test kit (Randox), Serum Bilirubin, Urea, Creatinine and Cholesterol test kits (Biosystem), Sodium, Potassium, Calcium, Magnesium and Chloride test kits (Teco, USA). All chemicals and reagents used were of analytical grades.

Plant materials

Apparently healthy fresh, ripe and edible fruits of *I. gabonensis* were collected from a local plantation in Ugiri-Ike, Ikeduru Local Government Area of Imo State, Nigeria.

Scientific classification	
Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Rosids
Order:	Malpighiales
Family:	Irvingiaceae
Genus:	*Irvingia*
Species:	*I. gabonensis*
Binomial name	
Irvingia gabonensis **(Aubry-Lecomte ex O'Rorke) Baill.**	

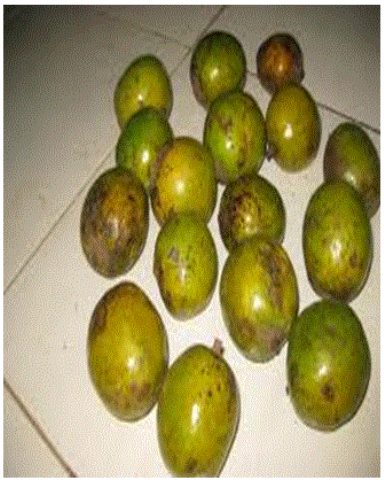

Figure 1: Overview on *Irvingia gabonensis*.

The plant material was authenticated by Dr. F. N. Mbagwu, a plant taxonomist at the Department of Plant Science and Biotechnology, Imo State University, Owerri, Imo State. Plant specimens were deposited in the institution's herbarium with voucher no. IMSUH 0198. These fruits were obtained fresh as at when needed (Figure 1).

Fruit juice preparation

The ripe and edible fruits of *I. gabonensis* were washed with clean tap water and peeled, seeds removed and the succulent pulp cut into sizeable bits. These were weighed and 250 g portion of the fleshy part of the fruits was extracted with 250 ml of distilled water in a juice extractor (Sinbo SJ3138, China) to obtain the fruit juice. The resultant juice was then stored in a freezer (≤ -4.0°C) until needed. Fresh juice of the fruit was prepared each day of administration.

Qualitative phytochemical screening

The methods described by [22] were used to evaluate the qualitative phytochemical content of the fruit juice.

Animals

Twenty four healthy, male albino Wistar rats (*Rattus norvegicus*) weighing 120-150 g were used for the study. The animals were purchased from the animal house of the Department of Veterinary Medicine, University of Nigeria, Nsukka. They were housed in stainless steel cages under standard laboratory conditions of light, temperature (21 ± 2°C) and relative humidity (55 ± 5%). The animals were fed standard rat pellets (Vital finisher, Nigeria) and tap water ad libitum and allowed for a period of two weeks to acclimatize before commencement of the study. The Ethical committee of the University approved the study protocol prior to commencement of study and treatment of the animals was in accordance with the Principles of Laboratory Animal Care (NIH Publication, 1985 to 1993; revised, 1985).

Grouping of animals

The rats were randomly divided into four (4) experimental groups of six (6) animals each and treatments administered for 35 days as follows:

Group I served as normal control (NC), which received standard pelletized diet and water only throughout the treatment period.

Group II served as intoxicated control (NaFC), which received standard diet and water ad libitum and sodium fluoride toxicant (20 mg/kg b wt) by gavage daily.

Groups III served as intoxicated test group (I.G), which received standard diet and water ad libitum, in addition to *I. gabonensis* fruit juice (IG) and sodium fluoride toxicant (20 mg/kg b wt) daily.

Group IV served as intoxicated standard group (Q +Vit E), which received standard diet and water ad libitum, in addition to Quercetin (15 mg/kg b wt) plus α-tocopherol (100 mg/kg b wt) and the toxicant sodium fluoride (20 mg/kg b wt) daily.

Blood collection

At the end of thirty five days of daily intoxication and treatment with *I. gabonensis* juice and standard for amelioration, animals were fasted overnight. They were then lightly anaesthetized with

dichloromethane, sacrificed by cervical dislocation and blood collected by cardiac puncture. The blood sample (5 ml) of each animal was taken and allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 600 × g for 15 min and analyzed for various biochemical parameters.

Biochemical analyses

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities [23], ALP activities [24], protein [25] and albumin concentrations [26] were assayed using commercial kits (Randox, UK). Serum total bilirubin [27], total cholesterol [28], urea [29] and creatinine [30] concentrations were determined using commercial kit (Biosysytem). Sodium was determined by the modified method described by [31] and [32], potassium by the method described by [33], chloride by modified method described by [34], magnesium as described by [35] and calcium as described by [36] using commercially available test kits (Teco, USA).

Statistical analyses

Statistical software "Analyze-it" for Microsoft excel was used for the analysis. Differences between the various groups and the control group were tested at $p \leq 0.05$ using one-way analysis of variance (ANOVA) statistic followed by Tukey post-Hoc test.

Results and Discussion

Phytochemical screening revealed the presence of phenolics, alkaloids, flavonoids, phytosterols, phenols, phlobatanin, tannins and saponins in *I. gabonensis* fruit juice.

Results shown on Figure 2 indicated that serum ALT and AST activities were significantly ($p<0.05$) elevated by 82% and 88% respectively in NaF-exposed rats compared to controls. Treatment of exposed rats with *I. gabonensis* fruit juice and reference standard resulted in a reduction in serum ALT and AST activities by 35.8% & 21.4% and 48.23% and 22.5% respectively. For AST activity, there was a significant reduction from 175.12 ± 10.63 U/L in the NaF control group to 137.68 ± 12.66 U/L in the *I. gabonensis* group and 135.69 ± 10.66 U/L in the standard group (Q+Vit. E). For ALT activity, it was 50.52 ± 2.42 U/L in the NaFC group, 32.43 ± 3.66 U/L in the *I. gabonensis* group, 26.15 ± 0.45 U/L in the standard and 27.74 ± 4.55 U/L in the normal control.

Similarly, bilirubin concentration was reduced from 11.54 ± 0.21 g/L in the NaFC to 8.76 ± 0.10 g/L in I.G group and 8.96 ± 0.21 g/L in standard group. However, serum total protein and albumin concentrations remained relatively unchanged (Figure 2). Figure 2 also showed that total cholesterol was significantly ($p<0.05$) increased in the NaF control rats, and in exposed rats treated with the reference standard and *I. gabonensis* fruit juice compared to the normal control.

From these results, it was observed that oral administration of NaF for 35 days to adult male rats resulted in a significant alteration of liver function. The liver is a primary site for xenobiotics detoxification, its metabolism is readily altered by toxicity. Xenobiotic hepatotoxic action is usually expressed by cell respiration disorders that interfere with oxidation and reduction mechanisms; either through impairment in protein, carbohydrate and lipid metabolism or by disturbances in intra- and extracellular transport. Consequently, whole cell or its cytoplasmic organelles can be damaged.

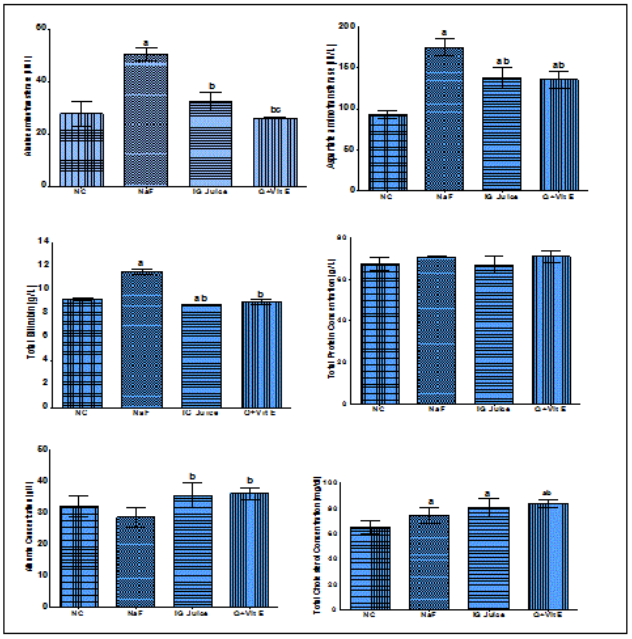

Figure 2: Effect of *I. gabonensis* juice administration on liver function of male Wistar albino rats administered 20 mg/kg b wt NaF for 35 days. Values are mean ± standard deviation of 6 determinations. *a* Significantly ($p<0.05$) different from Normal control (NC); *b* Significantly ($p<0.05$) different from sodium fluoride (NaF) control; *c* Significantly ($p<0.05$) different from *I. gabonensis* (IG) juice.

Most frequently the damage occurs as parenchymal vacuolar degeneration, necrosis of hepatocytes or disorders in the activity of metabolic enzymes [37-39] as seen from our study. Adverse effects of NaF on liver function have been reported with marked elevation of ALT and AST in mice [40].

These results are consistent with earlier reports on the hepatotoxicity of NaF. One study [41] reported that NaF induced morphological changes in rat hepatocytes and promoted cells vacuolar degeneration. Pale, granular hepatocytes, compatible with parenchymal degeneration, were observed in mice administered 0.95 mg fluoride/kg/day in drinking water for 7-28 days [42]. Also, liver congestion was observed in sheep given a single intragastric dose of fluoride as low as 9.5 mg fluoride/kg. Mild serum increases of liver enzymes glutamate dehydrogenase (GDH) and gamma-glutamyl transferase (γ-GT) also occurred in sheep administered 38 mg fluoride/kg body weight [43]. However, these alterations in liver enzymes were normalized by *I. gabonensis* fruit juice. Furthermore, the corresponding increase in total bilirubin in our study revealed a deleterious effect of NaF on liver metabolism in line with the elevation of serum transaminases. Hyperbilirubinaemia is characteristic of impaired bilirubin metabolism involving metabolic disturbances in the liver. This could be as a result of defective conjugation, transport and/or excretion of bilirubin, or overproduction of bilirubin caused by an excessive breakdown of red blood cells due to the toxins from the administered chemical. Breakdown of red blood cells frequently occur in humans, as a result of severe falciparum malaria, sickle cell disease, haemolysis associated with glucose-6-phosphate dehydrogenase

deficiency and toxins from bacteria or snake venoms etc. Administration of *I. gabonensis* fruit juice resulted in amelioration of liver damage as ALT, AST and total bilirubin levels were significantly reduced in these animals.

Our result showed no significant changes in serum protein and albumin metabolism by *I. gabonensis* juice and our reference standard administration to NaF exposed animals. Albumin is a key metabolite in liver detoxification function. Depletion of albumin concentration usually occurs in correspondence with reduction in total protein as this form the bulk of available serum and liver protein. On the other hand, a decreased albumin concentration could be explained by inflammatory reactions. These inflammatory reactions have been confirmed by histological sections realized on liver of NaF-treated mice in the works of [40]. They observed infiltration of leucocytes in hepatocytes of mothers and their pups, which however was more pronounced in the mothers [40].

Also, result obtained demonstrated a significant increase in total cholesterol of animals exposed to NaF. The *I. gabonensis* juice and reference standard also produced mild increases in cholesterol level. These increases may be an indirect pointer to the ability of these amelioratives to promote cholesterol synthesis. *I. gabonensis* fruit has been widely reported to exert lipid lowering effect by induction of HDL-cholesterol synthesis [8-10]. Hence the increase in total cholesterol observed in our study is believed to be as a result of increased HDL-cholesterol synthesis. Lipid profile studies were not carried out in this study to ascertain the exact cholesterol lipoprotein that was increased, but we had earlier observed a hypolipidemic effect of the fruit juice on sodium fluoride induced dyslipidemia in rats, as a result of increased HDL-C synthesis [12].

Our result in Figure 3 revealed a significant ($p<0.05$) increase in urea concentration in NaF-treated rats compared to normal control. Administration of the reference standard resulted in a significant ($p<0.05$) reduction of urea in exposed rats, but this reduction was non-significant in *I. gabonensis* treated rats. Similarly, serum creatinine was significantly ($P<0.05$) elevated in NaF control rats compared to normal control. This was significantly reduced by both *I. gabonensis* juice and reference standard administration showing a protective effect on the kidney. The effect of *I. gabonensis* on serum urea and creatinine levels were very much similar to that of the standard (Q+Vit E) group, but were not reduced to the level of the normal control. It is well known that ureamia paralleled by elevated serum creatinine is characteristic of intrinsic renal failure [44].

The electrolyte profile results (Figure 3) showed that potassium concentration was not altered by NaF administration. On the other hand, NaF exposure resulted in a significant ($P<0.001$) elevation of sodium concentration in NaF control rats and among *I. gabonensis* juices and reference standard treated groups compared to control.

The observed increase in sodium concentration may be attributed to an increased retention of sodium ion or may be contributed by Na^+ ion resulting from the administered NaF. Also, our result demonstrated a significant ($p<0.05$) increase in chloride ion concentration across all groups exposed to NaF compared to control. The elevated serum concentration of Na^+ and Cl^- ions indicate reduced ability of the kidney to eliminate the toxic metabolic substances and reabsorb the metal and non-metal ions. Increase in chloride concentration may be attributed to retention or a decreased clearance of chloride ion which may be due to a preferential excretion of F^- anion instead of Cl^- anion. The observed increase in chloride ion concentration is consistent with observed increase in sodium ion concentration.

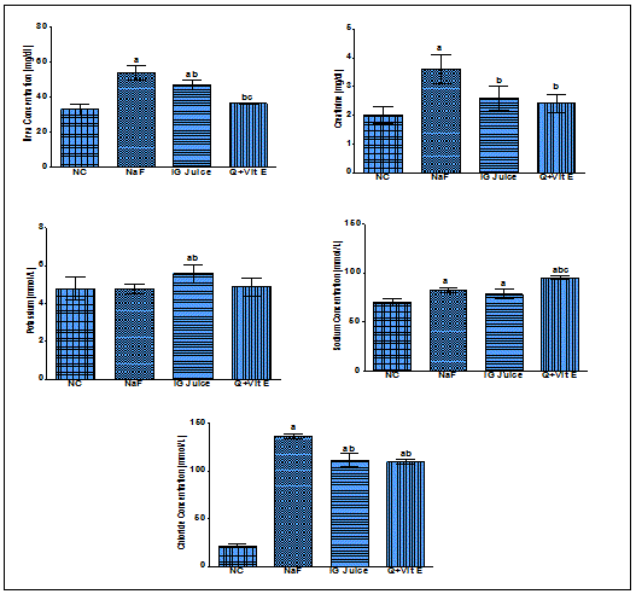

Figure 3: Effect of *I. gabonensis* juice administration on kidney function of male Wistar albino rats administered 20 mg/kg b wt NaF for 35 days. Values are mean ± standard deviation of 6 determinations. *a* Significantly ($p<0.05$) different from Normal control (NC); *b* Significantly ($p<0.05$) different from sodium fluoride (NaF) control; *c* Significantly ($p<0.05$) different from *I. gabonensis* (IG) juice.

Potassium ion concentration was not altered by sodium fluoride administration in the NaF control group. However, *I. gabonensis* juice administration resulted in a significant ($p<0.05$) increase in potassium ion concentration. The observed increase in K^+ ion may be as a result of *I. gabonensis* juice serving as a source of this cation. Changes in serum urea, creatinine, Na^+, and Cl^- are associated with impairment of renal function [45] and the major route of excretion of fluoride is by the kidneys. The result of our study showed that NaF administration may have caused kidney damage resulting in altered kidney metabolism, dyshomeostasis of electrolyte profile and impairment in kidney clearance of urea and elevated creatinine. Our observed alteration in kidney function by NaF administration is consistent with previous reports [46-49].

NaF exposure had no effect on calcium concentration as can be seen in NaF control group when compared to normal control. However, administration of *I. gabonensis* juice resulted in a 30% increase in serum calcium concentration. We suggest that the ameliorative effect of our juice may be via mechanisms involving calcium retention, or may be as a result of the juice serving as a source of calcium. Similarly, *I. gabonensis* juice and our reference standard resulted in a significant ($P<0.05$) increase in magnesium concentration compared to both normal and NaF control. These showed that the fruit juice of *I. gabonensis* could serve as a potential source of these important minerals (Figure 4). *I. gabonensis* has been reported to be a rich source of these minerals [50].

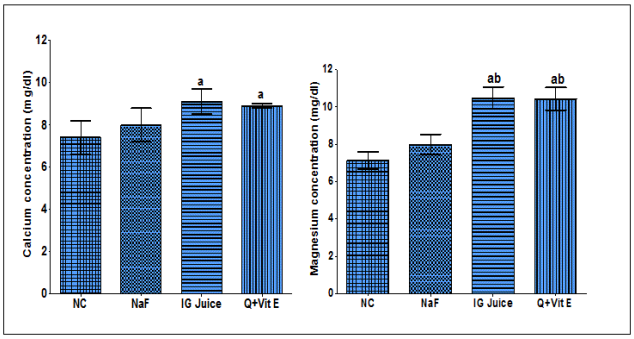

Figure 4: Effect of *I. gabonensis* juice administration on serum calcium and magnesium concentrations of male Wistar albino rats administered 20 mg/kg b wt NaF for 35 days. Values are mean ± standard deviation of 6 determinations. *a* Significantly ($p<0.05$) different from Normal control (NC); *b* Significantly ($p<0.05$) different from sodium fluoride (NaF) control; *c* Significantly ($p<0.05$) different from *I. gabonensis* (IG) juice.

Magnesium ion concentration in the NaF exposed rats was low, but was significantly elevated by administration of *I. gabonensis* and our reference standard. Fluoride is known to reduce absorption of calcium and magnesium from the gut [51,52]. These cations are needed by metalloenzymes in transcription, translation and enzymatic cascade mechanisms, acting as secondary messengers [53]. Hypocalcaemia may lead to muscle spasms and weakness, convulsions and cardiac dysrhythmias, coma and respiratory failure may also occur. Hence it is a great advantage that Mg^{2+} concentration was restored in the *I. gabonensis* treated group.

Conclusion

It can be deduced from our study that *I. gabonensis* fruit juice has some hepatoprotective potential which may be as a result of the presence of some secondary plant metabolites. The fruit is also a good source of Ca^{2+} and Mg^{2+} ions. Further studies are thus required in this area of research as the use of *I. gabonensis* is sustainable and environmentally friendly. Increased domestication of the plant is also encouraged.

Conflict of Interest

The authors declare no conflict of interest whatsoever.

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