Hypolipidemic effect of *Irvingia gabonensis* fruits juice on sodium fluoride induced dyslipidemia in rats

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Effect of *Irvingia gabonensis* fruit juice administration on serum lipid profile of sodium fluoride (NaF)-intoxicated rats was investigated. Twenty-four (24) male Wistar rats divided into four groups of six (6) animals each - one control and three intoxicated groups were used. The normal control (NC) group received only standard pelleted diet and water. All three intoxicated groups received 20 mgkg⁻¹ bodyweight of NaF daily by gavage for 35 days. While the first group (NaFC group) received only NaF, the second in addition was treated with *I. gabonensis* fruit juice (*I. gabonensis* group). The third received NaF plus 15 mgkg⁻¹ body weight Quercetin + 100 mgkg⁻¹ bodyweight vitamin E (Q+Vit E group). Result showed that LDL-C was significantly elevated, while HDL-C was markedly reduced in the NaFC group. In the *I. gabonensis*-treated group, lipoprotein phenotypes were normalized, with HDL-C increasing from 38.92±9.28 mgdl⁻¹ in NaF intoxicated group (NaFC) to 65.14±5.33 mgdl⁻¹, which was even higher than 60.83±4.56 mgdl⁻¹ obtained in the standard (Q+Vit E) group. Low density lipoprotein-cholesterol concentration also reduced from 17.3±3.2 mgdl⁻¹ in NaF-intoxicated group to 7.5±1.0 mgdl⁻¹ in *I. gabonensis*-treated group, which compared favourably with that of the standard. Furthermore, NaF toxicity resulted in the elevation of atherogenic index in the NaFC group. This was significantly (p<0.05) lowered in all other groups. The total non-HDLcholesterol and LDL/HDL ratio were significantly reduced in *I. gabonensis*-treated rats. This tends to suggest that the juice of *I. gabonensis* may be useful in alleviating and preventing cardiovascular diseases.

**Key words:** Sodium flouride, *Irvingia gabonensis*, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL).

**INTRODUCTION**

Tropical Africa's rainforests hold a wide array of plants and biodiversity. About ¼ of all the medicines used around the world are from rainforest plants (Palande, 2010). Many of these plants have been identified, scientifically classified and widely applied for known therapeutic benefits (Fennell et al., 2004). Among the classified, some still possess many
unidentified beneficial pharmacological properties. Some of these plants have long been used by the local people either for food or applied in the treatment of various ailments. With changing life pattern and increase in predisposing factors, atherosclerosis and cardiovascular diseases are fast becoming common decimals in both developed and underdeveloped nations. This is often as a result of changes in life style. For instance, with continuous increase in distances from homes to offices, most people resort to eating outside their homes, very often eating junk meals laden with saturated fats. This has led to the increased need for search for effective and natural hypolipidemic agents to reduce cholesterol in the blood. Atherosclerosis is usually linked to increased cholesterol, especially of LDL phenotype, as well as with triglycerides which is the main carrier of cholesterol in plasma (Nelson and Cox, 2005). LDL influences the plasma cholesterol concentration and is also closely implicated in the aetiology of atherosclerosis (Phil-Sun et al., 2006). Research in many countries show that increasing concentration of blood cholesterol is associated with a progressively increasing risk of coronary heart disease (CHD), even from concentrations regarded as absolutely normal such as 200 mg/100 ml (Tamas et al., 2002). Dyshomeostasis in serum lipids resulting in increased Non-HDL- cholesterol is also a risk factor. Diet plays a significant role in the onset of hypercholesterolemia, atherosclerosis and CHD, especially high saturated fat diets.

The plant *Irvingia gabonensis* is a tropical tree of the genus *Irvingia*. It is indigenous to west and central Africa. It is known by the common names ‘bush mango’ or ‘wild mango’ and indigenous names of *Ugiri, Ado, Dika* etc. It is widely distributed in the rain forest of Ghana, Nigeria, Cameroon, Ivory Coast, Uganda and Democratic republic of Congo (Ayivor et al., 2011). The tree *I. gabonensis* is about 15-40 m in height and 1 m in diameter; it may occur in gregarious clusters. They produce edible fruits similar to the mango fruit from which it derives the common name “bush mango”. Different parts of the plant are used in traditional and modern medicine for the treatment of several illnesses and in industrial processes (Anegbeh et al., 2003). The fruits are nearly spherical, green when ripe with a bright orange pulp. This fleshy and succulent pulp is edible or can be processed into jelly, jam, juice or wine (Akubor, 1996). 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 (Ndoye and Tchamou, 1994), dysentery (Okolo et al., 1995), scabby skin (Ndoye and Tchamou, 1994) and a potent anti-inflammatory agent (Okolo, et al., 1995). Leaf decoction of *I. gabonensis* and the seed extract have been reported to possess hypoglycaemic and hypolipidemic effect (Dzeufiet et al., 2009). In addition, antidiabetic effects of its bark and leaves on streptozocin-induced diabetic rats have been reported (Ngondi et al., 2006). *I. gabonensis* has also been shown to improve the haematopoietic system of rats (Omonkhuwa and Onoagbe, 2012). Since this fruit is relatively available but often less frequently consumed by people, and given the fact that available information on the hypolipidemic properties of the plant were on other plant parts (leaves, seed extract) and were all done outside the locality of this research (Dzeufiet et al., 2009; Oben, 2010), the present study was therefore designed to investigate the effect of *I. gabonensis* fruit juice on sodium fluoride-induced dyslipidaemia in Wistar albino rats.

Sodium fluoride is a well-known toxicant. Earlier studies showed that fluoride can produce abnormalities in the liver including degenerative and inflammatory changes (Chinoy et al., 1993; Parihar et al., 2013). Its ability to increase oxygen free radicals and consequent oxidative stress has been well demon-strated (Eşsiz et al., 2008). The neurotoxic effects of sodium fluoride in rats have been established since 1995 (Mullenix et al., 1995; Connett, 2012). More recently, low glucose utilization (Jiang et al., 2014), cognitive deficits and anxiety-depression-like behaviors have been described in mice treated with NaF (Liu et al., 2014). Use of sodium fluoride is on the increase in our world today and a lot of debate going-on on its continuous usage or non-use especially in fluoridation of water.

Children also consume a lot of fluoride through tooth pastes. Increased risk of fluorosis due to high water-borne fluoride concentrations is threatening to many parts of the world (Vasant and Narasimhacharya, 2012), and black children are disproportionately affected (CDC, 2005; Connett, 2012). This is probably due to biologic susceptibility or greater fluoride intake (CDC, 2005). It therefore becomes pertinent to assess the effect of this fruit extract on sodium fluoride-induced toxicity.

**MATERIALS AND METHODS**

**Plant materials**

Ripe and edible fruits of *I. gabonensis* were collected from a local plantation in Ugiri-Ike, Ikeduru Local Government Area of Imo State. The plant material was authenticated by a plant taxonomist, at the Department of Plant Science and Biotechnology, Imo State University, Owerri, Imo State. These fruits were obtained fresh as when needed.

**Animals**

Twenty-four healthy, male albino Wistar rats (*Rattus norvegicus*) weighing 115-150 g (averaging 6 weeks old) were used for this study. They 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 given standard rat pellets (Vital finisher) and tap water *ad libitum* and were left for a period of two weeks to acclimatize before commencement of the study. The rats were randomly divided into four (4) experimental groups and used for the determination of sodium fluoride toxicity and normalisation effect of *I. gabonensis*. The ethical committee of the university approved the study protocol prior to commencement of study and the study was carried out according to the guidelines of the Animal Welfare Act.
Preparation of fruit extracts

The ripe fruits of *I. gabonensis* were washed with clean tap water and peeled, seeds removed and the succulent pulp cut into small pieces. This was weighed and 250 g portion of the fleshy portion of the fruits was extracted with 250 g of distilled water in a juice extractor; Sinbo SJ3138 (Sinbo, China), to obtain the fruit juice (I.G). The resulting juice was then stored in a freezer (≤ 4.0°C) until needed. A fresh juice of the fruit was extracted each day of administration.

Grouping of animals

Twenty four (24) healthy, male albino Wistar rats were divided into four groups of six (6) animals each for the amelioration of sodium fluoride toxicity studies. Animals (rats) were separated into groups and treatments were as follows: Group I served as normal control (NC) which received standard pelletized diet and water *ad libitum* (NC) which received standard diet and water *ad libitum* and sodium fluoride toxicant (20 mg/kgbwt) daily; Group II served as intoxicated control (NaFC) which received standard diet and water *ad libitum* in addition to *I. gabonensis* fruit juice (IG) and sodium fluoride toxicant (20 mg/kgbwt) daily; Group IV served as intoxicated standard (Q +Vit E) which received standard diet and water *ad libitum*, in addition to Quercetin 15 mg/kgbwt + α-tocopherol 100 mg/kgbwt and toxicant sodium fluoride (20 mg/kgbwt) daily.

At the end of thirty five days of daily intoxication and treatment with *I. gabonensis* juice and standard for amelioration, the animals were fasted for 24 h after which they were lightly anaesthetized with dichloromethane and sacrificed by cervical dislocation and blood collected by cardiac puncture. Blood samples 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 the determination of serum total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides (TG) levels using commercial diagnostic kits (Biosystem, Spain).

Biochemical estimations

Total cholesterol was determined by the enzymatic (cholesterol esterase/oxidase/peroxidase) method of Allain et al. (1974). Triglycerides was determined by the glycerol phosphate oxidase/peroxidase method as described by Bucalo and David (1973). Low-density lipoprotein –cholesterol (LDL-C) was determined according to the method of Assman et al. (1984) while high-density lipoprotein-cholesterol (HDL-C) was determined using the phototungstate/Mg-cholesterol oxidase and peroxidase method by Grove (1979) and Burstein et al. (1980).

Data analysis

Data was analyzed using the statistical software “Analyze-it” for Microsoft Excel. Results were presented as mean ± SD and differences between the various groups and the control group were tested at (P<0.05) using one-way analysis of variance (ANOVA) statistic followed by Tukey test.

RESULTS

Results show that NaF toxicity caused significant (p<0.05) elevation of serum total cholesterol in all the treatment groups (NaF control group (NaFC = 74.86±6.26 mgdl⁻¹), I. *gabonensis* treatment group (I.G = 80.8±7.09 mgdl⁻¹) and standard (Q+Vit E = 107.20±9.73 mgdl⁻¹)) as compared to normal control (NC = 64.96±5.13 mgdl⁻¹) (Figure 1). Although IG administration in NaF exposed rats resulted in significant (p=0.008) elevation of total serum cholesterol as compared to the control, no significant difference was obtained between the cholesterol levels of NaF control and I.G groups.

HDL cholesterol concentrations (Figure 2) were 44.02±8.18, 38.92±9.28, 65.14±5.33 and 60.83±4.56 mgdl⁻¹ in NC, NaFC, I.G and Q+Vit E respectively. HDL-cholesterol was significantly (p<0.05) elevated in exposed groups administered with I.G and Q+Vit E as compared to NaF control and NC. Figure 3 shows the serum triglycerides concentration of the exposed animals. No significant (p>0.05) alteration in serum triglycerides concentration was seen across the groups.

LDL concentration (Figure 4) was 10.2±1.1 mgdl⁻¹ in NC group, 17.3±3.2 mgdl⁻¹ in NaFC group, 7.5±1.0 mgdl⁻¹ in I.G group and 10.5±1.2 mgdl⁻¹ in Q+Vit E-treated group. Result obtained demonstrated that NaF exposure resulted in a significant (p<0.05) elevation of LDL-cholesterol in NaFC as compared to NC, I.G and Q+Vit E groups. Also, *I. gabonensis* administration significantly (p<0.05) lowered LDL-cholesterol in exposed subjects and was even lower than that of the standard – treated group.

Effect of *I. gabonensis* fruit juice administration on LDL/HDL ratio in NaF-induced dyslipidemia (Figure 5) showed that NaF toxicity resulted in an elevation of LDL/HDL ratio in NaF control (0.44 ± 0.02) which was significantly lower than that of standard treated group (0.25±0.02). 

![Figure 1. Effect of *I. gabonensis* fruit juice administration on serum total cholesterol in sodium fluoride (NaF)-induced dyslipidemia. Results are mean ± SD of 6 determinations, values with different superscript show significant difference at p<0.05. a=significantly (P<0.05) different from normal control (NC); b=significantly (P<0.05) different from NaF; c=significantly (P<0.05) different from Vit E + Quercetin.](image-url)
Figure 2. Effect of *I. gabonensis* fruit juice administration on serum high density lipoprotein (HDL)-cholesterol in sodium fluoride (NaF)-induced dyslipidemia. Results are mean ± SD of 6 determinations, values with different superscript show significant difference at *p*<0.05. *a* = significantly (*P*<0.05) different from normal control (NC); *b* = significantly (*P*<0.05) different from NaF; *c* = significantly (*P*<0.05) different from VitE + Quercetin.

Figure 3. Effect of *I. gabonensis* fruit juice administration on serum triglyceride in sodium fluoride (NaF)-induced dyslipidemia. Results are mean ± SD of 6 determinations, values with different superscript show significant difference at *p*<0.05. *a* = significantly (*P*<0.05) different from normal control (NC); *b* = significantly (*P*<0.05) different from NaF; *c* = significantly (*P*<0.05) different from VitE + Quercetin.

Figure 4. Effect of *I. gabonensis* fruit juice administration on serum low density lipoprotein (HDL)-cholesterol in sodium fluoride (NaF)-induced dyslipidemia. Results are mean ± SD of 6 determinations, values with different superscript show significant difference at *p*<0.05. *a* = significantly (*P*<0.05) different from normal control (NC); *b* = significantly (*P*<0.05) different from NaF; *c* = significantly (*P*<0.05) different from VitE + Quercetin.

Figure 5. Effect of *I. gabonensis* fruit juice administration on low density lipoprotein/high density lipoprotein (LDL/HDL) ratio in sodium fluoride (NaF)-induced dyslipidemia. Results are mean ± SD of 6 determinations, values with different superscript show significant difference at *p*<0.05. *a* = significantly (*P*<0.05) different from normal control (NC); *b* = significantly (*P*<0.05) different from NaF; *c* = significantly (*P*<0.05) different from VitE + Quercetin.
Acute and chronic exposure to NaF may differentially change in different species (kotodziezyk et al., 2000). Abnormal metabolic function as well as histopathological changes in the liver (Anamika et al., 2012) and is known to result in cardiovascular function (Bera et al., 2007), induce testicular damage and lipid peroxidation in mice and disrupt other biological functions (Ghosh et al., 2002). It is also known that acute fluoride intoxication leads to the progressive fall in arterial blood pressure responsible for cardiovascular damage (Strubelt, 1982). The present study examined the ability of I. gabonensis fruit juice to normalise serum lipid profile in NaF-induced dyslipidemia. Result of our study shows that NaF of 20 mg/kg bwt daily for 35 days induced a deranged lipid profile on our experimental animals. It is well known that dyslipidemia is a common feature of chemical toxicity-induced damages especially those affecting the liver (Alisi et al., 2011). Identification and management of dyslipidemia have gained importance for both primary as well as secondary prevention of recurrent events in atherogenesis.

In our study, serum total cholesterol was significantly (p<0.05) elevated in NaF challenged animals as compared to normal controls. Similar elevations were observed in the standard group treated with a combined dose of 20 mg/kg bwt Quercetin+100 mg/kg bwt α-Tocopherol, as well as the I. gabonensis group. Recall that the primary event in atherogenesis is cholesterol deposition in the arterial walls. Cholesterol originates from circulating plasma lipoproteins which contain both free and cholesteryl esters. LDL and lipolytic products of chylomicrons as well as very low density lipoproteins (VLDL) also contribute greatly to atherogenesis (Tabas, 2009).

It is important to note that increased TC in the NaFC group was contributed immensely by elevated LDL-C level which is a major risk factor in cardiovascular events. The I. gabonensis group and the standard group had HDL-C contributing highly to their elevated TC, sinceno changes were seen in their triglyceride levels. Conventional treatment of atherogenic lipid profile targets reduction in total cholesterol levels and LDL-cholesterol, or increasing HDL-cholesterol. Treatments often include statins, fibrates, niacin, resins and lifestyle modification (Sharma and Garg, 2012).

Lipid-lowering effect of plant extracts has been known to occur via reduced gastrointestinal absorption which is often reflected in the concurrent increase in fecal lipid load of the animals (Miettinen et al., 1995). This was however not investigated in our study. The hypolipidemic effect would have been exerted through increased catalolism of LDL or reduced activation (inactivation) of acetyl CoA carboxylase (McCarthy, 2001), thus leading to reduced cholesterol synthesis. On the other hand, the elevated total cholesterol level may suggest that the extract contains ingredients capable of enhancing the activities of hepatic lipogenic and cholesterologenic enzymes like malic enzyme, fatty acid synthase, glucose 6-phosphate dehydrogenase and HMG-CoA reductase (Vega et al., 2003) which are required for cholesterol synthesis.

HDL-cholesterol level in I. gabonensis juice-treated group was elevated more than that of the standard group receiving combined treatment of 20 mg/kg bwt quercetin + 100 mg/kg bwt α-tocopherol. A significant (p<0.05) lowering effect on LDL-cholesterol was also evident in I. gabonensis fruit juice-treated group as compared to the other three groups. This significant reduction of LDL cholesterol and increased HDL is indicative of a lowered

**DISCUSSION**

Fluoride toxicity in animals can be multifarious. It is implicated in inflammatory and degenerative changes in the liver (Anamika et al., 2012) and is known to result in abnormal metabolic function as well as histopathological changes in different species (kotodziezyk et al., 2000). Acute and chronic exposure to NaF may differentially affect cardiovascular function (Bera et al., 2007), induce testicular damage and lipid peroxidation in mice and disrupt other biological functions (Ghosh et al., 2002). It is also known that acute fluoride intoxication leads to the progressive fall in arterial blood pressure responsible for cardiovascular damage (Strubelt, 1982). The present study examined the ability of I. gabonensis fruit juice to normalise serum lipid profile in NaF-induced dyslipidemia. Result of our study shows that NaF of 20 mg/kg bwt daily for 35 days induced a deranged lipid profile on our experimental animals. It is well known that dyslipidemia is a common feature of chemical toxicity-induced damages especially those affecting the liver (Alisi et al., 2011). Identification and management of dyslipidemia have gained importance for both primary as well as secondary prevention of recurrent events in atherogenesis.

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negative atherogenic index shown as reduced LDL/HDL ratio in the *I. gabonensis* treated group. This could be related to presence in the plant of alkaloids, saponins, flavonoids and polyphenols commonly known to reduce serum lipids in animals (Ezekwe and Obioha, 2001).

The total non-HDL-cholesterol was significantly elevated in the NaF control group when compared to the others. Dyshomeostasis in serum lipids resulting in increased non-HDL-cholesterol is a risk factor in the onset of atherosclerosis. Non-HDL cholesterol (which usually includes cholesterol in VLDL, VLDL remnants, IDL and LDL) may actually be more predictive of CAD risk than LDL cholesterol (Goldberg, 2013).

Total non-HDL cholesterol was however significantly reduced by our juice. This was indeed remarkable and underscores the efficacy of *I. gabonensis* juice in CAD. Results of our study are in agreement with earlier reported studies on hypolipidemic properties of *I. gabonensis* carried out in other parts of Africa (Dzeufiet et al., 2009; Oben, 2010). Although a clearly reduced total cholesterol level may not have been seen from our study, the significantly reduced total non-HDL cholesterol makes up for it showing that increased HDL-C may have been contributory. Oben (2010) reported that *I. gabonensis* reduced total cholesterol and increased HDL-cholesterol and adiponectin levels in their study. This is indeed similar to our findings.

Plant polyphenols and ascorbic acid possess strong antioxidative potency responsible for their protective effect against various toxicants. Ascorbic acid-deficient diets have actually been associated with increased aortic accumulation of cholesterol. Our result showed an ameliorative potency of *I. gabonensis* fruit juice on NaF-induced lipidaemia in rats. The ameliorative effect observed may be due to its reportedly rich vitamin C content and plant polyphenolics (Anil, 2007; Ebimieowei, 2012).

In conclusion, NaF which is usually incorporated as a component of dental products, drinks, dietary supplements and foods in general may not be as safe as it is considered. It has been identified to cause systemic toxicity in the liver, induce testicular damage and lipid peroxidation in mice and disrupt other biological functions. Our study reveals that aqueous fruit juice extract of *I. gabonensis* has hypolipidemic effect on NaF induced dyslipidemia. The extract was able to normalise lipoprotein phenotype altered by NaF-induced toxicity in albino rats by enhancing HDL-C concentration and lowering serum LDL-C concentration and atherogenic index. Further studies are however required to determine the exact mechanism by which *I. gabonensis* may have affected cholesterol biosynthesis and in general lipoprotein metabolism.

**Conflict of Interest**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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