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The phytoconstituents and the comparative effects of aqueous extract of *Irvingia gabonensis* seeds and proviron on the biochemical parameters of male guinea pigs

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ABSTRACT

Objective: To investigate the phytochemical screening and the effects of the aqueous extracts of the seeds of *Irvingia gabonensis* on the biochemical parameters of male guinea pigs. **Methods:** The biochemical parameters were assayed using Randox Diagnostic kits, Phenolphthalein method and colorimetric method. The phytochemical screening was carried out using standard procedures. **Results:** Phytochemical investigations revealed the presence of flavonoids, tannins, carbohydrate, alkaloids, terpenoids, steroids, volatile oils, saponins and cardiac glycosides. The aqueous extract of *Irvingia gabonensis* seeds (50 – 400 mg/kg) caused a statistically significant ($P < 0.05$ ANOVA) decrease in the levels of total cholesterol, urea, uric acid, total protein, prostatic, alkaline, and acid phosphatases. The highest reduction effect was obtained with uric acid at 400 mg/kg of *Irvingia gabonensis* extract while the least effect was observed in total cholesterol. These effects were dose- and time- dependent. **Conclusions:** This shows that the seeds of *Irvingia gabonensis* have hepatoprotective, nephroprotective and cardio protective properties. The study therefore, supports the claims on the use of the seeds of this plant by traditional medicine practitioners as a hepatoprotective and nephroprotective agent. Although further studies need to be done to isolate, identify and characterize the active principles in the seeds of this plant.

1. Introduction

Irvingia gabonensis (family of Irvingiaceae) is a large tree with dense compact crown of evergreen large leaves with edible seeds and sweet edible fruit pulp. It is found in rainforests, widely distributed in tropical West Africa and represented by two varieties: the fruit with sweet edible scanty fibrous pulp, fluted or cylindrical holes and fruits with bitter in edible, very fibrous pulp with buttressed whole [1,2]. *Irvingia gabonensis* trees usually reach maturity and begin flowering at 10 – 15 years old.

Irvingia gabonensis is commonly known as African Mango, Dikanut, or bush Mango. The seeds are known as Ogbuno in Ibo, Apon in Yoruba Nigeria. The paste from the kernel is known as Dika bread in Gabon and Etima in Cameroon [3,4].

Irvingia gabonensis is very useful to man. The bark of *Irvingia gabonensis* is used for diarrhea or dysentery, the fruit is rich in vitamin C and is consumed as a desert fruit throughout Western and Central Africa [5, 6]. The pulp is used for making jelly, jam and juices [5, 7]. The seeds are used as thickener for soup, stew or as additive for flavouring [6]. Traditionally the

Irvingia gabonensis bark is given to women to shorten their breast feeding period. It is also used for colic and dysentery [7], for hernias, yellow fever and as anti- poison [3]. It also has antimicrobial properties; the decoction of the bark is used for treating scabies, toothache and skin diseases [7]. *Irvingia gabonensis* has hypoglycemic effect hence its use as an anti-diabetic agent [8, 9].

Even though *Irvingia gabonensis* has been used extensively in Nigeria in the treatment of various diseases traditionally, there has never been a scientific report on its safety and effects on biochemical parameters. It is on the light of this that this study seeks to establish for the first time, scientific information on its effects on the biochemical parameters of male guinea pigs.

2. Materials and Methods

2.1. Plant materials

The seeds of *Irvingia gabonensis* were collected in June 2008 from the eastern part of Nigeria. The plant was authenticated by H.D Onyeachusim, a taxonomist at Botany Herbarium of University of Port Harcourt Nigeria, where voucher specimen was deposited. All the chemicals used

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were of analytical grade.

2.2. Preparation and extraction of plant sample

The plant seeds were dried under the oven at temperature of 28 °C for 2 hours. The dried seeds were ground with hammer mill and the fine powder were extracted using Soxhlet apparatus. The yields of the extract were obtained after removal of solvent. The extracts were stored in the refrigerator for subsequent reconstitution and use.

2.3. Animals

Adult male guinea pigs of average weight 300–600 g were obtained from the animal house of University of Port Harcourt. They were housed in a cage of five animals and were allowed to acclimatize to the new environment for 10 days. The animals were properly feed on elephant grass throughout the experimental period.

2.4. Phytochemical screening

Chemical tests were carried out on the extracts and on the powdered specimens using standard procedures to identify the constituents^[10, 11] by characteristic colour changes as described by Sofowora A. et al^[12, 13]. Briefly, formation of brownish green coloration on addition of 3 drops of ferric chloride to sample confirmed presence of tannins; formation of yellow coloration which disappears on standing when 5 mL of dilute ammonia solution and concentrated sulphuric acid were added sequentially to portion of the extract confirms the presence of flavonoids; presence of steroids was confirmed by colour change from violet to blue on addition of 2 mL acetic anhydride and 2 mL sulphuric acid to 0.5 g plant extract; terpenoids were confirmed by formation of reddish brown colouration of the interface on addition of 2 mL chloroform and concentrated sulphuric acid, 3 mL to 5 mL of the extract; saponins were confirmed in the plant by the frothing test; cardiac glycosides were confirmed by formation of brown ring of interface on addition of 2 mL glacial acetic acid containing 1 drop of ferric chloride solution and 1 mL concentrated sulphuric acid; presence of alkaloids were confirmed by Dragendorff reagent which formed a reddish brown precipitate with the sample.

2.5. Evaluation of biochemical parameters

The animals were divided into ten groups of five animals

each. Group 1–5 were used for time–dependent studies for a period of 28 days. Group 6–10 were used for dose–dependent studies. The animals from group 6–10 were administered different doses of the extract (50–400 mg/kg/day) for 96 hours after they were sacrificed. While group 1–5 were administered a fixed dose of the extract (400 mg/kg/day) over a period of 7, 14, 21, 28 days respectively. At the end of each treatment period, the animals from different groups were anesthetized with diethylether. The blood samples were collected by cardiac puncture with 21G needle fixed on 5 mL syringe, for serum liver enzyme makers and blood enzyme assays. These were assayed using Randox Diagnostic kits,^[17] Phenolphthalein method^[15], and colorimetric method^[16, 19]. Sample serum was separated from the cells, centrifuged at 3 400 r for 10 minutes and used for the assays.

2.6. Statistical analysis

Data were expressed as mean±standard error of mean (SEM) of five observations. Statistical analysis of data was performed using analysis of variance (ANOVA). Results were subjected to Graph Pad prism 5 demo (software) analyses, the differences between mean accepted as significant at $P < 0.05$ (ANOVA).

3. Results

Phytochemical screening revealed the presence of steroids, flavonoids, alkaloids, steroids, cardiac glycosides, volatile oils, terpenoids, tannins and saponins.

This study showed that the aqueous extract of *Irvingia gabonensis* causes a dose– and time– dependent decreases in the biochemical parameters such as: Urea, Uric acid, Creatinine, Total cholesterol, Protein, Alkaline, Acid, and Prostatic Phosphatases (Figure 1 and 2; Table 1 and 2). These effects were statistically significant at $P < 0.05$ (ANOVA). The observed effects of *Irvingia gabonensis* on the biochemical parameters of male guinea pigs were comparable to that of Proviron (Table 2).

The administration of cadmium caused significant increases in the levels of all the biochemical parameters assayed except total protein. And this effect was inhibited by pre–treatment with *Irvingia gabonensis* (Figure 1 and 2; Table 1). But there was no significant inhibitory effect observed in Urea, Uric acid and alkaline phosphate by pre–treatment with *irvingia gabonensis* (Figure 1 and 2).

Table 1

The dose–dependent comparative effects of *Irvingia gabonensis* and Proviron on the biochemical parameters of male guinea pigs.

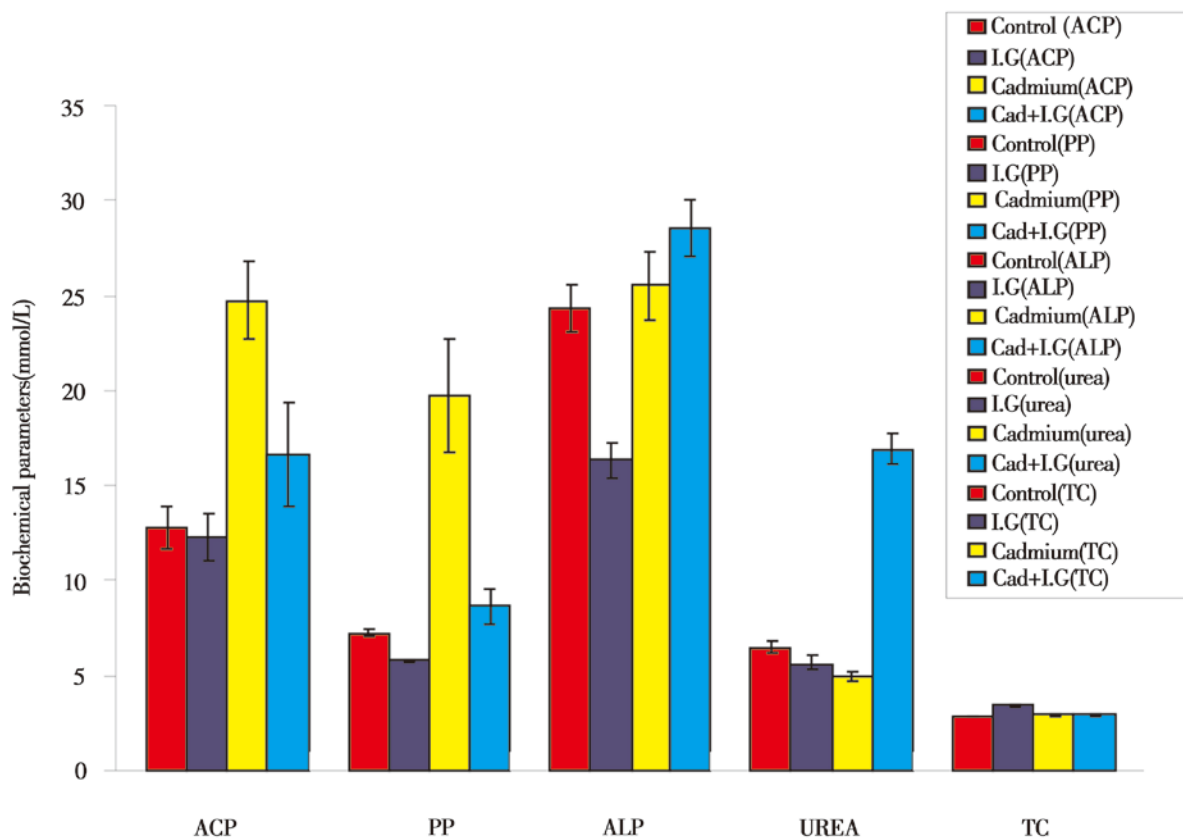
Dose (mg/kg)	ACP	PAP	ALP	UA	TP	Urea	Creatine	TC
Control	12.7±1.1	7.2±0.2	24.3±1.2	399.0±6.0	52.5±3.8	6.4±0.3	63.7±6.1	2.80±0.01
50	12.2±1.2	5.7±0.1 ^a	16.3±1.0 ^a	390.0±6.0	55.3±3.9	5.6±0.4 ^a	48.3±3.1 ^a	3.40±0.09
100	22.2±2.2	12.9±1.1	25.0±2.5	435.0±7.0	50.3±2.0	4.9±0.3 ^a	59.0±4.5	2.80±0.01
200	13.8±1.9	7.0±0.3	23.3±2.1	435.3±6.8	52.3±2.2	4.4±0.3 ^a	48.0±3.0 ^a	2.70±0.02
400	27.0±3.5 ^a	16.4±2.7 ^a	28.8±1.5	446.5±6.7 ^a	55.0±3.8	6.0±0.4	58.7±4.0	2.90±0.03
Cd	24.7±2.0	19.7±3.0 ^a	25.5±1.8	510.5±27.2	51.5±1.9	4.9±0.2	136.0±14.2 ^b	2.90±0.03
Cd +I.G	16.6±2.8	8.6±0.9	28.5±1.5	482.5±6.9	51.3±1.8	16.9±0.8	66.3±6.5 ^a	2.90±0.03

Results expressed as Mean ± SEM; n = 5; a: $P < 0.05$; b: $P < 0.001$ (ANOVA); Cd represents cadmium; I.G *Irvingia gabonensis*; ACP acid phosphatase, PAP prostatic acid phosphatase, and ALP alkaline phosphatase; UA uric acid; TP total protein; TC total cholesterol respectively.

Table 2The time-dependent comparative effects of *Irvingia gabonensis* and Proviron on the biochemical parameters of male guinea pigs.

Time (days)	ACP	PAP	ALP	UA	TP	Urea	Creatine	TC
0	12.7±1.1	7.2±0.2	24.3±1.2	399.0±6.4	52.5±1.5	6.4±0.5	63.7±6.2	2.80±0.01
7: I.G	13.8±1.3	7.0±0.2	23.3±1.2	390.0±6.0	52.3±2.2	4.4±0.3 ^a	48.0±3.2 ^a	2.70±0.02
PV	14.3±1.9	9.8±0.2	22.7±1.1	418.0±7.0	55.7±3.9	7.2±0.6	59.7±4.5 ^a	2.70±0.02
14: I.G	10.2±2.7 ^a	5.8±0.2 ^a	25.7±1.0	399.0±6.4	49.7±1.4	9.7±0.9	78.7±13.5	2.70±0.02
PV	9.8±2.6 ^a	4.9±0.1 ^a	26.0±1.1	383.0±6.1	50.0±2.1	15.8±1.5	70.0±11.0	2.60±0.02
21: I.G	16.2±2.8	9.3±1.2	27.7±1.3	372.0±5.1 ^a	50.7±2.0	15.3±1.4	80.0±13.2 ^b	2.70±0.01
PV	14.4±2.6	9.0±1.2	31.0±1.5	403.0±7.1	53.3±2.3	67.0±0.5	84.0±13.5	2.70±0.01
28: I.G	19.3±3.0 ^a	11.5±1.8	26.0±1.1	504.8±9.0	58.8±4.0 ^a	5.8±0.3	64.0±6.0	3.10±0.04
PV	33.3±3.9 ^a	15.9±2.2	34.0±2.0	521.7±7.0	81.0±7.1 ^a	5.5±0.3	56.0±4.5	3.40±0.05

Results expressed as Mean ± SEM; n = 5; a: P < 0.05; b: P < 0.001 (ANOVA). Cd represents cadmium; I.G *Irvingia gabonensis*; ACP acid phosphatase, PAP prostatic acid phosphatase, and ALP alkaline phosphatase; UA uric acid; TP total protein; TC total cholesterol respectively.

**Figure 1.** The effects of 50 mg/kg of *Irvingia gabonensis* on the biochemical parameters of male guinea pigs.

4. Discussion

The flavonoids, as an anti-oxidant in this plant may contribute to the effects of this plant as hepatoprotective, nephroprotective, antimicrobial, anti-inflammatory, and anti-carcinogenic effect [14,17]. Alkaloids in this plant may be responsible for its analgesic effects and its use as an antimicrobial agent [17]. This is consistent with the past works [14, 18–20]. Alkaloids and their synthetic derivatives

are used as basic medicinal agents for their antispasmodic and bactericidal effects [17, 19]. Tannins have astringent properties, hasten the healing of wounds and inflame mucous membranes. This may be responsible for its use in cleansing and treatment of wounds [17–19].

By reducing the total cholesterol levels, it indicates that this plant can exert anti-diabetic, anti-hypertensive, and anti-lipidemic properties. This is consistent with the past works [14, 15, 17]. By lowering the levels of Phosphatases and Creatinine, it indicates that *Irvingia gabonensis* has

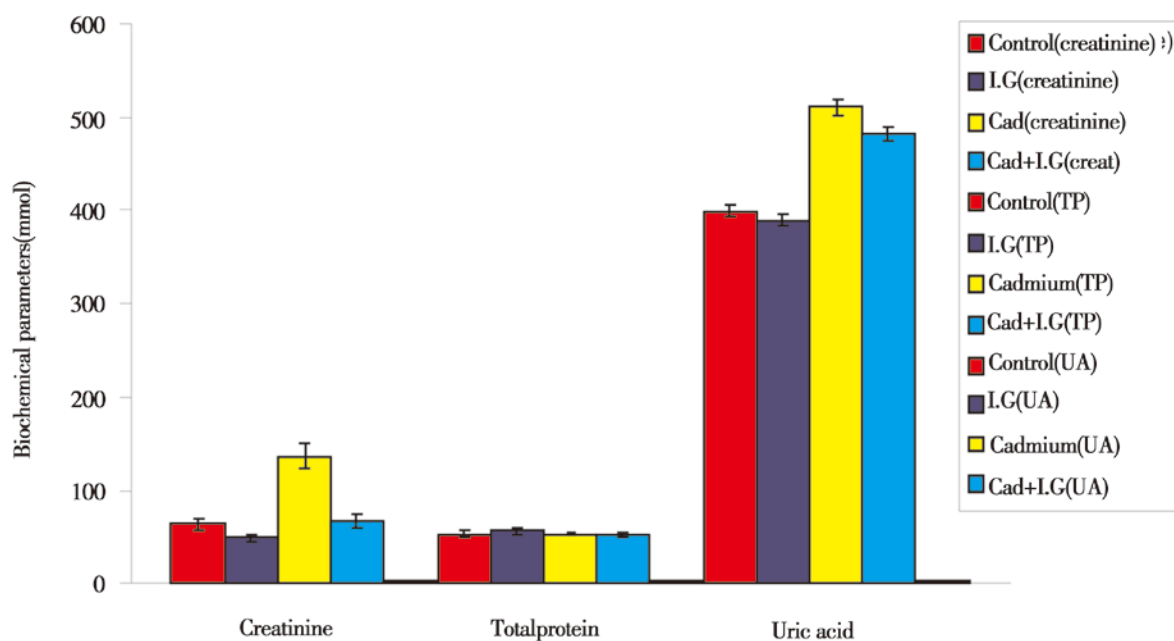


Figure 2. The effects of 50 mg/kg of *Irvingia gabonensis* on the biochemical parameters of male guinea pig.

hepatoprotective and nephro-protective effects. This is also consistent with the past works [14, 17, 20].

The Uric acid, total cholesterol and protein lowering effect of these plants indicate that the plant can have anti-hypertensive, anti-inflammatory and anti-nociceptive effects. This is also consistent with the past works [14, 17–20].

This study therefore supports the claims on the folkloric use of *Irvingia gabonensis* as a hepatoprotective and nephroprotective agent. It also supports the potentials of the plant as anti-carcinogenic, anti-lipidemic, analgesic and anti-inflammatory agent. Further studies are on the way to isolate, identify and characterize the active principles in the seeds of *Irvingia gabonensis* as well as identification of mechanism of action of *Irvingia gabonensis*.

Conflict of interest statement

We declare that we have no conflict of interest.

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