GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Effect of *Irvingia gabonensis var. wombolu* kernel powder and oil on serum protein profile and enzymatic activities of *Wistar* albino rat

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GSC Biological and Pharmaceutical Sciences, 2022, 21(02), 172–179

Publication history: Received on 24 July 2022; revised on 15 November 2022; accepted on 17 November 2022

Article DOI: https://doi.org/10.30574/gscbps.2022.21.2.0364

Abstract

Irvingia gabonensis Kernel powder and the oil extracts (±testa) were mixed with commercial animal feed (50:50)and fed to groups of animals. Animals in groups A and B were fed with the powder at an equivalent concentration of 67mg/kg body weightwhile those in groups C and D were fed with the equivalent4ml of the oil extract in the mixture. Animals in group E, the control, were fed on the commercial feed only. All the animals were fed in an aluminum cage for 21 days and sacrificed the following day. Blood from the jugular vein was collected in universal bottles and used for determination of protein and enzymatic profiles of these animals. Groups A and B showed appreciable elevation of albumin. Elevated transaminases were indicative of early signs of liver damage. The observation was attributed principally to the phytochemical component of the testa present in these sample types A and C fed to the animals under the restricted dietary laboratory condition. The study has shown that under unrestricted dietary condition, incorporation of *Irvingia gabonensis* kernel in animal feed had beneficial effects.

Keywords: *Irvingia gabonensis*; Testa; Transaminase; Phytochemical

1. Introduction

Irvingia is a non- timber forest tree comprising the stem, leaves, roots and fruits([1]). Commonly referred to as wild bush mango and African bush mango, the tree is named after a Royal Navy surgeon and botanist, George Irving([2]). It is of the order *Malpighiales*, family *Irvingianceae* and comprises seven species. In the family *Irvingiaceae*, *Irvingia gabonensis* and *Irvingia wombolu* are well known. *Irving*ia is native to Central and West African Countries. Specifically, the species *wombolu* and *gabonensis* span through the humid forest zones of West and Central Africa.

The flesh and the kernel of *I. gabonensis var.gabonensis* are edible while only the kernel of var. *wombolu* is edible. The former is for this reason called sweet bush mango and the latter bitter bush mango. Some local names for the kernels of African bush mango include*: ogbono* among the Ibos, *dikanut* among the Camerounians, *oro –egili* and *oro –aikpele* among the Igala people of Kogi State, Nigeria.

Indigenous African tribes have used the fruits in various ways including its usage as a thickening agent for traditional soups. When squeezed, the oil from the fruit can also be used for cooking. The western world, however, consider it a super fruit because of studies showing that extracts from its seeds and the fruit as a whole could help in loss of weight, control of blood cholesterol as well as reduction in blood glucose ([3])

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1.1. Justification For the Study

Irvingia gabonensis has been known to cause reduction in weight, waist circumference and systolic blood pressure. In addition, the presence of appreciable fibre content in *I. gabonensis* makes it a very good option in reduction of blood sugar and hence management of diabetes mellitus ([3]).

Although literature reports abound on the role of *Irvingia gabonensis* in weight and glucose control, they are rather scanty on enzymatic and protein profile changes that occur following administration of kernel fractions. The current study aimed to provide this knowledge.

Aim and Objectives

The aim of the study was to determine the effect of *I. gabonensis* (var. *wombolu*) kernel powder and oil extracts (with and without testa) on some enzymatic activities and protein profile of *wistar* albino rat.

The specific objectives of the study were to determine the effects of *I .wombolu* kernel powder and oil extracts (with and without testa) on certain enzymatic activities (alanine transaminase, aspartate transaminase and alkaline phosphatase) and protein profile(albumin and globulin)of *wistar* albino rat.

2. Material and methods

2.1. Collection and Treatment of Kernel Samples

Mature *I.wombolu* fruits were plucked from the tree in Aloko, Bassa Local Govt., Kogi State, Nigeria. The fruits were then split open with a stainless steel knife; the kernels were removed from the shell and then divided in to two equal portions. The testa of one of the portions wasleft intact (Iwkt) while the other was removed (Iwk).

Oil was extracted from part of Iwk and Iwkt by Soxhlet extraction method as described by Pearson([4]). The oil samples so extracted were labeled Iwot and Iwo respectively. The Iwkt or Iwk kernel powder sample was undefatted in this study.

2.2. Purchase of Reference Commercial Feed

The commercial feed used in this study was pelletized growers mash procured from a commercial shop located opposite Grimard Hospital. Anyigba, Kogi State, Nigeria. The composition of the feed, according to the manufacturers was as follows; crude potein (15%), fat,(7%), crude fibre (10%), calcium (1.0%), available phosphorus (0.35%) and metabolisable energy (2550 Kcal/Kg).

2.3. Oil Extraction

Oil was extracted using Soxhlet extraction method as described by Pearson ([4]}. Boiling flasks, 250 ml, were dried in oven at a temperature of 110 ^{0}Cfor thirty minutes and transferred into desiccators to cool. Respective samples (800 g) of Iwkt and Iwk were later weighed into labeled thimbles. Correspondingly labeled cooled boiling flasks were then weighed and filled with petroleum ether (40- 60 ^{0}C) The extraction thimble was then plugged lightly with cotton wool, the Soxhlet apparatus assembled and allowed to reflux for six hours. The thimble was then removed carefully, the petroleum ether in the top container of the set-up collected and drained into a flask for re-use.

The flasks were later removed, when it was free of petroleum ether, and dried at 110 ^{0}C for 1 hr after which they were transferred into desiccators, allowed to cool and then weighed.

2.4. Experimental Animals

The experimental animals used were *wistar* albino rats with weight range of 120 – 180g obtained from the animal house of Kogi State University, Anygba. The animals were kept in standard aluminum cubicles at the animal house of Biochemistry Department, Kogi State University, Anyigba.

Twenty-five animals were grouped into five with each cubicle containing five rats. The cubicles were labeled A, B, C, D and E.

2.5. Treatment Protocol

Kernel powder and the oil extracts (±testa) were mixed with commercial animal feed (50:50) and fed to groups of animals. Animals in groups A and B were fed with the powder at equivalent concentration of 68 mg per kilogram body weight while those in groups C and D were fed with the equivalent 4ml of the oil extract. Group E, the control, was fed only with commercial animal feed. They were also provided with potable water *adlibitium.*

The treatment lasted for a total of twenty-one days. On the twenty-second day, the animals were sacrificed using a sharp surgical blade; part of the blood was collected in in universal bottles.

Table 1 Protocol For Animal Treatment

Sample Types	Animal groups (5/ group)
(Iwkt)	A
(Iwk)	B
(Iwot)	C
(Iwo)	D
Control	E

Iwkt = *Irvingia* Kernel Powder (+testa)
Iwk = *Irvingia* Kernel Powder (-testa)
Iwot = *Irvingia* Kernel Oil (+testa)
Iwo= *Irvingia* Kernel Oil (-testa)
Control = Commercial Animal feed

2.6. Assaying For Selected Serum Enzymes

The plasma levels of alanine amino transferase (ALT) and aspartate amino transferase (AST) were measured according to the method described by Ignatius *et al.*([5]) using Randox kit cat N0 AL100 and AS101 (RandoxLaboratories, Crumlin, Antrim UK) respectively by monitoring the concentration of oxaloacetate hydrazone formed with 2,4 dinitrophenylhydrazine in the case of AST;

$$\alpha - \text{oxoglutarate} + \text{L} - \text{aspartate} \xrightarrow{\text{SGOT}} \text{L} - \text{glutamate} + \text{oxaloacetate},$$

while ALT was measured by measuring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine

$$\alpha - \text{oxoglutarate} + \text{L} - \text{alanine} \xrightarrow{SGPT} \text{L} - \text{glutamate} + \text{pyruvate}$$

2.7. Aspartate Amino Transaminase (AST)

A known volume (100µl) of the working reagent was added to 1.0ml of the samples in cuvettes and the content was thoroughly mixed and incubated at 25^{0}C for 1minute.The absorbance (A) of the samples were then read against the blank at 1 minute interval for 3 minutes.

Calculation;

$$(\Delta\, A/\text{min}) = A_3\text{-}A_0 / T_3\text{-}T_0 \ldots\ldots\ldots\text{equation 1}$$

Where;

A_o = Absorbance at 1 minute
A_3 =Absorbance at 3 minutes
T_0=time of first absorbance reading

T_3 = time of last absorbance reading
AST (U/L) = ΔA/min x1768……equation 2

2.7.1. Alanine Amino Transferase

The same reaction principle holds for alanine aminotransferase (SGOT) and aspartate amino transaminase (SGPT). (See equation above).

2.8. Alkaline Phosphatase

This was done according to the method described by Ignatius *et al.*([5]) using p-Nitrophenyl phosphate kinetic reaction.

2.9. Procedure

Reagent, 1.2 ml, was added to 20µl of samples in a cuvette and the contents thoroughly mixed and incubated at 25 ^{0}C for 5 minutes. The absorbance (A) of the sample were read against the blank at 405 nm for 3 minutes at 1 minute interval.

Calculation

$$(\Delta A/\min) = \frac{A_3 - A_0}{T_3 - T_0} \text{equation 1}$$

Where;

A_0= Absorbance at 1 minute
A_3= Absorbance at 3 minutes
T_0= time of first absorbance reading
T_3= time of last absorbance reading

Alp (U/L) = ΔA/min x 3300……..equation 2

2.10. Determination of Protein

The serum total protein was determined by Biuret colorimetric reaction according to the method described by Plumer([6])

2.11. Procedure

Samples, 25 µl, were carefully withdrawn and mixed with equal volumes of distilled water and calibrator and 1ml of working reagent. The mixture was incubated at 25 ^{0}C for 10 minutes. The absorbance (A) of the samples and the standard were read against the blank at 540 nm.

Table 2 Protocol for Determination of Serum Total Protein

	Blank 1.0	**Standard 1.0**	**Sample 1.0**
Distilled Water(µl)	25	-	-
Working reagent(ml)	1.0	1.0	1.0
Standard (µl)	-	25	-
Sample (µl)	-	-	25

Calculation;

$$\text{Total Protein (g/dl)} = \frac{\text{(A) Sample}}{\text{(A) standard}} \times \text{Concentration of standard}$$

2.12. Albumin

This was determined by bromocresol green colorimetric reaction, according to the method described by Dumas *et al*[7]. Sample, 5µl, was mixed with equal volumes of distilled water and standard and 1 ml of the reagent. The well mixed content was incubated at 25 ^{0}C for 10 minutes. The absorbance (A) of the samples and standard were read against the blank at 630 nm.

Table 3 Protocol for Determination of Serum Albumin conc

	Blank 1.0	**Standard 1.0**	**Sample 1.0**
Distilled Water(µl)	25	-	-
Working reagent(ml)	1.0	1.0	1.0
Standard (µl)	-	5.0	-
Sample (µl)	-	-	5.0

$$\text{Albumin (g/dl)} = \frac{\text{(A) sample X 5g/dl}}{\text{(A) standard}}$$

Where 5g/dl = concentration of standard.

2.13. Globulin

Concentration was arrived at by deducting the albumin concentration from the total protein concentration.

$$\text{Globulin conc (g/dl)} = \text{Total protein conc(g/dl)} - \text{Albumin conc (g/dl)}$$

3. Results

3.1. Enzyme and Protein profiles of Wistar Albino Rats Fed with *Irvingia gabonensis* Kernel Powder and oil (± testa) on 22nd Day

Table 4 The following parameters were analyzed as presented below

Parameter	**A Powder (+t)**	**B Powder (+t)**	**C Oil (+t)**	**D Oil (+t)**	**E Control**	**LSD**
ALT (IU/L)	29±1.03^a	31.70±0.50^a	38.41±5.80^b	46.60±0.32^c	24.00±1.30^a	7.67
ALP (IU/L)	131±5.00^b	144.70±2.43^c	147.32±1.60^a	144.20±2.01^c	122.70±1.70^a	7.91
AST (IU/L)	36.10±0.10^a	39.00±0.92^b	36.20±0.14^a	38.20±0.62^c	36.34±0.22^a	1.44
Total protein(g/l	10.30±0.30^a	9.50±0.30^a	9.04±0.50^a	9.20±0.13^a	9.63±0.30^a	0.99
Albumin(g/l)	6.12±0.08^d	5.63±0.10^c	5.00±0.01^b	4.42±0.20^a	5.70±0.12^c	0.32
Globulin(g/l)	4.20±0.32^a	3.90±0.32^a	3.90±0.34^a	4.80±0.13^b	3.94±0.30^a	0.81

Results are expressed as mean± standard error of mean for n=3; A –D refer to animals treated with I.wkt, I.wk, I.wot and Iwo respectively and E is the control; Values with similar alphabets in a row show no significant value at P>0.0; ALT = Alanine transaminase; ALP = Alkaline phosphatase; AST = Aspartate transaminase

- **ALT:** Samples A, B and E showed relatively lower ALT activity than samples C and D. Sample D showed the highest activity.
- **ALP:** Sample E showed the least ALP activity compared with all the treated groups ($p \leq 0.05$)
- **AST**: Samples A, C and E had relatively lower AST activity than B and D($p \leq 0.05$)
- **Total protein**: There were no significant statistical differences ($P \geq 0.05$) among the groups, including the control. However, samples A, B and E had similar proportion of albumin: globulin of 6 : 4 while samples C and D had inverse ratios of 5 : 4 respectively.

- **Albumin:** The treated groups had significantly differing concentrations of albumin ($p \leq 0.05$).
- **Globulin:** There appeared to be confounding effect of sample treatment on globulin content, even though sample E had similar lower concentration with samples B and C.

4. Discussion

In this study, powdered forms of *Irvingia gabonensis* var. *wombolu* and their oil extracts, with and without testa, were analysed for their effects on the enzymatic and protein profiles of *wistar* albino rats, previously fed for 21 days. A commercial feed as stated in the methodology, was used as control (E) while 50 : 50 mixture of the commercial feed and the processed *wombolu* kernel powder were fed to some groups (A and B) ; the equivalent amount of the oil replaced the kernel powder in the feeding of groups (C and D).

4.1. Protein and Selected Enzymes

4.1.1. Total Proteins

A rise in serum total protein is indicative of a wide range of pathologies from malignancies to infection or allergy. This work did not show any significant difference in total protein between the control and the treated groups ($p \geq 0.05$). An implication is that *Irvingia wonbolu* kernel is safe for consumption. Irrespective of treatment, it was observed that groups A, B and E had similar proportion of albumin: globulin of 6 : 4 while between C and D there was inverse ratios of 5 :4 respectively .

4.1.2. Albumin

This is a component of protein produced in the liver. It is the predominant component of total protein, accounting for up to 60% of total protein, with a normal range of 3.4 – 5.4 g / dl ([8]). This work has shown significantly differing concentrations of albumin ($p \leq 0.05$) in the treated groups, and not exceedingly out of the upper range stated. The powdered groups have shown higher concentrations of albumin than the oil groups, most probably due to higher concentration of essential nutrients needed for its production. It also proves the point that consuming the kernel whole is more nutritious than consuming the oil alone.

4.1.3. Globulin

The normal range in the blood is 2.6 – 4.6 g /dl. This work showed confounding effect of sample treatment on globulin content even though group E had similar lower value with groups A, B and C

4.1.4. Albumin/Globin Ratio

The normal range is 1.7-2.2. A ratio < 1.7 may mean liver pathology while a value > 2.2 may indicate hypothyroidism or hypoglobulineamia([9]). The albumin /globulin ratio of about 1.0-1.5 was observed in this study.

4.1.5. Serum Enzymes

These are organic catalysts needed for several biochemical reactions.

4.1.6. Alanine transaminase (ALT)

This is a transaminase enzyme as the name suggests. Its other synonyms are alanine transferase (ALAT) and serum glutamic pyruvic transaminase (SGPT). This study showed elevated ALT activities in oily groups C and D than groups A, B and E ($p \leq 0.05$), indicative of liver pathology. The observation alluded to possible higher solubility of polyphenolic compounds in the oil extracts([10]) had reported the damaging effect of *Irvingia gabonensis* seed and flesh extracts on the kidney using 200mg/kg body weight of the extracts for a period of 21days.

4.1.7. Alkaline Phosphatase (ALP)

This is a group of enzymes found primarily in the liver and bone although small amounts are produced by cells lining the intestines, the placenta and the proximal convoluted tubules of the kidney (Jeremy, 2015).The range in adult is 50 – 75 mg/ dl or (0.09-1.3IU/L). Increased levels were observed in the treated groups of animals. Though not too specific an index of pathology, the result, in combination with ALT and AST, could be indicative of liver and / or kidney damage.

4.1.8. Aspartate amino transferase (AST)

This enzyme abounds in large amount in the liver, heart and muscle cells. Its other names are aspartate amino transaminase and serum glutamic oxaloacetic transaminase (SGOT). The normal range of the enzyme is 10 – 34 IU/L (Dugdale, 2013). Higher values of 36-39IU/L were observed in this study. The control (E) and the treated groups A and C with testa showed similar lower values than those without testa ($p \leq 0.05$). It implied that there were components of the testa that positively promoted AST activity as discernable with ALT activity and the ALP activity on the powdered samples.

5. Conclusion

This study has shown that animals fed with the kernel powder samples exhibited higher levels of enzyme activity, implying early onset of liver and kidney damage; this was probably due to strict restriction to mono-diet under laboratory conditions. It also demonstrated a higher level of albumin among the animals treated with the powdered samples compared to the oil groups.

From this study, it is safe to conclude that *irvingia gabonensis* products are safe and good for health when consumed in moderation; and are better consumed whole Excessive and restrictive consumption of the product over a long time on the other hand has negative health implications. Those who indulge in excessive consumption of formulated *irvingia* oil (tablets) are advised to take caution

Compliance with ethical standards

Acknowledgments

I appreciate the contributions of my supervisor, Prof. Arogba, S.S for his painstaking efforts throughout the period of this study. He has, through his uncmon persistence and meticulousness, ignited in me a passion for scholarship. May his days be long.

I remain eternally grateful to our indefatigable Professor of Biochemistry and former Head of the Department, Prof. Obidoa, O for his fatherly role. I cannot forget the brotherly roles played by the current Head of Department, Prof. Omale, J in the course of this work. You were a source of encouragement and I beseech Allah to reward you accordingly. I equally recognize the contributions of our P.G Co-ordinator, (Dr. Olajide.E.J), Dr. Sanni, M (Reader), and Dr. Daikwo, M of the Department of Biochemistry. May the almighty be your strength today and forever more.

My gratitude also goes to Dr. Friday and Mr Obaje, G of the College of Health Sciences, Kogi State University, Mr. Olusegun, O of Biochemistry Laboratory, Mr. Ejembi, D and Mr. Ayeni, G of Department of Biochemistry and all those who either directly or indirectly had a role to play during the course of this research.

Disclosure of conflict of interest

There was no conflict of interest in this study.

Statement of ethical approval

Ethical approval for this study was obtained from the ethical committee of the university before the study was embarked upon.

References

[1] Umoh, I.B, Eka, O.U and Ekpe, O.O (2007). Effects of a Typical Rural Processing Method on the Proximate Composition and Amino Acid Profile of Bush Mango Seeds. African Journal of Food, Agriculture, Nutrition and Development. Rural Outreach Program. 7(1);1-12

[2] Gledhill, D (1989). The Names of Plants, 4th Edn. Cambridge University press, Cambridge .U.K

[3] Ngondi, J.L, Oben, J.E and Minka, S.R .The Effects of Irvingia gabonensis Seed on Body Weight and Blood Lipids of Obese Subjects in Cameroon. Lipids Health. 4;12. The open access publishers (Posted May 25th, 2015, sighted Aug. 2015).

[4] Pearson, D (1976) The Chemical Analysis of Foods.7th Edn. R.K .Churchill

[5] Ignatius, C.Maduka, Emeka, E.N, Ebele, J.I, Samuel, U.C and Ebele, E (2009). Assessment of Liver Enzymes in Asymptomatic HIV-Seropositive Patients, Journal of Biological Sciences;4(3); 360-362.

[6] Plumer, D.T (1978) An Introduction to Practical Biochemistry. 2nd Edn. McGrawHill, London.

[7] Dumas, B.T., Waston, W.A., and Brigg, H.G (1971) .Albumin Standards And the Measurement of Serum Albumin with Bromocresol Green. Clinic Chim, Acta, 258(1); 21-30.

[8] Dugdale, C.D (2013) .Aspartate transaminase Blood Test. MedlinePlus Medical Encyclopedia (Editted 2013, sighted 11th April, 2015)

[9] Jeremy, K.E (2015). Alkaline Phosphatase. drkaslow.com (last edited Oct. 2015, sighted 6th Nov. 2015).

[10] Sule, J.O, Onyije, F.M and Onoru, G.F (2015) The Effects of Irvingia gabonensis Flesh and Seed Extracts on the Biochemical and Histological Parameters of the Liver in Wistar Albino Rats. Journal of Pharmaceutical and Biomedical Sciences. 5 (01); 25-3