Histopathological and Biochemical Effects of Methanol and Aqueous Extracts of the Leaf of Irvingia gabonensis (African wild mango) on the Liver of Albino Wistar Rat

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How to cite this article: Ezinwa, Chijioke Noris, Onyemelukwe, Anulika Obianuju. Histopathological and Biochemical Effects of Methanol and Aqueous Extracts of the Leaf of Irvingia gabonensis (African wild mango) on the Liver of Albino Wistar Rat. International Journal of Contemporary Pathology/Volume 10 No. 1, 2024.

Abstract

Background: The use of Irvingia gabonensis locally for different purposes from eating down to drinking its extracts and concoctions for treating various ailments has been a common practice over the ages. The liver is a very vital metabolic organ and the central site for food and drug metabolism and subsequent excretion. In that case, the understanding of the effects of these foods and drugs both orthodox and herbal is ever invaluable both to the clinicians and the patients. This research work aims to investigate the effects of its leaf extract on the liver using histological and biochemical parameters.

Methods: The experimental rats (n=20) used were divided into five (5) groups of four (4) each. Group one served as control and received only water and standard pellets. Groups two and three were administered with the methanolic extract of 150 mg/ kg and 300 mg/kg respectively. Groups four and five were administered with the aqueous extract of 250 mg/kg and 500 mg/kg respectively. The liver samples were harvested, fixed and processed in an automated tissue processor. The blood sample obtained through retro-orbital puncture was made to clot. The serum was then used to analyze the ALT, AST, ALP and Bilirubin content of the liver. Results were obtained and analyzed statistically, showing a significant ($p < 0.05$) increase in the AST and ALP levels in groups three (3) and five (5). The Serum bilirubin and ALT levels showed insignificant ($p > 0.05$) increases in all the groups. The photomicrograph indicates mild fibrosis and sinusoidal dilations and cellular infiltrations in groups three and five with no visual changes in the rest of the groups. **Conclusion:** The findings suggest that at high doses of both methanolic and aqueous extracts (300 and 500mg/kg respectively), the leaf extract is mildly toxic to the liver.

Keywords: Irvingia gabonensis, biochemical parameters, liver, albino rats, tissue processor.

Introduction

Background

Irvingia gabonensis belongs to the family Irvingiaceae and genus Irvingia, commonly called dika nut, African wild mango, bush mango, and Ugili amongst other names[1]. It is well spread in tropical African countries including Nigeria and most West African countries. The inedible fruit pulp has a turpentine flavour and is harsh and peppery. Known as "ogbono" in Igbo tradition, Nigeria, where the seed is utilised as a food thickening agent, and also serves primarily as a source of food for them

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[2]. Also, the seeds have been shown to be rich in oil (54-67%) calculated on dry kernel. This is known as "dika" fat, which has become evaluated and used now as a lubricant [2].

Generally, there is an increasing interest in using supplements containing *Irvingia gabonensis* for weight loss, lowering cholesterol levels, and improving the control of diabetes [4,5]. However, there is limited scientific evidence to support these uses. On the other hand, a systematic review previously documented has shown that multiple parts including the leaves, seeds and barks of *Irvingia gabonensis* have been employed in traditional medicine and recorded a great success too[3]. The ripe fruit has been made into juice and has been documented to have anti-diarrheal, anti-diabetic, anti-ulcer, hepatoprotective, antimicrobial, and anti-inflammatory properties while the seeds have folkloric uses for weight loss and are popular as blood thinners and anti-diabetics[8]. While the review recorded all these positive effects and uses including the bark of *Irvingia gabonensis,* they only suggested that the leaf of the plant has the "potential" to enhance renal and hepatic functions, safeguarding these vital organs against the detrimental effects of toxic substances [3]

Another study on the ethnomedicinal uses, phytochemistry, and pharmacological activity of the *Irvingia species* has suggested that they have pharmacological activities such as antiprotozoal, antimicrobial, antioxidant, antidiabetic, anti-inflammatory, and hepatoprotective activities[5]. These multiple reports suggest greatly that the plant has some sort of effect on the liver but without substantiation and most importantly, with little or no pointer that it is the leaf extracts that achieves these effects on the liver.

The liver is the largest gland in the body, weighing about 1.5kg with functions ranging from secretory, excretory, metabolic, storage, and haemopoietic functions down to the inactivation of drugs and metabolites. [6]. This organ is identified as the metabolic engine room of the body where almost all the drugs, foods and water constituents are metabolized and detoxified, and as such it is often exposed to maladies resulting in several clinical syndromes or diseases [7]. On the other hand, liver disease is any condition that may cause liver inflammation or tissue damage and affects liver function[8].

Natural products that are found in vegetables, fruits, plant extracts, herbs, insects, and animals have been traditionally used for treating liver diseases [9]. Botanical medicines have been used traditionally by herbalists and indigenous healers worldwide for the prevention and treatment of liver disease and more so, clinical research in this century has confirmed the efficacy of several plants in the treatment of liver disease, while basic scientific research has uncovered the mechanisms by which some plants provide their therapeutic effects. [10]. On the other hand, some of these herbal or plant products have exerted some toxic effects on the liver and yet are being consumed daily by individuals both educated and otherwise[11].

Justification

There have been quite a few reviews and researches about the methanolic and aqueous leaf extracts of *Irvingia gabonensis* (Ugili) in relation to its prophylactic, therapeutic or toxic effects on the human liver and its associated diseases with most research focusing on the ethanolic extracts [12,13]. While this might be attributed to the toxic nature of methanol to the human body as compared to ethanol, several pieces of research have shown that due to the higher dielectric constant of methanol than that of ethanol, it can be used to extract more polar substances and thus, has been recommended as the optimal solvent to obtain high content of phytochemical constituents as well as high antioxidants from plant sources[14, 15].

Nevertheless, in recent times it has drawn a lot of attention as it is among the very few plants whose entire content from the seed to the leaf and bark has been implicated in the treatment of one medical condition to another. The oil extract of its seed has also been used as a biodiesel making it a potential source of fuel for diesel engines [16]. This subtle variation in the prevention and treatment of various disease conditions using various parts of *Irvingia gabonensis* with little or nothing on the therapeutic, prophylactic and toxic effects of its leaf extract on the human liver and its associated diseases has been a major attraction for this very research work. For example, the leaf extract has been known to be used to treat diarrhoea [17], and on the other hand, its bark has also been shown to have hypoglycemic

and antidiabetic effects [18]. Some previous studies also show that *Irvingia gabonensis* seed extracts might reduce bad cholesterol levels and increase good cholesterol levels in people who are overweight. But this research is low quality [4]. While these studies have focused on the effects of the leaves as well as other parts of the plant, on other parts of the body, its effect on the engine house of the body (the liver) is yet to be established. Consequently, it is very important that research be conducted on the effects of the methanol and aqueous leaf extracts of *Irvingia gabonensis* on the liver.

Aim

This study aims to investigate the biochemical and Histopathological effects of aqueous and methanolic leaf extracts of *Irvingia gabonensis* on the liver of Albino Wistar Rats.

Specific objectives

1. Determination of the effects of Irvinga gabonensis on the body weights of the Albino rats.
2. Estimation of liver marker enzymes; alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST) and Total Bilirubin content of the livers of the Albino rats.
3. Gross and microscopical examination of the liver tissues.

Methodology

Materials and Methods

Plant material and collection:

The fresh leaf of *Irvingia gabonensis* was collected from various sites in Awkuzu, Oyi Local Government Area of Anambra State, Nigeria and authenticated by a botanist at the herbarium section of the Department Of Plant Sciences And Biotechnology, University Of Nigeria Nsukka with the voucher number-UNH NO221

Preparation of extracts:

The fresh leaves of Irvingia gabonensis were collected and dried at room temperature for three (3) weeks in preparation for grinding. The dried leaves were then pulverized three different times using a Crestor high-speed milling machine into a fine powder. The pulverized leaves were then measured using a sensitive weighing balance to get one hundred (100) grams each for aqueous and methanol extractions.

Aqueous Extraction: One hundred (100) grams of leaf powder was soaked and homogenized in five hundred (500) millilitres of water, covered tightly and made to stay overnight in a refrigerator. The homogenate was sieved using a muslin cloth. The filtrate obtained was stored at 4°C in a refrigerator until required. The concentration of the filtrate (aqueous extract) was determined by evaporating 2ml (two millilitres) of the aqueous extract using an evaporating dish of known weight in an oven to dryness. The weight of the dried filtrate was obtained by deducting the weight of the evaporating dish and the average weight taken. The extractive value for the aqueous extracts was determined as 410mg/ml.

Methanol Extraction: One hundred (100) grams of powdered *Irvingia gabonensis* leaves were soaked and homogenized in 500ml (five hundred millilitres) of 80% methanol in an air-tight container. The homogenate was shaken intermittently and left for 24 hours. Afterwards, the extract was filtered using Whatman filter paper and the resultant filtrate was evaporated to dryness using a rotary evaporator. The extractive value for the methanol extract was then determined to get a concentration of 260mg/ml. The residue was then reconstituted with 500mls (five hundred millilitres) of distilled water and stored in a refrigerator until when needed.

Animal housing and setting:

Twenty (20) apparently healthy albino rats were obtained from the animal holding unit of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka and were stored in Panacea Diagnostic Research Laboratory under standard conditions of temperature (27 ± 2°C) with twelve hours light and dark periodicity. The rats were weighed and divided into five (5) groups of four (4) animals each. These animals were kept in clean gauze cages in groups and fed with standard pellets (Grand Cereals ® Ltd, Enugu) and water ad-libitum. The rats were then left to acclimatize for two (2) weeks.

Ethical considerations

The handling of animals in this study was done in accordance with the protocols approved by institutional guidelines on animal care and use committee and also conform to established guidelines set by the National Institute Of Health on experiments involving the use of animals. Furthermore, the Animal Welfare and Ethics Committee Department of Animal Science at the University of Nigeria, Nsukka after cross-examining the research protocols gave their approval on the 26th of July 2021 with the reference number [UNN/AREC/0721-15-200573ML].

Experimental design and study:

After acclimatization for two weeks, administration of both aqueous and methanol extracts commenced.

Group 1 served as the control and received water only.

Group 2 received 150mg/kg of the aqueous leaf extract of *Irvingia gabonensis*.

Group 3 received 300mg/kg of the aqueous leaf extract of *Irvingia gabonensis*.

Group 4 received 250mg/kg of the methanol leaf extract of *Irvingia gabonensis*.

Group 5 received 500mg/kg of the methanol leaf extract of *Irvingia gabonensis***.**

Sample collection and biochemical assay:

Upon completion of extraction and administration, blood samples were obtained by retro-orbital puncture of the medial cantus of the eye under chloroform anaesthesia using a capillary tube and then dispensed into well-labelled plain tubes. The blood samples were kept to clot for about 45 minutes and afterwards were centrifuged at 3000rpm (three thousand revolutions per minute) for 15 minutes. The sera were then separated from each sample for biochemical analysis. Serum levels of Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP) and Total Bilirubin were estimated using standard commercial reagent kits.

Determination of relative organ weight:

The rats were sacrificed under chloroform anaesthesia and the liver tissue were excised from each rat. A gross examination was carried out on the excised liver to observe the presence of lesions or any other abnormality.

The liver of each rat was blotted with filter paper and weighed on a balance. The relative organ weight (ROW) was calculated thus:

ROW = Absolute Organ Weight ÷ Body Weight of Rat on Sacrifice × 100

Tissue processing:

The excised liver tissues from the sacrificed rats were fixed in 10% forma saline before histological processing using paraffin wax embedding technique for light microscopical examination. The tissues were then taken through the process of dehydration, clearing and wax impregnation using an automated tissue processor. The tissues were then embedded in molten paraffin wax and tissue sections of five (5) micrometre were cut using the rotary microtome and were further stained according to the Haematoxylin and Eosin(H & E) staining technique as described by Baker and Silverton (2014). The sections were then examined using an Olympus Binocular Microscope with inbuilt lighting system. Photomicrographs of the sections were also obtained.

Statistical analysis:

The statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 20.0. Data obtained were expressed where appropriate as mean ± standard error of the mean (SEM). Differences between mean values were determined using one-way analysis of variance (ANOVA) followed by Tukey's post hoc tests. $P<0.05$ was considered significant.

Results

Table 1. Weights of the rats before and after the administration of extracts expressed as mean ± standard error of the mean.

Groups	Mean Weight of Rats Before	Mean Weight of Rats After
GROUP 1 (control)	119.75 ± 2.462	145.50 ± 7.632
GROUP 2	131.75 ± 1.250	154.50 ± 4.992
GROUP 3	235.75 ± 5.543*	234.75 ± 5.677*
GROUP 4	268.25 ± 3.198*	252.25 ± 4.308*
GROUP 5	229.50 ± 32.214*	202.25 ± 29.576
Significance	0.00 P<0.05	0.00 P<0.0

Table 2. Statistical grouping of the relative organ weight expressed as mean ± standard error of the mean.

GROUPS	RELATIVE ORGAN WEIGHT
GROUP 1 (control)	0.0637 ± 0.00691
GROUP 2	0.0531 ± 0.00512
GROUP 3	0.0429 ± 0.00285
GROUP 4	0.0335 ± 0.00245
GROUP 5	0.0516 ± 0.00694
SIGNIFICANCE	0.11 P>0.05

Table 3. Statistical grouping of the liver function biomarkers expressed as mean ± standard error of the mean.

GROUPS	TOTAL BILIRUBIN	AST	ALT	ALP
GROUP 1(control)	0.480 ± 0.2142	55.80 ± 1.2049	32.45 ± 1.0500	75.58 ± 1.6854
GROUP 2	0.165 ± 0.0065	48.73 ± 0.6860*	25.35 ± 0.7399*	71.68 ± 1.2665
GROUP 3	0.423 ± 0.1527	63.90 ± 0.9840*	29.13 ± 0.5391	97.95 ± 2.7777*
GROUP 4	0.323 ± 0.0805	59.13±0.0479	32.50 ± 1.1811	82.98 ± 1.0765
GROUP 5	0.208 ± 0.0450	66.75 ± 1.1558*	27.23 ± 0.8148	100.50 ± 0.294*
SIGNIFICANCE	**0.363** **P>0.05**	0.000 P<0.05	0.000 P<0.05	0.000 P<0.05

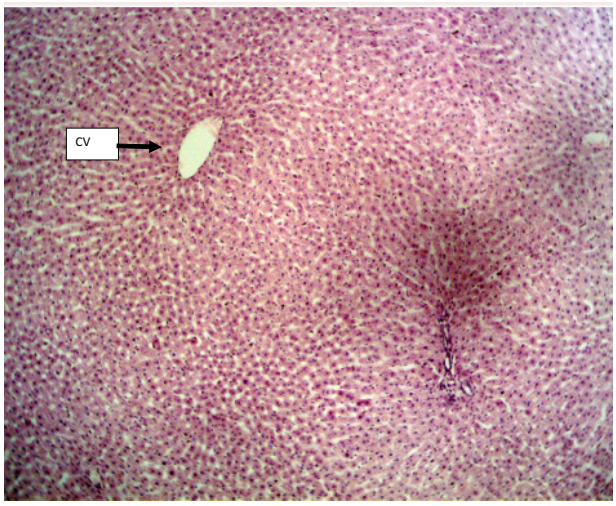

Figure 1. Histological features of the processed liver

Representative liver micrograph for the control group with normal histological features. The hepatocytes, central veins (CV) and portal triad (PT) are all normal. Stain: haematoxylin and eosin. Magnification: X 100.

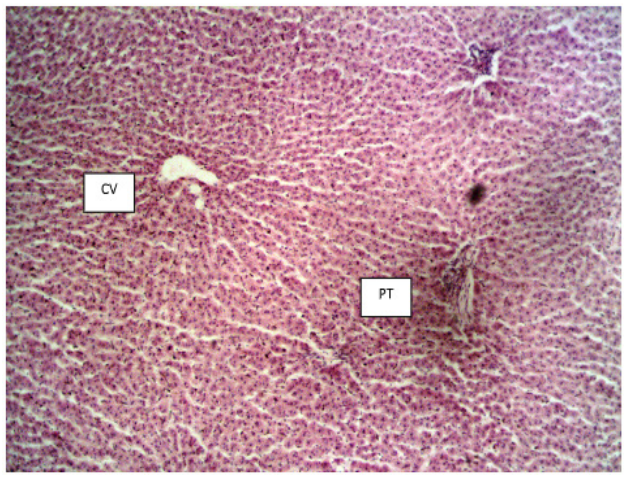

Fig 2. Histological features of the processed liver

Representative liver micrograph for group 2 with normal histological features. The hepatocytes, central veins (CV) and portal triad (PT) are all normal. Stain: haematoxylin and eosin. Magnification: X 100.

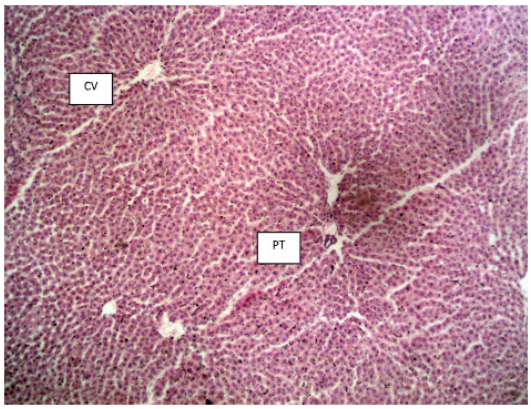

Fig 3. Histological features of the processed liver

Representative liver micrograph for group 3. The hepatocytes, central veins (CV) and portal triad (PT) are all normal; some sinusoids appear mildly dilated (arrow). Stain: haematoxylin and eosin. Magnification: X 100.

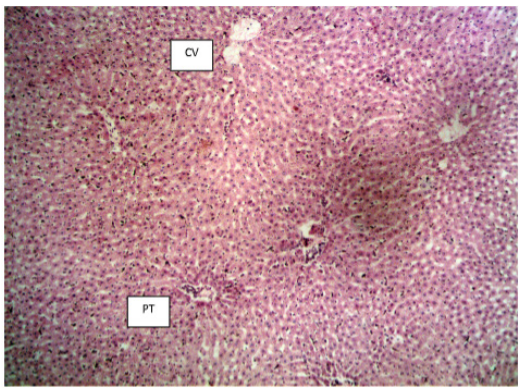

Fig 4. Histological features of the processed liver

Representative liver micrograph for group 4 with normal histological features. The hepatocytes, central veins (CV) and portal triad (PT) are all normal. Stain: haematoxylin and eosin. Magnification: X 100.

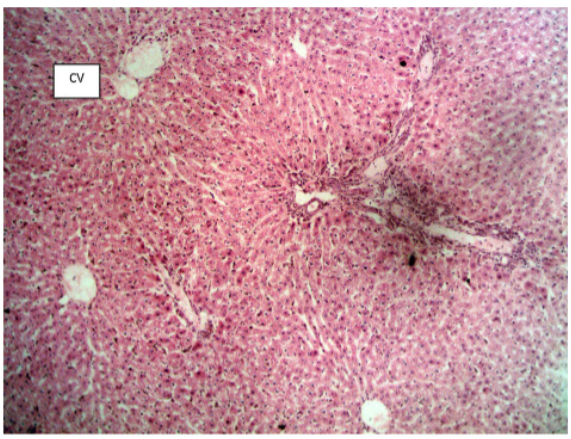

Fig. 5. Histological features of the processed liver

Representative liver micrograph for group 5. The hepatocytes and central veins (CV) appear normal; there is fibrosis and mild cellular infiltration of the portal triad pictured (arrow). Stain: haematoxylin and eosin. Magnification: X 100.

Discussion

There has been a relative increase in the use of herbal medicines in the treatment of several diseases locally, with particular interest in *Irvingia gabonensis*. This has led to massive scientific research into the therapeutic utilization of different plants. It is now generally known that most therapeutic effects of medicinal plants owe to their phytochemical constituents and as such in recent years, secondary plant metabolites also referred to as phytochemicals have been investigated extensively as sources of medicinal agents [19]. On the other hand, the incessant cases of self-medication using herbal extracts, particularly in Africa, has also led to researches to understand the toxic effects of these herbal drugs on the liver and the body generally [20].

Aspartate aminotransferase (AST) and Alanine aminotransferase (AST) are members of the transaminase family of enzymes. ALT and AST are found in large amounts in the liver and also small amounts are found in the heart, kidney and muscles [21]. When the liver is injured or inflamed as the case may be via its exposure to various forms of toxic substances, the level of ALT and AST in the blood is usually elevated[13]. Elevation of serum ALP activity indicates the presence of diseases such as liver and bone diseases. Elevated serum ALP levels may also be indicative of bile duct obstruction. Serum ALP activity may also be elevated due to primary neoplasm at sites other than liver and non-neoplastic hepatobiliary diseases, such as in xenobiotic-induced hepatoxicity [22]

The result obtained from this study showed a significant increase in ALP and AST levels at higher doses of both methanolic and aqueous extracts (300 and 500mg/kg respectively). [13] also agrees to this although the author worked on an ethanol extract. The author showed that administering the ethanolic extract alone at a high dose of 500 mg/kg body weight produced significant ($p<0.05$) higher levels

of serum AST and ALT activities. This is as opposed to the report given by [19] who suggest that the AST levels are reduced in a dose-independent manner. This is probably because of Cadmium-induced hepatotoxicity in Ewere's experimental model and it also suggests that taking the herbal extract at high doses in the absence of a hepatic disorder will result in liver toxicity.

The photomicrographs showed histological backup to this, as there were signs of mild dilation in group three (3) and mild fibrosis and cellular infiltration in group five (5). This could be due to oxidative stress posed by the extract at higher doses thereby distorting the membrane integrity of the liver tissue. On the other hand, terpenoids which have cytotoxic activities and are being used in trials as an anti-cancer agent [23,15] are found at higher concentrations in methanolic extraction than in aqueous or ethanolic extractions, this gives credence why the group five (5) rat models were affected most histologically. The bilirubin and ALT levels showed no significant increase in comparison with the control.

Conclusion

It can be deduced that higher doses of 300 and 500mg/kg of aqueous and methanolic extracts of the leaf of *Irvingia gabonensis* respectively, have a mild level of toxicity on the liver.

Recommendation

Dose-dependent study is recommended to ascertain the effect of the leaf extract at higher doses on the liver and the relative organ weight of the liver.

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