

## Icacinia Manni Impact on Metabolism in Male Rats

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DOI: [10.36348/sjm.2021.v06i06.009](https://doi.org/10.36348/sjm.2021.v06i06.009)

| Received: 23.03.2021 | Accepted: 14.05.2021 | Published: 26.06.2021

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### Abstract

The liver as a major organ of metabolism is one of the first predisposed organs to food chemicals. *Icacinia manni* tuber, the examined agent in this study has claims of direct and indirect consumption by man as it is observed to be a source of energy which contains high amount of carbohydrates among other nutritional and anti-nutritional constituents. Four (4) weeks old Male Wistar rats were used for this study. They were fed with rat chow from vital feeds and allowed free access to drinking water throughout the experimental period. *Icacinia manni* tuber was washed with water to remove sand, cut into pieces and sun dried. The dried specimen was extracted with 80% Ethanol. After two (2) weeks of drying, the tuber was reduced into powder. The powder was divided into two parts. One part was macerated in 80% ethanol for 72hrs to give the crude ethanolic extract. The other part was successively macerated for 72hrs in n-hexane and ethanol to give the corresponding gradient fraction of these solvents. The liquid filtrate was concentrated and evaporated to dryness using rotary evaporator. The median lethal dose (LD50) of the extract was estimated using albino Wistar mice by intra peritoneal (ip) route. The rats which weighed between 60 and 100gm were randomly assigned four (4) groups. Group A served as the control and was given distilled water by mouth and allowed liberal food and water throughout the experimental period which was 28 days. Group B, C and D had low, intermediate and high dose of extract. Group B received 1/10 of LD50 by feeding tube i.e.  $1/10 \times 894.43 \text{ mg/kg} = 89.44 \text{ mg/kg}$ . Group C received 2/10 of LD50 by mouth through a feeding tube i.e.  $178.88 \text{ mg/kg}$ . Group D received 3/10 of LD50 by mouth through a feeding tube i.e.  $268.32 \text{ mg/kg}$ . Stock concentration was 50mg/ml. On the twenty-eighth (28th) day, the animals were anaesthetized with chloroform. Blood samples were obtained by cardiac puncture. Serum obtained for biochemical analysis. The livers were harvested for histological analysis. The results showed significant higher levels ( $p < 0.01$ ) of Mean Total Cholesterol and High Density Lipoprotein in treated rats compared to the untreated rats. Significant lower levels ( $p < 0.01$ ) of Mean Triglycerides and Low Density Lipoprotein were observed in untreated rats compared to treated rats. The Mean Total Protein, Mean Albumin and Mean Globulin levels for treated rats were significantly lower ( $p < 0.01$ ) than that of the untreated rats. The histologic photomicrographs of liver of treated rats showed moderate area cellular abnormalities with area of vascular congestion and degeneration, cellular degeneration, vacuolization and pyknotic nuclei as compared to untreated rats. This study shows the hepatotoxicity of *Icacinia manni* tuber and its associated risk of metabolic syndrome.

**Keywords:** *Icacinia manni*, Liver, Metabolism, Lipid Profile, Serum protein.

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### 1. INTRODUCTION

None of the body systems can function in isolation. The inter-dependency property of the body systems with metabolism at the central point of energy supply divulges the danger of metabolic anomalies.

Metabolism influences the general body function and in a way affects other systems and organs. In our recent work, pregnancy outcome was observed to be influenced by lipids and serum protein synthesis and metabolism [1].

Liver as a major organ of metabolism functions in many other physiological capacities including vascular, immunological, secretory and excretory function. This portrays the danger of liver disease which is rated as the major cause of death every year [2]. The most common causes of liver disease worldwide are chronic hepatitis B and C, alcohol and non-alcoholic steatohepatitis associated with obesity and metabolic syndrome [3].

The detoxicative and metabolic property of the liver makes it the first predisposed organ to food chemicals. In this study, *Icacinia manni* tuber which is

observed to contain nutritional and anti-nutritional compounds is the examined agent.

*Icacinia manni* is a wild shrub abundantly found in most part of Africa with a unique ball-shaped underground tuber giving it its vernacular name, Earth Ball. The tuber is observed to be a source of energy as it contains high amount of carbohydrates among other nutritional constituents. The tuber is being experimented to be used as an alternative for energy source in animal feed and claims of its direct and indirect consumption by man are reported.

Apart from containing carbohydrates, proteins, lipids, etc, *Icacinia manni* tuber has been observed to contain many other anti-nutrients: cyanide, alkaloids, phytic acid, oxalic acid and tannins [4]. The anti-nutritional component of *Icacinia manni* tubers has been shown to impair metabolism [5-8].

### 1.1. Purpose of the study

This study tries to determine the metabolic fate of the body using the liver statue as the criteria in *Icacinia manni* tuber treated rats. The following parameters were examined;

1. Lipid profile
2. Serum protein
3. Liver histology

### 1.2. Significance of the Study

The liver is a major organ in the body with many vital functions including metabolism. Therefore this study will confer the health statue of the body on account of *Icacinia manni* consumption using metabolic view point.

## 2. METHODOLOGY

### 2.1. Collection, Identification of *Icacinia manni*

*Icacinia manni* has two parts - The leafy shrub on the outside and the tuber underground. The plant with tuber, leaves and stem was harvested from the bush in Uyo, Akwa Ibom state of Nigeria, and identify by the Department of Botany, University of Uyo, Nigeria. This research work made use of the tuber. The leaves and stem were discarded.

### 2.2. Preparation and Extraction of *Icacinia manni*

The tuber was washed with water to remove sand, cut into pieces and sun dried. The dried specimen was sent to department of pharmacognosy, University of Uyo, Nigeria for extraction with 80% Ethanol.

#### 2.2.1. Maceration

After two (2) weeks of drying, the tuber was reduced into powder. The powder was divided into two parts. One part was macerated in 80% ethanol for 72hrs to give the crude ethanolic extract. The other part was successively macerated for 72hrs in n-hexane and ethanol to give the corresponding gradient fraction of these solvents. The liquid filtrate was concentrated and

evaporated to dryness using rotary evaporator. The pure extract was stored in a refrigerator at 4°C pending when it will be used for the proposed study.

### 2.2.2. Preparation of Stock Concentration

The stock solution was prepared using std procedures.

### 2.3. Experimental Animals

Four (4) weeks old Male Wistar rats were used for this study. The rats were obtained from the animal house of the Department of Physiology, University of Calabar and kept in a well-ventilated experimental section of the animal house of the faculty of Pharmacy, University of Uyo, Uyo.

The animals were fed and kept for 7 days to acclimatize before the experiment began. They were kept in wooden cages and fed with rat chow from vital feeds. They were allowed free access to drinking water throughout the experimental period.

### 2.4. Determination of Median Lethal Dose (LD50)

The median lethal dose (LD50) of the extract was estimated using albino Wistar mice by intra peritoneal (ip) route using the method of Lorke (1983) [9].

### 2.5. Experimental Procedures

A total of forty (40) male rats weighing between 60 and 100gm were randomly assigned four (4) groups.

Group A served as the control and was given distilled water by mouth and allowed liberal food and water throughout the experimental period which was 28 days.

Group B, C and D had low, intermediate and high dose of extract. Group B received 1/10 of LD50 by feeding tube i.e.  $1/10 \times 894.43 \text{ mg/kg} = 89.44 \text{ mg/kg}$ .

Group C received 2/10 of LD50 by mouth through a feeding tube i.e. 178.88mg/kg.

Group D received 3/10 of LD50 by mouth through a feeding tube i.e. 268.32mg/kg.

Stock concentration was 50mg/ml. Due to small variation in body weight of the animals, the average dose per group was used for all the animals in that group.

### 2.6. Sample Collection

On the twenty-eighth (28th) day, the animals were anaesthetized with chloroform. Blood samples were obtained by cardiac puncture. Serum obtained for biochemical analysis. The livers were harvested for histological analysis.

Approval was gotten from the Local Research Ethical Committee of the University of Uyo, Uyo, Akwa Ibom State, Nigeria.

## 2.7. Analysis

### 2.7.1. Biochemical Analysis

The collected blood was allowed to clot and centrifuged at 300 revolutions per minutes for 20mins. The serum was collected with the aid of a micropipette for lipid profile and serum protein levels analysis.

The plasma concentration of Total Cholesterol, Triglycerides, High Density Lipoprotein Cholesterol and Low Density Lipoprotein Cholesterol were measured using spectrophotometric method.

Laboratory kit reagents (Randox Laboratory Ltd, UK) were used for all biochemical analysis and their absorbance were read using a UV-Vis spectrophotometer (DREL 3000 HACH); using standard protocols for serum albumin and globulin.

### 2.7.2. Histological Analysis

The livers were immediately immersed in Bouin's solution for fixation and processed until embedded in paraffin for histological analysis. Five micron thick sections were prepared using microtome (microTecLaborgerate GmbH Rudolf-Diesel-Straße, Walldorf, Germany) and stained using Hematoxylin and Eosin (H&E) method. The specimens were examined under Olympus/3H light microscope-Japan.

### 2.7.3. STATISTICAL ANALYSIS

Data obtained were analyzed using Mean, Standard Error of Mean and Analysis of Variance followed by Duncan's test which was used to determine the direction of significance.

The results were reported in the form mean  $\pm$  SEM and statistical significance was established at 0.01 level of significance with  $p < 0.01$  signifying significance. Data were analyzed using the Statistical

Package for Social Sciences (SPSS version 22.0) and GraphPad Prism 5.0.

## 3. RESULTS

### 3.1 Effect of oral administration of *Icacinia manni* extract on lipid profile of treated versus untreated male Wistar rats

#### Total cholesterol

The mean total cholesterol for the treated rats was significantly higher ( $p < 0.05$ ) than that of untreated rats.

#### Triglycerides - TG

The mean TG values obtained for untreated rats was significantly higher ( $p < 0.01$ ) than that of treated rats.

#### High Density Lipoprotein - HDL

Findings of this study showed that treated rats had a significant higher ( $p < 0.01$ ) HDL than the untreated rats.

#### Low Density Lipoprotein - LDL

The LDL for the treated rats was significantly lower ( $p < 0.05$ ) than that of untreated rats. See Figure 1.

### 3.2 Effect of oral administration of *Icacinia manni* extract on serum proteins of untreated versus treated male Wistar rats Total protein

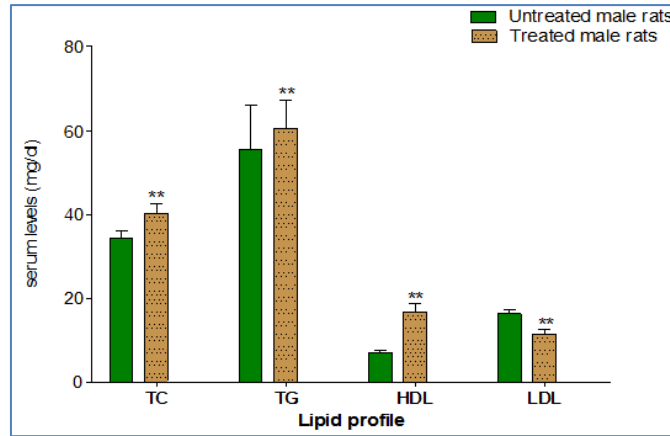
The mean total protein for treated rats was significantly lower ( $p < 0.01$ ) than that of the untreated rats.

#### Albumin

The mean albumin for untreated rats was significantly higher ( $p < 0.01$ ) than that of the treated rats.

#### Globulin

The mean globulin for the untreated rats was significantly higher ( $p < 0.01$ ) than that of the treated rats. See Figure 2.



**Fig-1: Effect of oral administration of Icacinia manni extract on lipid profile of untreated versus treated male Wistar rats. \*\* = significantly different from untreated male (p>0.01)**

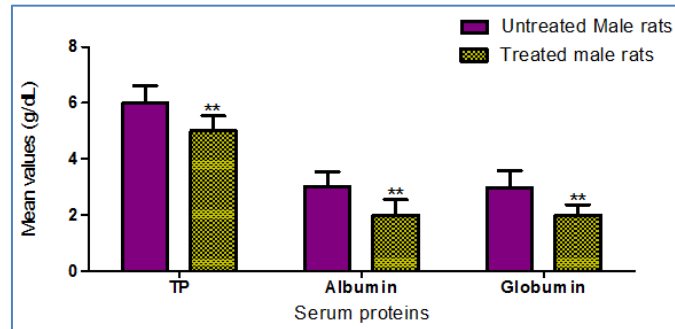
**Keys:**

**TC-** Total Cholesterol

**TG-** Triglycerides

**HDL-** High Density Lipoprotein Cholesterol

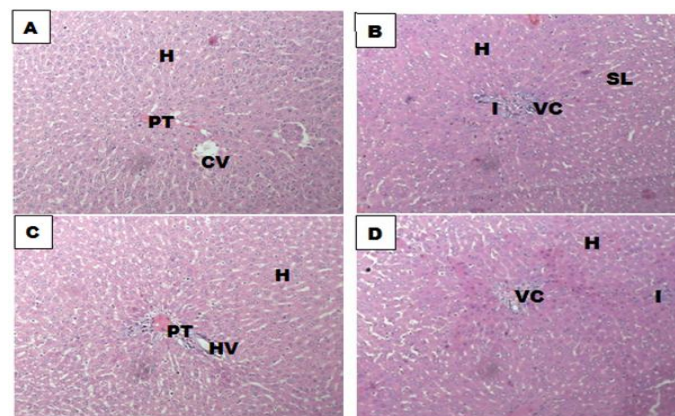
**LDL-** Low Density Lipoprotein Cholesterol



**Fig-2: Effect of oral administration of Icacinia manni extract on serum proteins of untreated Vs treated male Wistar rats. \*\* = significantly different from untreated male (p<0.01).**

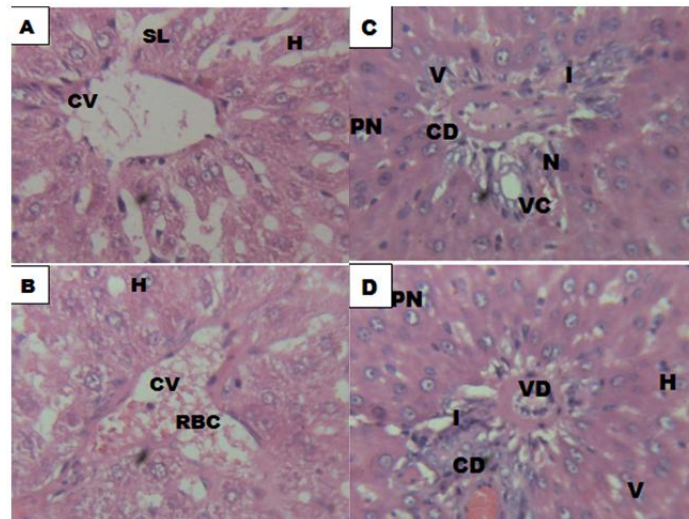
**Keys:**

**TP-** Total Protein



**Fig-3: Histologic photomicrographs of liver of A, C- control group; B, D-treated group of male rats at magnification (X100) stained with H & E technique**

**H** =Hepatocytes; **CV**= Central vein  
**I** = Inflammation; **HV**= Hepatic vein  
**VC** = Vascular congestion  
**SL** = Sinusoidal layer  
**V**= vacuolation  
**PT**= Portal triad



**Fig-4: Histologic photomicrographs of liver of A, B- control group; C, D-treated group of male rats at magnification (X400) stained with H & E technique**

H =Hepatocytes; RBC= Red blood cells  
 I = Inflammation; CV=Central vein  
 VC = Vascular congestion; SL = Sinusoidal layer  
 V= vacuolization; PN = Pyknotic  
 CD = Cellular degeneration; N = necrosis

Liver of control group rats: A,C (X100) of Figure 3; A,B (X400) of Figure 4: Liver show area of normal cellular pattern of central vein, portal triad, containing red blood cells, biliary epithelium, bile duct, and hepatocytes radiating from the sinusoidal layer all within normal limit.

Liver of Treated group rats: B, D (X100) of Figure 3; C, D (X400) of Figure 4: Liver show moderate area cellular abnormalities with area of vascular congestion and degeneration, cellular degeneration, vacuolization and pyknotic nuclei as compared to untreated group.

#### 4. DISCUSSION

The liver plays an important role in metabolism which includes synthesis, catabolism and circulation. Therefore, impairment in the liver would as well result in metabolic disorder and may also affect other body functions.

An abnormal lipid profile is usually associated with severe liver dysfunction. In this current study, *Icacinia manni* tuber causes dyslipidemia. High levels of Total Cholesterol, Total Glycerides and High Density Lipoprotein are recorded with decrease Low density Lipoproteins (Figure 1).

Though, it is believed that the level of plasma lipids and lipoproteins tends to decrease with the severity of liver disease [10, 11]. But in this study, the reverse was the case. However, the reason for the discrepancy of the results could be due to the different etiology of liver injury.

Hypercholesterolemia has been shown to be associated with other metabolic disorders and several studies have implied that cholesterol metabolism is affected by the metabolism of other nutrients [12-14]. Elevated Cholesterol level in this study is possibly as a result of metabolic dysfunction in the liver, making it unable to break down Cholesterol.

High level of Tryglycerides as shown in this study is implicated in metabolic syndrome as well as in cardiovascular disease [15]. There are associated risk factor in hypertryglyceridemia and include obesity, metabolic syndrome, proinflammatory and prothrombotic biomarkers, type 2 diabetes mellitus and increase risk of acute pancreatitis. Evidences of increase fat accumulation in the liver as a result of hypertryglyceridemia have also been reported.

Elevated High Density Lipoprotein Cholesterol and the decrease Low Density Lipoprotein Cholesterol observed in the study may be a compensatory measure to get rid of the excess Cholesterol in the arteries. This which will prevent buildup of plaque in the arteries may be an indication that *Icacinia manni* tuber may cause less risk of Coronary heart disease.

*Icacinia manni* tuber is observed to contain high carbohydrates and carbohydrates from starchy foods are associated with hyperlipidemia. Carbohydrate intake from starchy foods contributes more strongly to metabolic disorders [16]. Hyperlipidemia has been shown to be the major effects of alcohol consumption on lipid metabolism [17]. However, this could suggest high alcohol content in the Carbohydrates in *Icacinia manni* tuber.

The histologic photomicrographs of the liver (Figure 3 and 4) of the male treated rats show moderate area cellular abnormalities with area of vascular congestion and degeneration, cellular degeneration, vacuolization and pyknotic nuclei as compared to male untreated group. This indicates the hepatotoxic nature of this plant tuber.

Albumin and Globulin are synthesized in the liver. Decrease levels of serum protein are recorded in this study (Figure 2). Protein synthesis is influenced by many factors which include iron and amino acid availability, insulin, growth hormones, enzymes, cellular function etc. In this study, the decrease levels of serum protein could be attributed to the cellular defect in hepatocytes as observed in the liver histology.

## CONCLUSION

This study shows that *Icacinia manni* tuber consumption is a risk factor of metabolic syndrome due to its hepatotoxicity.

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