Phytochemical screening , Anti-obesity and Hepatoprotective activities of ethanol leaf extract of *Jatropha tanjorensis* in Wistar rats.

Abstract

Aim: The use medicinal plants has been in existence for decades. The practice started in Africa before the discovery of orthodox medicines. The aim of this research is to evaluate the phytochemical constituents, anti-obesity and hepatoprotective activities of *Jatropha tanjorensis*

Methods: Thirty-five (35) albino Wistar rats were divided into five (5) groups of seven(7) rats each and treated thus: Group A served as the control group, group B administered dose(100mg/kg.) and group C administered dose (150mg/kg.) of extract respectively, group D administered dose (200mg/kg.) and group E were administered dose (250mg/kg.) of *Jatropha tanjorensis* extract respectively. The phytochemical constituents, anti-obesity and hepatoprotective activities of *Jatropha tanjorensis* were determined using standard procedures.

Results: The qualitative phytochemical screening of *Jatropha tanjorensis* revealed that it contains biochemical principles such as alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones and saponins. The result revealed a significant ($P<0.05$) decrease in cholesterol in all the groups except group C compared with control. There was a significant ($P<0.05$) increase in the HDL concentration levels of group A compare to the other groups. Administrations of the extract resulted in a significant ($p<0.05$) decrease in ALP levels in treated group D (8.70±6.54) compared with the normal control (15.00±8.00). The ALT activity was found to be much lower in the treatment group E (5.33±1.33) but there was a significant decrease in other groups. The AST activity was found to have a mixed difference in levels whereby treatments group B (14.00±3.61) and E (14.00±3.61) had a slight significant decrease whilst treatment groups C (24.33±3.53) and D (36.67±17.34)

Conclusion: The study suggests that the plant extracts possess hepatoprotective effects and lipid lowering potentials which may be employed in the management of metabolic disorders such as obesity and cardiovascular diseases and also the phytochemicals may be used as drug precursors, templates for synthetic modification, and pharmacological probes.

Key words: lipid profile, cardiovascular diseases, obesity, Cholesterol, liver enzymes and plant extract.

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Introduction

Immense benefits have been derived by man from using medicinal herbs in disease management because they are relatively safer, more affordable and sometimes offer better therapeutic value than synthetic drugs. The increasing discovery of more medicinal plants has demanded for increased scientific scrutiny of their bioactivity so as to provide data that will help physician and patients make wise decision before using them. African traditional medicine is the oldest, and perhaps the most assorted, of all therapeutic systems. Africa is considered to be the cradle of mankind with a rich biological and cultural diversity marked by regional differences in healing practices [1]. New species of plants with medicinal properties are constantly being brought to light. Such plants have limited data and research that confirm their potential. One of such plants is *Jatropha tanjorensis*. The genus Jatropha that belongs to tribe Joannesieae in the Euphorbiaceae family contains approximately 170 known species. The name Jatropha is derived from the Greek word ‘‘jatros’’ meaning ‘doctor’ and ‘‘trophe’’ meaning ‘food’, which indicates its medicinal uses [2].*Jatropha tanjorensis* Ellis & Saroja is a common weed of field crops. The leaf is a commonly consumed vegetable in many parts of Southern Nigeria. It is commonly called ‘hospital too far’, catholic vegetable, ‘Iyana-Ipaja’ or ‘lapalapa’. It is also popular as a natural remedy against diabetes in this region. *Jatropha tanjorensis* (L) (Fam – Euphorbiaceae), which has been consumed as leafy vegetable and as medicinal plant in Nigeria has shown hematological, antimalarial, antimicrobial, hypoglyceamic, hypolipidemic and antihypertensive activities. This review focuses on valuable knowledge of traditional uses, phytochemistry, and pharmacological activities of Jatopha tanjorensis against some tropical diseases, in order to highlight its therapeutic potentials with a view to integrate it into conventional medical use. Several classes of phytochemical compounds such as the polyphenols, saponins, tannins and

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alkaloids have been associated with the plant. It has also been shown to possess a wide spectrum of biological activities such as antiplasmodial (antimalarial), antimicrobial, antiparasitic, antioxidant, anti-diabetic, antihypertensive, antihyperlipidemic and as remedy for anaemia. [3].

Methodology

Sample Collection And Extraction of ethanol leaf extract of *Jatropha tanjorensis*

Fresh leaves of *Jatropha tanjorensis* were harvested from local garden in Abuja, Nigeria. The plants specimens were identified and authenticated in the Department of Biology, Veritas University, Abuja. The leaves were thoroughly washed, then air-dried at room temperature. 100g of homogenize leaves of *Jatropha tanjorensis* was macerated with 100ml of ethanol and allowed to stand for 48hours. Each preparation was shaken every 30 minutes for 6 hours and allowed to stand for 48 hours. Each preparation was filtered with No 1 wattman filter paper. They were placed separately in a beaker and put into a water bath to be evaporated to dryness at37°C for 48 hours to get the dried extract.

Experimental Animals

Twenty-five (35) adults male Wistar rats used for the research were purchased from Kaduna state Nigeria. The animals were acclimatized for one week. The animals were maintained under laboratory conditions of humidity, temperature (23 to 25°C) and 12 hours light-dark cycle in the Animal House of Department of Biochemistry, Veritas University, Abuja and allowed free access to standard grower's mash and water ad libitum.

Experimental Design

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Chart 1 : List of dosage of extract used for the study

Groups	Dosage of extract administered	
A	Control group	
B	250mg/kg of *Jatropha tanjorensis extract*	
C	350mg/kg of *Jatropha tanjorensis extract*	
D	450mg/kg of *Jatropha tanjorensis extract*	
E	550mg/kg of *Jatropha tanjorensis extract*	

At the end of the administration, the Wistar rats were weighted using weighing balance, euthanized under chloroform. The abdominal region was opened long the linear Alba, dissected using surgical blade to expose the organs. Blood sample was collected through cardiac puncture using a sterile needle. A syringe was used to collect the blood and transferred into a properlylabeled plain sample bottles. It was centrifuged at 3000rpm for 10 minutes. A sterile Pasteur pipette was used to transfer the serum from the clotted blood into a serum container.

Screening For Qualitative Phytochemical Properties

Test For Saponins

10ml of distilled water was added to 2m1 each of the extracts in a test tube. The mixture was shaking vigorously. It was examined for persistent frothing even after heating which indicates the presence of saponins .

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Test For Anthroquinone

To about 2m1 each of the extracts, 5m1 of 10% ammonia was added and mixed by shaking vigorously. It was observed for a color change which is an indication of a positive test (the color change is from its original color to another color). No color change is an indication of a negative test

Test For Phenol

10ml of each of the extracts was mixed with 8m1 of distilled water in a test tube. 6m1 of ferric chloride solution was added to the mixture. A color change to light brown was checked for, which is an indication of a positive test[1] .

Test For Tannins

To 1ml each of the extracts 1% ferric chloride was added. It was checked for a color change which indicates positive test

Test For Phylobatanning

To 2m1 each of the extracts, 1% aqueous hydrochloric acid was added and boiled. It was observed for the presence of white precipitate which indicates positive test

Test For Alkaloids

Each extract of 0.5 g was dissolved in 3 drops of Dragendoffs reagent. An orange precipitate indicates the presence of Alkaloid .

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Test For Flavonoids

Each extract of 0.2 g was dissolved in 2 ml of sodium hydroxide solution. The occurrence of a yellow solution which disappears on addition of HCl acid indicates the presence of flavonoids (Unegbu *et al*., 2019).

Test For Glycosides

Legal's test

To a small portion of the extracts, sodium nitropruside in pyridine and sodium hydroxide was added and observed .

The anti-obesity was carried out using kits.

Statistical Analysis

Data were recorded as mean and standard error of the Mean. Statistical difference between the means was determined by one-way ANOVA using SPSS 16.0. Any significant difference between means was assessed by and $P<0.05$ was accepted as the significant level.

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Results

Phytochemical composition of *Jatropha tanjorensis*.

Phytochemical Components	(leaf)
Flavonoids	+
Saponins	-
Anthraquinone	-
Phenol	+
Phylobataning	-
Alkaloids	-
Tannins	-
Glycosides	+

+ = positive

- = negative

Table 1 shows the phytochemical constituents of *Jatropha tanjorensis* (Hospital too far). The ethanol extracts of the leaf contain flavonoids, phenols and glycosides with absence of alkaloids, saponins, anthraquinones, phylobataning and tannins.

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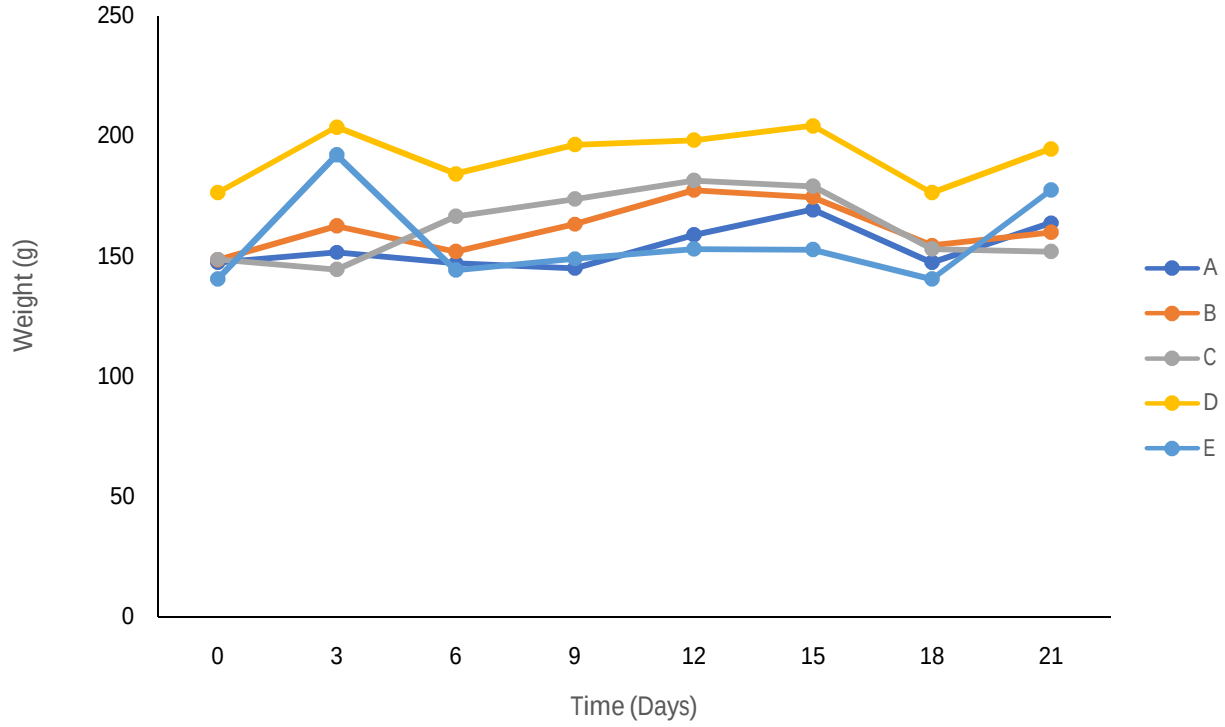

fig.1 : Effect of Jatropha leaves Extract on the Weight of wistar rats

Fig.2 : Effect of ethanol *Jatropha tanjorensis* leaf extract on some anti-obesity parameters in Wistar Rats

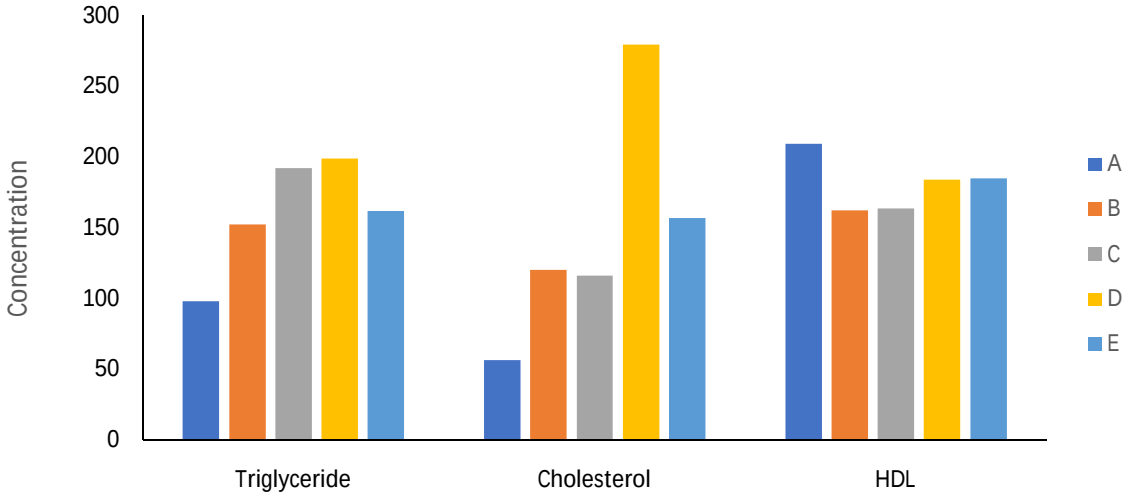

Table 2: Effect of Ethanol Leaves Extract of *Jatropha tanjorensis* on Liver Function of Wistar Rats

GROUPS	Concentration mg/kg of body weight	AST (µ/l)	ALT (µ/l)	ALP (µ/l)
A	Control	15.00±8.01[a]	22.67±14.84	15.00±8.00
B	250mg/kg	14.00±3.61[a]	8.33±4.33	25.18±1.65
C	350mg/kg	24.33±3.53[a]	11.00±3.79	25.36±12.51
D	450mg/kg	36.67±17.34[a]	17.00±11.06	8.70±6.54
E	550mg/kg	14.00±3.61[a]	5.33±1.33	41.55±7.68

Values are represented as Mean±SEM of triplicate determinations. Values with different alphabetic superscript are significantly different along the column at $p<0.05$

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Discussion

The effect of Jatropha leaves extract on the weight of rats at an interval of three days for twenty-one days is shown as a graph below in figure 1. The rats in group D showed higher weight (ranging from 176.43±10.51 g at day 0 to 204.29±15.71 g at day 15) throughout the treatment period than those in the other groups. A progressive increase in weight was observed from day 6 to 12 for rats in groups B, C, D and E while group A progressively increased from the 9th day through day 15 of treatment. At day 18, there was a decline in the of weight rats in all the groups with subsequent increase on the 21st day except in group C (152.00±4.99 g) that reduced. Rats in groups A, B and C did not show significant ($p<0.05$) difference in weight through all treatment time. Moreso, the weight of rat in group D was significantly ($p<0.05$) higher than was observed in group A through all the experimental time.

Lipids are group of naturally occurring molecules that include fats, waxes, sterols, fat-soluble vitamins, monoglycerides, diglycerides, triglycerides, phospholipids and others. The main biological functions of lipids include storing energy, signaling and acting as structural component of cell membranes. Lipids may be broadly defined as hydrophobic or amphiphilic small molecules. Although humans and other mammals use various biosynthetic pathways to both break down and synthesize lipids, some essential lipids cannot be made this way and must be obtained from the diet. In order to ensure that the body lipid concentration is normal, lipid profile test is done[4]. Many studies of either gender have desmonstrated that the risk of atherosclerosis is inversely related to blood levels of high density lipoproteins [5].

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The amount of High-density lipoprotein, Low-density lipoprotein, and Triglycerides in the body is measured in a full Lipid Profile test. Cardiovascular disease, primarily owing to atherosclerosis, is a major consequence of obesity and a leading cause of death worldwide (hardening of the arteries) [6].

In recent years, many people have been unaware of the benefits of knowing one's profile. Lipid profile is a panel of blood tests that serves as an initial screening tool for abnormalities in lipids, such as cholesterol and triglycerides. There are two common concerns people have about lipids in their diet. One is their high caloric level which may result in undesirable weight gain. The other is their association with high cholesterol level which is a risk factor for cardiovascular diseases. The effect of ethanol extracts of *Jatropha tanjorensis* on total cholesterol, triglycerides, high density lipoprotein cholesterol, low density lipoprotein cholesterol, and very low-density lipoprotein cholesterol of Wistar rats were investigated. The non-toxicity of the ethanol extracts of *Jatropha tanjorensis* leaves was observed up to 1800 mg/kg-1 b.wt (highest dose), suggesting the safety of the extracts for human and animal consumption and complements. However, non-toxicity was observed in ethanol extracts of Jatropha tanjorensis leaves up to 2900 mg/kg-1b.wt.

Cholesterol is the principal sterol synthesized by all animals and occurs mainly in the cell membrane due to its amphipathic nature. Its synthesizes begins with the mevalonate or HMG-CoA reductase pathway, the target of statin drugs, which encompasses the first 18 steps, then followed by 19 additional steps to convert the resulting lanosterol into cholesterol via either of two pathways, the Bloch Pathway, or the Kandutsch-Russell Pathway. It is reportedly a major cause of cardiovascular derangements such as atherosclerosis, myocardial infarction and coronary heart diseases. In this study, the plant extracts produced a decreased in serum cholesterol which might be due to a reduce absorption from the intestine by binding with bile acid within the intestine and increasing bile acid secretion, or due to the presence of saponins, a phytochemical which forms insoluble complexes with cholesterol or their bile salt precursor, thus making them unavailable for absorption. Therefore, it implies that the plant extracts possess anticholesterolaemic activities[7].

Triglyceride is an ester derived from glycerol and three fatty acids and the most common type of lipid in the body. Triglycerides are the main constituents of body fat in humans and other

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vertebrates, as well as vegetable fat. They are also present in the blood to enable the bidirectional transference of adipose fat and blood glucose from the liver and are a major component of human skin oils. It is not cholesterol but it is measured because when it is high and high-density lipoprotein cholesterol (HDL-c) is low, it may result in atherosclerosis and coronary heart diseases. Hypertriglyceridemia is a high level of triglyceride in the blood and could results in cardiovascular disease. In this study, it was observed in all the test groups that the plant extracts elevate triglyceride levels [8].

Low density lipoprotein cholesterol (LDL-c) transports cholesterol form the liver to the exact site where it is going to be utilized. If there is excess of LDL-cholesterol, it may initiate the process of atherosclerosis. It transports about 60-70 % of total cholesterol. Therefore, an increase in TC level consequently increase LDL-c. The plant extracts administered at high doses appeared to have a decreased in serum LDL-c level, hence a non-predisposition to atherosclerosis and other cardiovascular related diseases. Atherosclerosis narrows the area where blood flows through the vessels. This reduces the supplied with the blood, and it is perfect place for clot formation. If there is too much of LDL-cholesterol, it can lead to many other illnesses such as angina, coronary heart diseases, heart attacks, stroke and hypercholesterolemia

High density lipoprotein cholesterol (HDL-c) is an anti-atherogenic lipoprotein which transports cholesterol from peripheral tissues back to the liver where it is broken down to bile acids, as revealed in the group that was administered with high dose of *Jatropha tanjorensis* only. The inhibition of HMG-CoA reductase (a microsomal enzyme which catalyze the rate of limiting step in cholesterol synthesis pathway), reduces LDL-c and concurrently increase HDL-c. Increased level of HDL-cholesterol observed is associated with healthy heart thereby reduce risk for cardiovascular diseases development and related complications such as stroke, myocardial infarction and death. Also, this could possibly be due to increasing activity of lecithin-cholesterol acyl transferase (LCAT), an enzyme responsible for incorporating free cholesterol into HDL-c, thereby promoting reverse cholesterol transport and competitively inhibiting the uptake of LDL-c by endothelial cells and preventing the generation of oxidized LDL-c. Previous studies revealed that one out of three deaths would be due to cardiovascular disease and the prevailing factors remain elevated levels of serum total cholesterol (TC), low density lipoprotein cholesterol (LDL-c), triglyceride (TG) and decreased level of high density lipoprotein cholesterol (HDL-c).

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These Prevailing factors predisposing to cardiovascular disease was not observed in the study. The effect was dose dependent with respect to *Jatropha tanjorensis*.

Hypolipidemic activities: The leaf extract of Jatropha tanjorensis has been shown to cause significant reduction in serum total lipids, total cholesterol and LDL cholesterol with no significant difference in the levels of serum triglycerides and HDL cholesterol between rats in the test groups and the control. This observation of significant reduction in serum total lipids, total cholesterol and LDL cholesterol by the extract, implies that it can be used to prevent cardiovascular complications arising from hyperlipidemia. This might explain the traditional use of the leaf extract as a natural remedy against heart diseases in the West Africa sub- region. High levels of LDL cholesterol promote health problems and cardiovascular disease, and are often called “bad cholesterol” as opposed to HDL particles, which are referred to as “good cholesterol” or “healthy cholesterol”. It was reported that those with higher levels of HDL cholesterol seem to have fewer problems with cardiovascular diseases, while those with low HDL cholesterol levels have increased rates of heart disease. The results of these studies suggest that the medicinal properties attributed to *Jatropha tanjorensis* as a useful herb in the treatment of heart diseases could be based on effects of phytoconstituents present in the plant on serum lipid profile in albino rats.

Conclusion: The study suggests that the plant extracts possess hepatoprotective effects and lipid lowering potentials which may be employed in the management of metabolic disorders such as obesity and cardiovascular diseases and also the phytochemicals may be used as drug precursors, templates for synthetic modification, and pharmacological probes.

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References

[1] Mahomoodally MF. Traditional Medicines in Africa: An Appraisal of Ten Potent African Medicinal Plants.2013

[2] Sabandar CW, Ahmat N, Jaafar FM, Sahidin I. Medicinal property, phytochemistry and pharmacology of several *Jatropha* species (Euphorbiaceae): A review, *Phytochemistry*. 2013;**85**: 7–29.

[3] Idu M, Igbafe, G. and Erhabor, J. Anti-anaemic activity of *Jatropha tanjorensis* Ellis & Saroja in Rabbits, *Journal of Medicinal Plants Studies*. 2014;**2**(1): 64-72).

[5] Dennis Amaechi, Udosen EO, Ekam V S, and Ekpe IP. (2018) . Effects of Methanolic Root Extract of Holarrhena floribunda on the Lipid Profile and Sex Hormones in Wistar Rats. Asian Journal of Research in Biochemistry, 3(3): 1-7

[6] Ekpe, IP, Dennis Amaechi, Yisa, BN, and Osarhiemen, OG..Evaluation of the Effects of Aqueous Fruit Extract of Tamarindus indica on Body Weight, Lipid Profile, Some Electrolytes and Urea of Wistar Rats. Journal of Pharmaceutical research International. 2021; 33(59A):444-451

[7] Oluwole IO, Oluwaseun TO, Bukola VA. Assessment of renal and hepatic functions in rats administered methanolic leaf extract of J. tanjorensis. Annals of Biological Research.2013; 3(2): 837-41.

[8] Davis, C.D.(2003). Low dietary copper increases fecal free radical production, fecal water alkaline phosphatase activity and cytotoxicity in healthy men. Journal of Nutrition.133(2): 522-527.

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