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Original Research Article

# Acute Toxicity Study and Serum Lipids Profile of Pet-Ether Extract of Leave, Stem Bark and Root of *Jatropha curcas* in Wister Rats

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## Abstract

This study was carried out to evaluate the toxicity and serum lipid profile of pet- ether leave, stem bark and root extract of *jatropha curcas* in male albino rats. Fourty-nine (49) adult wister rats weighing between 160-240mg/kg was purchased and 14 wister rats were used for acute toxicity study while the remaining 35 rats were randomly divided into 7 groups of 5 rats each. The normal control (group 1) received normal saline, while groups 2 to 7 administered with leave, stem bark and root extracts of *jatropha curcas* at low dosse (200 mg/kg body weight) and high doses (400 mg/kg body weight). The extracts were administered orally for seven consecutive days, while the animals were sacrificed on the 8th day; blood samples were collected, allowed to stand for fifteen minutes and then centrifuged to obtained the serum for lipid profile analysis. This result showed that the oral administration of the leave, stem bark and root extract of *Jatropha Curcas* possess hypolipidemic activity and may be useful in the management of cardiovascular disease. While acute toxicity (LD50) of the Leave, Stem Bark and Root Extract of *Jatropha Curcas* is greater than 5000mg/kg hence its declared practically non-toxic to the experimental animals.

**Keywords:** Albino rats, Blood, *jatropha curcas*, aqueous extract, acute toxicity, Lipid profile.



## INTRODUCTION

*Jatropha curcas."* The genus name Jatropha derives from the Greek word *jatr´os* (doctor) and *troph´e* (food), which implies medicinal uses [1]. This plant belongs to the Euphorbiaceae family, a drought resistant shrub or tree which is widely distributed in the wild or semi-cultivated areas in Central and South America, Africa, India and South East Asia [2]. All parts of *Jatropha* (seeds, leaves, bark, etc) have been used in traditional medicine and for veterinary purposes for a long time [3, 4]. Extensive public interest and expansion in the use of herbal medicine have led to new emphasis and drive in medical plant research. The research approaches taken recently include activities to develop herbal medicines into quality, efficacious and safe products for human consumption. *Jatropha curcas* have played major role in the treatment of various diseases, including bacterial and fungal infections. The scientific name of physic nut is "*Jatropha curcas."* The genus name Jatropha derives from the Greek word *jatr´os* (doctor) and *troph´e* (food), which implies medicinal uses [1]. This plant belongs to the Euphorbiaceae family, a drought resistant shrub or tree which is widely distributed in the wild or semi-cultivated areas in Central and South America, Africa, India and South East Asia [2, 5]. All parts of *Jatropha* (seeds, leaves, bark, etc) have been used in traditional medicine and for veterinary purposes for a long time [3, 4]. Extensive public interest and expansion in the use of herbal medicine have led to new emphasis and drive in medical plant research. The research approaches taken recently include activities to develop herbal medicines into quality, efficacious and safe products for human consumption. This can be an advantage for *J. curcas* to expand its potential as herbal medicines to cure many illnesses and diseases. The cure for these illnesses and diseases lies in the chemical compositions isolated from different parts of the plant.

Lipid profile is the collective term given to the estimation of typically, total cholesterol, high-density lipoprotein (HDL) cholesterol, Low-density lipoprotein (LDL) cholesterol and triglycerides. An extended lipid profile may include very low — density lipoprotein (VLDL). This is used to identify hyperlipidemia (various disturbances of cholesterol and triglyceride levels), many forms of which are recognized risk factors for cardiovascular diseases and sometimes pancreatitis [6].

Acute toxicity tests measure the adverse effects that occur within fourteen days after administration of a single dose of a test substance. This is performed principally on rodents (mice or rats) and is usually done early in the development of a new chemical or product to provide information on its potential toxicity. Traditionally, acute oral toxicity testing has focused on the immediate determination of the dose that kills half of the animals (i.e., the median lethal dose or $LD_{50}$), the timing of lethality following acute chemical exposure, as well as observing the onset, nature, severity and reversibility of toxicity. But in recent times, after the immediate observation is done, toxicological parameters (biochemical, haematological and histopathological) to assess potential adverse effects are carefully chosen and measured. The fixed single dose, at which signs of toxicity but no deaths are detected is used to classify the test compounds according to their toxic potential [7]. This paper is aimed to evaluate the toxicity and serum lipids profile of leave, stem bark and roots of *jatrpha coccus* aqeuous extracts.

## MATERIALS AND METHODS

### Plant Sample Collection and Preparation of Extract

The leave, stem bark and root were collected at Dawakin Tofa Local Government area, Kano State.Nigeria and were identified by a Botanist (Anas Abba) in Pharmacognocy Department, Bayero University Kano, Nigeria and was given Herbarium accession No: BUKHAN 223. The leaves, stem bark and roots were clean with water and dried at room temperature, after which it was pulverized to coarse powder using mechanical grinder. *Jatropha curcas* methanolic leaves extract was prepared according to Mittal *et al*;[8] and Fernando *et al;* [9] method. One thousand grams (1000g) of the powder *Jatropha curcas* leave, stembark and roots were separately mixed and soaked in 2000$cm^3$ pet-ether in a 2 litre conical flask, the content of the flask was mixed vigorously. The mixture was then shaken and top covered with aluminium foil and kept for 48 hours. The extracts were obtained by filtration using whatman No1 filter paper and concentrated using vacuum evaporator at 60$^oC$ in water bath (OSL200 water bath and shaker Grand instrument, Cambridge). The concentration and total yield of the pet-ether leave root and stem bark extract of *Jatropha curcas was* determined and stored in air tied container for further analysis.

### Experimental animals

Fourty-nine (49) adult wister rats weighing between 160-240mg/kg was obtained from the animal house of Physiology Department, Bayero University Kano and were kept in cages at room temperature for two (2) weeks to acclimatize and were allowed access to food and water *ad libitum.*

### Acute toxicity study (ld $_{50(oral, rats)}$) determination

The $LD_{50(Oral,rats)}$ was determined by Lorke,[10] in two phases, in the first phase the rats were divided into three groups of three rats each and administered with 10 , 100, and 1000mg/kg of the pet-ether extracts of *jatropha curcas* orally. The rats were observed for mortality and general behaviour. In the second phase, the rats were grouped into five group of one rat each, they were administered with pet-ether extracts of *Jatropha curcas* at varying dose of 1250, 2000, 2750, 3750 and 5000 mg/kg. The rats were observed for 24hours for mortality and other signs of toxicity.

### SERUM LIPID PRIFILE DETERMINATION

***Experimental design***

A total of thirty-five (35) wister rats were used and divided into seven group of five (rats) each

Group 1- The normal control (they were administered normal saline only).

Group 2- Administered 200 mg/kg body weight of pet-ether leaf extract.

Group 3- Administered 400 mg/kg body weight of pet-ether leaf extract.

Group 4- Administered 200 mg/kg body weight of pet-ether stem bark extract.

Group 5- Administered 400 mg/kg body weight of pet-ether stem bark extract.

Group 6- Administered 200 mg/kg body weight of pet-ether root extract.

Group 7- Administered 400 mg/kg body weight of pet-ether root extract.

The pet-ether extracts were administered to the experimental animals orally for two weeks, 14 consecutive days.

### Blood Sample Collection and Serum Preparation

All the animals were sacrificed using light chloroform and 5ml of blood sample was collected into specimen bottle and allowed to clot and separated by centrifugation at 3000g for 10 minutes using centrifuge hitachi 32. The supernatant obtained were used for the determination of lipid profile such as total cholesterol level, TAG, VLDL, LDL and HDL.

### Serum lipid profile analysis

Measurement of serum or plasma total cholesterol according to Roeschlau *et al*.,[11] Measurement of Serum triglycerides according to McGowon *et al.* [12] Measurement of Serum or Plasma HDL — Cholesterol according to Lopes — Virella *et al.* [13]. Measurement of Scram or Plasma VLDL and LDL— Cholesterol according to Friedewald *et al.* [14].

## STATISTICAL ANALYSIS

The data was statistically analyzed at P-value ($p<0.05$) significantly accepted and a comparison between the groups was performed using one-way analysis of variance (ANOVA) by Graphpad instat3

software (2000) version 3.05 by Graphpad Inc. The data are given as the mean ± standard deviation.

## RESULT AND DISCUSSION

**Acute Toxicity Study Pet Ether Extract of Leave, Stem Bark and Root of *Jatropha Curcas***

The acute toxicity study of the pet ether extract of leave, stem bark and root of *jatropha curcas* were conducted in two phases. In the first phase (Tables 1.1a) no mortality, toxic symptom and change of behavior was observed in all the groups orally administered with 10, 100 and 1000mg/kg of all the three extracts. In the second phase (Tables 1.1b) no mortality, toxic symptom and change of behavior, no any symptoms of weakness and decrease in moving activities were observed in the groups administered with doses up to 5000mg/kg body weight for group administered with leave, stem bark and root of *jatropha curcas*. The absence of death at all doses up to 5000mg/kg showed that the $LD_{50}$ of the leave, stem bark and root of *jatropha curcas* may be greater than 5000mg/kg body weight.

The lack of death 24hours after oral administration of up to 5000mg/kg body weight of leaf, stem bark and root of *Jatropha Curcas* pet-ether extracts suggests that the plant parts are practically non-toxic [15] and they are therefore safe up to 5000mg/kg for oral use in ethno-therapeutic management of disease. This higher safety profile obtained may have been responsible for wide spread used of *Jatropha Curcas* pet-ether extracts can be used in different ethno-therapeutic activities. This is similar to the report of Yakubu *et al*[16] on the acute lethal effect on ethanol root extract of *Erminalia Macroptera Guill. and Perr. (Combretaceae)* induced intraperitoneally showed that no animal died after 24 hours. The major sign of toxicity noticed was general weakness and loss of appetite. The sign became increasingly pronounced as the dose increased towards 5000 mg/kg. The signs were not noticed in 10 and 100 mg/kg doses respectively. The $LD_{50}$ being greater than 5000mg/kg body weight is thought to be safe as suggested by Lorke [10]. Again, the absence of death among rats in all the dose groups throughout the two weeks of the experiment seems to support this claim.

Lorke [10] reported that $LD_{50}$ values could be measured exactly and reproducively, the knowledge of its precise numerical value would barely be practically important, because an extrapolation from the experimental animals to man may not be possible. However, it serves as first guides to the safety or toxic potential of a substance whose toxicity profile is not known [17].

**Table-1.1a: First Phase $LD_{50}$ (Oral, rat) of Pet Ether Extract of Leave, Stem Bark and Root of *Jatropha Curcas***

| Doses in (mg/kg) | Leave extract | | Stem bark extract | | Root extract | |
|---|---|---|---|---|---|---|
| | No: of Rat | Mortality | No: of Rat | Mortality | No: of Rat | Mortality |
| 10 | 3 | 0/3 | 3 | 0/3 | 3 | 0/3 |
| 100 | 3 | 0/3 | 3 | 0/3 | 3 | 0/3 |
| 1000 | 3 | 0/3 | 3 | 0/3 | 3 | 0/3 |

**Table-1.1b: Second Phase $LD_{50}$ (Oral, rat) of Pet Ether Extract of Leave, Stem Bark and Root of *Jatropha Curcas***

| Doses in (mg/kg) | Leave extract | | Stem bark extract | | Root extract | |
|---|---|---|---|---|---|---|
| | No: of Rat | Mortality | No: of Rat | Mortality | No: of Rat | Mortality |
| 1250 | 1 | 0/1 | 1 | 0/1 | 1 | 0/1 |
| 2000 | 1 | 0/1 | 1 | 0/1 | 1 | 0/1 |
| 2750 | 1 | 0/1 | 1 | 0/1 | 1 | 0/1 |
| 3750 | 1 | 0/1 | 1 | 0/1 | 1 | 0/1 |
| 5000 | 1 | 0/1 | 1 | 0/1 | 1 | 0/1 |

$LD_{50}$ Oral, rats of Pet Ether Extract of Leave, Stem Bark and Root of *Jatropha Curcas* is > 5000mg/kg body weight of rats.

**Serum Lipid Profile of Pet-Ether Leave, Stem Bark and Root Extract of *Jatropha Curcas* for Fourteen Days Oral Administration**

The concentration of plasma lipid profile of the experimental albino rats administered with Pet-Ether Leave, Stem Bark and Root Extract of *Jatropha Curcas* for fourteen days showed that the total plasma cholesterol and LDL-cholesterol of rats administered with low and high doses of leave, stem bark and roots pet-ether extracts decreased across the group when compared with control group with exception of group 2 treated rats for total plasma cholesterol which increased at the lower dose of 200mg/kg of the leaf extract. Also, there was significant decreased (P >0.05) in plasma HDL-cholesterol across the groups when compared with the normal control group, whereas the plasma VLDL-cholesterol increased significantly across the groups compared to the control. However, there was no significant changes was observed in the plasma triglycerides in all the group administered with both low and high doses of the pet –ether extract of the leaf, stem bark and roots for fourteen days when compared to the control group (Table 2.1).

**Table-2.1: Serum Lipid Profile of Pet-ether Leave, Stem Bark and Root Extract of *Jatropha Curcas* for fourteen days' oral administration**

| GROUPING | DOSES | TOTAL CHOLESTEROL (mmol/L) | TRIGLYCERIDES (mmol/L) | VLDL (mmol/L) | LDL (mmol/L) | HDL (mmol/L) |
|---|---|---|---|---|---|---|
| GROUP 1 | (CONTROL) Administered with normal saline | 3.42±0.07[a] | 0.85±0.07[a] | 0.30±0.15[a] | 0.47±0.10[a] | 2.81±0.15[a] |
| GROUP 2 | Administered with 200mg/kg of pet-ether leaf extract | 3.43±0.17[e] | 0.92±0.10[e] | 0.45±0.07[e] | 0.44±0.13[e] | 2.43±0.12[e] |
| GROUP 3 | Administered with 400mg/kg of pet-ether leaf extract | 3.19±0.10[e] | 0.81±0.06[e] | 0.43±0.04[e] | 0.36±0.0[e] | 2.55±0.05[e] |
| GROUP 4 | Administered with 200 of p et-ether stem bark extract | 3.33±0.16[e] | 0.86±0.14[e] | 0.40±0.07[f] | 0.44±0.1[e] | 2.65±0.11[e] |
| GROUP 5 | Administered with 400 of pet-ether stem bark extract | 3.05±0.07[f] | 0.90±0.16[e] | 0.50±0.09[e] | 0.41±0.05[f] | 2.45±0.04[f] |
| GROUP 6 | Administered with 200 of pet-ether root extract | 3.14±0.10[g] | 0.92±0.10[e] | 0.43±0.14[e] | 0.43±0.13[e] | 2.55±0.12[e] |
| GROUP 7 | Administered with 400 of pet-ether root extract | 3.17±0.33[e] | 0.84±0.10[e] | 0.45±0.10[g] | 0.30±0.00[g] | 2.43±0.10[g] |

Results are expressed as mean ± standard deviation (n=5). Values in the same column with the different alphabet as a superscript are significantly different ($p<0.05$) when compared with the test control group (I) HDL = High density lipoprotein, LDL= Low density lipoprotein, VLDL = Very low density lipoprotein

Cardiovascular diseases are global challenges todays associated with coronary heart diseases, stroke and hypertension. High level of serum or plasma lipids was reported to be the risk factor of cardiovascular related problems. Elevation of plasma triglycerides and cholesterol level were the most common factors to be considered in diagnosis of most cardio-related problems [18]. Lipids are transported in the blood with the proteins complexes called lipoproteins [19]. Hyperlipidemia is identified when the increased LDL cholesterol and reduced HDL-cholesterol are observed [20]. Therefore, lowering serum concentrations of LDL and increase HDL concentration is considered as one of the strategies that can hinder or delay the on-set of chronic disorders related to hyperlipidemia in humans [21].

In this study, the effects of oral administration of Pet-Ether Leave, Stem Bark and Root Extract of *Jatropha Curcas* for fourteen days on seven groups of wister rats were evaluated. It was found that the levels of Total plasma cholesterol and LDL-cholesterol decreased across the groups (except group 2) compared to the normal control (group 1). This indicated that there is reduction of cholesterol transported by LDL-cholesterol from extracellular fluids to the blood vessels, which in turn would reduce accumulation of plasma cholesterol in the blood vessels in a process that can lead to atherosclerosis. There was general decrease in the levels of HDL-cholesterol across the groups compared to the control (group 1), this might be due to the effects of administration of Pet-Ether Leave, Stem Bark and Root Extract of *Jatropha Curcas* for Fourteen Days Oral administration on the reduction of synthesis of HDL in rats. However, increase concentration of HDL is associated with decrease accumulation of atherosclerosis within the walls of arteries. This is because atherosclerosis results in sudden plaque ruptures, cardiovascular disease, stroke and other vascular diseases. Additionally, the decrease in HDL-cholesterol observed in this study across the treatments could be similar with the study conducted on mice that showed HDL-lacking mice still have the ability to transport cholesterol to bile, suggesting that there are alternative mechanisms for cholesterol to be removed from the blood [22]. The levels of plasma VLDL-cholesterol was found to be increased non-significantly ($P > 0.05$) across groups when compared to the control group. VLDL is lipoprotein that is synthesized by the liver; hence it could mean that Pet-Ether Leave, Stem Bark and Root Extract of *Jatropha Curcas* extracts may have difference influence on its synthesis. VLDL-cholesterol and LDL-cholesterol are referred to as bad cholesterol, since they transport cholesterol from extracellular body fluids to the blood vessels [23]. However, the highest value was shown by group 5 rats that had 0.50±0.09 mmol/l, while the lowest value was recorded for normal control (group 1) 0.30±0.15mmol/l. There was no definite trend in the levels of plasma triglycerides across treatment groups compared to the control. This observation may be due to different in synthesis of triglycerides in the rat's liver of the groups administered the extracts of *Jatropha Curcas.* The highest level of triglycerides in this study was obtained in groups 2 and 6 experimental rats while the lowest was recorded for group 3 rats. However, there was no significant changes was observed in the plasma triglycerides in all the group administered with both low and high doses of the pet –ether extract of the leaf, stem bark and roots for fourteen days when compared to the control group. Triglycerides are the most common type of lipid synthesized in animals. The body

converts any form of excess calories into triglycerides for long term storage. High levels of triglycerides are related to a higher risk of heart and blood vessels [6].

## CONCLUSION

The study showed that oral administration of Pet-Ether Leave, Stem Bark and Root Extract of *Jatropha Curcas* led to a significant improvement in the levels of serum lipid profile, which showed significant decrease in LDL as well as total cholesterol level by some treated groups. However, no significant differences (P >0.05) were observed on the levels of serum triglycerides and VLDL among treated groups. It was found that the blood lipids of the rats were within normal range, hence administration of extracts of Leave, Stem Bark and Root Extract of *Jatropha Curcas* may have no deleterious effects on the serum lipid profile of the animals. This research work showed that the oral administration of the leave, stem bark and root fruit extract of *Jatropha Curcas* possess hypolipidemic activity and may be useful in the management of cardiovascular disease. Also acute toxicity (LD50) of the Leave, Stem Bark and Root Extract of *Jatropha Curcas* is greater than 5000mg/kg hence it's declared practically non-toxic to the experimental animals.

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