Global Journal of Biotechnology & Biochemistry 10 (1): 11-15, 2015
ISSN 2078-466X

DOI: 10.5829/idosi.gjbb.2015.10.01.1116

The Effect of Ethanol Leaf Extract of *Jatropha curcas* on Chloroform Induced Hepatotoxicity in Albino Rats

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Abstract: In this study, ethanol leaf extract of *Jatropha curcas* were selected for their hepatoprotective activity on experimentally induced hepatotoxicity in rats. The leaves of *Jatropha curcas* were sun-dried, ground and then extracted with ethanol. LD_{50} was carried out and found to be between the ranges of 1900mg/kg to 2600mg/kg body weight and thus, the lethal dose was calculated to be 2222.6 mg/kg. Wistar strain albino rats were used for assessment of hepatoprotective activity and hepatotoxicity was induced by the administration of chloroform. Ethanol extract was administered on the rats by oral intubation method for 14 days, also chemiron was the standard drug administered. Blood was collected by ocular puncturing and various biochemical parameters alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) were studied. Treatment with ethanol leaf extract of *Jatropha curcas* showed a significant decrease ($p<0.05$) in the levels of elevated ALT, ALP and AST when the results of the experimental groups were compared with the negative control (group 4). The results suggest that the leaves of *Jatropha curcas* could serve as a promising source of drug for the treatment of liver related complications of oxidative stress.

Key words: *Jatropha curcas* · Oxidative stress · AST · ALP and ALT

INTRODUCTION

The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction [1-7]. Therefore, maintenance of a healthy liver is essential for the overall wellbeing of an individual. Liver cell injury caused by various toxicants such as certain chemotherapeutic agents, carbon tetrachloride, thioacetamide etc., chronic alcohol consumption and microbes is well-studied. Since time immemorial, mankind has made use of plants in the treatment of various ailments. The Indian Traditional Medicine like Ayurveda, Siddha and Unani are predominantly based on the use of plant materials. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. The association of medical plants with other plants in their habitat also influences their medicinal values in some cases. One of the important and well-documented uses of plant-products is their use as hepatoprotective agents [1-5].

Hence, there is an ever increasing need for safe hepatoprotective agent [1-3]. In spite of tremendous strides in modern medicine, in 2004, the U.S. National Centre for complementary and Alternative Medicine of the National Institutes of Health began funding clinical trials into the effectiveness of herbal medicine. For this reason, various medicinal plants have been studied using modern scientific approaches which have shown that due to their various biological components, many of these medicinal plants posse a number of properties such as anti-diabetic, antioxidant, anticancer and anti-inflammatory effects, etc. and can be used to treat a wide range of various diseases.

The medicinal properties of plants are due to the presence of certain specific substances, referred to as bioactive principles which may be stored in organs like roots, leaves, stem bark, fruits and seeds [1]. Many herbs have shown positive results *in-vitro*, animal models, or small-scale clinical tests [7-12]. Plants and their extracts

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have immense potentials for the management and treatment of wounds. The phytomedicine for wound healing are not only cheap and affordable, but are also safe as hypersensitive reactions are rarely encountered. These natural agents induce healing and regeneration of tissues by multiple mechanisms. However, there is need for scientific validation, standardization and safety evaluation of plants of traditional medicine before recommendation for the healing of wounds.

Jatropha curcas leaves can be used as tea against malaria; the seeds are used as contraceptives in South Sudan and also used against constipation. The watery sap is put onto fresh cuts and sores at the corner of the mouth and can also be used as antidotes for venomous stings and bites [8-12]. In addition to the above claims, a large set of data suggest that *Jatropha curcas* might have hepatoprotective activity. This research is aimed at determining the effect of ethanol leaf extract of on chloroform induced hepatotoxicity in albino rats.

MATERIALS AND METHODS

Materials

Plant Materials: The leaves of *Jatropha curcas* were purchased from Oba market in Anambra State and identified by Mr. A. Ozioko of the Department of Plant Science and Biotechnology, University of Nigeria Nsukka. A voucher specimen was deposited in the Department's *Herbarium*.

Animals: Wistar albino mice (20- 30g) of both sexes and female rats (134 -206g) bred in the faculty of Veterinary Medicine, University of Nigeria Nsukka, were used in the experiment. The animals were kept under Room temperature and were acclimatized in the new environment for a period of 7 days with free access to food and water before the commencement of the experiment.

Methods

Preparation of Plant Material: The leaves of *Jatropha curcas* were collected, dried and milled to powder using the grinding machine.

Extraction of Plant Material: A known quantity, 500g of ground leaves of *Jatropha curcas* were macerated in 1500ml of ethanol with thorough shaking at regular interval for 72h at room temperature (26-28°C). The resulting solution was filtered using Whatman No. 1 filter paper. The filtrates were concentrated using rotary evaporator to obtain slurry of the extract. The semi-pastry extract was stored in the refrigerator and used for the study.

Experimental Design: Twenty five (25) Wistar albino rats were used in this study. They were randomly distributed into five (5) groups of 4 rats each. Oxidative stress was induced in the rats and this was performed by intraperitoneal injection of chloroform (100 mg/kg b/w). The rats were fed graded doses of ethanol extract of *Jatropha curcas* through oral intubation method. The groups and doses administered are summarized below.

Group 1: Negative control rats without Chloroform intoxication were treated with 0.5 ml of normal saline.

Group 2: Chloroform intoxicated rats were treated with 100 mg/kg b.w. of ethanol extract of *Jatropha curcas*.

Group 3: Chloroform intoxicated rats were treated with 200 mg/kg b.w. of ethanol extract of *Jatropha curcas*.

Group 4: Positive control rats with Chloroform intoxication were treated with 0.5 ml of normal saline.

Group 5: Standard control rats with Chloroform intoxication were treated with 5 mg/kg body weight of standard drug Chemiron.

Collection of Blood Sample: Blood sample of the rats were collected through ocular puncture for biochemical analysis.

Experimental Protocol for Acute Toxicity Studies: Median Lethal Dose (LD_{50}) is the log dose of a drug that kills 50% of the population to which the drug is administered. Investigation on the acute toxicity study LD_{50} of the extract was determined using the method described by Lorke (1983).

Phase I:

Group 1: Mice were administered with 10 mg/kg of body weight of the ethanol leaf extract of *Jatropha curcas*.

Group 2: Mice were administered with 100 mg/kg of body weight of the ethanol leaf extract of *Jatropha curcas.*

Group 3: Mice were administered with 1000 mg/kg of body weight of the ethanol leaf extract of *Jatropha curcas.*

Group 4: Mice were administered with 1000 mg/kg of body weight of distilled water.

Acute Toxicity (LD_{50})

The LD_{50} of the ethanol extract in mice was found to be more than 1900mg/kg and less than 2600 mg/kg body weight. Four animals died and the other remaining two animals in the group showed signs of toxicity as illustrated below within 24 hours of constant observation.

PHASE 1

Group	Dosage	Mice 1	Mice 2	Mice 3
Group 1	10 mg/kg	ND and NST	ND and NST	ND and NST
Group 2	100 mg/kg	ND and NST	ND and NST	ND and NST
Group 3	1000 mg/kg	ND and NST	ND and NST	ND and NST
Group 4	Standard Control	ND and NST	ND and NST	ND and NST

PHASE 2

Group	Dosage	Mice 1	Mice 2	Mice 3
Group 1	1,900 mg/kg	ND and NST	ND and NST	ND and NST
Group 2	2,600 mg/kg	ST	ST	D
Group 3	5,000 mg/kg	D	D	D
Group 4	Standard Control	ND and NST	ND and NST	ND and NST

ND = No Death, NST = No Signs of Toxicity, D= Death, ST= Signs of Toxicity

The LD_{50} was then calculated as square root of the product of the lower lethal dose and the highest non-lethal dose that is the geometric mean of the consecutive doses for which 0 and 100% survival rates were recorded in the second phase. The LD_{50} was thus, v1900 x 2600 =2222.6 mg/kg

Phase II

Group 1: Mice were administered with 1900 mg/kg of body weight of the ethanol leaf extract of *Jatropha curcas*.

Group 2: Mice were administered with 2600 mg/kg of body weight of the ethanol leaf extract of *Jatropha curcas*.

Group 3: Mice were administered with 5000 mg/kg of body weight of the ethanol leaf extract of *Jatropha curcas*.

Group 4: Mice were administered with 5000 mg/kg of body weight of distilled water.

The mice were monitored closely for 24 hours for signs of toxicity and lethality.

Assay Methods: Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) were assayed by the method of Thapa and Walia, (2007).

Statistical Analysis: Data were reported as means ± SEM, where appropriate. Both one and two way analyses of variance (ANOVA) were used to analyze the experimental data and Duncan multiple test range was used to compare the group means obtained after each treatment with control measurements. Differences were considered significant when $p \leq 0.05$.

RESULTS AND DISCUSSION

The present study assessed the effect of ethanol leaf extract of *Jatropha curcas* on chloroform induced hepatotoxicity in albino rats. The ethanol leaf extract of *Jatropha curcas* was tested for hepatoprotective activity as per the method by Hander and Sharma (1990) [4]. The liver plays a pivotal role in glucose and lipid homeostasis and is severely affected in oxidative stress conditions (Seifter and England, 1982) [8].

The LD_{50} of the ethanol extract in mice was found to be more than 1900mg/kg and less than 2600 mg/kg body weight. Four animals died and the other remaining two animals in the group showed signs of toxicity as illustrated below within 24 h of constant observation.

Figure 1 shows a significant increase ($p < 0.05$) increase in ALP activity of group 4 (positive control) when compared to group 1(normal control) and this increase is an indication that the animals were oxidatively stressed. The treated with graded doses of ethanol extract of *Jatropha curcas* in groups 2 and 3 significantly ($p<0.05$) decreased the levels of ALP activities when compared to group 4 (untreated group). The same pattern of reduction was also noticed in group 5 (treatment with standard drug).

Figure 2 shows a significant increase ($p < 0.05$) increase in AST activity of group 4 (positive control) when compared to group 1(normal control) and this increase is an indication that the animals were oxidatively stressed. The treated with graded doses of ethanol extract

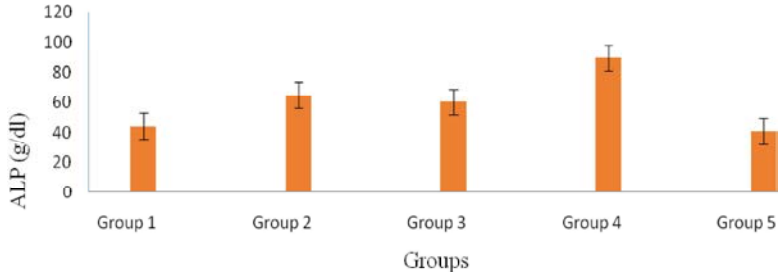

Fig. 1: The Effect of *Jatrophacurcas*on ALP level of chloroform intoxicated rats.

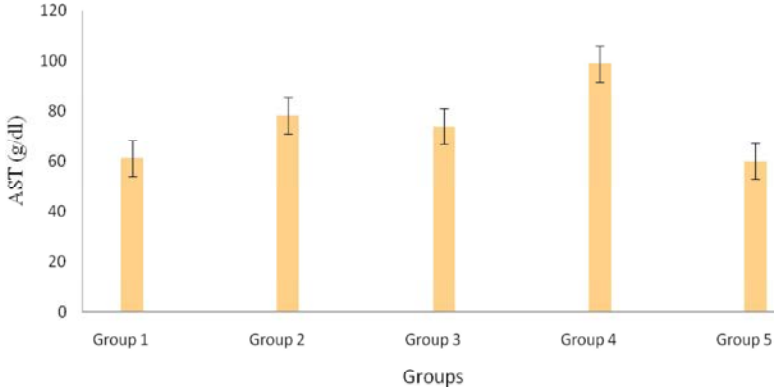

Fig. 2: The Effect of *Jatrophcurcas*on AST level of chloroform intoxicated rats.

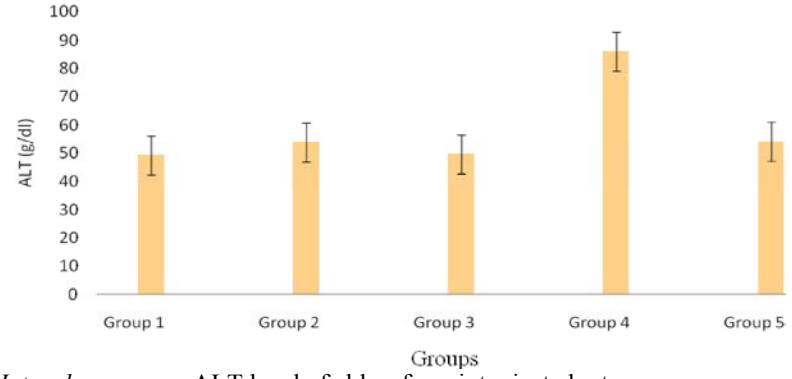

Fig. 3: The Effect of *Jatrophacurcas*on ALT level of chloroform intoxicated rats.

of *Jatropha curcas* in groups 2 and 3 significantly ($p<0.05$) decreased the levels of AST activities when compared to group 4 (untreated group). The same pattern of reduction was also noticed in group 5 (treatment with standard drug).

Figure 3 shows a significant increase ($p < 0.05$) increase in ALT activity of group 4 (positive control) when compared to group 1(normal control) and this increase is an indication that the animals were oxidatively stressed. The treated with graded doses of ethanol extract of *Jatropha curcas* in groups 2 and 3 significantly ($p<0.05$) decreased the levels of ALT activities when compared to group 4 (untreated group). The same pattern of reduction was also noticed in group 5 (treatment with standard drug).

In this study, chloroform administration to experimental rats caused a marked elevation in the levels of serum AST, ALP and ALT which is indicative of hepatocellular damage. This might possibly be due to the release of these enzymes from the cytoplasm into the blood circulation rapidly after rupture of the plasma membrane and cellular damage. Several studies have reported similar elevation in the activities of serum AST, ALP and ALT during toxicants administration [4] and [7]. The level of hepatotoxicity developed can be observed by the elevated levels of the biomarkers' activities which are attributed to the generation of free radicals during metabolism by hepatic microsomes which in turn cause peroxidation of lipids of cellular membrane. The study of serum markers such as AST, ALT and ALP has been

found to be of great value in assessing clinical and experimental liver damage (Moore *et al*., 2005) [3]. In the present investigation, the rats suffered significant hepatic damage from treatment with chloroform, as indicated by elevated levels of serum liver markers. A rise in AST is usually accompanied by an increase in ALT, which plays a vital role in the conversion of amino acids to keto acids (Vaishwanar and Kowale, 2006) [11]. From the results shown (Fig. 1-3), it is clear that chloroform induced liver toxicity as indicated in the elevation in ALP, AST and ALT activity in the group 4 (Induction + no treatment) as compared with the other test groups (the groups that received treatment). However, on treatment with graded doses, the result indicated that the ethanol leaf extract of *Jatropha curcas* significantly reduced ($p<0.05$) the elevated level of liver marker enzymes' activities.

CONCLUSIONS

The administration of the ethanol extracts of *Jatropha curcas* showed a significant reduction in the activities of the enzyme markers, thus, supporting the folk information regarding its hepatoprotective activity but caution need to be taken in its administration due to its toxicity at certain doses. Thus *Jatropha curcas* could serve as a promising source of drug for the treatment of liver related ailments.

REFERENCES

1. Agarwal, S.S., 2001. Development of hepatoprotective formulations from plant sources. In: Pharmacology and Therapeutics in the New Millennium. Edited by Gupta SK, Narosa Publishing House, New Delhi, pp: 357-358.
2. Ashok, M., B. Bendre and P.C. Pande, 2006. Medicinal plants. In: Introductory Botany 4th edition revised RasTogi Publication, pp: 149.
3. Etuk, E.U. and B.J. Muhammed, 2010. Evidence based of chemical method of induction of diabetes mellitus in experimental animals. Asian Journal of Experimental Biological Sciences, 1(2): 331-336.
4. Handa, S.S. and A. Sharma, 1990. Hepatoprotective activity of andrographlide from *Andrographispaniculata* against CCL_4. Indian Journal of Medical Research, 92(8): 137-140.
5. Lorke, D., 1983. A New Approach to Practical Acute Toxicity Testing. Archives of Toxicology, 55: 275-287.
6. Moore, M., H. Thor, G. Moore, S. Nelson, P. Moldeus and S. Correnius, 2005. The toxicity of acetaminophen and N-acetyl P-benzoquinone imine in isolated hepatocytes is associated with thio depletion and increased cytosolic Ca2+. Journal of Biological Chemistry, 260: 13035-13040.
7. Owolabi, O.J., F.C. Amaechina and M. Okoro, 2011. Effect of ethanol leaf extract of *Newbouldia Laevis* on blood glucose levels of diabetic rats. Tropical Journal of Pharmaceutical Research, 10(3): 249-254.
8. Seifter, S. and S. England, 1982. Energy Metabolism, In: Arias, I., Popper, H. and Schacter, D. (Eds.) The Liver: Biology and Pathology, New York Raven Press, pp: 219-249.
9. Srinivasan, K., 2005. Spices as influencers of body metabolism: an overview of three decades of research. Food Research International, 38(1): 77-86.
10. Thapa, B.R. and A. Walia, 2007. Liver function tests and their interpretation. Indian Journal of Paediatrics, 74: 663-671.
11. Vaishwanar, I. and C.N. Kowale, 2006. Effect of two ayurvedic drugs Shilajeet and Eclinol on changes in liver and serum lipids produced by carbon tetrachloride. Indian Journal of Experimental Biology, 34: 58-61.
12. Ward, F.M. and M.J. Daly, 1999. Hepatic Disease. In: Clinical Pharmacy and Therapeutics (Walker R.andC.Edwards Eds.). Churchill Livingstone, New York, pp: 195-212.