Research Article

Effect of Ethanol Extract of *Jatropha gossypifolia* (POHL) on Hematological and Plasma Biochemical Parameters in Male Wistar Rats

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ABSTRACT

This study aims at investigating the effect of Ethanol Extract of *Jatropha gossypifolia* (EEJG) on hematological and plasma biochemical parameters in male rats. Six hundred grams of air-dried *Jatropha gossypifolia* leaves were cold macerated in 70 % ethanol and concentrated using water-bath. Eighteen Swiss male mice (20-25 g) were used for acute toxicity study. Twenty male rats (80-150 g) were divided into control (distilled water) and EEJG-treated (62.5, 125, 250 mg/kg) groups (5 per group) for hematological and biochemical assays. The animals were orally treated on daily basis for 30 days. Red Blood Cell (RBC) count and Total White Blood Cell (TWBC) count were determined using hemocytometer. Activities of plasma Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP), as well as levels of total protein, globulin, albumin, creatinine and urea were determined by spectrophotometry. Data were analyzed using descriptive statistics and ANOVA at p=0.05. The EEJG (250 mg/kg) significantly ($p<0.05$) increased neutrophil value relative to the control. The extract (125 and 250 mg/kg) significantly ($p<0.05$) increased AST and ALT activities relative to their respective controls. However, EEJG (62.5 mg/kg) significantly ($p<0.05$) decreased total protein level relative to the control. It can therefore be concluded that *Jatropha gossypifolia* probably has a little toxic effect and overwhelming beneficial potentialities on the hematological functions and blood chemistry of male rats.

Keywords: *Jatropha gossypifolia*, Rats, Total white blood cell counts, Red blood cell counts, Total protein.

INTRODUCTION

Jatropha gossypifolia(Pohl) belongs to the family of Euphorbiaceae, which occur preferentially in tropical and subtropical environment[1]. It is commonly called Bellyache bush in English language, "Faux manioc" in French language and "Lapalapa pupa" by the Yoruba language speaking people of Nigeria.

The plant is used medicinally as an anti-inflammatory, anti-hemorrhagic, analgesic, antianemic and antimicrobial agents[2,3].

Pharmacologically, it is used as an antihypertensive[4], antineoplastic[5], wound healing[6], contraceptive in female rodents[7] and antioxidant agent[8].

This plant has been reported to have anti-hemorrhagic function medicinally[2]; however, due to paucity of information from literature on the effects of extracts of this plant on hematological and plasma biochemical parameters in male rats, this study therefore aims at investigating the effect of ethanol extract of this plant on these aforementioned parameters.

MATERIALS AND METHODS

Experimental animals

Adult male rats weighing between 80-150 g bred in the Pre-Clinical Animal House of the College of Medicine and Health Sciences, AfeBabalola University were used. They were housed under standard laboratory conditions and had free access to feed and water. They were acclimatized for two weeks to laboratory conditions before the commencement of the experiments. All experiments were carried out in compliance with the recommendations of AfeBabalola University Ethics Committee on guiding principles on care and use of animals.

Plant material

Fresh samples of *Jatropha gossypifolia* plants were collected from the Botanical Garden of the University of Ibadan, and were authenticated in the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan where a voucher specimen (No. FHI.110178) was deposited in their Herbarium.

Preparation of ethanol extract of *Jatropha gossypifolia* (EEJG)

Large quantity (1.5 kg) of fresh specimens of the leaves of *Jatropha gossypifolia* were washed free of debris and air-dried. The dried leaves were pulverized using laboratory mortar and pestle.

Weighted portion (600.0 g) of the pulverized specimen was macerated with 70 % ethanol (1:2 wt./vol.) for 72 hours at room temperature.

The resulting solution was then filtered using a wire-gauze and a sieve with tiny pores (0.25 mm). The 70 % ethanol was later evaporated using water-bath to give a percentage yield of 10.96 % of the starting material.

The dried material was reconstituted in distilled water to make up test solutions of known concentrations.

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Acute toxicity test

The method described by[9] was used to determine the LD_{50}, which is the index of acute toxicity. Male Swiss mice (20-25 g) were used. This method involved an initial dose finding procedure, in which the animals were divided into three groups of three animals per group. Doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg were administered orally, one dose for each group. The treated animals were monitored for twenty-four hours for mortality and general behavior.

From the results of the above step, seven different doses (2000 mg/kg, 3000 mg/kg, 4000 mg/kg, 5000 mg/kg, 6000 mg/kg, 7000 mg/kg, 8000 mg/kg) where chosen and administered orally to eight groups of animals of one mouse per group respectively. The treated animals were monitored for twenty - four hours. The LD_{50} was then calculated as the geometric mean of the lowest dose showing death and the highest dose showing no death.

The dosages of EEJG administered in this study were obtained from the result of the acute toxicity test.

Experimental design

Twenty male rats (80-150 g) were randomly divided into four groups, with each consisting of five animals. The four groups were subjected to the following oral treatments once a day for thirty (30) days:

Group I: received 0.5 ml/100 g of distilled water as control group

Group II: received 62.5 mg/kg of EEJG

Group III: received 125 mg/kg of EEJG

Group IV: received 250 mg/kg of EEJG

Collection of blood samples

Twenty four hours (day 31) after the last dosing of all the groups, blood samples were collected from all the animals through the medial cantus with heparinized capillary tubes into EDTA bottles for hematological and plasma biochemical analyses. Before assays, the blood was centrifuged for 5 minutes using a bench top centrifuge (Centromix) and the plasma were used for the determination of the biochemical parameters.

Determination of hematological Parameters

The red blood cells (RBC) and white blood cells (WBC) counts were determined by the Improved Neubauerhemocytometer method. The hemoglobin (Hb) concentration was determined according to[10] using the cyanomethemoglobin method. The packed cell volume (PCV) was determined by the micro-hematocrit method according to[11]. Schilling method of differential leukocyte count was used to determine the distribution of the various white blood cells[12]. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were computed according to[10].

Determination of plasma biochemical parameters

The total protein concentration was determined using the Biuret method[13] and the albumin concentration by the method of[14]. The globulin concentration was calculated by subtracting the albumin concentration from the total protein concentration. Activities of plasma alanine transaminase (ALT) and aspartate transaminase (AST) were determined according to the method of[15]. The level of creatinine, urea and alkaline phosphatase were determined using the method of[16]. All the above biochemical parameters were determined in the plasma using the Randox kits.

Statistical analysis

The mean and standard error of mean (S.E.M.) were calculated for all values. Comparison between the control and experimental groups was done using one-way analysis of variance (ANOVA) with Duncan's Multiple Range Test. Differences were considered statistically significant at $p<0.05$.

RESULTS

The LD_{50} of the crude extract was found to be 6500 mg/kg *per os.* The effects of varying doses of EEJG on hematological and plasma biochemical parameters after treatment of rats for 30 days are shown in Tables 1 and 2 respectively. Treatment of rats with all the treatment doses of EEJG (62.5 mg/kg, 125 mg/kg and 250 mg/kg) produced no significant ($p>0.05$) changes in PCV, Hb, RBC, TWBC, platelet, lymphocyte, monocyte, eosinophil, MCV, MCHC and MCH values relative to their respective controls. Treatment of rats with 250 mg/kg of EEJG caused significant ($p<0.05$) increase in neutrophil values relative to the control, while 62.5 mg/kg and 125 mg/kg produced no significant ($p>0.05$) changes in neutrophil value relative to the control.

Treatment of rats with 62.5 mg/kg of EEJG caused significant ($p<0.05$) decrease in total protein level relative to the control, while treatment of the rats with 125 mg/kg and 250 mg/kg of EEJG caused no significant ($p>0.05$) changes in total protein levels relative to the control. Administration of all the treatment doses of EEJG (62.5 mg/kg, 125 mg/kg and 250 mg/kg) to the rats produced no significant ($p>0.05$) changes in albumin and globulin levels relative to their respective controls. Treatment of rats with 125 mg/kg and 250 mg/kg of EEJG caused significant ($p<0.05$) increments in ALT and AST activities relative to their respective controls, while 62.5 mg/kg caused no significant ($p>0.05$) change in AST and ALT activities relative to their respective controls. Treatment of rats with all the doses of EEJG (62.5 mg/kg, 125 mg/kg and 250 mg/kg) produced no significant ($p>0.05$) changes in ALP activity relative to the control. Treatment of the rats with all the doses of EEJG (62.5 mg/kg, 125 mg/kg and 250 mg/kg) produced no significant ($p> 0.05$) changes in urea and creatinine levels relative to their respective controls.

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Table 1: Effect of 30 days treatment with varying doses of EEJG on hematological parameters

Parameters	Control	62.5 mg/kg	125 mg/kg	250 mg/kg
PCV (%)	46.00 ± 2.00	42.00 ± 1.83	46.50 ±1.19	44.25 ± 1.65
Hb (g/dL)	13.99 ± 0.73	14.00 ± 0.85	15.28 ± 0.43	14.68 ± 0.51
RBC ($\times10^6$/µL)	7.89 ± 0.37	7.13 ± 0.36	7.81 ± 0.25	7.50 ± 0.34
TWBC ($\times10^3$/µL)	5.38 ± 1.31	4.63 ± 0.38	6.84 ± 0.48	5.1 ± 0.71
Platelets ($\times10^5$/µL)	0.86 ± 0.22	0.82 ± 0.08	1.20 ± 0.05	0.81 ± 0.15
Lymphocytes (%)	71.00 ± 3.06	68.75 ± 1.38	69.50 ± 1.56	65.00 ± 1.47
Neutrophils (%)	25.67 ± 2.19	27.75 ± 0.48	26.00 ± 1.83	31.00 ± 1.47*
Monocytes (%)	1.33 ± 0.33	1.75 ± 0.48	2.25 ± 0.48	1.75 ± 0.48
Eosinophils (%)	2.00 ± 1.00	1.75 ± 0.85	2.00 ± 0.58	2.25 ± 0.25
MCV (FL)	58.30 ± 0.29	59.00 ± 0.75	59.58 ± 0.90	59.07 ± 0.51
MCHC (g/dL)	33.66 ± 0.44	33.33 ± 0.35	32.85 ± 0.96	33.18 ± 0.25
MCH (pg)	19.62 ± 0.17	19.67 ± 0.38	19.57 ± 0.31	19.60 ± 0.29

*Significant different as compared with control group at p<0.05

Table 2: Effect of 30 days treatment with varying doses of EEJG on Plasma Biochemical Parameters

Parameters	Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Total Protein (gm %)	7.90 ± 0.21	7.08 ± 0.82*	7.35 ± 0.19	7.58 ± 0.13
Albumin (gm %)	4.57 ± 0.33	4.03 ± 0.42	4.23 ± 0.19	4.45 ± 0.17
Globulin (gm %)	3.00 ± 0.38	3.10 ± 0.15	3.13 ± 0.13	2.50 ± 0.35
AST (µ/L)	37.33 ± 1.45	39.50 ± 1.56	45.50 ± 1.26*	43.50 ± 2.06*
ALT (µ/L)	28.33 ± 0.33	29.00 ± 1.47	32.25 ± 0.48*	31.75 ± 0.95*
ALP (IU/L)	113.33 ± 6.06	123.50 ± 2.60	115.00 ± 2.97	115.75 ± 4.33
Urea (mg/dL)	15.33 ± 0.33	14.00 ± 0.41	14.50 ± 0.50	15.00 ± 0.58
Creatinine (µmol/L)	0.63 ± 0.03	0.53 ± 0.06	0.58 ± 0.06	0.65 ± 0.09

*Significant different as compared with control group at p<0.05

DISCUSSION

This plant extract exhibits low toxicity and wide safety margins which is reflected by its high LD_{50}, since it has been reported that any compound or drug with an oral LD_{50} estimate greater than 1000 mg/kg could be considered of low toxicity and safe[17]. Similar result was reported by[18] in *Physalisalkekengi* extract treated rats.

The result has shown that the extract caused no significant change on the PCV, RBC and indices relating to it (Hb, MCV, MCH and MCHC). This could indicate that the extract does not have the potential to stimulate erythropoietin release from the kidneys which is the humoral regulator of RBC production[19]. It could also indicate that there were no changes in the oxygen carrying capacity of the blood and the amount of oxygen delivered to the tissues since RBC and hemoglobin are very important in transferring respiratory gases[20]. It has been reported that values of RBC and associated parameters lower than normal ranges are indicative of anemic conditions while higher values are suggestive of polycythemia[21]; thus, the extract may not have the potential to induce anemia or polycythemia. Also, the extract may not have adverse effect on the bone marrow, kidney and hemoglobin metabolism, since it has been reported that only substances which significantly affect the values of red blood cells and associated parameters would have effects on the bone marrow, kidney and hemoglobin metabolism[22]. The extract caused no significant change in TWBC values which probably indicates that it has no effect in the ability of the body to defend against invading organisms[23]. Contrary result was reported by[24] in *Viscum album* extract treated rats. The extract caused no significant change in the platelet value which probably indicates that it has no effect on the hemostatic function of the body. Contrary result was reported by[25] in *Fadogiaagrestis* extract treated rats. The extract caused no significant change in lymphocyte value which probably indicates that it has no effect in the acquired immune response of the body. Similar result was reported by[26] in isolated ergosterol treated rats. The extract caused a significant increase in the neutrophil count which probably indicates an increase in the ability of the body to attack and destroy invading bacteria, viruses and other injurious agents (phagocytosis). Similar result was reported by[27] in *Dennettiatripetala* extract treated rats. The extract caused no significant change in the monocyte value which probably indicates that it has

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no effect in the phagocytic function of the body[28]. Contrary result was reported by[29] in *Saccharomyces cerevisiae* extract fed hens. The extract caused no significant change in eosinophil value which could indicate that it has no effect in the anti-allergic and anti-parasitic infectious responses of the body. Contrary result was reported by[30] in *Arctotisactotoides* extract treated rats and mice.

The results of the plasma biochemical study have shown that treatment of rats with the extract caused significant decrease in total protein level. This could indicate a reduction in the buffering capacity of the blood as well as a decrease in colloid osmotic pressure which could cause loss of fluid from the capillaries, since plasma proteins have been reported to be responsible for 15 % of buffering capacity of blood[23] and that osmotic pressure caused by the plasma proteins (called colloid osmotic pressure) tends to cause fluid movement by osmosis. Contrary result was reported by[31] in *Euphorbia heterophylla* extract treated rats. The extract caused no significant changes in albumin level which probably indicates that it has no effect in the plasma levels of metals, ions, fatty acids, amino acids, bilirubin and enzymes; since it has been reported that albumin serves as a carrier for metals, ions, fatty acids, amino acids, bilirubin, enzymes and drugs[23]. Contrary result was reported by[32] in *Enicostemmaaxillare* extract treated rats. The extract produced no significant change in globulin level which probably indicates that it has no effect in both the natural and acquired immunity of the body against invading organisms, since it has been reported that globulins are principally responsible for the body's both natural and acquired immunity against invading organisms[28]. Similar result was reported by[33] in *Portulacaoleracea* extracts treated rats.

The significant increase in the activity of AST caused by the extract could indicate an induction of tissue necrosis, since it has been reported that elevation in the activity of AST can be associated with cell necrosis of many tissues, which allows leakage of large amounts of this enzyme into the blood[34]. Similar result was reported by[35] in *Sidarhombifolia* extract treated mice and rats. The extract caused a significant increase in the activity of ALT which probably indicates an induction of hepatic damage, since it has been reported that ALT is present in the liver and other cells and is particularly useful in measuring hepatic necrosis, especially in small animals[15]. Similar result was reported by[36] in *Moringaoleifera* extract treated rats. The extract caused no significant change in ALP level. This probably indicates the absence of cholestasis, since ALP has been reported to be a marker of cholestasis[37]. Similar result was reported by[38] in *Mangiferaindica* extract treated rats. The extract caused no significant change in urea level. This probably indicates absence of nephrotoxicism, since urea and creatinine have been reported to be markers of kidney functions[39]. Contrary result was reported by[40] in *Passifloraedulis* extract treated rats. The extract caused no significant change in creatinine level. This probably indicates absence of induction of renal impairment, since creatinine level is used to measure the extent of renal impairment. Contrary result was reported by[41] in rats treated with *Mucunapruriens* extract.

CONCLUSION

In conclusion, this study has shown that the crude extract of *Jatropha gossypifolia* has a little toxic effect and overwhelming beneficial potentialities on the hematological functions and blood chemistry of male rats. However, the effect of the crude extract of this plant on human hematological functions and blood chemistry are unknown; nevertheless, considering these findings in animal model, it is recommended that moderation should be exercised in consumption of *Jatropha gossypifolia* by the locals for its hematological usefulness in order to prevent tissue necrosis and hepatotoxicity.

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