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Effects of Oral Administration of Aqueous Stem Extract of *Jatropha gossypiifolia* on Weight-Gain and Some Haematological Parameters in Rats

B. U. Shamaki[*1], B. M. Agaie[2], P. O. Ajagbonna[2], A. T. Elsa[3] and A. A. Ebbo[2].

[1]Nigerian Institute for Trypanosomiasis and Onchocerciasis Research,
P. M. B. 03, Vom, Nigeria; [2]Department of Veterinary Physiology and Pharmacology,
[3]Department of Veterinary Surgery and Reproduction, Faculty of Veterinary Medicine,
Usmanu Danfodiyo University, Sokoto, Nigeria

ABSTRACT

The aqueous stem extract of *Jatropha gossypiifolia* var. *gossypiifolia* was obtained and reconstituted daily and orally administered to animal according to weights. In acute toxicity study, a single oral dose of 5000 mg/kg body weight was administered and toxic signs and behaviour were observed and the weight, monitored for 14 days. In repeated dose, test extract was administered daily for 28 days at various doses of 250 mg/kg, 500 mg/kg, 750 mg/kg and 1000 mg/kg respectively. The packed cell volume (PCV), red blood cell count (RBC), differential leucocyte counts. total white blood cell count (WBC), Haemoglobin concentration (Hb), and mean corpuscular haemoglobin concentration (MCHC) plasma proteins, plasma bilirubin, albumin, liver enzymes such as aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP), plasma electrocytes (Na^+, K^+, Cl^- and urea) of the rats were determined on days 14 and 28. While the weight of the rats were simultaneously monitored in both set of the experiments. There were no significant changes in the PCV, RBC, Hb and MCHC, while significant increase ($p<0.05$) was observed in total WBC. Similarly, there were no significant change in values of serum urea, Na^+, Cl^- and K^+. The values of total protein, albumin, bilirubin were not altered, liver enzymes such as AST, ALT are not significantly($p<0.05$) altered, but ALP significantly increased ($p<0.05$). Rats in all the groups of the two experiments showed remarkable increase in weights throughout the study period. It is concluded that extract of *Jatropha gossypiifolia var gossypiifolia* does not have toxic effect on blood parameters and stimulate weight increases in rats and therefore can be medicinally useful on further investigation.

Key words: *Jatropha gossypiifolia* var. *gossypiifolia,* weights, haematology, plasma biochemistry and Wistar rats

INTRODUCTION

Jatropha gossypiifolia var. *gossypiifolia* is an ornamental shrub that belongs to the family Euphobiaceae. It is a native plant to tropical America, Mexico, Paraguay and naturalized in Africa and Asia. (Vincentz, 2006). The plant synonyms include: Bellyache, cotton leaf, physic nut, sibidigua and tua-tua. In Nigeria the shrub is grown around houses and variously called "Bini-da-zugu-baki" (Hausa), "kwolkwolaje" or "kolakolaje" (Ful-fulde),"botiye", "lobotiye" or "lapalapa" (Yoruba), "olulu-idu" or "owulu-idu" (Igbo),'igadam-gbara "(Tiv) and "Duzu" (Gbagyi), and the shrub is well adapted and spread in Nigeria especially in the arid northern region of the Country. It sheds its purple-red leaves in dry season and blossom during rainy season except when there is constant water supply.

The plant rarely grows more than 1 metre high with the leaves stalk covered with dark brown hairs, the flowers are purple and the fruits and seeds are small in size.

The distinctive colouration of this plant differentiates it from other poisonous variant such as *Jatropha curcas, J. podagrica, J. multifida* and *J. aceroides*.

Therapeutic and other uses of *J. gossypiifolia* which were reported by various workers include, management of ailments such as pains, and sores (Morton, 1981), Newcastle disease infection in chicken (Zakari, 2004),

*Author for correspondence; *E-mail:*shamaki@yahoo.com

Antimalarial effect (Gbeassor *et al*., 1989), Immune modulatory effect in HIV infected persons (Lolade, 2005) and the leave extract as an anticoagulant for biochemical and haematological analyses (Oduola *et al*., 2005).

Jatropha are generally considered to contain toxoalbumin as the toxic constituent and toxicity is reported to be due to dehydration and cardiovascular collapse as a result of haemorrhagic gastroenteritis. (Jourbert *et al*., 1984). However, this toxicity are reported for other variant of *Jatropha* other than *J. gossypiifolia* from which there is dearth of information on the effect of acute and repeated dose test of the extract of this plant or its products on weight changes and blood chemistry. These studies were therefore undertaken to evaluate the effects of the extract on weights, haematological and biochemical changes in rats..

MATERIALS AND METHODS

Animals

Thirty (30) albino rats of Wistar strain were used for this study. The rats were of different sexes. There weights range from 95.4 - 173.8 g. The blood of each animal was screened for the presence of haemoparasites by examination of wet smear and Giemsa stained smear of blood on a ×40 magnification as described by Herbert and Lumsden (1976). The animals were kept in groups of five rats/group and animals in a particular group are kept in the same cage which was cleaned three times in a week. They were fed with commercially formulated pelleted chick growers' mash (Vital feeds Nig. Ltd., Bukuru, Jos, Nigeria) and water was given *ad libitum*. Two weeks acclimatization period was allowed before the commencement of the experiments.

Extract

Two hundred (200 g) grams of the pulverized fresh stem of the plant were boiled in 1000 ml of distilled water for 10 minutes at 100ºC. This was allowed to cool for 24 hours and filtered using muslin cloth and finally Whatman's filter paper No.1. The filterate was kept in a clean conical flask. The residue was weighed to get the percentage yield of the plant. Evaporation of the filterate to get the extract was done below ambient temperature as described by Abdulkadir *et al*. (2005). A dark brown sticky paste was collected at the end of evaporation and this was kept dry in a closed glass jar until required for the experiment.

Studies on haematology and biochemistry

Five rats were used in acute toxicity study and they were placed in a single cage, each rat was marked according to its weight on specific sites on its body using picric acid for identification. Each animal received a single dose of 5000 mg/kg/body weight of the *J. gossypiifolia* var. *gossypiifolia* stem extract. Weights were monitored daily for 14 days as described by WHO (1991) in up and down acute oral toxicity method of OECD/OCDE test guidelines

Twenty five rats were used for repeated oral dose study. They were placed in five groups (A, B, C, D and E) of five (5) rats per group. Each animal in groups A, B, C, and D received 250, 500, 750 and 1000 mg/kg orally of the reconstituted aqueous stem extract of *Jatropha gossypiifolia* var. *gossypiifolia* respectively. Animals in group E serves as untreated control. The extract was administered daily for 28 days in repeated dose test. The experiment was terminated after 28 days. The animals were sacrificed on 29 day and blood and serum samples were collected for analysis.

Haematology.

Haematological parameters such as packed cell volume(PCV) white blood cell(WBC) counts, and red blood cell(RBC) counts haemoglobin concentration,(Hb)were determined by standard technique as described by Schalm *et al.* (1975).while mean corpuscular haemoglobin concentration (MCHC) was calculated, following daily administration of the extract and on days 14 and 28 of the experiment.

Blood biochemistry

Plasma electrolytes were determined before administration of the extract and on days 14 and 28 following daily administration of the extract. Plasma sodium, potassium and chloride ions were determined using the flame photometric method (Corning Model 410, corning scientific Ltd., England), as described by Van Slyke (1923). The plasma total protein, albumin and globulins levels were determined by the biuret method as described by (Cornal *et al*., 1949). Liver enzymes such as AST, ALP and ALT were also determined using calorimetric method as described by Reitman and Frankel (1957).Plasma bilirubin was determined by methods described by Jendrassik *et al*. (1938) and blood urea was estimated by diacetylmonoxine method as described by Marsh *et al.*,(1965).

Studies on weight changes

Weight changes were monitored concurrently with acute and repeated dose toxicity test. Initial weights of individual animals were observed before oral administration of the extract in both experiments and subsequently

three times per week, after which the total average weight of individual rat was used. The animals' weight in repeated dose toxicity study was compared to the control group.

Statistical analysis

Differences in the means (± SEM) between groups were assessed using analysis of variance(ANOVA). A p-value of 0.05 was considered significant.

RESULTS

Palatability test indicated that the extract of *Jatropha gossypiifolia* var. *gossypiifolia* may either be sweet or tasteless, following oral administration; the animals were active and shows no sign of irritant effect of the extract on the oral cavity. The extract was found to be tasteless after reconstitution with distilled water.

The oral administration of extract of *J. gossypiifolia* did not produced any significant changes in PCV, RBC, Hb and MCHC at the administered doses of 250 mg/kg, 500 mg/kg, 750 mg/kg and 1000 mg/kg respectively, it equally dose not produced significant ($p<0.05$) changes in WBC $(10)^3$ on day 1, 14 and 28 following oral administration of all the doses except 500 mg/kg which showed little effect on white blood cell value when compared to control (Table 1). However, the white blood cells showed slight increases which appeared not dose dependent but neutrophils produced significant ($p<0.05$) increase on day 14 and 28 at 500 mg/kg, while monocyte, eosinophils and basophils showed no changes in values when compared to control. (Table 2). Serum values of total protein, bilirubin and albumin were not affected (Table 3). Aspartate amino transferase (AST) and Alanine amino transferase (ALT) were equally not affected but Alkaline phosphatase (ALP) increased significantly ($p<0.05$) in the groups treated with 500 mg/kg, 750 mg/kg and 1000 mg/kg respectively (Table 3). Urea showed significant increase ($p<0.05$) at doses 250 mg/kg, 500 mg/kg and 1000 mg/kg while Na^+ and K^+ only showed increases at 750 mg/kg and 1000 mg/kg, respectively (Table 4). There was increase in weights as monitored weekly in the study period, (Tables 5 and 6) and all rats survived the study period. They were sacrificed on day 15 in acute toxicity studies and day 29 in repeated oral dose toxicity studies.

Table 1. The effect of the aqueous stem extract of *Jatropha gossypiifolia* var. *gossypiifolia* on haematological parameters of Wistar rats

Parameters	Dose (mg/kg per os)	Days		
		1	14	28
PCV(%)	Control	38.4 ± 0.5	33.4 ± 0.4	36.4 ± 1.7
	250	35.2 ± 1.1	35.2 ± 0.4	35.4 ± 0.5
	500	34.0 ± 0.3	33.0 ± 0.2	37.4 ± 0.2
	750	34.0 ± 3.5	38.0 ± 1.8	36.0 ± 0.7
	1000	38.2 ± 2.4	37.0 ± 0.3	31.6 ± 0.7
		0.8	11.9 ± 0.8	8.5 ± 1.4
WBC($10^3/\mu$l)	Control	11.1 ±		
	250	9.2 ± 0.8	18.3 ± 0.8*	5.6 ± 0.4
	500	7.7 ± 0.8	15.2 ± 0.3*	6.9 ± 1.0
	750	10.0 ± 1.0	11.9 ± 0.4	6.2 ± 1.0
	1000	15.5 ± 0.2*	17.9 ± 0.6*	4.9 ± 0.6
RBC($10^6/\mu$l)	Control	8.3 ± 1.4	7.7 ± 1.3	5.9 ± 6.4
	250	8.2 ± 0.4	5.5 ± 0.4	6.7 ± 0.1
	500	7.5 ± 0.3	6.5 ± 0.2	7.9 ± 1.0
	750	7.5 ± 1.4	8.7 ± 0.7	6.9 ± 0.2
	1000	7.0 ± 1.7	7.7 ± 0.9	6.4 ± 0.2
Hb (g/dl)	Control	0.446 ± 0.0	0.332 ± 0.03	0.421± 0.01
	250	0.441 ± 0.05	0.321 ± 0.01	0.405± 0.4
	500	0.329 ± 0.03	0.318 ± 0.03	0.392± 0.03
	750	0.441 ± 0.05	0.321 ± 0.01	0.405± 0.4
	1000	0.352 ± 0.04	0.335 ± 0.0	0.307 ± 0.01

Table 1. (Continued)

Parameters	Dose (mg/kg per os)	Days		
		1	14	28
MCHC (g/dl)	Control	1.19 ± 0.4	4.2 ± 0.4	7.2 ± 0.6
	250	1.3 ± 0.1	5.8 ± 0.3	6.1 ± 0.6
	500	0.97 ± 0.2	5.2 ± 1.3	5.1 ± 1.0
	750	1.32 ± 0.3	4.1 ± 0.6	5.3 ± 0.2
	1000	0.92 ± 0.1	4.4 ± 0.6	5.4 ± 0.2

*Statistically, significant at ($p<0.05$) when compared to control All result presented as mean standard deviation.

Table 2. The effects of the aqueous stem extract of *Jatropha gossypiifolia* var. *gossypiifolia* on differential leucocyte counts of Wistar rats

Parameters	Dose (mg/kg per os)	Days		
		1	14	28
Lymphocytes (10^9u/l)				
	Control	11.5	11.4	11.4
	250	11.8	12.0	11.5
	500	12.8	11.6	10.7
	750	12.3	10.7	10.3
	1000	11.5	11.5	11.5
Neutrophil (10^9u/l)				
	Control	4.7	5.3	4.6
	250	4.8	4.0	4.6
	500	3.0	5.3	5.0
	750	4.4	5.9	6.2
	1000	4.3	5.2	5.2
Monocytes (10^9u/l)				
	Control	1.3	1.5	1.5
	250	1.0	1.6	1.5
	500	1.5	0.8	1.8
	750	0.8	1.2	1.0
	1000	1.6	1.1	1.3
Eosinophils (10^9u/l)				
	Control	0.8	0.3	0.2
	250	0.6	0.5	0.5
	500	0.4	0.5	0.9
	750	0.6	0.3	0.4
	1000	0.6	0.5	0.4
Basophil (10^9u/l)	Control	0.0	0.0	0.0
	250	0.0	0.0	0.0
	500	0.0	0.0	0.0
	750	0.0	0.2	0.0
	1000	0.0	0.5	0.0

Statistically, significant at ($P<0.05$) when compared to control All result presented as mean standard deviation.(absolute values calculated from total WBC of 18.2x10^9u/l)

Table 3. The effects of the aqueous stem extract of *Jatropha gossypiifolia* var. *gossypiifolia* on serum liver enzymes, protein and bulirubin of Wistar rats

	Experimental groups (Dose mg/kg)				
Parameters	Control	250	500	750	1000
Total bilirubin (mg/dl)	0.93±0.2	0.9±0.1	0.9±0.2	0.8±0.1	1.1±0.04
Cngugated bilirubin(mg/dl)	0.4±0.2	6.8±0.6	7.4±0.8	6.6±6.7	0.4±0.7
Total protein (g/dl)	6.7±0.6	6.8±0.6	7.4±0.8*	6.6±0.7	7.3±0.7
Albumin(g/dl)	4.5±0.3	4.6±0.2	4.7±0.2	4.4±0.2	4.5±0.2
AST(I.U/l)	28.0±0.8	28.0±0.5	34.0±0.9*	27.0±0.4	31.0±0.2*
ALT (I.U/l)	17.2±0.5	17.2±0.4	20.0±0.6*	16.0±0.4	19.0±0.2*
ALP(I.U/l)	34.5±1.7	34.9±0.8	72.5±1.3*	58.0±0.2*	462.3±1.4*

*Statistically, significant at ($p<0.05$) when compared to control

Table 4. The effects of the aqueous stem extract of *Jatropha gossypiifolia* var. *gossypiifolia* on blood urea and electrolytes of Wistar rats

	Experimental groups (Dose mg/kg)				
PARAMETERS	Control	250	500	750	1000
Urea (mmol/l	1.4±0.1	7.6±0.9*	7.7±0.4*	7.4±0.8	7.0±0.6*
Na^+ (mmol/l)	126.0±0.1	130.0±0.4	130.0±0.1	137.0±0.8*	131.0±0.05*
Cl^+ (mmol/l)	100.0±0.1	50.0±0.9	90.1±0.1	95.0±0.01	102.0±0.1
K^+ (mmol/l)	3.0±0.2	3.7±1.3	3.5±0.7	6.3±1.1	4.5±1.3

*Statistically, significant at ($p<0.05$) when compared to control

Table 5. Weight changes and survival of the experimental rats in acute toxicity studies

S/No.	Sex	Initial weight (g)	Final weight (g)	Dose of extract (mg/kg)	Amount administered orally (mg)	Volume administered orally (mg)	Survival after two weeks
1	M	108.1	120.2 ± 0.31	5000	540.4	1.80	Survived
2	F	104.4	116.9 ± 0.06	5000	522.0	1.74	Survived
3	M	101.1	112.9 ± 0.47	5000	505.5	1.70	Survived
4	M	95.4	104.2 ± 0.00	5000	477.0	1.59	Survived
5	F	109.1	110.5 ± 0.75	5000	545.5	1.81	Survived
6	F	103.2	112.2 ± 0.01	5000	521.1	1.71	Survived

All doses are based on initial weight

DISCUSSION

The aqueous stem extract of *J. gossypiifolia* var. *gossypiifolia* did not produce any remarkable alterations in blood parameters but a time-related increase in WBC values in treated rats, as was observed in differential

leucocytic counts. Where a slight icrease in neutrophils was observed. Toxicity which was reported in other variants of *Jatropha* (Ojewole and Odebiyi, 1989; Jourbert *et al*., 1984) was not observed although other reports on phytochemical studies (Shamaki *et al*., 2008) indicated the presence of cardiac glycosides, alkaloid and steroids, reported case of toxicity associated with this extract (Aplin, 1976) was not observed from onset of administration where there was absence of symptoms of oral cavity irritation. These is due to the method of extraction using heat in which some phytotoxins, which is reported to be thermolabile (Kingsbury, 1964) are denatured. Shamaki *et al*. (2008) reported no presence of this phytotoxins in extract from fresh plant of *Jatropha gossypiifolia*. Reports of poisoning in *Jatropha* is generalized among the variants (Aplin, 1976). However, despite its similarity to *J. curcas* (toxic variant), the only toxic sign observed in this work was polydipsia and this was not extreme as reported (Aplin, 1976). This polydipsia is equally associated with polyuria suggesting absence of cytotoxic effect of the extract of *Jatropha* on the kidney and glomerular filtration time, hence the steady weights increases in the rats within the study period. This phenomenon was equally related to the normalcy of plasma electrolytes and slight increases in the plasma urea values coupled with unaltered values of liver enzymes levels.

Table 6. Effects of the aqueous stem extract of *Jatropha gossypiifolia* var. *gossypiifolia* on weekly weight of the rats in repeated dose tests

Groups	Wk 1 (g)	Wk 2 (g)	Wk 3 (g)	Wk 4 (g)	Weight differences Final - initial weight (g)	Survival after 28 days
100 mg/kg	141.1	150.2	148.4	165.0	23.9	All survived
7850 mg/kg	173.8	177.4	185.9	187.3	13.5	,,
500 mg/kg	131.8	153.7	163.0	17.7	40.9	,,
250 mg/kg	131.7	146.9	156.1	161.8	30.1	,,
Control	125.9	141.9	151.9	155.5	29.6	,,
Mean					27.6	
Mean ± SEM					27.6 ± 0.0	

Values are statistically significant at $p<0.05$ when compared to control

The anticoagulant properties of the leave extract of this plant studied by Oduala *et al*. (2005) was not observed in this work, however, phytochemical studies of the stem extract of this plant (Shamaki *et al*., 2008) showed presence of lots of organic and inorganic elements that can be co-factors to RBC formation and as co-enzymes in biochemical reactions, although various parts of same plants can present difference in phytochemical components (Sofowora, 1993).

Weights of rats monitored simultaneously with the studies showed constant and steady non dose dependent increases until termination of the work in both sets of experiments. This was associated with the increase in feed and water consumption of the treated rats, which was equally related to the presence of unclassified steroid content of this plant (Shamaki *et al*., 2008). This steroid might be stimulating feeding centres or acting similar to curcin II, which is reported to be active in protein synthesis. (Stirpe *et al*., 1976). The increase in fat deposits observed in the subcutaneous tissues and mesentery of the intestines after sacrificing the rats, suggested this findings. The steroids may also complements the oncotic pressure exerted by albumin in controlling dehydration since administration of this extract did not produce any alteration in plasma albumin levels. In addition to the alkaloid contents that was reported to have antimicrobial and anti irritant effect in the gastrointestinal tract (Idowu *et al*., 2003) increase in neutrophils may also add to the antimicrobial activity of this extract, hence, the absence of enteritis.

The results from this study provides haematological basis, in addition to Oduala *et al*. (2005) for further investigation into the medicinal potential of this plant. The absence of related changes in blood parameters together with the absence of any serious toxic sign, although, doses of 250 mg/kg - 1000 mg/kg was administered for 28 consecutive days in repeated dose toxicity study, and 5000 mg/kg body weight single oral administration in acute toxicity study, suggested non toxic effect of this plant extract. In further studies, attention should be particularly given to the steroid component that might be responsible for the water retention property of this plant, and a healthy source of plant steroid that can be exploited for fattening programmes in animals.

In conclusion, the extract is not toxic on blood parameters and can be safely exploited for plant source of steroids and antimcrobials.

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