
HAEMATINICS AND WEIGHT REDUCTION PROPERTIES OF ETHANOL EXTRACT OF JATROPHA CURCAS SEEDS IN RATS**Koker Ayesha¹, *Samai Mohamed¹, Onome T. Abiri¹, Mohamed Bawoh¹, and Kwanashie O. Helen².**

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ABSTRACT

Despite the potential uses of *Jatropha curcas* (JC) very little is known about its effects on the blood cells. The current study was therefore undertaken to investigate the effect of JC seed from Sierra Leone on different haematological parameters.

Ethanol extract of JC was administered daily to adult male and female Wistar rats (120-200g) either intraperitoneally or orally for 3 days or 7 days respectively. For each treatment route the rats were divided into six sub-groups as follows: group 1 rats (control group) received 0.9 % physiological saline (i.p.) or de-ionised water (p.o.) at 10 ml/kg, while rats in groups 2, 3 and 4 received 50, 100 and 200 mg/kg dose of the extract respectively; and groups 5 and 6 rats received iron (5 mg/kg) and EDTA (40 mg/kg) respectively. The rats were weighed before and after the treatment period, and at the end of the treatment period blood samples were collected by cardiac puncture. The following haematological parameters were evaluated: RBC, WBC, lymphocytes, neutrophil counts, Hb, HCT concentrations (with MCV, MCH, MCHC values calculated). Data obtained were analysed by one way ANOVA using the statistical software Graph Pad Prism version 6; with $p < 0.05$ taken to be statistically significant.

The extract decreased the body weights of the rats in a dose, route and sex dependent manner; with i.p. > p.o., male > female. The extract also significantly ($p < 0.05$) increased the mean Hb, HCT, MCV, MCH, MCHC, RDW values and neutrophil counts; while WBC and lymphocyte counts were decreased by the extract. The aforementioned increases in the mean Hb and RBC-related indices were to a large extent dose-dependent, with peak effects recorded at 100 mg/kg. It was concluded that *J. curcas* seeds could be exploited for haematinics benefits, provided its detailed pharmacokinetic and safety profiles have been documented.

Key words: Haematinics, Plant extract, *Jatropha curcas*, Wistar rats, Weight, Obesity.

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INTRODUCTION

Sierra Leone, like many other African countries, has a diverse healthcare system with traditional medicines forming part of the primary healthcare, though informally. It is known that there is a strong coexistence between western medical system which is based on modern scientific medicine and a variety of non-conventional therapies, including a multiplicity of local indigenous systems founded on traditional beliefs and practices. African traditional healing forms part of African culture and today traditional healers remain essential for the health and well-being of a large proportion of the population particularly the black population (Van der Linde, 1997). A survey conducted by WHO in 2008, indicated that about 80% of the Sierra Leonean population rely on, and resort to traditional medicine practitioners (WHO AFRO, 2008).

Jatropha curcas is one of several species of *Jatropha* plants cultivated globally including across West Africa. The plant *Jatropha curcas* (JC), produces seeds that are a source of biodiesel and also contain several metabolites of pharmaceutical importance (Okoli et al., 1994). For instance the seeds have been shown to be useful in the management of dropsy, gout and paralysis, and the seed oil have been applied to treat skin diseases such as eczema and to soothe rheumatic pain (Okoli et al., 1994). Okoli and colleagues in their publication concluded that the linoleic acid content in JC seed oil could be of interest for the skincare industry (Okoli et al., 1994). Despite these and other potential uses of JC seeds and other parts of the plant, the effects of JC on the blood cells have not been investigated and documented.

Blood being a specialized body fluid that supplies the tissues with nutrients, oxygen and removes harmful waste is composed of red blood cells (RBCs), white blood cells (WBCs) and platelets. Anaemia which is characterised by low levels of RBCs, and therefore lack of oxygen-carrying capability of the blood, is the most common haematological disorder globally. It is particularly a very big problem in Sierra Leone affecting at least 45% women and young children (DHS, 2008). Deficiency of WBCs (defence system against bacterial and viral infections) can lead to many immune diseases such as rheumatoid arthritis, German measles or rubella (Liang, 1999). Derangement of platelet levels affects blood

clotting while those of serum electrolyte destabilizes homeostasis.

Since JC is known for its extensive traditional uses, it may be a good precursor for a potential drug source with known safety profiles (Gadir et al., 2003). As there are known variations in the composition of JC from across the world the effect of JC therefore varies in different parts of the world (King et al., 2009). Moreover, based on the relevance of the blood and the potential uses of JC seed, it is clear that investigations into the effect of JC seed on haematological indicators, will address a clear research need. Thus as very little has been published or reported on the haematological effect and toxicity of the seeds, the current study aims to investigate the effect of JC seed from Sierra Leone on the different haematological parameters and to establish whether JC has any haematinics property.

MATERIALS AND METHODS

Experimental Animals

A total of one hundred and twenty Wistar rats (120-200g) of both sexes purchased from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria were used for the study. The animals were allowed free access to standard rodent diet (brand) from vital feeds and clean drinking water throughout the experimental period. They were housed in cages (about 12 to a cage) in a room kept at room temperature which was relatively humid. Male rats were kept in cages separate from the female rats to avoid the females getting pregnant during the experimental period. The animals received humane care and the study protocol was in compliance with the university's guidelines for the use of laboratory animals. The experimental protocol was approved by the Ahmadu Bello University's Ethics Committee.

Plant extraction process

Dried JC fruits were collected on the 21st. May 2012 at Calaba Town in the Eastern part of Freetown, Sierra Leone and were authenticated by a Taxonomist Alhaji B.M.S. Turay, Head, Department of Pharmacognosy and Dean, Faculty of

Pharmaceutical Sciences, College of Medicine and Allied Health Science.

Jatropha curcas fruits were de-shelled to obtain the seeds and were pulverized using a mortar and pestle. 350.04g of the plant material was defatted for 72 hours at room temperature via Soxhlet apparatus using petroleum ether (60-80°C) as the extraction solvent. The petroleum ether extract was evaporated using a heating mantle and the oil was collected and weighed.

The defatted marc was dried at room temperature for 4 hours, placed in a separating funnel for cold extraction using 1L of 70% ethanol for 48 hours. The ethanol extract was collected in a beaker, the marc rinsed with 500 mL of 70% ethanol and collected as well. Two crucibles were weighed and the filtrate was poured into them. These crucibles containing the filtrate were heated in a water bath at 55°C to evaporate the marc filtrate. Thereafter, the crucibles were weighed and the ethanol extract stored in an airtight container for future use. The extract came out as semi-solid dark brownish paste-like material with a total yield of 14g of the dried extract (4% of the plant material).

Dosing of the experimental animals

The ethanol extract of JC was administered to adult male and female (120-200g) wistar rats either intraperitoneally or orally. For each treatment route of drug administration, the rats were subdivided into six groups.

Intraperitoneal treated Rats

A total of 72 adult Wistar rats (120 -200g) of both sexes were treated via intraperitoneal (IP) route and were divided into 6 sub-groups. The JC extract dissolved in normal saline (NS) was administered daily as a single dose to the first, second and third groups of rats at 50, 100, and 200 mg/kg respectively for three days. The fourth group (control group) received equal volume of the NS daily for three days; whilst the fifth and sixth groups received single daily doses of iron (5 mg/kg) and EDTA (40 mg/kg) respectively for three days.

Orally treated Rats

A total of 48 adult Wistar rats (120 to 200 g) of both sexes received single daily doses of the extract and the other drugs via oral route daily for 7 days. They were also divided into six groups and treated orally as described above.

Haematological studies

At the end of the experimental period, the rats were anaesthetized using light chloroform and blood was collected from each rat by cardiac puncture into labelled heparinised bottle. The blood samples were taken to the haematology department of the Sick Bay at Ahmadu Bello University and the following haematological parameters were determined using an auto analyser: RBC count, MCV, RDW, MCH, MCHC, Hb, HCT or PCV; Platelet count; and WBC and differential count.

In addition to the haematological analysis, the rats were weighed prior to administration of JC extract and other experimental drugs (day 0) and at the end of the experimental periods, day 4 for IP route and day 8 for the oral route. The weights of the rats at the end of the experimental periods were compared to day 0 for the two routes.

Statistical analysis

Results are expressed as mean \pm SEM. Means were obtained from multiple experiments performed in triplicates. Data were analyzed and graphed with the commercially available statistical software graph pad prism version 6 (Graphpad software, San Diego, CA, USA). Differences between mean values within the groups were determined by one way analysis of variance (ANOVA) followed by Dunnett's (post hoc) test for comparison of multiple means. Differences between mean values for different groups were tested for using the unpaired, two-tailed Student's t-test. The level of significance was set at $p < 0.05$.

RESULT

Body weight of the Rats

IP administration of JC (50, 100 and 200 mg/kg) to the rats produced a dose dependent reduction in their body weights; and the reductions were statistically significant at 100 and 200 mg/kg ($p < 0.05$; Figure 1A). However, when the rats were disaggregated by sex, statistically significant reductions in weight were still noted with 100-200 mg/kg dose of the extract for the males ($p < 0.05$; Figure 1B) but only 200 mg/kg dose of JC produced a significant reduction in body weight for the female rats ($p < 0.05$; Figure 1C). It was also found that iron and EDTA at the doses used had no effect on body weights of the rats irrespective of the route of administration {Figures 1(A-C)}. Unlike the IP route, when similar doses of the JC extract were administered to the rats daily for 7 days orally, there were no statistical significant effects on their body weights (result not shown).

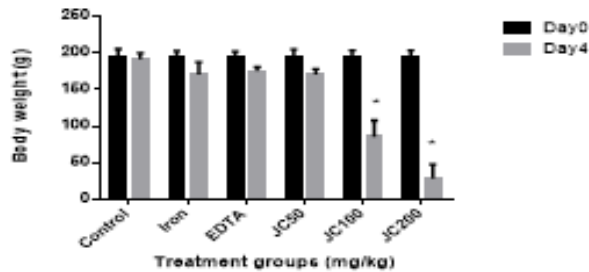


Fig. 1A

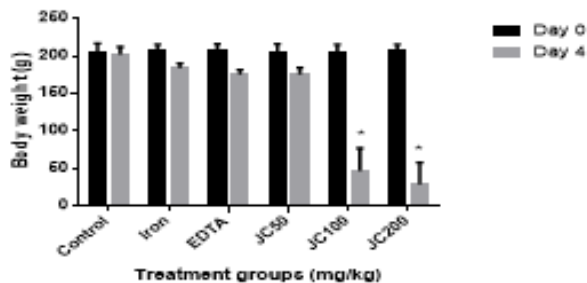


Fig. 1B

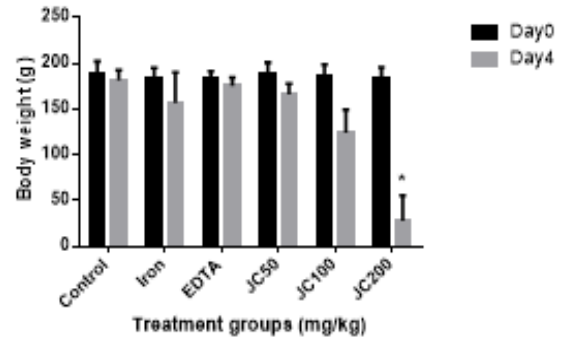


Fig. 1C

Figure 1(A-C): The effect of ethanol extract of JC on the mean body weight of rats (A) both sexes, (B) males and (C) Females. The rats were treated with NS, JC (50, 100 and 200 mg/kg), iron (5 mg/kg) and EDTA (40 mg/kg) intraperitoneally for 3 days and their mean weight computed on day 4. N=8 for each sub-group. * $p < 0.05$ vs. control group.

Haematological Indices

Hematological parameters such as RBC, MCV, RDW, MCH, MCHC, Hb, HCT, Platelets, WBC and differential count (neutrophils and lymphocytes) were determined in each treatment group after IP and oral administration of the experiment drugs on days 4 and 8 respectively.

Irrespective of the route used, the doses of JC extract administered had no statistical significant effect on the RBC count of the rats when compared to the control group ($p > 0.05$; Tables 1-2). Nevertheless, following the IP administration of the JC extract a fairly dose dependent increase in Hb, HCT, MCV and MCH was noted up to the 100 mg/kg dose. The increase produced by the 100 mg/kg dose of the JC extract was found to be statistically significant ($p < 0.05$; Table 1). Increase in the dose of the extract to 200 mg/kg did not produce further increase in these RBC parameters. Furthermore, 100 mg/kg IP dose of the JC extract significantly reduced platelet count when compared to the control group ($p < 0.05$; Table 1). With regards to WBC and differential counts, the extract

administered via IP route at 50 mg/kg, significantly reduced WBC and lymphocytes but significantly

increased neutrophils when compared to their respective controls ($p < 0.05$; Table 1).

A statistically significant increase in Hb, HCT, MCV and MCH were noted in rats treated via the IP route with 5 mg/kg iron and 40 mg/kg EDTA when compared their respective control groups ($p < 0.05$; Table 1). The platelet count was significantly reduced by IP administration of 5mg/kg dose of iron ($p < 0.05$) whereas IP administration of 40 mg/kg EDTA had no significant effect on the platelet count (Table 1).

effect on the haematological parameters of the rats when compared to the control groups (Table 2) except for MCH count, which was significantly reduced by the JC extract at 50 mg/kg (15.55 ± 2.23) and 100 mg/kg (16.00 ± 2.31) dose ($p < 0.05$; Table 2). Furthermore, with the exception of MCH which was significantly reduced by the oral administration of 40 mg/kg EDTA ($p < 0.05$), oral EDTA and iron at the doses used had no significant effect on the haematological parameters of the rats (Table 2).

Unlike the IP administration of the JC extract, oral administration produced no statistically significant

Table 1: The Effect of IP Ethanol Extract of JC on Haematological Indices in Rat

Parameters	Control	JC 50 mg/kg	JC 100 mg/kg	JC 200 mg/kg	Iron	EDTA
RBC ($\times 10^6/\mu\text{L}$)	8.00 \pm 0.16	7.67 \pm 0.25	8.19 \pm 0.24	7.31 \pm 0.00	8.38 \pm 0.30	7.32 \pm 0.43
HCT (%)	42.18 \pm 0.23	45.45 \pm 1.44	49.69 \pm 1.54	40.60 \pm 0.00	49.40 \pm 1.86	43.24 \pm 1.84
Hb (g/dl)	12.93 \pm 0.09	14.41 \pm 0.35	15.65 \pm 0.39	13.10 \pm 0.00	15.29 \pm 0.47	13.65 \pm 0.51
MCV (fl)	52.89 \pm 1.13	59.38 \pm 0.73	60.66 \pm 0.88	55.50 \pm 0.00	58.89 \pm 0.38	59.45 \pm 1.07
MCH (pg)	16.25 \pm 0.44	18.89 \pm 0.32	19.11 \pm 0.21	17.90 \pm 0.00	18.26 \pm 0.11	18.84 \pm 0.45
MCHC (g/dl)	30.65 \pm 0.18	31.76 \pm 0.25	31.55 \pm 0.25	32.30 \pm 0.00	30.99 \pm 0.31	31.60 \pm 0.25
PLT ($\times 10^3/\mu\text{L}$)	646.38 \pm 9.48	622.50 \pm 72.13	393.50 \pm 29.07	474.00 \pm 0.00	521.75 \pm 33.66	636.88 \pm 41.03
WBC ($\times 10^3/\mu\text{L}$)	16.73 \pm 1.07	8.90 \pm 0.44	9.84 \pm 0.80	15.50 \pm 0.00	12.99 \pm 2.00	11.44 \pm 1.36
Lymphocytes ($\times 10^3/\mu\text{L}$)	87.10 \pm 0.94	64.69 \pm 3.03	52.64 \pm 6.12	29.70 \pm 0.00	70.73 \pm 1.71	67.66 \pm 4.83
Neutrophils ($\times 10^6/\mu\text{L}$)	12.90 \pm 0.94	35.31 \pm 3.03	47.36 \pm 6.12	70.30 \pm 0.00	29.28 \pm 1.71	32.34 \pm 4.83

The rats were treated with NS, JC (50, 100 and 200 mg/kg), iron (5 mg/kg) and EDTA (40 mg/kg) intraperitoneally for 3 days and their RBC count, HCT, Hb, MCV, MCH, MCHC, WBC and differential count, and platelets were determined on day 4. $N=8$ for each sub-group and results were expressed as mean \pm SEM. * $p < 0.05$ vs the control.

Table 2: The Effect of Oral Ethanol Extract of JC on Haematological Indices in Rat

Parameters	Control	JC 50 mg/kg	JC 100 mg/kg	JC 200 mg/kg	Iron	EDTA
RBC ($\times 10^6/\mu\text{L}$)	7.11 \pm 0.16	6.59 \pm 0.95	6.32 \pm 0.92	7.27 \pm 0.42	7.58 \pm 0.18	6.91 \pm 1.04
HCT (%)	43.25 \pm 0.52	37.86 \pm 5.50	36.61 \pm 5.31	42.34 \pm 2.05	45.03 \pm 0.75	40.91 \pm 6.14
Hb (g/dl)	13.48 \pm 0.13	11.70 \pm 1.69	11.51 \pm 1.66	13.34 \pm 0.60	13.89 \pm 0.21	12.56 \pm 1.86
MCV (fl)	57.13 \pm 3.86	50.26 \pm 7.20	50.79 \pm 7.30	58.44 \pm 0.96	52.36 \pm 7.50	51.83 \pm 7.41
MCH (pg)	24.28 \pm 5.29	15.55 \pm 2.23	16.00 \pm 2.31	18.45 \pm 0.40	18.34 \pm 0.26	15.98 \pm 2.29
MCHC (g/dl)	29.69 \pm 1.58	27.06 \pm 3.87	27.55 \pm 3.95	31.68 \pm 0.16	29.60 \pm 1.31	27.09 \pm 3.88
PLT ($\times 10^3/\mu\text{L}$)	744.75 \pm 38.87	594.50 \pm 92.89	690.50 \pm 104.90	606.50 \pm 33.86	730.38 \pm 53.57	595.38 \pm 99.23
WBC ($\times 10^3/\mu\text{L}$)	17.09 \pm 2.80	16.83 \pm 3.30	12.54 \pm 2.20	14.76 \pm 2.76	21.61 \pm 2.59	15.83 \pm 2.88
Lymphocytes ($\times 10^3/\mu\text{L}$)	85.43 \pm 1.50	74.26 \pm 10.79	64.51 \pm 9.71	64.78 \pm 10.80	82.34 \pm 0.75	71.19 \pm 10.21

The rats were treated with NS, JC (50, 100 and 200 mg/kg), iron (5 mg/kg) and EDTA (40 mg/kg) orally for 7 days and their RBC count, HCT, Hb, MCV, MCH, MCHC, WBC and differential count, and platelets were determined on day 8. $N=6$ for each sub-group and results were expressed as mean \pm SEM. * $p < 0.05$ vs the control

The rats were treated with NS, JC (50, 100 and 200 mg/kg), iron (5 mg/kg) and EDTA (40 mg/kg) intraperitoneally for 3 days and their RBC count, HCT, Hb, MCV, MCH, MCHC, WBC and differential count, and platelets were determined on day 4. N=8 for each sub-group and results were expressed as mean \pm SEM. * $p < 0.05$ vs the control.

DISCUSSION

The JC seed extract significantly reduced the body weights of the rats in a dose and route dependent manner. This was evidenced by a progressive and significant reduction in body weight following IP treatment of the rats with varying doses of the JC extract (50, 100 and 200 mg/kg). A greater reduction in the body weights of the male rats was produced by the JC extract when compared to their female counterparts, indicating a gender dependent reduction. However, at the end of the experimental period, three of the female rats were found to be pregnant, which may have accounted for the gender variation. This may also explain in part why females are excluded from most clinical trials as pregnancy may influence the outcome of most studies or the drug may influence the pregnancy.

The route dependent difference in weight reduction may be due to the differences in the pharmacokinetic profile following IP and oral administration such as, incomplete absorption, hepatic first-pass effect and therefore bioavailability. Since the pharmacokinetic profile of the JC extract has not been fully documented, it is plausible that the oral bioavailability of the JC extract was reduced due to a combination of factors. Furthermore, one would have expected the protracted oral administration of the extract to reduce the mean body weight of the rats. However, this was not the case, possibly because the serum concentration achieved and/or the exposure period following oral administration was not sufficient enough. In a separate and independent study in our laboratory by our group, in which phytochemical analysis of the JC extract was conducted, JC was shown to contain tannins which significantly reduce food intake (Glick, 1981 and Frutos et al., 2004). Thus, the reduction in body weights may not be unrelated to the presence of tannins in the extract and therefore the reduction of food intake by the rats. Unlike the JC extract, the conventional haematinic (iron) and the known chelating agent (EDTA) at the doses used had no

effect on the body weights of the rats irrespective of the route of administration.

The results also show that IP administration of the JC extract had no effect on RBC count, increased RBC indices and neutrophils, but reduced WBC and lymphocytes. The RBC count was not affected partly because the balance between the rate of production and destruction of the RBC was not altered (Adebayo et al., 2010). Although the extract had little or no effect on the RBC count, it significantly increased Hb, HCT, MCV, MCH, MCHC and RDW levels. Since MCV, MCH and MCHC are all related to individual red blood cells whilst Hb and HCT are associated with the total population of red blood cells in a sample, it is possible that the extract increases the production of the red cells which was nullified by a concomitant increase in the destruction of the RBCs. Hence there was no difference in the total RBC counts. Furthermore, these parameters were progressively increased by the extract up to a dose of 100 mg/kg. Thereafter, a further increase in dose did not increase the levels of these parameters. Thus, in this study, the optimal dose for increasing these parameters is 100 mg/kg. The effects produced by the extract on the RBC indices were similar to that produced by iron which is a conventional haematinic. Since anaemia is characterized by reduced haemoglobin, and or haematocrit levels (Provan, 1999), the extract could be a very useful haematinic agent in correcting anaemia. Unlike iron and the extract, EDTA had no effect on the RBC indices.

J. curcas extract reduced WBC and lymphocytes, but increased neutrophil counts. JC extract was shown in a separate and independent study in our laboratory by our group to contain phytochemicals such as saponins, tannins, flavonoids and alkaloids which have deleterious effects in animals (Adedaop and Abatan, 2005; Miles et al., 1993). Although the effects of these phytochemicals on WBC counts and its differentials have not been documented, the reduction of WBC and lymphocyte counts may not be unrelated to the direct or indirect actions of these phytochemicals. Since JC extract is useful in the management of several disease conditions such as arthritis, parasitic skin diseases (Heller, 1996), jaundice (Oliver-Bever, 1986), treatment with JC product may elevate neutrophil level leading to a false impression of bacterial infection. Furthermore, Swenson and Reece (1993) noted that toxic plants do not produce a direct effect on white blood cells

such as neutrophils and lymphocytes, thus the alterations in WBC and its differentials may not necessarily be due to the direct toxic or harmful effect of the extract. In addition, the effect of the known chelating agent (EDTA) and the conventional haematinic (iron) on WBC and its differentials were similar to those produced by the extract.

With regards to the platelet counts, the extract produced a dose dependent reduction in platelet counts after IP administration. Although the effects of the extract on bone marrow suppression and platelets reduction have not been documented, this reduction may not be unrelated to the phytochemicals contained in this extract.

In concluding, the study shows that ethanol seed extract of JC increases HCT, Hb and other RBC parameters except RBC count. These haematinic properties may be exploited in the management of anaemia provided its safety profile particularly on platelet count is well investigated and documented. In addition, the weight reduction property of this extract could also be explored in the treatment of obesity.

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