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THE EFFECT OF ETHANOL EXTRACT OF JATROPHA TANJORESIS ON HAEMATOLOGICAL AND HISTOPATHOLOGICAL PROPERTIES OF PHENYL HYDRAZINE INDUCED ANAEMIC RAT

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ABSTRACT

Background: Herbal medicine has become an indispensable means of the health care system globally. Jatropha tanjorensis is a commonly consumed leafy green plant in Nigeria and used in the treatment of anaemia and other ailments.
Objectives: This study investigated acute toxicity and the effect of ethanol extract of Jatropha tanjorensis on haematological and histopathological properties of phenyl hydrazine induced anaemic rats.
Methods: Fresh leaves of Jatropha tanjorensis were obtained from Sango otta, authenticated and identified in the department of Botany University of Lagos. The dried leaves were subjected to soxhlet extract using 70% ethanol. A total of fifteen mice and fifty-four male Wistar rats weighing 18-23 gram and 74-131gram respectively were used for the research. Lorke's method was used to determine acute toxicity. They were divided into six groups of nine rats. Groups, I, II, III, V and VI are induced with anaemia using phenylhydrazine. Groups I, II, and III were treated with 200, 400 and 800 mg/kg of the extract respectively. Group IV served as control and was not induced nor treated. Rats in group V were treated while those in group VI received orthodox drug orheptal. The rats were sacrificed after treatment by cervical dislocation and blood collected by ocular puncture and kept in an anti-coagulant bottle and organs liver and spleen were fixed in formal saline for histological analysis. The blood samples were analyzed using an auto Haematological analyzer.
Results: The result showed a significant increase in RBC, Hb, PCV, WBC and PLT in the group treated with 200mg/kg, 400mg/kg and 800mg/kg. There were no significant changes in MCH, MCHC in all treated groups. However, at 200mg/kg, the spleen formed a follicle. The surrounding sinus is not congested. There was no abnormality discovered in the hepatocyte.
Conclusions: The extract has LD50 of 3162 mg/kg body weight and shown to reverse the anaemic status of the induced rats.

Keywords: Anaemia, *Jatropha tanjorensis*, Haematological, Histopathological, Phenyl hydrazine

INTRODUCTION

Herbal Medicine (HM) is the fulcrum of complementary and alternative medicine, which in recent times is increasingly gaining widespread popularity all over the world and gradually streaming toward integration into the mainstream health care system. It can be defined as plant-derived product used for medicinal and health purposes (Bauer, 2000).
The use of herbal medicine appears to be universal in a different culture, however, the plant used for the same ailments and the modes of treatment may vary from place to place. There are many plants been used in traditional medicine for many years without scientific data to back their efficacy. In those cases, the plants are called crude drugs of natural or biological origin. (Tabui, 2006, Anoka, 2012). The herbs could be in different forms including leaves, stems, flowers, roots and seeds. Where the plant is macerated with water, alcohol, or other solvents to extract, some of the chemicals. The resulting products contain dozens of chemicals including fatty acids, steroids, alkaloids, flavonoids, glycosides, saponins and others (Rotblah and Ziment, 2000). The plant *Jatropha tanjorensis* has been reported to treat anaemia, Krishan, 2018.

Therefore such plants should be investigated to understand their properties, safety and efficacy, (Naseimento, 2000). Any plant organ such as a root, stem, leaf, flower or fragrant has medicinal properties (Krishan, 2018). Medicinal plants are used both as crude or modern medicine after purification smith *et al.,* 2012).

However, the knowledge of herbal medicine for the treatment of diseases continued to be the practicing of herbalists of plant scientists with the belief that herbal medicine will lose its potency if revealed to other people. Although some herbs may have medicinal value sometimes the medicine preparation inflict certain side effect. Today, many studies focused on the knowledge of the medicinal uses of plants and the scientific investigation to confirm their medicinal value (Oladeji, 2016). The majority of the world in habitat rely mainly on traditional medicine for their primary health care (Owolabi *et al.,* 2007). Healing with the medicinal plant is as old as mankind itself. Leaf extract of *Jatropha tanjorensis* has been reported to possess blood cholesterol-lowering properties hence useful in the treatment of cardiovascular diseases caused by hyperlipidemia (Oyewole and Akingbala, 2011). Its antibacterial activities have been shown (Iwalewa *et al*., 2005).

Furthermore, its antioxidant properties have been reported (Atansuyi, *et al.,* 2012). As well as anticancer, hepatoprotective and pesticidal activity (Anwar, 2007).

Numerous studies are revealing the association between herbal medicine and its adverse effect (Huxable, 1990, De Smet, 2004).

Anaemia is a condition in which there is a scarcity of healthy red blood cells that transfer the proper amount of oxygen to all parts of the body. The concentration of haemoglobin in men who have anaemia is below 13g/dl and in young girls more than 15years it is below 12g/*dl* (Contaaldo *et al*, 2019). It is curable at an early stage and in acute state it is serious and may be fatal (Estcourt *et al.,* 2016). Anaemia is a health problem affecting both developing and developed countries worldwide (Meser *et al.,* 2015) and continues to burden a large segment of the global population. An estimated 29% of non-pregnant women and 43% of preschool-age children world-wide have anaemia (Seven *et. al*., 2013. And iron deficiency anaemia is the single largest cause of years lived with disability in children and adolescent worldwide.

Between 1990 and 2013 the global prevalence of anaemia declined by 21% (from 33.3% to 27.0% Kasserbaum, 2016). There was an observed disparity in anaemia prevalence observed disparities in anaemia prevalence among the region, sexes and age groups (Kassebaum *et al.,* 2014).

There are various types of anaemia notably among other are sickle cell anaemia, pernicious Anaemia, drug-induced anaemia. The treatment of drug-induced anaemia will depend on the condition of the patient and the responsible drug should be stopped or adjustment of the dose (Poirot *et al.,* 2015). *Jatropha tanjoresis* is well known herbal plant for the treatment of Anaemia, in Yoruba commonly called Iyana Ipaja (Iwalewa, *et al.,* 2005). It is useful in herbal medicine, prepared locally in most of southern Nigeria by collecting the leaves and squeezing out the juice (Manthey *et al*., 2013). The leaf is a commonly consumed vegetable in many parts of southern Nigeria that is used as a natural remedy against diabetes (Olayiwola *et al.,* 2004).

2.0 MATERIALS AND METHODS

Chemical and reagents: All chemicals and reagents used were of analytical grades. Dr. Meyer's Orhepthal elixir manufactured by Farmax Meyer LTD was obtained from Julie Pharmacy.

Plant Collection and authentication

Fresh leaves of *Jatropha tajorensis* were purchased in sufficient quantity from Idiroko Road, Sango Ota, Ogun State. And was identified and authenticated at the herbarium of the Botany department of the University of Lagos with Voucher No. LUH 8729.

2.3 Preparation of plant material

Jatropha Tanjorensis leaves obtained were cleaned make free of stone and pebbles, and dried at room temperature for 14 days before being ground into powder with a waring blender. The 400g of crude sample of the powdered leaves obtained were subjected to extraction using 500ml, 70% Ethanol in

Soxhlet extractor for 72 hours before filtration using Thimble Filter paper and then concentrate in an evaporator at 45^0C the 60 g of caked brown residue was obtained. The percentage yield was obtained and the extract was kept in a labelled sterile container inside a bio-freezer before use.

2.4 **Experimental Animals**
A total of 45 albino Wistar rats of the age of 8-10 weeks with an average weight of 97.5 gram. Fifteen mice with average age of 7 weeks and an average of the weight of 20 gram were used. They were allowed to acclimatize for two weeks in a well-ventilated cage and fed with standard rat feed. With free access to clean drinking water, their beddings were changed every two days. They were maintained under standard conditions (27 ± 2°C, 12h dark and light cycle). The study conformed to the guide for the care and use of laboratory animals published by the US National Institute of Health (NIH publication number 85-23) revised for studies involving experimental animals.

2.5 **Acute Toxicity Study**
The method of Lorke was used to evaluate the median lethal dose of the extract. This was done in two phases using 15 mice. In the first phase, 12 mice were grouped into 4 with three rats each. Group 1 served as control and received distilled water while groups 2, 3 and 4 received 10, 100 and 1000 mg/kg body weight of the extract. The treated mice were observed for 24 hours during which no mortality was observed. In the second phase, 3 mice were randomized into groups 5, 6 and 7 with one mouse per group. Groups 5, 6 and 7 received 1700, 2000 and 5000 mg/kg body weight of the extract respectively. The mice were closely monitored after the second phase one death was recorded in the 5000 mg/kg group.

2.6 Induction of anaemia and treatment
A dose of 40 mg/kg body weight of phenylhydrazine (PHZ) was given intra peritoneal administration to the rats for three days to induce anaemia using the method of Yang and Woo, 2011. Rats that developed anaemia with haemoglobin concentration lower than 10 g/dl were used for the study. Anaemic rats were randomly allocated to 5 groups as follows I, II, III, V and VI.

2.7 Haematological analysis
Haematological parameters namely packed cell volume (PCV), red blood cells (RBC), haemoglobin (Hb), mean cell volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and white blood cell counts (WBC) were determined using haematology analyzer.

2.8 Histological analysis
The liver and spleen were harvested from the rats and fixed in formal saline for histological analysis.

Table 1: Lethal dose determination of *Jatropha tanjorensis* extract

Group	Dosage (mg/ kg)	Mortality D/T after 24hrs
Phase 1	Control	0/3
	10	0/3
	100	0/3
	1000	0/3
Phase II	1700	0/1
	2000	0/1
	5000	1/1

D/T = $\frac{\text{Number of dead mice}}{\text{Total number of Mice}}$

LD50 = $\sqrt{D_0 x D_{100}}$

Where Do = Highest dosage at 0% mortality

Do = Lowest dosage that gave 100% mortality.

LD_{50} = √2000 x 5000

= 3162mg/Kg

In all the groups, only one death was recorded at the highest dose of 5,000 mg/kg

TABLE 2: The weight of phenyl hydrazine-induced anaemic rat before and after the treatment.

GROUP	DOSE mg/kg	WEIGHT (g) BEFORE TREATMENT	WEIGHT (g) AFTER TREATMENT	WEIGHT (g) GAIN
1.	(200)	94.5 ± 7.02	102.2 ± 13.42	7.7
2.	(400)	106.6 ± 9.89	116.4 ± 12.56	9.8
3.	(800)	103.2 ± 11.29	123.8 ± 7.32	20.6
4.	No treatment	88.8 ± 7.82	110.0 ± 6.59	21.2
5.	Negative control Induced not treated	114.8 ± 3.97	115.2 ± 8.22	0.4
6.	Positive control Treated with Orheptal	144.4 ± 12.99	149.8 ± 11.95	5.4

Table 3: Haematinic effect of the aqueous extract of *Jatropha tanjorensis* phenylhydrazine induced anaemic rats at different dosages

Parameters	Group 1 (200mg/kg)	Group 2 (400mg/kg)	Group 3 (800mg/kg)	Group 4 Negative control	Group 5 Induced and treated with orheptal and the positive control	Group 6 positive control
WBC uL	9.07 + 2.21[a]	10.73 + 4.31[a]	6.03 + 0.15[a]	115.17 + 23.51[b]	9.63 + 5.51[a]	10.30 + 2.70[a]
PCV %	41.40 + 0.26[a]	42.40+ 2.16[a]	40.93 + 1.70[a]	22.00 + 1.44[b]	40.00 + 3.60[a]	40.53 + 4.80[a]
HB g/dL	13.63 + 0.25[a]	14.10 + 0.78[a]	13.90 + 0.36[a]	9.03 + 0.75[b]	13.47 + 1.19[a]	13.70 + 1.45[a]
RBC mL	5.88 + 0.10[a]	6.21 + 0.31[a]	6.06 + 0.13[a]	2.48 + 0.26[b]	5.65 + 0.44[b]	7.64 + 1.15[a]
PLT uL	606.67 + 68.41[a]	671.00 + 242.76[a]	595.00 + 52.68[a]	909.00 + 159.50[b]	750.00 + 135.88[a]	933.00 + 215.01[a]
MCHC g/dL	32.9 + 0.61[a]	33.23 + 0.59[a]	33.60 + 0.44[a]	40.97 + 1.10[b]	33.63 + 0.67[a]	33.77 + 0. 42[a]
MCH g/L	23.10 + 0.10[a]	22.67 + 0.72[a]	22.77 + 0.15[a]	36.37 + 0.71[b]	23.8 + 0.46[a]	17.97 + 1.20[a]
MCV uM[3]	73.43 + 525[a].	68.33 + 0.84[a]	68.07 + 1.00[a]	89.03 + 3.93[b]	70.83 + 1.00[a]	53.33 + 3.00[a]

Note: Values represent the mean ±SD, n=3, values with the same alphabet in each column are not statistically different ($p>0.05$) from the control for each of the parameters in the table. Whereas values with the different alphabet are statistically significantly different ($p<0.05$) from the control group in each column for each parameter.
WBC (White Blood Cell), PCV (Packed Cell Volume), HBC (Hemoglobin Concentration), RBC (Red Blood Cell), PLT (platelets) MCHC (Mean Corpuscular Haemoglobin Concentration), MCH (Mean Corpuscular Haemoglobin), MCV (means corpuscular value

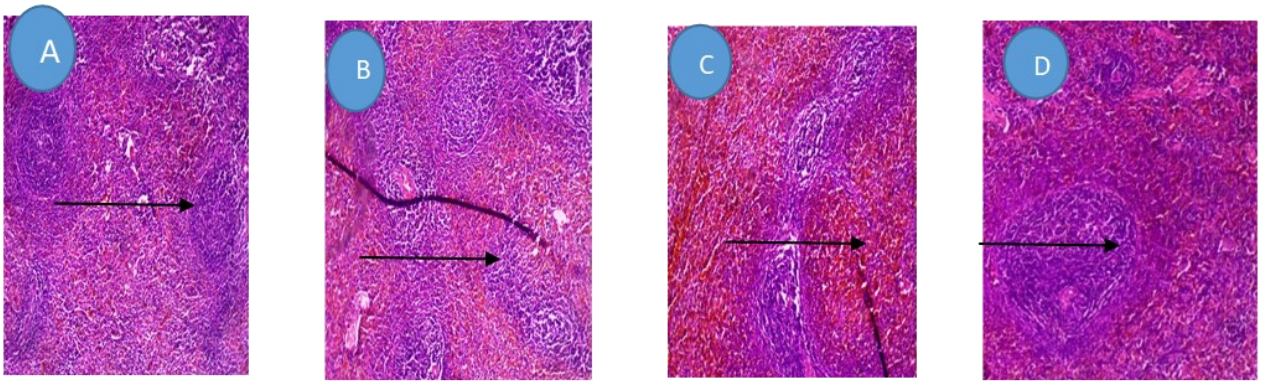

Plate 1: Photomicrograph the of a liver section of anaemic rats treated with varying doses of *Jatropha tanjorensis* extract (×200) (A) Liver section from control group showing normal hepatocytes (B) Liver section from anaemic rats treated with 200 mg/kg of the extract showing lymphoid aggregate which formed follicles. (C) Liver section from anaemic rats treated with 400 mg/kg body weight of the extract showing hepatocyte with oval nuclei and moderate eosinophillic cytoplasm (D) Liver section of anaemic rats treated with 800 mg/kg body weight of the extract showing normal hepatocytes.

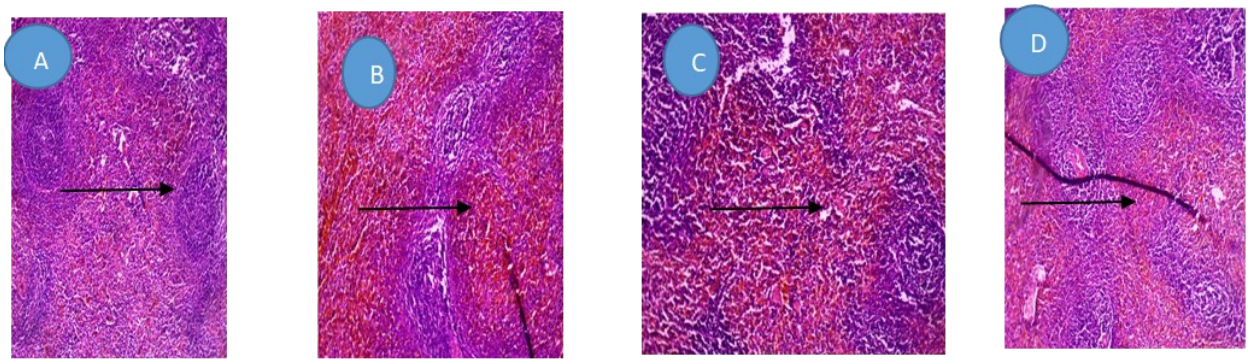

Plate 2: Photomicrograph of the spleen section of anaemic rats treated with varying doses of *Jatropha tanjorensis* extract (×200) (A) Spleen section from control group showing lymphoid aggregates with follicles (B) Spleen section from anaemic rats treated with 200 mg/kg of the extract showing congestion with aggregate of red blood cells (C) Spleen section from anaemic rats treated with 400 mg/kg body weight of the extract showing mild inflammation, surrounding sinuses with aggregates of red blood cells (D) Spleen section of anaemic rats treated with 800 mg/kg body weight extract showing lymphoid aggregates with form follicles.

5.1 DISCUSSION

The acute toxicity determination result indicates that at varying concentration dosages the ethanol extract proved to be non-toxic to the test animals via the oral route of administration one mouse died at 5000 mg/kg was recorded in the phase 1 and 2 concentration dosage administered.

Table 1 showed how acute toxicity was carried out using different dosages in both phases (1& 2). The mice were administered with a different dosage ranging from 100-5000 mg/kg respectively. However, one death was recorded at 5000 mg/kg body weight. The acute toxicity was determined to be 3162 mg/kg. This value showed the extract is slightly toxic as acute toxicity of 500-5000 mg/kg body weight is considered as slightly toxic (Looms and Hayes, 1996). Table 2 showed the result of the effect of the ethanol extract of *Jatropha tanjorensis* on the weight of the phenylhydrazine-induced anaemic rats in which there was an increase in weight in all the groups of rats treated with the ethanol extract of *Jatropha tanjorensis* as well as the control group but the group that was induced with anaemia and not treated showed a significant loss in weight which is an indication of disease condition in the rats.

A study by Ezeonu *et al*., (2017) reported that supplementation with a flavonoid-rich fraction of *Jatropha tanjorensis* ameliorated the induced depletion of blood in the test animals. It was previously reported that *Jatropha tanjorensis* is rich in iron (Fe) and this increases the amount of iron available for erythropoiesis (Wardlaw and Kessel, 2002). In Table 3 Studies on the ethanol extract of the leaves of *Jatropha tanjorensis* showed a statistically significant ($p < 0.05$) elevation in the PCV and Haemoglobin concentrations of both male and female Wistar rats, thereby justifying the local claim of the plant's use as a blood tonic (Omigie *et al.,* 2013). The flavonoid-rich fraction of *Jatropha tanjorensis* may, therefore, be used in physiological conditions such as pregnancy and menstruation when there is a drop `in Hb and PCV. It may also be employed in the stress-induced depletion of blood cells as a hematinic (Ezeonu *et al*., 2017). The significant increase in white blood cell and the differential leukocyte counts in the treated animals shows that the extracts of *Jatropha tanjorensis*, may increase immunity.

Plate 1, the Liver at 800 mg/kg shows a histologic section of liver tissue that showed parallel plates of hepatocytes with oval nuclei and moderate eosinophilic cytoplasm. No abnormalities were seen in Groups 1-3.

Plate 2 revealed the effect of the phenylhydrazine on the organ spleen of the induced anaemic rat. Group 1 & 2 were administered varying doses of 200 mg/kg & 400 mg/kg extract respectively, and also those in Group iv that were (induced not treated) resulted in the lymphoid aggregated to form follicles. The surrounding

sinuses were also congested with an aggregate of red blood cells leading to spleen inflammation.

The extract has been shown to be non-toxic to vital metabolic organs liver and the spleen even at a higher dosage of 800 mg/kg. The histological examination also revealed a non-toxicity effect and have shown to reverse the anaemic status of the induced rats

5.2 CONCLUSION

The ethanol extract of *Jatropha tanjorensis* should be taken with caution and also has therapeutic properties of anti anaemic. However, we advocate proper toxicological studies by purification and isolation of the bio-actives, since the extract has been shown to reverse the anaemic status of the induced rats.

We would like to recommend further research into the phytochemical composition of the ethanol extract of *Jatropha tanjorensis* to identify and isolate the possible constituent responsible for some of the therapeutic properties of the plant. In addition, we also recommend further research into the effect of the plant extract on the kidney functions.

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