ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4

Journal of Coastal Life Medicine

Phytochemical Evaluation, Nephroprotective and Hematological Modulating Effects of Ethanol leaf extract of Jatropha tanjorensis on Wistar Rats

Received: 18 October 2022, **Revised:** 22 November 2022, **Accepted:** 24 December 2022

Ini. P. Ekpe
Department of Biochemistry, Veritas University, Abuja.

Dennis Amaechi
Department of Biochemistry, Veritas University, Abuja

Benjamin.N. Yisa
Department of Biochemistry, Veritas University, Abuja

Martin Onukwuba
Department of Religion and Intercultural studies, Veritas University Abuja.

Judith. Z. Malachy
Department of Biochemistry, Veritas University, Abuja

Keywords:
Hematology, renal marker,phytochemical screening, packed cell volume

Abstract

Aim: This study is aimed at invstigating the effect of Jatrophatanjorensis leaves extract on modulating hematology, renal function of wistar rats and phytochemicals.

Method: A total of thirtyfive adult wistar rats were randomly divided into five groups, with 7 rats each respectively in each of the group. Normal control (group A) was administered distilled water, Group B, C, D & E was administered 250, 350, 450, and 550mg/kg b.w, respectively of the plant extract. The extraction and all biochemical analysis were carried out using standard laboratory techniques.

Results: The results showed that Jatrophatanjorensis extract caused significant changes at ($P < 0.05$) in the packed cell volume (PCV) of the administered groups compared to the normal control group due to the extract. However, the administered group showed no significant increase at ($P < 0.05$) in white blood cell count (WBC) of administered groups compared to the control group. The results of the renal was significantly reduced at ($P < 0.05$) sodium chloride potassium and no significant difference ($P < 0.05$) in urea concentration in comparison to the normal control groups of sodium, chloride, potassium,and urea respectively. Phytochemical screening revealed the presence of tannins, quinone, terpenonid, steroid, phenols, anthraquinones and flavonoids in Jatrophatanjorensis leaf extract.

Conclusion:Jatrophatanjorensis improve the blood volume and may be used in the management of anaemic conditions in patients , It has no toxic effects on the renal function and it also contains phytochemicals, which may be used as pharmacological probe.

1. Introduction

Plants are excellent sources of active phytochemicals with importance in prevention of various diseases. Wild and cultivated food plants may have medicinal, nutritional, and pharmaceutical health benefits [1]

Medicinal plants are plants that possesstherapeutic properties and commonly used for prevention or treatment of illness with herbal remedies. Medicinal plants have long been utilized in traditional medicine and are used widely in non-industrialized or developing countries in Africa, Asia, and southern America, mainly because they are thought to be more effective cheaper than modern medicine, and also readily available[2]

In modern medicine, about one quarter of the drug prescriptions are derived from medicinal plants and are rigorously tested[3]. Herbal medicines have been in usage long before the existence of modern

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

medicine, and there was usually little or no existing knowledge of the pharmaceutical and biochemical basis of their actions and mechanisms including risk associated with toxicity and some other effects on human health [2].

Medicinal plants also contain chemical compounds that dictate their therapeutic potency. Researchers have shown different bioactive components at different concentrations. The higher the amount of the important phytochemical in medicinal plants, the greater the therapeutic potency. There are more than 300 known medicinal plants in Nigeria; though the applications vary from plants to plants, culture to people believes, whether and other factors.

The root *Jatrophatanjorensis*commonly known as 'Hospital too far' or "miracle leaves "belongs to a family of Euphorbiaceae and is a commonly consumed green leafy plant found in field crops at about 1.8m in height. The plant thrives best in rainforest and vegetative areas of West Africa where it also grow best during rainy season
Jatropha leaves is known to be consumed locally as vegetables in food primarily 'traditional soups' and used as traditional herbal remedies to treat some health conditions like anaemia, diabetes mellitus, hypertension in Nigeria etc. as it is said to possess anti-oxidant and anti-hyperglycemic properties. It is also known to boost fertility in both male and female and increases blood flow in pregnant woman because of sufficiency in iron and calcium in *Jatropha* leaves.

2. Methodology

Plant Extraction

Fresh and healthy leaves of *Jatrophatanjorensis* were harvested from *Bwari* area of Abuja.

The leaves were washed in running water to remove contaminants and air-dried at room temperature in open laboratory space and blended into powder using an electric blender. The blended leaves were exhaustively extracted by macerating in absolute ethanol for 48 hours and filtered using a muslin cloth and Whatman filter paper. Maceration in ethanol continued until a faint filtrate was obtained. The filtrate was concentrated using a rotary evaporator at 50 °C and further concentrated using a thermostatic water bath at 40°C. Once the process was complete, the extract was transferred in a sterile sample bottle and kept in a refrigerator at 4ºC until required for use.

The percentage yield of the crude extract was calculated as

$$\% \text{ Yield} = \frac{\text{Weight of crude extract}}{\text{Weight of the dried sample}} \times 100$$

Qualitative Phytochemical Screening

The crude extracts of *Jatrophatanjorensis* were subjected to preliminary qualitative phytochemical screening for secondary metabolites such as saponins, tannins, flavonoids, terpenoids, alkaloids, steroids, phenols, and glycosides.

Test for saponins

Two ml of the extracts was added to 6ml of distilled water in a test tube. The mixture was shaken vigorously, allowed to stand. There was no formation of persistent foam on the surface of the mixture lasting for more than 10 minutes indicating the presence of saponins.

Test for tannins

Two ml of the extract was treated with 4ml of 10% alcoholic $FeCl_3$ solution in a test tube and observed. There was a formation of dark green color solution indicating the presence of tannins.

Test for flavonoids

Two ml of the extract was mixed with Fehling A and Fehling B separately and observed. There was a formation of a green color with Fehling A and brown color with Fehling B indicating the presence of flavonoids.

Test for terpenoids

Three ml of concentrated H_2SO_4 and 2ml of chloroform were added to 5ml of each of the extracts in a test tube. A monolayer of reddish-brown coloration of the interface was observed indicating the presence of terpenoids.

Test for steroids

Two ml of each of the extract was treated with 2ml of chloroform, 10 drops of acetic anhydride and 2 drops of conc. H_2SO_4. The solution was observed for the

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

formation of red coloration indicating the presence of steroids.

Test for phenols

Two ml each of the extracts was added 1% ferric (III) chloride in methanol/water (1:1). A dirty green precipitate was formed in the ethyl acetate and methanol extract solution indicating the presence of phenols.

Test for Anthraquinones

To the extract 1ml of the extract, 10ml benzene was added and filtered. 0.5ml of 1% Ammonium solution was then added and shaken. Pink, red, or violet colour in the ammoniac lower phase indicates the presence of Anthraquinones

Test for Glycosides

***One* ml of the extract in a boiling tube, 5ml Sulphuric acid was added. The mixture was then heated in boiling water for 15minutes. Fehling's solution A and B were added and the resulting mixture was heated to boil. A brick red precipitates indicates the presence of glycosides.**

Test for Phlobatannins

A 0.2 grams of the extract was boiled with equal volume of 1% hydrogen chloride, the deposition of a red precipice indicates the presence of phlobactannin.

Animals

Male and female Albino Wister rats of weight range 119-225g were used in this study. The wister Rats were obtained from an animal farm in Abuja, Nigeria. They were acclimatized for 3 weeks in the animal house of the Department of Biochemistry, Veritas University, Abuja. Wister rats were fed with normal Rat pellets and distilled water *ad libitum* and kept at standard laboratory condition of 12-hour light and 12-hour dark periodical alterations, **temperature ranging 22-28°C.**

The rats were randomly selected a day to the commencement of the experiment and allocated into five (5) groups. The first groups were control group which were fed with normal feed and distilled water, second to the fifth groups, were given extract according to their body weight. They were 7 each per Group. The rats in each group were fed with Growers' feed mash and water ad libitum.

Extract Administration

On day one of the experiment, the five groups were allocated into their various group in such a way that the difference in body weight within and between members of a particular group does not exceed ±20% of the average weight of sample population of rats. The five groups rats were orally given specific measurements of required feed, water and extract.

Table 1: Experimental Design

Rat groups	Group title	Extract administered
Group A	Normal control	distilled water
Group B	Administration group one	250mg/kg of *Jatrophatanjorensis*leaf extract.
Group C	Administrator group two	350mg/kg of *Jatrophatanjorensis*leaf extract.
Group D	Administration group three	450mg/kg of *Jatrophatanjorensis*leaf extract.
Group E	Administration group four	550mg/kg of *Jatrophatanjorensis*leaf extract.

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

Animal Sacrifice and Blood sample collection for analysis

At the end of the 21 days, food was withdrawn from the rats and they were fasted overnight but had free access to water. They were then euthanized under chloroform vapor and sacrificed. A midline incision was made through the anterior abdominal wall of the rats. Whole blood was collected using cardiac puncture using sterile syringes and needles. 5ml of blood samples were collected, from the descending abdominal aorta, in Heparin anticoagulated tubes for Hematological analysis and plain tubes for kidney function test.

Evaluation of Hematological parameters

Hematological parameters white blood cell and packed cell volume (PCV) count were measured using manual method of evaluation. The obtained results are precise (error of measurement is lower than 1%) and are manually compared with standard values for individual parameters.

White Blood cells (WBC)

Blood was collected using a syringe from the descending abdominal aorta from wistar rats into a Heparinized sample bottle 0.02ml of the blood was diluted into 0.38ml of Turks solution and that gave one in twenty dilutions (1:20) using a Thomas pipette. The Thomas pipette is marked to give 0.02ml of blood after diluting the blood, the improved Neubauer counting chamber is then changed with a drop diluted blood and covered with a glass cover slip and then placed on the microscope and viewed using x10 objective lens. The four large squares placed at the corner of the chamber was counted using a tally counter since the concentration of the counteraction of white blood cells are lower than red blood cells so a larger area will be used.

Calculations for white blood cells

For white Blood cells, the number of cells counted was calculated as thus;

Number of cells counted = 1/Area of counting chamber x 1/depth of counting chamber x dilution factor

Number of cells counted = A cells counted x a known constant

Number of cells counted = A cells/ mm^3

Note: The improved Neubauer counting chamber has a known depth and area a given constant is given

Packed cell volume (PCV)

Blood was collected using a syringe from the heart of wistar rats into Heparinized sample bottle. The blood was then collected using a Heparinized capillary tube to about ¾ full of the Heparinized capillary tube. After collection the Heparinized tube was then sealed by passing it through flame using a burden burner. When sealing was completed the capillary tube was then placed on the hematocrit centrifuge and spun at 5000 revolution per minute for 5 minutes. When spinning was completed, the capillary tube then separated into their components; the buffy coat, plasma and the red cells, it's the uppermost part of the red cells that was read using a hematocrit reader.

Statistical Analysis

The results obtained from this study were analysed by one-way analysis of variance (ANOVA), followed by Duncan post-hoc test to evaluate the significance of the difference between the mean value of the measured parameters in the respective control groups using SPSS version 23. Differences between means were considered significant at $P < 0.05$.

3. Result:

Table 2: Phytochemical Screening of *Jatrophatanjorensis*

PHYTOCHEMICALS	RESULT
Saponins	-
Tannins	+

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

Flavonoids	+
Terpenoids	+
Steroids	+
Phenols	+
Glycosides	-
Phlobatanin	+
Anthraquinone	+
Quinone	+
Alkaloids	-
Balsams	-

+ = present

- = absent

Table 3: Effect of ethanol *Jatrophatanjorensis*leaf extract on hematology indices

	HEMATOLOGICAL INDICES							
Group	PCV	Hb	RBC	WBCX1 0^3	Neut	Lym	MID	MCV
A(ctrl)	39.00±0.55^a	13.04±0.41^a	5.60±0.25^a	4.94±1.47^a	19.00±1.27^a	63.00±1.97^a	18.20±1.36^b	69.60±3.70^a
B	50.00±1.46^c	15.96±0.33^c	7.10±0.15^c	6.69±0.70^a	15.29±3.39^a	72.29±3.86^a	12.71±1.02^a	71.71±1.80^a
C	51.17±1.08^c	16.10±0.36^c	7.07±0.13^c	4.82±0.48^a	21.00±5.03^a	70.33±4.26^a	13.33±1.67^a	71.50±1.54^a
D	49.50±1.23^c	15.42±0.36^c	6.80±0.10^c	6.78±1.17^a	16.50±2.32^a	68.00±3.79^a	15.50±1.54^{ab}	70.33±2.40^a
E	44.83±1.87^b	14.22±0.42^b	6.18±0.15^b	5.57±0.76^a	12.50±1.48^a	72.50±2.49^a	15.50±1.02^{ab}	70.67±1.56^a

Values are represented as Mean±SEM of triplicate determinations.

a = Values are significantly different across the row at $p<0.05$.

ab = Values are significantly different across the row at $p<0.05$.

b = Values are significantly different across the row at $p<0.05$.

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

c = Values are significantly different across the row at p<0.05.

Table 4: Effect of ethanol *Jatrophatanjorensis*leaf extract on Renal markers

Treatments	Sodium (Na^+) (mmol/L)	Postassium (K^+) (mmol/L)	Chloride (Cl^-) (mmol/L)	Urea (mmol/L)
A (control)	592.61±70.99^{c}	4.50±1.21^{a}	172.22±22.89^{a}	74.52±17.52^{ab}
B	222.13±87.63^{a}	5.11±1.07^{a}	130.77±12.34^{a}	73.56±9.36^{ab}
C	296.72±84.02^{a}	3.89±0.01^{a}	155.77±12.12^{a}	101.24±5.39^{b}
D	368.69±88.69^{ab}	3.33±0.76^{a}	138.43±12.38^{a}	93.10±2.24^{ab}
E	336.25±53.02^{ab}	3.59±1.39^{a}	133.40±6.69^{a}	64.75±3.67^{a}

Values are represented as Mean±SEM of triplicate determinations. Values with different alphabetic superscript are significantly different along the column at p<0.05.

a = values has significantly difference within and down the group at p<0.05.

b = values has significantly difference within and down the group at p<0.05.

ab = values has significantly difference within and down the group at p<0.05.

c = values has significantly difference within and down the group at p<0.05.

4. Discussion

Herbal medicines have received greater attention as an alternative to clinical therapy and the demand of these remedies has currently increased. Experimental screening method is important in order to ascertain the safety, efficacy and properties of traditional and herbal products and also its effect on animal species. Plant's medicinal properties are dependent on the plant secondary metabolite contained in the plant and these metabolites that possess medicinal properties are found only in a few species of plant. This study was carried out to evaluate the phytochemical properties, hematological and kidney function activity of the ethanol leaf extract of *Jatrophatanjorensis* in rodent model.

The present study showed that after preliminary phytochemical test on the powdered leaf of *Jatrophatanjorensis* indicated the presence of tannins, quinone, terpenonid, steroid, phenols, anthraquinones, phlobatannins and flavonoids. The positive (+) level of Terpenoid, Tannins, Quinine, Phenols, Anthraquinones, phlobatannins, Steroids and Flavonoids indicate the presence of these compounds in high quantity in the plant. While the negative (-) level of Alkaloids, Balsalms, Gycosides, and Saponins in the plant indicates a low quantity of the compound in the plant as presented in Table 2. These bioactive constituents may be responsible for the observed therapeutic effect of the plant [4]. For example, tannins possess astringent properties that hasten the healing of wounds and inflamed mucus membrane. Plant that contains tannins as major constituent are used for the treatment of intestinal disorders like diarrhea and dysentery. The absence of tannins is an indication that the intake of the extract has no effect on inhibition of minerals in the body [4].

Flavonoids have also found to be water soluble polyphenolic molecules with antioxidant activity which prevent cardiovascular and heart related ailments. Apart from their anti-fungal and anti-bacterial properties, they are known to prevent the oxidation of low-density lipoproteins and reduce the

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

risk of atherosclerosis hence flavonoids are capable of lowering cholesterol and triglyceride levels in the blood. Saponins are also found to lower blood cholesterol levels, while cardiac glycosides are known diuretics and heart tonics which help in the prevention of heart failure. According to [5]phlobatannins are anti-inflammatory, analgesic and antioxidant agents known to have the ability to heal wounds while the steroids, are currently used for treating symptoms of uterine cramps, abdominal colic and menstrual irregularity. Phenolic compounds have the ability to reduce risk for development or treatment of cancers, cardiovascular disorders, obesity, diabetes, aging diseases, urinary tract infections, and periodontal disease that the richness of the polyphenolic content of green tea and red wine has made them popular choices for associated anticancer and cardiovascular health benefits.

Quinone on the other hand plays an important role in oxidative stress when there is an imbalance between the production and quenching of free radicals from oxygen species and have diverse role in medicine including, anti-cancer agent and anti-aging and arteriosclerosis . Terpeniods also have been found to be useful in the prevention and therapy of several diseases, including cancer, antimicrobial, antifungal, antiparasitic, antiviral, anti-inflammatory antiallergenic, and immunomodulatory.

Hematology refers to the study of the numbers and morphology of the cellular components of the blood and the use of these results in the diagnosis and monitoring of disease [6].

The assessment of the hematological indices can be used to detect or determine the extent of deleterious effect of foreign compounds including plant extracts and product on the blood constituent of an animal. The aim of this study was to evaluate the hematological indices (such as white blood cell count and packed cell volume) of rats treated with *Jatrophatanjorensis*varying doses of (250, 350, 450, and 550 mg/kg per body weight).

The outcome of this study showed that packed cell volume of wistar rats treated with *Jatrophatanjorensis* has great significant difference at ($p<0.05$) in the administration groups which are B, C, D and E (ranging 50.00±1.46c to 44.83±1.87b) compared to the normal control group (39.00±0.55a) as presented in Table 3. This could be attributed to the presence of phytochemical compounds. This compounds is a good anti-anaemic, which prevent anemia due to its high iron content and facilitate the production of red blood cells. High amount or concentration of iron in the extract increases the amount of iron available for erythropoiesis. The result of this study is in conformity with [7], who stated that it is possible that some of the chemical constituents of the extract may have erythropoietin-like effect on the bone marrow leading to the increase in the rate of erythropoiesis and a resultant increase in packed cell volume and hemoglobin concentration. Extracts of *Jatrophatanjorensis* can, therefore, be used in building up the blood level in physiological conditions like pregnancy and during menstruation when there is drop in hemoglobin concentration and packed cell volume.

The results in the study also revealed there was no significant ($p<0.05$) difference in the amount of WBC, neutrophil, lymphocytes as well as the MCV concentration in all the treatment groups. This indicates the non-toxic nature of the plant to the white blood cells. This can imply as they may be no tendency of developing infections or diseases; production of WBC will only increase if there is risk to infections in the immune system. Although according to [8] revealed that *Jatrophatanjorensis*may show mild toxicity to the liver and lungs.

Serum or plasma electrolyte concentrations are the net result of intake, excretion (mainly alimentary and renal), and shifts between intra- and extracellular fluids. Shifts can occur in vivo or in vitro. Electrolyte concentrations in serum/plasma essentially represent concentrations in all extracellular fluid. Serum/plasma electrolyte concentrations must be interpreted with knowledge of the animal's hydration status and consideration of the extracellular volume. While reference ranges for electrolytes (sodium, potassium, and chloride) are fairly wide, the range of results in a well-controlled study is generally quite narrow.

The result showed significant difference at ($P < 0.05$) that sodium was significantly lower in the administration groups which are B, C, D and E (ranging 222.13±87.63a to 336.25±53.02ab) after 21 days of consumption of aqueous extract of *Jatrophatanjorensis*when compared to the normal

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

control group(592.61±70.99c) as presented in Table 4. Similar trends may also be observed in chloride concentration showing slightly significant difference in the administration groups which are B, C, D and E (ranging 130.77±12.34a to 133.40±6.69a) when compared to the normal control group(172.22±22.89a). This result may suggest that *Jatrophatanjorensis* aqueous extract the wistar rats to electrolyte imbalance and metabolic acidosis. Control of electrolyte levels is based on H_2O and pH balance and is enacted by the renal glands through processes such as active transport in the proximal convoluted tubules, osmosis, and passive diffusion. Decrease in sodium and chloride concentrations may also be influenced by fluid loss (dehydration) and environmental factors.

Urea concentration showed no significant difference in the administration groups and normal control group expect for group C possessing a higher content of Urea as presented in table 4. This result suggest that potential risk to kidney damage is little or non-existent.

5. Conclusion:

Jatrophatanjorensis improve the blood volume and may be use for the management of anaemic conditions in patients. It has no toxic effects on the renal function and it also contains phytochemicals, which may be used as pharmacological probe.

References

[1] Awuchi, C. G. (2019). Medicinal plants: the medical, food, and nutritional biochemistry and uses. *International Journal of Advanced Academic Research*, *5*(11), 220-241.

[2] Hall, J. E., & Hall, M. E. (2020). *Guyton and Hall textbook of medical physiology e-Book*. Elsevier Health Sciences.

[3] Anhwange, B. A., Agbidye, I. G., Kyenge, B. A., &Ngbede, P. O. (2019). Phytochemical Screening, Antimicrobial Activities and Nutritional content of Jatrophatanjorensis Leaves. *Nigerian annals of pure and applied sciences*, *2*, 108-113.

[4] Dennis Amaechi , I. P. Ekpe , E. D. Edet and M. C. Madu . Hepatoprotective and Hematological Effects of Solanummelongena (Garden Egg), Solannumlycopersicum (Tomato) and Daucus carrots Subsp. Sativus (Carrot) Extracts against Lead Induced Toxicity in Wistar Rats. Asian Journal of Biochemistry, Genetics and Molecular Biology 7(2): 10-18, 2021

[5] Wadood, A., Ghufran, M., Jamal, S. B., Naeem, M., Khan, A., & Ghaffar, R. (2013). Phytochemical analysis of medicinal plants occurring in local area of Mardan. *Biochem anal biochem*, *2*(4), 1-4.

[6] Falodun, A., Igbe, I., Erharuyi, O., &Agbanyim, O. J. (2013). Chemical characterization, anti inflammatory and analgesic properties of Jatrophamultifida root bark. *Journal of Applied Sciences and Environmental Management*, *17*(3), 357-362.

[7] Oyewole, O. I., &Akingbal, P. F. (2011). Phytochemical analysis and hypolipidemic properties of Jatrophatanjorensis leaf extract. *European Journal of Medicinal Plants*, *1*(4), 180.

.