

Effect of *Jatropha curcas* Leaf Extract on Castor Oil Induced Diarrhea in Albino Rats

Fatima U. Maigari, Musa Halilu, M. Maryam Umar, Rabiu Zainab

Abstract—Plants as therapeutic agents are used as drug in many parts of the world. Medicinal plants are mostly used in developing countries due to culture acceptability, belief or due to lack of easy access to primary health care services. *Jatropha curcas* is a plant from the *Euphorbiaceae* family which is widely used in Northern Nigeria as an anti-diarrheal agent. This study was conducted to determine the anti-diarrheal effect of the leaf extract on castor oil induced diarrhea in albino rats. The leaves of *J. curcas* were collected from Balanga Local government in Gombe State, north-eastern Nigeria; due to its bioavailability. The leaves were air-dried at room temperature and ground to powder. Phytochemical screening was done and different concentrations of the extract was prepared and administered to the different categories of experimental animals. From the results, aqueous leaf extract of *Jatropha curcas* at doses of 200mg/Kg and 400mg/Kg was found to reduce the mean stool score as compared to control rats, however, maximum reduction was achieved with the standard drug of Loperamide (5mg/Kg). Treatment of diarrhea with 200mg/Kg of the extract did not produce any significant decrease in stool fluid content but was found to be significant in those rats that were treated with 400mg/Kg of the extract at 2hours (0.05±0.02) and 4hours (0.01±0.01). A significant reduction of diarrhea in the experimental animals signifies it to possess some anti-diarrheal activity.

Keywords—Anti-diarrhea, Diarrhea, *Jatropha curcas*, Loperamide.

I. INTRODUCTION

IN Nigeria, about 80% of the population living in rural areas rely on herbal medicine [1]. Ancient discovery of some new plant food that relieves pain might have been the beginning of folk knowledge, which was passed down to generations [2]. Several plants and their products have been used in treating both domestic animals and human. Such plants are used as therapeutics or as precursors for the synthesis of several drugs [3].

The increased awareness of the significance of herbal medicine, particularly the desire to integrate it with orthodox medicine still leaves much to be achieved [3]. Over 85% of the population in sub-Saharan Africa including Nigeria depends on herbal traditional medicine for their healthcare [4]. *Jatropha curcas* is a shrub of the *Euphorbiaceae* family, which grows up to a height of 3-4 metres. It has thin, often

F. U. Maigari is with the Department of Biochemistry, Gombe State University Nigeria (phone: +234-703-786-5969; e-mail: fatimamaigari@gmail.com).

Musa Halilu is also with the Department of Biochemistry, Gombe State University Nigeria.

M. Maryam Umar is with the School of Sciences, Federal College of Education Gombe Nigeria (e-mail: maryam.maigari@yahoo.com).

Rabiu Zainab is with the Department of Biochemistry, North-West University Kano Nigeria (e-mail: lalabrabiu@yahoo.com).

greenish bark which exudes copious amounts of watery sap when cut [5]. *Jatropha curcas* is dispersed and neutralized throughout the tropics; and propagated by cutting the seeds. Its habitat is mostly Guinean or Sudanese areas [6]. Climatically, *Jatropha curcas* is native to tropical America but is now cultivated widely in tropical countries throughout the world, the plant likes heat and does well even in lower temperatures and can withstand a light frost [7].

A. Medical Uses of *Jatropha curcas*

- The plant is known to be toxic when ingested, but applied as a remedy for external injuries. Its internal uses are always prescribed with caution under medical supervision [7].
- The leaves are used either as a homeostatic agent, to hasten fermentation of cassava, to induce breast milk in women, regarded as anti-parasitic, applied to scabies, hard tumors, rheumatism.
- The latex is used to treat skin disease such as ring worm, scabies, eczema [6], strongly inhibiting to watermelon mosaic virus Colombians and Costa Ricans apply the latex to burns [8].
- The roots are used to make an antidote for snake bites [9]. Also, the root as used as decoction in mouthwash for bleeding gums, toothache and for eczema, scabies and ringworm.
- The stem of the young leaves has been used to treat urinary infections successfully, and the twig as a toothbrush [9].
- The seeds as well as the fruits are used as a contraceptive, and as well as purgative, anti-helminthic and abortifacient, and other skin diseases. The seed oil of the plant has been used as an ingredient in the treatment of rheumatic conditions and a mouth wash, gonorrhoea, as a diuretic agent, otitis, and herpes [8].

B. Other Uses of *Jatropha curcas*

The wood and fruits of *Jatropha curcas* can be used for numerous purposes including fuel. The seed oil has been used for illuminations, soap, candles, adulteration of olive oil and making turkey red oil, nuts can be strung on grass and turned like candle nuts [8].

Diarrhea is associated with increased frequency of bowel movements with the production of soft or watery stool, or the passage of more than 300ml of liquid feces in 24hours [10]. Diarrhea can be caused due to infection caused by viruses, bacteria, idiopathic inflammatory bowel diseases, defective intra-luminal digestion etcetera [11]. Intra-cellular cyclic nucleotides are important regulators of absorptive and

secretory process. Stimuli enhancing cAMP or cGMP cause net loss of salt and water, both by inhibiting NaCl absorption in villous cells and by promoting anion secretion (Na^+ accompanies) in the crypt cells which are primarily secretory in nature. Many bacterial toxins activate adenylcyclase, which enhances secretion that reaches its peak after 3-4 hours and persists until the stimulated cells are in the normal turnover i.e., 36 hours after a single exposure. Concurrent inhibition of absorption adds to the rate of salts and water loss. Prostaglandins and intracellular Ca^{2+} also stimulate the secretory process. All acute enteric infections produce secretory diarrhea [10].

Ingested castor-oil contains the active ingredient of retinoic acid [12] which causes irritation and inflammation of the intestinal mucosa and subsequent release of prostaglandins [13]. It also alters permeability of the intestinal mucosa to electrolytes and water thereby causing diarrhea [14].

Some general measures for the treatment of diarrhea may either involve treatment of fluid deflation, shock and acidosis, maintenance of nutrition or through drug treatment.

Reference [15] reported that anti-diarrheal drugs act on bowel muscle to delay the passage of gut contents, so allowing the time for more water to be absorbed.

Loperamide, a drug used in the treatment of diarrhea is chemically related to haloperidol and diphenoxylate, a weak analogue of meperidine. Their mechanism of action on the gut is similar to that of opioids and involves inhibition of acetylcholine release through post-synaptic opioid receptors in the enteric nervous system. Loperamide is less sedative than diphenoxylate [16].

II. JUSTIFICATION OF STUDY

In Nigeria, traditional and herbal medicine is still practiced largely by many. This is due to belief, culture or lack of easy access to primary healthcare services. Even among the highly educated and rich families, this medical practice is widely accepted.

Jatropha curcas leaves are mostly obtained soaked in water and consumed to treat diarrheal diseases in both adults, children and infants. Hence, this study determined the effect of the leaf extract as anti-diarrheal treatment in castor-oil induced diarrhea in albino rats.

III. METHODOLOGY

A. Definition of Study Area

This study was conducted in Gombe State. It is one of Nigeria's 36 states with an area of 20,265 km² and a population of around 2,353,000 people as of 2006; having its state capital Gombe. The State nicknamed the 'Jewel in the Savannah' was carved out in October 1996 from part of the old Bauchi State. The state is being located in the northeastern zone; right within the expansive savannah allows the state to share common borders with the states of Borno, Yobe, Taraba, Adamawa and Bauchi states. Gombe has two distinct climates, the dry season (November-March) and the rainy season (April-October) with an average rainfall of 850mm.

B. Collection and Preparation of Plant Material

Jatropha curcas leaves was obtained from Balanga local government in Gombe State Nigeria and taxonomical identification was done at the department of Biological Sciences, Gombe State University. The leaves were then thoroughly washed with distilled water to remove sand and dirt. They were then air-dried at room temperature and grounded to fine powder. 100g of the powdered material was macerated in 500ml distilled water and the water was added to a level of 1000ml. This was left for 24hours after which it was filtered with a No.1 Whatman filter paper. The filtrate was then dried in a water-bath to yield 9.84% w/w. The prepared extract was then stored in a refrigerator at 4°C protected from light until time of usage.

C. Experimental Animals

Albino rats weighing 100-150g were obtained from the animal house at the department of Physiology, College of Medical Sciences, Gombe State University. The animals were allowed to acclimatize for 7days with free access to food and water. They were then divided into four groups of five rats each. The animals were given the treatment orally as:

- Group I- negative control (normal saline 2mg/kg)
- Group II- positive control (standard loperamide 5mg/Kg)
- Group III- aqueous extract of *J. curcas* (200mg/Kg)
- Group IV- aqueous extract of *J. curcas* (400mg/Kg)

D. Phytochemical Screening Test

Acute toxicity test was also conducted based on the limit test recommendations of [18]. Apparently healthy adult albino rat of Wister strain was allowed to fast for 4h and then the extract was administered orally in a single dose of 2000mg/kg. General behaviour of the mouse was observed continuously for 24h. Additional 4 mice were giving same dosage and kept under observation. Observation was continued for 14days for any sign toxicity and mortality.

E. Test for Alkaloids

Extract (5g) was stirred with 3ml of 1% aqueous hydrochloric acid on the water-bath and filtered; 2-3ml of the filtrate was treated with a few drop of dragendorff's reagent and was observed for orange brown precipitate.

F. Test for Flavonoids

Extract (0.5g) was added to 2ml of dilute NaOH solution in test tube and shake to dissolve. A few drop of conc. H_2SO_4 and was added and then observed for a colorless solution.

G. Test for Tanins

To 10ml distilled water in a test tube, 0.5g of the extract was added, and was then stirred and filtered through a whattman no.1 filter paper. To the filtrate, 2-3ml of 5% ferric chloride solution was added gradually and a deep green color (olive-green) was observed.

H. Test for Anthraquinones

To 10ml of dilute H_2SO_4 in a test tube, 5g of the extract was added and boiled for a few minutes. It was then filtered

through whatman no.1 filter paper while the mixture was still hot. It was then allowed to stand until the ether and aqueous layers was then separated, and 2.5 of 10% ammonia solution was added and either a pink or red violent coloration was observed in the aqueous layers indicating absence of anthraquinones.

I. Test for Glycosides

To 10ml distilled water in a test tube, 5g of the extract was added, and was then stirred and filtered through a whatman no.1 filter paper. To 2ml of the filtrate, a few drops of conc. HCL was added and boiled for a few minutes to hydrolyze any glycosides present. 2-2 drops of aqueous ammonia solution were added to make the mixture alkaline. 2-3 drops of the mixture were then added to 2ml of Benedict's qualitative reagent and boiled for a few minutes. A reddish brown precipitate was not observed.

J. Effect on Antidiarrheal Activity on Castor-oil Induced Diarrhea

Diarrhea was induced in the experimental animals as described by [19] by oral administration of 0.5ml castor oil, 30minutes before the treatment. The number of both dry and wet feces was counted. All results were subjected to statistical analysis using the SPSS statistical package.

IV. RESULTS

A. Phytochemical Screening

After screening for phytochemical constituents. *Jatropha curcas* leave extract indicated the presence of alkaloids, tannins, saponins, flavonoids, steroids and glycosides. It however tested negative for anthraquinones.

TABLE I
RESULT FOR PRELIMINARY PHYTOCHEMICAL TEST

Phytochemical Test	result
Alkaloids	+
Saponins	+
Steroids	+
Flavonoid	+
Tannins	+
Glycosides	+
Anthraquinones	-

+: present, - : absent

B. Results for Castor-Oil Induced Diarrhea

Table II showed the mean± standard error of mean stool score for the different groups of animals. Those treated with the standard drug has the least mean stool score with the group treated with 200mg/kg having the highest mean stool score.

TABLE II
CASTOR-OIL INDUCED DIARRHEA

Group of Animals	Mean± SE Stool Score	Number of wet faeces	% inhibition
Group I	3.27± 0.13	12.7±0.13	---
Group II	2.01±0.02	2.1±0.08	77.9
Group III	2.78±0.07	9.6±0.31	24.4
Group IV	2.52±0.02	5.2±0.05	56.7

C. Results for Effect of *J. curcas* on Weight Composition of Wet and Dried Stool on Castor-Oil Induced Diarrhea in Albino Rats

TABLE III
STOOL FLUID CONTENT OF DIARRHEAL INDUCED CASTOR-OIL ANIMALS

Group of animals	1hr Stool fluid content in weight (g)	2hr Stool fluid content in weight (g)	3hr Stool fluid content in weight (g)
Group I	0.08±0.003	0.07±0.005	0.07±0.005
Group II	0.07±0.050	0.05±0.030	0.06±0.050
Group III	0.33±0.100	0.33±0.007	0.32±0.007
Group IV	0.27±0.008	0.05±0.004*	0.01±0.001

Values are mean± SE (n=5)

*p< 0.05 across the row

V. DISCUSSION

The result of the preliminary phytochemical screening of the leaves of *J. curcas* plant indicated the presence of saponins, tannins, flavonoids, steroids, alkaloids and glycosides but no evidence of the presence of anthraquinones. This is also in agreement with the report of [18].

Highest percentage inhibition is seen the group of mice that received standard drug of loperamide, the group of mice that 400mg extract also showed more antidiarrheal activity. Reference [19] showed that the anti-diarrheal properties of some medicinal plants were due to the presence of some phytochemicals of tannins, alkaloids, saponins and reducing sugars. *J. curcas* may be of therapeutic benefit such as in the treatment of diarrhea as well as effective against multiple microorganisms due to its flavonoid composition [20]. Reference [21] reported that tannins and polyphenols are attributed to the reduction in enzyme activities of bacteria, and that saponins and alkaloids have been shown to act against gram positive and gram negative bacteria [22].

A reduction of diarrhea in rats treated with extract of *J. curcas* showed the extract to possess an anti-diarrheal property. A study of phytochemicals in plants found in the Mediterranean coast which are used to treat diarrhea reported that constituents responsible for such activity propulsive motor activities, predominantly in the jejunum were tannins and flavonoids [23].

None of the animals during the observation period showed behavioral changes, physical changes, any neurological changes nor death occurred at the limit dose of 2000mg/Kg of the aqueous extract of *J. curcas*. Hence, the LD50 for *Jatropha curcas* leaves was considered to be above 2000mg/Kg

Loperamide (a standard anti-diarrheal drug) is a synthetic opiate analogue developed specifically for use against diarrhea. Loperamide regulates the gastrointestinal tract by inhibiting the propulsive motor activities. It also reduces colonic flow, and consequently increasing colonic water absorption [24].

The ability of *J. curcas* to reduce stool fluid flow in the group administered with 400mg/kg may be due to its phytochemical contents of flavonoid, tannins and alkaloids. It was reported by reference [25] that tannins and flavonoids increase water and electrolyte reabsorption in the colon while other phytochemicals act by inhibiting intestinal motility.

Jatropha curcas aqueous extract was also reported to have an antibacterial activity against *S. aureus* and *E. coli* [26], it was also reported to have antimicrobial activity against 12 bacterial species consisting of both gram positive and gram negative organisms [27].

VI. RECOMMENDATIONS

For further research, the quantitative assessment of the phytochemical constituents of *Jatropha curcas* leaves should be analyzed. Also, comparative assay of efficacy of the leaves obtained from different locations is also recommended.

ACKNOWLEDGMENT

The authors of this paper thank the Vice Chancellor Gombe State University, Nigeria Prof Ibrahim Musa Umar for giving us the conducive environment to carry out this research.

REFERENCES

- [1] A.T. Oladele, O. C. Cyril, O.C. and O. E. Taiwo (2012). Ethnobotanical survey of plants used as memory enhancer and anti-aging in Ondo State, Nigeria. *International journal of pharmacy* 2(1):26-32.
- [2] L. S. Latha and P. N. Reddy (2009). Antimicrobial, antidiarrheal and analysis of phytochemical constituents of *Shaeranthus amaranthoides*. *Indian J Sci Technol* 2(3):45-48.
- [3] A. Soforowa (2008) medicinal plants and traditional medicine in Africa (3rd edition). Spectrum books ltd. Ibadan, Nigeria. Pp 199-205
- [4] R. L. Guerrant, T. Van Gilder, T.S. Steiner, M.N. Thielman, L. Slutsker, and R. V. Tauxe (2001) practice guidelines for the management of infectious diarrhoea. *Clin. Infect Dis* 32:331-335.
- [5] R. Audu, S. R. Umilabug, J. K. Renner, J. A Awodiji (2000) Diarrhoea management. *J Nigerian Infect Contr Asso.* 3:15
- [6] C. O. Okujagu, C. O. Kadiri, E. U. Akueshi, S. E. Agina, and C. Ngurukwem (2005) Anti-microbial potential of *Hyptis suaveolens* (Lamiaceae). *Nig J Botany* 15:31-41
- [7] J. de jongh (2006) General data on *Jatropha curcas*. In handbook on *Jatropha curcas* (ed. W. Rijssenbech), pp 4-9, FACT Foundation, Eindhoven.
- [8] J. M. Watt and M. G. Breyer-Brandwijk (1962) the medicinal and poisonous of southern and eastern Africa, 2nd edn. Livingstone, London UK
- [9] O.M. Agbogidi, S.O. Akparobi and P.G. Eruotor (2013) health and environmental benefits of *Jatropha curcas* Linn. *App. Sci. Rep 1(2):36-39*
- [10] J. R. Shah (2011) investigation on the Gastroprotective and anti-diarrheal properties of *Mimosa pudica* leaves, Karnataka Bangalore, India. Published MSc thesis, Ragiv Gandhi University of Health Sciences, India.
- [11] J. A. Crawford (1999). *The gastrointestinal tract*. In: R. S. Cotran, V. Kumar and T. Collins (ed). Pathologic basis of diseases (6^{edn}). WB Saunders Company. Pp 805-807.
- [12] W. T. Tadesa, A. E. Hailu, A. E. Gurm, and A. F. Mechesso (2014) Experimental assessment of antidiarrheal and antisecretory activity of 80% methanolic leaf extract of *Zehneria scabra* in mice. *BMC Complementary and Alternative Medicine* 14:460
- [13] N. C. Chaulya, P. K. Halder, and A. Mukherjee (2011) Anti-diarrhoeal activity of methanol extract. *International Journal of Pharmacy and Pharmaceutical sciences* 3:133.
- [14] B. Kumar, K. Divakar, P. Tiwari, M. Salhan, and D. Goli (2010) Evaluation of antidiarrheal effect of aqueous and ethanol extracts of fruit pulp of *Terminalia bellerica* in rats. *Int J Drugs Dev Res* 2: 769-779
- [15] D. F. Altman (2001) Drugs used in gastrointestinal disease. In: B. G. Katzung. 'Basic and clinical pharmacology' 8th edition. Lange Medical Books/ Mc Graw Hill, New York. Pp 1070-1071.
- [16] OECD Guidelines (2008) Guidelines for testing of chemicals; Acute Oral Toxicity: Up and Down procedures. OECD publishing. <http://www.oecdlibrary>.
- [17] C. S. Franca, F. S. Menezes, L. C. Costa, E. S. Niculau, P.B. Alves, J. E. Pinto, R. M. Marcal (2008) Analgesic and anti-diarrheal properties of *Ocimum selloi* essential oil in mice. *Fitoterapia* 79(suppl 7-8): 569-573
- [18] R. A. Ahirao, M. R. Patel, D. M. Pokal, d. K. Patil and H. P. Suryawanshi (2011) Phytochemical screening of the leaves of *Jatropha curcas* plant. *International Journal of Research in Ayurveda and pharmacy* 2:1324
- [19] B. G. Katzung (2004) basic and clinical pharmacology. Boston McGraw Hill, 50
- [20] E. T. Williams, J. T. Barminas, J. Akinniyi, and A. Williams (2009) Antidiarrheal effects of *Jatropha curcas* in male mice. *African Journal of Pure and Applied Chemistry* 3(8):152-157
- [21] A. B. Sallau, G. C. Njaki, A. R. Olokisi, A.U. Wurochekke, A. Abdulkadir, S. Isah, M. S. Abubakar, and S. Ibrahim (2005) Effects of *Jatropha curcas* leaf extracts on some *Echis carinatus* venom enzyme. *J Med Sci* 5(4):2880-283.
- [22] M. W. Iwu, A.R. Duncan, and C.O. Ukunji (1999) New antimicrobials of plant origin In: Perspective on new crops and new uses (Janick, J. Editor) ASHS press, Alexandria, V A. pp 457-462.
- [23] A. H. Atta, and S. M. Moueir (2005) Evaluation of some medicinal plants extracts for antidiarrheal activity. *Phytother Res* 19: 303-309
- [24] Shettima et al 2012
- [25] E. A. Palombo (2006) Phytochemicals from traditional medicinal plants used in the treatment of diarrhea; modes of action and effects on intestinal function. *Phytother Res* 20:717-724
- [26] E. O. Ekundayo and J. N. Ekekwue (2013) Antibacterial activity of leaves of *Jatropha curcas* and *Euphorbia heterophylla*. *African Journal of Microbiology Research* 7(44): 5097-5100
- [27] O. O. Igbinosa, E. O. Igbinosa, O. Aiyegoro (2009) antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* Linn. *Afr J phar Pharmacol* 3(2): 058-062.