



Studies on the Effect of *Tephrosia linearis* (Wild) Pers. Aqueous Extract on *Jatropha curcas* Oil Induced Hemorrhoids in Rats

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ABSTRACT

Treatments of hemorrhoids by traditional medical practitioners using various plant species has been a long standing practice in various parts of Nigeria. This project work was carried out to ascertain and to evaluate the claim of traditional medical practitioners in the North-Eastern Nigeria on the effect of the plant species, *Tephrosia linearis* (Wild) Pers. in the treatment of hemorrhoids. Twenty Wister rats both male and female were used in this research work. A hemorrhoid model was prepared by the means of application of *Jatropha* oil into the rectum of rats. A cotton swab soaked with the inducer for 60 seconds was inserted into the anus of rats for 60 seconds. The rats were randomly divided into five groups: Group 1 (healthy rats), Group 2 (induced and untreated), Group 3 (induced and treated with standard, Daflon 10mg/kg b.w), Group 4 (induced and treated with aqueous extract of *T. linearis* 200mg/kg b.w) and Group 5 (induced and treated with aqueous extract of *T. linearis* 600mg/kg b.w). The results of the study observed were as follows; Group A; 1.52 ± 0.29 , Group B; 3.85 ± 0.05 , Group C; 1.95 ± 0.48 , Group D; 1.70 ± 0.14 and Group E; 1.70 ± 0.40 . The results obtained indicated that the rectoanal tissues of the rats in Group C,D, and E increased when compared to the control, Group A. The rectoanal tissues of the rats in Group B however experienced significant increase. From the study, extract of *T. linearis* presented the strongest anti-hemorrhoid activity in rats than Daflon 500. The use of this plant as treatment for hemorrhoids by traditional medicine practitioners in North-East Nigeria is justified by this study.

Keywords: Hemorrhoids, *Tephrosia linearis*, *Jatropha* oil, Daflon, Rectoanal

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INTRODUCTION

Hemorrhoids or piles are a common ailment among adults, more than half of men and women aged 50 years and older will develop hemorrhoid symptoms during their lifetime (Ali and Shoeb, 2017). According to the Merck Manual definition hemorrhoid is "Varicosities of the veins of the hemorrhoidal plexus, often complicated by inflammation, thrombosis, and bleeding" (Chopane *et al.*, 2020). Hemorrhoids are abnormal masses of dilated blood vessels in swollen tissue that occurs internally in the anal canal or externally around the anus which may be bleeding, pain or itching. It has been a common and painful human disease for decades (Cletus *et al.*, 2017; Gardner *et al.*, 2020).

The growing problem associated with this ailment especially among the local populace in North-east Nigeria States, results into various dilations of the internal hemorrhoids as well as psychological dilations which exist at infancy, is presumed to have developed into varicosities under the influence of wide range of factors (Cletus *et al.*, 2017). The predisposing factors of hemorrhoids include heredity age, anal sex, and prolonged labour at pregnancy as well as the stony dietary intake which put extra pressure on the intestinal mucosa linings of the small intestine (Cletus *et al.*, 2017). Scientific evidence supports that rationale of using native plants and traditional formulations in health care, in this modern era, all medical systems rely on synthetic medicine, but the plant materials remain an important resource for combating illnesses, including infectious disease (Agyemang *et al.*, 2021). Out of the native plants used in traditional medical systems, many have been investigated for potential drugs, alternative medicine, Food additives, agrochemicals and industrial chemicals (Kori *et al.*, 2020).

T. linearis. is a perennial shrub and legume species which are traditionally medically used as leaves for relieving earache and pain from fractures, bark for removing ticks

from camel ears (Jongbloed *et al.*, 2003). The plant possesses insecticidal and anti-cancer properties (Cheruth *et al.*, 2017). The plant possesses medicinal properties and has significant antibacterial properties; the leaves and the root have been used to treat bronchitis, cough, earache, wounds and bone fractures by herbalists in countries like Oman (Cheruth *et al.*, 2017; Ali *et al.*, 2019).

The prevalence of hemorrhoid is a problem amongst the local populace particularly in northern Nigeria mainly due to dietary habits and lifestyle (Ray-Offor and Amadi, 2019). Treatment of hemorrhoids by traditional medical practitioners using various plants species has been a long standing practice in various parts of Nigeria. This project work was carried out to ascertain and to evaluate the claim of traditional medical practitioners in the North-eastern part of Nigeria on the effect of the plant species *T. linearis* in the treatment of haemorrhoids.

MATERIALS AND METHODS

Plant Materials

The Medicinal plant was sourced from Vintim village, Mubi North LGA, Adamawa State. It was identified by Dr. M.T. Akase of the Department of Botany, Adamawa State University, Mubi, Nigeria. The voucher number of the plant is ADSU/BT/21/287.

Instruments/Equipment

The instruments used for the study include; Razor blade, Meter rule, Marker/Pen, Mechanical blender, Weighing balance, Rotary evaporator, Autoclave, Oven, Magnetic stirrer, vacuum and pressure pump.

Chemicals/Reagents

All chemicals and reagents in this study were of analytical grades and products of Sigma Aldrich Ltd. (U.S.A). They include; Whatman no.1 filter paper, Normal Saline,

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extract ointment, Silver sulfadiazine (standard), Diethyl ether, Methylated Spirit produced by Triveni chemicals.

Animals

Albino rats of either sex weighing 150-200g were purchased from Veterinary Research Institute, Vom, Jos, Plateau State, Nigeria and were taken to the animal house of the Department of Biochemistry and Molecular Biology, Nasarawa State University, Keffi, Nigeria. The experimental animals were kept in standard cages and maintained under standard condition of ambient temperature of 20-25°C with 12hrs day light cycles. The rats were provided with water and fed with standard pelletized feed *ad libitum*. Twenty rats were grouped into five with four rats in each group based on the following treatment Group A (Control), Group B (Untreated), Group C (Daflon, 10mg), Group D (*T. linearis* 200mg), Group E (*T. linearis* 600mg). The animals were handled in accordance with the guidelines and recommendations of the ethical committee on the use of laboratory animals for research.

Preparation of the Plant Extract *T. linearis*

The plant samples were chopped into small pieces and air dried at room temperature until dry. The dried samples were ground into fine powder using steel blender. Two hundred (200) gram of powdered plant material was placed in 200ml conical flask and 100ml of water was added. The mouth of the conical flask was covered with aluminum foil and kept in a reciprocating shaker for 24 h for continuous agitation at 150 rpm in order to ensure proper mixing and to dissolve in the respective solvent. Then, extract was filtered using muslin cloth followed by Whatman no. 1 filter paper. The solvent from the extract was removed by using rotary vacuum evaporator RE52 at the temperature of 50°C. Finally, the residues were collected and used for the experiment.

Induction of Hemorrhoid Using *J. curcas* Oil

Haemorrhoids were induced in the rats by the application of *Jatropha* oil into the anus of the rats for five days (Cletus *et al.*, 2019) with slight modifications. Hemorrhoids were induced to all the groups, except normal control group, by applying *J. curcas* oil preparation. On each of the five (5) days of induction, each rat was immobilized in a restrainer cage. The tampon of an ear cotton bud was dipped in 10ml of the above inducer contained in 10 ml vial for 60 s. Then, it was inserted into the rectum and allowed to contact the mucosa to a depth of 2 cm for 60 s and then removed smoothly. The animals were freed from their restrainer and placed in back into their cages.

Experimental Design and Administration of Extract

The rats were randomly grouped into five (5) groups of 4 rats each based on treatment plan. Group 1 Normal (not induced and untreated), Group 2 Induced and untreated, Group 3- induced treated with standard (Daflon 500mg, 10mg/kg), Group 4— Induced and treated with Exact (200mg /kg), Group 5 induced and treated with extract (600mg/kg). Before the administration of the extract, hemorrhoid was induced in the rats through the use of *Jatropha* oil for five (5) days. A day after the induction, all the rats were subjected to their respective treatment as designated once daily for a period of five days.

Recto-anal Coefficient

The animals were then sacrificed by an overdose of diethyl ether. The lower abdomen was opened, all of the reproductive organs removed, the rectum and the lower rectum were cut from any adhering tissue. Then a rectum-anus portion measuring 2 cm was cut from each rat starting from the circular hairline on the anus epithelium using a pair of compass. The cut piece was then opened longitudinally, blotted on a piece of tissue paper and weighed immediately. The weight of tissue corresponding to 150g body weight was then calculated in mg. Similarly, the percentage ratio of the whole 2cm piece relative to the total body weight of the animal was also calculated. The percentage reductions in the weights of the rectum of the treated animals in relation to the control weights were then calculated. The Rectoanal coefficient was calculated using the Formula;

$$\text{Rectoanal coefficient} = \frac{\text{weight of recto-anal tissue (mg)}}{\text{Body weight (g)}}$$

Statistical Analysis

The results were statistically analyzed using one way analysis of variance (ANOVA). Data were analysed using statistical package for social science (SPSS) version 23 and presented as Mean \pm SD, where SD= standard deviation. A value $p \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

Effect of *J. curcas* oil on the body weights of rats

Animals in the control group (untreated) experienced a steady increase in body weight. For the group Induced and untreated, there was significant ($p \leq 0.05$) reduction in the mean body weights of the animals in all the experimental groups after hemorrhoids was induced as shown in figure 1 below and after treatment with 10mg/kg b.w std drug, 200mg/kg b.w polar extract and 600mg/kg b.w polar extract, the body weights were observed to increased significantly.

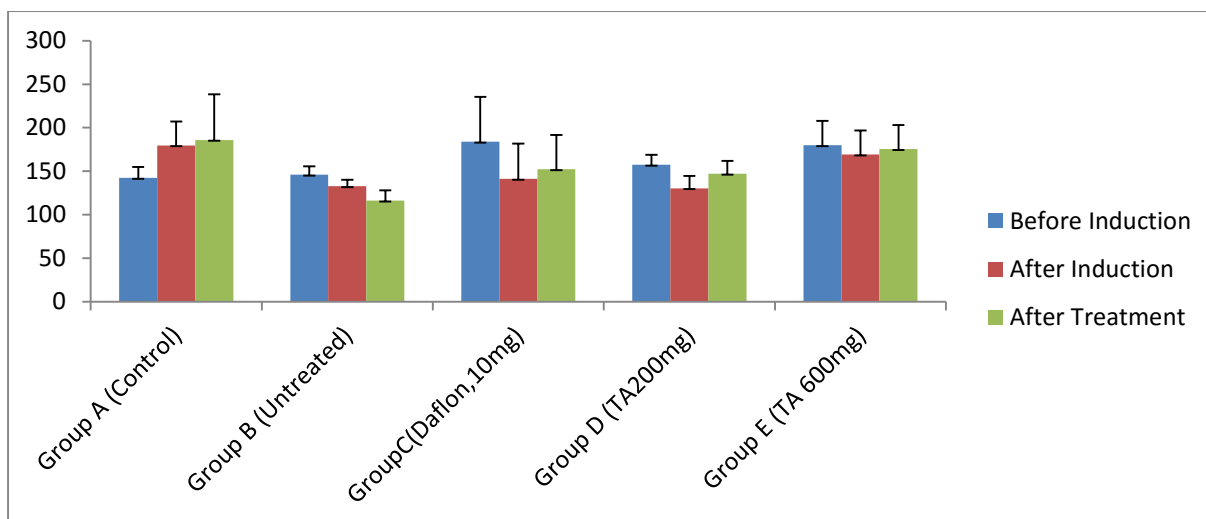


Figure 1: Effect of *J. curcas* oil on the body weights of rats

Effect of *T. linearis* extract of the Recto-Anal tissue of rat

As presented in the figure 2, the recto anal tissue of the rats induced and administered 10mg/kg b.w. std drug, 200mg/kg b.w. aqueous extract and 600mg/kg b.w.

aqueous extract shows there was no significant difference ($p \leq 0.05$) when compared to the control group. However, the rectoanal tissue of rats induced and untreated (Group B) was observed to have significant difference ($p < 0.05$) when compared with that of the control group.

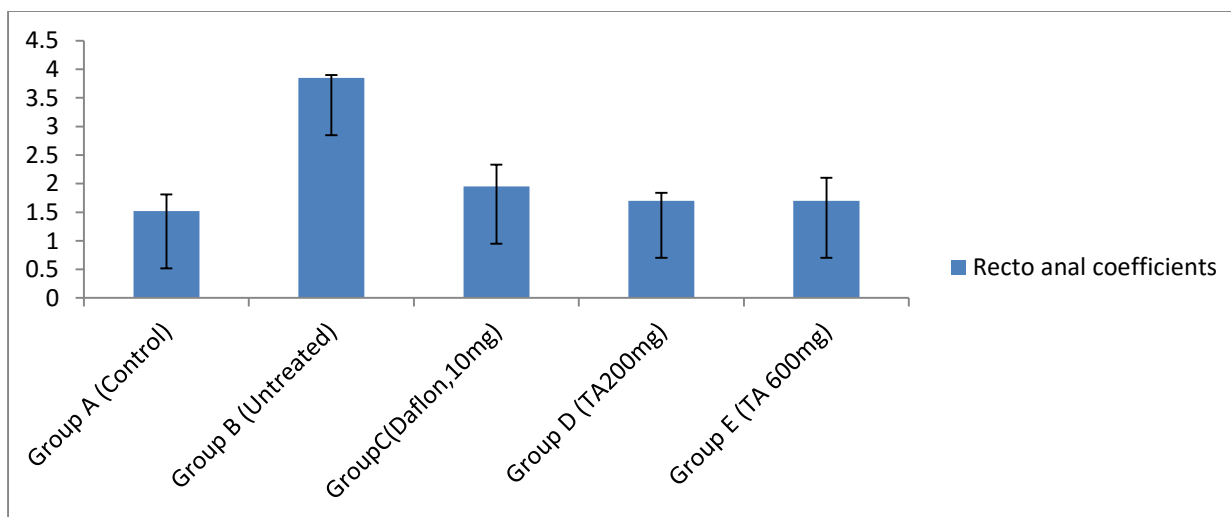


Figure 2: Effect of *T. linearis* on the recto anal tissue of rats

DISCUSSION

Haemorrhoids are pathological condition characterized by severe vasodilation at the recto-anal region, leading to inflammation of the surrounding tissues (Cletus *et al.*, 2017). This may lead to secondary complications such as extravasations of fluid into interstitial space mainly due to increased vascular permeability and migration of large quantity of inflammatory white blood cells (granulocytes and monocytes) (Sireeratawong *et al.*, 2012). In this study, *Jatropha* oil from the seeds of *Jatropha curcas* was used to induce experimental hemorrhoids in rats, it is known that *Jatropha* oil causes inflammation due to the release of soluble factors involving inflammatory lipid metabolites (Soweminio *et al.*, 2007). Other factors may have combined with the previously stated factors to regulate the resident cells (mast cells, endothelial cells, macrophages) and newly recruited inflammatory cells (monocytes, lymphocytes, eosinophils and neutrophils), activating them and leading to systematic response to inflammation (Kologrivova *et al.*, 2021). The fact that the oil from

Jatropha causes inflammation in the rectum of rats and mortality as witnessed on the third day and fourth day of induction suggests that the oil extract is toxic in higher doses and is a potential carcinogen in cancer research (González Fernández, 2020).

Animals in Group A (control) were not induced and experienced a steady weight gain throughout the duration of the study (Figure 1). The rats in Groups B-E experienced a weight drop after the induction of hemorrhoids using *Jatropha* oil as shown in Table 1 after 5 days of treatment, the rats in Groups C, D, and E experienced gain in body weight that brought them close to their initial weights before the induction. On the other hand, rats in Group B experienced further loss of body weights as they were not administered any treatment. In this case, loss in body weight in the animals after induction served as a symptom of haemorrhoids due to loss of blood and severe inflammation in the recto-anal region of the

rats, coupled with anal itching as seen in the animal groups (Kori *et al.*, 2020).

Intraperitoneal administration of the standard drug (Daflon 500) and plant extract *T. linearis* showed that the recto-anal tissue of the rats in groups C, D and E indicated no significant difference ($p \leq 0.05$) when compared with the rats in the control group A. The recto-anal tissue of the rats in group B however showed a significant increase ($p \leq 0.05$) when compared to the control, group A (Table 2). The standard drug Daflon 500 exerted healing effects on the experimental animals in Group C at 10mg/b. w. as evidenced by the reduction in the weight of their rectal tissues. The greatest healing of the rectum amongst the treated groups were shown in Group D and Group E, to which were administered 200 mg/b. w. and 600 mg/b. w. respectively of the plant extract *T. linearis*. This may suggest that the dosages given were equally effective and the plant extracts have been used for healing wounds, correct disorders internally and to promote immune responses (Kori *et al.*, 2020). *T. linearis* has been used to treat wounds and polio (Abu-Rabia, 2015), it is known to possess anticancer properties (Cletus *et al.*, 2017) and other various medicinal properties (Jongbloed *et al.*, 2003). Therefore, the extract from the plant may have exerted similar effect on the experimental animals in a dose-dependent fashion. The extract from *T. linearis* produced better recto-anal coefficient values than the first line drug Daflon 500.

CONCLUSION

Medicinal plants are a source of many biological ingredients that cannot be ignored. This study therefore shows that the aqueous extract of *Tephrosia linearis* possesses anti-hemorrhoid properties in rats. From the study, extract of *Tephrosia linearis* presented the strongest anti-hemorrhoid activity in rats than Daflon 500. This plant therefore is a definite source for the development and production of conventional medicine for the treatment of hemorrhoids (pile) other than the present alternatives available. The use of this plant as treatment for hemorrhoids by the Fali tribe traditional medicine practitioners in North-East Nigeria is justified.

RECOMMENDATION

Further studies should be undertaken to understand what compound causes the anti-hemorrhoid activity of the plant extract and the exact biochemical mechanism behind it.

REFERENCES

Abu-Rabia, A. (2015). *Indigenous medicine among the Bedouin in the Middle East*. Berghahn Books.

Agyemang, A. O., Amponsah, I. K., Mensah, M. L. K., Turkson, B. K., Baidoo, M. F., Orman, E., and Bayor, M. T. (2021). Utilization of Plants for Medicinal Purposes and Concerns with Endangered Plant Species from Ghana. *Traditional and Integrative Medicine*. pISSN: 2476-5104, eISSN: 2476-5112.

Ali, S. A., and Shoeb, M. F. R. (2017). Study of risk factors and clinical features of hemorrhoids. *International Surgery Journal*, 4(6): 1936-1939.

Ali, M. A., Mosa, K. A., El-Keblawy, A., and Alawadhi, H. (2019). Exogenous production of silver nanoparticles by *Tephrosia apollinea* living plants under drought stress and their antimicrobial activities. *Nanomaterials*, 9 (12): 1716.

Chopane, S. M., Dhonde, P., Thalkari, A. B., and Karwa, P. N. (2020). Time to think about Hemorrhoids. *Research Journal of Pharmacology and Pharmacodynamics*, 12 (1): 39-46.

Cheruth, A. J., Al Baloushi, S. A., Karthishwaran, K., Maqsood, S., Kurup, S. S., and Sakkir, S. (2017). Medicinally active principles analysis of *Tephrosia apollinea* (Delile) DC. growing in the United Arab Emirates. *BMC research notes*, 10(1): 1-6.

Cletus, A. U., Dibal, M. Y., Malgwi, T. S., Hadiza, M. I., Adama, Y. A., and Abubakar, U. S. (2017). Anti-hemorrhoid evaluation of selected medicinal plants used in bali north-east nigeria for the treatment of hemorrhoids (pile). *Journal of Pharmaceutical Research International*, 1-6.

Gardner, I. H., Siddharthan, R. V., and Tsikitis, V. L. (2020). Benign anorectal disease: hemorrhoids, fissures, and fistulas. *Annals of Gastroenterology*, 33(1): 9.

González Fernández, M. J. (2020). In vitro antitumor activity of arachidonic and docosahexaenoic acids as both monoacylglycerols and free fatty acids on colorectal cancer cells. PhD Dissertation submitted to the Department of Agronomy University of Almeria 225 pp

Jongbloed, M., Feulner, G., Böer, B., and Western, A. R. (2003). Environmental Research and Wildlife Development Agency. *The comprehensive guide to the wild flowers of the United Arab Emirates*. Abu Dhabi: Environmental Research and Wildlife Development Agency.

Kologrivova, I., Shtatolkina, M., Suslova, T., and Ryabov, V. (2021). Cells of the immune system in cardiac remodeling: Main players in resolution of inflammation and repair after myocardial infarction. *Frontiers in Immunology*, 12: 1056.

Kori, Y. S., Yogi, B., and Gupta, S. (2020). Antihaemorrhoid activity of Isolated and semi-synthesized Rutin Derivative from *Euphorbia hirta* Linn. *Research Journal of Pharmacy and Technology*, 13(3): 1333-1338.

Ray-Offor, E., and Amadi, S. (2019). Hemorrhoidal disease: Predilection sites, pattern of presentation, and treatment. *Annals of African medicine*, 18(1): 12.

Sireeratawong, S., Jaijoy, K., Panunto, W., and Soonthornchareonnon, N. (2012). Anti-inflammatory activity and toxicity of the water extract of *Terminalia chebula* Rezt in rats. *Planta medica*, 78(11): P112.

Sowemimo, A. A., Fakoya, F. A., Awopetu, I., Omobuwajo, O. R., and Adesanya, S. A. (2007). Toxicity and mutagenic activity of some selected Nigerian plants. *Journal of ethnopharmacology*, 113(3): 427-432.