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AN ASSESSMENT ON THE PROTECTION OF GYMNEMA SYLVESTRE EXTRACTS AGAINST CASTOR OIL – INDUCED DIARRHOEA IN RATTUS NOVERGICUS

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ABSTRACT

Diarrhoea is one of the main causes of death worldwide in children and adult alike. In view of this problem, the World Health Organization has encouraged studies for treatment and prevention of diarrhoea diseases depending on traditional medicinal practices. Anti-diarrhoeal effect of crude aqueous extracts of the stem, leaf and flower of *Gymnema sylvestre* was assessed in castor oil-induced diarrhoea at doses of 700, 1500 and 2000 mg/kg b.w, using *Rattus novergicus*. At these doses the extracts inhibited castor oil-induced diarrhoea in dose dependent fashion. There was significant ($p<0.05$) reduction in defecation severity when compared with the reference drug and control. The flower extract is more potent followed by the stem and the leaf. The result obtained in this study indicates that the aqueous extracts of *Gymnema sylvestre* may contain varied pharmacologically active ingredients with potent anti-diarrhoeal activity.

Key words: Castor oil, *Gymnema sylvestre*, anti-diarrhoea, *Rattus novergicus*

INTRODUCTION

Diarrhoea is the condition of having three or more loose or liquid bowel movements per day [1]. It is a common cause of death in developing countries and the second most common cause of infant deaths worldwide. The loss of fluids through diarrhoea can cause dehydration and electrolyte disturbances such as potassium deficiency or other salt imbalances. In 2009, diarrhoea was estimated to have caused 1.1 million deaths in people aged 5 and over 1.5 million deaths in children under the age of 5 years[1] Diarrhoea remains the second leading cause of death and one of the leading causes of mortality in developing countries and the major cause of this malnutrition.

The World Health Organization (WHO) has encouraged studies for treatment and prevention of diarrhoeal diseases depending on traditional medicinal practices [2, 3, 4, 5]. Healing with medicinal plants is as old as mankind. A large amount of archaeological evidence exist which indicates that humans were using medicinal plants during the Palaeolithic, approximately 60,000 years ago. Furthermore, other non-human primates are also known to ingest medicinal plants to treat illness [6]. Awareness of medicinal plant usage is a result of the many years of struggles against illness due to which man learn to pursue drugs in barks, leaves, seeds, fruits and other parts of plant. The use of medicinal plants as fundamental component of the African traditional health care system is

perhaps the oldest and the most assorted of all the therapeutic systems. In many parts of rural Africa, traditional healers prescribing medicinal plants are the most easily accessible and affordable health resources available to the local community and at times, the only therapy that subsists [7].

Gymnema sylvestre (G. sylvestre), Asclepiadaceae has been used as a traditional medicinal plant in Africa, Australia and Asia, especially in India [8].

G. sylvestre R.Br. is a perennial, woody climber [8]. It is a potent anti-diabetic plant and used in folk, ayurvedic and homeopathic systems of medicine. It is also used in the treatment of asthma, eye complaint, family planning, snake bite, urinary complaints, stomach problems, piles, chronic cough, colic pain, cardiopathy, constipation, dyspepsia and haemorrhoids. In addition ,it also possesses antimicrobial, anti-hyper cholesterolemic, anti-inflammatory and sweet suppressing activities and it also acts as feeding deterrents to caterpillar [9].

The leaves of *G. sylvestre* contain triterpene saponins belonging to oleanane and damarane classes. The major constituents like gymnemic acids and gymnema saponins are members of oleane type of saponins while gymnemasides and damarane saponins [10, 11]. Other phyto-constituents include anthraquinones, flavones, stigmasterol, butyric acid, phytin, resins, lupeol, calcium oxalate, ß-amyrin related glycosides [12].

Numerous bioactive compounds isolated from the plant both as pure compound or as crude extracts possess medicinal properties and clinically tested in animal model systems for scientific validation [13].

The aim of this study was to assess the anti-diarrhoeal effect of aqueous stem, leaf and flower extracts of *G. sylvestre* in *Rattus novergicus*.

MATERIALS AND METHODS

Plant Material Collection:

Stems, leaves and flowers of *G. sylvestre* were collected green and fresh from a farm land very close to Ajaokuta Steel Company in Ajaokuta Local Government Area in Central Senatorial District of Kogi State, Nigeria. The plant had already been authenticated by Dr. William D. Hawthrone of James Martin Research Fellow, plant for the 21st century, Department of Plant Sciences,University of Oxford. The plant sample was rinsed with clean water to remove dirt particles. After that, the leaves were detached, and the flowers were carefully plucked from the stem and were separately spread and air-dried under shade at room temperature for three weeks.

Preparation of Plant Extract:

The air-dried, leaves and flowers were pulverized into powder using an electric blender, while the stem was first pounded into semi-powder using mortar and pestle, which was further pulverized into fine powder using a mechanical grinder.

Cold extraction method was used to obtain aqueous extracts of the samples. Portions (197.7g) of powdered leaf, 271.15g of stem and 103.89g of flower were soaked in different containers (small rubber bucket) with 1000ml of distilled water each. They were properly stirred and left for four days with occasional stirring each day. The mixtures were then filtered using the high pressure vacuum pump machine. The filtrates in the beakers were concentrated by evaporation at 60^{o}C to dryness in a water bath.

Experimental Animals:

Rattus novergicus of either sex (167.28 – 205.10g), obtained from a breeder at Kogi State University Staff Quarters, were housed in wired cages in the animal house of the Department of Biochemistry, Faculty of Natural Sciences, Kogi State University, Anyigba, Nigeria and were acclimatized for two weeks prior to the commencement of the experiments. The animals were housed under standard laboratory

conditions, light and dark cycles of 12h, and were provided with standard rodent pellet diet and water *adlibitum*. The animals were categorized into control and experimental groups. The experimental groups were administered, in addition to feed and water, aqueous extracts of *G. sylvestre* (AEGS) or standard drug (Loperamide hydrochloride) for a period of three (3) days. Appropriate authority has consented to the use of these animals in the Department for experimental purpose.

Drugs:

Castor oil, Loperamide hydrochloride (5mg/kg) were obtained from Kuzak Pharmacy, Anyigba, Kogi State, Nigeria.

Anti-Diarrhoeal Activity Evaluation:

Castor oil-induced diarrhoea in rats: Castor oil-induced diarrhoea was carried out as described by Awouters [14, 15]. A total number of 20 wistar rats of either sex (167.28 – 205.10g) were randomly distributed into five groups of four rats each. Group 1 served as the control administered distilled water (1ml/kg) orally by gavage and groups 2-5 as the experimental groups. Groups 2, 3 and 4 were administered AEGS orally by gavage at doses of 700, 1,500 and 2000 mg/kg b.w. respectively, while group 5 was administered the standard drug, Loperamide hydrochloride (5 mg/kg b.w.).

After two days of AEGS or Loperamide hydrochloride administration, all the animals (groups 1-5) were fasted for 18h before the third day treatment. After 1h of treatment with distilled water, AEGS or standard drug on the third day, diarrhoea was induced by administration of 2ml of castor oil orally to each rat. Each animal was placed in an individual cage, the floor of which was lined with absorbent paper and observed for 4h. The frequencies of characteristic diarrhoeal droppings were noted on the absorbent paper placed beneath the individual rat [16, 17, 18]. The percentage inhibition of diarrhoea by the extract was calculated as follows [19, 20, 21).

% of inhibition = 100 – {(FNE/FNC) x 100}
Where:
FNE = Mean fecal number of each experimental group
FNC = Mean fecal number of the control group.

Statistical Analysis:

In all the above experiments, the results have been expressed as mean (S.E.M). Statistical significance testing was performed by ANOVA and P – values were calculated by comparing with respective controls. $P<0.05$ implies significance [22].

RESULTS

Castor oil-induced diarrhoea: The aqueous stem extract of *G. sylvestre* significantly ($p<0.05$) reduced the number of wet faeces dose

Table-1. Efect of aqueous *G. sylvestre* stem extract on Castor oil-induced diarrhoea in *Rattus novergicus*.

Group	Treatment/dosage (mg/kg)	Number of wet faeces	% inhibition of diarrhoea
1	Distilled water (1ml/kg)	10.00±1.00	-
2	700	5.25±0.95	47.50*
3	1500	2.50±0.29	75.00*
4	2000	2.25±1.04	75.20*
5	Loperamide hydrochloride (5mg/kg)	3.00±1.29	70.00*

n = 4, values are means ± SEM; * = $P<0.05$ significant difference when compared with control using one way ANOVA test for significance.

dependently and are more effective than the reference drug (Loperamide hydrochloride (5mg/kg) (Table 1).

Table 2 shows the effect of aqueous *G sylvestre* leaf extract on Castor oil-induced diarrhoea in rats. The leaf extract significantly inhibited diarrhoeal defecation in dose dependent manner and much more comparable to the standard drug used (Loperamide hydrochloride).

The results in table 3 indicate the effect of aqueous *G. sylvestre* flower extract on Castor oil-induced diarrhoea in rats. The flower extracts inhibited diarrhoea in dose related fashion and is more effective than the reference drug in diarrhoea prevention.

DISCUSSION

The age long use of herbal medicines in the treatment of diarrhoeal disease is a common practice in many countries across the globe including Nigeria. Therefore, the need to substantiate or otherwise the claim on *G. sylvestre* as an anti-diarrhoeal agent using animal model of diarrhoea cannot be overemphasized. The results showed that there was statistically significant reduction not only on the onset of diarrhoea but also on its severity as revealed by the Castor oil-induced diarrhoea.

Castor oil was used in this experiment to induce diarrhoea. It is well documented that castor oil produces diarrhoea due to its most active metabolite, ricinoleic acid by hypersecretory response, which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa [23, 24, 25]. Its action also stimulates the release of endogenous prostaglandins E and F which cause stomach cramp and diarrhoea due to the

Table 2: Effect of aqueous *G. sylvestre* leaf extract on Castor oil-induced diarrhoea in *Rattus norvegicus* .

Group	Treatment/dosage (mg/kg)	Number of wet faeces	% inhibition of diarrhoea
1	Distilled water (1ml/kg)	9.00±1.23	-
2	700	4.25±1.32	52.78*
3	1500	3.25±1.38	63.89*
4	2000	3.01±1.10	63.98*
5	Loperamide hydrochloride (5mg/kg)	2.50±0.65	72.22*

n = 4, values are means ± SEM; * = P<0.05; ** = p<0.01); significant difference when compared with control using one way ANOVA test for significance. Values were considered significant when p<0.05 and non-significant when p>0.05.

Table 3: Effect of aqueous *G. sylvestre* flower extract on Castor oil-induced diarrhoea in *Rattus norvegicus*

Group	Treatment/dosage (mg/kg)	Number of wet faeces	% inhibition of diarrhoea
1	Distilled water (1ml/kg)	9.00±0.58	-
2	700	4.50±0.65	50.00*
3	1500	1.51±0.87	83.33*
4	2000	1.49±0.05	83.59*
5	Loperamide hydrochloride (5mg/kg)	2.58±0.67	79.89*

n = 4, values are means ± SEM; ** = p<0.01); * = p<0.05. Significant difference when compared with control using one way ANOVA test for significance.

effect on the smooth muscle and secretion [26, 27]. Ricinoleic acid initiates diarrhoea via several mechanisms such as: causing irritation and inflammation which stimulates secretory diarrhoea [28].

It increases the volume of intestinal content by preventing the re-absorption of water as well as interfering with oxidative metabolism and thus an effect on adenylate cyclase or mucosal adenosine 3, 5 – cydic monophosphate content; and being cytotoxic to intestinal epithelial cells and causing histological abnormalities and mucosal permeability [29].

These sequences of events may be related to the release of eicosanoids, prostaglandins, nitric oxide, platelet activating factor, c AMP and tachkinins by the intestinal mucosal, which consequently could give rise to diarrhoea.

The inhibition of experimental diarrhoea and the reduction in fecal output by a substance are the basis of the pharmacological evaluation of a potential anti-diarrhoeal agent [30, 31].

In this experiment, loperamide hydrochloride was utilized as the standard drug, which is currently one of the most efficacious and widely employed anti-diarrhoeal drugs [32, 14, 33]. Loperamide, apart from regulating the gastro intestinal tract, is also reported to slow down transit in the intestine, reduce colon flow rate, and consequently any effect on colonic motility [34]. The therapeutic effect of loperamide is believed to be due to its anti-motility and anti-secretary properties [35].
As presented in table 1, 2 and 3, the significantly ($p<0.05$) prolonged time of induction of diarrhoea, decreased frequency of stool and fecal parameters (total number of wet faeces) following the administration of the extracts of *G. sylvestre* suggest anti-diarrhoeal activity at these doses.

This assertion was further supported with the increased inhibition of defecation. The high percentage of inhibition of defecation in the extracts (at doses of 700, 1500 and 2000 mg/kg b.w.) and reference drug (Loperamide hydrochloride 5mg/kg) suggest that the anti-diarrhoea activity of the extracts may proceed via the same mechanism as that of the reference drug, Loperamide hydrochloride (5mg/kg).

The extracts might have exerted anti-diarrhoeal activity via secretory mechanism as evident from reduction in total number of wet faeces (Table 1, 2 and 3). Furthermore, this anti-diarrhoeal activity could have resulted from the inhibitory activity of aqueous stem, leaf and flower extracts of *G. sylvestre* on prostaglandins synthesis, nitric oxide and platelet activating factors production, as inhibitors of prostaglandins and nitric oxide synthesis are known to delay diarrhoea induced by castor oil [36].

In this study, there was a statistically significant ($p<0.05$) reduction in the incidence and severity of diarrhoeas stool produced in the experimental animals. In all the extracts (stem, leaf and flower) (Table 1, 2 and 3) significantly inhibited the frequency of defecation (number of wet faeces) in dose dependent fashion. All the doses of extracts like the standard drug (5mg/kg loperamide) significantly reduced the frequency of defecation droppings compared to control. This result is in accordance with previous claims in respect of anti-diarrhoeal herbs[25]. Anti-diarrhoeal plants are known to reduce number of wet stools as reported for *Eremomastax speciosa* and *Momordica charantia* Linn [25, 37].

CONCLUSION

The result obtained in this study strongly suggests that *G. sylvestre* extract has inhibitory effect on castor oil-induced diarrhoea and justify the use of the plant in folk medicine and its use as a non-specific anti-diarrhoeal agent. In conclusion, this plant material from this preliminary study may be claimed as a potent anti-diarrhoeal agent. The anti-diarrhoeal potency may be related to its phytoconstituents.

Further studies are needed to determine the exact bioactive component of the plant contributory to its anti-diarrhoeal activity.

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CONFLICT OF INTERS STATEMENT

I hereby declare that there is no conflict of interest in this work.

REFERENCES

1. WHO (2009). The evolution of diarrhoeal and acute respiratory disease control at WGO. WGO, Geneva 1999. http//whqlibdoc.who.nq/WGO-CHI_CAH_99.12pdf.

2. WHO (1978). Ama Ata Declaration. Primary Health Care, Health for all series No. 1.

3. WHO (1999). WHO Monographs on selected medicinal plants: Bulbus Allii capae. World Health Organization, Geneva, Pp. 289, ISBN: 92415.

4. WHO (2011). World Health Statistics. World Health Organisation, Geneva.

5. Cynthia, B.P., Lana, V., Kenji, S. (2008). Estimating child mortality due to diarrhoea in developing countries: A meta-analysis review. Bull. World Health Organization, 86(9): 710-717.

6. Sumner, J. (2000). The natural history of medicinal plants. Timber press, p. 16, ISBN: 0-88192-483-0.

7. Fawzi, M. (2013). Traditional medicine in Africa: appraisal of ten potent African medicinal plants. Evidence-Based Complementary and Alternative Medicine. Article ID617459. DO1.org/101155/2013/617459.

8. Kanetkar, P., Singhal, R and Kamat, m. (2007). "*Gymnema sylvestre*: a memoir", *Journal of Clinical Biochemistry and Nutrition*, 41(2): 77-81.

9. Gurav, S., Gulkari, V., Duragkar, N., Patil, A (2007). A systematic review: Pharmacognosy, photochemistry, pharmacology and clinical applications of *Gynmema sylvestre* R.Br. Phamacognosy Reviews. 1:338-343.

10. Foster, S. (2002). "*Gymnema sylvestre*" In: Alternative Medicine Reviews Monographs, 205-207. Thorne Research Inc.

11. Khramov, V., Spasv, A.A and Samokhina, M.P. (2008). Chemical composition of dry extracts of *Gymnema sylvestre*. Pharmaceutical Chemistry Journal, 42(1): 30-32.

12. Suisheimer, J.E., Rao, G.S. and Mclhenny, H.M. (1970). Constituents from *Gymnema sylvestre* leaves: Isolation and preliminary characterization of gymnemic acids. Journal of Pharmaceutical Sciences, 59(5): 622-628.

13. Baskaran, K; Ahmath, B.K; Shanmugasundaram K.R and Shanmugasundaram, E.R.B. (1990). Antidiabetic effect of a leaf extract from *Gymnema sylvestre* in non-insulin dependent diabetes mellitus patients, JNournal of Ethnopharmacology, 30(3): 295-305.

14. Awouters, F., A. Megens, M., Verlinden, J., Schuurkes, C., Niemegeers, C.J.E. and Janssen, P.A. (1993). Loperamide: Survey of studies on mechanism of its anti-diarrhoeal activity. *Dig. Dis. Sci.*, 38: 977-995.

15. Awouters, F; Niemegeers, C.J.E; Lenaerts, F.M. and Janssen, P.A. (1978). Delay of castor oil diarrhoea in rats: A new way to evaluate inhibitors of prostaglandin biosynthesis. *Journal of Pharmacol.* 30:41-45.

16. Izzo, A.A; Nicoletti, M; Giannattasio, B. and Capasso, F. (1992). Anti-diarrhoeal activity of terminalia sericea Burch ex. DC extract.

In: Capasso, F. and N. Mascolo (Eds.), Natural Drugs and the Digestive Tract. EMSI, Rome, Pp. 223-230.

17. Mukherjee, P.K; Das, J., Balasubramanian, R., Saha, K., Pal, M., Sah. B.P. (1995). Anti-diarrhoea evaluation of Nelumbo nucifera rhizome extract. *Indian J. Pharmacol.*, 27: 262-264.

18. Karim, A., Makhfi, H., Ziyyat, A., Legssyer, A., Bnouham, M. (2010). Anti-diarrhoeal activity of crude aqueous extract of Rabra tintoruml. Roots in rodent. *J. Smooth Muscle Resource*; 46(2): 119-123.

19. Sale, M.L., Njinga, N.S., Musa, A.M., Magaji, M.G., Abdullahi, A. (2009). Phytochemical and anti-diarrhoea studies of the stem bark of *Ceiba pentandra* (Bombacaceae). *Nige. J. Pharm. Sci.,* 8(1): 143-148.

20. Akuodor, G.C., Idris-Usman, M.T., Ugwu, C., Akpan, J.L., Irogbeyi, L.A., Iwuanyanwu, T.C., Osunkwo, U.A. (2010). Ethanolic leaf extract of verbena hastate produces anti-diarrhoeal and gastro intestinal motility slowing effects in albino rats. *J. Med. Plant – Res.*, 4(16) 1624-1627.

21. Akuodor, G.C., Muazzam, I., Usman-Idris, M., Megwas, U.A., Akpan, J.L., Chilaka, K.C., Okorafor, D.O., Osunkwo, U.A. (2011). Evaluation of the anti-dirrhoeal activity of methanol leaf extract of Bombax buonopozense in rats. *Ibnosina J. Med. Biomed. Sci.*, 3(1): 15-20.

22. Woodson, R.F. (1987). Statistical methods for the Analysis of Biomedical Data, Wiley, Chechester; p. 315-316.

23. Zavala, M.A., Perez, S., Vargal, R. and Perez, R.M. (1998). Anti-diarrhoeal activity of *Walteria* Americana, Coumelins cuelestries and alternathra repens. *J. Ethnopharmacol*; 61: 41-47.

24. Hardman, J.G. and Limbird, L.E. (2001). Drugs affecting gastrointestinal function. In: Goodman, L.S. and Gilman, A. (Eds.), the Phamarcolonical Basis of Therapeutics. 10th Edn., McGraw Hill, New York, Pp. 1023-1024.

25. Bakare, R.I., Magbagbebla, O.A., Akinwade, A.I., Okunowo, O.W. and Green, M. (2011). Anti-diarrhoeal activity of aqueous leaf extract of Monordica charantia in rats. *J. Pharmacogn. Phytother*; 3(1): 1-7.

26. Galvez, J., Zarzuelo, A., Crespo, M.E., Lorente, M.D., Ocente, M.A. and Jimenez, J. (1993). Anti-diarrhoeal activity of Euphorbia hirta isolation of an active flavonoidal constituent. Planta Med., 59: 33-36.

27. Saito, T., Mizutani, F., Iwangga, Y., Morikawa, K., Kato, H. (2002). Laxative and anti-diarrhoeal activity of polycarbophil in mice and rats. *Japanese J. Pharmacol*; 89: 133-141.

28. Mbagwu, H.D.C., Adeyemi, O.O. (2008). Anti-diarrhoeal activity of the aqueous extract of Mezoneuron Benthamianum Baill (caesalpinaceae). *J. Ethnopharmacol*; 116: 16-20.

29. Mascolo, N., Izzo, A.A., Barbato, F., Capasso, F. (1993). Inhibitors of nitric oxide synthelase prevent castor oil induced diarrhoea in rats. *Br. J. Pharmacol.* 108: 861-64.

30. Watson, W.C., Godon, R. (1962). Studies on the digestion, absorption and metabolisms of castor oil. Biochem. Pharmacol; 11: 229-236.

31. Ammon, P.J., Thomes, P.S. (1974). Effect of oleic and recinoleic acids net jejunal water and electrolyte movement. *J. Clin. Investigat;* 53: 374-379.

32. Niemegeers, C.I.L., Lenaerts, F.M., Janseen P.A.J. (1974). Loperamide (R-18553): A novel type of anti-diarrhoeal agent. Part 1. In: Vitrodrab pharmacology and acute

toxicity comparing with morphine, codeine and difenoxine. Atzneimittelforsch, 24: 1633-1636.

33. Balogun, S.O., Tanayen, J.K., Ajayi, A.M., Ibrahim, A., Ezeonwumelu, J.O.C., Oyewale, A.A., Oloro, O.J; Goji, A.D.T; Kiplagat, D.M. and Adzu, B. (2011). Preliminary evaluation of anti-diarrhoeal, ulcer – protective and acute toxicity of aqueous ethnanolic stem bark extract of ficus trichopoda in experimental rodents. *Asian J. Med. Sci.,* 3(1): 37-42.

34. Theoderan, V., Floramont, J., Hachet, T., Bueno, L. (1991). Absorptive and motor components of anti-diarrhoeal action of Loperamide: An *invitro* study in pigs. Gut, 32: 1355-1359.

35. Couper, I.M. (1987). Inhibition in intestinal motility and the secretion by flavonoids in mice and in rats: Structural activity relationship. *J. Pharmacy and Pharmacol.*, 45: 1054-1059.

36. Capasso, F., Mascolo, N., Izzo, N., Gaginella, A.A. (1994). Dissociation of castor oil-indcued diarrhoea and intestinal mucosal injury in rat: Effect of NG-nitro-l-arginine methyl ester. *Br. J. Pharmacol;* 113: 1127-30.

37. Oben, J.E., Assi, S.E., Agbor, G.B., Musoro, D.F. (2006). Effect of Remomastax speciosa on experimental diarrhoea. *African J. Trad. Complementary.* 3(1): 95-100.

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