ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in
Gandhimathi R et al /J. Pharm. Sci. & Res. Vol.1(2), 2009,61-66

Pharmacological studies of anti-diarrhoeal activity of *Guettarda speciosa* (L.) in experimental animals.

Gandhimathi R*., Saravana Kumar A[1]., Senthil Kumar K.K[2]., Kusuma Praveen Kumar[3], Uma Maheswari J[4].

*Department of Pharmaceutical Chemistry, Sree Vidyanikethan College of Pharmacy, Sree Sainath Nagar, Chandragiri (M), Tirupati, Andhra Pradesh, India-517102.

[1]Department of Pharmacology, Sree Vidyanikethan College of Pharmacy, Sree Sainath Nagar, Chandragiri (M), Tirupati, Andhra Pradesh, India-517102.

[2]Department of Pharmaceutical science, St.Peter's College Of Pharmacy, Madikonda, Kazipet, Warangal Dt, , Andhra Pradesh India-506 001

[3]Department of Pharmaceutical chemistry, Vaagdevi Institute of Pharmaceutical Sciences, Bollikunta, Warangal Dt, Andhra Pradesh India-506 002

[4]Department of Pharmaceutical science, SRR College of Pharmaceutical Science, Valbapur (V), Elkathurthi (Mdl), Karimnagar Dt., Andhra Pradesh, India-505476.

Abstract

Aims: The purpose of the present study was to evaluate scientifically the anti-diarrhoeal effects of ethanolic (90%) extract of inner bark of *Guettarda speciosa.* Linn (EEGS) was studied against castor oil-induced-diarrhoea model in rats.

Methods: Antidiarrhoeal activity of 90% ethanol extract of *Guettarda speciosa* was investigated in this study using castor oil-induced-diarrhoea, enteropooling and Small intestinal transit models in rats. The weight and volume of intestinal content induced by castor oil were studied by enteropooling method.

Results: Standard drug diphenoxylate (5 ml/kg, p.o) was significant reductions in fecal output and frequency of droppings whereas EEGS at the doses of 200 and 400 mg/kg p.o significantly (P<0.001) reduced the castor-oil induced frequency and consistency of diarrhoea and enteropooling. The gastrointestinal transit rate was expressed as the percentage of the longest distance travelled by the charcoal divided by the total length of the small intestine. EEGS at the doses of 200 and 400 mg/kg significantly inhibited (P<0.001) the castor oil induced charcoal meal transit.

Conclusion: The EEGS showed marked reduction in the number of diarrhoea stools and the reduction in the weight and volume of the intestinal contents, as well as a modest reduction in intestinal transit. The results obtained establish the efficacy and substantiate the folklore claim as an anti- diarrheal agent. Further studies are needed to completely understand the mechanism of anti-diarrhoeal action of *Guettarda speciosa.*

Keywords: Antidiarrhoeal Activity, *Guettarda spe ciosa*, Traditional medicine, Castor Oil- induced diarrhoea, Enteropooling Method, Small intestinal transit

Introduction

Guettarda sp eciosa Linn. (Family: Rubiaceae) is widely distributed from East Africa to India and throughout to Malaysia into the South Pacific. This plant is common along the seashore, sea cliffs, beach thickets and low land forest. It is a spreading and much branched tree up to 20m height. In Tahiti, the plant has antidiarrheic, febrifugal and anticholinergic applications. In New Guinea, a preparation of the bark is drunk to cure dysentery. The native practitioners in and around Tirunelveli District, India, have claimed that the inner bark of this plant are being traditionally used in diarrhoea **[1, 2].** In the previous studies, we have investigated Antiepileptic activity of guettarda speciosa. **[3].** Upon literature review it was found that the plant contains loganic acid and secologanin **[4, 5].** However there are no reports on the antidiarrheal activity of the plant. Hence, the present study was designed

***For Correspondence**

Mobile no : +91-9959203901
E-mail: sarganjune1@gmail.com

to verify the claims of the native practitioners.

Materials and Methods

Plant collection

The Plant material of inner bark of *Guettarda Spe ciosa* used for investigation and it was collected from Tirunelveli District, in the Month of August 2008. The plant was authenticated by Dr.V.Chelladurai, Research Officer Botany. C.C.R.A.S., Govt. of India. The voucher specimen (CHE-SA-GS-01) of the plant was deposited at the college for further reference.

Preparation of extracts

Inner bark of the whole plants were dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (60gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. Percentage yield of ethanolic extract of *G. sp eciosa* was found to be 17.5 % w/w.

Preliminary phytochemical screening

The phytochemical examination of ethanolic (90%) extract of inner bark of *Guettarda speciosa* was performed by the standard methods **[6]. (Harbone 1973).**

Animals used

Albino wistar rats (150-230g) of either sex were obtained from the animal house in C.L. Baid Metha College of Pharmacy, Chennai. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given *ad lib itum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Ref No. IAEC / XIII / 01 / CLBMCP / 2008 - 2009).

Castor oil-induced diarrhoea

Diarrhoea was induced by Nwodo and Alumanah (1991) and Nwafor *et al*., (2005) **[7, 8].** Animals were fasted for 24 h but allowed free access to water. Rats were divided into four groups of six animals each, diarrhoea was induced by administering 2 ml of castor oil orally to rats. Group I treated as control (2 ml/kg, p.o. saline), group II received diphenoxylate (5 mg/kg p.o) served as standard and group III and IV received EEGS (200 and 400 mg/kg, p.o) 1 h before castor oil administration. Then observed for consistency of faecal matter and frequency of defaecation for 4 hrs.

Castor oil-induced enteropooling

Intraluminal fluid accumulation was determined by the method of Robert *et al .,* (1976) and DiCarlo *et al .,* (1994) **[9, 10].** Animals were fasted for 24 h but allowed free access to water. Rats were divided four groups of six animals each. Group I received normal saline (2 ml/kg, p.o served as a control, group II received diphenoxylate (5.0 mg/kg p.o.) and groups III and IV received EEGS 200 and 400 mg/kg p.o respectively 1hr before the oral administration of castor oil. Two hours later the rats were sacrificed, the small intestine was removed after tying the ends with thread and weighed. The intestinal contents were collected by milking into a graduated tube and their volume was measured. The intestine was reweighed and the difference between full and empty intestines was calculated.

Small intestinal transit

Rats were fasted for 18 h divided into five groups of six animals each, Group I received 2 ml normal saline orally, group II received 2 ml of castor oil orally with saline 2 ml/kg p.o, group III received atropine (3 mg/kg, i.p.), group IV and V received EEGS 200 and 400 mg/kg p.o respectively, 1 h before administration of castor oil. One ml of marker (10% charcoal suspension in 5% gum acacia) was administered orally 1 h after castor oil treatment. The rats were sacrificed after 1h and the distance traveled by

charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum **[11].**

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the groups was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test *P* values less than 0.05 were considered as significance.

Results

Phytochemical Screening

The results of preliminary phytochemical screening of the ethanolic extract of inner bark of *Guettarda speci osa.* Linn revealed that presence of alkaloids, flavonoids, triterpinoids, carbohydrates, tannins, phenols, gums and mucilage and absence of saponins and steroids.

Castor oil-induced diarrhoea

After 30 min administration of castor oil the diarrhoea was clinically apparent in all the animals of control group, for the next 4 h. This was markedly reduced by diphenoxylate (5 mg/kg p.o) (67.79%). A similar marked reduction in the number of defecations over four hours was achieved with *G.speciosa* at the doses of 200 or 400 mg/kg p.o. EEGS 200 and 400 significantly inhibited the defecation (41.45% and 61.19%) EEGS 200 and 400 mg/kg, p.o. dose of extract delayed the onset of diarrhoea and only 30% of animals showed diarrhoea at first hour (P<0.001) (Table 1 and Figure 1)

Castor oil-induced enteropooling

Castor oil caused accumulation of water and electrolytes in intestinal loop. Castor oil-induced enteropooling is not influenced by diphenoxylate (5 ml/kg p.o) in rats. EEGS 200 and 400 produced a dose-dependent reduction in intestinal weight and volume. EEGS 200 and 400 mg/kg, p.o dose produced 34.43% and 54.77% inhibition of volume of intestinal content respectively with significance (P<0.001). The weight of intestinal content was also reduced significantly at both the doses. **(**Table 2 and Figure 2**).**

Small intestinal transit

The percent intestinal transit was increased with castor oil (90.77%), but it was reduced in both doses of extract, and much more markedly by atropine (37.02%). EEGS 200 mg/kg, p.o dose of extract produced 62.54% intestinal transit induced by castor oil respectively. Whereas, EEGS 400 mg/kg, p.o dose produced 48.80% of castor oil induced charcoal meal transit (Table 3 and Figure 3).

Discussion and Conclusion

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, accompanied by hurry, resulting in an excess loss of fluid in the faeces. At doses of 200 and 400 mg/kg, the ethanol extract of *Guettarda speciosa* showed significant anti-diarrhoeal activity against castor oil-induced diarrhoea as compared with the control

Table 1: Effect of *EEGS* on castor oil-induced diarrhoea in rats.

Group	Treatment	Mean Defecation in 4hr	% Inhibition of Defecation
I	Castor oil (2ml p.o) + saline (2ml/kg p.o)	25.33±1.70	---
II	Castor oil (2ml p.o) + diphenoxylate (5 ml/kg p.o)	8.16±0.30**	67.79
III	Castor oil (2ml p.o) + EEGS (200mg/kg p.o)	14.83±0.91*	41.45
IV	Castor oil (2ml p.o) + EEGS (400mg/kg p.o)	9.83±0.80**	61.19

Effect of EEGS on castor oil-induced diarrhoea in rats: EEGS was administered p.o 1 h before castor oil administration. Values are expressed as mean ± SEM from the experiments. *P<0.01, **P<0.001 when compared with *Castor oil* + saline-treated group.

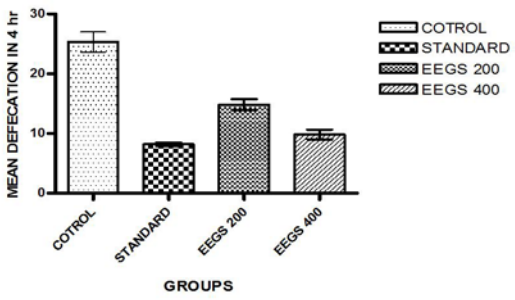

Figure 1: Effect of *EEGS* on castor oil-induced diarrhoea in rats.

group it significantly(P<0.001) reduced the frequency of diarrhoea and consistency of defecations. **(Table 1 and Fig ure 1).** The EEGS also showed a dose related decrease in castor oil-induced diarrhoea. Several mechanisms have been supposed to be involved in the diarrhoeal effect of castor oil **[12].** These include Castor oil is decreases fluid absorption, increases secretion in the small intestine and colon, and affects smooth muscle contractility in the intestine. Castor oil produces diarrhoeal effect due to its active component of recinoleic acid **[13],** inhibition of intestinal Na^+,K^+-ATPase activity to reduce normal fluid absorption **[14, 15],** activation of adenylyl cyclase **[13],** stimulation of prostaglandin formation **[16],** platelet-activating factor and recently nitric oxide was contribute to the diarrhoeal effect of castor oil **[17, 18 , 19].** Despite the fact that number of mechanisms has been involved for the diarrhoeal effect of castor oil, it has not been possible to define its correct mechanism of action **[11].**

EEGS may act an above any one of the mechanism.

It is also noted that EEGS significantly inhibited castor oil induced intestinal fluid accumulation and the volume of intestinal content. **(T able 2 and Fi gure 2).** The secretory diarrhoea is associated with an activation of Cl^- channels, causing Cl^- efflux from the cell, the efflux of Cl^- results in massive secretion of water into the intestinal lumen and profuse watery diarrhoea **[20].** The involvement of muscarinic receptor effect was confirmed by increased production of both gastric secretion and intraluminal fluid accumulation induced by castor oil. The EEGS may inhibit the secretion of water into the intestinal lumen and this effect is partly mediated by both α_2-adrenoceptor and muscarinic receptor systems. The significant inhibition of the castor oil-induced enteropooling in mice suggests that the extract of *Guettarda sp eciosa* produced relief in diarrhoea by spasmolytic activity in vivo and anti-enteropooling effects. **[10].**

The EEGS significantly reduced the castor oil induced intestinal transit as compared with control group **(T able 3 and Figure 3).** In this study, atropine increased intestinal transit time possibly due to its anti-cholinergic effect **[20].** In castor oil induced diarrhoea, the liberation of ricinoleic acid results in irritation and inflammation of the intestinal mucosa,

Table 2: Effect of *EEGS* on castor oil induced enteropooling in rats.

Group	Treatment	Weight of Intestinal Content	% Inhibition of Weight Intestinal Content
I	Castor oil (2ml p.o) + saline (2ml/kg p.o)	2.41±0.12	---
II	Castor oil (2ml p.o) + diphenoxylate (5 ml/kg p.o)	1.62±0.12**	32.78
III	Castor oil (2ml p.o) + EEGS (200mg/kg p.o)	1.58±0.05*	34.43
IV	Castor oil (2ml p.o) + EEGS (400mg/kg p.o)	1.09±0.12**	54.77

Effect of EEGS on castor oil-induced enteropooling in rats: EEGS was administered p.o 1 h before castor oil administration. Values are expressed as mean ± SEM from the experiments. *P<0.01, **P<0.001 when compared with Castor oil + saline-treated group.

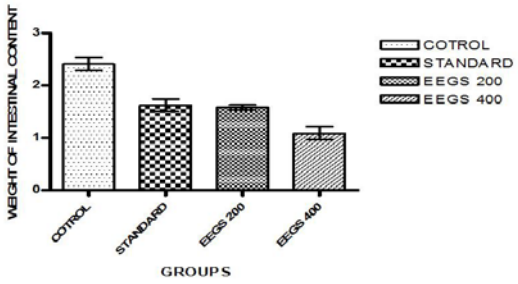

Figure 2: Effect of **EEGS** *on castor oil induced enteropooling in rats.*

leading to release of prostaglandins, which results in stimulation of secretion **[21]** by prevents the reabsorption of NaCl and water **[16].** Probably EEGS increased the reabsorption of NaCl and water by decreasing intestinal motility as observed by the decrease in intestinal transit by charcoal meal.

Anti-dysentric and antidiarrhoeal properties of medicinal plants were found to be due to tannins, alkaloids, saponins, flavonoids, sterols and/or triterpenoids and reducing sugars **[22].**

Table 3: Effect EEGS on castor oil-induced small intestinal transit in rats.

Group	Treatment	Total Length of Intestine	Distance Travelled By Marker	% Intestinal Transit
I	saline (2ml/kg p.o)	87.8 ± 1.69	45.58 ± 1.76	51.91
II	Castor oil (2ml p.o) + saline (2ml/kg i.p)	80.21 ± 2.92	72.81 ± 1.21	90.77
III	Castor oil (2ml p.o) + atropine (3mg/kg i.p)	96.91 ± 2.84	35.88 ± 1.36**	37.02
IV	Castor oil (2ml p.o) + EEGS (200mg/kg i.p)	83.51 ± 1.82	52.23 ± 1.78*	62.54
V	Castor oil (1ml p.o) + EEGS (400mg/kg i.p)	86.06 ± 1.21	42.1 ± 1.33**	48.80

Effect of EEGS on castor oil-induced small intestinal transit in rats: EEGS was administered p.o 1 h before castor oil administration. Values are expressed as mean ± SEM from the experiments. *P<0.01, **P<0.001 when compared with Castor oil + saline-treated group.

The phytochemical analysis of EEGS revealed the presence of alkaloids, flavonoids, triterpinoids carbohydrates, tannins, phenols, gums and mucilage. These constituents may mediate the anitdiarrhoeal property of the EEGS.

In conclusion, the present study has shown that *Guettarda s peciosa* is a potential therapeutic option in the effective management of diarrhoea, thus justifying its widespread use by the local population for these purposes. Concerted efforts are being made to fully investigate the mechanisms involved in the pharmacological activities of *Guettarda sp eciosa* and phytochemical studies are also in progress to isolate and characterize the active constituents of *Guettarda speciosa*. The isolated compound may serve as useful prototypes of anti-diarrhoeal drugs of natural origin possessing the desired pharmacological activities while lacking certain untoward effects.

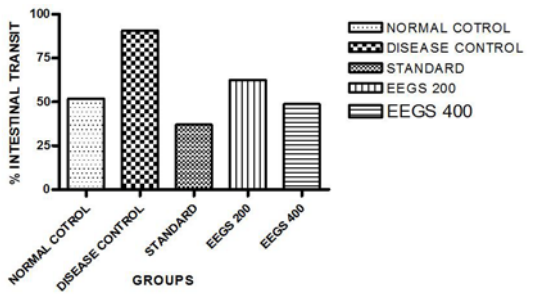

Figure 3: Effect EEGS on castor oil-induced small intestinal transit in rats.

Acknowledgement

Authors are sincerely thankful to Dr. S. Venkatraman, M.Sc., M.D., Ph.D., Director and Mr. P. Muralidharan, M.Pharm. (Ph.D), Department of Pharmacology, C.L. Baid Metha Foundation for Pharmaceutical Education and Research, for their contribution and facilities provided regarding our Research work.

References

[1] Weiner, M.A., Ethnomedicine in Tonga. *Econ. Bot*. 1971, 25, 423-450.

[2] Weiner, M.A., Secrets of Fijian Medicine.Govt. Printer, Suva, Fiji. 1984, 93.

[3] Saravanakumar, A., Amutha, P., Gandhimathi, R., Dhanapal, R., *Iranian J pharmacol therapeut,* 2009, (2) 8. (article in press)

[4] Cambie, R.C., Ash, J., Fijian Medicinal Plants, CSIRO, Australia. 1994, 255.

[5] Harbone, J.P., Phytochemical Methods, A Guide to modern technique of plant analysis, (Chapmann and Hall, London), 1973, pp. 1-271.

[6] Nwodo, O.F.C., Alumanah, E.O., *J Ethnopharmacol*, 1991, 31, 395–398.

[7] Nwafor, P.A., Jacks, T.W., Ekanem, A.U., Ching, F.P., *Nigerian J of N atl Pro d Med,* 2005, 9, 66–70.

[8] Robert, A., Nezamis, J.E., Lancaster, C., Hanchar, A.J., Klepper, M.S., *Prostaglandins,* 1976, 11, 809-828.

[9] DiCarlo, G.D., Mascolo, N., Izzo, A.A., Capasso, F., Autore, G., *Phytotherapy Research*, 1994, 8, 42–45.

[10] Mascolo, N., Izzo, A.A., Avtore, G., Barboto, F., Capasso F, *J Pharma col Exp ther,* 1994, 268, 291-295.

[11] Izzo, A.A., *Phytotherapy R esearch*, 1996, 10, S109–S111.

[12] Capasso, F., Mascolo, N., Izzo, A.A., Gaginella, T.S., *British J Pharmacol*, 1994, 113, 1127–1130.

[13] Phillips, R.A., Love, A.H.G., Mitchell, T.G., Neptune, E.M*., Nature 206*, 1965, 1367–1368.

[14] Nell, G., Rummel, W., 1984. Action mechanism of secretagogue drugs. In: Csaky, T.Z. (Ed.), Pharmacology of Intestinal Permeation, second ed. Springer-Verlag, Berlin, 1984, pp. 461–508.

[15] Galvez, A., Zarzuelo, M.E., Crespo, M.D., Lorente, M., Ocete, A., Jimenez, J., *Planta Medica,* 1993, 59, 333–336.

[16] Mascolo, N., Izzo, A.A., Gaginella, T.S., Capasso, F., *Naunyn Schmi edebergs Arch Pharmacology,* 1996, 353, 680–684.

[17] Ammon, H.V., and Soergel, K.H.,. Diarrhea: In Berk, J.E., Haubrich, W.S., Kaiser, M.H., Roth J.L.A., Schaffner, F., Bockus Gastroenterology, eds. 4th ed. Philadelphia, Saunders, 1985, pp. 125-141.

[18] Horton, E.W., Main, I.H.M., Thampson, C.J., and Wright, P.M., 1968, *Gut*; 9, 655-658.

[19] Greenbargena, N.J., Arwanitakis, C., and Hurwitz, A., Azarnoff, D.L., (eds), *In dr ug development of ga strointestinal disorders*, Churchill Livingstone, NewYork, 1978 pp 155-156.

[20] Brown, J.A. and Taylor, P., Muscarinic receptor agonists and antagonist. In: Hardman, J.G., Limbird, L.E., (Eds), Goodman and Gilman's the pharmacological Basis of therapeutics 10th Edition, MacGraw Hill, New York, 2000, pp 115-158.

[21] Pierce, N.F., Carpenter, C.C.J., Elliot, H.Z., and Greenough, W.B., *Gastroenterol,* 1971, 60, 22-32.

[22] Longanga_Otshudi A, Vercruysse, A., and Foriers, A., J *Ethnopharmacol*, 2000, 71(3), 411 -23.