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ORIGINAL ARTICLE

# Evaluation Of The Antidiarrhoeal Activity Of The Leaves Of Ixora Coccinea Linn. In Rats

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## ABSTRACT

**Purpose:** Ixora coccinea Linn (Rubiaceae), a small shrub which is cultivated throughout India, has been reported to possess a number of medicinal properties. The purpose of the present study was to evaluate the antidiarrhoeal activity of the aqueous extract of the leaves of Ixora coccinea linn which is used traditionally as folk medicine by using a castor oil induced diarrhoea model.

**Methods:** The aqueous extract of the leaves of Ixora coccinea were studied against a castor oil induced diarrhoea model in rats. The gastrointestinal transit rate was expressed as the percentage of the longest distance which was traversed by the charcoal, divided by the total length of the small intestine. The weight and the volume of the intestinal content induced by castor oil were studied by the enteropooling method. Loperamide was used as a positive control.

**Result:** The plant-extract showed significant ($P<0.001$) inhibitor activity against castor oil induced diarrhoea and castor oil induced enteropooling in rats at the dose of 400 mg/kg. There was significant reduction in gastrointestinal motility by the charcoal meal test in rats.

**Conclusion:** The results obtained by this study substantiate the antidiarrhoeal effects of the aqueous extract and its use by the traditional practitioners in the treatment of diarrhoea.


**Keywords:** Antidiarrhoeal activity, Ixora coccinea linn. Traditional medicine, castor oil induced diarrhoea, Enteropooling method, small intestinal transit.

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## Introduction

Millions of people in developing countries use herbal medicines because they are locally available and are prescribed by traditional practitioners of medicines who are a part of their community. Diarrhoea is a common and a potentially serious illness of early childhood. It is one of the major determining factors leading to malnutrition as the principal cause of death, if left untreated without proper medication, especially in developing countries.

Diarrhoea is defined by the WHO as having 3 or more loose or liquid stools per day, or as having more stools than is normal for that person. According to the World Health Report, diarrhoea is the cause of 3.3% of all deaths. The worldwide distribution of diarrhoea accounts for more than 5-8 million deaths each year in children less than 5 years. The use of traditional medicine to combat the consequences of diarrhoea has been emphasized by the WHO in its diarrhoea control programme (CDD) [1-6].The approach towards evaluating medicinal plants has been based on the chemical extraction of plants which are then tested on various experimental models. Morphine, quinine, emitine, digitalis glycosides, ergot alkaloids and vinca alkaloids which are in wide use today, were originally

obtained from plants. Several studies have shown the beneficial effects of traditional medicines in diarrhoea.[7-10].

Ixora Coccinea Linn. is a small shrub which is cultivated throughout India.(Flame of Woods in English, Rangan in Hindi and Bengali, Kisukare in Kannada.) Its roots and flowers are used for dysentery, dysmenorrhea, leucorrhoea, haemoptysis and catarrhal bronchitis. The leaves are used for diarrhoea. Its roots are also used for hiccups, nausea and loss of appetite and externally for the treatment of sores, eczema and chronic ulcers. Its roots contain an aromatic acrid oil, tannin and fatty acids. Its leaves yield flavonol, kaemferol, quercetin, proanthrocyanidines, phenolic acids and ferulic acids. Its flowers yield cyanidins, flavonol and cooling materials which are related to quercitin.[11-15]. Its roots are ground into pulp, mixed with water and are used as tincture is used for diarrhoea and dysentery [11-15]. However, there is limited scientific evidence to verify these claims.

Hence, the present study was undertaken to evaluate the possible antidiarrhoeal activity of the leaf extract of Ixora Coccinea Linn which is used commonly in Indian traditional medicine, by using various validated models and to find out if the folk medicinal use has a scientifically justified basis.

## MATERIALS AND METHODS

### *Plant material*

The leaves which were used for the study were collected from the Belgaum District of Karnataka in the month of November-December. The plant was authenticated by Prof: A. P. Kore, Dept of Botony, R.L.S. College, Belgaum. The voucher specimen of the plant has been deposited at the college for further reference.

After collecting the leaves, they were dried under shade for a period of four weeks. After drying, they were finely powdered. Cold extracts of the leaves were prepared according to the method described by Rawlins [16]. The powder was dissolved in water in the ratio of 1:3 (250mg of powder in 750ml of distilled water [17] and this mixture was shaken three to four times a day, for a period of seven days. After filtration, the filtrate was concentrated and dried under reduced pressure. The extract was brown in colour, in a semisolid form and the yield was 18.6% (w/w). The extract was stored in desiccators until use.

### *Animals used*

Albino Wistar rats of either sex, weighing 150-200gm, were used. They were housed in standard polypropylene cages under room temperature (24 ± 2˚C) and were exposed to 12:12 h light and dark cycles. The rats were fed with a standard diet (Gold Mogr Lipton India Ltd.) and water ad libitum. The study protocol was approved by the Institutional animal ethical committee (reg.no.627/02/a CPCSEA) of Ethical committee IAEC-Jawaharlal Nehru Medical College, Belgaum.

### *Phytochemical screening*

The freshly prepared extract was subjected to a standard phytochemical screening test for various constituents (Trease and Evans, 1993) [18].The extract was screened for alkaloids, glycosoids, tannins, saponins, sterols and flavanoids.

### *Castor oil induced diarrhoea*

This was studied by the method described by Niemegeers et al [19]. The animals were kept in fasting for 24hrs before the test, with free access to water. The rats were divided into five groups of six animals each. Diarrhoea was induced by administering 1ml of castor oil orally. Group I was taken as the control (2ml/kg, i p saline), Group II which received loperamide (5mg/kg), served as the standard and Groups III, IV and V received the extract (100, 200 and 400mg/kg i p) 1hr before castor oil administration. The consistency of the faecal matter and a number of both wet and dry diarrhoeal droppings were counted every hour for a period of 4hrs.

### *Castor oil induced enteropooling*

Intra luminal fluid accumulation was determined by the method of Robert et al (1976) [20], and Dicarlo et al (1994) [21]. The rats were divided into five groups of six animals each and they were kept in fasting overnight, but were allowed free access to water. Group I was treated as the control (2ml/kg i p saline) and Group II which received loperamide (5mg/kg ip) was treated as the standard. Groups III, IV and V received

the extract (100, 200, and 400mg/kg ip). Then, 1hr later, 2ml of castor oil was administered orally to the above groups for induction of diarrhoea. Two hours later, the rats were sacrificed, their small intestines were ligated at both the pyloric sphincter and the ileocaecal junction and they were dissected. Their small intestines were weighed. Then intestinal contents were collected by milking into a graduated tube and thus, the volume was measured. The intestines were reweighed and the difference between the full and the empty intestines was calculated.

### *Small intestinal transit*

The method described by Jansen and Jageneau was used [22]. The rats were kept in fasting for 18 hrs and were divided into six groups of six animals each. Group I received 2ml/kg of normal saline orally. Group II received 2ml of castor oil orally with 2ml/kg of normal saline intraperitoneally. Group III received loperamide (5mg/kg, ip) and Groups IV, V, and VI received 100, 200 and 400 mg/kg of the plant extract intraperitoneally, 1hr before the administration of castor oil. 1ml of marker (10% charcoal suspension in 5% gum acacia) was administered orally, 1hr after the castor oil treatment. The rats were sacrificed after 1hr and the distance travelled by the charcoal meal from the pylorus to the caecum was measured and was expressed as the percentage of the whole length of the intestine.

### *Statistical analysis*

The experimental results were represented as mean± SE (standard error of mean). Student's t- test was used for the evaluation of the data and P values < 0.05 were considered as significant.

## RESULTS

### *Castor oil induced diarrhoea*

Diarrhoea was apparent in all the animals of control group, thirty minutes after the administration of castor oil, for the next 4hrs. This was largely eliminated by the intraperitoneal injection of 5 mg /kg (48.12%) loperamide.

[Table/Fig 1] The effect of the extract was not as potent as loperamide in the dose of 100 mg/kg, but in the doses 200 mg/kg and 400 mg/kg, the extract produced a significant dose dependent reduction in the number of defection over four hours (P<0.001).

**[Table/Fig 1]:Effect of aqueous extract of leaves of Ixora coccinea on castor oil induced diarrhoea in rats**

| Group | Treatment | Mean defecation in 4 hr | % of Inhibtion of defecation |
|---|---|---|---|
| 1. | Castor oil( 1ml. p.o)+ saline (2 ml/ kg i.p) | 23.51± 0.349 | - |
| 2. | Castor oil (1ml p.o) +loperamide (5 mg /kg ip) | 12.20 ±1.7?? | 48.12 |
| 3. . | Castor oil (1 ml p.o)+ extract (100 mg / kg i.p) | 21.91 ± 0.737? | 6.8 |
| 4. | Castor oil (1 ml p.o) )+ extract (200mg/ kg i.p) | 16.81 ± 0.51?? | 28.49 |
| 5. | Castor oil (1 ml p.o) + extract (400 mg / kg i.p) | 11.88 ±0.25?? | 49.46 |

**Extract was administrated i.p , 1 hour 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.**

### *Castor oil induced enteropooling*

Castor oil caused the accumulation of water and electrolytes in the intestinal loop. The treatment with the extract (100,200 and 400 mg/kg) produced a significant and dose dependent reduction in the intestinal weight and volume [Table/Fig 2]. Significant results (P values < 0.001) were observed with doses of 200 and 400 mg/ kg.

**[Table/Fig 2]:Effect of aqueous extract of leaves of Ixora coccinea on castor oil induced enteropooling in rats**.

| Group | Treatment | Wt. of intestinal content | % Inhibtion of weight of intestinal content |
|---|---|---|---|
| 1. | Castor oil (2 ml p.o) + saline (2 ml / kg i.p) | 2.51 ±0.122 | ---- |
| 2. | Castor oil ( 2 ml p.o) +loperamide ( 5 mg / kg i.p) | 2.41 ± 0.2054 | 3.98 |
| 3. . | Castor oil ( 2 ml p.o) + Extract ( 100 mg / kg i.p) | 1.87 ± 0.1163? | 25.49 |
| 4. | Castor oil ( 2 ml p.o) + Extract ( 200 mg / kg i.p) | 1.478± 0.049?? | 41.11 |
| 5. | Castor oil ( 2 ml p.o) + extract(400 mg /kg i.p) | 1.094 ± 0.1172?? | 56..4 |

**Extract was administrated i.p , 1 hour 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**

### *Small intestinal transit*

The aqueous extract of Ixora coccinea decreased the propulsion of the charcoal meal through the gastrointestinal tract significantly as compared to the control group. Loperamide (5mg/kg) produced a marked decrease in the propulsive movement and the intestinal length travelled by charcoal. (100,200 and 400 mg/kg) produced a significant and dose

dependent reduction in the intestinal weight and volume [Table/Fig 3].

**Table 3:Effect of extract of of leaves of Ixora coccinea on Castor oil induced small intestinal transit in rats.**

| Group | Treatment | Total length of intestine | Distance travelled by marker | %of intestinal transit |
|---|---|---|---|---|
| 1. | Saline (2 ml/ kg p.o) | 87.8 ± 1.69 | 45.38 ± 1.76 | 51.91 |
| 2. | Castor oil(2ml p.o) + saline (2 ml / kg i.p) | 80.21 ± 2.92 | 72.81 ±1.21 | 90.77 |
| 3. | Castor oil( 2 ml p.o)+loperamide (5 mg / kg i.p) | 96.91 ± 2.84 | 35.88 ±1.36?? | 37.02 |
| 4. | Castor oil ( 2 ml p.o) + Extract ( 100 mg / kg i.p) | 83.51 ± 1.82 | 52.23 ±1.78? | 62.54 |
| 5. | Castor oil ( 2 ml p.o) + Extract ( 200 mg / kg i.p) | 86.06 ± 1.21 | 42.1 ±1.33?? | 48.84 |
| 6. | Castor oil ( 2 ml p.o) + extract (400 mg /kg i.p) | 87.03 ± 1.71 | 40.3 ±0.73?? | 45.98 |

**Extract was administrated i.p , 1 hour 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.**

## DISCUSSION AND CONCLUSION

The results of this study suggest that the aqueous extract of the leaves of Ixora coccinea in graded doses of 100, 200 and 400 mg /kg of body weight, reduced diarrhoea by inhibiting intestinal motility, intestinal fluid accumulation and by significantly reducing the frequency of defaecation. This signifies the use of the aqueous extract of Ixora coccinea as folk medicine.

Castor oil is known to produce changes in the intestinal mucosal permeability to electrolytes and water, thus leading to diarrhoea [23],[24]. The antidiarrhoeal activity of this extract may be due to one of these mechanisms.

a) The extract may increase the reabsorption of NaCl and water by decreasing the intestinal motility by the charcoal meal. b) The presence of tannins in the extract may make the intestinal mucosa more resistant and may reduce the secretion [25],[26]. Tannic acid and tannins are water soluble polyphenols that are present in many plants [27]. c) The liberation of recinoleic acid by castor oil results in the irritation and the inflammation of the intestinal mucosa, thus leading to the release of prostaglandins [28],[29]. The extract may reduce prostaglandin secretion. d) Flavoniods and alkaloids are known to inhibit the release of autocoids and prostaglandin, thereby inhibiting the secretion induced by castor oil [30],[31]. The phytochemical analysis of the aqueous extract of Ixora coccinea showed the presence of flavonoids, alkaloids and tannins. The antidiarrhoeal and antidysentric properties of medicinal plants were found to be due to the presence of tannins, alkaloids, saponins, flavonoids, sterols and reducing sugars [32]. Sesquiterpenes, diterpenes, terpenes, flovonoids and terpenoid derivatives are known to inhibit the release of autocoids and prostaglandis, thereby inhibiting the motility and the secretion induced by castor oil [33],[34].Loperamide, a synthetic opiate analogue, regulates the gastrointestinal tract by inhibiting the propulsive motor activity, predominantly in the jejunum and this effect is partially inhibited by an opiate antagonist. Loperamide is also reported to reduce the colonic rate of flow and to consequently increase colonic water absorption, but it does not have an effect on colonic motility [35].

This study has intentionally been undertaken by using a crude aqueous extract, as it is our belief that the different biological activities assayed herein, may not be due to a single constituent. This has also been highlighted by Mayer Manga et al [36], who have stated that the crude extracts contain several compounds acting on different mechanisms. In addition, the interplay of the constituents in the crude extract may result in better activity due to synergism or may lead to a decrease in toxicity and it is possible that pure compounds may not necessarily behave in the same manner as the natural extracts [37],[38]

To conclude, the present study supports the claims made by traditional medical practitioners about the use of the aqueous extract of Ixora coccinea Linn. in the treatment of diarrhoea. The active constituent which is responsible for the antidiarrhoeal activity remains to be identified and further studies are required to understand the mechanism of action of the antidiarrhoeal activity of Ixora coccinea Linn.

## REFERENCES

[1] Inayathulla, Shariff, W, R., Karigar Asif, A. Sikarwar. Mukesh, S. Evaluation of Antidiarrhoeal activity of Crataeva nurvala root bark in experimental animals. International Journal of pharmacy and Pharmaceutical Sciences; 2010;2:158-161.
[2] World Health Organization, World Health Report, WHO, Geneva, 2004;120-125.
[3] Sunilson, J.A., Anand arajagopal, K., Kumari, A.V.A.G. and Mohan, S. Antidiarrhoeal activity

of leaves of Melastoma malabathricum Linn. Indian Journal of Pharmaceutical Sciences, 2009; 71(6); 691-695.
[4] Chitme HR, Ramesh,C,Sadhna.K., Study of antidiarrhoeal activity of Calatropis gigantean in experimental animals. J pharmacol. Pharm Sci. 2004;7;70-75.
[5] Syder,JD. and Merson,M.H, The magnitude of the global problem of acute diarrheal disease; a review ofactive surveillance data . Bulletin of the World Health Organization, 1982;60: 605-13.
[6] Lutterodt, G,D; Inhibition of gastrointestinal release of acetylcholine by quircetin as possible mode of action of Psidium guajava leaf extracts in the treatment of acute diarrhoeal disease. Journal of Ethnopharmacololgy.1989;25:235-47.
[7] Offiah,V.N. and Chikwender,U. A., Antidiarrhoeal effect of Ossium gratissium leaf extract in experimental animals. Journal of Ethnopharmacololgy, 1999;68: 327-330.
[8] Mukharjee,P.K., Saha,K., Murage san, T, Mandal, S,C., Pal, M. Saha,B.P., Screening of Antidiarrhoeal Profile of some plant extract of aspecific region of West Bengal, India. Journal of Ethnopharmacololgy.1998; 60: 85-89.
[9] 9. Rani, S., Ahemad, N., Rajaran, S., Saluja, R., Thenmozhi, S. and Muragesan, T., Antidiarrhoeal evaluation of Clerodendrum phlomidis Linn. leaf extract in rats. Journal of Ethnopharmacololgy,1999; 68: 315-319.
[10] 10. Zavata,M,A., Perez, S., Perez, C. Vargus, R, and Perez, R.M., Antidiarrhoeal activity of Waltheria Americana, commelina coelestis and Alternenthera repens, Journal of Ethnopharmacololgy 1998; 61; 41-47.
[11] Medicinal plants of India ; Ixora Coccinea Linn. ICMR,1976; 1: 92-95
[12] Nadakarni, K.M., The Indian Materia Medica Popular prakashan Pvt Ltd,
a. Mumbai.2005;1:698-699.
[13] Theodore cooke, C, I.E. The flora of presidency of Bombay. Botanical survey of India, Ixora Coccinea Linn.1901;2:40.
[14] Chopra R.N., Chopra I.C., Handa K.L., Indigenous Drugs of India, U.N.Dhur and Sons Pvt Ltd, Culcatta.1958;1:288-289.
[15] Kirtikar K.R, Basu BD, Indian Medicinal plants, Vol 1,2nd editon Dehradun, International book publisher,2005.
[16] Rawlins, E.A., Bantley's Textbook of Pharmaceutics Pub, Bailliere. Tindall London: 1992 ;8 :173-176.
[17] Mukharjee P. K. Quality control of herbal drugs, Vol.1 ., New Delhi Business horizone; 2002;195 -196.
[18] Trease,G.E.,Evans,M.C.(1983)Textbook of pharmacognosy,12th ed.,Bailliere,Tindail, London . pp; 343-384.
[19] Awouters, F., Niemegeers, C.J.E. Lenaerts, F.M.Jansen, P.A.J., Delay of castor oil diarrhoea in rats; a new way to evaluate inhibitors of prostaglandin biosynthesis. Journal of pharmacy and pharmacology,1978; 30: 41- 45.
[20] Robert A., Nezamis, J.E., Lancaster, C., Hanchar A.J., Klepper, M.S., Enteropooling assay; a test for diarrhoea produced by prostaglandins. 1976;11: 809-828.
[21] DiCarlo, G.D., Mascolo, N., Izzo, A.A., Caparso, F., Autore,G., Phytotherapy Research, 1994;8:42-45.
[22] Mascolo, N., Izzo, A.A., Autore,G., Barboto,F., Caparso, F., Nitric oxide and castor oil induced diarrohoea, journal of Pharmacology and Experimental therapeutics, 1994;268:291-295.
[23] Bruton,L., Agent affecting gastroinestinal water flux and motility, digestants and bile acids. Pharmacological Basis of Therapeutics; Mc. Graw Hill, New York, 1996;9:916.
[24] Galves, J., Zavzuelo, A., Craspo, M.,Lorente,M., Ocete, Jimenez ,J. Antidiarrhoeal activity of Euphorbia hirta extract and isolation of an acute flavonoid constituent. Plant Med, 1993;59:333-336.
[25] Tripati, K.D., Essential of Medical Pharmacology, New Delhi, Jaypee Brothers medical publishers,1994,775.
[26] Das, S.R., Prakash, Devaraj, S.N. Antidiarrhoeal effect of methanolic extract of Hemidesmus indicus (Indian sarsaparilla) an in vitro and in vivo study. Ind. J. Exp. Biol,.2000;41:363-366.
[27] Ofuji, K., Hara.H., Sukamoto, T., Yamashita, S. Effect of antidirrhoeal containing an exract from Geranium herb on astringent action and short circuit current across jejunal mucosa. Nippan Yakurigaku Zasshi,1998;111:265-275.
[28] Pierce, N., Carpenter, C., Elliot,H., Green ough,W. Effect of prostaglandin, theophylline and cholera exotoxin upon transmural water and electrolyte movement in canine jejunum,Gasteroenterology,1971;60:22-32.
[29] Ramakrishna, Mathan, B.M, Mathan,V. Alteration of colonic absorption by long chain unsaturated fatty acids. Influence of hydroxylation and degree of unsaturation. Scand.J.Gastroenterol,1994;29:54-58.
[30] Vimala,R., Nagarjun, S., Alam, M., SusanT., Joy,S. Antiinflammatoey and antipyretic activity of Michelia champaca Linn., (white variety), Ixora brachiata Roxb, and Rhynchosia cana(wild) D.C.flower extract, J. Exp. Biol.,1997;35:1310-1314
[31] Veiga, V., Zunino,L., Calixto, J., Patitucci,M., Pinto,A.Phytochemical and antioedematogenic Studies of commercial copaiba oils available in Brazil., Phytother.Res.,2001;15:476-480
[32] Longanga Otshudi,A., Vercruysse, and Fories, A. Contribution to the ethnobotanical, phytochemical and pharmacological studies of traditionally used medicinal plants in the treatment of dysentery and diarrhoea in Lomela area Democratic republic of Congo(DRC), J.Ethnopharmcology, 2000;71: 411-423.
[33] Milanova, Rhan,K., Moore,M. Oxidation and glucose conjugation of synthetic abietane

diteterpines by Cunninghamella SP.II. Novel routes to the family of diterpenes from Tipterygium wilfrdi;, J. Nat. Prod, 1995;%8:68-73.

[34] Nikiema, J.B., Vanhaelen_ Fastre, R., Vanhaelen, M. Fontaine, J., De_Graef. C.and Heenen, M., Effect of anti inflammatory triterpines isolated from Leptadenia hastate on keratinocyte proliferation, Phytother Res, 2001;15, 2; 131-4

[35] Theoderau, V., Fiora mont,J., Hachet, T., Bueno, L. Absorptive and motor components of antidiarrhoeal action of loperamide; an in vivo study in pigs. Gut; 1991;32: 1355-1359.

[36] MavarManga,H.,Haddad,M.,Pieters,L.,Baccelli, C.,Penge,A.,Quetin ledercq,J.Antiinflammatory compounds from leaves and bark of Alchornea cardifolia(Schumach and Thonn),Mull.Arg.J.Ethnopharmacol 2008;115;25-29.

[37] Kicklighter, C.E., Kubanek, J., Barsby, T., Hay.M.E. Palatability and defence of some tropical in faunal worms; alkyl pyrrole sulfates as deterrents to fish feeding. Mar Ecol Prog Ser 2003; 263; 299-306.

[38] Liu, R.H.Potential synergy of phytochemicals in cancer prevention, mechanism of action. J. Nutr 2004; 134; 3479s-3485s.