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Research Article

PRELUDE STUDIES OF ANTI-DIARRHOEAL ACTIVITY OF ETHYL ACETATE EXTRACT OF AERIAL PART OF *INDIGOFERA PURPUREA* ON ISOLATED RABBIT ILEUM

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ABSTRACT

In Present study we aimed to investigate the anti-diarrhoeal activity of ethyl acetate extract of *Indigofera purpurea* aerial part, by using castor oil-induced diarrhoeal model in mice. The effects of these extract on perfused isolated rabbit ileum were also evaluated. The ethyl acetate extract produced a dose dependent relaxation of the rabbit ileum. The ethyl acetate extract of aerial parts produced a dose dependent protection against the castor oil-induced diarrhoea with the highest protection (85%) at a dose level of 600mg/kg comparable to that of loperamide (6mg/kg) the standard agent. The preliminary phytochemical analysis revealed that the extract contained tannins, steroids, alkaloids, saponnis and flavonoids. The acute toxicity test revealed no mortality upto the dose of 4500mg/kg. The results revealed that the ethyl acetate extract of *Indigofera purpurea* aerial part possess pharmacological activity against diarrhoea.

Keywords: *Indigofera purpurea,* Antidiarrhoeal studies, Ethyl acetate extracts.

INTRODUCTION

Diarrhoeal disease is one of the leading causes of childhood morbidity and mortality in developing countries. An estimated 1000 million episodes occur each year in children under 5 years of age. Diarrhoeal causes an estimated 5 million death in children fewer than 4 years of age per year[1]. Incidence of diarrhoeal diseases still remains high despite intervention of government agencies and international organisation to halt the trend. The use of herb drugs in the treatment of diarrhoeal diseases is a common practice in many African countries[2]. Despite immense technology advancement in medicine, many people in developing countries still rely on traditional healing practices and medicinal plant for their daily health care need [3]. The world health organisation (WHO) encouraged studies for the treatment and prevention of diarrhoeal diseases depending on traditional medical practices[4]. There is therefore an urgent need for the intensification of research into medicinal plant claim to be effective in the management of diarrhoeal diseases.

A number of medicinal plant have been used traditionally in the management of diarrhoeal diseases, and one of such medicinal plant is *Indigifera purpurea.* It belongs to the family fabaceae. It is an annual non climbing herb or shrub that can grow up to 1m tall. It is widely distributed throughout south india[5]. In ethno medicine, the leaves are used to treat infected wound while the decoction of the aerial part is used as prophylactic against snake-bites[6] and as anti-inflammatory[7]. Recent studies have shown that it also possesses anti diabetic activity[8].

The present study was carried out to test the potential effects of *indigofera purpurea* ethyl acetate extract on gastrointestinal (GIT) motility in mice and intestinal smooth muscle using rabbit ileum with particular emphasis on the anti-diarrhea effects.

MATERIALS AND METHODS

Collection of plant materials

Indigofera purpurea sample was collected from forest area in and around Kurnool district in the month of October 2009 and authenticated by Botanical survey of india, Agricultural university, Coimbatore, T,N.

Preparation of plant materials

The aerial parts of *Indigofera purpurea* was air dried under shade and then grinded it. 3kg of *Indigofera purpurea* aerial part grinded powder was taken in a bucket and 2 litres of ethyl acetate (75%) and (25%) of water mixture was poured into it and it was allowed to macerated for 5 days. Then it was filtered. The filtrate was then transferred to an evaporating dish and was evaporated using a water bath. The extract obtained after evaporation of solvent was stored at refrigerator till the day of the experiment. Solutions of the extracts were prepared freshly for each study.

Animals

Albino rabbit (800g) and albino mice (16-30g) maintained in the Animal house from the Department of Pharmacology, Santhiram Medical College, A.P, India were used in these experiments. The animals were maintained on standard animal feed and water *ad libitum.* This research was carried out in Santhiram Medical College in accordance with the rules governed by CPCSEA, New delhi, India.

Drugs

Acetylcholine and Adrenaline (Sigma chemical, India), Castor oil (Castor Ambuja Solvex Pvt. Ltd, India) and Loperamide (Tablets india Pvt Ltd., India).

Phytochemical procedure

The preliminary Phytochemical screening of the crude extract of *Indigofera purpurea* was carried out in order to ascertain the presence of its constituents by utilizing standard conventional protocols[9].

Acute toxicity study

The method previously described by[10] was adopted using 13 mice. In the first phase, three doses of the ethyl acetate extract (10, 100 and 1000mg/kg were administered to three groups each containing three mice). In the second phase, more specific doses were administered to four groups each containing one mouse. The median lethal dose (LD_{50}) was determined as the geometric mean of the highest non lethal dose and lowest lethal dose of which there is 0/3 and 0/1 survival.

Effects on isolated rabbit ileum

The rabbit was made unconscious by a powerful strike at the back of the neck (stunning). The abdomen was immediately opened using forceps and part of the ileum was quickly removed.

The ileum was introduced immediately into a Petri dish of saline containing Tyrode solution and each end of the ileum was tied with a thread. The ileum was then suspended into the organ bath, which contains supply of oxygen and air to the ileum in organ bath. One

end of the ileum was attached to the transducer which measured the mechanical impulse of the tissue and converted it to electrical impulses which was then recorded on microdynamometer. The temperature of the inner bath was kept at temperature 37^0c by the thermo regulator. Subsequently, solutions of acetylcholine and adrenaline.

Indigofera purpurea extracts were added at intervals to the isolated perfuse chamber. After application of each drug, the tissue was washed three times with the Tyrode solution to remove every trace of the drug.

Effects of castor oil induced diarrhea in mice

The mice were fasted for 12hours prior to the commencement of the experiment and were randomly divided into five groups of five mice each. The mice in the first group received 15mlkg^{-1} normal saline intraperitoneally while the mice in the second received 6mlkg^{-1}. Loperamide as a standard positive control, the third, fourth and fifth 150, 300 and 600mg/kg, respectively. After 30 minutes of administration of the extract, castor oil 0.3ml/mouse were administered orally. The animals were placed on individual special cages over white clean whatman filter, three hours after castor oil challenge. The cages were inspected for the presence of the characteristic diarrhoea droppings. The absence was recorded as a protection from diarrhoea[11] and the percentage protection was calculated[12].

Statistical analysis

The results were analysed by chi square (X^2). Values were considered significant with $P< 0.05$ for both isolated tissue and castor oil induced diarrhoea.

RESULTS

Phytochemical analysis:

The preliminary phytochemical screening of the extract revealed the presence of alkaloids, saponins, flavonoids, tannins and steroids.

Acute Toxicity study

The median lethal dose of the extract was found to be greater than 4500mg/kg bodyweight.

Effect of ethyl acetate extract of *Indigofera purpurea* on isolated rabbit ileum

Figure-2 showed the effects of the plant ethyl acetate extracts on the isolated rabbit ileum were dose related the extract relaxed the spontaneous contraction of the rabbit ileum.

Effect of *Indigofera purpurea* on castor oil induced diarrhoeal

The extract produced a dose dependent protection against the castor oil- induced diarrhoeal with the highest protection (85%) obtained at the highest dose tested (600mg/kg) comparable to that of loperamide, the standard anti diarrhoeal agent.

DISCUSSION

The median lethal dose of the extract was greater than 4500mg/ kg .bodyweight Castor oil causes diarrhea due to its active metabolite, ricinoleic acid[13,14] which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin[15].

Acetylcholine (Ach) cause increased contraction of the isolated jejunum as shown in Figure - 1. However, *Indigofera purpurea* significantly reduced intestinal transit time as observed by the decrease in intestinal motility of isolated rabbit jejunum as shown in Figure - 2. The relaxation effect of the extract is also similar to adrenalin as shown in Figure - 3. The effects of both the extract and adrenalin were blocked by propranolol, which is a beta blocker as shown in Figure - 4.

This suggests that the extract may be acting via beta receptors. Phytochemical screening revealed the presence of alkaloids, tannins and sterols. Earlier studies showed that anti-dysenteric and antidiarrhea properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids and sterols.

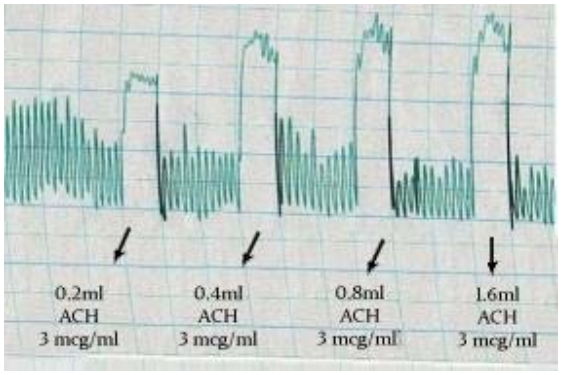

Fig. 1: Effects of Acetylcholine (mcg/ml) on isolated rabbit ileum.

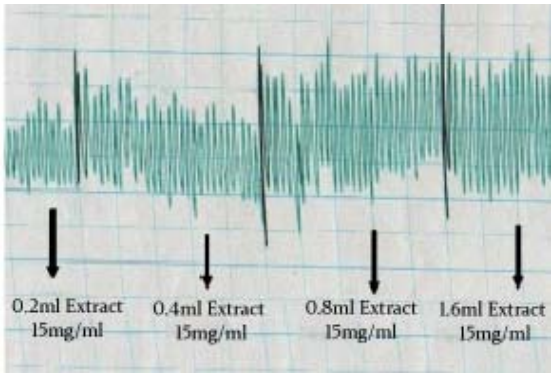

Fig. 2: Effect of Ethyl Acetate extract of aerial part of *Indigofera purpurea* on isolated rabbit ileum

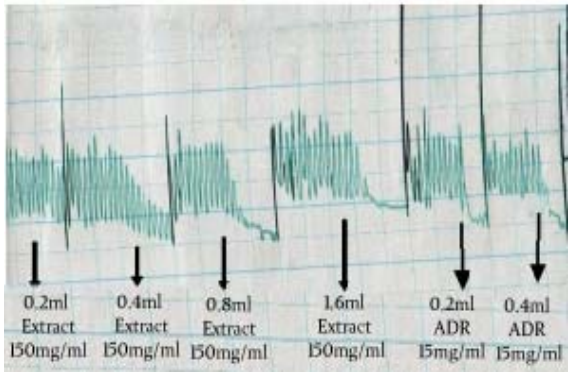

Fig. 3: The effect of Ethyl Acetate extract of aerial part of *Indigofera purpurea* and Adrenaline on rabbit ileum

Hence, tannins, sterols, alkaloids may be responsible for the mechanism of action of *Indigofera purpurea* anti-diarrhea activity[16, 17, 18]. The anti-diarrhea activity of this extract may also be due to the presence of denatured proteins, which form protein tannates. Protein tannates make the intestinal mucosa more resistance and hence, reduce secretion.

This can be due to the fact that the extract increased the reabsorption of by decreasing intestinal motility in isolated rabbit ileum.

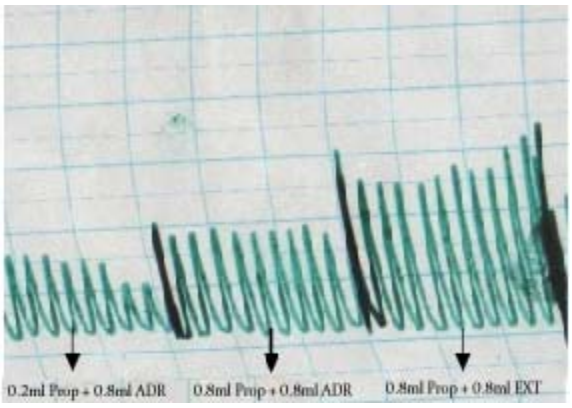

Fig. 4: Effects of Propanolol, Adrenaline and *Indigofera purpurea* Extract on isolated Rabbit Ileum

Table 1: Effects of ethyl acetate extract of *Indigofera purpurea* on castor oil Induced diarrhoeal in mice

Treatment	Dose (mg/kg)	No of mice with Diarrhoea	Protection (%)
Normal saline	15ml/kg	5/5	0.0
Extract	150mg/kg	3/5	50.0
Extract	300mg/kg	2/5	65.5
Extract	600mg/kg	1/5	85.0
Loperamide	6mg/kg	0/5	100.0

Results were analyzed by Chi-square ($X2$). Values were considered significant when $P<0.05$ compared with Normal saline group, n=5

Mice administered with 150, 300 and 600mg/kg ethyl acetate extract of *Indigofera purpurea* had diarrhea in 3/5, 2/5 and 1/5 respectively (50, 65, and 85% protection respectively) as shown in Table 1.In this study, ethyl acetate extract of *Indigofera purpurea* exhibited a significant anti-diarrheal activity. Its effect depended on the dose. The results were similar to that of the standard drug Loperamide 6mg/kg with regard to the severity of diarrhea. The observed relaxation exhibited by the leaves extract further explains its ability to protect the mice against diarrhea induced by castor oil.

REFERENCES

1. Carlos, C.C. and M.C. Saniel, (1990) Etiology and Epidemiology of diarrhoeal. Philipp. J. M icrobiol. Infect. Dis., 19(2): 51-53.
2. Agunu, A., S. Yusuf, G.O. Andrew, A.U. Zezi and E.M. Abdulrahman, (2005), Evaluation of five medicinal plants used in diarrhoeal treatment in Nigeria. J. Ethnopharmacol., 100: 27-30.
3. Ojewole, J.A.O ., (2004), Evaluation of the Antidiabetic, Anti inflammatory and Antidiabetic properties of sclerocarya birrea (A. Rich) Hochst. Stem bark aqueous Extract in Mice and Rats. Phytother. Res., 18: 601-608.
4. Atta, A.H. and S.M. Mouneir, (2004), Antidiarrhoeal activity of some Egyptian medicinal plant extracts. J. Ethnopharmacol., 92: 303-309.
5. Herper, F.N., (1976), The West African Herbaria of Isert and Thoning. Bentham-moxin trust in association with Carlsberg foundation, Kew, England. pp: 92.
6. Sule, M.I., U.U. Pateh, A.K. Haruna, M. Garba, A.A. Ahmadu and A.K. Adamu, (2003), Plants used in Hausa traditional medicine in N orthern Nigeria. J. Trop. Biosci., 3: 17-20.
7. Abubakar, M.S., A.M. Musa, A. Ahmed and I.M. Husaini, (2007). The perception and practice of traditional medicine in the treatment of cancers and inflammations by the Hausa and Fulani tribes of Northern Nigeria. J. Ethnopharmacol., 111(3): 625-629.
8. Tanko, Y., M.M. Abdelaziz, A.B. Adelaiye, M.Y. Fatihu and K.Y. Musa, (2008), Effects of N-Butanol portion of *Indigofera pulchra* leaves extract on blood glucose levels of alloxan-induced diabetic and normoglycemic Wistar rats. Eur. J. Scientific Res., 22: 501-507.
9. Trease, G.E. and M.S. Evans, (1989), Textbook of Pharmacognosy. 14th Edn., Balliere Tindall, London, pp: 81-90, 269-275, 30.
10. Lorke, D., (1983), A new approach to practical acute toxicity testing. Arch. Toxicol., 54: 275-287.
11. Diurno, M.V., A.A. Izzo, B. Mazonni, A. Bologgnese and F. Caspasso, (1996), Anti diarrhea activity of new thiazolinones related to Loperamide. J. Pharmacy Pharmacol., 48: 760-762.
12. Akah, P.A. and V.N. Offiah, (1996), Gastrointestinal effect of *Allamanda cathartica* leaf extract. Int. J. Pharmacogn., 30, 213-217.
13. Ammo, P., J. Thomas and S. Philips, (1974), Effects of oleic and recinoleic acids net jejunal water and electrolyte movement. J. Clin. Invest., 53: 374-379.
14. Watson, W.C. and R. Gordon, (1962), Studies on the digestion absorption and metabolism of castor oil. Biochem. Pharmacol., 11: 229-236.
15. Galvez, J., A. Zarzuelo, M.E. Crespo, M.D. Lorento, M.A. Ocete and J. Jimenez, (1993), Antidiarrhoeic activity of Euphorbia hirta extract and isolation of an active flavonoid constituent. Planta Medica, 59: 333-336.
16. Galvez, J., A. Zarzuelo and M.E. Crespo, 1991. Antidiarrhoeal activities of scleroarya birrea bark extract and its active tannin constituent in rats. Phytother. Res., 5: 276-278.
17. Loganga, O.A., A. Vercruysse and A. Foriers, (2000), Contribution to the ethanobotanical, Phytochemical and pharmacology studies of traditionally used medicinal plant in the treatment of dysentery and diarrhoeal in Lomela area, Democratic Republic of Congo (DRC). J. Ethnopharmacol., 71(3): 41-423.
18. A Mohammed, H Ahmed, A D T Goji, A O Okpanachi, I Ezekiel and Y Tanko, (2009), Preliminary Anti-diarrhoeal Activity of Hydromethanolic Extract of Aerial Part of *Indigofera Pulchra*.in Rodents, Asian J. Med. Sci., 1(2): 22-25.