PRELUDE STUDIES OF ANTI-DIARRHOOEAL ACTIVITY OF ETHYL ACETATE EXTRACT OF AERIAL PART OF INDIGOERA PURPUREA ON ISOLATED RABBIT ILEUM

N RAVEENDRA KUMAR*, G R VIJAYASANKAR1, R PREMA2, S JEEVANANDHAM3, G LAKSHMANA MURTHY4, M SEKAR5

*Department of Pharmacology, Santhiram Medical College, Nandyal, Vishwabharathi College of Pharmaceutical Sciences, Guntur, 2,3,4,Santhiram College of Pharmacy, Nandyal, India Email: jeeva1983@gmail.com

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, saponins 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. Diarrhoea causes an estimated 5 million death in children fewer than 4 years of age per year1. 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 countries2. 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 practices4. 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 Indigofera 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 India5. In ethno medicine, the leaves are used to treat infected wound while the decoction of the aerial part is used as prophylactic against snake-bites6 and as anti-inflammatory7. Recent studies have shown that it also possesses anti diabetic activity8.

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 Amhiba 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 protocols9.

Acute toxicity study
The method previously described by10 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 (LD50) 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°C 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 12 hours 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 15 ml/kg of normal saline intraperitoneally while the mice in the second received 6 ml/kg of Loperamide as a standard positive control, the third, fourth and fifth 150, 300 and 600 mg/kg, respectively. After 30 minutes of administration of the extract, castor oil 0.3 ml/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 and the percentage protection was calculated.

**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 4500 mg/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 (600 mg/kg) comparable to that of loperamide, the standard anti diarrhoeal agent.

**DISCUSSION**

The median lethal dose of the extract was greater than 4500 mg/kg bodyweight. Castor oil causes diarrhea due to its active metabolite, ricinoleic acid 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 prostaglandins.

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 antidyserteric and antidiarrhoea properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids and steroids.

**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-diarrhoea activity. The anti-diarrhoea 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.
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


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

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

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