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# *IN VIVO* EVALUATION OF ANTIDIARRHOEAL ACTIVITY OF *RHUS SEMIALATA* FRUIT EXTRACT IN RATS

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## Abstract

*Rhus semialata* Murr. (Anacardiaceae) is a deciduous tree of north eastern India. The fruit of this plant is traditionally used to control diarrhoea and dysentery. The Present study was undertaken to evaluate anti-diarrhoeal potency of methanol extract of fruits of *R. semialata* using Wister albino rats to substantiate folklore claims. The extract at graded doses (100, 200, 400 and 600 mg/kg body weight) was investigated for anti-diarrhoeal activity in term of reduction in the rate of defecation in castor oil induced diarrhoea. To understand the mechanism of its anti-diarrhoeal activity, the gastrointestinal transit and $PGE_2$-induced intestinal fluid accumulation (enteropooling) were further evaluated. At graded doses, the extract showed a remarkable anti-diarrhoeal activity evidenced by the reduction in the rate of defecation up to 80.70 % of control diarrhoeal animals at the dose of 600 mg/kg body weight. Results are comparable to that of standard drug diphenoxylate (50 mg/kg body weight). Extract produced profound decrease in intestinal transit (8.02 – 47.05 %) at selected doses comparable to that of single intraperitoneal injection of standard drug atropine sulphate at doses of 0.1 mg/kg body weight. It significantly inhibited $PGE_2$ - induced enteropooling (21.98 – 56.03 %). The results indicated that the methanol extract of the fruits of *R. semialata* possesses significant anti-diarrhoeal effect and substantiated the use of this herbal remedy as a non-specific treatment for diarrhoea in folk medicine.

**Key words:** Atropin sulphate, Castor oil, Diarrhoea, Diphenoxylate, *Rhus semialata*.

## Introduction

Diarrhoea is a symptom marked by rapid and frequent passage of semisolid or liquid feacal material through the gastrointestinal tract. Diarrhoea has long been recognized as one of the most important health problems in the developing countries (Syder and Merson, 1982). More than 5-8 million annual deaths in diarrhea for infants and small children of less than 5 years have been reported worldwide (Park, 2000). In developing countries, the majority of people living in rural areas almost exclusively use traditional medicines to treat all sorts of diseases including diarrhoea. Many plants, namely *Andrographis paniculata, Asparagus racemosus, Cinnamomum bejolghota, Astragalus verus, Ficus bengalensis, Prunus cerasoides, Nelumbo nucifera, Acacia catechu, Acacia chandra, Terminalia chibula, Pterocarpus marsapium, Cassia auriculata* etc. available in India are used in traditional folklore medicine for the treatment of diarrhoea (Chopra et al., 1956).

*R. semialata* Murr. (Anacardiaceae) is a deciduous tree (syn. *R. chinensis* Mill.; *R. javanica* Linn.) found in the outer Himalayan ranges at an altitude of 3,000-7,000 ft, the hills of Assam, Khasia, Naga and Sikkim in

India (Gurung, 2002; Rai and Sharma, 1996; Bhattacharjee, 1998), upper Burma, China and Japan (Kiritikar and Basu, 1987). The fruits are edible with sharp acidic taste. The infusion of fruits is traditionally used to control diarrhoea and dysentery. The fruit contains tannin, gallic acid and the potassium acid salts, together with small amount of aluminium, calcium, magnesium and iron, acid salts of mallic, tartaric and citric acids (Anonymous, 2003). Exhaustive literature survey indicated that systematic pharmacological work has so far not been done with regard to this plant. Hence, the present work was undertaken to investigate the anti-diarrhoeal activity of the fruits of *R. semialata* to substantiate folklore claims.

## Materials and Methods

### Plant material and preparation of extract

The fruits of *R. semialata* (JU/PT/Pcog/04/07) were collected from local area of Pandam, East Sikkim, India and authenticated by Botanical Survey of India, Gangtok. The voucher specimen is kept in the Division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata.

The fruits were dried in an incubator for two days at 40°C, reduced to a coarse powder. 500 g of powdered fruits was extracted in a Soxhlet apparatus with methanol and solvent was removed under vacuum and finally lyophilized to obtain a solid mass. The extract was dissolved in 2% v/v aqueous Tween 80 solution. On preliminary screening the methanol extract showed positive results for carbohydrate and tannin (Kokate, 1994; Harborne, 1998).

### Selection of animals and animal care

Wister albino rats (150 – 180 g) of either sex were used for the experiments. Animals were allowed to be acclimatized for a period of 2 weeks in our laboratory environment prior to the study. Animals were housed in polypropylene cages (4 animals per cage), maintained under standard laboratory conditions (i.e. 12:12 hour light and dark sequence; at an ambient temperature of 25 ± 2 ºC; 35 - 60% humidity); the animals were fed with standard rat pellet diet (Hindustan Liver Ltd. Mumbai) and water *ad libitum*. The principles of Laboratory Animal Care (NIH, 1985) were followed and instructions given by Jadavpur University, Kolkata, India animal ethical committee were maintained throughout the experiments.

### Chemicals and Reagents

Atropine sulphate was purchased from Samarth Pharma. Pvt. Ltd., Mumbai, India. Diphenoxylate was procured from Maiden Pharma. Pvt. Ltd., Delhi, India. $PGE_2$ was purchased form Astra-IDL Limited, India.

### Toxicity study

An acute toxicity study was performed using different oral doses of the extracts on experimental Swiss albino mice according to the method described by the method of Ghosh (1984).

### Castor-oil induced diarrhea

Overnight fasted thirty six rats were divided into six groups equally as follows. Group I: (Control group) Rats of this group received 1 ml 2% v/v aqueous Tween 80 orally. Group II, III, IV and V: (Extract treated groups) Rats of these groups were treated with methanol extract of *R. semialata* fruits at the doses of 100, 200, 400, and 600 mg/kg body weight by oral route respectively suspended in 2% v/v aqueous Tween 80. Group VI: (Standard drug treated group). Rats of this group were treated with the reference drug, diphenoxylate at the dose of 50 mg/kg body weight, orally. After one h of dosing, all the rats were treated with 1 ml of caster oil orally by gavage and observed for consistency of faecal material. The numbers of wet faecal droppings were measured for four hrs after castor oil administration. Characteristic diarrhoeal droppings were noted in transparent plastic dishes placed beneath the individual perforated rat cages (Awouters et al., 1978; Gnanasekar and Perianayagam, 2004). The total number of diarrhoeal faeces of the control group was considered 100%. The results were expressed as percentage of inhibition of diarrhoea.

### Gastrointestinal motility tests

This experiment was done by using charcoal meal as a diet marker (Boominathan et al., 2005). Albino rats

were fasted for 18 h and divided into six groups containing six animals each. Each animal was administered with 1.0 ml of charcoal meal orally (3% deactivated charcoal in 10% aqueous Tween 80) and subsequent treatments were as follows Group I: (Control group) Rats of this group received 1.0 ml 2% v/v aqueous Tween 80 orally. Group II, III, IV and V: (Extract treated groups) Rats of these groups were treated with methanol extract of *R. semialata* fruits (suspended in 2% v/v aqueous Tween 80) at the doses of 100, 200, 400, and 600 mg/kg body weight by oral route respectively. Group VI: (Standard drug treated group) Rats of this group were treated with the reference drug, atropine sulphate at the dose of 0.1 mg/kg body weight, intraperitonealy. After 30 mins, all rats were sacrificed under ether anesthesia. The peritoneal cavity was cut and the distance traversed by charcoal meal from the pylorus towards caecum was measured and expressed as a percentage of the distance from the pylorus to the caecum (Rani et al., 1999).

**$PGE_2$- induced enteropooling**

In this method rats were deprived of food and water for 18 h and divided into six groups of six animals in each group as follows. Group I: (Control group); Rats of this group received 1.0 ml 2% v/v aqueous Tween 80 orally. Group II, III, IV and V: (Extract treated groups); Rats of these groups were treated with methanol extract of *R. semialata* fruits at the doses of 100, 200, 400, and 600 mg/kg body weight by oral route respectively suspended in 2% v/v aqueous Tween 80. Group VI: (Standard drug treated group); Rats of this group were treated with the reference drug, atropine sulphate at the dose of 3.0 mg/kg body weight, intraperitonealy. Immediately afterwards, $PGE_2$ was administered orally to each rat (100 µg/kg) in 5% v/v ethanol in normal saline. After 30 mins, each rat was killed and the whole length of the intestine from the pylorus to the caecum was dissected out and its contents were collected in a test tube and measured (Mandal et al., 1997).

**Statistical Analysis**

The experimental results are expressed as the mean ± S.E.M of six determinations. Statistical tests were performed by Student's 't' test and p value was calculated by comparison with control groups (Woodson, 1987).

## Results

**Toxicity studies**

Acute toxicity study of methanol extract of *R. semialata* fruits showed even at an oral dose of 3.2g/kg body weight., no death of experimental mice occurred.

**Inhibition of castor-oil induced diarrhoea**

A single oral administration at various doses of *R. semialata* extract produced a significant decrease in the severity of diarrhoea in terms of reduction in the rate of defecation in Wister albino rats. The percentage inhibition for the number of wet faeces indicates the presence of antidiarrhoeal activity in extract as compared with that of control group. Experimental result reflects that the activity is more pronounced at the dose of 600 mg/kg body weight (Table 1). The percentage of inhibition of number of wet faeces was 80.70 %, $p < 0.01$ at the dose of 600 mg/kg body weight while that of standard drug diphenoxylate (50 mg/kg) was 99.53 % control of castor oil-induced diarrhoea.

**Effects on gastrointestinal motility**

The extract produced profound decrease in intestinal transit of 8.02 – 53.63 % at the dose range of 100 - 600 mg/kg body weight (Table 2) and while that of atropine sulphate produced 54.42 % inhibition of intestinal transit at dose of 0.1 mg/kg body weight.

**Anti-enteropooling activity**

The extract also inhibited significantly $PGE_2$ induced enteropooling in term of volume of intestinal content (Table 3). The extract at the dose range of 100 - 600 mg/kg body weight showed percentage inhibition range of

21.98 – 56.03 % while atropine sulphate at the dose of 3 mg/kg body weight produced 66.67 % inhibition of $PGE_2$-induced enteropooling.

## Discussion

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied with an excess loss of fluid in the faeces. Castor oil causes diarrhoea due to its active metabolite, ricinoleic acid (Ammon et al., 1974; Watson and Gordon, 1962), which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates and

**Table 1:** Effect of the methanol extract of *Rhus semialata* fruits at different dose levels on castor oil-induced diarrhoea.

| Group | Treatment | Number of wet faeces | Percentage of inhibition |
|---|---|---|---|
| I | 2% v/v aqueous Tween 80 (1 ml/ kg) + Castor oil (1 ml) | 4.30 ± 0.54 | |
| II | Extract (100mg/kg) + Castor oil (1 ml) | 2.12 ± 0.25* | 50.70 |
| III | Extract (200mg/kg) + Castor oil (1 ml) | 1.92 ± 0.45* | 55.34 |
| IV | Extract (400mg/kg) + Castor oil (1 ml) | 1.41 ± 0.38* | 67.21 |
| V | Extract (600mg/kg) + Castor oil (1 ml) | 0.83 ± 0.31** | 80.70 |
| **VI** | Diphenoxylate (50 mg/ kg) + Castor oil (1 ml) | 0.02 ± 0.32** | 99.53 |

Data are expressed as mean ± S.E.M., n = 6. *p < 0.05, **p <0.01 when compared with vehicle-control.

**Table 2:** Effect of the methanol extract of *Rhus semialata* fruits at different dose levels on charcoal-induced gut transit changes.

| Group | Treatment | Percentage of distance travel by charcoal meal | Percentage of inhibition |
|---|---|---|---|
| I | Charcoal meal + 2% v/v aqueous Tween 80 (1 ml/ kg) | 78.50 ± 2.35 | |
| II | Charcoal meal + Extract (100mg/kg) | 72.20 ± 1.86* | 8.02 |
| III | Charcoal meal + Extract (200mg/kg) | 65.66 ± 2.11* | 16.36 |
| IV | Charcoal meal + Extract (400mg/kg) | 56.52 ± 2.26* | 28.00 |
| V | Charcoal meal + Extract (600mg/kg) | 36.40 ± 2.14** | 53.63 |
| VI | Charcoal meal + Atropine sulphate (0.1mg/kg) | 35.78 ± 2.68** | 54.42 |

Data are expressed as mean ± S.E.M., n = 6. *p < 0.05, **p <0.01 when compared with vehicle-control.

irritates intestinal mucosa and ensures the release of endogenous prostaglandin (Galvez et al., 1993).

In this study, the methanol extract of *R. semialata* fruits exhibited a significant dose-dependant anti-diarrhoealactivity. The results were comparable to that of the standard drug diphenoxylate (50 mg/kg) with regard to the severity of diarrhoea. The extract also significantly reduced intestinal transit as observed by the decrease in

**Table- 3:** Effect of the methanol extract of *Rhus semialata* fruits at different dose levels on $PGE_2$- induced enteropooling.

| Group | Treatment | Volume of intestinal fluid ml | Percentage of inhibition |
|---|---|---|---|
| I | 2% v/v aqueous Tween 80 (1 ml/ kg) + $PGE_2$ in ethanol (100 µg/kg) | $2.82 \pm 0.18^{*}$ | |
| II | Extract (100mg/kg) + $PGE_2$ in ethanol (100 µg/kg) | $2.20 \pm 0.14^{*}$ | 21.98 |
| III | Extract (200mg/kg) + $PGE_2$ in ethanol (100 µg/kg) | $1.92 \pm 0.09^{**}$ | 31.91 |
| IV | Extract (400mg/kg) + $PGE_2$ in ethanol (100 µg/kg) | $1.60 \pm 0.12^{**}$ | 43.62 |
| V | Extract (600mg/kg) + $PGE_2$ in ethanol (100 µg/kg) | $1.24 \pm 0.08^{*}$ | 56.03 |
| VI | Atropine sulphate (3.0 mg/kg) + $PGE_2$ in ethanol (100 µg/kg) | 0.94 ± 0.45 ** | 66.67 |

Data are expressed as mean ± S.E.M., n = 6. *p < 0.05, **p <0.01 when compared with vehicle-control.

transit motility of charcoal meal. This may be due to the fact that the extract may increase the reabsorption of water by decreasing intestinal motility as observed in the decrease of intestinal transit by charcoal meal. The extract also led to a marked reduction in the volume of the intestinal contents on $PGE_2$-induced enteropooling. Thus the inhibiting effect of the extract justifies the use of the plant in folk medicine as a nonspecific anti-diarrhoeal agent. Preliminary phytochemical investigations indicated presence of tannins in the fruits of *R. semialata.* Tannins are known for their anti-diarrhoeal activity (Kokate, 1988). Infact, tannins are responsible for the denaturation of proteins and form protein tannate, which makes the intestinal mucosa more resistant and reduces secretion (Tripathy, 1994). Thus the antidiarrhoeal activity may be due to tannins present in the fruits.

**Conclusions**

The results indicate that the methanol extract of *R. semialata* fruits possess significant anti-diarrhoeal activity due to its inhibitory effect both on gastrointestinal propulsion and fluid secretion. The data obtained are consistent with literature report on antidiarrhoeal activity of *R. semialata* fruits using gastrointestinal motility test and castor-oil induced diarrhoea and intraluminal accumulation of fluid in rats. The inhibitory effect of the extract justified the use of the plant as a non-specific anti-diarrhoeal agent in folk medicine. So, we can conclude that the present study seems to support the claims of a traditional medicine practioners on the use of *R. semialata* in diarrhoea.

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