http://dx.doi.org/10.4314/ajtcam.v11i2.11

# INVESTIGATION ON ANTIDIARRHOEAL ACTIVITY OF *ARISTOLOCHIA INDICA* LINN. ROOT EXTRACTS IN MICE

**Senthil Rajan Dharmalingam[a], Rajkumar Madhappan[b], Srinivasan Ramamurthy[a], [1]Kumarappan Chidambaram[a], Meka Venkata Srikanth[a], Suresh Shanmugham[a], Senthil Kumar K. L.[b]**

[a]School of Pharmacy, International Medical University, Bukit Jalil, Kuala Lumpur, Malaysia.
[b]Department of Pharmacognosy, Padmavathi College of Pharmacy and Research Institute, India.
***Email:** dsenthilrajan@yahoo.co.in

## Abstract

**Background:** The present study aimed at investigating the effect of ethanolic extract (EtAI), and aqueous extract (AqAI) of *Aristolochia indica* Linn roots on castor oil-induced diarrhoea and study on small intestinal transit. Phytochemical analysis of extracts was performed as per standard procedure.
**Materials and Methods**: The oral toxicity study using Swiss albino mice was performed in accordance with OECD guidelines. The EtAI and AqAI extracts of *Aristolochia indica* Linn were studied for antidiarrhoeal property using castor oil-induced diarrhoeal model and charcoal-induced gastrointestinal motility test in *Swiss albino* mice.
**Results:** Among the tested doses of 200 and 400 mg/kg body weight, the extracts reduced the frequency and severity of diarrhoea in test animals throughout the study period. At the same doses, the extract delayed the intestinal transit of charcoal meal in test animals as compared to the control and the results were statistically significant**.**
**Conclusion:** Experimental findings showed that ethanol extract of *Aristolochia indica* Linn root possess significant antidiarrheal activity and may be a potent source of anti-diarrhoeal drug in future.

**Key words:** Antidiarrheal activity, *Aristolochia indica* Linn, ethanol extract, small intestinal transit

## Introduction

Diarrhoea is characterized by increased frequency of bowel movement, wet stool and abdominal pain (Ezekwesili et al., 2004). Diarrhoea is the first runner-up killer of children under 5, years of age, which accounts for 10% of child deaths every year (Liu et al, 2012). *Aristolochia indica* Linn commonly known as Indian birthwort (family: Aristolochiaceae) is an important medicinal plant which is a perennial climber with greenish white woody stems found in low hills and plains of India, Nepal and lower Bengal to Chittagong in Bangladesh.
*Aristolochia indica* Linn has been reported for anti-fertility effect (Pakrashi and Pakrasi, 1979), anti-diabetic activity (Sanjay *et al.*, 2012), anti-venom (Meenatchisundaram *et al.*, 2009), and antioxidant properties (Thirugnanasampandan et al., 2008).

Adeyemi et al. (2012) reported that *Aristolochia ringens* belonging to Aristolochiaceae family has anti-diarrhoeal activity. There is apparently no available scientific report regarding the antidiarrhoeal effects of *Aristolochia indica* Linn in any animal model Hence, we undertook this study to investigate the effect of *Aristolochia indica* Linn roots on castor oil-induced diarrhoea and study on small intestinal transit in mice.

## Materials and Methods

### Collection of Plant Material

The roots of *Aristolochia indica* Linn were collected from Salem district, Tamil Nadu, India. The plant was identified and authenticated by Dr. P. Jayaraman, Botanist, Plant Anatomy Research Centre (PARC), Chennai, India. Voucher specimen (PARC/2009/112), has been deposited at the herbarium of the Department of Pharmacognosy, Padmavathi College of Pharmacy and Research Institute, India.

### Preparation of Extract

*Aristolochia indica* Linn root was dried in shade and powdered by means of a wood-grinder, and the powder was passed through the sieve no.60. About 50 g of root powder was macerated in 95% ethanol (200ml) for 72 hours. For aqueous extract, 50 g of root powder was extracted with distilled water using soxhlet at boiling temperature (100 °C) up to 10 h. The liquid filtrates obtained were concentrated under controlled temperature using rotary evaporator. The extracts were stored in desiccators until further use (Mothana *et al.*, 2012).

### Phytochemical screening

The freshly prepared AqAI and EtAI extracts were qualitatively tested for the presence of phytochemical such as carbohydrates, alkaloids, steroids, flavonoids, volatile oil, tannins, saponins, Phytosterols and terpenoids chemical constituents.

### Experimental animals

Healthy male Swiss albino mice (weighing 20-25 g), were selected for evaluation of anti-diarrhoeal activity. Six mice were taken for each group. The mice were used after acclimatization under controlled conditions of temperature of 24 ± 2°C, humidity of 50 ± 5% and 10–12 hrs, of light and dark cycles respectively for 7, days. The animals were housed individually in polypropylene cages containing sterile paddy husk as bedding throughout the experiment and had free access to sterile animal chow and water *ad libitum.* The study was commenced after obtaining the authorization of Institutional animal ethics committee and Institutional Ethics Committee (IAEC), and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (IAEC /CPCSEA/2011/069).

http://dx.doi.org/10.4314/ajtcam.v11i2.11

**Acute toxicity studies**

The acute oral toxicity study was performed for AqAI and EtAI extracts of *Aristolochia indica* Linn root using fixed dose method of OECD guideline (OECD, 2008).

**Castoroil-induced diarrhoea model**

The diarrhoea was induced in S*wiss male albino* mice (weighing 20-25 g), by previously described method (Vareishang and Arun, 2004), with slight modification to suit experimental needs. The animals were randomly placed into six groups each of six animals and housed in separate cages. Animals of first group received 1ml castor oil orally, while those in groups II and III were pre-treated orally at a dose of 200mg and 400mg /kg body wt. of EtAI extract respectively and groups IV and V were pre-treated orally at a dose of 200mg and 400mg /kg body wt. of AqAI extract and the last group VI was pre-treated with diphenoxylate Hcl (sigma aldrich), raw reference standard drug (5mg/kg, p.o). Animals of groups II to VI received 1 ml castor oil orally using the orogastric cannula 30 min after pre-treatment with the extracts or standard drugs or distilled water. Group VII was treated with only 0.5% CMC (Vehicle control). Following treatment with castor oil, the animals were then placed in individual cages over clean filter paper and were examined up to four hours for the presence of the characteristic diarrheal droppings; their absence was recorded as a protection from diarrhoea (Diurno et al., 1996). The total number of normal faeces and diarrhoeal faces excreted in record time were scored and compared with control group. The total score of diarrhoeal faeces of vehicle control group was considered that of 100%. The results were expressed in percentage of inhibition (Zaval et al., 1998).

**Study on small intestinal transit**

Swiss male albino mice weighing 20-25 g were divided into six groups of 6, animals each. First group served as the vehicle control (0.5%w/v CMC). The second and third groups received orally with 200mg and 400mg /kg body wt. of alcoholic extract respectively.
The fourth and fifth groups received orally with 200mg and 400mg /kg body wt. of AqAI extract respectively. The sixth group received orally the standard drug, atropine sulphate in the dose of 100µg/kg (i.p.). Half an hour after treatment, individual animals were administered orally with 1 ml of charcoal meal (10%w/v activated charcoal in 0.5% CMC) suspension as a marked by oral route.
After the observation of 40min, each mouse was sacrificed and intestinal distance travelled by the charcoal meal from pylorus to caecum was estimated and expressed as percentage of distance moved (Mujumdar, 1998).

**Statistical analysis**

The statistical analysis of data was analysed by one way ANOVA, followed by Dunnett multiple comparison test. Values of $p<0.05$ were considered significant.

**Table 1:** Effect of ethanol and AqAI extract of *Aristolochia Indica* Linn roots on castor oil- induced diarrhoeal model (mean ± SD, n = 6)

| Groups/Treatment | Mean frequency of normal faecal drops ± SEM | Mean frequency of wet diarrhoeal faeces | Percentage of diarrhoea protection |
|---|---|---|---|
| **Group I:** Control (Castor oil, 1mL, p.o) | 1.83± 0.75[b] | 17.50±2.16[b] | 0% |
| **Group II:** EtAI extract (200mg/kg, p.o) + Castor oil | 3.50± 1.04 | 7.33±1.21[a, b] | 58.11% |
| **Group III:** EtAI extract (400mg/kg, p.o) + Castor oil | 5.66±1.03[a] | 4.83±0.75[a, b] | 72.38% |
| **Group IV:** AqAI extract (200mg/kg, p.o) Castor oil | 2.83±0.75[b] | 8.33±1.63[a,b] | 54.4% |
| **Group V:** AqAI extract (400mg/kg, p.o) + Castor oil | 5.5±1.04[a] | 6.66±1.36[a,b] | 61.94% |
| **Group VI:** Diphenoxylate (5mg/kg, p.o.) + Castor oil | 6.83±1.16[a,b] | 2.83±0.75[a,b] | 83.81% |
| **Group VII:** Control (Vehicle) | 4.66±1.03[a] | 0.00 | 100% |

[a] $P < 0.05$ statistically (Mean ± SEM) significant from castor oil control group (n=6); [b] $P < 0.05$ statistically (Mean ± SEM) significant from vehicle control group (n=6)

**Table 2:** Effect of extracts of A*ristolochia Indica* Linn. roots on gastrointestinal motility

| Group | Treatment | Mean movements of charcoal meal (cm) ± SD | % Movement of charcoal meal |
|---|---|---|---|
| I | Vehicle Control | 84.87±1.83 | 79.15±1.52 |
| II | EtAI extract 200mg/kg | 76.65±2.87[a] | 72.29±1.99[a] |
| III | EtAI extract 400mg/kg | 54.31±2.87[a] | 51.15±2.84[a] |
| IV | AqAI extract 200mg/kg | 79.22±3.47[a] | 74.46±3.36 |
| V | AqAI extract 400mg/kg | 68.15±4.12[a] | 64.93±1.77[a] |
| VI | Atropine sulphate (100ug/kg, i.p.) | 31.30±2.70[a] | 29.16±2.24[a] |

01, statistically (Mean ± SEM) significant from vehical control group (n=6).

http://dx.doi.org/10.4314/ajtcam.v11i2.11

## Results and discussion

### Acute toxicity studies

EtAI extract and AqAI extract of *Aristolochia indica* Linn roots was studied for oral acute toxicity at dose of 2000mg/kg by oral route. The extract was found devoid of neither mortality of the animals nor any visible clinical signs of general weakness in the animals. Hence, 2000mg /kg was considered as LD 50 cut-off value. So the doses selected were 200 mg/kg ($1/10^{th}$ of 2000 mg/kg) and 400 mg/kg ($1/5^{th}$ of 2000 mg/kg) based on fixed dose method of OECD guidelines.

In the present investigation, anti-diarrheal activity was evaluated by castor oil-induced diarrhoeal model. It was observed that both the extracts (Group II to V), at both dose levels significantly reduced number of defecation and total weight of wet faecal matter in comparison to control (Group I).

The percentage inhibition of ethanol extract and aqueous extract at higher dose level 400 mg/kg was found to be 72.38% and 61.94% respectively. Results were compared with that of standard drug, diphenoxylate Hcl in Table 1. Subsequently the EtAI extract of *Aristolochia indica* Linn roots successfully inhibited the castor oil-induced diarrhoea; the extract might have exerted its antidiarrheal action via anti-secretory mechanism which was also evident from the decrease of total number of wet faeces in the groups II-V in the experiment (Table 1). Flavonoids present in the plant extract are claimed to inhibit release of autacoids and prostaglandins, thereby inhibit motility and secretion induced by castor oil (Veiga et al., 2001) and also to inhibit contraction caused by spasmogenes (Macauder, 1986).

EtAI extract and atropine sulphate (100µg/kg), decreased the propulsion of the charcoal meal through the gastrointestinal tract when compared with the control (0.5% CMC), whereas even though AqAI extract decreased the intestinal transit, the results were statistically insignificant (Table 2). Loperamide, a standard antidiarrheal drug is also reported have no effect on colonic motility and incline to slowdown small intestinal transit, reduced colonic rate of flow, and consequently increased colonic water absorption (Theoderau, 1991). EtAI extract also significantly decreased intestinal transit as observed by the decrease in transit motility of charcoal meal. Previous investigation revealed that tannins, triterpenes, reducing sugars and sterol may be responsible for the mechanism of action of antidiarrheal activity (Longanga Otshudi et al.,2000). This can be due to the fact that the steroid present in EtAI extract increased the reabsorption of water and Na + and water by decreasing intestinal motility as observed in the decrease of intestinal transit by charcoal meal (Longanga et al., 2000). This investigation establishes the use of *Aristolochia indica* Linn as an antidiarrhoeal as claimed by the traditional medicine (Umamaheshwari et al., 2012). Further studies on chemistry of *Aristolochia indica* Linn extracts and on other pharmacological factors influencing antidiarrheal activity may provide more insights on its activity.

**Conflict of interest statement:** We declare that we have no conflict of interest.

## Acknowledgements

The authors are grateful to acknowledge Management of Padmavathi College of Pharmacy and Research Institute, India.

## References

1. Adeyemi, O. O., Aigbe, F. R., and Badru, O.A. (2012). The antidiarrhoeal activity of the aqueous root extract of *Aristolochia ringens* (Vahl.) Aristolochiaceae. Nig Q J Hosp Med., 22 (1):29-33.
2. Diurno, M.V., Izzo, A.A., Mazzoni, B., Bologgnese, A. and Capasso, F. (1996). Antidiarrhoeal activity of new thiazolidinones related to loperamide. J Pharm Pharmacol., 8: 760-762.
3. Ezekwesili, C.N., Obiora, K.A. and Ugwu O. P. (2004). Evaluation of Anti-Diarrhoeal Property of Crude Aqueous Extract of *Ocimum gratissimum* L. (Labiatae) in Rats. Biokemistr., 16(2): 122-131.
4. Liu, L., Johnson, H.L., Cousens, S., Perin. J., Scott, S., Lawn, J.E. (2012). Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet, 379: 2151-61.
5. Longanga, O. A., Vercruysse, A., and Foriers, A. (2000). Contribution to the ethnobotanical, phytochemical and pharmacological studies of traditionally used medicinal plant in the treatment of dysentery and diarrhoea in Lomela area, Democratic Republic of Congo (DRC). J Ethnopharmacol., 71(3): 411-423.
6. Longanga, O.A. Vercruysse, A. and Foriers, A. (2000). Contribution to the ethnobotanical, phytochemical and pharmacological studies of traditionally used medicinal plant in the treatment of dysentery and diarrhoea in Lomela area, Democratic Republic of Congo (DRC). J Ethnopharmacol., 71(3): 411- 423.
7. Meenatchisundaram, S., Parameswari. G. and Michael. (2009). Studies on antivenom activity of *Andrographi spaniculata* and *Aristolochia indica* plant extracts against Daboiarusselli venom by *in vivo* and *in vitro* methods. Indian J Sci Tech., 2: 76-79.
8. Jessy, E.M., Srinivasan, K.K., Seekarajapuram, D. V. and Magi, J. (2011). Anti-inflammatory, Antipruritic and Mast Cell Stabilizing Activity of *Aristolochia Indica*. Iran J Basic Med Sci., 14 (5): 422-427.
9. Macauder, P.J. (1986). Flavonoid affect acetylcholine, prostaglandin E and antigen mediated muscle contraction. Prog Clin Biol Res., 213: 489-492.
10. Mothana, R.A.A., Salah. A.A.A., Sidgi. H., Faisal. M.N.A., Sama. A.Z.A. and Ulrike. L. (2012). Antimicrobial, Antioxidant and Cytotoxic Activities and Phytochemical Screening of Some Yemeni Medicinal Plants. eCAM, 7(3): 323–330.
11. Mujumdar, A. M. (1998). Antidiarrhoeal activity of *Azadiachta indica* leaf extract. Ind. Drug., 35 (7): 417-420.
12. Organization for Economic cooperation and development (OECD). OECD Guidelines for testing of chemicals acute oral toxicity. OECD 2008; 425.
13. Pakrashi, A and Chakrabarty, B. (1978). Anti-oestrogenic & anti-implantation effect of aristolic acid from *Aristolochia indica* (Linn). Indian J Exp. Biol., 16: 1283–1285.
14. Sanjay Kumar, K., Sagar Kumar, M., Dilipkumar, P. and Arijit, M. (2012). Isolation of β-sitosterol and evaluation of antidiabetic activity of *Aristolochia indica* in alloxan-induced diabetic mice with a reference to *in-vitro* antioxidant activity, J Med Plant Res., 6(7): 1219-1223.
15. Theoderau,V., Fioramont, J., Hachet, T., and Bueno, L. (1991). Absorptive and motor components of anti-diarrhoeal action of loperamide: an *in vivo* study in pigs. Gut., 32: 1355 - 1359.
16. Thirugnanasampandan, R,, Mahendran, G. and Narmatha Bai, N. (2008). Antioxidant properties of some medicinal Aristolochiaceae species. Afr J Biotechnol.,7 (4):357-361.
17. Umamaheshwari, S., Murthy, S. and Mahadeva. (2012). An Evaluation And Comparative Study of Root Extract of *Aristolochia Indica* and Antibiotics on *Escherichia Coli*, J Pharm Sci Innov., 1(6): 61.
18. Vareishang, T. and Arun, K.Y. (2004). Antidiarrhoeal activity of *Rhus javanica* ripens fruit extract in albino mice. Fitoterapia., 75: 39–44.
19. Veiga, V.F., Zunino, L., Calixto, J.B., Pititucci, M.L. and Pinato, A.C. (2001). Phytochemical and anti-oedematogenic studies of commercial copaiba oils available in Z.S Brazil. Phytother Res., 15 (6): 476-480.
20. Zaval, M.A., Pera, Z.S., Perez, P., Vargan, R. and Perz, R.M. (1988). Antidiarrheoal activity of *Waltheria anorlana*, *Commelina coelestis* and *Alternanthera repens*. J Ethnopharmacol., 61: 41-47.