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## ANTIDIARRHOEAL ACTIVITY OF LEAVES OF *ACORUS CALAMUS*

M.D. Kapadia\* and A.R. Kharat

Rofel, Shri G.M.Bilakhia College of Pharmacy, Vapi-396191, Gujarat, India

### ABSTRACT

#### Keywords:

Antidiarrhoeal activity, *Acorus calamus* leaves, Loperamide, Gastrointestinal motility test, Inhibition of Castor-oil induced diarrhoea

#### Correspondence to Author:

**Mayur D. Kapadia**

B-48, Miranagar Society, Opp. Zansi ki Rani Garden, Udhna, Surat, Gujarat-394210

E-mail : mayur.kapadia25@gmail.com

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In developing countries, the majority of people living in rural areas almost exclusively use traditional medicines to treat all sorts of diseases including diarrhea. Many plants namely *Andrographis paniculata*, *Acacia catechu*, *Acacia chandra*, *Terminalia chebula*, *Pterocarpus marsapium*, *Cassia auriculata* etc. available in india are used in traditional folklore medicine for the treatment of diarrhea. Diarrhoea is a common cause of illness resulting in high mortality in developing countries worldwide distribution of diarrhoea accounts for more than 5-8 millions deaths each year. It thus becomes important to identify and evaluate scientifically the antidiarrhoeal effects of leaves of *Acorus calamus* against castor oil induced diarrhoea. Methanolic Extract was studied for antidiarrhoeal potential using wistar rats and loperamide as a standard. Extracts were administered orally to test animals. Stools were collected upto 4 hrs in transparent plastic dishes of uniform weight. Urine was drained off by gravity every 15 mins. during the 4 hrs. diarrhoeal free period, acute diarrhoea and late diarrhoeal excretions were noted. Gastrointestinal motility test also performed. Methanolic extract showed promising antidiarrhoeal activity which valid its traditional claim. Methanolic extract (400mg/kg) showed control wet faeces (0.98±0.35) and more diarrhoeal period.

**INTRODUCTION:** Diarrhea, which could be infectious or non-infectious, is one of the principal causes of death, particularly in the malnourished infants. In order to combat the problems of diarrhoea globally, the World Health Organization in its Diarrhoeal Disease Control programme has given a special emphasis on the use of traditional folklore medicines in the control and management of diarrhea.

*Acorus calamus* Linn. is a leaf, found more or less everywhere throughout India. It was traditionally used to treat diarrhoea, dysentery, leucorrhoea, hemorrhoids, wounds, Chemical investigation of the plant showed the presence of Glycosides and saponins. *Acorus calamus* is the botanical name of the plant more commonly known as calamus.

Other common names of calamus include calamus root, flag root, muskrat root, sweet calomel, sweet flag, sweet sedge, and many other names. Both the leaves and rhizome are apparently psychoactive, with the rhizome being more potent.

Some users report mild hallucinations when sufficient quantities of calamus are ingested. In lesser amounts it can have a stimulating or sedative effect on the user. According to Arabic, Roman, and later European folk botany, the plant is also an aphrodisiac. It is said that calamus will keep people young, boost their health, and strengthen their sexual life. For aphrodisiac purposes, higher doses are recommended. A herbal bath with calamus is said to increase sexual desire.

The oil of *Acorus calamus* is used as an ingredient in flavors, particularly in liquors. It is used a great deal in the making of alcoholic drinks and in perfume to give a bitter tang to the former and those special nuances to the perfumes; it is also used in toothpaste. The rootstalks were at one time used to make candy. If boiled in water for about an hour, with several changes of water, then simmered in syrup, they can be a sweet treat. The plant has a branched and aromatic root or rhizome (underground horizontal stem of a plant that produces roots) from which rise its long erect leaves.

The roots have a sweet fragrance (they have been used to flavor candy) and the leaves smell similar to lemon. The sword-like leaves of the plant resemble those of other similar plants so much, that before the *Acorus calamus* is in flower, it is difficult to recognize it simply by the appearance of its leaves. In late spring, green flowers appear in 2 to 4 long spadices (plural form of spadix) below the leaf tips. The flowers eventually give way to small berries. Calamus is found in both temperate and sub-temperate areas of the globe. The sheathing leaves of this perennial are from 2 to 6 feet in height and about 1 inch in width. They are sharp pointed and have a ridged midrib running their entire length. Studies have shown that calamus is mutagenic in bacteria. There is also a risk of hypertensive reactions if taken with monoamine oxidase inhibitors (MAOI's).

The use of calamus in digestive medicines has been discontinued in most countries because of possible toxic and carcinogenic effects. Toxicity is ascribed to beta-asarone. This compound may cause duodenal and liver cancer. TMA can be synthesized from *Acorus calamus*. TMA (trimethoxyamphetamine) is a drug in the MDMA (commonly known as ecstasy) class of drugs. TMA, like ecstasy, can be described as a psychedelic amphetamine. However, calamus is not converted to TMA in the human body.

#### MATERIALS AND METHODS:

**Collection:** Leaves of *Acorus calamus* are collected from medicinal garden of Rofel Shri GM Bilakhia College of Pharmacy, Vapi.

**Authentication:** Leave of *Acorus calamus* was authenticated by Dr. Minoo Parabia, Head, Department of Bioscience, VNSGU, Surat and Herbarium was

deposited in Department of Pharmacognosy, Rofel Shri G. M. Bilakhia College of Pharmacy, Vapi in year of 2011.

**Preparation of Plant Extract:** Leaves were air dried powdered and macerated with methanol for 7 days. Solvent was filtered and concentrated by rotary vacuum evaporator. Extract was stored at 4°C until use. The yield of the dried extract obtained was 6.74±0.64%. A stock solution of the dried powder was reconstituted in distilled water at a concentration of 800 mg/ml and different doses 100, 200 and 400 mg/kg were prepared from the stock solution and administered orally to the animals.

**Experimental Animals :** Albino Wistar rats (weighing 200 - 280 g) of male sex, were housed in standard metal cages. They were provided standard pellet diet and water. They were allowed a one-week acclimatization period prior to the study. The Animals are maintained under standard laboratory conditions (i.e., 12:12 hour light & dark sequence, at an ambient temperature of 25±2°C, 35-60% Humidity). The equipment, handling and sacrificing of the animals were in accordance with the Animal Ethics committee for protection of animals.

**Drugs & Chemicals:** Atropine sulphate and loperamide, castor oil, normal saline (NaCl 0.9%) and charcoal meal (10% activated charcoal) were used.

#### Antidiarrhoeal Activity tests:

1. **Castor-oil Induced Diarrhoea:** Overnight fasted 30 rats were divided into 6 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, and IV: (Extract treated groups) rats of these groups were treated with methanol extract of *Acorus calamus* at the doses of 100, 200, and 400 mg/kg body weight by oral route, respectively suspended in 2 % (v/v) aqueous Tween 80.

Group V: (Standard drug treated group) Rats of this group were treated orally with the reference drug, loperamide at the dose of 3 mg/kg body weight. After 1 h of administration, all the rats were treated with 1 mL of castor oil orally by gavage and observed for consistency of faecal material. The numbers of wet faecal droppings were measured for 4 h after castor oil administration.

Characteristic diarrhoeal droppings were noted in transparent plastic dishes placed beneath the individual perforated rat cages. The total number of diarrhoeal faeces of the control group was considered 100 %. The results were expressed as percentage of inhibition of diarrhoea.

2. **Gastrointestinal Motility Test** : This experiment was done by using charcoal meal as a diet marker. Albino rats were fasted for 18 h and divided into 5 groups containing 6 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, and IV: (Extract treated groups) rats of these groups were treated with methanol extract of *Acorus calamus* (suspended in 2 % (v/v) aqueous Tween 80) at the doses of 100, 200, and 400 mg/kg body weight by oral route, respectively. Group V: (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, intraperitoneally. After 0.5 h, all rats were sacrificed under ether anesthesia. The peritoneal cavity was

cut and the distance travelled by charcoal meal from the pylorus towards caecum was measured and expressed as a percentage of the distance from the pylorus to the caecum.

3. **Statistical analysis**: All experiments were repeated at least six times and the results expressed as mean  $\pm$  S.E.M. The statistical analysis of data was done using one-way ANOVA (Analysis of Variance) with level of statistical significance taken as  $p < 0.05$ .

## RESULT :

### Antidiarrhoeal Activity:

**Inhibition of castor oil induced Diarrhoea** : A single oral administration at various doses of *Acorus calamus* extracts produced a significant decrease in the severity of diarrhoea in terms of reduction in the rate of defecation in Wistar 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 (**Table 1**).

**Effects on gastrointestinal motility**: The *Acorus calamus* extracts produced profound decrease in intestinal transit of 9.51-38.15 % at the dose range of 100, 400 mg/kg body weight (**Table 2**).

**TABLE 1 : EFFECT OF THE METHNOL EXTRACT OF LEAVES OF ACORUS CALAMUS ON CASTOR OIL INDUCED DIARRHOEA**

| Group | Treatment   | No. of Wet Faeces | Inhibition (%) |
|-------|---|-------------------|----------------|
| I     | 2% (v/v) aqueous Tween 80 (1mL/kg) + Castor oil (1mL) | 3.90 $\pm$ 0.68   | -              |
| II    | Extract (100mg/kg) + Castor oil(1mL)                  | 1.74 $\pm$ 0.28*  | 55.84          |
| III   | Extract (200mg/kg) + Castor oil(1mL)                  | 1.59 $\pm$ 0.39*  | 59.24          |
| IV    | Extract (400mg/kg) + Castor oil(1mL)                  | 0.98 $\pm$ 0.35** | 74.85          |
| V     | Loperamide (3mg/kg) + Castor oil(1mL)                 | 0.03 $\pm$ 0.34** | 99.96          |

[Data are expressed as mean  $\pm$  SEM, n = 5. \*p < 0.05, \*\*p < 0.01 when compared with vehicle-control]

**TABLE 2 : EFFECT OF THE METHNOL ACORUS CALAMUS EXTRACT AT DIFFERENT DOSE LEVELS ON CHARCOAL INDUCED GUT TRANSIT CHANGES**

| Group | Treatment   | Distance Travel by charcoal meal | Inhibition (%) |
|-------|---|----------------------------------|----------------|
| I     | Charcoal Meal + 2% (v/v) aqueous Tween 80 (1mL/kg)    | 84.29 $\pm$ 1.92                 | -              |
| II    | Charcoal Meal + Extract (100mg/kg) + Castor oil (1mL) | 76.28 $\pm$ 1.78*                | 9.51           |
| III   | Charcoal Meal + Extract (200mg/kg) + Castor oil (1mL) | 71.21 $\pm$ 1.32*                | 15.52          |
| IV    | Charcoal Meal + Extract (400mg/kg) + Castor oil (1mL) | 52.14 $\pm$ 1.66**               | 38.15          |
| V     | Charcoal Meal + Atropine Sulphate (0.1mg/kg)          | 35.25 $\pm$ 2.67**               | 57.59          |

[Data are expressed as mean  $\pm$  SEM, n = 5. \*p < 0.05, \*\*p < 0.01 when compared with vehicle-control]

## DISCUSSION :

### Antidiarrhoeal Activity:

**Inhibition of castor oil induced Diarrhoea**: Experimental result reflects that the activity is more pronounced at the dose of 400 mg/kg body weight

(**Table 1**). The percentage of inhibition of number of wet faeces was 74.25%,  $p < 0.01$  at the dose of 400 mg/kg body weight while that of standard drug loperamide (3 mg/kg) was 99.96 % control of castor oil-induced diarrhoea.

**Effects on Gastrointestinal Motility:** The *Acorus calamus* extracts produced profound decrease in intestinal transit of 9.51-38.15 % at the dose range of 100, 400 mg/kg body weight (Table 2) and while atropine sulphate produced 57.59% inhibition of intestinal transit at dose of 0.1 mg/kg body weight. In this study, Preliminary phytochemical investigations indicated presence of glycosides and saponins present in Methanolic extract of *Acorus calamus*. Saponins are known for their antidiarrhoeal activity. Infact, saponins are responsible for the denaturation of proteins, which makes the intestinal mucosa more resistant and reduces secretion.

Thus, the antidiarrhoeal activity may be due to saponins present *Acorus calamus*. The present study sought to assess the antidiarrhoeal activity of the plant. Our results showed that the extract inhibited significantly ( $p < 0.01$ ) castor oil-induced diarrhoea in rats. Several mechanisms had been previously proposed to explain the diarrheal effect of castor oil. These include inhibition of intestinal Na<sup>+</sup> K<sup>+</sup> ATPase activity, thus reducing normal fluid absorption, activation of adenylate cyclase or mucosal cAMP-mediated active secretion, stimulation of prostaglandin formation and platelet activating factor. Most recently nitric oxide has been claimed to contribute to the effect of castor oil.

However, it is well documented that castor oil produces diarrhoea due to its most active component ricinoleic acid through a hypersecretory response. Therefore it can be assumed that the antidiarrhoeal action of the extract was mediated by an antisecretory mechanism. This was also evident from the inhibition of castor oil-induced fluid accumulation by the extract. The results were comparable to those of the standard drug, loperamide.

Futhermore, the extract significantly reduced intestinal transit as evidenced by the decrease in the distance travelled by charcoal meal. These results also show that extract suppressed the propulsion of charcoal meal thereby increasing absorption of water & electrolytes.

**CONCLUSION:** This results of this investigation revealed that *Acorus calamus* contains pharmacological active substances with antidiarrhoeal activity. These attributes may provide the rationale for the use of *Acorus calamus* in diarrhoea management. Further research is needed to evaluate and purify the extract in order to find out molecule responsible for the Antidiarrhoeal Activity observed.

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