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IN-VIVO ANTIDIARRHOEAL ACTIVITY OF EXTRACTS FROM STEM BARK OF *Saraca Asoca Roxb*. PREPARED BY DIFFERENT EXTRACTION METHODS

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ABSTRACT

Dried stem bark extracts of Saraca asoca Roxb. Prepared by different extraction methods (Soxhlet, Ultrasonication assisted & Microwave assisted extraction) with ethanol 90%, ethanol 60% and acetone. The acetone extract prepared by Ultrasonication assisted extraction, showed significant antidiarrhoeal property in castor-oil induced diarrhoea in albino rats at a dose of 200 mg/kg, b.w. as compared to standard drug loperamide at a dose of 3 mg/kg, b.w. The preliminary phytochemical screening of extracts of stem bark of Saraca asoca results with the presence of alkaloids, tannins, flavonoids and phenolic compounds etc. The result obtained establishes the efficacy and substantiate the folklore claim as an antidiarrhoeal agent. Further studies are needed to completely understand the mechanism of antidiarrhoeal action of Saraca asoca Roxb.

KEYWORDS

Antidiarrhoeal activity, Saraca asoca, Castor-oil, Ultrasonication assisted extraction, Microwave assisted extraction.

INTRODUCTION

Diarrhoea has long been recognized as one of the most important health problems in the developing countries (Synder and Merson, 1982). According to WHO estimation for the year 1998, there were about 7.1 million deaths due to diarrhoea (Park, 2000). Secretary diarrhoea is most dangerous symptoms of gastrointestinal problems and it is characterized by increased frequency of bowel movement, wet stool and abdominal pain (Maiti *et. al.,* 2007). In developing countries, diarrhoea is almost universally infectious in origin. The two bacterial groups causing traveler's diarrhoea are diarrhoegenic *E. coli*, mainly enterotoxigenic and enteroaggregative (Adachi *et. al.,* 2001) and invasive bacterial pathogens like *Shigella, Campylobactor* & *Salmonella* (Hoge *et. al.,* 1998). Amongst the viral agents, *rotavirus* is the most common (Daswani *et. al.,* 2010). Appropriate clinical management of diarrhoea includes oral rehydrate therapy and chemotherapy (Irfan *et. al.,* 2001). A vast majority of people of developing countries relies on herbal drugs for the management of diarrhoea. Considering this fact the WHO has constitute a diarrhoeal disease control programme, which includes studies of traditional medicine practice together with the elevation of health education and prevention approaches (Das *et. al.,* 1999).

Saraca asoca Roxb. De Wilde, Syn. *Saraca indica* acut non L. (Ashoka) is an evergreen tree belonging to the Caesalpiniaceae subfamily of the legume family (Sivrajan, 1994). It occurs almost throughout India up to an altitude of 750 m, in the central and eastern Himalayas and in the Khasi, Gao and Lushai hills (Satyavati, 1970). The stem bark of *S. asoca* contains glycosides, n-octacosanol, tannin, catechin, (+)-catechol, (-)-

epicatechin, (-)-epicatechol, leucocyanidin, leucopelargonodin, procyanidin (Khare, 2007). It also contains flavonoids and sterols (Vijai, 1976; Behari, 1977). Ashoka is wonderful herb that claims to cure several diseases. According to Ayurvedic medicine, it is the herb that stands out as especially useful for treating excessive uterine bleeding. It is extensively used in the Ayurvedic system of medicine for a variety of ailments (Kirtikar, 1918; Adhya, 1940) as a blood purifier, in stomach ache (Satyavathi, 1969), and as a hypothermic and diuretic (Dhawan, 1977). Ayurvedic texts describe more than 50 preparations of Ashoka for the treatment of variety of ailments in which the stem bark is mainly used (http: /www. divineremedicines.com). The bark of *S. asoca* is used in Indian traditional medicines as an antidiarrhoeal drug. It is used as an astringent to treat hemorrhagic dysentery, diarrhoea, diabetes, syphilis and as a uterine tonic as it has a stimulating effect on the endometrial and ovarian tissue (Yoganarasimhan, 2000). The bark is also used in treating gynecological problems, especially decoction of bark in mixture with milk and water used to prevent leucorrhoea and menorrhea as stated in Indian folklore medicine. It has cosmetic application also, such as removing black spots on face (Kurian, 1995).

MATERIALS AND METHODS

Collection and Authentification of drug

The stem bark of *Saraca asoca* Roxb. Was collected from rural areas of Udaipur (Rajasthan), in the month of Sep-Oct 2008. Sample was identified by Dr. S.S. Katewa, College of Science, MLSU, Udaipur (Raj.) and authentified from Botanical Survey of India, Dehradun (UK) and the no. is BSI/NRC/Tech (Indent)/2011/166 (113548).

Preparation of extracts

Dry coarse powder (40 mesh sieve) of stem bark of *Saraca asoca* was subjected to extraction with ethanol 60% in Soxhlet extraction method and acetone in Ultrasonication assisted and Microwave assisted extraction method. The different extracts were evaporated under reduced pressure using rotary evaporator at 38°C.

Preliminary Phytochemical Screening

The Preliminary Phytochemical Screening of different extracts of *Saraca asoca* was carried out in order to ascertain the presence of its constituents (Mukherjee, 2000).

Procurement and Selection of animals

The experimental animal, albino rats (*Wistar* Strain) of either sex (150-200g) were used in the study. Animals were housed in polypropylene cages in controlled temperature (25±2°C), relative humidity (60±5%) and light. The study was carried out in accordance with the guidelines given by CPCSEA, New Delhi and the Registration no. is 870/ac/08/CPCSEA.

Experimental Protocol

Group I: Kept as normal untreated control.

Group II: Kept as treated control i.e. treated with castor oil (1ml/animal, p.o.).

Group III: Treated with castor oil (1ml/animal) + standard drug loperamide (3 mg/kg, p.o.).

Group IV: Treated with castor oil (1ml/animal, p.o.) + 200 mg/kg hydroalcoholic extract of *Saraca asoca* Roxb. (stem bark) prepared by soxhlet extraction method.

Group V: Treated with castor oil (1ml/animal, p.o.) + 200 mg/kg acetone extract of *Saraca asoca* Roxb. (Stem bark) prepared by ultrasonication extraction method.

Group VI: Treated with castor oil (1ml/animal, p.o.) + 200 mg/kg acetone extract of *Saraca asoca* Roxb. (stem bark) prepared by microwave extraction method.

Table:Effect of *Saraca asoca* Roxb. De Wilde extracts prepared by different extraction methods in castor oil induced diarrhoea

Group	Treatment	Total no. of diarrhoeal faeces (within 4 hrs)	Consistency of faeces	Total wt.(gm) of faeces (after 4 hrs)	Percentage protection
I(Normal untreated control)	Vehicle only	7.00±0.36	Solid	2.01±0.16	-
II(Treated control)	Castor oil (1ml/kg)	19.67±0.61	Semisolid with excess of water	8.05±0.27***	-
III (Standard)	Castor oil (1ml/kg)+Loperamide (3mg/kg)	7.33±0.33	Solid	2.22±0.13***	72.42%
IV (Test I)	Castor oil (1ml/kg)+Extract I(200mg/kg)	14.00±0.51	Semisolid with lumps	4.62±0.20***	42.60%
V (Test II)	Castor oil (1ml/kg)+Extract II(200mg/kg)	10.33±0.42	Semisolid with water	3.48±0.17***	56.77%
VI (Test III)	Castor oil (1ml/kg)+Extract III(200mg/kg)	12.00±0.36	Semisolid	4.14±0.19***	48.57%

Values are mean ± SEM, (n=6); $p<0.05$ considered as significant, *** $p<0.001$, One way ANOVA followed by Dunnett's test as compared to control.

Castor Oil Induced Diarrhoeal Method: Rats were fasted 24 hr before the test with free access to water. Rats were treated orally with vehicle and extracts of *Saraca asoca* Roxb. (stem bark) and standard (Loperamide). One hour after drug treatment, each rat received castor oil (1 ml each orally). Each rat was then housed separately in cage over clean butter paper. Then diarrhoea episodes were observed for a period of 4 hours. During this period, consistency of faeces, total number of diarrhoeal faeces within 4 hr and total weight of faeces after 4 hr were recorded (Venkat Rao, 2006; Bheemachari, 2007; Viswanatha, 2007; Doherty, 1981).

Stastical analysis: Data were analyzed statistically by one-way ANOVA followed by Dunnet's *t*-test using computerized Graph Pad Prism version 5.4 (Graph Pad Software, U.S.A.). The data are expressed as mean ± S.E.M. P-values less than 0.05 imply significance.

RESULTS

Preliminary Phytochemical Screening

Phytochemical investigation of extracts showed the presence of glycoside, steroid, tannins and phenolic compounds, carbohydrates and reducing sugar. The results obtained were comparable and satisfied the standard literature.

Castor Oil Induced Diarrhoea

After 30 min administration of castor oil, the diarrhoea was clinically apparent in all the animals of control group, for the next 4 hour. This was markedly reduced by Loperamide (3 mg/kg, p.o.) at 72.42%. A similar marked reduction in the number of defecations over

four hours was achieved with *Saraca asoca* (stem bark) extracts at the dose of 200 mg/kg, p.o. The percentage inhibition for the number of wet faeces within 4 hours as well as total weight (gm) of wet faeces after 4 hrs of treatment indicates the presence of antidiarrhoeal activity in extracts as compared with that of control group. The average total number of wet faeces and average total weight of wet faeces in the control group were 19.67±0.61 and 8.05±0.27 gm respectively. The defecation after 4 hours of treatment significantly ($p<0.001$) inhibited by hydro-alcoholic extract (soxhlet method) is 42.60%, acetone extract (UAE method) is 56.77% and acetone extract (MAE method) is 48.57%.

DISCUSSION

Oral administration of castor oil (1ml/animal) to the control group causes significant diarrhoea (8.05 ± 0.27). 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. The liberation of ricinoleic acid results in irritation and inflammation of intestinal mucosa leading to release of prostaglandin (Galvez *et al.,* 1993; Pierce *et al.,* 1971).

Antidiarrhoeal properties of medicinal plants might be ascribed to tannins, alkaloids, saponins, flavonoids, sterols and reducing sugars (Longaga *et al.,* 2000). The antidiarrhoeal activities of flavonoids have been ascribed to their ability to inhibit intestinal motility and hydro electrolytic conditions (Venkatesan *et al.,* 2005). Flavonoids present in the plant extracts are reported to inhibit release of autocoids and prostaglandins, thereby may inhibit motility and secretion induced by castor oil (Veiga *et al.,* 2001). Tannins and tannic acid present in antidiarrhoeal plants denature proteins in the intestinal mucosa by forming protein tannates which make the intestinal mucosa more resistant to chemical alteration and reduce secretion (Havgiray *et al.,* 2004). In phytochemical screening of extracts of *Saraca asoca* showed the presence of alkaloids, tannins, flavonoids, phenolic compounds and sterols. The presence of these constituents may mediate the antidiarrhoeal property of the extract.

CONCLUSION

In conclusion, the results of this investigation revealed that stem bark of *Saraca asoca* contains pharmacologically active substances with anti-diarrhoeal properties. These properties confirm the use of *Saraca asoca* as an antidiarrhoeal drug as proposed by traditional healers. Further research is to be carried out to fractionate and purify the extract in order to find out the molecules responsible for the antidiarrhoeal activity observed.

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