STUDIES ON ANTI-DIARRHEAL ACTIVITY OF *DALBERJIA SISSOO* ROXB. IN EXPERIMENTAL ANIMALS.

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Summary

The successive solvent ether, ethanol, and aqueous extracts of *Dalberjia sissoo* bark were studied for anti-diarrhoeal properties in experimental diarrhea, induced by caster oil in rats. As the dosage 200 – 400 mg/kg per oral, the solvent ether extract showed significant and dose dependent anti-diarrhoeal activity. The extracts also significantly reduced the intestinal transit time in charcoal meal when compared with atropine sulphate (1 mg/kg ip) The solvent ether extract was found to be equipotent of atropine. The result showed that the extracts of *Delberjia sissoo* bark have significant anti-diarrhoeal activity and supports its traditional uses in herbal medicine.

***Key words*: *Dalberjia sissoo*,** anti-diarrhoeal property, intestinal transit, castor oil.

Introduction

Worldwide diarrhoeal diseases constitute a major cause of morbidity and mortality, especially in developing countries. More than 5 million children under the age of 5 years die every year of diarrhoea[1]. A vast majority of the people in these developing countries rely on herbal drugs for the management of diarrhoea. WHO has encouraged studies for treatment and prevention of diarrhoeal diseases using traditional medical practices[2]. Thus it is become important to identify and evaluate commonly available natural drugs as alternative to currently used anti-diarrhoeal drug, which are not completely free from adverse effect. In India several drugs are mentioned in traditional system of medicine and widely used by traditional healers, however the most of the drugs has not evaluated on scientific basis.

Dalberjia sissoo is among the most important Indian timber tree. Various part of *Dalberjia sissoo* plant were being used for various therapeutic purposes. The plant is very less explored for its phytoconstituents and pharmacological activity. The plant shows presence of flavonoids, it posses good antioxidant activity. The plant also shows its efficiency as anti-inflammatory agent. The bark has astringent property[3]. Traditionally plant bark is used for trading the diarrhoea; however, detail investigations of this aspect had not been carried out so far hence this led us to study the anti-diarrhoeal activity of *Delberhia sissoo* barks.

Methods

Plant material: Bark of *Dalberjia sissoo* were collected from Shirur, in the month of June 2007. A voucher specimen has been deposited at C. T. Bora College, Shirur, Pune and authenticated. The plant bark were cleaned, washed, dried under shade, dried material were coarsely powdered. The 500gm of powdered material was extracted by successive extraction process using soxhlet apparatus with different solvent in increasing order of polarity such as solvent ether, ethanol and water. The extracts were concentrated under vacuum and residues were used for experiment purpose. The percent extract found 1.62%w/w, 5.98%w/w and 6.93%w/w respectively.

Phytochemical screening: The freshly prepared extracts were subjected for preliminary phytochemical study for identification of different phytoconstituents. The aqueous extract shown the presence of carbohydrates, Tannins and saponines. The ethanol extract shows presence of flavonoids, tannins, terpenoids and alkaloids, while solvent ether extract contains fixed oil alkaloids, terpenoids and saponines[4].

Animals: Albino Swiss rat of either sex weighing 150-180 g were used for castor oil induced diarrhoea and intestinal transit activity. All animals were fed standard fed and tap water *ad libitium* before the experiment. Each animal group consist of six animals housed in separate cadge.

Acute Toxicity: Acute toxicity was performed according to the OECD-423 guide lines. Swiss mice (20 – 25 g) of either sex were used. The animals were administered with distilled water (10 ml/kg), the vehicle or different extract (1 - 5 g/kg) of *Dalberjia sissoo* bark orally (p.o). The animals were observed for 6 h within the first 24 h, then for further 14 days for deaths and manifestation of toxic effects. The toxic effects observed included agility, muscular tonus, tremors, and convulsions, breathing patterns, water and food intake. When no mortality was observed, the procedure was repeated up to the highest dose of 5 g/kg.

Caster oil induced diarrhea: The 18 hr fasted albino rats of either sex 150-200gm are divided into 8 groups six each. One ml of caster oil was given to the group 1 orally and serves as control. While group 2 received loperamide (1mg/kg) as standard. solvent ether extract at dose of 200 and 400mg/kg was administered to group 3 and 4 respectively. Alcoholic extract at 200-400mg/kg was administered to group 5 and 6 respectively and aqueous extract at as dose 200-400mg/kg was administered to group 7 and 8 respectively. One hour after treatment, each rat received 1ml of castor oil orally and then observed for defecation up to 4hr. after treatment castor oil challenge. The presences of characteristic wet and dry diarrhoeal dropping were noted on non-wetting paper sheet of uniform weight for period of 4 hr[5]. (Table 1.)

Gastrointestinal motility test: Animals fasted for 18 h prior to the experiment with tap water *ad libitum* were randomly divided into five groups of six animals each. Each rat was administered orally with 1ml of charcoal meal (3% deactivated charcoal in 2 % aq. gum acacia). Group 1 received vehicle (0.2ml, 2% w/v aq. gum acacia) and serves as control. Group 2 treated with Atropine (1mg/kg IP) as standard drug. The group 3, 4 and 5 received 400mg/kg of solvent ether, ethanol and aqueous extract respectively. Thirty min. later, each rat was scarified and intestinal distance moved by the charcoal meal from pylorus sphincter was measured and expressed as mean distance from pylorus to caecum[6]. (Table 2).

Results

The castor oil induced diarrhoea:

In castor oil induced diarrhoea experiment, the rat which doesn't receive plant extracts and standard drug produced diarrhoea after 30 min. and continue up to 4 hr. with sign of watery and frequent defecation. All the extract of *Dalberjia sissoo shows* marked reduction in number of defecation over four hours in dose dependent manner ($p<0.01$), the ethanol and aqueous extract at dose200 mg/kg dose not significantly affect of onset of diarrhea while solvent ether extract shows marked anti-diarrhoeal effect in rats. Both doses of *Dalberjia sissoo* solvent ether extract significantly decreases ($p<0.01$) the total number of wet faces produced by administration of castor oil (0.22 gm at dose of 200mg/kg and0.7gm at dose of 400mg/kg)

The effect of *Dalberjia sissoo* solvent ether extract was more potent than of standard drug loperamide (3mg/kg) which produced an inhibition of 84.61% (table 1) the average weight of faces in control was 4.55gm and treatment with both doses of solvent ether extract significantly reduces ($p<0.01$) the weight of faces to0.07gm.

Table No.1 Comparison of Antidiarrhoeal Activity of *Dalbergia sissoo* Bark extracts in castor Oil Induced Diarrhoea.

Treatment	Dose (mg/kg)	Mean weight of stool.± S.E after 4 h (gm)	Percentage of inhibition (%)
Control(Castor oil)	-	4.59±0.178*	--
Loperamide	1	1.52±0.143*	65.71
Solvent ether extract	200	2.31±0.192*	51.21
	400	0.70±0.145*	84.61
Ethanolic extract	200	3.03±0.131[a]	30.11
	400	1.30±0.134*	72.09
Aqueous extract	200	3.23±0.165[a]	30.77
	400	1.21±0.133*	75.60

- mean ±S.E.M (n=8) $p<0.01$compared with control.
- [a]$P < 0.05$,significantly different from the control, one-way ANOVA.

b. Small intestine transit time:

The castor oil treated rats shows marked decrease in gastric transit time, while atropine (1mg/kg) markedly increases gastric time of charcoal feed. All the extract retards the gastric / intestinal transit of charcoal meal as compare to control. Among all the extract solvent ether extract at dose 400mg/kg significantly delayed intestinal transit time of charcoal meal in test animals. (table 2)

Table No. 2. Comparison of *Dalbergia sissoo* Bark Extracts On Gastro-intestinal Motility.

Treatment	Dose (mg/kg)	Mean Distance Traveled By Charcoal ±SEM
Control (vehicle)	-	84.3±3.67
Atropine sulphate	1	21.0±1.84
solvent ether extract	400	12.6±1.06
Ethanolic extract	400	62.4±2.64
Aqueous extract	400	41.1±2.15

Values are mean ± SEM of six experiments.

Discussion

The diarrhea is mostly occurred as hypersecretion of certain components or by hyper motility. The use of caster oil induced diarrhea model is logical, as caster oil hydrolyze to recinolic acid in duodenum, results in irritation and inflammation of intestinal mucosa, leads to release of autocoids and prostaglandins which stimulate motility and secretions[7-10]. In present study *Dalberjia sissoo* all the extracts significantly inhibited caster oil induced diarrhea in dose dependent manner, it can be assumed that the antidiarrhoeal action was exerted by anti-secretary mechanism, this can be evident by reduction in total number of wet feces produced by test extract treated animals. The solvent ether and aqueous extract may increased the absorption of water and electrolyte form gastrointestinal tract, because these increased the gastrointestinal transit time as compared to control and atropine, they might have anti-motility property. The non-polar and polar phytoconstituent are responsible for anti-secretary and anti-motility property. The preliminary phytochemical investigation shows the presence of alkaloids, steroids and sterols, saponins and fixed oils in solvent ether extract, while tannins, flavonoids, saponins and carbohydrates and proteins. earlier studies showed that anti-dysenteric and antidiarrhoeal properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids, sterol and/or triterpenes and reducing sugars[11,12]. Hence sterols triterpenoids and alkaloids may be responsible for mechanism of action of solvent ether extracts anti-diarrhoeal activity, while presence of tannins and reducing sugars may be responsible for the mechanism of action of aqueous extracts anti-diarrhoeal activity. The presence of tannins produces protein tannates makes the intestinal mucosa more resistant and hence reduces secretion[13].

Acute toxicity

The aqueous extract at the doses of 1 - 8 g/kg showed no signs of toxicity in the mice within and after 24 h following oral administration. No deaths were recorded even at the highest dose of 8 g/kg body weight.

Conclusion

In conclusion, the results of this investigation revealed that solvent ether and aqueous extract of *Dalberjia sissoo* contains pharmacologically active substance(s) with anti-diarrhoeal properties. These properties confirm the use of *Dalberjia sissoo* as an anti-diarrhoeal 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 anti-diarrhoeal activity observed.

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