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Anthelmintic activity of different extracts of D*albergia sissoo* Roxb. on Indian Adult Earthworms

Hood M. M.*, Tembhurne S. V. and Sakarkar D. M

Department of Pharmacology and Physiology, Sudhakarrao Naik Institute of Pharmacy, Pusad (M.S), India
Sudhakarrao Naik Institute of Pharmacy Pusad, Dist: Yavatmal (M.S.), India

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ABSTRACT

Dalbergia sissoo Roxb. popularly known as Sisam in India belongs to the family Fabaceae. It is an important plant of great economic importance. The objective of the present work was to compare different extract for the in-vitro anthelmintic potency of Dalbergia sissoo Roxb. leaves using Indian earthworms (Pheretima posthumad). The various extracts of Dalbergia sissoo roxb. includes petroleum ether, carbon tetrachloride, benzene and ethanol were used in different concentrations viz. 10, 25, 50 and 100 mg/ml of respectively for in-vitro testing of anthelmintic potency by determination of time of paralysis and time of death of worm. Piperazine citrate was used as standard. The result of present study of indicates Dalbergia sissoo potentiate to paralyze earthworm and also caused its death after some time. The shortest time of paralysis was observed at 100 mg/ml of carbon tetrachloride extract of Dalbergia sissoo which was 19min 14 sec and 48 min 15sec respectively. The results of the study were comparable to standard Piperazine citrate. The results of the present study demonstrate that carbon tetrachloride extract of Dalbergia sissoo leaves took less time to cause paralysis of the earthworm than other three extracts. Thus the present study demonstrates the traditional claim of Dalbergia sissoo Roxb. as an anthelmintic has been confirm as the ethanolic, benzene, petroleum ether and carbon tetra chloride extracts of leaves displayed activity against the earthworm used in study. While carbon tetrachloride extract demonstrated to highly potent anthelmintic activity compared to other three extract of Dalbergia sissoo Roxb.

Key word: Sisam, paralysis of earthworms and death of earthworm.

INTRODUCTION

Parasitic helminthes affects animals and men, causing considerable hardship and stunned growth. Most diseases caused by helminthes are of a chronic; debilitating nature they probably cause more morbidity and greater economic and social deprivation among humans and animals than

any single group of parasites. Immature forms of the parasites invade human beings via the skin or gastrointestinal tract (GIT) and evolve into well differentiated adult worms that have characteristic tissue distribution. Anthelminthics are drugs that may act locally to expel worms from the GIT or systemically to eradicate adult helminthes or development forms that invade organs and tissues (Mcload 1995; Monterio 1997; Goodman et al., 2001).

Parasitic helminthes affects animals and men, causing considerable hardship and stunned growth. Most diseases caused by helminthes are of a chronic; debilitating nature they probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites. The major control strategy adopted against helminthes parasite is the use of anthelminths. However, the high cost of modern anthelmintics has limited the effective control of these parasites. Herbal drugs have been in use since ancient times for the treatment of parasitic diseases in human and could be of value in preventing the development of resistance (Coles et al., 1992; Hammond et al., 1997).

Shisham (*Dalbergia sissoo* Roxb), a deciduous tree of family Fabaceae, is an important plant of great economic importance. Shisham wood is used in furniture, construction work, agricultural implements, plywood industries and fuel purposes. According to Greeks, Shisham plant has some medicinal properties. It provides financial support to the farmers as it is considered as cash plant. *Dalbergia sissoo* Roxb. (Fabaceae), known as Indian Rosewood, is reported to be useful in many conditions including fever, ulcers, digestive disorders, and skin diseases (Kirtikar et al., 1933; Sharma et al., 2001). Traditionally, it is used as a anthelmintic, bitter and stimulant. It is also used in hemorrhages, leprosy, boils, eruptions and to allay vomiting. It is also known to be effective against helminthes, diarrhoea and dysentery (Kapoor 2000; Kirtikar et al., 1933; Sharma et al., 2001). Recently an antidiarrhoeal activity leaf of Dalbergia sissoo is scientifically proved (Brijesh et al., 2006). To the best of our knowledge, no experimental evidence is available to prove its anthelminthics activity. Thus the present study was performed to prove scientifically the traditional claim of *Dalbergia sissoo* Roxb as an anthelmintic as well as to compare the potency between different extracts of it.

MATERIALS AND METHODS

Plant: The fresh leaves of *Dalbergia sissoo* Roxb. were collected in the month of June 2010 from its natural habitat at Seminary hills area in Nagpur region, Maharashtra, India. The plant was authenticated by Dr. Alka Chaturvedi of Botany Department; RTM Nagpur University, Nagpur India. A voucher specimen (No: 9530) was deposited at Herbarium, Department of Botany, RTM Nagpur University Nagpur.

Experimental worms:
All the experiments were carried out in Indian adult earthworms (*Pheretima posthumad*) due to its anatomical resemblance with the intestinal roundworm parasites of human beings.They were collected from moist soil and washed with normal saline to remove all fecal matter were used for anthelmintic activity.

Material: Ethanolic, petroleum ether, benzene and carbon tetrachloride extracts of *Dalbergia sissoo* Roxb. leaves, Piperazine citrate.

Preparation of Extracts: The collected leaves of *Dalbergia sisso* were dried under shade and undergone crushing in electric blender to form powdered and subjected to extraction by using maceration in a air tight closed container by using ethanol, petroleum ether, benzene and carbon tetrachloride as a solvent. The extracts were concentrated by evaporation at room temperature and used for testing anthelmintic activity. The percent yield for petroleum ether extract was 0.59%, for carbon tetrachloride it was 25, 1.8% for benzene and 3.6% for ethanol.

Administration of Extracts:
The suspension of all extracts of *Dalbergia sissoo* Roxb of different concentration (10, 25, 50 and 100mg/ml) were prepared by using 0.5% w/v of CMC as a suspending agent and final volume was made to 10 ml for respective concentration. Piperazine citrate was used as standard. Groups of approximately equal size worms consisting of three earthworms individually in each group were released into in each 10 ml of desired concentration of drug and extracts.

Administration of Piperazine citrate:
Piperazine citrate (10 mg/ml) was prepared by using 0.5% w/v of CMC as a suspending agent as administered as per method of extract.

Experimental Design:
Indian adult earthworms (*Pheretima posthumad*) collected from moist soil and washed with normal saline to remove all fecal matter were used for anthelmintic activity. Different concentration (10-100 mg/ml) of various extracts of *Dalbergia sissoo* Roxb were prepared by using 0.5% w/v of CMC as a suspending agent and final volume was made to 10 ml for respective concentration of *Dalbergia sissoo* Roxb. A Piperazine citrate (10 mg/ml) was used as standard. Different groups of approximately equal size worms consisting of three earthworms individually in each group were released into 10 ml of desired concentration of drugs.

The anthelmintic assay was carried out as per the method of (Ajaiyeoba et. al, 2001; Dash 2002) with minor modification. The adult earthworms were divided into seventeen groups containing three earthworms each. Different concentration of extracts and standard piperazine solution were poured in different petri dishes containing worms respectively. Observations were made for the time taken for paralysis (Paralysis was said to occur when worm did not revive in normal saline) and death (Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when shaken vigorously nor when dipped in warm water (50^0c), followed with their body colors fading away). All the results were expressed as Mean ±S.E.M. of three worms in each group.

RESULTS AND DISCUSSION

Helminthiasis or infections with parasitic worms are pathogenic for human beings. Immature forms of the parasites invade human beings via the skin or gastrointestinal tract (GIT) and evolve into well differentiated adult worms that have characteristic tissue distribution. Anthelmintics are drugs that may act locally to expel worms from the GIT or systemically to eradicate adult helminthes or development forms that invade organs and tissues (Jain et al., 1972; Okon et al., 1980).

Table no.1: Anthelmintic potency of different extracts of *Dalbergia sissoo leaves*

Treatment	Concentration	Average Time of Paralysis (min) (Mean±S.E.M)	Average Time of death (min) (Mean=±S.E.M)
Piperazine citrate	10 mg/ml	5. 23±0.72	20.45±2.33
Petroleum ether extract	10 mg/ml	55. 23± 4.33	93. 44±7.54
	25 mg/ml	45.32±6.23	86. 55±4.55
	50 mg/ml	30.49±4.55	79.34±6.34
	100 mg/ml	25.54±4.21	70.23±6.32
CCl_4 extract	10 mg/ml	30.57±4.78	94. 23±8.46
	25 mg/ml	27.12±3.26	80.27±7.21
	50 mg/ml	23.53±3.89	50.36±4.35
	100 mg/ml	19.14± 2.78	48.15±3.23
Benzene extract	10 mg/ml	50. 23±7.87	78.34±6.65
	25 mg/ml	30.12±5.43	62.45±6.32
	50 mg/ml	35. 08± 5.33	55.35±4.67
	100 mg/ml	40.17±6.21	59.45±4.32
Ethanolic extract	10 mg/ml	40.43± 4.37	88.45±5.32
	25 mg/ml	38.19± 4.76	72.36±6.12
	50 mg/ml	30. 43±3.43	60.12±7.32
	100 mg/ml	25.56±3.54	54. 48±5.43

All the values are given in mean ± s.e.m; N=3 earth worms individually in each group were released into 10 ml of desired concentration of respective drugs in Petri plate.

The *Dalbergia sissoo* leaves extracts were found to show anthelmintic activity when compared to standard drug Piperazine citrate. Ethanolic extract of *Dalbergia sissoo* leaves (10, 25, 50 and 100 mg/ml) showed paralysis of earthworm at 40.43, 38.19, 30.43 and 25.56 min respectively, whereas death time found up to 54.48 min at highest permissible dose (100mg/ml). The carbon tetrachloride extract (10, 25, 50 and 100 mg/ml) was found to showed paralysis at 30.57, 27.12, 23.53 and 19.14 min. respectively whereas death time found upto 48.15 minute at higher permissible dose. The petroleum ether extract (10, 25, 50 and 100 mg/ml) was found to showed paralysis at 55.23, 45.32, 30.49, and 25.54 min. respectively whereas death time found upto 70.23 minute at higher permissible dose. The benzene extract (10, 25, 50 and 100 mg/ml) was found to showed paralysis at 50.23, 30.12, 35.08 and 40.17 min. respectively whereas death time found upto 59.45 minute at higher permissible dose. From the above result, it is clear that all extracts of *Dalbergia sissoo* demonstrate to possess anthelmintic activity in dose dependent manner when compared with standard piperazine citrate. Data in the table 1 reveals the carbon tetra chloride extracts of *Dalbergia sissoo* leaves took less time to cause paralysis of the earthworm than that other extracts. Thus from results the traditional claim of *Dalbergia sissoo* as an anthelmintic have been confirm as a leaves extract displayed activity against the worm used in present study. The possible mechanism of the anthelmintics activity of *Dalbergia sissoo* cannot be explained on the basis of our present results. However, it may be due to its effect on inhibition of glucose uptake in the parasites and depletion of its glycogen synthesis. While there need further study to isolate and revealed the active compound contained in the crude extract of *Dalbergia sissoo* as well as to establish mechanism(s) of action.

CONCLUSION

From the results it conclude that, both extracts of *Dalbergia sissoo* (ethanolic, petroleum ether, benzene and carbon tetrachloride) demonstrate to possess dose dependant anthelmintic activity when compared to piperazine citrate. The results also revealed that the carbon tetra chloride extracts of *Dalbergia sissoo* leaves took the less time to cause paralysis of the earthworm than that of other extracts of *Dalbergia sissoo* leaves thus it conclude that carbon tetrachloride extracts of *Dalbergia sissoo* possess potent anthelmintic activity compared to other extracts. From results the traditional claim of *Dalbergia sissoo* as an anthelmintic have been confirm as a it displayed activity against the worm used in present study.

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