ANTIFILARIAL POTENTIAL OF STEM WOOD OF *DIOSPYROS MONTANA*

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Summary

The effect of petroleum, and alcoholic extract of stem wood of *Diospyros Montana (D.montana)* was observed on the spontaneous movements of the whole worm (w.w) preparation and nerve muscle (n.m) complex of cattle filarial parasite *Setaria cervi (S.cervi)* and there effect on survival of microfilariae was also studied. Petroleum extract of stem wood could inhibit the spontaneous activity in both the w.w and the n.m preparation whereas alcoholic extract of stem wood had effect only on the n.m preparation.The lethal concentration 50 (LC50) and lethal concentration 90 (LC90) for petrol extract were 25ng/ml and 35ng/ml respectively.

Key words : Antifilarial activity, Diospyros, S.cervi

Introduction

Diospyros is the largest genera of family *Ebenaceae* having about three hundred fifty species consisting both trees and shrubs and are widely distributed in the both hemispheres[1,2]. 41species of *Diospyros* genus commonly grow in India in Assam, Daccan, Bangal and few in North India [3,4].A large number of *Diospyros* species are used as folk medicine in India. The bark of *Diospyros montana* is used to treat jaundice , fruits for boils as well as cracks in sole of feet and root as abortifacient [5] .*D. montana* is also used as anticancer[6]. Diospyrin isolated from the stem bark has been shown to possess anti *leishmanial* activity in vitro [7]. Phytochemical constituents isolated from fruit pulp are ursolic acid, β-sitosterol and α-amyrin [8], lupeol and iododiospyrin form wood [9].

Seteria cervi (Nemetoda; Filariodiea) is naturally occurring filarial parasite of water buffalow (*Bubalis bubalis linn*) resembles closely to human filarial worm in its responses to drugs and can be used for the evaluation of antifilarial potential [10,11]. *Setaria cervi* as well as its nerve-muscle complex [12] shows vigorous rhythmical movements which can be recorded on a kymograph by suspending the worm in an isolated bath at 37^0C .

Methods

Plant material

The plant *Diospyros montana* was procured from village Rampur, Bulandshahr, (UP) and the plant was identified by taxonomist Prof. Wazahat Hussain, Department of Botany, AMU., Aligarh (India) its voucher specimen was deposited in the same department.

Preparation of extract

The shade dried and powdered stem wood of *D. montana* was taken in a round bottom flask and steeped in desired solvent. The ethyl alcohol was used as a solvent for alcoholic extract, and petrol for petroleum extract.The flask contents were refluxed over steam bath for 18-24 hours. The solvents (Petrol and ethyl alcohol) were removed by distillation under reduced pressure,. After the complete removal of the solvent, the residual material obtained was diluted with distilled water to make a stock solution of 1mg/ml for screening of antifilarial activity.

Collection of filarial worm Setaria cervi

Motile adult *S.cervi* (*Nematoda: Filarioidea)* of average length 6.0±1.0 were collected from the peritoneal cavity of freshly slaughtered cattle and brought to the laboratory in a vacuum flask containing modified Ringer's solution (NaCl 9.0g, KCl 0.42g, $CaCl_2$ 0.24g $NaHCO_3$ 0.5g, glucose 0.25 per liter) at 37 ^{o}C [11].

Whole worm (w.w.) preparation

Adult *S. cervi* were suspended in an ideal isolated organ bath of 20 ml capacity, in modified Ringer's solution at 37 ^{o}C. Spontaneous movements of the worm were recorded on a slow moving kymograph drum [10]. Air or Oxygen was not bubbled through the solution, as it did not improve the movements of the worm. Approximately 15mins were allowed for the movements of worm to stabilize before eliciting the response of the extract. The extract was added in increasing concentration to the bath fluid and allowed to remain in contact for 15mins, the effect was observed for 6 hrs. If there was no response within 15 min it was considered inactive.

Nerve-muscle (n.m) complex

A worm was placed in a petri-dish containing modified Ringer's solution (37^oC). Two dissecting needles were inserted into the worm at one end, and the cuticle was split longitudinally. The intestine and uterus were cut at both ends and removed. The anterior 1 cm of the worm was removed to eliminate the influence of the nerve ring and cephalic ganglia. The remaining part was tied at either end and suspended in an isolated organ bath, containing modified Ringer's solution at 37^oC. The preparation served to expose the n.m. complex directly to the action of the drugs, and also could exhibit spontaneous rhythmical movements similar to those of the whole worm. The drug concentrations were

tested for their response as with whole worm preparation. The concentration of extract, which modified the movements, was tested in at least six preparations and the duration of observation in each case was 6 hrs.

Collection of microfilariae (m.f.)

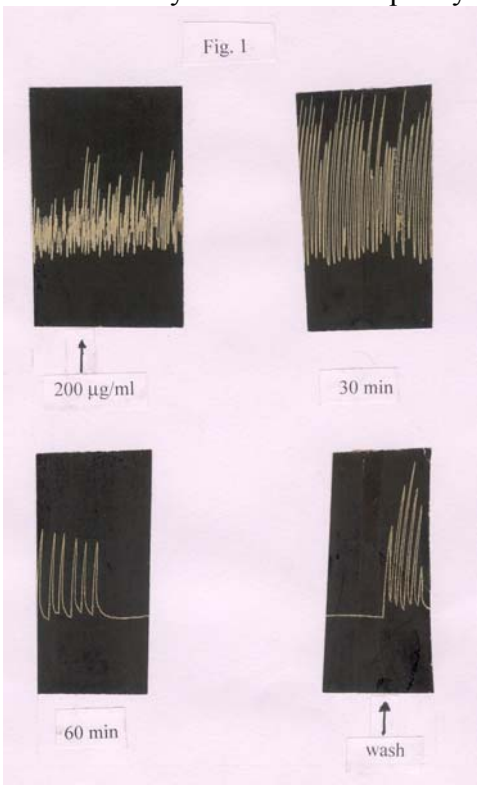
The uterus of a female *S. cervi* was cut at its junction with the vagina just below the bifurcation, and removed from the worm. It was teased with a fine needle in the Ringer solution and microfilariae (mf) were freed. The microfiliariae were suspended in a human serum : Ringer mixture and the mf count was adjusted to 100/ml. 0.5 ml aliquots of the microfilariae suspension were placed in sterilized screw capped bottles containing extract of *Diospyros montana* in equal serum : ringer mixture (v/v). Extract was added in doubling concentration from 5ng/ml. The bottles were kept in an incubator at 37^{o}C and examined under a microscope every 30 min till 6 hours to observe the survival / mortality of microfilariae. The LC 50 and LC 90 were calculated from a concentration vs death graph. In a preliminary set of experiment it was ascertained that the concentration of alcohol / petrol in the suspending medium did not influence the survival / mortality of the m.f. and also the petroleum and alcoholic extracts of *Diospyros montana* were added to m.f. in concentration of 5, 10, 15, 20, 25 ng/ml to determine the limits of activity within 6 hours at 37 ^{0}C, within these limits six concentrations were selected to observe the survival of m.f. The effect of each dose was observed 10 times. The mean of the values were plotted on a graph

Results

Effect of petroleum extract of stem wood of *D. montana* on whole worm:

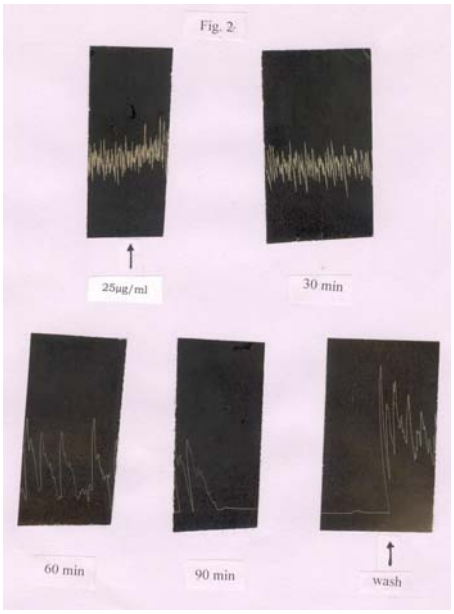
Stimulant effect was observed on addition of 200µg/ml of petroleum extract, characterized by increase in amplitude and tone of contractions (Fig. 1 Effect of Petroleum extract of stem wood of *D. montana* on w.w., showing initial stimulation

followed by reversible paralysis at a concentration of 200 µg/ml.).



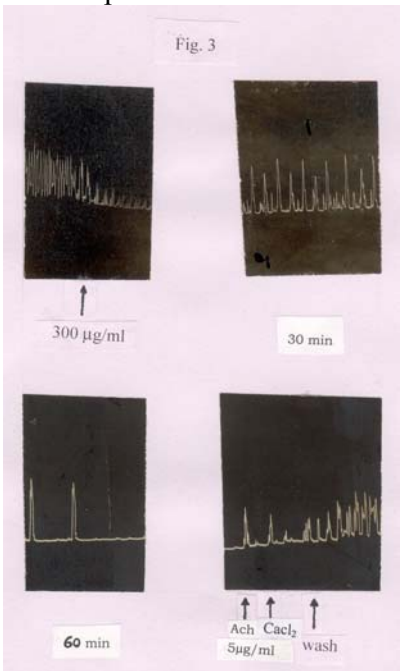
The stimulant effect lasted for 30 min, followed by decrease in amplitude and rate of contractions. At 60 min the activity ceased completely resulting in paralysis of the worm. The repeated change of bath fluid restored the movements of the worm indicating a reversible paralysis.

Effect of petroleum extract of stem wood of *D. montana* on nerve-muscle complex:
The stimulant effect of petroleum extract was evident immediately after addition of extract at a concentration of 25µg/ml which was characterized by increase in the tone and amplitude of contractions with no change in the rate (Fig. 2 Stimulant effect followed by reversible paralysis of petroleum extract on n.m complex of *S. cervi*. at a concentration of 25µg/ml).



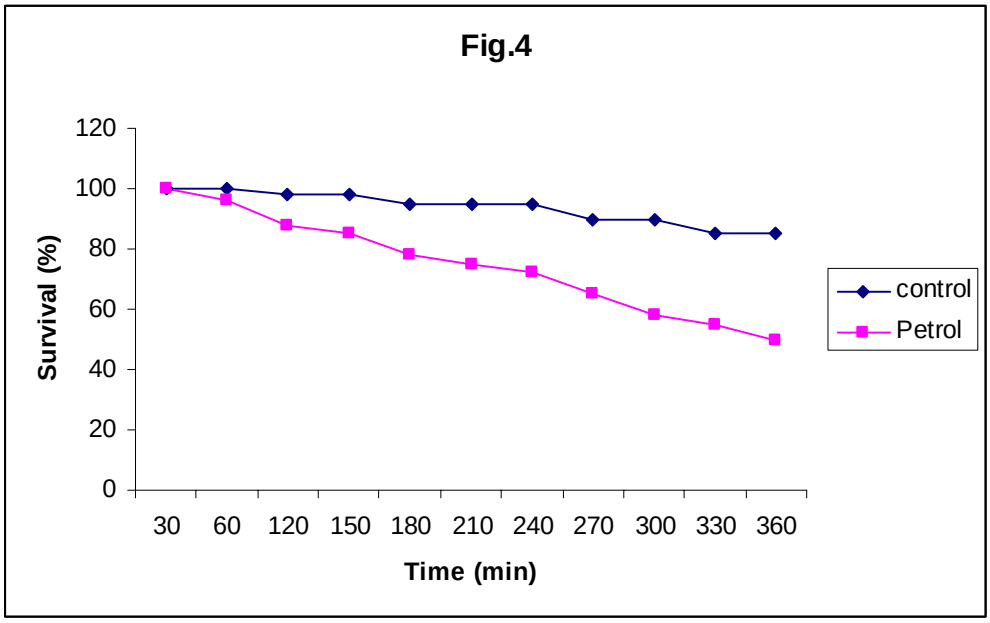
The effect on the tone was short lived and was restored to pre- drug level after 15 min. The rate started to decline after 30 min resulting in complete cessation of contractions at 90 min. Restoration of movements after repeated washing indicates reversible paralysis.

Effect of alcoholic extract of stem wood of *D. montana* on nerve-muscle complex: There was no effect of alcoholic extract on w. w. preparation up to 10 mg/ml concentration. While on n. m. complex of *S. cervi* 300µg/ml of extract, produced a depressant effect, characterized by decrease in amplitude, rate and tone of contractions (Fig. 3 Depressant effect of alcoholic extract of stem wood of *D. montana* on n.mcomplex at a concentration of 300µg/ml, followed by reversible paralysis).



The amplitude returned to pre-drug level at 30 min but the rate continued to decline leading to complete cessation of movements and reversible paralysis at 60 min.

Effect of petroleum extract 0f stem wood of *D. montana* on survival of microfilariae: Shows effect of petroleum extract on survival of *microfilariae* at a concentration of 25ng/ml.



The LC_{50} and LC_{90} as observed after 6 hours were 25ng/ml and 35ng/ml respectively.

Discussion

The petroleum extract of stem wood produced initial stimulation of both the whole worm and the nerve muscle complex characterized by increase in force of contraction in w.w. , whereas increased tone in n.m. complex, ultimately leading to reversible paralysis in both preparations. Marked increase in the amplitude of the w.w . could be due the irritation of the outer cuticular membrane as has been seen with other substances as well, which cause irritation to the worm, this effect is not seen in n.m. complex where the cuticle is stripped [13]. Alcoholic extract of stem wood caused paralysis of the n.m. complex only, the activity of the w.w. was not modified even at a higher concentration. Similar are the results produced by the petroleum, alcoholic and aqueous extracts from the roots of *D. montana*. It might be possible that the active principle in alcoholic extract of stem wood and all the three extracts of root are not able to penetrate the cuticular barrier due to low lipid solubility [12] and once the cuticle is removed the effect could be seen. During the paralysant phase the stimulant effect of acetylcholine and calcium chloride was observed suggesting that the effect is not due to the blockade of cholinergic receptors or calcium channels in w.w. and n.m. complex of *S. cervi* [14]. It is possible that the response of the compound is similar in nature to a known antifilarial agent diethylcarbamazine (DEC), where effect is characterized by initial stimulation followed by paralysis [15]. DEC is a

voltage sensitive potassium channel antagonist. The petroleum extract of stem wood reduced the survival time of microfilariae of the *S. cervi* in a concentration dependent manner. If this concentration can be achieved *in vivo*, it could prove to be a useful tool in the treatment of filariasis. Further studies are in progress to isolate the active principle involved in the causation of the observed effect and its mechanism of action.

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