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ANTIFILARIAL ACTIVITY OF PLUMERIA ALBA LINN BARK

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ABSTRACT

The aqueous extract of Bark of *Plumeria. alba* was tested for its effect on the spontaneous movements of the whole worm (w.w) preparation and nerve muscle (n.m) complex of *Setaria cervi* a cattle filarial parasite, and on the survival of microfilariae . The aqueous extract could inhibit the spontaneous movements of *S.cervi*, characterized by initial stimulation followed by reversible paralysis. The concentration required to produce similar effect on nerve muscle complex was less as compared to the whole worm. The lethal concentration 50 (LC_{50}) and lethal concentration 90 (LC_{90}) for aqueous extract were 80 ng/ml and 105 ng/ml respectively.

Keywords: *Plumeria. Alba*, Antifilarial activity

INTRODUCTION:

Plumeria alba (white champa), commonly known as White Frangipani in English, Veyvi in Telgu, Perumal arali in Tamil, is a small tree, 4-5 m high, occasionally grown in gardens as ornamental plant throughout India. The fruit is edible, the latex is applied to ulcers and scabies, and the seeds are said to possess haemostatic properties, roots are cathartic and branches are used as abortifacient.(1). The bark contains α and β- amyrins and their acetates, plumieride, scopoletin and β- sitisterol,. The medicinal properties of this plant have yet to be thoroughly investigated.

MATERIALS AND METHODS

The dried bark was ground in an electric grinder; the powder obtained was filtered through a fine muslin cloth and was transferred to thimbles of Whatman filter paper No.1 in Soxhlet apparatus, distilled water was used as solvent. The apparatus was allowed to run for 48-72 hours. Later the solvent was allowed to evaporate in a vacuum dessicator and after the complete evaporation of the solvent, the residual material obtained was diluted with saline to make a stock solution of 1mg/ml. 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 9g, Kcl 0.42g, $CaCl_2$ 0.24g $NaHCo_3$ 0.5g, glucose 0.25 per liter) at 37 ^oC.

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. 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 drug. The extract was added in increasing concentration to the bath fluid and allowed to remain in contact for 15mins. If there was no response it was considered inactive.

Nerve-muscle (n.m) complex

A worm was placed in a petridish containing modified Ringer's solution (37^{o}C). 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^{o}C. 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 extract 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.

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

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 *Plumeria. alba* 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 aqueous extract of *Plumeria. alba* was 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 aqueous extract of bark of *P.alba* on w.w : At a concentration of 600 µg/ml there was stimulation in the form of increase in the rate and amplitude of contraction. This effect started to decline at 60 min and within 90 min there was no contraction – paralysis of worm which lasted for two hours (duration for which observation was made). The paralysis was reversible in nature as it could be restored by repeated changes in bath fluid. (fig. 1)

Effect of aqueous extract of bark of *P.alba* on n.m preparation :Concentration required to elicit the effect on the n.m (250 µg/ml) was less than half of the w.w. The effect was depressant in nature characterized by decrease in the tone and amplitude which lasted for about 45 min and at 60min there was complete cessation of movements leading to paralysis (2 hours) that could be reversed by repeated changes in bath fluid. (fig.2)

Table No.1 Effect of aqueous extract of *P.alba* on the survival of m.f of *S. cervi* at 6 hours.

Aqueous Extract (LC)	Conc. (ng/ml)
LC_{50}	80
LC_{90}	105

Effect of aqueous extract of bark of *P.alba* on the survival of microfilariae (m.f) : The aqueous extract produced a concentrated related effect on the survival of the m.f. The time related lethal effect at a concentration of 25ng /ml is shown in Fig. 3.The LC_{50} and LC_{90} as observed after 6 hours are shown in table No.1

Fig. 1: Shows the effect of aqueous extract of *P.alba* on whole worm of *S.cervi*.

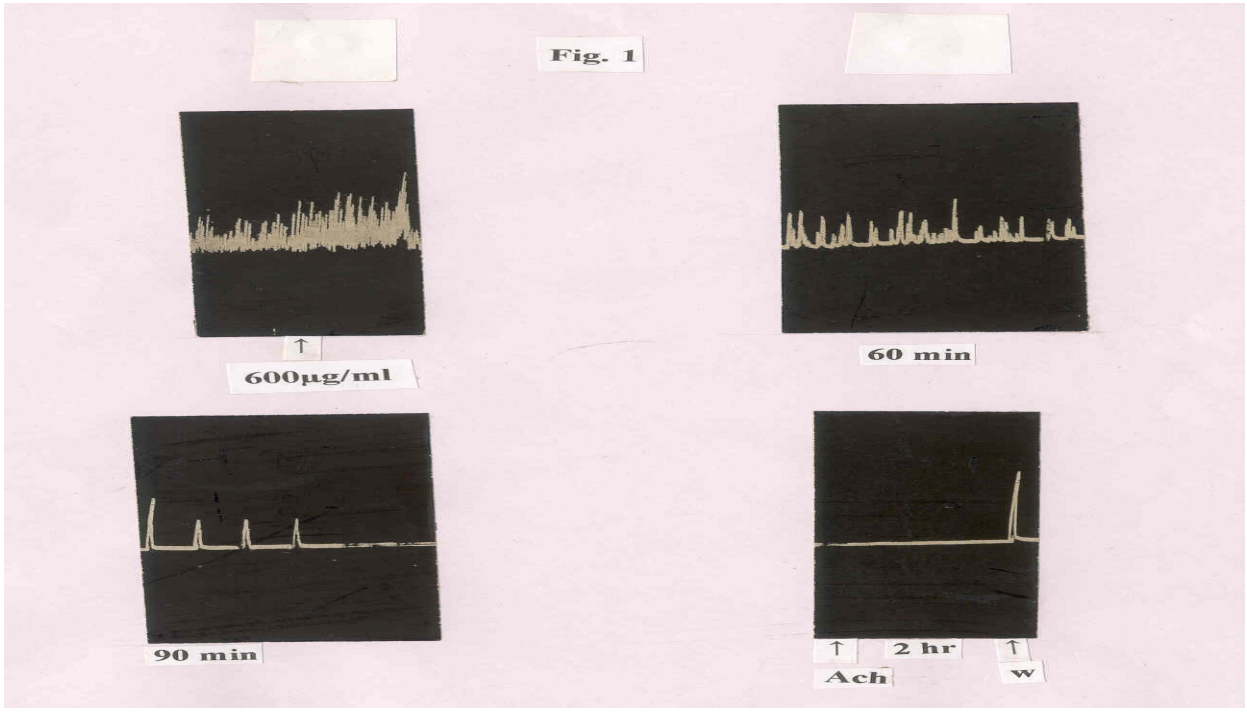


Fig. 2: Shows the effect of aqueous extract of *P.alba* on nerve muscle preparation of *S.cervi.*

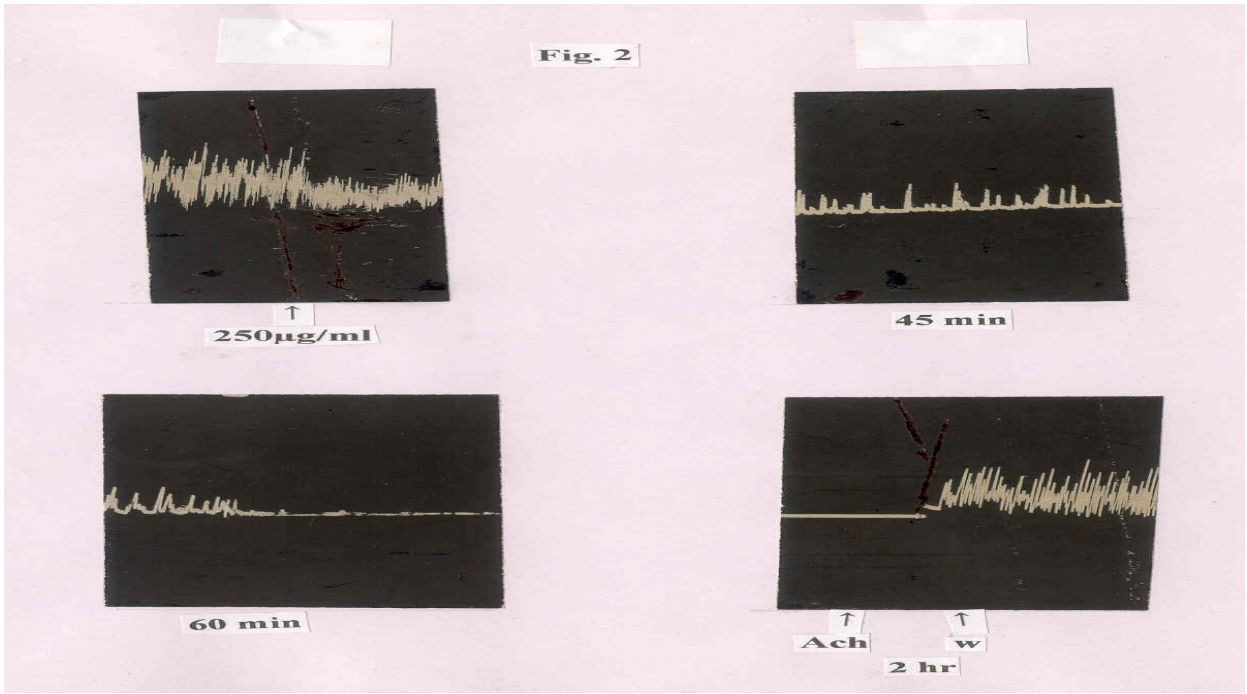
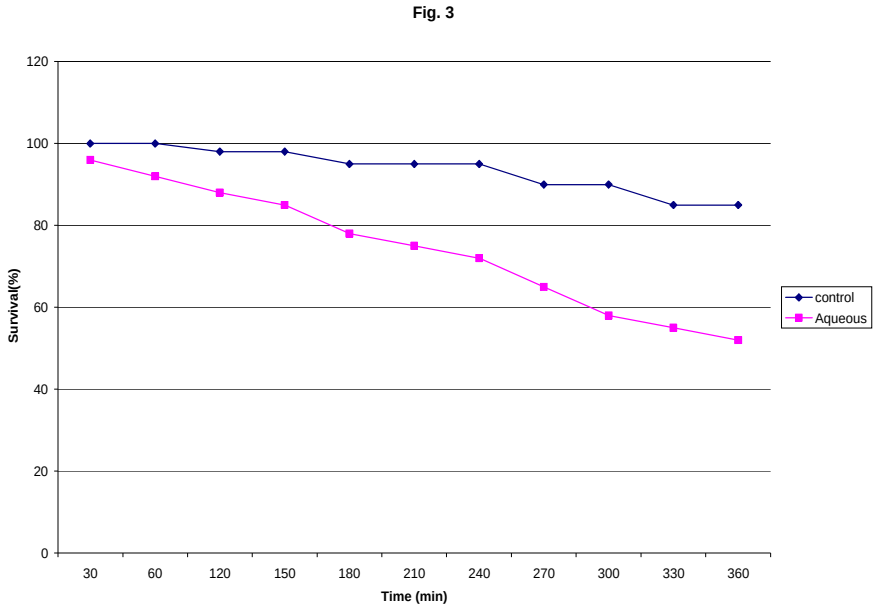


Fig.3 : Effect of aqueous extract of *P.alba* on the survival of microfilariae of *S.cervi* at a Concentration of 25ng/ml.



DISCUSSION:

The effect produced by aqueous extract of bark of *P.alba* on *Setaria cervi* was inhibitory in nature. The onset was rapid in both preparations with a difference that there was a phase of initial stimulation in case of w.w, this could be due to the cuticular irritation as has been shown by Christ, et al (2). The concentration that produced inhibition in n.m was less than half of that required for w.w suggesting a cuticular permeability barrier. Penetration depends on lipid solubility, substance with poor lipid solubility will penetrate to a lesser extent across the cuticle, as shown for Ascaris. Fetterer (3) and Dipetalonema vitae. Christ et al (2). Inhibitory response of the spontaneous movements could result either by blocking the effect of excitatory

neurotransmitter acetylcholine Singhal K.C et al.(4), or by facilitating the inhibitory neurotransmitters 5- hydroxytryptamine (5-HT) and gamaaminobutyric acid (GABA) Singhal et al (5). Piperazine a known anthelmintic is GABA mimetic and causes hyper polarization of Ascaris and S.cervi muscle cell Singhal et al (5), Aubry ML et al., (6), Del Castillo et al (7). A piperazine derivative diethylcarbamazine (DEC) causes initial stimulation followed by dose dependent reversible paralysis by blocking voltage sensitive potassium channels Martin BJ, (8). P.alba also produced initial stimulation leading to reversible paralysis- response similar to DEC Singhal et al, (9). We can conclude that the bark of P.alba posses potential antifilarial activity which can be useful clinically.

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