

**IN-VITRO SCREENING FOR ANTIFILARIAL POTENTIAL IN LEAVES OF  
CALOTROPIS GIGANTAE**

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**Summary**

The effect of alcoholic extract of leaves of *Calotropis gigantea* was studied on the spontaneous movements of the whole worm (w.w) preparation and nerve muscle (n.m) complex of cattle filarial parasite *Setaria cervi* and on the survival of microfilariae. The alcoholic 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 n.m complex was less as compared to the w.w. The lethal concentration 50 (LC50) and lethal concentration 90 (LC90) for alcoholic extract were 58ng/ml and 70ng/ml.

**Key words** : Calotropis Setaria cervi Filariasis Microfilariae

**Introduction**

Is an erect perennial shrub, growing chiefly in waste lands. It ascends to an altitude of 3,000 ft on the Himalayas, and extends from the Punjab to south India, Assam. [1]  
Chemical composition – The latex contains two isomeric resinols,  $\alpha$ -calotropeol and  $\beta$ -calotropeol, and amyriins, traces of glutathione are also found. The stem bark contains terpene and traces of sterols in addition to  $\alpha$  and  $\beta$ -calotropeol.[1]

The Hindu physicians consider the root bark as a valuable remedy in skin diseases, enlargement of abdominal viscera, intestinal worms, cough, ascites etc. the milky juice is regarded as a drastic purgative. The flowers are considered digestive, stomachic, tonic and useful in cough and asthma. The root bark reduced to a paste with rice vinegar is applied to legs and scrotum in patients of elephantiasis. [2].

## Methods

### **Plant material**

The shrub was collected during hot, and dry weather and identified by the Taxonomist at Aligarh Muslim University Aligarh and the specimen was deposited in the department.

### **Preparation of extract**

The dried leaves were ground in an electric grinder, the powder obtained was transferred to thimbles of Whatman filter paper No.1 in Soxhlet apparatus, ethyl alcohol was used as a solvent. The apparatus was allowed to run for 48-72 hours. Later the solvent was allowed to evaporate in a vacuum dessicator (yield was 12%) 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  $6.0\pm 1.0$  cm 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<sub>2</sub> 0.24g NaHCO<sub>3</sub> 0.5g, glucose 0.25 per liter) at 37 °C.

### ***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 °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°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°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 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.

***Collection of microfilariae (m.f.)***

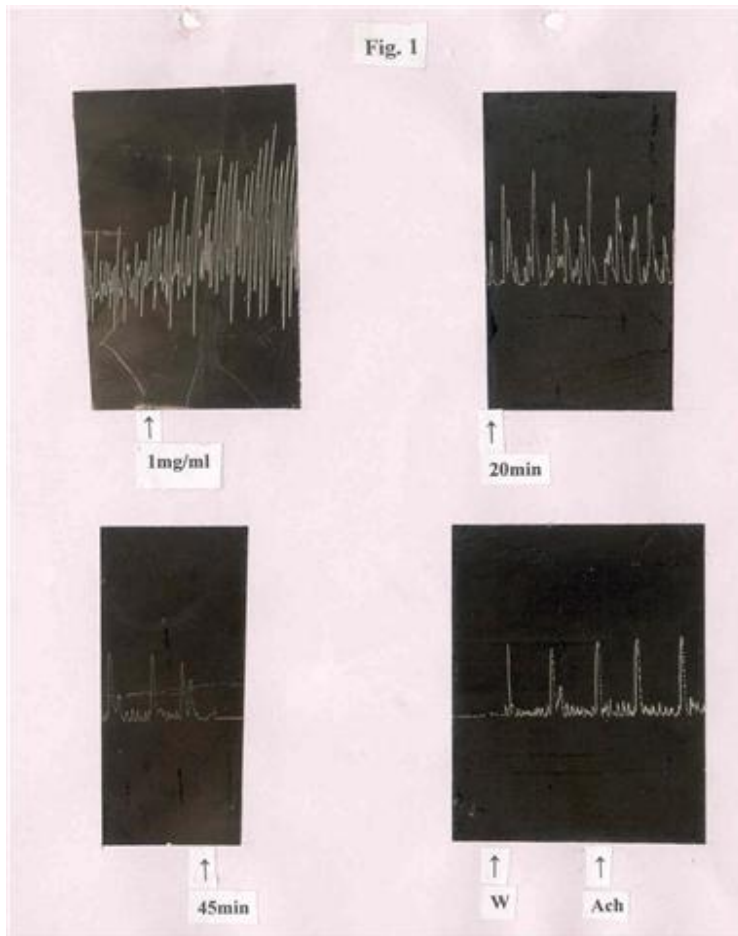
The uterus of a female *S. cervi* was cut at its junction with the vagina just below the bifurcation, and removed from the worm. It was teased with a fine needle in the solution and microfilariae (mf) were freed. The microfilariae were suspended in a human serum : Ringer mixture and the mf count was adjusted to 100/ml. 0.5 ml aliquots of the microfilariae suspension were placed in sterilized screw capped bottles containing alcoholic extract of *Calotropis. gigantea* 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°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 in the suspending medium did not influence the survival / mortality of the m.f.

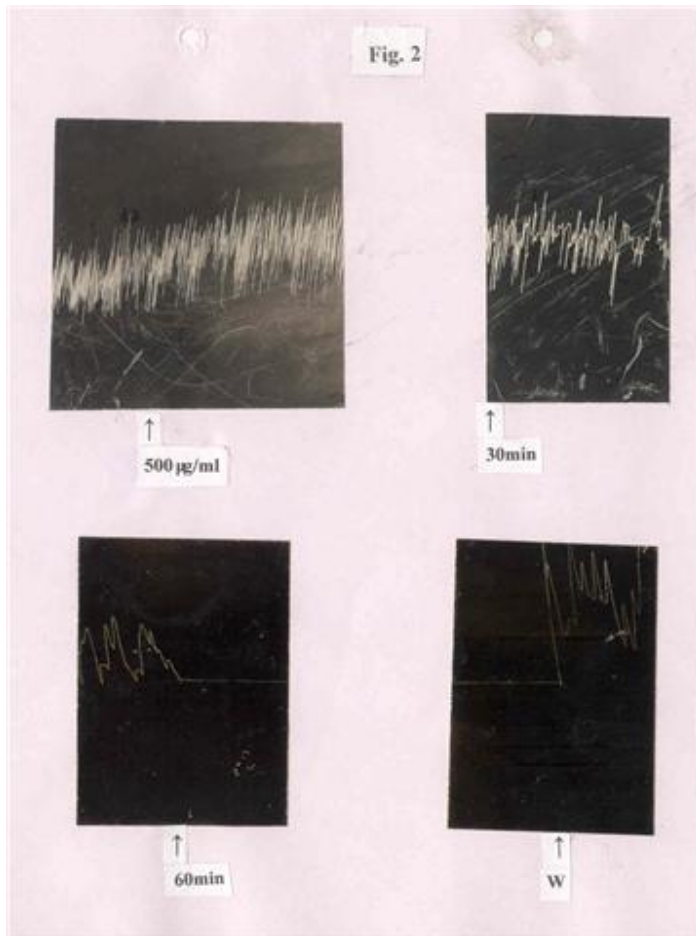
In a preliminary experiment, alcoholic extract of *Calotropis. gigantea* were added to m.f. in concentration of 5, 10, 15, 20, 25 ng/ml to determine the limits of activity within 6 hours at 37 °C, within these limits six concentrations were selected to observe the survival of m.f. The effect of each dose was observed 10 times. The mean of the values were plotted on a graph.

**Results*****Effect of alcoholic extract on whole worm (w.w):***

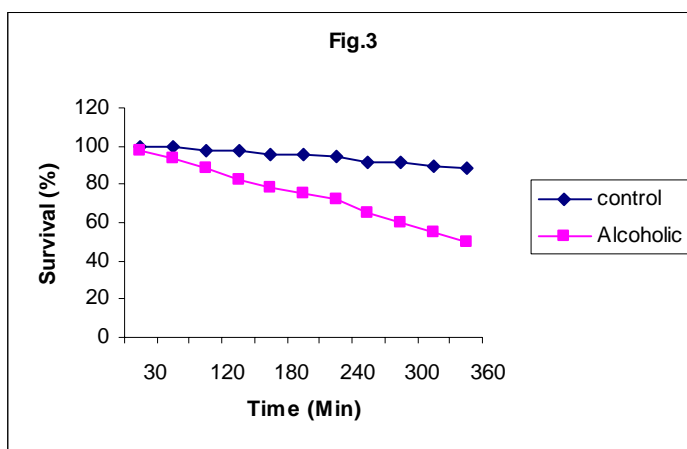
At a concentration of 1mg/ml there was increase in tone and force of contraction which lasted for about 20 minutes followed by decrease in rate and force of contraction leading to complete cessation of movements that could be restored by repeated washing of the bath that is reversible paralysis (Fig 1. Shows the effect of 1mg/ml of extract on w.w of *S. cervi*).



*Effect of alcoholic extract on nerve muscle preparation (n.m.):*  
Only 500 $\mu$ g/ml of extract was required to produce initial stimulation characterized by increase in force of contraction that lasted for 30 mins leading to reversible paralysis. (Fig 2. Shows the effect of 500 $\mu$ g/ml on n.m preparation of S. cervi)



Effect of alcoholic extract Of leaves of *Calotropis. gigantea* on survival of microfilariae: Shows effect of petroleum extract on survival of microfilariae at a concentration of 25ng/ml(Fig 3)



The lethal concentration 50 (LC50) and lethal concentration 90 (LC90) for alcoholic extract were 58ng/ml and 70ng/ml.

### Discussion

The alcoholic extract of leaves produced inhibition of both the whole worm and the nerve muscle complex characterized by increase in force of contraction in w.w., whereas increased force of contraction and tone in n.m. complex, leading to reversible paralysis in both cases. 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 [3,4], which cause irritation to the worm, this effect is not seen in n.m. complex where the cuticle is stripped [5]. The difference in pattern of activity could also be explained on the basis of lipid solubility of the active principle, it might be possible that the active principle in alcoholic extract is not able to penetrate the cuticular barrier due to low lipid solubility [6] and once the cuticle is removed the effect becomes more prominent.

During the paralyzant phase the stimulant effect of acetylcholine was observed suggesting that the effect is not due to the blockade of cholinergic receptors in w.w. [7]. 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 [8]. DEC is a voltage sensitive potassium channel antagonist.

The alcoholic extract of leaves 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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