International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Evaluation of In Vitro *Antifilarial* Potential in Alcoholic Extract of Bark of *Albizzia Lebbeck*

Waseem Rizvi[1*], Syed Shariq Naeem[1], Anil Kumar[1]

[1]Department of Pharmacology, J. N. Medical College, A.M.U, Aligarh (U.P.)

[*]Correspondence Email: waseemnakhat@gmail.com

Received: 05/11//2012 *Revised: 27/11/2012* *Accepted: 01/12/2012*

ABSTRACT

The effect in alcoholic extract of Bark of *Albizzia lebbeck* was studied for antifilarial activity using *Setaria cervi (S. cervi) worm procured from the freshly slaughtered buffalo.*We used the whole worm (w.w.) preparation and nerve muscle (n.m.) complex of *Setaria cervi (S. cervi)*. The n.m. complex is made by stripping the cuticle of the worm. The effect was stimulant in nature in both the preparations but the dose required to produce the effect was less in case of n.m preparation as compared to w.w. Microfilariae was used to determine the lethal concentration 50 (LC50) and lethal concentration 90 (LC90) which was 65ng/ml and 80ng/ml respectively.

Key Words: Albizzia, Antifilarial activity, Setaria cervi

INTRODUCTION

Albizzia lebbeck. (Family: *Mimosaceae*) is a deciduous tree which possesses several medicinal properties and has been traditionally used for the therapy of several ailments. According to indigenous system of medicine it is useful in respiratory problems, snakebite, and malaria fever. The bark is used in leucoderma, skin diseases, and bronchitis. It is also claimed to be effective anthelmintic and anti-inflammatory remedy. It is reported to possess nootropic,[1,2] anxiolytic,[2] anticonvulsant,[3,4] Antifertility[5] and Antidiarrhoeal activity.[6]

Different phytochemicals have been isolated from beans which include albigenic acid - a triterpenoid sapogenin [7] and albigenin - a triterpene. [8] Albiziahexoside - a bioactive saponin isolated from bark. [9] *Seteria cervi (Nemetoda; Filariodiea)* is naturally occurring filarial parasite of water buffalo (*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]

In this study, we have evaluated the antifilarial activity of ethanol extract of *Albizzia lebbeck* to confirm the folklore claims.

METHODS

Plant material

The plant *Albizzia lebbeck* was procured from the gardens of Botany Department, Aligarh Muslim University,

Aligarh. The plant was identified by taxonomist Prof. Wazahat Hussain, Department of Botany, AMU., Aligarh (India) its voucher specimen has been deposited in the same department.

Preparation of extract

The shade dried and powdered bark of *Albizzia lebbeck* was taken in a round bottom flask and steeped in desired solvent. Ethyl alcohol was used as a solvent for alcoholic extract. The flask contents were refluxed over steam bath for 18-24 hours. The solvent was 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 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 9.0g, KCl 0.42g, CaCl2 0.24g NaHCO3 0.5g, glucose 0.25 per liter) at 37^0 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 37^0 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 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 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 *Albizzia lebbeck* 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^oC 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. and also the alcoholic extract of *Albizzia lebbeck* 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 alcoholic extract of bark of *A. lebbeck* on w.w: At a concentration of 800 µg/ml there was initial stimulation i.e. increase in the rate and force of contractions. This effect lasted for 60 mins and at 90 min there was no contraction – paralysis of worm. The paralysis was reversible in nature as it could be restored by repeated changes in bath fluid. (fig. 1)

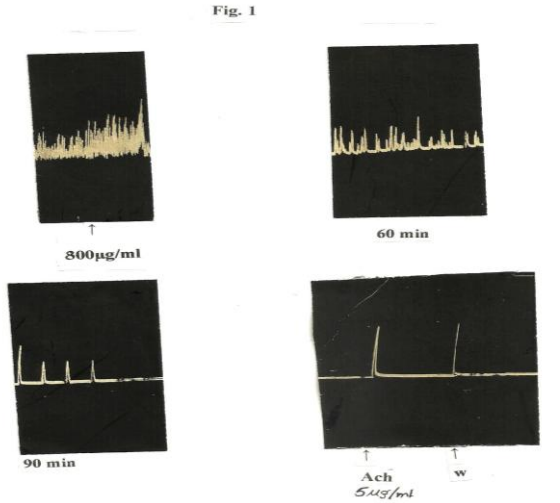

Figure 1: Shows the stimulant effect of alcoholic extract of A. lebbeck on whole worm (w.w) at a concentration of 800 µg/ml.

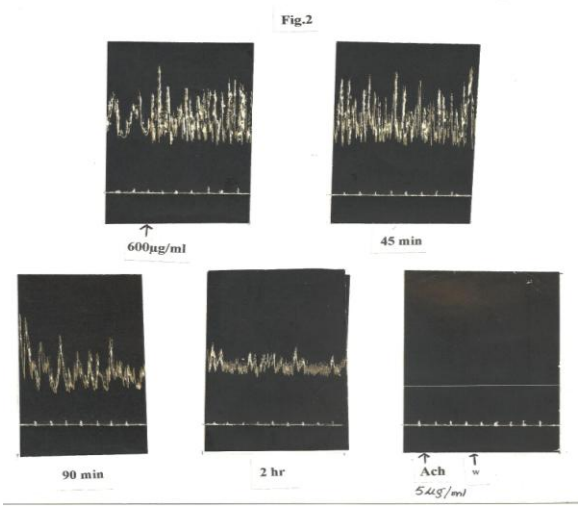

Figure 2: Shows the stimulant effect of alcoholic extract of A. lebbeck on nerve muscle (m.n) preparation at a concentration of 600 µg/ml.

Effect of alcoholic extract of bark of *A. lebbeck* on n.m preparation: Lesser concentration (600 µg/ml) was required to produce the effect on the n.m. as compared to w.w. The effect was stimulant in nature characterized by increase in the force of contractions which lasted for about 60 min and near 2hours there was complete cessation of movements leading to paralysis that could not be reversed by repeated changes in bath fluid. (fig.2)

Effect of alcoholic extract of bark of *A. lebbeck* on the survival of microfilariae (m.f): The ethanol extract produced a concentration related effect on the survival of the m.f. The time related lethal effect at a concentration of 25 ng/ml is shown in Fig. 3 The LC_{50} and LC_{90} as observed after 6 hours were 65ng/ml and 80ng/ml respectively.

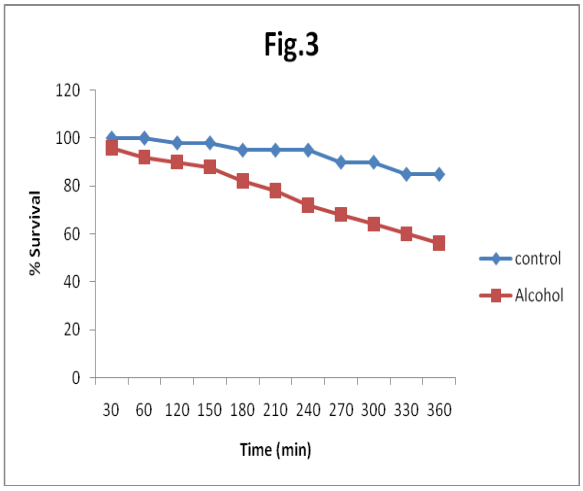

Figure 3: Shows effect of alcoholic extract on survival of *microfilariae* at a concentration of 25 ng/ml.

DISCUSSION

Setaria cervi is a filarial nematode which exhibit spontaneous rhythmic activity which can be recorded on a kymograph. Nerve muscle preparation is made by removing the cuticle so that the nerve muscle is in direct contact with the testing agents making it more sensitive to the action of drugs. [12]

The alcoholic extract of bark of *A. lebbeck* produced stimulation of both the whole worm and the nerve muscle complex characterized by increase in rate and force of contraction in w.w., whereas increased force of contraction in n.m. complex, ultimately leading to paralysis which was reversible in nature in case of w.w but irreversible in n.m preparation. 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] The difference in nature of effects produced on two preparations could possibly be due to the extent of penetration of the active principle across the cuticle which is determined by the lipid solubility. [13,14] During the paralysant phase the stimulant effect of acetylcholine could not be elicited suggesting that the effect could be due to the blockade of the cholinergic receptors. [12,15]

CONCLUSION

The alcoholic extract of bark of *A. lebbeck* 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.

REFERENCES

1. Chintawar, S.D. Somani, R.S. Kasture Veena, S. Kasture, S.B. 2002. Nootropic activity of *Albizzia lebbeck* in mice. Journal of Ethnopharmacology. 81:299-305.
2. Une, H.D. Sarveiya, V.P. Pal, S.C. Kasture, V. Kasture, S.B. 2001. Nootropic and anxiolytic activity of saponins of Albizzia lebbeck leaves. Pharmacology Biochemistry and Behavior. 69: 439-444.
3. Kasture, V.S. Chopade, C.T. Deshmukh, V.K. 2000. Anticonvulsive activity of Albizzia lebbeck, Hibiscus rosa sinesis and Buteamonosperma in experimental animals. Journal of Ethnopharmacology. 71: 65-75.
4. Kasture, V.S. Kasture, S.B. Pal, S.C. 1996. Anticonvulsant activity of Albizzia lebbeck leaves. Indian J of Exp Biol. 34:78-80.
5. Gupta, R.S, Kachhawa, J.B. Chaudhary, R. 2004. Antifertility effects of methanol pod extract of Albizzia lebbeck (L.) Benth in male rats. Asian J Androl. 6: 155-159.

6. Besra, S.E. Gomes, A. Chaudhury, L. Vedasiromoni, J.R. Ganguly, D.K. 2002. Antidiarrhoeal activity of seed extract of Albizzia lebbeck Benth. Phytotherapy Research. 16: 529-533.
7. Barua, A.K. Raman Pattabi, S. 1959. Triterpenoids—X: The constitution of albigenic acid—a new triterpenoid sapogenin from Albizzia lebbeck benth. Tetrahedron. 1: 19.
8. Barua, A.K. Raman, S.P. 1962. Triterpenoids- XII: The constitution of albigenin- a new triterpene from Albizzia lebbeck benth. Tetrahedron. 18: 155-159.
9. Ueda, M. Tokunaga, T. Okazaki, M. Satan, N.U. Ueda, K.Yamamura,S.2003.Albiziahexoside: a potential source of bioactive saponin from the leaves of Albizzia lebbeck. Nat. Prod. Res. 17: 329-335.
10. Singhal, K.C. Madan, B.R. Saxena, P.N. 1977. Effect of drugs on nerve-muscle complex of *Setaria cervi*. Indian J. Med. Res. 66 - 517.
11. Singhal, K.C. Saxena, P.N. Johri, M.B.L. 1973.Studies on the use of *Setaria cervi* for in vitro antifilarial screening . Jap. J. Pharmacol: 23-793.
12. Singhal, K.C. Madan, B.R. Saxena, P.N. 1977. Effect of drugs on the nerve muscle complex of Setaria cervi. Indian J. Med. Res. 66: 517-521.
13. Christ, D. Goebel, M. Saz, H.J. 1990. Actions of acetylcholine and GABA on spontaneous contractions of filarid Dipetalonema viteae. Br. J. Pharmacol.101: 971-977.
14. Fetterer, R.H.1986. Transcuticular solute movement in parasitic nematodes: relationship between non polar solute transport and partition coefficient. Comp. Biochem. Physio. 84A: 461-466.
15. Del Castillo, J. De Mello, W.C. Morales, T.1963. The Physiological role of acetylcholine in the neuromuscular system of Ascaris lumbricoides. Arch Int Physiol Biochem.71:741-757.

How to cite this article: Rizvi W, Naeem SS, Kumar A. Evaluation of in vitro *antifilarial* potential in alcoholic extract of bark of *albizzia lebbeck*. Int J Health Sci Res. 2013;3(1):23-27.

****