Asian J. Pharm. Res. Vol 5, Issue 3, 191-193, 2015.

e-ISSN 2231 – 363X
Print ISSN 2231 – 3621

Asian Journal
of
PHARMACEUTICAL RESEARCH
Journal homepage: - www.ajprjournal.com

INVITRO ANTHELMINTIC ACTIVITY OF STEM BARK OF *AILANTHUS EXCELSA*, ROXB BY LARVAL MIGRATION INHIBITION ASSAY (LMIA)

G.Prakash Yoganandam*[1] and K.Periyanayagam[2]

[1]Department of Pharmacognosy, College of Pharmacy, Mother Theresa Post graduate and Research Institute of Health Sciences (MTPG&RIHS), A Government of Puducherry Institution, Puducherry -605006, India.
[2]Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai-625 020, Tamil Nadu, India.

ABSTRACT

Objective to explore the *invitro* effect of various extracts of stem bark of *Ailanthus excelsa* (Fam: Simmaroubaceae) on third stage larvae of gastro intestinal nematodes of sheep and goat using larval migration inhibition assay. Anthelmintic activity by Larval Migration Inhibition Assay (LMIA). The method is to incubate L_3Stage in serial concentrations of the test drug on a nylon mesh, thus simulating the gut mucosal layer through which the larvae would normally migrate. The petroleum ether extract significantly inhibit larval migration 94.66% at 80 ppm/ml concentration and it is comparable to standard drug Levamizole in the same concentration, while the ethanolic and aqueous extracts showed 74% and 70.65% inhibition at the same concentration. The LMI_{50} values for petroleum ether, ethanolic and aqueous extracts were 45, 49 and 60 ppm/ml respectively. Further investigation by isolating the active principle responsible for the activity may provide molecule for the new potent anthelmintic drug from the plant origin with lesser side effects and can be practical and affordable way to reduce the incidence of infection by HIV and *M.tuberculosis*.

Key words: *Ailanthus excelsa*, Anthelmintic, Larval migration inhibition assay (LMIA).

INTRODUCTION

Haemonchosis and other common abomasal worm of small ruminants in India, is responsible for productivity losses in animals. This is controlled exclusively by the use of chemical anthelmintics. However, frequent indiscriminate use of chemical anthelmintics to control gastro intestinal parasitism has resulted in development of resistant strains [1]. Hence it is necessary to find alternative source of anthelmintics. Herbal drugs could be of immense value in minimizing the spread of anthelmintic resistance, when compared to synthetic drugs that are isolated chemical compounds. Phytotherapeutic drugs are safe, non-toxic, biodegradable and do not leave residues in animal products [2-3]. The plant *A .excelsa* (Family: *Simaroubaceae*) is a large deciduous tree. It is indigenous to Central and Southern India known for a wide range of therapeutic indications as anthelmintic, astringents, bitter, febrifuge and is used in indigenous veterinary practice. The stem bark contains quassinoids, ailanthic acid, dimethoxy benzoquinone, β-sitosterol, malanthin, triacontane and hexatriacontane [4-6]. In this investigation we prompted to study the anthelmintic activity of the stem bark of *A. excelsa* by LMIA (Larval migration inhibition assay).

Invitro methods that have been used to investigate the efficacy of anthelmintic towards nematode parasites are the Larval development (LD), Larval migration inhibition (LMI), Larval feeding inhibition (LFI) and Egg hatch assays (EHA). The larval migration inhibition assay has the advantages of being simple, rapid and provide accurate information on whether the bioactive compound inhibit the locomotion and this method has biological relevance with invivo conditions and simulates the invivo migration of the third stage larvae [7].

MATERIALS AND METHODS

Plant materials

The stem bark of *A. excelsa* was collected from Alagar koil hills, Madurai, Tamil nadu on August 2015. It was authenticated by taxonomist and a voucher specimen

Corresponding Author :- **G.Prakash Yoganandam** Email:- gprakashyoga@gmail.com

Asian J. Pharm. Res. Vol 5, Issue 3, 191-193, 2015.

(PCG/PAE/15/2006) was deposited in the herbarium. The materials were shade dried, powdered and passed through a fine sieve.

Preparation of bark extracts

The petroleum ether (60-80^{0}C) and ethanolic extracts were prepared by soxhlet extraction method. The aqueous extract is prepared by maceration process. The solvents are evaporated to yield a residue. The residue obtained above was used at the concentration of 40, 60 and 80 ppm/ml.

Larval suspension

The third (L_3) stage GI nematode larval suspension of sheep and goat were obtained from the Department of Parasitology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India.

Larval Migration Inhibition Assay (LMIA)

The aim is to incubate L_3 Stage in serial concentrations of the test drug on a nylon mesh, thus simulating the gut mucosal layer through which the larvae would normally migrate. Paralysis caused by susceptibility to the test drug has the effect of preventing the migration and causing the larvae to be held by the gauze [8]. In this assay the third stage larvae are incubated at 37^{o}C for 2 hours, in 40, 60 and 80 ppm/ml concentration of the test compound in a 24 well plate. For each and every concentration the test was done in triplicate. Following the incubation, the larvae were allowed to migrate through a fine mesh in a mini baermann's apparatus into well containing the same concentrations of the bark extracts. The number of larva retained by the mesh (Nr) and those that have migrated (Nm) through the mesh were counted by an inverted microscope and the percentage of migration inhibition is calculated as follows,

$$\%LMI = \frac{N_r}{Nm + N_r} \times 100$$

The above assay was performed using distilled water as control without bark extracts and levamizole as the standard drugs. The corrected percentage LMI was calculated by using abbot's formula.

$$Corrected\ \%LMI = \frac{Test\ LMI\ (\%) - Control\ LMI\ (\%)}{100 - Control\ LMI\ (\%)} \times 100$$

Statistical Analysis

The data were analyzed using one way ANOVA (Dunnet Multiple Comparison Test) to find out the significance of larval migration inhibition ($p< 0.05$) exposed to different concentration of stem bark of *A.excelsa*.

RESULTS

The petroleum ether extract at the concentration of 80 ppm/ml showed 94.66% of LMI which is comparable to the standard drug levamizole in the same concentration while the ethanolic extract and aqueous extract showed 74% and 70.65% inhibition at the same concentration (Table:1). The LMI_{50} values were calculated for all the extracts using regression line analysis. It shows that the LMI_{50} values for petroleum ether, ethanolic and aqueous extracts were 45, 49 and 60 ppm/ml respectively.

Table 1. Larval Migration Inhibition of Different Extracts of Stem Bark of *Ailanthus excelsa*

S.No	Name of Extract	Concentration (PPM)	% of LMI	LMI_{50}
1.	Aqueous	40	27.89	60
		60	51.80	
		80	70.65	
2.	Ethanol	40	40.88	49
		60	62.96	
		80	74.00	
3.	Pet. Ether	40	42.35	45
		60	72.24	
		80	94.66	
4.	Levamizole (STD)	40	52.27	-
		60	73.33	
		80	95.79	

DISCUSSION & CONCLUSION

In the present study the petroleum ether extract at the concentration of 80 ppm/ml significantly inhibited the third stage larval migration compared to the standard drug. A bitter principle malanthin was isolated and identified from the petroleum ether extract of stem bark of *A. excelsa* [9] and this malanthin may be responsible for inhibitory effect. A study using malanthin by the above method may give a safe, drug molecule to develop a potent anthelmintic from this plant for both in human and veterinary practice. The probable link of helminthiasis with HIV/AIDS and tuberculosis is that chronic helminthic infection down regulates the cellular immune response that is needed to prevent infection by HIV and *M.tuberculosis* [10-11]. So new, potent anthelmintic without side effect may be practical and affordable way to reduce the incidence of infection by HIV and *M.tuberculosis*, slowdown the

Asian J. Pharm. Res. Vol 5, Issue 3, 191-193, 2015.

progression of the disease they cause and to improve the efficiency of the vaccines against HIV/AIDS and TB. It is also optimistic that the investigation presented may give a potential contribution to the global programmes to eliminate helminthiasis. So that it may prevent the dominance of T2 immune responses which favour infection of HIV and *M.tuberculosis* and could impair vaccine trails. The stem bark of *A.excelsa* may provide better protection of HIV and *M.tuberculosis* where helminthiasis is high. It was also reported that leaves of *A.excelsa* satisfy the maintenance requirement of goats and sheep and are available at practically no cost and attention should be given to the utilization for rearing goats and sheep economically since they are rich in crude protein [12]. Since it is useful plants in many aspects further research is in progress in our laboratory.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

ACKNOWLEDGEMENT

The authors are very thankful to Dr. D. Stephan, Taxonomist, Department of Botany, The American College, Madurai, India for identifying and authentication of the plant. The authors are also thankful to T.J.Harikrishnan**,** Department of Parasitology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India.

REFERENCES

1. Yadav CL, Garg R. Anthelmintic resistance in gastrointestinal nematodes of sheep in Haryana. *J. Vet. Parasitol*, 18, 2004, 39-41.
2. Hammond JA, Fielding D, Bishop SC. Prospects for plant anthelmintics in tropical veterinary medicine. *Vet. Res. Com*., 21, 1997, 213-228.
3. Veerakumari L,Navaneetha Lakshmi K. *Invitro* effect of *Allium sativum* on lactate dehydrogenase activity of haemonchus contortus. *Vet.Parasitol*, 20(1), 2006, 93-96.
4. Ananymous. The Wealth of India, Raw materials. Vol I-A, CSIR. New Delhi. 1985, 116-117.
5. Nadkarni KM. (ed) 1927. The Indian Materia Medica. K.M Nadkarni, Bombay, 1927, 56.
6. Chopra RN, Chopra IC, Handa LK, Kapur ID. Indigenous drugs of India, U.N.Dhur and Sons, Calcutta, 1958, 493.
7. Athaniasiadou S, Kryiazakis I, Jaction F, Brant K. Determination of the most appropriate *invitro* test to assess the efficacy of substances extracted from bioactive forages. *Vet. Parasitol*, 99, 2000, 205-209.
8. Jackson F, Jackson E, Coop RL. 2002. Larval migration inhibition assay for determination of susceptibility of nematodes to levamizole in Halton, D.W.,Behnke,J.M., and Marshall,I.(eds)., Practical exercise in parasitology, Cambridge University Press. U.K.2002, 321-327.
9. Mahendra Kumar Jain. Chemical examination of *Ailanthus excelsa* dc. *Indian J Chem*, 2, 1963, 40.
10. John Fincham. Helminthes, HIV/AIDS and Tuberculosis. *http, //www.scienceinafrica.co.za/2001/December/worms.htm.*
11. Markus MB, Fincham JE. Worms and Paediatric Human Immuno Deficiency Virus Infection and Tuberculosis. *The Journal of Infectious Diseases*, 181, 2000, 1873-1874.
12. Mandal L. Nutritive values of tree leaves of some tropical species for goats. *Small Ruminant Research*, 24(2), 1997, 95-105.