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***In vitro* anthelmintic activity of stem bark of *Juglans regia* L.**

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ABSTRACT

Among the most common infections of digestive system in human beings are helminth infections. In developing countries they pose a large threat to the society. Such parasitic diseases cause severe morbidity, including lymphatic filariasis, onchocerciasis and schistosomiasis. These infections can affect most of the population in endemic areas with major economic and social consequences. Ayurveda provides many herbal preparations to overcome alimentary canal infections with negligible side effects. *Juglans regia* L, the valuable species from Juglandaceae family has a long history of traditional use as an anthelmintic. All parts of the plant are a rich source of medicinally useful components. Present study is an attempt to evaluate anthelmintic activity of different extracts of stem bark of *J. regia* L. Different concentrations of ethyl acetate, acetone, ethanol, methanol and aqueous extracts of the plant material were tested against *Eicinia foetida* as test worms. The bioassay involved determination of the time of paralysis and time of death control. Albendazole was included as standard reference and normal saline as control. The results of this study strongly support the traditional anthelmintic use of the plant material in medicines. It clearly indicates that the crude acetone and methanolic extracts significantly demonstrated paralysis and also caused death of worms in dose dependent manner as compared to standard reference Albendazole. Ethanolic extract shows quite weak anthelmintic effect. Further studies are in progress to isolate the active principle/s responsible for the activity.

Key words- *Juglans regia* L, *Eicinia foetida*, Albendazole, Anthelmintic activity.

INTRODUCTION

Helminthiasis, the condition resulting from worm infestation, is one of the major prevalent diseases in the world, particularly in the tropical countries. Lack of adequate sanitary facilities and supply of pure water coupled with poverty and illiteracy are some of the factors responsible for widespread nature of this disease in the developing countries. The commonest parasites

observed in India are roundworms, hookworms, thread worms, tapeworms, guineaworms and filarial worms. They can cause loss of blood, nutritional deficiencies, urticaria and other conditions. The parasites can be acquired by contact with a) infected water b) infected meal c) infected animal. Filarial worms are transmitted via blood sucking mosquitoes. The parasites usually enter the body in the form of either larvae or the egg form. The greatest danger of the worm infection lies in the injuries to the vessels and organs which the parasites produce by burrowing into the tissues. Anthelmintics are drugs which possess the property of ridding the body off parasitic worms. The effectiveness of anthelmintic may be due to the fact that they help in eliminating the parasites causing the disease in various ways. An ideal anthelmintic must have a wide margin between its toxicity to the worm and its toxic effect on the host. The drug must be effective in one dose. Anthelmintic drugs can be classified according to their chemical structure as well as to their action against the specific type of helminthes [1]. As per WHO, only few drugs are frequently used in the treatment of these parasite infections [2]. Family Juglandaceae includes a valuable, medicinally useful species *Juglans regia* L, growing in the forests of Himalayas in India. The root, stem bark, leaves, seeds, cotyledons and seed oil are used to treat a variety of health complaints. The stem bark is reported to be alterative, anthelmintic, astringent, bactericide, depurative, digestive, diuretic, laxative, detergent, stimulant, tonic and insecticidal [3]. The dried bark of the tree is used as a tooth cleaner [4]. An attempt is made to evaluate the anthelmintic activity of different extracts of *Juglans regia* L. Further isolation of active components is under progress.

EXPERIMENTAL SECTION

Plant material:

The plant is obtained from local market. It is authenticated at Agharkar Research Institute, Pune, Maharashtra, India. A voucher specimen (No. 14319) was submitted at Institute's herbarium department. Finely powdered plant material is extracted with solvents EtOAc, Acetone, EtOH and MeOH till completion and these extracts are screened for activity.

Screening for phytochemicals:

Qualitative assay of the extracts for the presence of phytoconstituents such as carbohydrates, alkaloids, glycosides, flavonoids, tannins etc were performed following Standard procedure[5,6].

Chemicals:

Albendazole, Normal saline were purchased from authorized pharmaceuticals. The solvents and other chemicals used during experimental protocol were of analytical grade.

Animal:

Indian Earthworm species *Eicinia feotida* was collected from Mahatma Phule Agriculture University, Pune. All earthworms were of approximately equal size (14 cm).

Anthelmintic Assay:

The anthelmintic assay was carried out as per the method of Nargund [7] with minor modifications. The assay was performed on adult Indian Earthworm *Eicinia feotida* due to its anatomical and physiological resemblance with the intestinal round worm parasite of human being [8,9]. Different dilutions of Albendazole with normal saline were used as standard. Same

dilutions of acetone, ethanol and methanol extracts in normal saline solution were used for the assay and normal saline served as control. The time taken for complete paralysis and death was recorded. External stimuli were applied to ascertain the paralysis time. The time taken by worm to become motionless was considered as paralysis time and lethal time was ascertained by death of motionless worm followed by fading away of their body color.

RESULTS AND DISCUSSION

In the present study it was observed that all the extracts of *Juglans regia* have exhibited positive response to certain degree of anthelmintic activity. Acetone extract of plant material exhibits significant activity at all dilutions as compared to the standard. Ethanol extract is the least active among four extracts. The rate of paralysis as well as death is higher at more concentrated extracts. It means at higher concentrations time of paralysis and death is less as compared to lower concentrations. Phytochemical screening of the crude extracts revealed the presence of flavonoids and polyphenolics components. Chemically polyphenolic compounds are tannins which accomplish anthelmintic activity [10]. It is possible that tannins present in the extracts of *J. regia* L. have, just like synthetic phenolic anthelmintics, interfered with energy generation in helminth parasites by uncoupling oxidative phosphorylation [11].

Table-1: Anthelmintic Activity of *J. regia* L. extracts

Sr. No.	Test Sample	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
1	Control (Normal saline)	---	---	
2	Albendazole (Standard)	10	147 \pm 0.192	230 \pm 0.21
		20	136 \pm 0.189	205 \pm 0.213
		50	115 \pm 0.19	152 \pm 0.22
3	Ethanol extract	10	135 \pm 0.16	223 \pm 0.14
		20	115 \pm 0.161	190 \pm 0.145
		50	96 \pm 0.16	120 \pm 0.141
4	Ethyl acetate extract	10	125 \pm 0.181	192 \pm 0.249
		20	99 \pm 0.178	163 \pm 0.243
		50	50 \pm 0.179	94 \pm 0.24
5	Acetone extract	10	52 \pm 0.202	114 \pm 0.142
		20	30 \pm 0.208	70 \pm 0.143
		50	21 \pm 0.201	30 \pm 0.142
6	Methanol extract	10	100 \pm 0.137	133 \pm 0.177
		20	85 \pm 0.125	107 \pm 0.175
		50	42 \pm 0.127	65 \pm 0.171

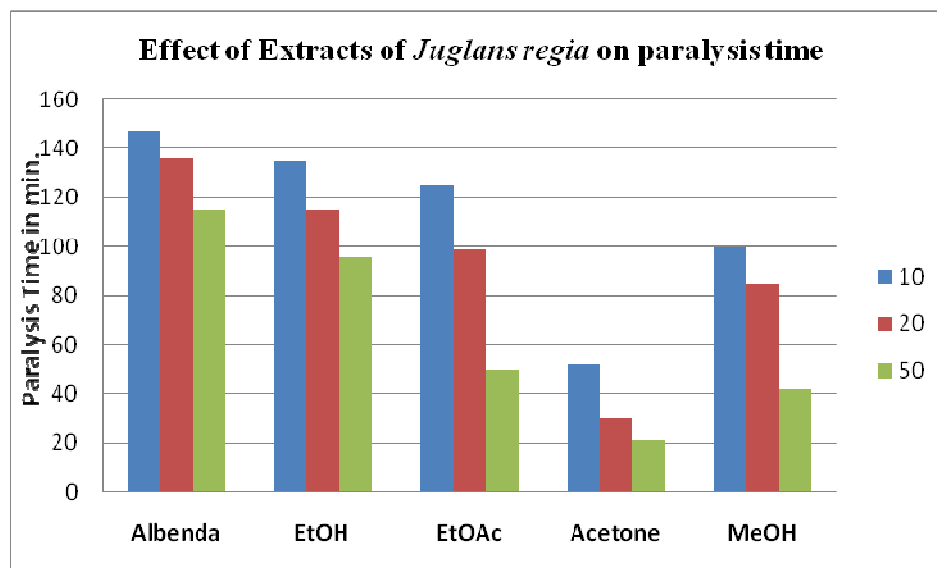
P < 0, 05 when compared to control. Values are expressed as mean \pm SEM

Tannins can bind to free protein in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death [12]. In conclusion *Juglans regia* L stem bark is powerful anthelmintic and acetone extract displayed profound anthelmintic activity in the assay. Further it

would be interesting to isolate the active phytoconstituents responsible for the anthelmintic activity. The results of anthelmintic activity are presented in **Table-1**.

The potent anthelmintic activity of various extracts under investigation can be viewed in **Fig. 1**.

Fig. 1 : Effect of extracts of *Juglans regia* L. on paralysis time



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