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Anthelmintic activity of leaves of different extracts of Gossypium herbaceum linn

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ABSTRACT

The N-hexane, ethyl ether and ethanol extracts of leaves of *Gossypium herbaceum* L was investigated for anthelmintic activity using earthworms (Pheretimaposthuma). Various concentrations (10,20,40,60,80 &100 mg/ml) of plant extracts were tested in the bioassay. Albendazole (10 mg/ml) was used as reference standard drug whereas 1% v/v tween 80 as control. Determination of paralysis time and death time of the worms were recorded. Theethyl ether and ethanol extracts exhibited significant anthelmintic activity at highest concentration of 60, 80 & 100 mg/ml compared to standard drug. The result shows that ethyl ether extract possesses potent vermicidal activity and found to be effective as an anthelmintic compared to ethanolic extract.

Keywords: Anthelmintic, *Gossypium herbaceum*, Albendazole, Earthworm and Pheretimaposthuma.

INTRODUCTION

In the recent years, the importance of Herbal drugs in Medicine has tremendously increased because of their fewer side effects. Consequently, the demand for the herbal formulation is increasing day by day. The phytochemical constituents and their standardization are accelerated with the development of instrumental analysis and this field becomes important and new for investigation.

Helminth infections are among the most widespread infections in humans, distressing a huge population of the world. As the half of world suffering from bacterial and Helminthes infection, the source of infection being very common due to poor sanitation, poor family hygiene, malnutrition, and crowded living conditions (Basu& Sharma 2005). Although the majority of infections due to helminths are generally restricted to tropical regions and cause enormous hazard to health and contribute to the

prevalence of undernourishment, anaemia, eosinophilia and pneumonia (Bundy1994). Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas (Tagbota&Townson2001). The gastro- intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases and treatment of helminthic infections becomes a major concern today (Sondhi&Shahu1994). Hence there is an increasing demand towards natural anthelmintics.

The disease is highly prevalent particularly in third world countries (Dhar et al 1982) due to poor management practices. Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world. However, increasing problems of development of resistance in helminthes (Geert et al 1995, Coles 1997) against anthel- mintics have led to the proposal of screening medicinal plants for their anthelmintic activity. The plants are known to provide a rich source of botanical anthelmintics (Satyavati et al 1976, Lewis 1977) A number of medicinal plants have been used to treat parasitic infections in man and animals (Nadkarnia 1954, Akhtar et al 200).

The Survey of literature reveals that the medicinal plant of *Gossypium herbaceum* Linn. Malvaceae is used in Ayurveda to treat various diseases. In Ayurveda the properties of Karpasa are Katu (Pungent), Kashaya (Astringent) in rasa; Laghu (Light), Tikshna (Penetrating) guna; Ushna (Hot) virya and Katu (Pungent) vipaka. It is used in Anartava (Amenorrhoea), Kashtartava (Dysmenorrhoea) and Prasutipashchatavikara (Purpueral disorders) (Chunekar et al 1982, Sharma et al 2001). Roots are thermogenic, emollient, abortifacient, emmenogougue, diuretic, haematopurative (Khare 2007)and root bark is anticancerous(Jain 1991). *Gossypium herbaceum* contains a Gossypol. Gossypol is a male contraceptive. It also assists menstrual flow and effectively inhibits egg implantation (Choudhry et al 1980, Krishna Reddy et al 1984). Gossypol and its derivatives have been shown to have significant antimicrobial activity as well as wound healing effect (Reddy et al 1981).In traditional system of medicine the tribals use various indigenous plants for the treatment of anthelmintic. One such plant drug used by tribals many parts of Andhra Pradesh is leaves juice of *Gossypium herbaceum* commonly called cotton plant is claimed by folklore for anthelmintic. With this view, the *Gossypium herbaceum* is studied for its anthelmintic activity.

MATERIALS AND METHODS

Plant materialprocurement of leaves

The leaves used for the investigation were collected from a medicinal garden at a place called sri Krishna chaithanya college of pharmacy, madanapalle in chittoor district, Andhra Pradesh. The leaves were shade-dried and powdered and stored in airtight containers further use.

Identification

The plant under investigation was identified with the help of taxonomists, Prof. Dr. T Damodharam, M.Sc, M.Phil, Ph.D, Assistant Professor, Sri Venkateswara University, Tirupathi.

Preparation of extracts

The collected leaves were shade dried completely. The dried leaf was then coarsely powdered and was sieved (sieve # 60) to get uniform powdered. The 1kg powder material extracted with solvent N-hexane, ethyl ether and80% ethanol in a Soxhlet apparatus. Final compound was concentrated by vacuum drying. The traces of the solvents were removed by keeping the dried extracts in to desiccators.

Preliminary phytochemical screening

The fractions of leaves of *Gossypium herbaceum*was screened for the presence of various phytoconstituents like alkaloids, flavonoids, saponin, tannin and glycosides etc. (Kokate 1986)

Methods of collection of earthworm (pheretimaposthuma)

The appropriate time for their collection was found early in the morning in the summer, and noontime during the winter. Freshly collected alive worms were stored in the plastic bags, filled with suitable quantity of wet compost soil.

Evaluation of anthelmintic activity

An Indian adult earth worm 4 -5 cm in length and 0.1-0.2 cm in width were used for the in vitro anthelmintic bio assay. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro (Sollmann 1918).The anthelmintic assay was carried out as per the method. (Ajaiyeoba et al 2001). The assay was performed in vitro using adult earthworm (Pheretimaposthuma) owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings for preliminary evaluation anthelmintic activity (Vigar 1984, Dash et al 2002, Shivakumar et al 2003)

The earthworms were divided into different groups containing six worms in each group.Control group received 1% v/v of tween80. The leaves of N-hexane, ethyl ether (EEEGH) and ethanol extracts (EEGH) of *Gossypium herbaceum* were dissolved in 1% w/v Tween 80, to give 10, 20, 40, 60, 80and 100 mg/ml respectively. Albendazole was used as the standard at 1, 2, 4, 6, 8 and 10mg/ml.10 ml of freshly prepared each extracts of *Gossypium herbaceum*, standard solution and 1% v/v tween 80 were poured into petridish. The worms were washed with saline and released into the petridish and the time taken for the worms to get paralyzed and killed was noted. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50^{o}C).

Statistical analysis

Experimental data are expressed as mean±standard error of mean (SEM). Statistical analysis was performed by one-way ANOVA followed by Dunnett's method of multiple comparisons was employed using Graph padInstat 3.0 software. Data were considered significant at $p< 0.05$.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical analysis of fractions of *Gossypium herbaceum*shows presence of steroids, alkaloids, flavonoids, glycosides, saponins, tannin and carbohydrate. (Table 1)

Anthelmintic activity

The results of anthelmintic activity are shown in Table 2 and Figure 1-3. The ethyl ether and Ethanolic extracts showed significant paralysis as well as death time at all concentrations. N-Hexane has no anthelmintic activity from 10 mg/ml to 60 mg/ml but 80 & 100 mg/ml shows effect but it is not significant when compared to standard drug albendazole. Among all the concentrations evaluated for the EEEGH 10, 20, 40, 60, 80 & 100 mg/ml solution, the 60, 80 and 100 mg/ml exhibited the most potent anthelmintic activity when compared with the positive control albendazole 10mg/ml. The 40 mg/ml of EEEGH revealed as like as standard. The same manner EEGH shows potent anthelmintic activity at the concentration of 80 and 100 mg/ml. The 60 mg/ml showed equal to standard but the ethyl ether extract is showed more potent anthelmintic activity than ethanol extract of *Gossypium herbaceum* with less concentration compared to ethanol extract.

Table 1: Phytochemical screening of methanolic and different fractions of *Gossypium herbaceum*

Extracts	Steroids	Alkaloids	Glycosides	Saponins	Flavonoid	Tannin	Carbohydrates	Phenolic compounds
N-Hexane	+	-	-	-	-	-	-	-
Ethyl ether	-	+	-	-	+	+	-	+
Ethanol	-	+	+	-	+	+	+	+

Table 2: *In vitro* anthelmintic activity leaves of various extracts of *Gossypium herbaceum*linn.

Groups	Treatment	Concentration in mg	Time taken for paralysis in minutes	Time taken for death in minutes
1	Control	1% v/v tween 80	-	-
2	Standard (Albendazole)	1	37±0.57	50±0.54
		2	32±0.46	47±0.24
		4	27±0.86	42±0.61
		8	21±0.22	34±0.86
		10	18±0.22	27±0.47
3	NHEGH	10	-	-
		20	-	-
		40	-	-
		60	- 108±1.20	- 129±1.57
		80	93±1.45	111±1.63
		100		
4	EEEGH	10	35±0.50	42±0.45
		20	24±0.12	36±0.28
		40	18±0.24	24±0.43
		60	14±0.81	19±0.17
		80	09±0.26	14±0.51
		100	06±0.18	10±0.34
5	EEGH	10	42±0.87	64±0.94
		20	31±0.71	50±0.10
		40	24±0.46	39±0.84
		60	18±0.23	27±0.61
		80	13±0.41	19±0.47
		100	11±0.58	16±0.78

Results are expressed as mean ± S.E.M. (n=6) for each group; significance at $p < 0.05$, as compared to Standard

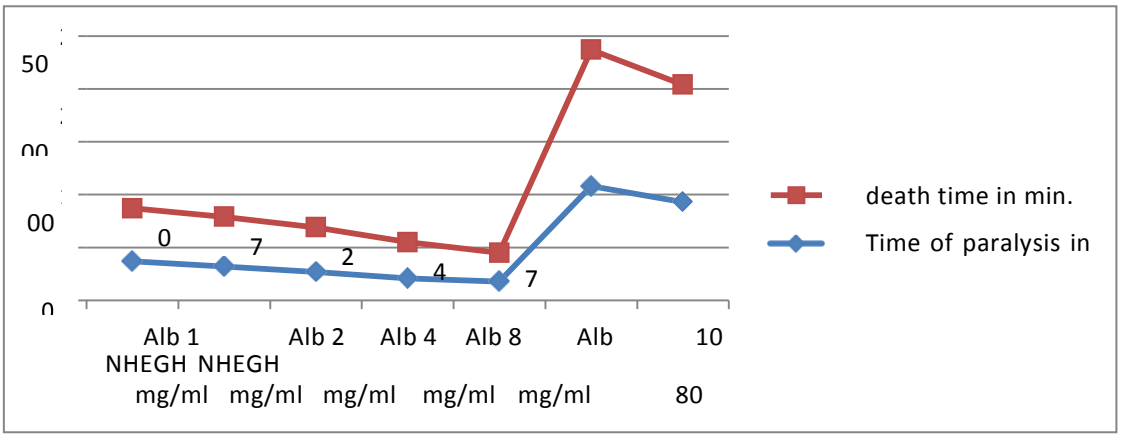

Figure 1 Paralysis and death time of N-Hexane extract of *Gossypium herbaceum* compare to Albendazole.

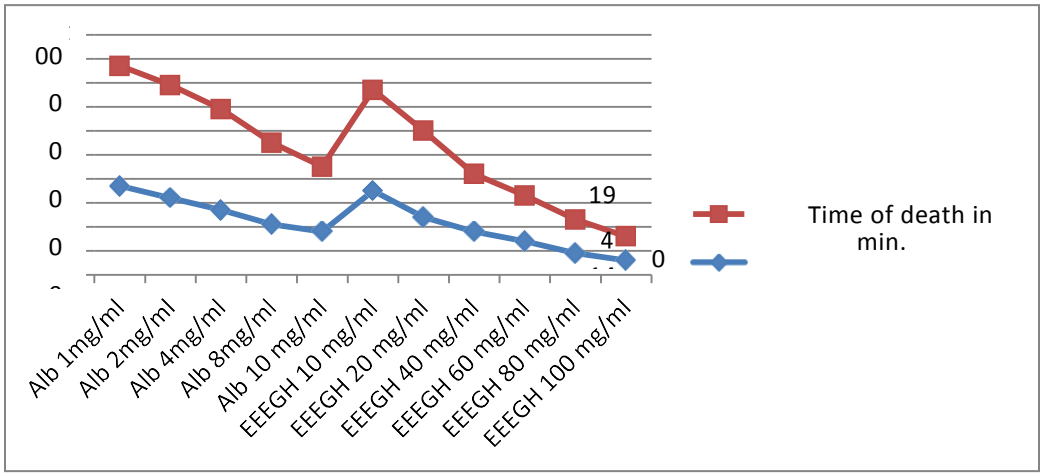

Figure 2: Paralysis and death time of ethyl ether extract of *Gossypium herbaceum* compare to Albendazole.

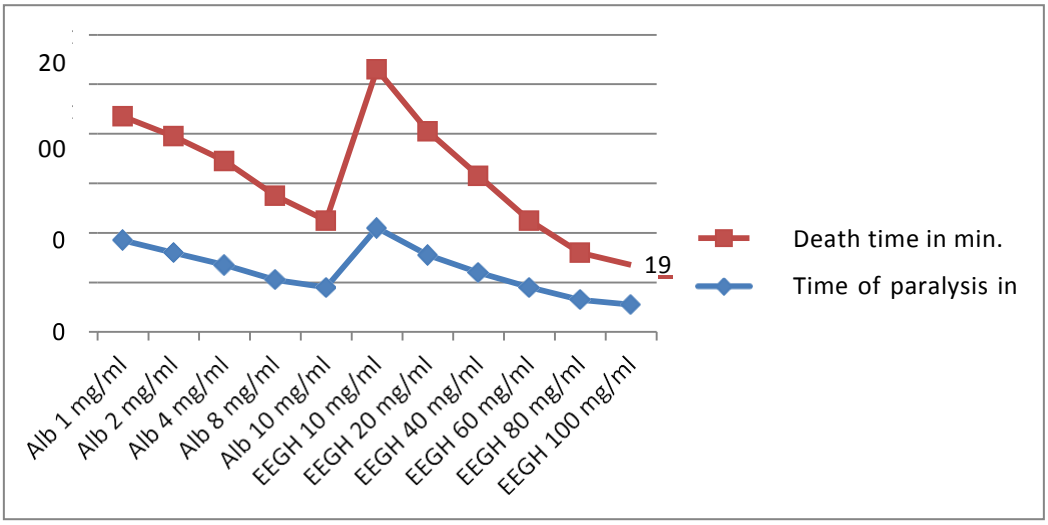

Figure 3: Paralysis and death time of ethanol extract of *Gossypium herbaceum* compare to Albendazole.

DISCUSSION

The predominant effect of albendazole on Primary action is binding to beta tubulin and thus inhibition of microtubule polymerisation. The action is more specific to parasitic beta-tubulin than that of host. Immobilisation& death of parasites occur slowly and they produce many biochemical changes in susceptible Nematodes Inhibition of higher concentration of 100 mg/ml. While hexane extract has shown no significant activity. Preliminary phytochemical screening of hexane extract revealed the presence of steroids while ethyl ether and ethanol extracts showed presence of glycosides, alkaloids, flavanoids, tannins and phenolic compounds. Earlier studies proved that the polyphenolic compounds show anthelmintic activity (Niezen et al 1995) and some synthetic phenolic anthelmintics e.g. niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation (Martin 1997). Polyphenols from bryophytes were shown to have anthelmintic activity against Nippostrongylusbrasiliensis (Gamenara et al 2001).mitochondrial fuma Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal (Athnasiadouet al 2001) or glycoprotein on the cuticle of the parasite (Thompson et al 1995) and cause death.

So the above point supported the anthelmintic activity of ethyl ether and ethanol extract of

Gossypium herbaceum might be due to phytoconstituents like tannins and phenolic compound once again it was confirmed by the hexane extract was not shown significant activity it may be due to absence of phytoconstituent like phenolic compound. The mechanism of EEEGH and EEGH interfere with polymerization of tubulin and energy production in helminthiasis.

CONCLUSION

The anthelmintic/wormicidal activity of various extracts of leaves of *Gossypium herbaceum*suggests that it is effective against parasitic infections of humans. Further, in future it is necessary to isolate the possible active phytoconstituents responsible for the anthelmintic activity mainly phenolic compound and study its pharmacological actions.

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