Determination of Anthelmintic Activity of the Leaf and Bark Extract of *Tamarindus Indica* Linn

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The aim of the present study was to evaluate the anthelmintic activity of ethanolic and aqueous extract of leaves and bark of *Tamarindus indica* Linn using *Pheretima posthuma* and *Tubifex tubifex* as test worms. The time of paralysis and time of death were studied and the activity was compared with piperazine citrate as reference standard. The alcohol and aqueous extract of bark of *Tamarindus indica* exhibited significant anthelmintic activity as evidenced by decreased paralyzing time and death time. The results thus support the use of *Tamarindus indica* as an anthelmintic agent.

Key words: Anthelmintic activity, *Pheretima posthuma*, piperazine citrate, *Tamarindus indica*, *Tubifex tubifex*

Helminth infections are among the commonest infections in man, affecting a large proportion of the world's population. In developing countries they pose a major threat to public health and contribute to the prevalence of malnutrition, anemia, eosinophilia, and pneumonia. Anthelmintics are drugs that either kill or expel infesting helminths and the gastrointestinal tract is the abode of many helminths, although some also live in tissues, or their larvae migrate into tissues. They harm the host by depriving him of food, causing blood loss, injury to organs, intestinal or lymphatic obstruction and by secreting toxins. Helminthiasis is rarely fatal, but is a major cause of morbidity[1].

Tamarindus indica is a tree belonging to the family Caesalpiniaceae whose different parts are used as traditional medicine as analgesic, antiinflammatory, diuretic, febrifuge, and anthelmintic, antifungal and in gastrointestinal problems[2]. The anthelmintic activity of leaves has been reported by Sampat *et al.*[3] but the anthelmintic activity of the bark extracts of *Tamarindus indica* has not been evaluated. Accordingly, this prompted us to investigate the anthelmintic activity of *Tamarindus indica* bark extracts in comparison to the leaf extract.

The reference standard drug used in this experiment is piperazine citrate. It causes hyperpolarization of muscle by its GABA agonistic action opening Cl^- channels that causes relaxation and depresses responsiveness to contractile action of acetylcholine thereby flaccid paralysis occurs. The worms recover if placed in a piperazine free medium[4].

The plant material was collected from the village Radhamadhabpur of District Bankura in September during morning hours between 09.00 AM and 10.00 AM. They were identified at the Botanical Survey of India, Shibpur, Howrah. Reference Number of the Authentication Letter is CNH/I-I/ (20)/2009/Tech.II/29. The shade-dried leaves and bark were pulverized into coarse particles and extracted with water by maceration (5% chloroform water) and with absolute ethanol using Soxhlet extractor for 72 h. Both the aqueous and alcohol extract were concentrated in a rotary evaporator at a temperature less than 45° and preserved in desiccators for further use. The yield for alcoholic extract and aqueous extract were 49.40% and 47.08%, respectively. The preliminary phytochemical analysis[5,6] were carried out to find out the phytoconstituents present in the crude extracts.

Indian earthworm *Pheretima posthuma* (Annelida) were collected from the water logged areas of soil, the average size of earthworm being 6-8 cm. They were washed with tap water for the removal of the adhering dirt. Aquarium worms *Tubifex tubifex* (Annelida) were collected from the local market. The average sizes of the worms were 1-1.5 cm. The anthelmintic assay was carried as per the method of Ajayieoba *et al*[7]. with minor modifications. The assay was performed on adult Indian earthworm *Pheretima posthuma*, due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. *Pheretima posthuma* worms are easily available and used as a suitable model for screening of anthelmintic drug. The assay was also performed on the aquarium worm, *Tubifex tubifex*, because they belong to same group of Annelida.

Briefly, 20 ml formulations containing three different concentrations, each of crude alcoholic and aqueous extract of bark and leaf (5, 10 and 15 mg/ml in double distilled water) were prepared and six earthworms (same size) and approximately 5 g of *Tubifex* worms were placed in it. Both the test solution and standard drug solution were freshly prepared and 'time for paralysis' was noted when no movement of any sort could be observed

TABLE 1: ANTHELMINTIC ACTIVITY OF ALCOHOLIC AND AQUEOUS EXTRACT OF LEAF AND BARK OF *TAMARINDUS INDICA*

Groups	Conc. (mg/ml)	*Pheretima posthuma*		*Tubifex tubifex*	
		Paralyzing time (min)	Death time (min)	Paralyzing time (min)	Death time (min)
Distilled water	-	-	-	-	-
Bark extract (alcohol)	5	60.66±0.67*	80.66±0.67*	63.00±2.08*	75.33±1.45*
	10	36.33±0.88*	66.33±0.88*	31.33±0.67*	39.33±0.88*
	15	22.33±0.88^{ns}	45.00±0.58*	14.66±0.88*	20.66±1.33*
Bark extract (aqueous)	5	>120	NA	67.66±2.60*	81.00±1.00*
	10	89.33±1.76*	115.33±0.88*	53.00±2.52*	74.33±2.08*
	15	58.33±1.45*	87.66±1.45*	23.00±1.73^{ns}	28.00±1.15*
Leaf extract (alcohol)	5	>120	NA	>120	NA
	10	>120	NA	>120	NA
	15	>120	NA	>120	NA
Leaf extract (aqueous)	5	>120	NA	>120	NA
	10	>120	NA	>120	NA
	15	>120	NA	>120	NA
Piperazine citrate	10	25.00±1.16	64±0.88	22.66±1.76	45.33±1.20

NA=Not applicable. Results are expressed as mean±SEM. N=3; *P<0.05, as compared to standard, ns= not significant.

except when the worms were vigorously shaken. The 'time for death' of worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50°. A maximum time period of 120 min was ascertained for the paralyzing as well as death time of *Pheretima posthuma* and *Tubifex tubifex* worms. Piperazine citrate (10 mg/ml) was used as reference standard with distilled water as the vehicle control. All experiments were repeated thrice. The mean and SEM were analyzed statistically by ANOVA followed by Dunnett's test, $P<0.05$ being considered as significant.

From the observations made, a dose dependent paralytic effect much earlier and the time of death was observed (Table 1). Although, both extracts showed anthelmintic activity in a dose-dependent manner but the alcoholic extract appeared to be more effective for both types of worms. Evaluation of anthelmintic activity was compared with reference standard piperazine citrate. The alcohol extract of the bark of *Tamarindus indica*, caused paralysis at 22.33 min. and time of death at 45.00 min. for *Pheretima posthuma* and 14.66 min as paralysis time and 20.66 min as death time for *Tubifex tubifex* worms respectively. With the aqueous fractions treatment of earthworm *Pheretima posthuma* and worm *Tubifex tubifex* resulted in a paralysis time of 58.33 and 23.00 min respectively while time of death was 87.66 and 28.00 min, respectively. The reference drug piperazine citrate showed the time of paralysis and time of death as 25.00 and 64.00 min, respectively. Considering the alcoholic extract of bark showed comparable activity, it would be important to identify the key phytoconstituents.

Phytochemical analysis of the crude extract revealed the presence of tannins along with other chemical constituents contained within them. Tannins have been reported to produce anthelmintic activities[8,9], as they can bind to free proteins in the gastrointestinal tract of host animal[10] or glycoprotein on the cuticle of the parasite and thereby cause deaths[11]. The wormicidal activity of the aqueous and alcohol extract against earthworms suggests that it is effective against parasitic infections of humans[12]. It would be interesting to identify the active principle responsible for the anthelmintic activity and to study its further pharmacological actions.

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