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In-Vitro Anti-Helmentic and Thrombolytic Activities of Ripe and Unripe Neem Fruit Pulp

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ABSTRACT

Reason: The present study is aimed to verify the anthelmintic and thrombolytic activities of ripe and unripe neem fruit pulp concentrates by using *In-vitro* pharmacological models as many tribes of our area are consuming these fruits to treat their ailments.

Main Findings: Three different concentrations of ripe (RNFP) and unripe (URNFP) neem fruit pulp i.e.; 10, 20 & 40 mg/ml solutions were tested for their anthelmintic & thrombolytic activities. The RNFP shows dose dependent anthelmintic action on the earth worms. High concentration of RNFP (40 mg/ml) shows significant anthelmintic activity than the standard, URNFP shows lesser anthelmintic action than the RNFP. Both the fruit pulps does not show any sort of thrombolytic action.

Conclusion: Anthelmintic activity of ripe and unripe neem fruit pulp was confirmed by examining the paralysis time and the death time of the Indian earth worms (*Pheretima posthuma*) were reported. High concentrated RNFP (40 mg/ml) shows more significant anthelmintic activity in the view of death time than the standard but Thrombolytic action of the fruit and unripe fruit of neem does not show any sort of clot lysis. This may be because of the usage of the whole pulp without extraction. So further we are planning to evaluate the both thrombolytic and anthelmintic activities by using different extracts of neem fruit pulp for the better activity.

KEY WORDS: *Azadirachta indica*, Anthelmintic activity, *Pheretima posthuma*, Albendazole, Thrombolytic activity, Streptokinase.

1. INTRODUCTION

WHO estimated that 80% of the population of developing countries based on plant based traditional medicines for their primary health care needs. Modern pharmacopoeia contains at least 25% of drugs derived from plants and some others are synthetic analogs of proto type compounds isolated from plants. Due to growing recognition of natural products demand of medicinal plants is increasing in both developing and developed countries (Lichterman, 2004). Neem tree *(Azadirachta indica)* is a famous tree and known to all the population as a medicinal source and it is belongs to the family *Meliaceae*. Even it is a native of Indomalaysian region, it is available most of the world, even cultivated in India as a fire wood and as a medicinal source. In Indian traditional medicine, the resin from the trees has been accredited with medical benefits. The active components of neem are effective as insecticide, anti-fungal, anti-bacterial. Some other traditional uses of different parts of neem are – seeds to treat head lice, neem oil as antiseptic, for treating furuncles, eczema and to treat intestinal worm infections (Forster, 2000). Moreover, the neem tree branches are used as one of the most effective forms of dental care in traditional medicines (Prashant, 2007). The entire neem plant is used to cure different ailments, such as stomach ulcers, jaundice and to overcome a variety of infectious and parasitic diseases, ranging from leprosy, chicken pox, and malaria. Infusions and teas made from leaves are used to alleviate malaria attacks, intestinal complaints, treat dental, headache, stimulating the appetite, heartburn and as insects repellent, in addition to that it was also used as a diuretic and for diabetes (Sujarwo, 2016). The neem fruits were consumed by many people to treat some of the worm infestations, so to give a scientific basis to the anthelmintic activity of this use we conducted this study.

2. MATERIALS & METHODS

Collection and Identification of Plant Material: The neem fruit & unripe fruits were collected from the neem trees present in the Mother Teresa College campus and they were identified by Dr. N. Dorababu, Head, Dept., of Pharmacognosy, Mother Teresa Pharmacy College, Sathupally.

Preparation of Pulp Concentrate: Both the ripe and un ripe neem fruits were collected, peeled off and pulp was made to grind by using mixer grinder without adding water and the juice/paste obtained was concentrated by spreading in the surface of petri dishes for a whole day. Then the concentrated layers/paste was collected and preserved in a refrigerator for the further usage. Three different concentrations of ripe (RNFP) and unripe (URNFP) neem fruit pulp i.e 10, 20 & 40 mg/ml solutions were prepared just before starting the assay.

Collection of Earthworms: The Indian earth worms (*Pheretima posthuma*) were collected from the vermi compost sheds present at Aswaraopeta. The average length of earthworm was maintained in between 6-8 cm. The adhering dirt of worms was removed by washing with tap water just before starting the experiment.

Anthelmintic Activity: Collected earth worms were washed to remove the clay sticks to its surface. Then prepare the test concentrations i.e. 10, 20 & 40 mg/ml of ripe fruit & the concentrations of unripe neem fruit just before

starting the experiment. Use six petri dishes for each concentration. Pour nearly 30ml of the prepared concentration of the fruit and unripe fruit pulp into the petri dishes and place a single earth worm into each petri dishes then note down the time of placing of earth worm in to the petri dish. Then observe a paralysis and death time of warms. The paralysis was conformed if the warm was not moving even with the vigorous shaking of the petridishes or no movement observed by dipping in to the normal saline. Death time was confirmed by fading of colour of the worm or after ascertain that the worms neither move with vigorous shaking nor when dipped in warm water (50°C). Distilled water was used as control & Albendazole (10, 20 mg/ml) used as standard. Both the paralysis and death times of the worms treated with the test and standard compounds were tabulated (Ajaiyeoba, 2001).

Streptokinase: Streptokinase (15,00,000 I.U.) used as standard which was collected from Sigma. Streptokinase was dissolved by using 5ml of sterile water for injection. 100µl (30,000 I.U) was used from the above solution as a standard for *in-vitro* thrombolytic activity.

Thrombolytic activity: Blood samples were collected from the healthy volunteers of among our study persons and 0.5ml sample was taken into pre weighed sterile micro centrifuge tubes (W1) and incubate at 37^0C for 45 min. Centrifuge if necessary. Separate the serum and weigh the micro centrifuge tube with clot (W2). Calculate clot weight (a=W2-W1). Then add 100µl of test solution to clot containing micro centrifuge tube (n=6), Distilled water was used as negative control and Streptokinase was used as standard i.e. positive control (30,000 IU). After addition of the compounds incubate the micro centrifuge tubes at 37^0C for 90 min. and observe the clot lysis then invert the micro centrifuge tube to collect the dissolved clot. Aspirate the dissolved clot and the added drug solutions completely without disturbing the clot and then weigh the clot remained with micro centrifuge tube (W3). The weight of dissolved clot was calculated as b= (a -W_3). The values were tabulated and percentage of thrombolytic activity was calculated (Ramjan Ali, 2014).

$$\% \text{ of clot lysis} = \frac{\text{Wt. of dissolved clot (b)}}{\text{Wt. of clot (a)}} \times 100$$

Both the anthelmintic and thrombolytic data was tabulated and analyzed by using one way ANOVA.

3. RESULTS AND DISCUSSION

Anthelmintic activity of ripe and unripe neem fruit pulp was confirmed by examining the paralysis time and the death time of the Indian earth worms (*Pheretima posthuma*) were reported in the table.1. As the neem tree & its parts like leaves, bark & stems are having wide pharmacological actions like anti-bacterial, antifungal, analgesic, antidiabetic etc. and the fruits are edible and used by the people to treat some gastro intestinal problems, we planned for the anti-helmentic activity by using *Pheretima posthuma* due to its anatomical & physiological resemblance with the intestinal roundworm parasite of human beings. Earth worms have been widely used for the primary evaluation of anthelmintic activity because of their ease availability.

Albendazole was used as standard drug in this bioassay; it acts by blocking the glucose uptake in the parasite and depletion of its glycogen stores. The site of action of the albendazole was appearing to be the micro tubular protein β-tubulin of the parasite. It binds to β-tubulin of susceptible worms with high affinity and inhibits its polymerization there by there is a gradual loss of intracellular microtubules in the cells of the worm.

Three different concentrations of ripe (RNFP) and unripe (URNFP) neem fruit pulp i.e. 10, 20 & 40 mg/ml solutions were prepared just before starting the assay. The RNFP shows dose dependent anthelmintic action on the earth worms. High concentrated RNFP (40 mg/ml) shows more significant anthelmintic activity in the view of death time than the standard. Results shown in the table.1 and Graph.1 & 2.

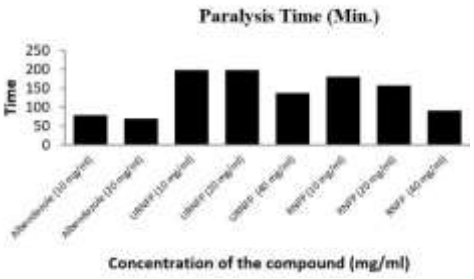
Thrombolytic activity: Platelets play a significant role in the development of atherothrombosis as well as damage the regions of endothelial surface (produced by reactive oxygen species). The stimulated platelets bind to leucocytes carrying them in to intricate process of plaque development and progression. Plasmin, a natural fibrinolytic agent, lyses clot by breaking down the fibrinogen and fibrin contained in a clot. Streptokinase forms a1:1 stoichiometric complex with plasminogen to plasmin (Ramjan Ali, 2014).

In the present study the thrombolytic activity of neem fruits were checked to prove their efficiency of dissolves the clot formed. There by it may give us the information related to the cardio protective activity of the selected fruit. The results were tabulated in table.2 & Graph.3.

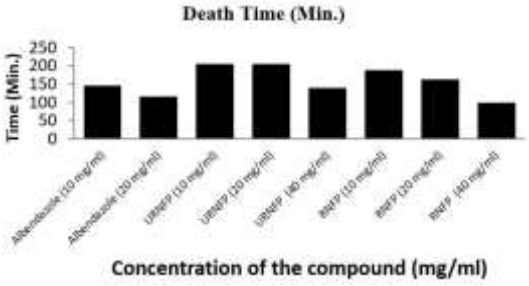
The results shows that the standard drug 100 µl Streptokinase (30,000 I.U) shows 39.05 percentage clot lysis at 37^0C, for an incubation period of 90 min. control group treated with the normal saline shows very negligible thrombolytic activity i.e. 2.6%. All the remaining test concentrations also shows very negligible % clot lysis.

Table.1. Paralysis & Death time of the compounds.

Name of the compound & Conc.	Paralysis Time (Min.)	Death Time (Min.)
Distilled Water	-	-
Albendazole (10 mg/ml)	78.33 ± 4.4	145.5±3.3
Albendazole (20 mg/ml)	70±5.06	117±3.69
URNFP (10 mg/ml)	198.3±2.05	203.8±1.99
URNFP (20 mg/ml)	198.5±1.83	205.1±1.02
URNFP (40 mg/ml)	138.1±2.19	138.6±1.49
RNFP (10 mg/ml)	181.2±1.34	187±4.7
RNFP (20 mg/ml)	158.2±1.21	161.6±1.48
RNFP (40 mg/ml)	92.16±1.34	99.2±1.89



Graph.1. Paralysis time of the neem fruit & Standard Drug

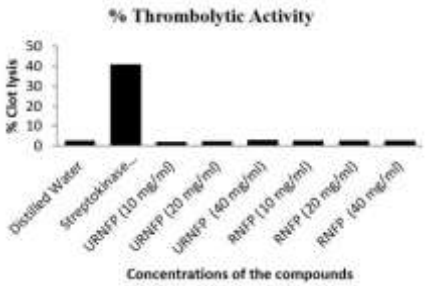


Graph.2. Death time of the neem fruit & Standard Drug

All the three concentrations of RNFP shows dose dependent action. High concentrated RNFP (40 mg/ml) shows more significant anthelmintic activity in the view of death time than the standard

Table.1. Thrombolytic activity of Neem fruit pulp & Standard drug

Name of the compound & Conc.	% clot lysis
Distilled Water	2.6
Streptokinase (30,000 I.U)	39.05
URNFP (10 mg/ml)	2.1
URNFP (20 mg/ml)	2.5
URNFP (40 mg/ml)	2.9
RNFP (10 mg/ml)	2.6
RNFP (20 mg/ml)	2.6
RNFP (40 mg/ml)	2.8



Graph.3. Percentage clot lysis of neem fruit pulp & Standard drug

Only the standard drug shows clot lysis, the fruit and unripe fruit of neem does not show any clot lysis.

4. CONCLUSION

Anthelmintic activity of ripe and unripe neem fruit pulp was confirmed by examining the paralysis time and the death time of the Indian earth worms (*Pheretima posthuma*) were reported. High concentrated RNFP (40 mg/ml) shows more significant anthelmintic activity in the view of death time than the standard but Thrombolytic action of the fruit and unripe fruit of neem does not show any sort of clot lysis. This may be because of the usage of the whole pulp without extraction. So further we are planning to evaluate the both thrombolytic and anthelmintic activities by using different extracts of neem fruit pulp.

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