SOME PHYSIOLOGICAL AND BIOCHEMICAL STUDIES ON THE EFFECTS OF THE FRUITS OF A PISCICIDAL PLANT Zanthoxylum armatum DC. (Z. alatum Roxb.) ON FRESHWATER FISHES

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ABSTRACT

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ABSTRACT

Many toxins both of natural and synthetic origin are being used in the modern fisheries to clear ponds from unwanted aquatic organisms prior to using them for fish nursery purposes, and to catch and transport fishes. The toxins which are used for killing the fishes are called as piscicides. The synthetic piscicides when used show both acute and chronic effects on non-target aquatic organisms. Their biodegradability is slower and residual toxicity is higher compared to natural piscicides. Therefore, the use of natural piscicides have been prefered in fishery management. One of the natural piscicides commonly used throughout the world is a phytotoxin called rotenone. Rotenone is obtained from the plants mainly belonging to the genus Derris. In India, rotenone is not available easily and is being imported for fishery purposes. Therefore, studies have been initiated on various plants/plant products to develop suitable alternative indigenous natural piscicides.

More than hundred plants available in this country have been cited to have piscicidal property. Only a few of these plants and their parts have been investigated to establish their piscicidal potentiality. However, no systematic approach has been made to investigate scientifically the nature of toxicity and mode of action of any indigenous plant or plant material. Therefore, the present study was undertaken to find out the toxicity of some of the potential piscicidal plants used in native fisheries of North-Eastern India and the mode of action of the most potent plant part on certain freshwater fishes.

A survey of the plants used for fish killing by the natives of Meghalaya was conducted. Ten plants were collected, identified and their toxicity was screened on two species of gill-breathing (Puntius shalynius and Danio dangila) and one species of hardy air-breathing (Heteropneustes fossilis) fish. The results of the exploratory studies indicated that among the plants/plant parts screened the fruits of Zanthoxylum armatum DC. (= Z. alatum Roxb.) was most potent as a piscicidal agent with minimum effective concentration and lethal time. Therefore, further studies on extraction, bio-assay and mode of action of Z. armatum fruit were conducted. The extraction of the piscicidal factors of Z. armatum fruit was done by the Medical Chemistry Division of Central Drug Research Institute, Lucknow. The concentrated water soluble alcoholic extract prepared had retained about 95% toxicity as observed in the bio-assay experiments.

The bio-assay studies were done following standard static bio-assay method to find out the lethal dose of the toxicant using both crude powder and alcoholic extract on P. shalynius and only alcoholic extract on two different weight groups of H. fossilis. The results of bio-assay experiments showed that the toxicity of Z. armatum fruit extract varied with the behaviour and size of the fish. The air-breathing fish were more resistant than gill-breathing fish and among the air-breathing fish, the higher weight group was more resistant. The effect of alcoholic extract and crude powder on P. shalynius indicated that the alcoholic extract had sharper action than

crude powder. The LC_{50} values were calculated following three different statistical methods (by graphical interpolation using regression formula Y = a + bX, semi-logarithimic method and probit analysis) to reach a near accurate point. The 6 hr LC_{50} value of Z. armatum for P. shalynius with crude powder was found to be 95 ppm and with alcoholic extract 6.4 ppm. The average values of 3, 6, 12 and 24 hrs LC_{50} for H. fossilis were 18.9, 17.2, 14.00 and 14.00 ppm in 8 - 12 g weight group and 30.35, 20.00, 17.45 and 16.95 ppm in 13 - 20 g weight group respectively.

The fishes showed a typical behavioural pattern on treatment with the toxin. The fish became more active in the initial phase of treatment and then showed erratic movements. The opercular movement increased along with the activity of the fish. As the fish approached lethal phase, the opercular movement decreased, the fish failed to reach the surface, lost the equilibriu and finally collapsed in an upside down posture at the bottom of the jar. If the fishes were transfered to freshwater at the beginning of lethal phase they recovered slowly.

The effect of the extract on the hematological paramters of H. fossilis was studied at different concentrations of the toxin. The relationship of hematological parameters with size of the fish were studied and was found to be quite variable. Therefore, it has been suggested that it is necessary to establish the intraspecific variations in hematological parameters in a particular species of fish before they are used for toxicity studies. The alcoholic extract of the fruits of Z. armatum did not show any effect on the hematological parameters studied in H. fossilis. Therefore, it was felt that the lethal

effect might be due to the damage done by the extract to some other physiological process(es).

The effect of the extract of the fruits of Z. armatum on oxygen consumption by the fish and the oxygen uptake at sub-cellular level were investigated. The experiments were conducted at organismal level using a weed fish Tilapia mossambica and at cellular level using liver and brain tissue homogenates of H. fossilis. The gross oxygen uptake was calculated from the changes in oxygen concentration of treated water estimated by Winkler's method. The effect on tissue oxygen uptake was studied in Warburg manometers using glucose as the substrate at two different temperatures. The results indicated that there was no effect of the extract on tissue respiration at sub-cellular level and the total oxygen consumption of fish was inhibited indirectly on treatment with the extract. The inhibition of oxygen uptake from water by the fish might be due to the paralysing effect of the toxicant on the different organs related to oxygen uptake. The general metabolic activity of the animal might have also been affected by the extract as total oxygen consumption of fish is indicative of general metabolism.

The results of the above experiments indicated that the extract of Z. armatum fruits might be affecting the nervous system and energy metabolism at sub-cellular level to result in different behavioural and physiological manifestations leading to death. Hence, studies on the effect of the extract on the activities of acetylcholinesterase (AchE), Mg^{2+} - and Na^{+}, K^{+} - ATPase in vivo and in vitro in different tissues of H. fossilis

were carried out. The activities of the enzymes were studied with relation to the concentration of the toxin and duration of treatment in different tissues of H. fossilis. The results showed definite inhibition of the enzymes studied by the extract. The inhibition were both related to the concentration of the extract and time of treatment. However, the maximum inhibition which was about 50% in the different enzymes studied did not change after a certain concentration of the extract. At sub-lethal concentration the enzyme activity showed gradual recovery to normalcy. Though the pattern of inhibition of different enzymes with concentration and time were similar, the initiation, the peak and the recovery were different for different enzymes and tissues. The AchE activity was inhibited maximally to about 40% in the three tissues. ATPase showed tissue specific variations in the rate of maximum inhibition in the following order (a) total ATPase gill > muscle > brain (b) Mg^{2+} ATPase - gill > muscle > brain (c) Na^{+}, K^{+} ATPase - gill > muscle > brain. In general, the activities of ATPase were maximally inhibited earlier than the AchE. However, at the initial stages the AchE was inhibited more than ATPase.

The in vitro studies with different concentrations of the extract indicated that the inhibition of the enzyme activities needed a higher concentration of the toxin than the in vivo inhibition. This could be due to the fact that the in vivo inhibition was the result of amplification of the effect of the extract through some other mechanism.

The kinetic studies on AchE and Mg^{2+} ATPase showed that the nature of inhibition was non-competitive for both the enzymes. However, the K_i of the extract for AchE was higher than for Mg^{2+} ATPase. The apparent K_m (Michaelis constant) of brain and muscle AchE for acetylthiocholine iodide was found to be 7.1×10^{-5}M and 8.85×10^{-5}M respectively. They decreased with eserine but did not change with Z. armatum fruit extract. The apparent K_m of brain, muscle and gill Mg^{2+} ATPase for ATP were found to be 9.72×10^{-4}M, 8.71×10^{-4}M and 7.1×10^{-4}M respectively. They decreased with KSCN but increased with Z. armatum fruit extract. The specific staining of AchE iso-enzymes on polyacrylamide gels showed only one band in brain and two bands in muscle of H. fossilis indicating that this tissue specific molecular variation in AchE might be the cause of the tissue specific differences in the enzymes activity and effect of the extract.

It is, therefore, concluded that the fruits of Z. armatum acts as a mild metabolic poison. It does not have significant effect on hematological parameters studied nor on cellular respiration process in fish. The primary modes of action seem to be through the blockade of neural transmission and supply of metabolic energy by inhibiting activities of AchE and ATPases. Though Z. armatum fruits showed a promise to be used as an indigenous substitute for rotenone, some work on its secondary toxicity and effects on plankton population are necessary before this could be tried in the field.