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ACRYLAMIDE TOXICITY STUDY ON NEUROPROTECTIVE EFFECTS OF *ERYTHRINA INDICA* USING *LUMBRICUS TERRESTRIS*

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ABSTRACT

Acrylamide (ACR) is a water soluble white crystalline solid commonly used in industries that has neurotoxic effects. ACR is found in food items that are cooked under high temperatures. *Lumbricus terrestris* (common earthworm) was selected as the target organism as it also has 'Central Nervous System'. *Erythrina indica'* was used as an inhibitory drug, as it has Anti-inflammatory properties and is beneficial for the nervous system. The present study attempts to assess the neurotoxic effects in cerebral ganglions along with the morphological, locomotory and neurological behavior of *Lumbricus terrestris* on Acrylamide intoxication along with the inhibitory effect of *Erythrina indica.*

KEY WORDS: Acrylamide, *Erythrina indica*, *Lumbricus terrestris*, Neurotoxin.

INTRODUCTION

Acrylamide: Acrylamide [C3H5NO] is a vinyl monomer and crystalline solid which is soluble in water. More recently, acrylamide was found to form naturally in foods that are cooked at a high temperature that is 120°C or above (Halford NG, et al., 2012). Heating of food will induce chemical reactions that lead to formation of heat-induced toxic components which are called thermal process contaminants (Mogol BA, et al., 2016). One such contamnant that received much scientific interest is acrylamide.

The Swedish National Food Administration (SNAF) announced that prolonged heat treatment of some foods, particularly starchy foods such as potato and grain products could create significant amounts of acrylamide (Arisseto AP, et al., 2007). After this announcement by SNAF, much attention was given on the Maillard Reaction which involves two natural components namely, the reducing sugar and the amino acid asparagine (Zhang Y, et al., 2009). Maillard reaction is a complex series of reactions that occurs during the thermal processing of food.

According to WHO reports, there is daily contact of people with dietary acrylamide with doses near to 0.3-2 micg/kg (Besaratinia A, et al., 2007). Major consequence of acrylamide is neurotoxicity when exposed to it. This compound is a cumulative neurotoxicant in rodents as well as in humans where it induces apoptosis and causes mitochondrial dysfunction. General symptoms of neurotoxicity in humans include Skeletal muscle weakness, weight loss, ataxia, degeneration of axons in central and peripheral nervous system (Calleman CJ, et al., 1994; Hagmar L, et al., 2001).

Erythrina indica: *Erythrina Indica* (Indian coral tree) is a showy, spreading tree. It is a part of the legume family(fabaceae). It is a nitrogen fixer, which uses atmospheric nitrogen and makes the soil around the tree nitrogen rich.

In a report it was mentioned that the seeds of the E.indica showed the presence of flavoneglycoside5,7,4'-trihydroxy-3'-methoxy-8-C-prenylflavone7-O-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranoside(Yadava RN, Reddy KIS., 1999).The tree grows up to 18m long and their leaves are trifoliolate and used traditionally for the treatment of various diseases. The leaves, flowers and bark are mostly used parts of the tree. Isoflavonoids are reported to be major phytoconstituents in stem and bark. Seeds yield an alkaloid, a fatty oil and saponaceous glycoside. The alkaloid has properties identical to hypaphorine(Irfan Ali Khan, Atiya Khanum.,2005).

E.indica tree's bark is used in Indian folk medicine for rheumatism, itching, joint pain, dysentery, burning sensation, fever, asthma, leprosy, convulsion as a diuretic and laxative(Nadkarni KM, Nadkarni AK., 1992; kirtikar KR, Basu BD., 1984; Joshi SG., 2000). Antioxidant activity of E.indica play a vital role in various pharmacological activities such as anti-aging, anti-atherosclerosis, anti-inflammatory and anti-cancer activities(Lee J, et al., 2004; Middleton E, et al., 2000).

Earthworm: Earthworm Lumbricus terrestris is a favourite model in neuro-science and behavioural studies because of known ventral nerve cord connections of the Central nervous system (CNS) and peripheral nervous system(PNS). Earthworms are sensitive and thus susceptible to soil chemicals especially agrochemicals because they don't have hard cuticles around their body(Lanna et al., 2004; Nahmani et al., 2007).Therefore, they are suitable bio-indicator of soil contamination.

Recent developments in national and international legislations have sharpened the need for reliable, sensitive indicator organisms to use in research, monitoring and regulatory testing. Earthworms can survive and reproduce in anthropogenically metal contaminated soil(Spurgeon, et al., 1994). Earthworms can be sampled easily, have a wide distribution range and strongly accumulate pollutants. However, accumulation of metals varies between ecological categories and species. In earthworm, cerebral ganglion functions as a simple brain. It is located above the pharynx and it is connected to the first ventral ganglion, the removal of which would result in uncontrolled movement of the worm.

Currently there exist no reports on this species of L.terrestris with regards to its response in locomotion and cerebral ganglionic features on impact by neurotoxins and also the inhibitory activity of E.indica towards Acrylamide. The study attempts to assess the neuropathological changes in cerebral ganglions along with the locomotion, morphological, and neuronal behaviour of Lumbricus terrestris on Acrylamide intoxication and the inhibitory activity of the drug E.indica towards the toxicity.

MATERIALS AND METHODOLOGY

Collection of samples: The Earthworm samples collected from 'Anushika Agri Products Vermicompost Garden Center' and the Erythrina indica leaf was authenticated.

Preparation of plant extract: Take 7 grams of leaf powder in a conical flask and Add 70 ml of 100% Ethanol . Keep it in shaker for 72 hours. After 72 hours Filter the leaf extract .pour the leaf extract into a Petri dish. (SS Sakat et al., 2010).

Toxicity test:

Filter paper test: Take 3 earthworms and measure it up. Based on the measurements Acrylamide is added for treatment and Induction 8 and 11 mg. Take 4 eppendorf tubes. Added 1ml of distilled water in 3 eppendorf tubes and 1 ml of ethanol in another tube. Take 40 mg/ml *E. Indica* extract, add into 1 ml of Ethanol and added 350ųL of it into the 8 mg Acrylamide and.Take 3 Filter papers, place it on petri plates , .Add the samples 1 ml in each petri plate suitably.put the earthworms in it.Keep it for observation (Pawar SS and Ahmad Shah ad., 2014).

Soil toxicity test: Take 3 containers. Add the 400 grams of soil . Take 9 earthworms (3 in each container). Measure it up . Based on the measurements acrylamide (5 mg) and *erythrina indica* (650ųL distilled water +350ųL) is weighed. These two mixed in Treated container. The ACR alone was added in Induction container .These process repeated for 7 days.After 7 days earthworms were measured.(Archana Jeyaprakasam et al., 2020)

Swimming Behaviour: Take a glass tank having 3 separate portions and fill water. Label it. Take the earthworms and add them into the glass containers as per labelled. Then observe it. (Drewes CD and Fourtner CR., 1993)

Specimen preparation for Histology test: Take 2 earthworms each from Glass containers. Cut head part of earthworm. Then put the cut part into eppendorf tubes and add 1 ml of 'Formaldehyde'and given for histology test. (Lian Duo et al., 2021)

Sample preparation for Biochemical assay for both Filter paper test and Soil toxicity test: Take the earthworms from the petri plates .cut and crush them .Take 3 eppendorf tubes and add 1 ml of Tris buffer solution and add the crushed earthworm .Then centrifuge at 16 C at 12,500 RPM for 20 minutes.After 20 minutes separate the supernatant and pellet.

Biochemical assay:

1 Protein estimation test: Take 8 test tubes label it Blank 1&2 ,Control 1&2, Treatment 1&2, Induction 1&2. Add 500 ml of Bradford reagent . Then add 500mL distilled water in blank .Take 50ųL of sample solution add into all the test tubes except blank. Then incubate for 30 minutes. After incubation keep it for absorbance test. wavelength at 595 nm in the UV Spectrophotometer. (Lowry OH et al., 1951).

2. Lipid Peroxidase test (LPO): Similarly take 8 testtubes. Add 950ųL of Fox Reagent. Add 50ųL of distilled water in blank and add 50ųL of sample solution in all test tubes except blank.Then incubate 30 minutes .After incubation keep it for absorbance test . wavelength at 560 nm . (Ohkawa H et al., 1979).

3. Lactate Dehydrogenase test (LDH): Take 8 test tubes . Add 1 ml of Glycine.Add 50ųl of sample solution in it except blank. Add 50ųL of Distilled water in Blank.Incubate for 15 minutes. After Incubation ,add 1 ml of Dinitrophenylhydrazine (DNPH) and incubate for 15 minutes . After the incubation keep it for absorbance test. Add 7 ml of NAOH (2N) in it simultaneously. wavelength at 420 nm.

4. Myeloperoxidase test: Take 8 test tubes. Add 50ųL of sample solution in test tubes except blank. Then Add 1.9ml of O.Dianisidine in all the 8 test tubes . Add 1 ml of H_2O_2 solution in all the test tubes .Incubate it for 30 minutes. After the incubation , keep it for absorbance test wavelength at 460 nm.

5. Catalase test: Take 8 test tubes.Add 500 ųL in H_2O_2 in each test tubes and then Add 50ųL of sample solution in all test tubes except blank and add 450ųL of Distilled water in it . Add 500ųL of Distilled water in Blank 1&2. Keep it for absorbance test wavelength at 240 nm (Aebi H., 1984).

RESULTS AND DISCUSSION:

Contact toxicity test: After 24 hours the earthworm in the 'Induction' petri plate was found dead. Its body was completely decomposed, and its body fluids came out. The earthworm's body was separated into 2 pieces. The earthworm in the 'control' petri plate was still alive and healthy. The earthworm in the 'treatment' labelled Petri plate was also dead. Its body only started to decompose.

Soil toxicity test:

Morphological observation: The earthworms were observed for Morphological effect. It was found that, in the earthworms that were kept in the 'Induction' there were constrictions and bulging on the body of the earthworm. The earthworms that were inside the 'Treated' containers didn't have any bulging or constrictions on their body. The earthworms that were kept in 'Control' were active and healthy.

Behavioral and locomotory observations: the behaviour of earthworms was observed by placing it in a piece of paper. The earthworms taken from the induction container were coiled and were dizzy and did not show any brisk movement. But it was alive only. Then the earthworms from the treatment container were observed, which showed slow locomotion. The earthworms in the control container were active and showed normal locomotive behaviors (Datta LG 1962).

Swimming behavior of earthworms: The observations were like the previous tests. The control earthworms were active and swimmed faster to remain on the surface. The earthworms from the 'Induction' container were coiled and did not swim and their movement was slow. The 'Treated' container earthworms were less brisk and showed less swimming behavior than 'Control' earthworms.

Figure 1: Contact toxicity test observation

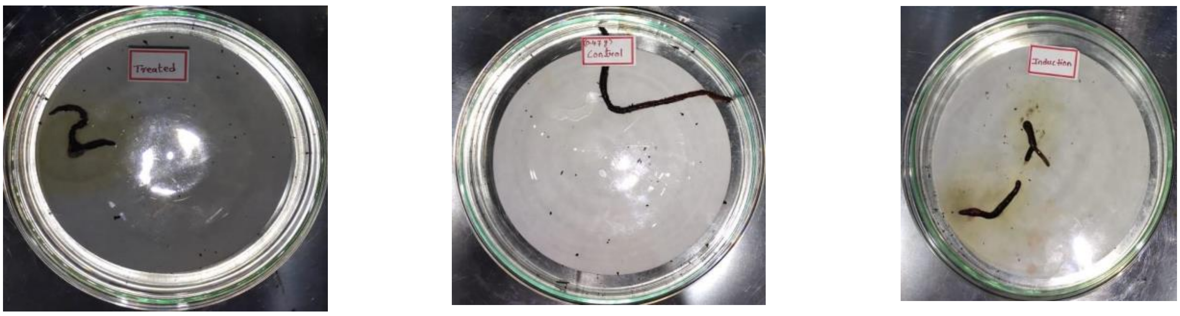
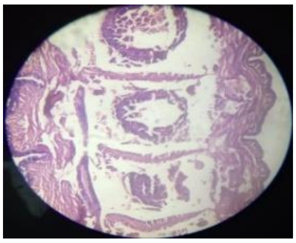


Figure 2 : Morphological changes

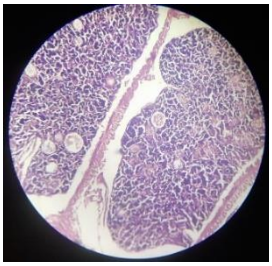
Figure 3: Swimming behavior

Figure 3: Histology test observation

Control



Treatment



Induction

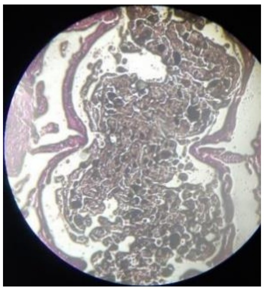


Table 1: Calculated values obtained through UV Spectrophotometer absorbance for contact toxicity test:

S.NO	EXPERIMENTAL GROUP	PROTEIN TEST µg/ml	LPO TEST µg/ml	MPO TEST µg/ml	LDH TEST µg/ml
1	CONTROL	44.032	7.474	1.545	1.222
2	INDUCTION	38.22	4.231	2.389	2.413
3	TREATMENT	44.55	4.321	2.235	1.742

Table 2 - Biochemical assay value of soil toxicity test

S.NO	EXPERIMENTAL GROUP	PROTEIN ESTIMATION µg/ml	LPO µg/ml	MPO µg/ml	LDH µg/ml	CAT µg/ml
1	CONTROL	18.508	6.974	2.119	2.383	6.868
2	INDUCTION	30.04	3.949	5.273	2.651	1.068
3	TREATMENT	38.46	5.974	1.373	2.001	5.089

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