E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2019; 7(2): 171-174
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www.fisheriesjournal.com
Received: 01-01-2019
Accepted: 05-02-2019

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Toxicity study of aqueous leaf extract of *Abutilon indicum* (Malvaceae) in fish *Cyprinus carpio*

S Rajeshwari and SP Sevarkodiyone

Abstract
This study was investigated the effect of cadmium exposure to common carp *Cyprinus carpio* as well as the effect of *Abutilon indicum* (Malvaceae) leaf aqueous extract of common carp *cyprinus carpio*. The study was to assess the effect of heavy metal agents on three enzyme activities, Gulatamata Oxalacetate Transaminase (GOT) Gulatamata Pyruvata transaminase (GPT), and Lactate Dehydrogenase (LDH) were increased significantly in gill, liver and muscle of treated fish compared to that of their control group. The various exposed periods (8, 16, 24, 32 Days) GOP, GPT, and LDH were measured both in control and experimental fish. During exposure periods (8, 16, 24, 32 days) the levels of GOT, GPT and LDH levels were (p<0.05) significantly elevated in the experimental fish. The result of the present investigation suggest that *Abutilon indicum* Leaf extracts affects the enzymological parameters of fish and alterations of these parameters can be useful in environmental biomonitoring of *Abutilon indicum* based products in freshwater environment.

Keywords: enzyme activity levels, GPT, GOT, LDH

Introduction
Aquaculture is increasingly becoming one of the fastest growing aspect of the agricultural industry worldwide [1]. In semi-intensive system of farming, the management of water pond weeds is one of the most important aspects of a successful production system [2]. Based on their known toxicological profiles in many animal models, it is certainly plausible that waterborne metals could alter physiological and biochemical parameters in fish. The survival of many aquatic species (even beyond fish) depends not only on the health status (at time of exposure) of the hosts, but also on the type/length of exposure to and inherent toxicities of the metal toxicants [3]. Cadmium is considered one of the most toxic contaminants present in polluted waterways, causing toxicity at each level of the ecologic stratum [4]. Even at sub-lethal concentrations, cadmium has a cumulative effect and causes serious physiologic disturbances in fish, such as induction of abnormal behavior, locomotor anomalies, or anorexia [5]. Numerous biochemical indices of stress have been proposed to assess the health of non-target organisms such as fish exposed to toxic chemicals in aquatic environments. Therefore, biochemical tests are routine laboratory tests useful in recognizing acute or chronic toxicity of insecticides [6] and can be a practical tool to diagnose toxicity effects in target organs and to determine the physiological status in fish. Medicinal plants are the nature's gift to human beings to make disease free healthy life. It plays a vital role to preserve our health. India is one of the most medico- culturally diverse countries in the world where the medicinal plant sector is a part of time- honored tradition that is a respected even today. Here, the main traditional systems of medicine include Ayurveda, Unani and Siddha [7]. With the emerging worldwide interest in adopting and studying traditional systems and exploiting their potential based on different health care systems, the evaluation of the rich heritage of traditional medicine is essential [8]. Hence the present study has been carried out the Toxicity study of aqueous leaf extract of *Abutilon indicum* (Malvaceae) in fish *cyprinus carpio.*

Materials and Methods
Maintenance of test organism
Live fishes (10± 1g) were collected from High-tech fish farm, Madurai, Tamil Nadu, India. The collected fishes were transported in a polythene bag containing oxygenated water. They were acclimatized to laboratory conditions for 20 days in plastic tanks.

The fishes were maintained in dechlorinated bore well water. Water was changed in alternate days. Proper aeration was maintained and fed with *ad-libitum.*

Experimental toxicant and its exposure
Technical grade cypermethrin (25%EC) was obtained from United Phosphorus Ltd., Bombay. After the normal process of acclimatization, a group of ten fish each were transferred to plastic tubs (15L capacity) containing 10L of water. Fish were exposed to 4 sub-lethal concentrations i.e., 5, 10, 15 and 20 % of 96hLC50 (3.31µg/l) for 5, 10 and 15 days along with the control. Control and exposed fishes were sacrificed at end of each day. The vital tissues like muscle, brain, liver, gill and kidney of the fish were taken for the estimation of nucleic acids (DNA & RNA).

Enzyme Preparation
The fish were sacrificed and the tissues like muscle, brain, liver, gill and kidney were quickly excised into cold solution. The excess blood is washed with 0.15M KCl (cold) solution. The tissues were homogenized (10% w/v) in 0.1M pH 8 tris HCl buffer using potter-Elvehjem homogenizer fitted with Teflon pestle. The homogenates were centrifuged at 5000rpm for 10 minutes. The resultant supernatant was again centrifuged at 5000rpm for 10 minutes. The resultant supernatants were stored in ice and were used as enzyme source for the estimation of AChE activity. All the enzyme preparations were carried out at 0-40°C. Protein content for enzyme preparations were estimated by the method of using Bovine serum albumin as standard.

Estimation of plasma glutamate oxalacetate transaminase (GOT)
Plasma GOT activity was estimated by 2, 4-DNPH Method of King. J [7] using Diagnostic Reagent Kit supplied by Span Diagnostics Pvt. Ltd., Surat, India.

Principle
GOT catalyses the following reaction:
A-Ketoglutarate + L-Aspartate = L-Glutamate + Oxalacetate
Oxalacetate so formed is coupled with 2, 4- Dinitrophenyl hydrazine (2, 4-DNPH) to give the corresponding hydrazone, which gives brown colour in alkaline medium.

Reagents
Reagent 1: Buffered Aspartate - *a* - KG Substrate, pH 7.4
Reagent 2: DNPH Colour Reagent
Reagent 3: Sodium hydroxide, 4N
Reagent 4: Working Pyruvate Standard, 2 mM

Procedure
For the estimation of GOT activity in plasma, 0.25 ml of Reagent - 1 was added to a test tube and incubated at 37°C for 5 min. Then 0.05 ml of plasma was added, mixed well and incubated at 37°C for 60 min., after which 0.25 ml of Reagent-2 was added. The contents were mixed well and allowed to stand at room temperature for 20 min. Then 2.5 ml of Solution -I was added mixed well and the contents were allowed to stand at room temperature for 10 min. The O.D. value was measured against distilled water at 505 nm using spectrophotometer (Spectronic - 20, Baush and Lomb, USA).

Calculation
The O.D. values were marked on the Y-axis of the standard curve and it was extrapolated to the corresponding enzyme activity on the X-axis.

Estimation of plasma glutamate pyruvate transaminase (GPT)
Plasma GPT activity was estimated by 2,4-DNPH method of Reitman and Frankel (1957) using Diagnostic Reagent Kit supplied by Span Diagnostics Pvt. Ltd., Surat, India.

Principle
GPT catalyses the following reaction:

a - Ketoglutarate + L- Alanine *====* L- Glutamate + Pyruvate

Pyruvate so formed is coupled with 2, 4-Dinitropbenyl hydrazine (2, 4-DNPH) to give the corresponding hydrazone, which gives brown colour in alkaline medium.

Reagents
Reagent 1: Buffered Alanine ***a*** - KG Substrate, pH 7.4
Reagent 2: DNPH Colour Reagent
Reagent 3: Sodium hydroxide, 4N
Reagent 4: Working Pyruvate Standard, 2 mM

Procedure
For the estimation of GPT activity in plasma, 0.25 ml of Reagent - l was added to a test tube and incubated at 37°C for 5 min. Then 0.05 ml of plasma was added, mixed well and incubated at 37°C for 30 min., after which 0.25 ml of Reagent - 2 was added. The contents were mixed well and allowed to stand at room temperature for 20 min. 2.5 ml of Solution -I was added, mixed well and the contents were allowed to stand at room temperature for 10 min. The O.D. value was measured against distilled water at 505 nm using spectrophotometer (Spectronic - 20, Baush and Lomb, USA).

Calculation
The 0.D. values were marked on the Y-axis of the standard curve and it was extrapolated to the corresponding enzyme activity on the X-axis.

Estimation of plasma lactate dehydrogenase (LDH)
LDH activity in plasma was estimated by 2, 4-DNPH Reitman.S [8] using Diagnostic Reagent Kit supplied by Span Diagnostics Pvt. Ltd., Surat, India.

Principle
Lactate dehydrogenase catalyses the following reaction:

LDH
Lactate + NAD ^====* Pyruvate + NADH

Products so formed are coupled with 2, 4- Dinitrophenyl hydrazine (2, 4-DNPH) to give the corresponding hydrazone, which gives brown colour in alkaline medium.

Reagents
Reagent 1: Buffered Lactate Substrate, pH 10.0
Reagent 2(A): NAD for Test
Reagent 2(B): NAD for Graph
Reagent 3: DNPH Colour Reagent
Reagent 4: NADH
Reagent 5: Sodium hydroxide, 4N

Reagent 6: Working Pyruvate Standard, 1 mM

Procedure

For the estimation of LDH in plasma, two test tubes marked as "Control" (C) and "Test" (T) were taken. To the 'Control' and 'Test' tubes, 0.5 ml of Reagent - l was added. To the tube marked 'Test' 0.05 ml diluted plasma was added and to the tube marked 'Control', 0.01 ml of distilled water was added. The contents in the tubes were mixed well and incubated at 37°C for 5 min. Then 0.1 ml of Solution –I (A) was added to the 'Test' tube, mixed well and both the 'Control' and 'Test' tubes were incubated for 37°C for 15 min. The tubes were taken out and 0.5 ml of Reagent – 3 was added to both the tubes. Immediately 0.05 ml of diluted plasma was added to the 'Control' tube alone. Once again the contents in both the tubes were mixed well and incubated at 37°C for 15 min., after which the tubes were taken out and 5.0 ml of Solution - III was added to both the tubes. The contents were mixed well by inversion and allowed to stand at room temperature for 5 min. The O.D. of 'Control' and 'Test' were measured against distilled water using spectrophotometer (Spectronic - 20, Baush and Lomb, USA).

Result

The activity of the enzymes GOT, GPT and LDH was increased (p<0.01) in treat fish of common carp, *Cyprinus carpio* 32 days when compared up to the control. GOT showed maximum increased of 208.66 ±0.57 in after 32 days exposed and minimum 205.0±1.00 in 8 days exposed and maximum increased of during on effect of *Abutilon indicum* (Malvaceae) leaf aqueous extract in *Cyprinus carpio* exposure to chromium toxicity observed overall increases in its activity than the control (Table-1). The GOT, GPT and LDH was directly proportional to the various exposure periods showing a minimum (GOT) decreased of (Native control) 202.33 ± 1.52 and increased 208.366±0.57 at the end of 32th day.The GPT and a maximum (control) of 59.3 ±1.52 and 61.61±1.52 at the end of 32d day. *Cyprinus carpio* the various exposure periods showing a minimum increase of 303.66 ±2.03 at the end of (Native control) and maximum increase 379.84±4.84at the end of 32 day of treatment respectively.

Table 1: Changes in enzymatical parameters of common carp *cyprinus carpio* exposed to varying periods concentration of cadmium aqueous leaf extract of *Abutilon indicum* (Malvaceae)

Parameter	Treatment	Positive control (Normal fish)	Native control (Cadmium)	Exposure period days			
				8	16	24	32
GOT(U/L)	C	206.33±0.57	202.33±1.52	205.0±1.00	207.0±1.00	207.66±1.15	208.66±0.57
	T	210.00±2.00	197.00±1.93	206.00±1.0	208.00±1.00	209.33±0.57	210.33±1.52
GPT (U/L)	C	52.00±2.00	47.33±1.52	54.66±2.08	55.6±1.57	58.00±1.00	59.3±1.52
	T	53.66±1.52	47.66±1.52	55.66±2.08	57.00±2.08	60.33±1.52	61.61.±1.52
LDH	C	347±1.15	303.66±2.03	356.33±0.66	364±1.00	372.33±1.45	380.66±1.20
	T	352.33±1.45	330±32.5	363±2.08	373.66±1.85	382±1.73	379.84±4.84

Discussion

Environmental stressors such as metal exposure [9, 10, 11] may change the biochemical parameters. Therefore the measurement of serum biochemical parameters can be useful as diagnostic tool in toxicology to find their general health status and target organs affected by toxicants [11, 12]. The present study that effect of *Abutilon indicum* (Malvaceae) leaf aqueous extract in *Cyprinus carpio* is also demonstrated that the common carp, the *Cyprinus carpio* exposed to concentrations of cadmium displayed a significant elevation in the level of blood glucose after all the exposure periods. The data of present study showed that the exposure of cadmium caused significant elevations in the activities of blood serum GOT and GPT levels. Several reporters showed that these blood enzymes were highly increased in the fish treated with cadmium [13, 14, 15, 16, 17], that the increase in blood enzymatic activity is either due to (i) leakage of these enzymes from hepatic cells and thus raising levels in blood, (ii) increased synthesis and (iii) enzyme induction of these enzymes [18]. Reported that these enzymes liberate to the blood stream when the hepatic parenchyma cells are damaged. There is an increase in the activities of GOT and GPT in *Cyprinus carpio* exposed to cadmium. The present study showed that cadmium altered the entire biochemical metabolism in *C. carpio* by changing the levels of GOT, GPT and LDH in serum. In the present study, increase in GOT and GPT transaminases might be attributed to tissue damage particularly liver. GOT and GPT enzymes activity were found to increase in response to heavy metals in different fish species [19]. In the present study, the significant increase in LDH activity may be due to damage of organs by cadmium nitrate which may find support from the work of [20]. The increase in LDH activity might be due to disruption of respiratory epithelium resulting in tissue hypoxia leading to shift in oxidative metabolism to anaerobic glycolysis. Similar studies are made in fish [21, 22]. Dependence on glycolysis increased in fish, *Labeo rohita,* on exposure to copper as evidenced by increased LDH activity in liver and muscle; this type of metabolic reorganization is strategic [23, 24] reported that the decrease in NAD+ dependent LDH may lead to a metabolic shift from aerobiosis 163 to anaenobiosis during cadmium exposure. Probably in the present study, the inhibition of LDH activity after first week of treatment may be due to impaired carbohydrate metabolism which may find support from the above workers. Such changes in biochemical levels under the effect of cadmium toxicity might results in impairment of energy requiring vital processes, and hence give an idea about the health status of the fish population.

Conclusion

The present study showed that cadmium altered the entire enzymological parameters in *Cyprinus carpio* by changing the levels of GOP, GTP and LDH in serum. Such changes in enzymological levels under the effect of cadmium toxicity might result in impairment of energy requiring vital processes, and hence give an idea about the health status of the fish population.

Acknowledgement

The authors thanks the Management, Principal and Head of the Department of Zoology, Ayya Nadar Janaki Ammal College, Sivakasi for providing facilities to carry out this research work.

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