Available online at www.journalijmrr.com

INTERNATIONAL JOURNAL OF MODERN RESEARCH AND REVIEWS

ISSN: 2347-8314

Int. J. Modn. Res. Revs.
Volume 2, Issue 11, pp 492-496, November, 2014

ORIGINAL ARTICLE

ALLEVIATE POTENTIAL OF *PISONIA ALBA* AND *CARDIOSPERMUM HALICACABUM* ON THE ANTIOXIDANT ENZYMES SUCH AS GLUTATHIONE-S-TRANSFERASE AND REDUCED GLUTATHIONE (GSH) ACTIVITY BY THE HERBICIDE ATRAZINE INDUCED HEPATIC TISSUE OF FRESHWATER FISH *LABEO ROHITA*.

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Article History: *Received 2nd October,2014, Accepted 31st October, 2014, Published 1st Novmber,2014*

ABSTRACT

Atrazine causes numerous toxic manifestations in many aquatic forms. The present experimental study is carried out to investigate atrazine induced alterations in the antioxidant enzymes such as glutathione-S-transferase (GST) and reduced glutathione (GSH) in liver tissue of fresh water fish, *Labeo rohita* exposed to atrazine before and after supplementation with *Pisonia alba* and *Cardiospermum halicacabum* (1g/fish). Fishes were exposed to atrazine at a sub lethal concentration of 20mg/L for 24, 48, 72, 96 and 120 hours. Significant decreased activity levels of GST and GSH were observed during atrazine exposure. With *P. alba* and *C. halicacabum* supplementation, a significant reversal in the oxidative stress enzymes was observed. Our findings clearly evidenced that the *C. halicacabum* supplementation is very effective in reducing the atrazine toxicity when compared to *P. alba* of supplementation to the freshwater fish *L. rohita*.

Keywords: Atrazine, *Pisonia alba, Cardiospermum halicacabum, Labeo rohita,* GST, GSH.

1.INTRODUCTION

Liver is a vital metabolic organ, which also has secretory and excretory functions. It has a paramount importance in the body because of its vital functions like detoxification of endogenous and exogenous substances like xenobiotics, viral infections, chronic alcoholism and bile secretion etc. exposure to all the above challenges liver overpowered, results in liver failure. All over the world the mortality and morbidity of liver diseases increases yearly. Nearly 20,000 deaths and 2, 50,000 new cases found each year (Meganathan and Madhana *et al*., 2011). In spite of tremendous advances in modern medicine, there are hardly any reliable drugs that protect the liver from damage and help in regeneration of hepatic cell. Many active plant extracts are frequently utilized to treat a wide variety of clinical diseases including liver disease.

Many freshwater ecosystems are contaminated with industrial, domestic and agricultural chemicals such as herbicides and insecticides, which are ubiquitous and can, spread regionally and globally (Jin *et al*., 2010b). Atrazine (ATR) is the most common herbicide pesticides in the freshwater ecosystems of the world (Dong *et al*., 2009). Field surveys performed in many countries have showed that ATR is common contaminants of surface and ground water (Gojmerac *et al*., 2006). Chemical pollution in the environment with pesticides has been increasing due to their extensive usage in agriculture (Trasande *et al*., 2011). Alterations in the chemical composition of natural aquatic environments may affect the freshwater fauna, particularly fish. Because, human consumption of fish, the effects of pesticides on fish have important significance in the evaluation of adverse effects of pesticides to human health (Begum and Vijayaraghavan, 1996). Recent studies have indicated that the pesticide toxicity in fish may be related to an increased production of reactive oxygen species (ROSs), leading to oxidative damage (Oruc, 2010).

The term "antioxidant" is used to define cells' own protective mechanisms. The antioxidant defense system includes enzymes such glutathione-S-transferase (GST) and low molecular weight scavengers reduced glutathione (GSH). The GST is a ubiquitous Phase II enzyme responsible for the detoxification of xenobiotics, toxins GST is involved in the detoxification of xenobiotics and highly reactive electrophilic components can be removed before they covalently bind to tissue nucleophilic compounds, which would lead to toxic effects and endogenous substrates including the toxic products of tissue damage (Storey, 1996).

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The reduced glutathione (GSH) antioxidant system is the principal protective mechanism of cells and is a crucial factor in the development of the immune response by immune cells reduced glutathione (GSH) comprise a system that maintains a reduced intracellular environment and acts as a primary defense against excessive generation of harmful ROS (Sheela *et al*., 2005).

Pisonia grandis (Synmyn: *Pisonia alba*, *Pisonia morindifolia*) commonly known as Leechikottai kerai in Tamil. In the alternative system of medicine *Pisonia grandis* leaves are used as analgesic, anti-inflammatory, diuretic, hypoglycemic agent and antifungal. It is also used in the treatment of ulcer, dysentery and snake bite. The leaves are edible and mostly used to treat wound healing, rheumatism and arthritis (Shubashini and Poongothai, 2010). *Cardiospermum halicacabum*, commonly known as Mudakkathan in Tamil. The whole plant has been used for several centuries in the treatment of rheumatism, stiffness of limbs, snake bite; its roots for nervous diseases, as a diaphoretic, diuretic, emetic, laxative, refrigerant, stomachic and sudorific; its leaves and stalks are used in the treatment of diarrhoea, dysentery and headache and as a poultice for swellings (Prabu *et al*., 2008).

2.MATERIALS AND METHODS

Experimental animal collection and maintenance

The freshwater fish *Labio rohita* were collected from the VGM fish farm located in Kurinjipadi, Cuddalore district. The fish were brought to the laboratory and transferred to the rectangular cement tanks (100 × 175) of 500 liters capacity containing chlorine free aerated well water. The fishes measuring 14-16 cm in length and 70-80 g in weight were selected irrespective of their sex for the experiments. During this time they were fed every 24 hour with a commercial diet. The physico-chemical parameters of the water were monitored throughout the acclimation period and remained constant (pH: 7.18 ± 0.5, conductivity: 118.25 ± 8.7 µS $cm^{-}1$, dissolved oxygen: 8.49 ± 0.9 mg O_2 L^{-1}, temperature: 21.96 ± 2.7 ^{0}C).

Experimental chemical

Experimental chemical atrazine was purchased from (TATA Atrataf 50% WP) manufacture by Rallis India Limited, Mumbai.

Supplementary feed

Healthy disease free leaves of *Cardiospermum halicacabum* and *Pisonia alba* were collected from in and around Chidambaram and Thiruvenkadu, the plant was identified. The leaves were washed in running tap water for 10 minutes leafs were dried, aerial parts (1kg) of *Cardiospermum halicacabum* and *Pisonia alba* were macerated thrice at room temperature and prepared in powdered condition and equal amount of rice brane mixed well and small amount water added and prepared small pellet as feed.

ENZYMATIC ASSAY

Glutathione – S – transferase (GST)

GST activity was measured with its conventional substrate 1-chloro, 2, 4-dinitro benzene (CDNB) at 340 nm as per the method of (Habig *et al*., 1974). The reaction was initiated by the addition of glutathione and the absorbance was read at 340 nm against reagent blank and the activity was expressed as µ moles of thioether formed / mg protein / min.

Reduced glutathione (GSH)

The GSH level was determined as described by Ellman (1959) and expressed as mg per gram of protein (mg/g prot). The method utilized metaphosphoric acid for protein precipitation and 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB) for color development and its density was measured at 412 nm.

EXPERIMENTAL DESIGN

Group- I: Fish exposed to tap water (control)
Group- II: Fish exposed to atrazine
Group-III: Fish exposed to atrazine along with *Pisonia alba*
Group- IV: Fish exposed to atrazine along with *Cardiospermum halicacabum*
Group- V: Fish exposed to *Pisonia alba* alone
Group- VI: Fish exposed to *Cardiospermum halicacabum* alone

STATISTICALLY ANALYSES

The data obtained in the present work were expressed as means ± SE, percentage changes and were statistically analyzed using student t-test (Milton and Tsokos, 1983) to compare means of treated data against their control ones and the result were considered significant at ($P<0.05$) and ($P<0.01$)level.

3.OBSERVATION

In the present experimental observed that the liver tissue antioxidant enzyme such as GST and GSH levels were decreased significantly at 5 % level ($p<0.05$) in the treated group II (Table 1and 2). At the end of 120 hours GST and GSH levels were decreased when compared to control group I. In the group III and IV GST and GSH levels were restored when compared to group II. In the group V and VI GST and GSH levels were increased significantly at 120 hours compared to group II and which was near to control group I.

4.DISCUSSION

Glutathione-S-transferases (GSTs), previously known as ligandins, comprise a family of eukaryotic and prokaryotic phase II metabolic isozymes best known for their ability to catalyze the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates for the purpose of detoxification (Allocati *et al*., 2009). Glutathione transferases play an important role in the detoxification and excretion of xenobiotics. In the present experimental result showed that the, levels of GST was decreased when the fish exposed to atrazine. But the group IV (atrazine along with *Cardiospermum halicacabum*) the GST level was gradually increases when compared to the group II. Moreover, the group III (atrazine along with *Pisonia alba*) also enhance the GST level very slowly than the group IV. A significant decline in GST level in liver under the present experimental study may be due to its utilization to challenge the prevailing oxidative stress under the influence of ROS generated from atrazine.

Table 1.Changes of glutathione S-transferase (GST) (µg/min/mg protein) activity in the liver tissue of freshwater fish *Labeo rohita* exposed to atrazine followed by the supplementary feed of *Pisonia alba* and *Cardiospermum halicacabum* exposed to 120 hours

Groups	Hours of exposure				
	24	48	72	96	120
Group-I Control	1.861 ± 0.020	1.853 ± 0.027	1.844 ± 0.034	1.831 ± 0.017	1.826 ± 0.030
Group-II atrazine	1.770* ± 0.025	1.737** ± 0.034	1.695** ± 0.042	1.614** ± 0.019	1.579** ± 0.022
% COC	-4.889	-6.260	-8.080	-11.851	-13.526
Group-III atrazine +. *P. alba*	1.824 ^{NS} ± 0.027	1.783* ± 0.016	1.727** ± 0.031	1.688** ± 0.025	1.625** ± 0.051
% COC	-1.988	-3.777	-6.344	-7.809	-11.007
% COT	3.050	2.648	1.887	4.584	2.913
Group-IV atrazine + *C. halicacabum*	1.847^{NS} ± 0.018	1.809^{NS} ± 0.033	1.778^{NS}± 0.019	1.743* ± 0.027	1.714* ± 0.041
% COC	-0.752	-2.374	-3.579	-4.806	-6.133
%COT	4.350	4.145	4.896	7.992	8.549
Group-V *P. alba*	1.867^{NS} ± 0.025	1.871^{NS} ± 0.029	1.873^{NS} ± 0.018	1.875^{NS} ± 0.050	1.876^{NS} ± 0.044
% COC	0.322	0.971	1.572	2.403	2.738
Group-VI *C. halicacabum*	1.871^{NS} ± 0.030	1.877^{NS} ± 0.035	1.880^{NS} ± 0.024	1.881^{NS} ± 0.026	1.883^{NS} ± 0.039
% COC	0.537	1.295	1.952	2.730	3.121

Values are mean ± S.E-Mean of six individual observations; and student t-test. Significant at *P<0.05; Significant at ** P<0.01 levels (+,-) denotes decreased and increased. % COC (change over control); % COT (change over treated), NS- Nonsignificant.

Table 2. Changes of reduced glutathione (GSH) (µg/min/mg protein) activity in the liver tissue of freshwater fish *Labeo rohita* exposed to atrazine followed by the supplementary feed of *Pisonia alba* and *Cardiospermum halicacabum* exposed to 120 hours

Groups	Hours of exposure				
	24	48	72	96	120
Group-I Control	3.704 ± 0.023	3.691 ± 0.021	3.687 ± 0.038	3.373 ± 0.027	3.665 ± 0.040
Group-II atrazine	3.618* ± 0.044	3.550** ± 0.034	3.486** ± 0.039	3.422** ± 0.035	3.216** ± 0.043
% COC	-2.321	-3.820	-5.451	-6.833	-12.251
Group-III atrazine+ *P.alba*	3.658^{NS} ± 0.041	3.607* ± 0.028	3.584** ± 0.020	3.509** ± 0.052	3.453** ± 0.044
% COC	-1.241	-2.275	-2.793	-4.465	-5.784
% COT	1.105	1.605	2.811	2.542	7.369
Group-IV atrazine + *C. halicacabum*	3.689 ^{NS} ± 0.037	3.633 ^{NS} ± 0.025	3.599 ^{NS} ± 0.033	3.555* ± 0.043	3.508** ± 0.037
% COC	-0.404	-1.571	-2.386	-3.212	-4.283
%COT	1.962	2.338	3.241	3.886	9.079
Group-V *P. alba*	3.709^{NS} ± 0.055	3.712^{NS} ± 0.051	3.717^{NS} ± 0.047	3.720^{NS} ± 0.039	3.724^{NS} ± 0.029
% COC	0.132	0.568	0.813	1.279	1.609
Group-VI *C. halicacabum*	3.715^{NS} ± 0.057	3.719^{NS} ± 0.063	3.725^{NS} ± 0.060	3.728^{NS} ± 0.054	3.731^{NS} ± 0.033
% COC	0.296	0.758	1.030	1.497	1.800

Values are mean ± S.E-Mean of six individual observations; and student t-test. Significant at *P<0.05; Significant at ** P<0.01 levels. (+,-) denotes decreased and increased. % COC (change over control); % COT (change over treated), NS- Nonsignificant.

The activity levels of GST progressively decreased in the present study with the increased exposure periods in the liver tissue. The decrement in GST activity could be explained by the high production of ROS induced by atrazine. In this similar work reported by Pretto *et al*. (2010). In addition, some investigators have also suggested that severe oxidative stress might suppress the activity of antioxidant enzymes due to oxidative damage and loss of the compensatory mechanisms (Atli *et al*., 2006; Liu *et al*., 2006; Khalindar Basha *et al*., 2013). Soundararajan *et al*. (2009) reported that the potential effect of arsenic induced GST level indices in liver tissue of fish *Tilapia mossambica*. Induced GST activity indicated the role of this enzyme in protection against the toxicity of xenobiotics (Leaver and George, 1998).

These results are in relation with the studies reported in the Egyptian catfish *Clarias lazera* subjected to dimethoate exposure and the study showed strong inhibition of GST in the exposed fish (Hamed *et al*., 1999).

Reduced Glutathione (GSH) is an important antioxidant in animals, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides (Pompella *et al*., 2003). GSH is known as a substrate in both conjugation reactions and reduction reactions, catalyzed by glutathione S-transferase enzymes in cytosol, microsomes and mitochondria. However, it is also capable of participating in non-enzymatic conjugation with some chemicals. In the present observation

showed that the, levels of GSH was decreased when the fish exposed to atrazine. But the group IV (atrazine along with *Cardiospermum halicacabum*) the GSH level was gradually increases when compared to the group II. Moreover, the group III (atrazine along with *Pisonia alba*) also enhance the GSH level very slowly than the group IV.

Sharma *et al*. (2013) reported that the significant concentration and time dependent decrease in the level of GSH. The decreased level of GSH was observed in the liver of *Oreochromis mossambicus* exposed to organophosphate (OP) insecticide (Rao, 2006). The decreased level of GSH levels in the liver tissues could account for the marked lipid peroxidation observed. The significant decrease in GSH levels was also observed by Achudume *et al*. (2010) and Jin *et al*. (2011). Enis Yonar and Fatih Sakin, (2011) also observed that the decrease in GSH level in liver of fish exposed to deltamethrin concentrations. Monteiro *et al*. (2006) pointed out that GSH enzyme activity can decrease by negative feedback from excess substrate or by damage induced by oxidative modification. A reduction in GSH activity in a given tissue could indicate that its antioxidant capacity was exceeded by the amount of hydroperoxide products generated. Thus, the reduced GSH level might reflect a possible failure of the antioxidant system in liver of deltamethrin exposed fish.

Ethanolic extract of *C. halicacabum* extract repressed the TNF-α induced DNA binding activity of NF-κB. These indicate the anti-inflammatory activity of the plant (Sheeba and Asha, 2009). These results suggest that ethanolic extract act as a natural antioxidant and anti-inflammatory mediator (Huang *et al*., 2010). Lately, the anti-inflammatory role of rutin has been recognized in this plant (Babu and Krishnakumari, 2005). The gamma-glutamyl trans-peptidase and phospholipase A2 activity to reduce the lipid peroxide content when compared to exposed group. At the same the *P. alba* having the certain important medicinal properties but it compared to the *C. halicacabum* was less. These bioactive compounds present in *C. halicacabum* and *P. alba* which may give recovery to fish in the presence of toxic stress.

5.CONCLUSION

In the present investigation concluded that the herbicide atrazine alter the GST and GSH level in the exposure group on the hepatic tissue of fish *L. rohita*. Besides, the recovery group (group III and IV) regain the GST and GSH level. Because of the *Pisonia alba* and *Cardiospermum halicacabum* having potential protective effect against atrazine toxicity. But, when compared to the both these plants the *C. halicacabum* having the some significant therapeutic properties compared to the *P. alba*.

6.ACKNOWLEDGEMENT

The authors wish to the express their gratitude to the University Grant Commission, New Delhi, for financial support in the form of a Major Research Project (F. No. 42-572/2013).

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