

Effect of Antioxidant status on liver following Atrazine exposure and its protection by *Andrographis paniculata*

Shirisha.K and Mastan.M

Dept of Biotechnology, Dravidian university, Kuppam-517426, chittoor district, A.P.

Abstract: The efficacy of *Andrographis paniculata* (AP) extract was studied on atrazine induced hepatic damage in rats. Ethanolic extract of AP (150mg/kg body weight) was found to protect the male wistar rats from hepato toxic action of atrazine as evidence by significant reduction in the level of lipid peroxidation and increased the antioxidant defense system activity in the atrazine intoxicated rats. However, AP treatment ameliorated the effects of atrazine suggesting it as potential antioxidant against atrazine induced oxidative stress.

Key words: *Andrographis paniculata*, Atrazine, Hepatoprotective, Antioxidant enzymes.

I. Introduction

Herbicides are a class of pesticides that are marketed specifically for the purpose of killing or inhibiting the growth of weeds. They constitute a large percentage of total pesticide use. Worldwide in 1997, there were 5.7 billion pounds of pesticides used, of which 2.2 billion pounds were herbicides. Because of wide spread use and easy accessibility, poisoning with herbicides has become a global environment and health problem (Sine (ed) (1998). Atrazine, 2-chloro-4-(ethyl amino)-6-(isopropyl amino)-s-triazine, is widely used herbicide for the control of grass and broad leaf weeds in crops such as sorghum, maize, sugarcane, lupins, pine and eucalypt plantations. Although it has been banned in the European Union in 2004 because of its persistent groundwater contamination, it is still one of the most widely used herbicides in the world (Frank, 2007). On administration of a single dose of atrazine (0.53 mg) to rats by gavage, 20% of it was excreted in the feces within 72 h and 80% was absorbed across the gastrointestinal tract into the blood stream (Hayes and Laws, 1990). Human exposure pathways for this chemical include occupational exposure through both inhalation and dermal absorption during its manufacture, its formulation and its application by spraying. Although, atrazine generally has low level of bioactivation in fish, it does accumulate in brain, gall bladder, liver and gut of some fishes (Eisler, 1989), therefore, consumption of contaminated fish can also contribute to human exposure. Earlier studies from our laboratory suggested that atrazine induces genotoxicity in liver and alters erythrocyte membrane structure in rats (Singh *et al.*, 2008a; 2008b).

Previously, Gojmerac *et al.* (1995) had reported hepatic degeneration in pigs following atrazine exposure. Pesticides and herbicides induce hepatotoxicity, as liver is major site for detoxification of these compounds. The detoxification reactions of atrazine can be divided into phase I and phase II reactions. Phase I reactions is the glutathione -S-transferase (GST) catalysed conjugation with glutathione (GSH) (Elia *et al.*, 2002). Herbicides such as paraquat are known to exert their effects by inducing oxidative stress in tissues of mammals and fish (Winston and Di Giulio, 1991). To prevent oxidation-induced damage, there are effective antioxidant systems in organisms, some components of these systems are GSH and certain antioxidant enzymes including free radical scavenging enzymes, such as glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase. Other associated antioxidant enzymes are glutathione reductase (GR) and GST.

Non enzymatic antioxidants such as α -tocopherol (vitamin E), ascorbate (vitamin C), β -carotene (vitamin A), flavanoids (quercetin, rutin etc.), selenium and thiol containing compounds such as glutathione (GSH) can also act to overcome the oxidative stress, being a part of total antioxidant system (Sies *et al.*, 1992). In modern medicine, plants play a crucial role in the controlling more diseases (Maiti *et al.*, 2006). In the liver disorders AP is mentioned a popular remedy (Maiti *et al.*, 2009). AP extracts acts as hepatoprotective, hepatostimulant, antioxidant effect (Trivedi *et al.*, 2001), anti platelet, anti thrombotic drug (Borhanuddin *et al.* 1994), anti-pyretic effect (Kanniappan *et al.*, 1999). In the present investigation we studied whether AP extract has the potential to attenuate atrazine induced oxidative stress.

II. Material and Methods

The entire chemicals including Atrazine were obtained from Sigma Aldrich chemicals.

Plant Material

The plant was collected from Herbal Garden Dravidian University, Kuppam. The Voucher specimen was identified with the help of local and regional floras. Sample was shade dried and pulverized in mechanical grinder and stored in an airtight container till further successive extraction.

Animals

Male rats (Wistar strain,) weighing about 200-230 g was used throughout the studies. The animals were housed in polypropylene cages provided with water and standard pellet diet ad libitum.

Preparation of Ethanolic extract

The dried powder extracted in a Soxhlet apparatus using ethanol at a temperature range of 55°C to 60°C. The filtrate was evaporated to dryness at reduced pressure in vacuum evaporator.

Experimental design

Animals were segregated in to four groups with each group having 6 animals. Animals in each group were treated with Atrazine and *Andrographis paniculata* daily for a period of 7 and 14 days as described below. Atrazine was given at a dose of 300 mg/kg body weight. The dose of *Andrographis paniculata* is 150 mg/kg body weight.

Control: Animals were given saline, orally

Atrazine treated: Animals were given Atrazine (300 mg/kg body weight) dissolved in saline, orally.

***Andrographis paniculata* treated:** Animals were given *Andrographis paniculata* (150 mg /kg body weight) dissolved in water orally. **Atrazine + *Andrographis paniculata* treated:** Animals were given Atrazine (300 mg/kg body weight) along with *Andrographis paniculata* (150 mg/kg body weight) dissolved in saline and water.

Sample collection

At the end of 7 days and 14 days, overnight fasted rats were sacrificed. Livers of the animals were removed, rinsed in ice cold isotonic saline (0.9% w/v NaCl), blotted dry, and weighed separately.

Preparation of samples

A 10% (w/v) tissue homogenate was prepared in 50mM Tris HCL (P^H 7.4) using homogenizer. Post mitochondrial supernatant (PMS) was prepared by centrifuging the homogenate at 10,000 rpm for 10 min at 4°C. The pellet was discarded and supernatant thus obtained was referred to as PMS. Various biochemical parameters were assayed in the homogenate and post mitochondrial supernatant of rat liver.

Protein estimation:

Protein content was determined according to Lowry method (1951) using bovine serum albumin as a standard.

Lipidperoxidation (LPO)

The level of lipid peroxidation in the tissues was measured in terms of malondialdehyde (MDA; a product of lipid peroxidation content and determined by using the thiobarbutaric acid reagent (TBA) reagent. The reactivity of TBA is determined with minor modifications of the method adopted by Hiroshi *et al* (1979). The organic layers was transferred in to a clear tube and its absorbance was measured at 532 nm. The rate of lipid peroxidation was expressed as μ moles of malondialdehyde formed/g wet wt. of tissue .

Antioxidant enzyme

Reduced glutathione (GSH): GSH activity in the selected tissue was assayed by the method of Ellaman *et al.* (1961). Samples were deprotenised by sulfosalicylic acid followed by the reaction of sulfhydryl groups of glutathione with DTNB (5,5'- dithiobis-2-nitrobenzoic acid) to produce a yellow coloured product 5-thio-2-nitrobenzoic acid. The reaction is read at 412nm. The results were expressed as n mol GSH/mg protein.

Superoxide dismutase (SOD) : SOD activity was determined by using the epinephrine assay of Misra and Fridovich (1972). At alkaline P^H, superoxide anion O₂⁻ causes the autooxidation of epinephrine adenochrome ; while completing the reaction of SOD decreased the adenochrome formation and changes in absorbance were recorded at 480 nm, measured at 10sec intervals for 1 min in spectrophotometer.

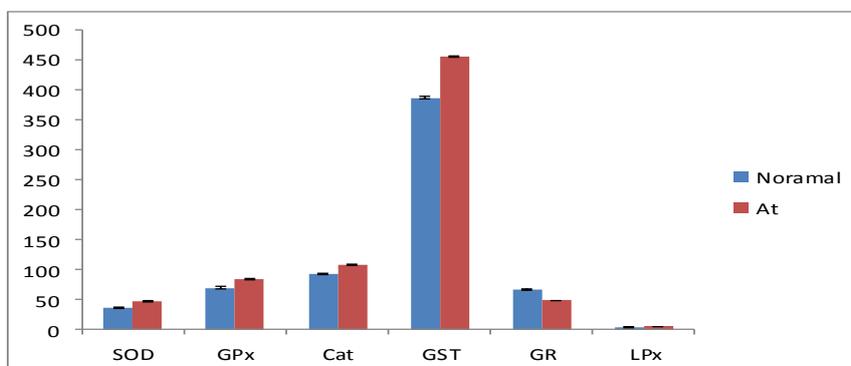
Catalase (CAT) : Catalase activity in the selected tissues was assayed by the method of Chance and Machly (1955). The decomposition of hydrogen peroxide was followed directly by measuring the decrease in absorbance at 240 nm, at 10 sec intervals for 1min in spectrophotometer (Hitachi model, U-2001). The catalase activity was expressed as n moles of hydrogen peroxide metabolised /mg ptn/min.

Glutathione peroxidase (GPx): It was measured by the method of Rotruck *et al.* To the enzyme source EDTA, sodium azide, glutathione reduced, hydrogen peroxide buffer is added at 37°C for 10 min. The reaction was arrested by adding TCA. The reaction was read at 412 nm in spectrophotometer.

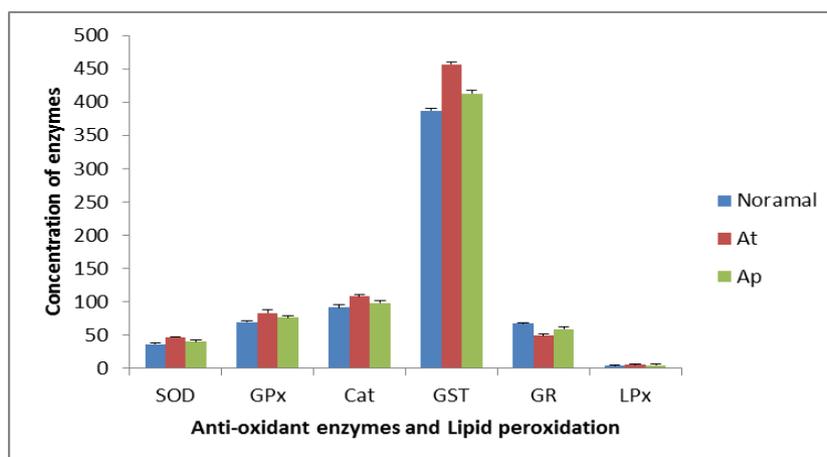
Glutathione-s-transferase (GST) : GST activity in the cytosol fraction of the selected tissues was assayed by using 1-chloro-2,4-dinitrobenzene (CDNB) at 340nm) substrate as described by Habig *et al* (1974).

Antioxidants enzymes in Liver

Activity	Mean ±SD	
	Normal	Atrazine
SOD	35.95±0.5	46.36±1.43
GPx	69.42±1.63	83.39±0.99
CAT	91.67±1.29	108.31±1.4
GST	387.01±2.2	456.78±0.77
GSH	67.23±0.63	48.76±0.23
LPO	3.37±0.25	4.8±0.22



S.No	Biochemical Parameter	Normal	Atrazine	<i>A.paniculata</i>
1	SOD	35.95±2.5	46.36±1.43	40.26±2.21
2	GPx	69.42±2.63	83.39±3.99	76.24±2.52
3	CAT	91.67±3.29	108.31±2.4	98.41±2.83
4	GST	387.01±4.2	456.78±2.77	412.52±5.24
5	GSH	67.23±1.63	48.76±2.23	58.63±3.45
6	LPO	3.37±1.25	4.8±1.22	4.21±1.48



III. Results

Effect on lipid peroxidation

Malondialdehyde (MDA) is the main oxidation product of peroxidized polyunsaturated fatty acids and denotes an important index of lipid peroxidation (Elia *et al.*,2002). Administration of atrazine increased hepatic MDA levels in rats and the effect was more pronounced with time. An increase by in MDA levels was observed in the liver of the atrazine administered rats after 14 days. Treatment with *Andrographis paniculata* resulted decrease in thiobarbituric acid reactive substances (TBARS) as compared to the control group after day 7 and day 14 of the treatment. Increased LPO in the liver of atrazine treated rats suggests that oxidative stress is induced by atrazine administration, which can be decreased by the administration of *Andrographis paniculata*.

Effect on reduced glutathione:

GSH shows remarkable metabolic and regulatory versatility. GSH/GSSG is the most important redox couple and plays crucial role in antioxidant defense system that the concentration of GSH in atrazine administered rats were decreased as compared to control group. The GSH content was decreases in atrazine treated group after 7days. However, the GSH content was found to be increased in rats treated with AP.

Effect on superoxide dismutase:

Variations in the activities of SOD of the liver of rats after administration of atrazine and/or AP. Administration of atrazine resulted in decreases the activity of the enzyme in the liver when compared to healthy controls. However decreased activity was after 7days of exposure. An increase in the activity of SOD was observed after the treatment of AP as compared to Atrazine treated rats.

Effect on catalase:

There are variations in the catalase activity after different treatments in rats. The catalase activity was decreased in the liver of rats treated with atrazine. The decrease was after 7days of atrazine administration, respectively. However, administration of AP will increase the catalase activity.

Effect on glutathione peroxidase

It is clearly evident that atrazine administration led to decrease in the activity of glutathione peroxidase (GPx) in the liver as compared to the control group. The increase in the activity of the enzyme after the treatment of rats with AP and was found to be dependent on duration of exposure. It was observed that there was an increase in the liver of the AP exposed group as compared to the control group. An improvement in the glutathione peroxidase activity was observed after 14 days treatment of atrazine exposed animals.

Effect on glutathione-s-transferase:

In the present investigation, glutathione-s-transferase (GST) activity decreased in the liver of the rats exposed to atrazine. Time dependent decrease was observed following atrazine exposure. Maximum increase was observed after 7days treatment. Administration of AP resulted in increase in the activity of enzyme after 14 days in the liver of the rats as compared to atrazine exposed group.

IV. Discussion

The present study investigates the propensity of atrazine to induce oxidative stress and its possible attenuation by AP in liver of male wistar albino rats. Experimental animals were administered atrazine and AP gavage for a period of 7 and 14 days. Exposure of experimental animals to pesticides is known to induce lipid peroxidation in various tissues, which is responsible for the adverse biological effects (Sharma *et al.*, 2005; El-Dermerdash *et al.*, 2004; Kamboj *et al.*, 2006). Mechanism of pesticide toxicity has been usually associated with the increase of lipid peroxidation of in liver (Sharma *et al.*, 2005; Datta *et al.*, 1994).The activity of the enzyme lipid peroxidation is increased. Therefore, the results indicated that the atrazine might generate free radicals that reacted with membrane lipids and induced oxidative damage of membrane structure. The present study reveals that AP treatment results decreased lipid peroxidation in the liver and protects it from atrazine induced oxidative stress.

A decrease in GSH content in the liver of rats after atrazine exposure indicated pro-oxidant conditions in the liver. Decrease in GSH levels after administration of various pesticides (Konstantinova and Russanov 1999 *et al.*). Results indicated that the GSH content and antioxidant of the hepatocytes of atrazine treated rats were decreased as compared to control .co-administration of AP along with atrazine restored the GSH content and antioxidant level of liver tissues nearly to control levels. Antioxidant is a substance that delays or inhibits oxidative damage to target molecules (Halliwell 1996).

Enzymes plays a major role in countering the oxidative stress induced due to the formation of reactive oxygen species. SOD provides the defense against oxygen derived free radicals. SOD activity decreases oxidative stress by dismutation of O₂ (Mccord and Fridovich, 1969). In the present study a decrease in the activity of SOD is observed in atrazine group suggest that an increased super oxide radical production and other reactive oxygen species there by induce oxidative damage (Fouche court, Riviere 1995). AP being an antioxidant reduces the oxidative stress and hence normalises the SOD activity to some extent.

Endogenous hydrogen peroxide may be converted either by catalase or GPx to water (Kehrer, 1993) or otherwise it may generate the highly reactive free hydroxyl radical by the fenton reaction (Harber and Weiss, 1934), which is widely belived to be responsible for oxidative damage (Fridovich, 1978; Chance *et al.*, 1979; Halliwell and Gutteridge, 1984). The elevated activity of catalase is an adaptive response like SOD against enhanced generation of free radicals (Koner *et al.*, 1998). Reciprocally SOD protects CAT against inhibition of

superoxide anion. In the present study catalase activity is decreases the effect of atrazine exposure. AP showed the ameliorating effect on the atrazine-induced increases in catalase activity.

GPx converts hydrogen peroxide or other lipid peroxides to water or hydroxyl lipids and in the process GSH is converted to oxidised glutathione (GSSG). We observed increase in GPx activity following atrazine exposure. Sharma *et al.*, (2005) reported an increased GPx activity in the liver of rats after treatment with dimethoate. However, Elia *et al.*, (2002) have reported increase in the activity of hepatic GPx but no change in the activity of GPx in the gills of fish following atrazine exposure. The results of study indicate that atrazine induced decrease in the GSH content. Administration of AP along with atrazine showed protective effect against atrazine-induced oxidative stress. Metabolic pathways of atrazine in humans have not been fully characterised, presence of atrazine mercapturates in human urine indicates that GSH conjugation is an important route of bitransformation of atrazine in humans (Lucas *et al.*, 1993; Jaeger *et al.*, 1998; Buchhloz *et al.*, 1999). In the present study, atrazine induced the activity of GST in the liver of rats. Increased activity of the GST suggested increased production of glutathione conjugates or metabolites that resulted in decreased content of the GSH in the liver. Pesticides are expected to induce the activity of GST as a potent protective mechanism of organism. Present investigations suggest that AP treatment trends to reduce the atrazine –induced toxicity from studies. In addition, atrazine treatment also increased hydrogen peroxide content, while reducing gene expression and enzymatic activities related to two major hydrogen peroxide- detoxification pathways (Ramel *et al.*, 2009).

V. Conclusion

From the above observations it can be concluded that to exposure of atrazine results in increased oxidative stress and altered antioxidant status of liver. Administration of AP along with atrazine resulted in normalization of the toxic effects of atrazine thus highlighting the protective action of AP.

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