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Mitigating Effect of Ginger against Oxidative Stress Induced by Atrazine Herbicides in Mice Liver and Kidney

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Abstract

Pesticide chemicals may induce oxidative stress leading to generation of free radicals and alterations in antioxidants or oxygen free radical (OFR) scavenging enzymes. Hence, the effect of sub-chronic atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) exposure was evaluated on lipid peroxidation, antioxidant power, glutathione and OFR scavenging enzymes in albino mice. Experimental animals were administered atrazine (1/8 LD_{50}) and/or ginger (120 mg/kg body weight, each alternative day) intraperitoneally for 14 days. There was higher level of malondialdehyde in liver and not in kidney of mice treated with atrazine than control animals. Superoxide dismutase and catalase activities were decreased in liver and kidney tissue in atrazine-treated animals compared to the control. A highly significant increase in glutathione-S-transferase activity was observed in liver of treated animals. Results indicated that the reduced glutathione (GSH) content of the liver only and not in kidney of atrazine-treated mice was significantly decreased as compared to the control group. Co-administration of ginger along with atrazine restored the hepatic GSH content nearly to control levels, decreased the level of lipid peroxidation and improved all the antioxidant enzymes as well as antioxidant power. In conclusion, results of the study demonstrated that atrazine induced oxidative stress in liver and kidney, in terms of decreased activities of the various antioxidant enzymes, increased of lipid peroxidation and decreased content of reduced glutathione and antioxidant power. However, ginger administration ameliorated the effects of atrazine, suggesting that ginger is a potential antioxidant against atrazine-induced oxidative stress.

Keywords: Atrazine; Ginger; Lipid peroxidation; Antioxidants; Superoxide dismutase; Catalase

Abbreviations: TBA: Thiobarbituric Acid; LDH: Lactate Dehydrogenase; ALP: Alkaline Phosphatase; GST: Glutathione S-transferase; GSH: Reduced Glutathione; ROS: Reactive Oxygen Species; SOD: Superoxide Dismutase; CAT: Catalase

Introduction

Modern agricultural practices often include the extensive use of a wide range of pesticides. Environmental contamination due to the excessive use of pesticides has become a great concern to the public and to environmental regulatory authorities [1]. Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) is a member of s-triazine group of herbicides. It, a chorotriazine herbicide, is used extensively in the United States. Degradation by-products, as well as the parent compound, have been detected in surface and ground water in areas of major usage [2]. Atrazine is almost non-volatile and its half-life in neutral condition is about 200 days but varies from 4-57 weeks [1] depending on various environmental factors like pH, moisture content, temperature and microbial activity [3]. It is used mainly for pre and post emergence control of annual grass and broad-leafed weeds in maize, sorghum, pineapple, sugarcane, macadamia nuts and many other crops and has been sold in different commercial names like Aatrex, Aatratol, Bicep, Gasaprim [4]. Although it is placed in toxicity class III by USEPA, which means it is slightly toxic, it has been classified as restricted use pesticide (RUP) due to its ground water contamination potential. Exposure to atrazine may cause detrimental effects and irritation to eyes, nose and throat [5]. Due to the toxicity behaviour of atrazine, German Government banned all atrazine-containing products in 1991 [6]. Atrazine has been shown to cause liver, kidney and heart damage in animals, and is therefore likely to cause the same in humans [7]. Singh. et al. [8] reported that reduced glutathione (GSH) content of the erythrocytes of atrazine treated rats was significantly decreased as compared to the control group. Co-administration of vitamin E along with atrazine restored the GSH content of erythrocytes nearly to control levels.

Oxidative stress in biological systems due to the effect of pesticides originates as the result of an imbalance between the generation of oxidizing species and cellular antioxidant defenses [9,10]. Numerous enzymatic and nonenzymatic mechanisms take place to protect the cell against oxidative damage. The radical chain reaction of lipid peroxidation appears to be a continuous physiological process. This process, if out of control, can alter essential cell functions and lead to cell death [11]. Reactive oxygen species (ROS) can be detoxified by an enzyme defense system, comprising superoxide dismutase (SOD), catalase (CAT), and selenium-dependent glutathione peroxidase, or non-enzymatic systems by the scavenging action of GSH, while organic peroxides can be detoxified by the activity of glutathione S-transferase (GST) [12].

Recently, great attention has been focused on the role of the antioxidative defense system in oxidative stress. Endogeneous antioxidants in medicinal herbs may play an important role in antioxidative defense against oxidative damage, possibly protecting the biological functions of cells [13]. There is increasing interest in the protective biological function of natural antioxidants contained

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medicinal herbs in Saudi Arabia and many other countries all over the world, which are candidates for the prevention of oxidative damage. Today many botanicals natural products are used in therapy of different diseases. Ginger (*Zingiber officinale* Roscoe, Zingiberacae) is example of botanicals which is gaining popularity amongst modern physicians and its underground rhizomes are the medicinally and winery useful part [14]. Many studies were carried out on ginger and its pungent constituents, fresh and dried rhizome. Among the pharmacological effects demonstrated are anti-platelet, antioxidant, anti-tumour, anti-rhinoviralis, anti-hepatotoxicity and anti-arthritic effect [15-17]. The antioxidant action of ginger has been proposed as one of the major possible mechanisms for the protective actions of the plant against a number of toxic agents such as carbon tetrachloride and cisplatin [18,19]. Recently, it has been shown that 6-gingerol is endowed with strong anti-oxidant action both *in vivo* and *in vitro*, in addition to strong anti-inflammatory and anti-apoptotic actions [20]. Ginger had ameliorative effects on mercury induced hepatoxicity in rats [21], mancozeb fungicide induced liver injury in albino rats [22] and can be used as both as prophylactic and therapeutic detoxificant on cadmium induced poison. Supplementation with ginger slightly attenuates the developmental toxicity of fenitrothion and/ or lead [23]. The goal of the present study was, therefore, to investigate the beneficial role of ginger against atrazine cytotoxicity in murine liver and kidney. Liver functions were determined by measuring the total protein (TP), total lipid (TL) and uric acid. Cellular damages were evaluated by measuring lactic dehydrogenase (LDH) and alkaline phosphatase (ALP) leakages in serum. The antioxidant power of hepatocytes was measured by ferric reducing/antioxidant power (FRAP) assay. In addition, the prooxidant–antioxidant status of liver and kidney tissue were determined by measuring (1) the level of end product of lipid peroxidation (LPO); (2) the activities of the intracellular antioxidant enzymes, namely, superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST), as well as by determining the (3) the level of reduced glutathione (GSH).

Materials and Methods

Chemicals

Atrazine (99.9% purity; bought from Sigma Chemical Co., St. Louis, MO) was prepared as a suspension in deionized water for the final concentration. Thiobarbituric acid (TBA), trichloroacetic acid (TCA), H_2O_2 (33%), ethylenediaminetetraacetic acid (EDTA), reduced glutathione (GSH), oxidized glutathione (GSSG), sodium azide, Tris–HCl, 5,50 dithiobis-(2-nitrobenzoic acid (DTNB), b-nicotinamide adenine dinucleotide phosphate (NADPH), potassium dihydrogenophosphate (KH_2PO_4), sulphuric acid, butanol, and sodium chloride (NaCl) of technical grade used in this study were supplied by Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals were procured from Merck Ltd., SRL Pvt., Ltd., Mumbai, India.

Extraction of ginger

Fresh *Zingiber officinale* was purchased from Saudi Arabia market. The ginger was washed and peeled, cut into pieces and sun dried for seven days; the dried ginger was crushed using an electric blender. The rhizomes of *Z. officinale* were shade dried at room temperature and were crushed to powder. 125 g of the powder were macerated in 1000 ml of distilled water for 12 h at room temperature and were then filtered to obtain the final aqueous extract. The concentration of the extract is 24 mg/ml equal to 120 mg/kg [24].

Animals and treatment protocol

Male mice (weighing 30–35 g) were purchased from King Fahed Medical Research Center in Jeddah (Saudi Arabia). The animals were maintained in solid-bottom shoebox-type polycarbonate cages with stainless steel wire-bar lids, using a wooden dust-free litter as bedding material. Animals were located in air-conditioned room and were allowed free access to pellet diet and tap water for a week before starting the experiment. The European Community Directive (86/609/EEC) and National rules on animal care have been followed. Animals were randomly divided into 4 groups with eight animals in each as follows:

(1) Group; control group: each animal in this group was given isotonic solution (0.1 ml of saline/animal) intraperitoneal (i.p) each alternative day for 14 days.

(2) Group; ginger group: mice were given 120 mg ginger extract/kg i.p (0.1 ml/ animal) each alternative day for 14 days.

(3) Group III; atrazine group, animals were given herbicide atrazine dissolved in water at a dose level of 1/8 LD_{50} (78.25 mg/kg body weight) where Stevens and Sumner [4] reported that the i.p.-LD_{50} of atrazine was 626 mg/kg in mice.

(4) Group IV; ginger/atrazine group, animals were given the same dose of aqueous extract of ginger i.p as in group II (120 mg/kg b.w.) followed by 0.1 ml of atrazine (78.25 mg/kg b.w.), each alternative day for 14 days.

Haematological parameters determination

Blood samples were collected from all animals from the retro-orbital plexus vein according to Sanford [25]. Blood samples were transferred to test tubes containing EDTA for haematological parameters [red blood cell (RBC) counts, haemoglobin (Hb), hematocrit (Hct), white blood cell (WBC) counts, lymphocytes counts, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and thrombocytes] using Sysemex KX21N hematology analyzer. Each sample was run in duplicate.

Serum enzymes activity

Serum samples were separated by centrifugation of the blood samples at 3000 rpm for 15 min and were kept at −20°C for subsequent use. Biomarkers for liver damage were determined using the commercial diagnostic kit of Stanbio Co., Spain. Enzyme activity was expressed in International Units per litre (IU/L) for serum lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) while total protein, total lipid and uric acid were expressed in mg/dL.

Tissue preparation

Liver and kidney were removed from mice under ether anaesthesia after 14 days of treatment and washed with cold saline buffer. Washed tissues were immediately stored at -80°C. To obtain the enzymatic extract, tissues were homogenized in ice-cold 50 mM sodium phosphate buffer (pH 7.0) containing 0.1 mole of Methylenediaminetetraacetic acid (EDTA) using a Potter-Elvehjem homogenizer to yield 10% (W/V) homogenate. The homogenates were then used for subsequent measurements.

Assay of antioxidant power of hepatocytes: ferric reducing/ antioxidant power (FRAP) assay

FRAP assay was carried out for the determination of the antioxidant power of hepatocytes. Briefly, 20 ìl of homogenated liver was added to 1.5 ml freshly prepared and pre-warmed (37°C) FRAP reagent (300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM

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$FeCl_3 \cdot 6H_2O$ in the ratio of 10:1:1) and incubated at 37°C for 10 min. Basically, it measures the change in absorbance at 593 nm due to the formation of a blue colored Fe^{II}-tripyridyltriazine compound from the colourless oxidized Fe^{III} form by the action of electron donating antioxidants [26]. The absorbance of the sample was read against reagent blank (1.5 ml FRAP reagent + 50 ìl distilled water) at 593 nm.

Lipid peroxidation assay

The extent of LPO was estimated as the concentration of thiobarbituric acid reactive product malondialdehyde (MDA) by using the method of Ohkawa et al. [27]. Two hundred fifty microlitres of tissue homogenate were added to 1.5 ml of 1% phosphoric acid (pH 2.0) and 1 ml of 0.6% of TBA in air–light tubes and were placed in a boiling water bath for 25 min. After incubation, the sample was cooled to room temperature and MDA-TBA was extracted with 2.5 ml of butanol. Organic phase was separated by centrifugation for 5 min at 2000g and measured at 532 nm. MDA concentrations were determined using 1,1,3,3-tetraethoxypropane as standard and expressed as µmol/g liver tissue.

Antioxidant enzymes

SOD activity was performed at room temperature according to the method of Misra and Fridovich [28]. Ten micro litres of tissue homogenate were added to 970µl of EDTA–Sodium carbonate buffer (0.05 M) at pH 10.2. The reaction was started by adding 20µl of epinephrine (30 mM) and the activity was measured at 480 nm for 4 min. A unit of SOD is defined as the amount of enzyme that inhibits by 50% the speed of oxidation of epinephrine and the results were expressed as U/min/g of tissue.

Catalase activity was measured by the H_2O_2 degradation assay [29]. In brief, 0.25 g of liver tissue was homogenated in 1 ml of 50 mM Tris–HCl and centrifuged at 2000 g for 15 min. Then 10µl of supernatant was added to a quartz cuvette containing 980µl of distilled water, and 10µl of 0.066 M H_2O_2 (dissolved in sodium phosphate buffer) was added to start the reaction. Catalase activity was determined by measuring the decrease in absorbance (H_2O_2 degradation) at 240 nm for 1 min. One unit of CAT activity was defined as 1µmol of H_2O_2 consumed/min/g of tissue.

Measurement of reduced glutathione and glutathione-S-transferase that involved in glutathione metabolism

Reduced glutathione assay: Reduced GSH estimation was performed by the method of Beutler et al. [30]. Livers were homogenized in 1 ml of 1.1% KCl cooled, then homogenate (100µl) was mixed with 750µl of precipitate solution (1.67 g of glacial meta-phosphoric acid, 0.2 g of EDTA and 30 g of NaCl in 100 ml of D.W) and 900µl of distilled water. Homogenate tissues were centrifuged at 2000g for 15 min to precipitate proteins. Protein-free supernatant (250µl) was added to 1 ml of Na_2HPO_4 (0.3 M) solution and the reaction was initiated by adding 125µl of DTNB (6 mM) and the absorbance of 5-thio-2-nitrobenzoic acid (TNB) formed was measured at 412 nm. The level of GSH was obtained by standard curve and expressed as mmole per g tissue.

Glutathione-S-transferase (GST): GST was measured by the spectrophotometric assay of Alin et al. [31]. It uses 1-chloro-2,4-dinitrobenzene as electrophilic substrate that binds to GSH with the participation of the enzyme and forms a colored GSH-substrate complex, detected at 340 nm. The activity of GST was expressed asµmol/mg protein.

Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test (level of significance = $P \leq 0.05$) using SPSS version 15, statistical program [32]. The results are expressed as mean ± SE, and were obtained from at least 5 mice in each treatment. Statistical analysis was based on comparing the values between the atrazine group and control group, while ginger-atrazine group compared to atrazine group.

Results

Haematological changes were shown in (Table 1). Treatments the animal with ginger only or in combination with atrazine improved the haematological parameter as compared to control or atrazine, respectively. A significant decrease was observed in Hct in atrazine-treated group compared to control group ($P < 0.01$). Percentage of lymphocytes and monocytes were increased significantly in atrazine group as compared to control group. Co-treatment of ginger with atrazine improved all the haematological parameters.

There were no significant difference in amount of total protein, total lipid and level of uric acid between control and ginger group (Figure 1). In the mice treated with atrazine, all the parameters that mentioned above were decreased by approximately 50% as compared to control and ginger groups. Treatment the animals with ginger in combination with atrazine restored the protein and lipid content and did not effect on the level of uric acid as compared to atrazine group.

Parameters	Control	Ginger	Atrazine	Ginger and atrazine
RBC (10^6 mm^{-3})	6.1 ± 0.04	8.2 ± 0.1[a]	5.7 ± 0.1	6.5 ± 0.1[b]
Hemoglobin (g/dl)	11.8 ± 0.04	14.5 ± 0.4[a]	11.8 ± 0.2	13.2 ± 0.4[b]
Hematocrit (%)	37.4 ± 0.1	47.6 ± 0.2[a]	30.9 ± 0.3[a]	38.8 ± 1.2[b]
MCH (pg)	19.4 ± 0.1	17.7 ± 0.2	16.3 ± 0.3[a]	21.2 ± 0.4[b]
MCV (fl)	61.3 ± 0.3	60.4 ± 0.2	49.2 ± 0.3[a]	52.5 ± 0.3
MCHC (g/dl)	32.1 ± 0.4	29.8 0.3	38.8 ± 0.5	41.7 ± 0.7
WBC (10^3 mm^{-3})	3.8 ± 0.1	6.5 ± 0.1[a]	4.4 ± 0.1	3.9 ± 0.4
Lymphocytes (%)	55.3 ± 0.2	79.2 ± 0.3[a]	83.1 ± 1.6[a]	81.9 ± 0.9
Monocytes (%)	4.5 ± 0.3	6.4 ± 0.1	8.3 0.1[a]	7.4 ± 0.2
Granulocytes (%)	6.2 ± 0.3	13.7 ± 0.2[a]	8.3 ± 1.4	9.3 ± 0.2
Thrombocytes (10^3 mm^{-3})	493.0 ± 1.8	688.7 ± 0.9[a]	389.5 ± 7.8	452.0 ± 3.9

Values expressed are mean ± SE (n = 5-6). Haematological parameters were determined using Sysemex KX21N haematology analyzer. Each sample was run in duplicate. Animals were killed 14 days after exposure to: control (isotonic solution), ginger (120 mg/kg), atrazine (78.25 mg/kg), and ginger (120 mg/kg) plus atrazine (78.25 mg/kg). [a]Significantly different from control group $P < 0.05$. [b]Significantly different from atrazine group $P < 0.05$.

Table 1: Haematological parameters and blood indices value of male mice after 2 weeks of atrazine treatment alone or in combination with ginger.

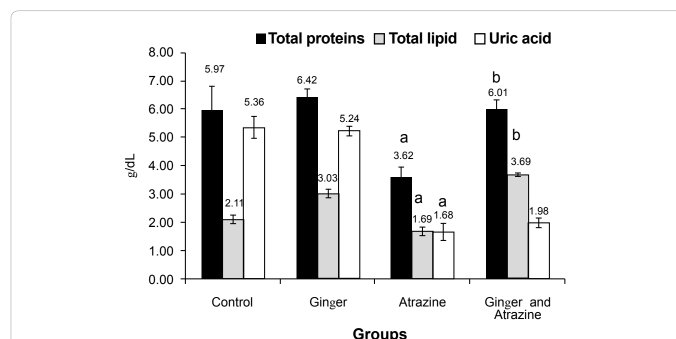

Figure 1: Effect of atrazine alone or in combination with ginger on serum total protein, total lipid and uric acid. Values expressed are mean ± SE (n= 5-6). Animals were killed 14 days after exposure to: control (isotonic solution), ginger (120 mg/kg), atrazine (78.25 mg/kg), and ginger (120 mg/kg) plus atrazine (78.25 mg/kg). [a]Significantly different from control group $P < 0.05$. [b]Significantly different from atrazine group $P < 0.05$.

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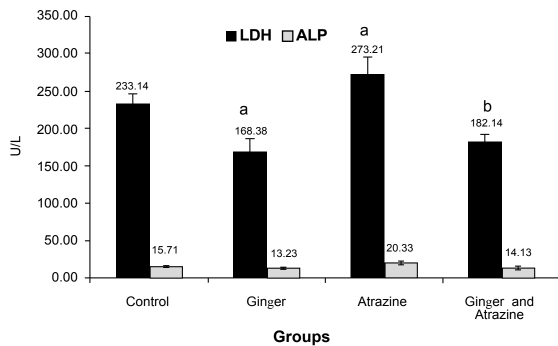

Figure 2: Effect of atrazine alone or in combination with ginger on serum lactate dehydrogenase (LDH) and alkaline phosphatase (ALP). Values expressed are mean ± SE of 5-6 separate animals in each group. Animals were killed 14 days after exposure to: control (isotonic solution), ginger (120 mg/kg), atrazine (78.25 mg/kg), and ginger (120 mg/kg) plus atrazine (78.25 mg/kg). [a]Significantly different from control group $P < 0.05$. [b]Significantly different from atrazine group $P < 0.05$.

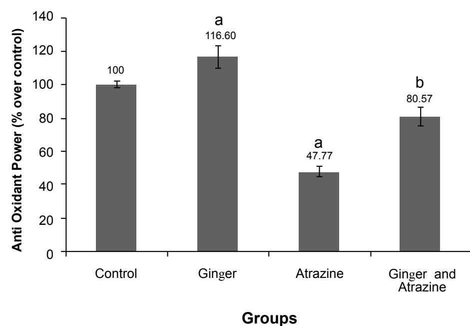

Figure 3: Effect of atrazine and ginger on intracellular ferric reducing/ antioxidant power (FRAP) in hepatocytes of mice. Animals were killed 14 days after exposure to: control (isotonic solution), ginger (120 mg/kg), atrazine (78.25 mg/kg), and ginger (120 mg/kg) plus atrazine (78.25 mg/kg). Values expressed are mean ± SE of 5-6 separate animals in each group. FRAP was determined by measuring the absorbance at 593 nm due to the formation of a blue colored Fe^{II}-tripyridyltriazine compound from the colourless oxidized Fe^{III} form by the action of electron donating antioxidants. About 20 µl liver homogenate was used (see Section 2.7). [a]Significantly different from control group $P < 0.05$. [b]Significantly different from atrazine group $P < 0.05$.

There are no significant differences in the activity of LDH and ALP in the sera of control group or animals group given ginger (Figure 2). Data shows that there was an insignificant elevation in ALP in the sera of animals treated with atrazine for 2 weeks. However, LDH exhibited significant increase after the second of atrazine treatment. On the other hand, animals treated with atrazine and ginger showed significant decrease in LDH activity in comparison with atrazine group.

(Figure 3) represents the effect of ginger or atrazine or ginger plus atrazine on intracellular antioxidant activity (FRAP) of hepatocytes. The FRAP increased in ginger and ginger-atrazine groups as compared to control and atrazine-treated animals, respectively.

A significantly pronounced release of MDA level was observed only in liver and not in kidney of atrazine-treated animals as compared to the control group (Figure 4). Co-administration of ginger along with atrazine significantly decreased the MDA.

SOD and catalase activities in liver and kidney of control and treated rats are presented in (Figure 5, Figure 6). SOD activity in liver and kidney of atrazine-treated mice (3.37 and 1.16 U/g tissue, respectively) was significantly lower as compared to control mice (7.39 and 2.14 U/g tissue, respectively). However, Co-administration of ginger along with atrazine significantly increased the SOD activity of liver tissue and did not effect on the SOD activity of kidney tissue as compared to atrazine-treated mice. Catalase activity of liver and kidney of atrazine-treated mice (1.45 and 3.5 U/g tissue, respectively) was significantly lower (Ñ < 0·001) than that in liver and kidney of control mice (6.35 and 4.59 U/g

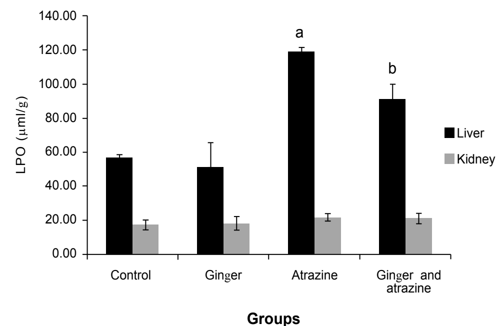

Figure 4: Degree of lipid peroxidation expressed as the content of malondialdehyde (MDA) determined in livers and kidney from mice submitted to different treatments. Control mice, ginger (120 mg/kg), atrazine (78.25 mg/kg) and ginger (120 mg/kg) plus atrazine (78.25 mg/kg). Values are expressed as mean ± SEM (n=5). [a]Significantly different from control $P < 0.001$. [b]Significantly different from atrazine group $P < 0.05$.

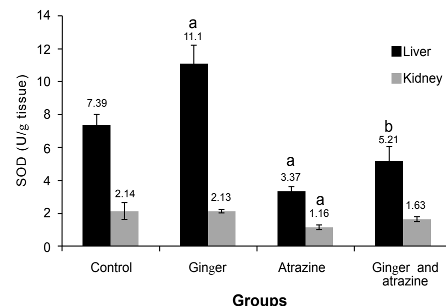

Figure 5: Effect of atrazine and ginger on the SOD activities in liver. SOD activity was determined by measuring the absorbance at 480 nm. 10 µl liver homogenate was used (see section 2.9) and activity was expressed as U min/g/ tissue. Values are expressed as mean ± SE (n=5). [a]Significantely different from control $P < 0.05$. [b]Significantly different from atrazine group $P < 0.05$.

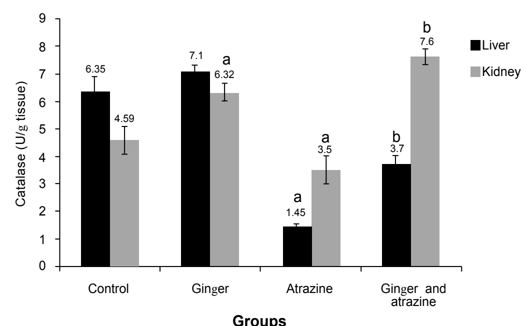

Figure 6: Effect of atrazine and ginger on the CAT activities in liver and kidney. CAT activity was determined by measuring the decrease in absorbance (H_2O_2 degradation) at 240 nm. In the assay 10 µl of liver homogenate was used (see Section 2.9) and activity was expressed as µmol of H_2O_2 consumed min/g/ tissue. Values are expressed as mean ± SE (n=5). [a]Significantely different from control $P < 0.05$. [b]Significantly different from atrazine group $P < 0.05$.

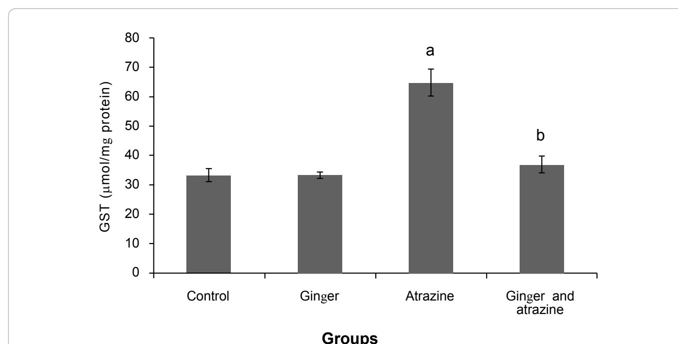

Figure 7: Effect of atrazine and ginger on the GST activities in liver. The activity of GST was expressed as µmol/mg protein and was detected at 340 nm. Values are expressed as mean ± SE (n=5). [a]Significantely different from control $P < 0.05$. [b]Significantly different from atrazine group $P < 0.05$.

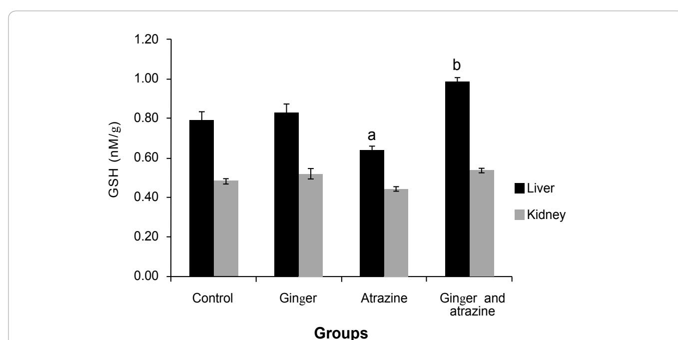

Figure 8: Effect of atrazine and ginger on the GSH content in liver and kidney. GSH content in liver and kidney were quantified in the supernatant obtained from liver and kidney homogenate and by measuring the absorbance at 412 nm. Values are expressed as mean ± SE (n=5). [a]Significantely different from control $P < 0.02$. [b]Significantely different from atrazine group $P < 0.0001$.

tissue, respectively). CAT activity restored in liver and kidney tissue of mice that has been administrated ginger followed by atrazine.

GST activity increased in liver tissue of the mice that treated with atrazine. GST activity decrease to the normal level in liver tissue of animal that treated with ginger followed by atrazine as compared to atrazine group (Figure 7).

GSH levels of kidney tissue did not effect in all treated groups. However, hepatocytes GSH decreased in atrazine-treated mice as compared to control group. GSH level improved to the normal level in treated mice with ginger and atrazine as compared to atrazine group (Figure 8).

Discussions

The present study investigates the propensity of atrazine to induce oxidative stress and its possible attenuation by ginger in liver and kidney of mice. Experimental animals were administered atrazine (78.25 mg/kg body weight) and/or ginger (120 mg/kg body weight, each alternative day) intra-peritoneal for a period of 14 days. Leucocytes are known to increase sharply in the face of infection or toxic substance, as the first line of defense of the body; the factor that led to the reduction in packed cell volume and white blood cell counts noticed in our study in the mice treated with atrazine. It also inhibited lipid and protein synthesis, increased the activity of LDH and ALP, increased the level of LPO and decreased some enzymatic and non-enzymatic antioxidant. Treating animals with ginger and atrazine led to an improvement in haematological parameters as well as in the liver function where a significant decrease in LDH and ALP activity were observed. Results indicated that the reduced glutathione (GSH) content and antioxidant power of the hepatocytes of atrazine treated mice were significantly decreased as compared to the control group. Co-administration of ginger along with atrazine restored the GSH content and antioxidant power level of liver tissue nearly to control levels. Antioxidant is a substance that delays or inhibits oxidative damage to target molecules [33]. The activities of antioxidant enzymes such as SOD and CAT were found to be decreased significantly in the liver tissue accompanied by an increase in the activity of GST, following atrazine exposure. In our study, one of the most prominent changes in CAT activity was noted in renal tissue. This effect might be due to increased H_2O_2 production and ROS generation which in turn induced oxidative stress [34]. GSH in the liver acts either by directly scavenging the free radicals or by acting as a substrate to GSH-Px and GST during the detoxification of hydrogen peroxides, lipid peroxides and electrophiles as well as by preventing oxidation of –SH groups of proteins [35]. SOD is the primary step of the defense mechanism in the antioxidant system against oxidative stress by catalyzing the dismutation of 2 superoxide radicals (O_2^-) into molecular oxygen (O_2) and H_2O_2. Hydrogen peroxide is neutralized by the combined action of CAT and glutathione peroxidase in all vertebrates [36]. These enzymes act in coordination and the cells may be pushed to oxidative stress state if any change occurs in the level of enzymes. In the present study, a significant decrease in the specific activity of SOD is observed in atrazine group suggests an increased superoxide radical production and other ROS thereby induce oxidative damage [37]. CAT activity was also decreased in atrazine-treated mice after 14 days of treatment, which is indicated the presence of superoxide radical [38]. The antioxidant enzyme CAT protects SOD against inactivation by hydrogen peroxide. Reciprocally, the SOD protects the CAT against inhibition by superoxide anion that could be formed during the treatment mice with atrazine.

Further, GST provides protection to the tissues by catalyzing the conjugation reactions between GSH and electrophilic xenobiotics [39]. The inverse correlation obtained in the present study between GSH and GST shows that, as the activity of the enzyme increased and consequently GSH concentration decreased. Therefore, the results indicated that the atazine might generate free radicals that reacted with membrane lipids and induced oxidative break down of membrane structure and decreased the enzymatic and non-enzymatic antioxidant.

On the other hand, when ginger was co-administered along with atrazine, activities of these enzymes were found to be restored significantly. Singh. et al. [8] reported that the activities of antioxidant enzymes such as SOD, CAT, glutathione peroxidase, and GST were found to be increased significantly in the erythrocytes accompanied by a decrease in the activity of the glucose-6-phosophate dehydrogenase, following atrazine exposure. The results in the present study showed that ginger was scavenging free radical by its potent antioxidant. This results were cleared by the data in which ginger reduced the level of hepatic malondialdhyde acting as lipid peroxidation marker and increased the hepatic level of antioxidant enzyme, SOD and CAT. Similarly, Siddaraju and Dharmesh [40] reported that ginger–free phenolic and ginger hydrolysed phenolic fractions exhibited free radical scavenging, inhibition of lipid peroxidation, DNA protection and reducing power abilities indicating strong antioxidant properties. Ansari et al. [41] showed that the ethanolic *Z. officinale* extract pre-treatment or 20 days in isoproternol treated rats induced oxidative myocardial necrosis in rats, enhances the antioxidant defense (CAT, SOD and tissue GSH) and

exhibits cardioprotection property. Bhandari et al. [42] studied the effect of an ethanol extract of ginger on country-made liquor (CML)-induced liver injury in rats. Their results showed that administration of ginger ethanolic extract (200 mg/kg) orally from day 15 to day 21 along with CML produced significant lowering of serum AST, ALT, ALP and tissue lipid peroxide levels. Ajith et al. [43] reported that ginger ameliorated cisplatin- induced nerphrotoxicity and this protection is mediated either by preventing the cispaltin – induced decline of renal antioxidant defense system or by their direct free radical scavenging activity. Amin and Hamza [18] demonstrated that *Z. officinal* increased the activities of testicular antioxidant enzymes, SOD, and CAT and reduced level of malondialdhyde. These results indicate the possible involvement of free radicals in atrazine-induced toxicity and highlight the protective action of ginger, an indigenous medicinal plant product. This makes it a very effective agent for prevention reactive oxygen species production.

In conclusion, results of the study demonstrated that atrazine induced oxidative stress in mice liver than kidney, in terms of decreased activities of the various antioxidant enzymes and decreased content of reduced GSH and antioxidant power. However, ginger administration ameliorated the effects of atrazine, suggesting that ginger is a potential antioxidant against atrazine-induced oxidative stress**.**

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