World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Effect of green tea supplementation on antioxidant enzymes in nephrotoxicity: study in rats

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Received: 04-06-2014 / Revised: 21-06-2014 / Accepted: 15-07-2014

ABSTRACT

Over last few decades Green tea has been subjected to many scientific and medical researchers to determine the extent of its long purported health benefits. This study was designed to investigate nephroprotective role of Green tea on thioacetamide treated rats. Green tea has ability to scavenge free radicals, which are generated by different metabolic pathways of certain medications and toxins, lead to oxidative damage of tissue. Thioacetamide is also a toxic agent, mainly renowned for its role in structural and functional change in liver. It also does alterations in kidney, spleen, thymus, small intestine and lungs. It induces centrilobular necrosis in hepatocytes, bile duct proliferation, and damages apical portion of proximal convoluted tubule of kidney. In current study, 24 male Sprague Dawley rats were divided into four groups (n=6). Group I was control (untreated healthy rats). Group II received TAA (at a dose of 200mg/kg b.w, i.p, twice a week, for 8 weeks). Group III received TAA (at a dose of 200mg/kg b.w, i.p, twice a week, for 8 weeks) and Green tea (500 mg/day, orally for 8 weeks). Group IV received only Green tea (500 mg/day, orally for 8 weeks). Biochemical analysis was evaluated by estimation of antioxidant enzymes SOD and Catalase and lipidperoxidation end product MDA. TAA-induced oxidative stress indicated by elevated levels of tissue MDA and reduced levels of SOD and Catalase. Marked increase in kidney weight was also observed in TAA-treated rats. Treatment with green tea efficiently reduced tissue MDA and restored SOD and Catalase concentration in TAA+Green tea treated rats. The results indicate the protective effect of Green tea for TAA-induced nephrotoxicity.

Key words: Oxidative stress, Green tea, Thioacetamide, MDA, SOD, Catalase.

INTRODUCTION

Camellia sinensis, the tea plant, is an evergreen laurel tree belongs to the family Theaceae. Primarily green tea was used in china (teaguardian.com 2010) but now it is an element of culture in every part of Asia. Green tea is also effective in different health problems and over few decades the research on its health benefits is in progress such as its effect on lowering the risk of cardiac diseases and different types of cancer (sciencedaily.com., 2003). Green tea does not itself increases the rate of metabolism which is enough for weight decrease, but the components of green tea polyphenols and caffeine carry out thermogenesis and which initiates fat oxidation. As a result of this, metabolic rate increases 4% without uplifting heart rate (dullo et al., 1999). Flavinoids present in a cup of green tea are greater than in other casual drinks, fresh fruits and wine (USDA database., 2007). Flavinoids are chemicals present in plants responsible for anticarcinogenic and antioxidative functions (USDA database., 2007). Green tea has different enzymes, carbohydrates, amino acids, sterols, lipids, caretenoids, polyphenols, caffeine, tocopherols, vitamins and its derivatives, plant chemicals and food minerals. There are diverse researches on the constructive effects of green tea on health in vitro and in animal studies but only few of them are proved for human health (Cabrera et al., 2006).

Polyphenols in green tea have catechins, theaflavins and thearubigins which have large numbers of health benefits. These polyphenols serve as antioxidants by scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions. Currently, the role of tea polyphenols in prevention of chronic diseases is specifically investigated (McKay et al., 2002). The

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prevention is done by many mechanisms but considerably the radical scavenging and antioxidant properties of tea polyphenols are thought to be the main spot of investigation (Higdon et al., 2003). Leaves of green tea contain catechins which are flavinol monomers. Different types of catechines present in green tea are: Epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG) s (*Graham et al., 1992*). EGCG is the most common catechin in tea. There are various studies enlightening that the tea polyphenols and catechins are strong scavengers of reactive oxygen and nitrogen specie in vitro, including superoxide [O·2−] (Nanjo et al., 1993; Nakagawa et al., 2002), peroxyl radicals, singlet oxygen (Guo et al., 1999), peroxynitrite [ONOO− (Haenen et al., 1997), and hypochlorous acid (Scott et al., 1993).

The potential of tea polyphenols to chelate metal ions, for example iron and copper, may take part to their antioxidant property by inhibiting redox-active transition metals from catalyzing free radical establishment (*Rice-Evans et al., 1997*). The ability of tea polyphenols to inhibit copper-mediated LDL oxidation and other transition metal-catalyzed oxidations in vitro are explained by these metal chelating properties (*Brown et al., 1998*).

The redox sensitive transcription factor, nuclear factor- κB (NF-κB) and activator protein (AP-1) can be introverted by green tea polyphenols and catechins because of their role as kinase Inhibitor in complex signaling pathways (*Yang et al., 2002*). Due to inflammatory cytokines, the inflammatory cells are stimulated and hence there is an increase in release of inducible nitric oxide synthase (iNOS) and production of nitric oxide (NO). These nitric oxides react with oxygen and form $ONOO^-$ and other derived oxidants which damage DNA and protein (surh et al., 2001).The catechins of green tea can inhibit lipopolysaccharide-induced iNOS gene expression and its activity in macrophages. Green tea catechins inhibit iNOS by decreasing NF- κB activation (lin et al., 1999). The lipoxygenases and cycloxygenases are able to co-oxidize molecules of other substrate too, due to their peroxidase activity, which increase oxidative stress in tissues (Parkinson., 1996). The polyphenols of green tea inhibit cycloxygenases (COX)-2 and 5-, 12-and 15- lipoxygenases activities in human colon cancer cells (Hong et al., 2001). Green tea supplement given to rats inhibits epidermal COX and lipoxygenase activities (Katiyar et al., 1997). The xanthine oxidase enzyme is inhibited by green tea polyphenols. Xanthine oxidase enzyme stimulates the synthesis of reactive oxygen species by catalyzing the hypoxanthine and xanthine to uric acid. Along with it also reduces O_2 to $O\cdot_2^-$ and H_2O_2 (Aucamp et al., 1997). The EGCG from green tea inhibits xanthine oxidase activity in leukemia cells (Lin et al., 2000).

Phase II enzymes increase the excretion of toxins and carcinogenic chemicals. These phase II enzymes have cis-acting regulatory elements i.e. antioxidant response elements (ARE). The ability of glutathione to damage nucleic acid and protein is reduced by Glutathione S-transferase (GST) which catalyzes the conjugation of glutathione to electrophile and GST is a family of phase II enzymes (Yu et al.,1997).

MATERIAL AND METHODS

Male Sprague Dawley rats weighing (200-250 g/kg b.w.) purchased from the animal house of Agha Khan University Hospital, Karachi, Pakistan for the study. Animals were acclimatized to the laboratory conditions one week before the start of experiment and caged separately in a quite temperature-controlled room (23`± 4^0C). Rats had free access to water and standard rat diet.

Ethical Guideline: The experiments were conducted in accordance with ethical guidelines of institutional FRB (ethical Review Board) and internationally accepted principles for Laboratory use and care in animal research (Health research extension Act of 1985).

Study Protocol and Drug Administration Schedule: 24 Male Sprague Dawley rats were randomly divided into four groups, each of six rats. The duration of the study was 8 weeks. Each group received following treatment;

Group I: the control (remain untreated)
Group II: TAA-treated
Group III: TAA+Green tea treated
Group IV: Green tea treated

Group I was the control group and remained untreated and was weighed every week. Group II was the TAA treated group, received thioacetamide at a dose of 200gm/ kg b.w, intraperitoneally, twice a week, for 8 weeks. Group III was the TAA+ Green tea treated group, received thioacetmide at a dose of 200mg/kg b.w, twice a week, for 8 weeks and received green tea 500mg/kg b.w, orally, for 8 weeks daily. Group IV received only green tea at a dose of 500mg/kg b.w,orally for 8 weeks and weighed every week. After 24 hours of last dose of treated groups, rats were decapitated and the blood was collected from the neck wound in the lithium heparin coated tubes. The collected blood was mixed gently and then transferred to centrifuged glass tubes and then centrifuged at 2000 rpm for 20 minutes. Serum was separated and collected in

eppindroff tubes and stored at-70°C until analysis. Liver was excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination dried by blotting with filter paper and weighed. The remaining tissues then kept in freezer -70°C until analysis.

Preparation of kidney Homogenate: (Ricardo et al, 2005): Kidneys were sliced into small pieces, homogenates were obtained using a tissue homogenizer Ultra Taurax T-25 Polytron at 4°C. The homogenates (1:10 w/v) were prepared by using a 100 mM KCI buffer (pH=7.0) containing EDTA 0.3 mM. All homogenates were centrifuged at 600 g for 60 minutes at 4ºC and the supernatant was used for biochemical assays (MDA, Catalase, SOD).

Estimation of Malonyldialdehyde (MDA) (Okhawa et al, 1979): The malonyldialdehyde (MDA) content, a measure of lipid peroxdation, was assayed in the form of thiobarbituric acid reacting substances (TBARS) by the method of Okhawa et al, 1979. The reaction mixture consisted of 0.2 mL of 8.1 % sodium dodecyle sulphate, 1. 5 mL of 20% acetic acid solution adjeusted to pH= 3.5 with sodium hydroxide and 1. 5 mL of 0.8% aqueous solution of thiobarbituric acid was added to 0.2 mL of 10% (w/v) of homogenate. The mixture was brought up to 4.0 mL with distilled water and heated at 95 °C for 60 minutes. After cooling with tap water, 1.0 mL distilled water and 5.0 mL of the mixture of n- butanol & pyridine (15:1 v/v) was added and centrifuged. The organic layer was taken out and its absorbance was measured at 532 nm on schimadzu-spectrophotometer UN 120-01 and compared with those obtained from MDA standards. The concentration values were calculated from absorption measurements as standard absorption in nM/g tissue.

Assessment of Antioxidant Status:

Estimation of Catalase (Sinha, 1972): Catalase activity was assayed by the method of Sinha et al, 1972, in a clean glass test tube, the assay mixture consisted of 1.96 mL of phosphate buffer (0.01 M, pH= 7.0) 1.0 mL of hydrogen peroxide (0.2 M) and 0.04 mL (10 mL of 2 m L of dichromate acetic acid reagent (5% of 50 mL dichromic acid + 150 mL of glacial acetic acid) was added in 1 mL of reaction mixture, boiled for 10 min, cooled. Changes in absorbance were recorded at 570 nm on Schimadzu-spectrophotometer UV 120-01. The concentration values were calculated from absorption measurements as standard absorption in mM/g tissue.

Estimation of Superoxide Dismutease (SOD) (Kono et al, 1978): Levels of SOD in the cell free supernatant were measured by the method of Kono et al, 1978. Briefly, 1.3 mL of solution A (0.1 mM EDTA containing 50 mM Na2CO3 pH= 10.0), 0.5 ml of solution B (90µM NBT-nitroblue tetrazolium dye) and 0.1 ml of solution C (0.6% triton-100 in solution A), 0.1 ml of solution D (20mM Hydroxylamine hydrochloride, pH= 6.0) were mixed and rate of NBT reduction was recorded for one minute at 560 nm on Schimadzu-spectophotometer UV120-01. 0.1 ml of the supernatant was added to the test and reference cuvettes, which do not contain solution D. Finally, the % inhibition in the rate of reduction of NBT was recorded as described above in U/g tissue. One enzyme unit was expressed as inverse of the amount of protein (mg) required inhibiting the reduction rate by 50% in one minute. The activity was calculated using the % inhibition in gram of tissue and expressed in Unit/g tissue.
Calculation of SOD activity

Statistical Analysis: Results are presented as mean ± SD. Statistical significance and difference from control and test values were evaluated by student's t-test. P-values of *P<0.01 and **P<0.05 were considered significant.

RESULTS

Effect of thioacetamide and Green tea treatments on kidney weight and kidney to body weight ratio in control and treated rats: Increased kidney weight and relative kidney weight was observed in TAA group after 8 weeks administration of thioacetamide as compare to control (0.74±0.07 P<0.01) (3.30±0.26 P<0.01) (Table 1). Whereas reduction in the kidney weight and relative kidney weight was observed in TAA+ Green tea group as compare to control (0.71±0.03 P<0.01) (3.10±0.15 P<0.01) respectively. An increase in relative kidney weight was observed in Green tea treated rats (0.70±0.15 P<0.01) as compare to control whereas relative kidney weight was almost normal (2.87±0.67 P<0.01) as compare to control.

Effects of thioacetamide and Green tea treatment on renal concentration of MDA in control and treated rats: Level of MDA was markedly increased in TAA-treated group as compare to control (19.28±6.98, P>0.05). Green tea administration in TAA+Green tea group decreased the concentration of MDA as compare to control rats. (8.22±5.38, P<0.05) while green tea treated group showed a slight increase in MDA level as compare to control (4.59±3.5, P<0.01) (Table 2).

Effects of thioacetamide and Green tea treatment on renal concentration of SOD in control and treated rats: Table 2 showed a significant decrease in SOD activity in TAA treated group as compare to control (2008.05±250.9, P<0.01). TAA + green tea group after green tea supplementation, showed normal level of SOD activity (3366.1±225.2, P<0.01) as compare to control. SOD activity reduced in green tea –treated group (3053.7±243.7, P<0.01) as compare to control.

Effects of thioacetamide and Green tea treatment on renal concentration of catalase in control and treated rats: Concentration of catalase was significantly decreased in thioacetamide treated group (0.9±0.65, P<0.01) as compare to control. Administration of green tea in TAA+Green tea group resulted in increased catalase level (2.10±0.12, P<0.01) as compare to control. Alone Green treatment had no effect on catalase level (3.11±0.73, P<0.01) (Table 2).

DISCUSSION

There is a number of certain medications such as acetaminophen and gentamycin and a few environmental toxic chemicals which can originate brutal kidney injuries by the generation of highly reactive oxygen and nitrogen species (Olagunju et al., 2009). Thioacetamide is a widely used chemical and industrial toxin which induces centrilobular hepatic necrosis, bile duct proliferation, liver cirrhosis, and hepatocellular carcinoma. It also damages the apical region of proximal convoluted tubule of kidney (Edward and Smuckler., 1974). Hence thioacetamide damages only selective regions of tissues like hepatocytes, cortical thymocytes, and renal proximal convoluted tubular cells (Edward and Smuckler., 1974).

The renal damage due to induction of thioacetamide results in the reduction of glomerular filtration rate due to production of secondary free radicals and/or obstruction or leakage of renal filtrate in backward direction (Leena and Balaraman, 2005). In vivo, TAA is metabolized into the sulfine (sulfoxide) and sulfene (sulfone). These metabolites approach all the vital organs of the body with blood and at last, excreted in the form of acetate by urine within 24 hours. Sulfine and sulfene are extremely reactive compounds which damage all the cellular biomolecules by the induction of lipid peroxidation, generation of free radicals and unbalancing the antioxidant enzyme system which consequently reduces the intracellular free radical scavengers (Abu et al., 2002). Treatment of rats with thioacetamide for the duration of 8 weeks in group II, produced toxicity in kidney which was diagnosed by estimation of lipid peroxidation end product i.e. MDA and antioxidant enzymes i.e. SOD and Catalase. There was marked increase in MDA concentration and considerable decrease in concentrations of SOD and Catalase as compare to control rats.

The reduction in SOD and Catalase activities indicated frequent production of free radicals in excess amount which results in cellular damage (Abu et al., 2005). TAA-induced toxicity principally occurs due to generation of free radicals (Mohammad et al., 2011) which results in oxidative stress due to altered antioxidant protective mechanism and the concentration of SOD and Catalase in kidney cells (Parlakpinar et al., 2005). This oxidative stress can be reversed by the intake of dietary antioxidants, preferably with natural products because of its low cost and absence of side effects. Most of the natural products contain natural polyphenols and free radical scavenger molecules (Brown et al., 1998). Green tea is natural plant which consists of great number of polyphenols which are powerful antioxidants. They contain catechins and it derivatives, which are absorbed, metabolized and distributed into all organs (Lee et al., 2000). The treatment of group III with green tea significantly reduced the MDA level which is end product of lipid per-oxidation of tissue membranes.

This reflects the effect of green tea in scavenging free radicals and in prevention of oxygen, peroxyl, hydroxyl and lipid radicals and superoxide anions (Guo et al., 1996; Jovanovic et al., 1997; Khan et al 1992). Membrane phospholipids are protected by lipid peroxidation due to free radical scavenging property of green tea; hence the lower rate of ROS formation results in stoppage of lipidperoxidation. Water soluble antioxidant catechins also decrease the transport of free radicals in lipid bilayer. The hydrophobic portion of catechins penetrates in lipid bilayer and influences the antioxidant ability of biological membranes. It reaches to the hydrophobic core of membranes and produces membrane stabilizing effect by altering the arrangement of lipid packaging (Arora et al., 2000). Additionally, they can also react with head groups of phospholipids, especially with the hydroxyl group containing head groups so they reduce the fluidity and polar surface of phospholipids (Chen et al., 2002). Moreover, catechins also chelate metal ions, particularly copper and iron which also reduce the production of free radicals (Guo et al., 1996). There was also significant increase in antioxidant enzymes SOD and Catalase by green tea in group IV of rats. These results reflect that intake of green tea catechins have biological considerations. Catechism and its derivatives such

as EGCG serve as defensive agents against oxidative stress by stimulating antioxidant enzymes and balancing free radical generation and antioxidant production (Aucamp et al., 1997).

Table 1: kidney weight, kidney to body weight ratio in control and treated rats.

Groups	Kidney weights	Relative kidney weights
Control	0.62±0.08*	2.69±0.35*
TAA-Treated	0.74±0.07*	3.30±0.26*
TAA+Green tea treated	0.71±0.03*	3.10±0.15*
Green tea treated	0.70±0.15*	2.87±0.67*

Values are mean±SD. Significant difference among control, thioacetamide and TAA+Green tea treated groups by t-test. *P<0.01.

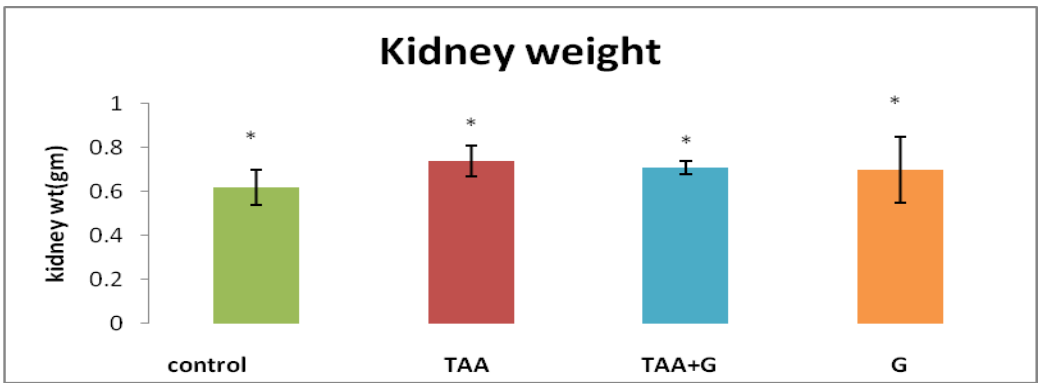

FIGURE 1: Effect of thioacetamide and Green tea treatments on kidney weight in control and treated rats.

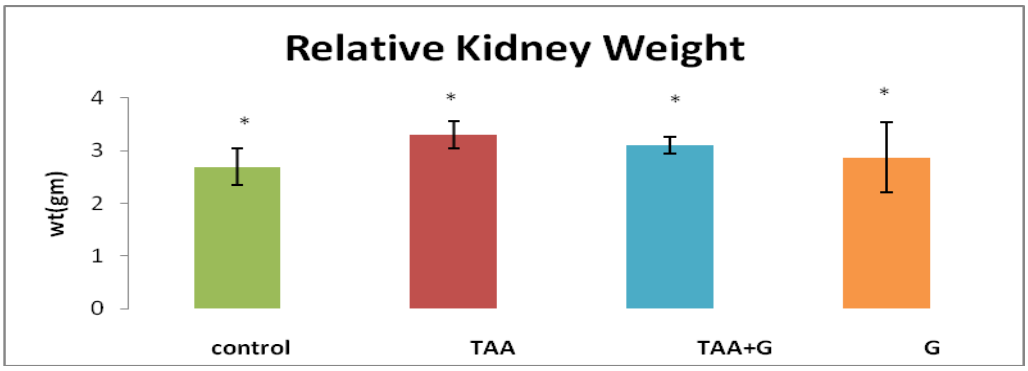

FIGURE 2: Effect of thioacetamide and Green tea treatments on kidney to body weight ratio in control and treated rats.

Table2: Effects of thioacetamide and green tea treatment on renal concentrations of malondialdehyde, superoxide dismutase and catalase.

Groups	MDA (nmol/gm)	SOD (U/g)	Catalase (mM/g tissue)
Control	16.80±7.58*	2928.6±158.4*	3.01±0.01*
TAA- treated	19.28±6.98	2008.05±250.9*	0.9±0.65*
TAA+Green tea treated	8.22±5.38*	3366.1±225.2*	2.10±0.12*
Green tea	4.59±3.5*	3053.7±243.7*	3.11±0.73*

Values are mean±SD. Significant difference among control, thioacetamide and TAA+Green tea treated groups by t-test. *P<0.01, *P<0.05.

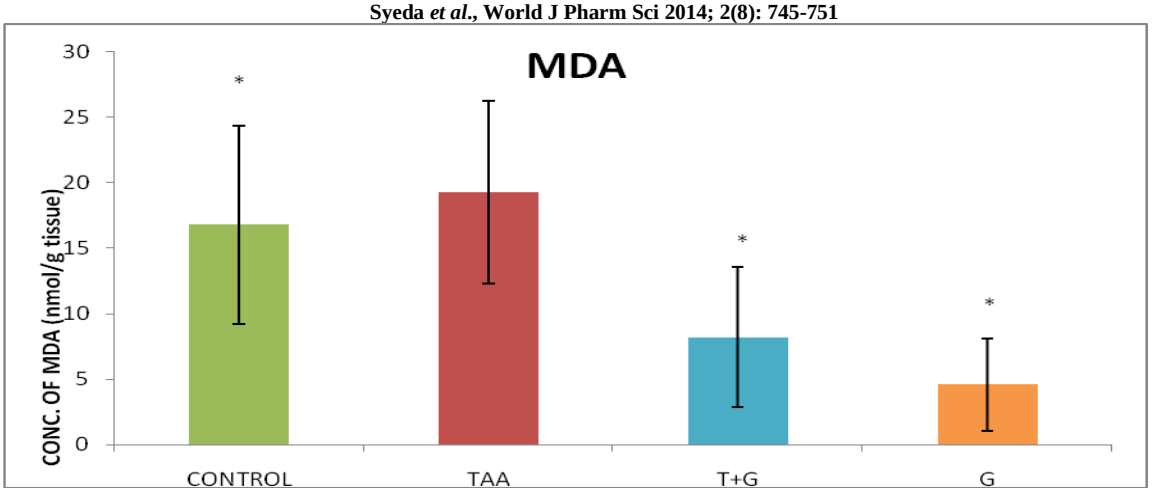

Figure 3: Effects of thioacetamide and Green tea treatment on renal concentration of MDA in control and treated rats

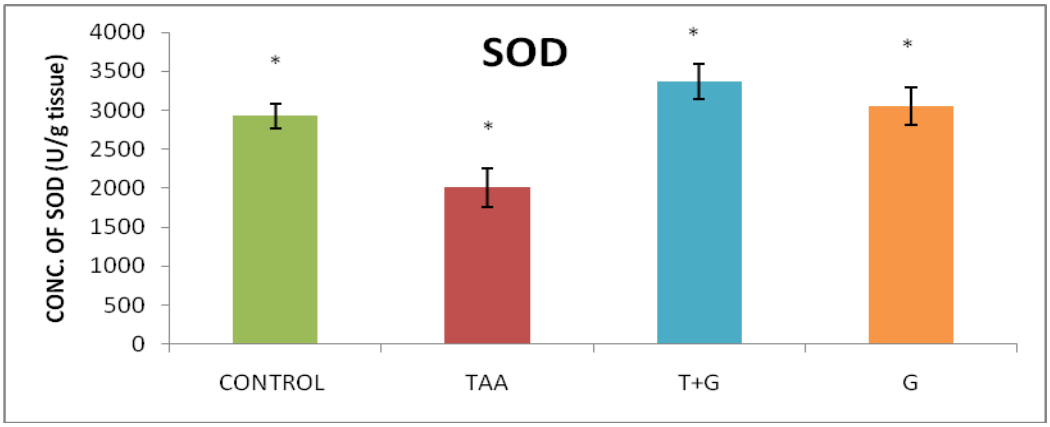

Figure 4: Effects of thioacetamide and Green tea treatment on renal concentration of SOD in control and treated rats.

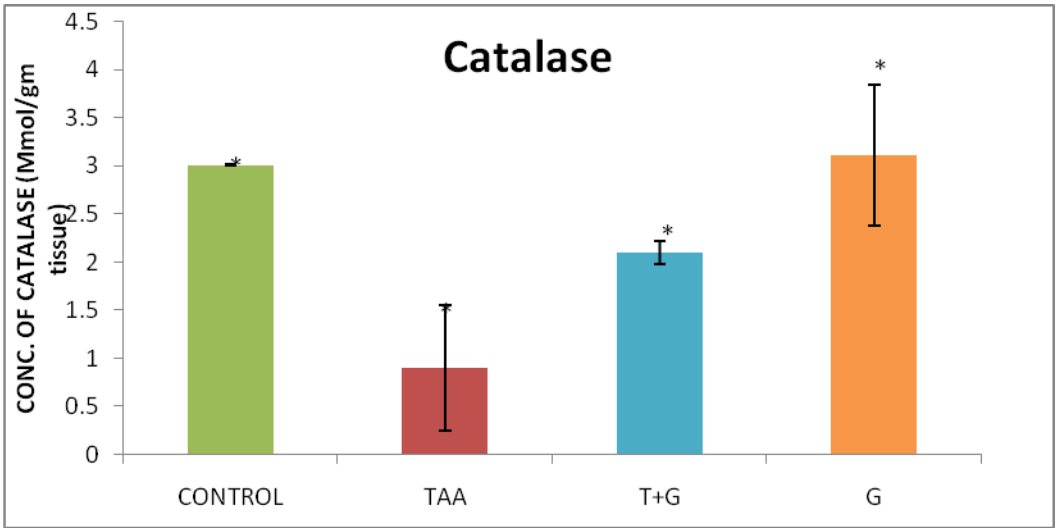

Figure 5: Effects of thioacetamide and Green tea treatment on renal concentration of catalase in control and treated rats.

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