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# The Effect of Green Tea (*Camellia sinensis*) Extract on Liver Tissue Injury Consequent Isoniazid Administration in the Rats

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**Abstract:** Tuberculosis continues to be a common health problem worldwide. Isoniazid, an antibiotic used routinely for tuberculosis chemotherapy is documented to be a potent hepatotoxicant. The aim of the present study was to assess the hepatoprotective activity of green tea (*Camellia sinensis*) extract (GTE) against isoniazid induced hepatotoxicity in the rats. Male Wistar rats were randomly allocated into 4 groups of 10 animals each including: normal healthy control rats, healthy rats receiving GTE, toxicant control and toxicant drug plus GTE treatment group. In groups 2 and 4 GTE (1.5%, w/v) was given as only source of drinking for 8 weeks. In the midst stage of experiment (4th and 5th weeks), Isonizid (50 mg $kg^{-1}$ b.w./day, i.p.) was administrated for groups 3 and 4 for a period of 2 weeks. At the end of experiment, serum biomarkers of liver tissue injury and product of lipid peroxidation (MDA), activities of Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPX) and Glutathione Reductase (GR) were assayed in liver homogenates. Finally, the biochemical findings were matched with histopathological verifications. Significant differences among the groups were determined by one-way analysis of variance followed by Tukey post-test. In group 4, GTE significantly ($p<0.05$) decreased the elevated levels of serum biomarkers of hepatic injury and total bilirubin and significantly ($p<0.05$) increased the reduced levels of serum albumin and total proteins (respectively $p = 0.001$, $p = 0.032$). In this group, GTE significantly ($p<0.05$) decreased the lipid peroxidation and elevated the decreased values of hepatic antioxidants. Histopathologically, the changes were in the same direction with biochemical findings. This study showed that the hepatoprotective effect of GTE in isoniazid-induced oxidative damage may be related to its antioxidant and free radical scavenging activity.

**Key words:** Isoniazid, hepatotoxicity, green tea extract, hepatoprotective activity, rats

## INTRODUCTION

Tuberculosis is an important worldwide common health concern in the daily live. Especially in adult people with AIDS. It is one of the main causes of death (Gajalakshmi *et al*., 2003). Isoniazid that is being used as a potent anti-tuberculosis agent considered a potent hepatotoxic drug where considered as the main site of its detoxification. So by different mechanism its administration will produce multiple metabolic and morphologic dysfunction and hepatitis. That depend on the cause of the illness is being identified by serum Albumin and Globulin decreasing. It has been shown that Endogen lipids Peroxidation is the main factor contributing in isoniazid cytotoxicity. Isoniazid induced oxidative damage give rise to highly active oxygen production that causes lipid peroxidation and destruction of lipid membranes (Georgieva *et al*., 2004). Reportedly, during isoniazid hepatotoxicity there is a change in various cellular defense mechanism including enzymatic and non-enzymatic mechanism (reduced glutathione, GSH) (Tasduq *et al*., 2005). The use of herbal drugs in treatment of wide variety of disease is rapidly developing. Now a days, there is a special attention to the protective effects of natural antioxidants against chemical agent's toxicity (Frei and Higdon, 2003). Today, tea as a source with biologic and pharmacologic activities, considered as a useful agent for human health. Therapeutic effects of tea extract and catechin polyphenols led to scientific studies on prevention and treatment of various diseases (Mandel *et al*., 2006; Ostrowska and Skrzydlewska, 2006). Crepsy and Williamson (2004) showed that green tea (*Camellia sinensis*) extract has antioxidative and free radical scavenging properties (Crespy and Williamson, 2004). Mohamadin *et al*. (2005) showed that green tea supplementation reduces hepatic damage due to cyclosporine-A administration (Mohamadin *et al*., 2005). Researchers know that green tea catechins, prevent

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lipid peroxidation from chemical agents in the liver and kidney of the animals (Sano *et al*., 1995). Moreover, researches have shown that green tea polyphenols have Antineoplastic effects in Humans (Lung *et al*., 2002; Yang *et al*., 1999). Due to various therapeutic benefits of green tea, seem that it will be able to protect the liver from toxic oxidative effect of isoniazid. However, there was no similar study about protective effects of green tea against isoniazid hepatotoxicity. So, present research has been designed for the first time to evaluate the protective properties of green tea against isoniazid hepatotoxicity.

## MATERIALS AND METHODS

### Extraction method

**Extract preparation:** The extract was prepared according to method of Maity *et al*. (1998). About 15 g fresh green tea powder was soaked in 1 L of boiling stilled water for 5 min. Then were filtered and a 1.5% extract were prepared. This extract was the only drinking water source available for treatment group (Maity *et al*., 1998).

**Chemical materials:** All chemicals used in this study were of analytical grade and were obtained from Nanjing Jiancheng Bioengineering Institute, Nanjing, China.

**Study pattern:** Present study was an interventional and experimental research. Forty male Wistar rats weighing 200±20 g were randomly selected with average of 9 weeks old. Feeding and caging conditions were the same for all groups and they were under 12 h light/dim and environmental temperature of 21±2°C were fed with standard ration and water *ad libitum*. After 1 week adaptation period, the experiment was started. All ethical consideration and experiment protocols approved by regional SPCA committee. Rats were randomly divided into four group of ten each: healthy control group, healthy group treated will extract, toxicant control group, group of treatment with extract and toxicant drug.

The experiment continued to week 8. Since, the day 1st till the end of week 8, group 1 and 3 merely received stilled water and group 2 and 4 received 1.5% green tea extract as drinking water in midst of the experiment (weeks 4 and 5), isoniazid solution in sterile distilled water (10 mL $kg^{-1}$) was administered intraperitoneally daily (50 mg $kg^{-1}$) (Prabakan *et al*., 2000; Sodhi *et al*., 1997). Simultaneously distilled water was administered to groups 1 and 2.

**Analysis of biochemical factors:** At the end of the experiment and 24 h after last treatment, blood sample were taken through Retro bulbar plexus for analyzing ALT, AST (Reitman and Frankel, 1957), LDH (Martinek, 1972), albumin, total protein (Lowry *et al*., 1951) and total bilirubin (Malloy and Evelyn, 1937). Blood samples were centrifuged at 2500 rpm at 30°C for 15 min and sera were derived.

**Evaluation of anti-oxidative activity:** All mice were euthanized, their liver excised and washed in cool saline. About 15% homogenate in 1.15% (w/v) potassium chloride prepared. Homogenate were centrifuged by 1000 rpm for 10 min in 4°C and supernatant liquid was taken to evaluate the malondialdehyde (rate of lipid peroxidation) superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase (rate of anti oxidative activity).

**Evaluation of malondialdehyde:** Malondialdehyde were evaluated according to Esterbauer and Cheeseman (1990) method as a criterion for lipid peroxidation. In summary a reactive solution comprised of 0/2 mL 8.1% lauryl sodium sulphate, 1.5 mL of 20% acetic acid (pH = 3/5) and 1.5 mL of 0/8% acetic acid aquatic solution in 0/2 mL of 10% PMS were prepared. Total volume of final solution by adding distilled was reached to 4 mL water and then were warmed to 95°C for 60 min. Then, let to be cooled in running water, 1 mL distilled water and 5 mL N-butanol and pyridine (15:1, V/V) mixture was added to and centrifuged. The organic layer was removed and absorbance rate was measured in 532 (nm) nanometer. TBARS levels were calculated by 1/56×105/M/cm coefficient and expressed in nanomole TBARS in milligram. Tissue protein was measured by Biureha Method and expressed as nanomol per milligram.

**Measurement of superoxide dismutase activity:** Superoxide dismutase activity was measured by Nishikimi *et al*. (1972) method and modulated by Kakkar and Viswanathan (1984) method. About 5 μg of total protein from each hepatic homogenate was mixed with pyrophosphate buffer (PMT and NBT). The reaction were started by adding NADH and incubated for 90 sec in 30°C then stopped by adding 1 mL glossal acetic acid. Absorptive rate resultant colorful material was measured in 560 nm under research conditions each until of superoxide dismutase activity were determined as needed concentration to prevent from color production up to 50% in 1 min.

**Measurement of catalase activity:** According to Claiborne (1985) Method and based on hydrogen peroxide decomposition catalase activity was measured in 240 nm. In summary, measured mixture volume was totally 10 mL

and comprised of 1/95 mL phosphate buffer (0/05 M, pH = 7), 1 mL hydrogen peroxide 0/019M) and 0/05 mL PMS (10%). Final results were calculated as catalase activity per minute.

**Measurement of glutathione peroxide activity:** Glutathione peroxidase activity was measured according to Rotruck *et al.* (1973) method by the following description:

$$H_2O_2 + 2GSH \text{ (Reductive Glutathione)}$$
$$2H_2O + GSSG \text{ (Oxide Glutathione)}$$

Glutathione is being oxidated by glutathione peroxidase meanwhile hydrogen peroxide is being reduced to water. This reaction is being stopped by acid trichloroacetic and remaining glutathione reactivated by DTNB solution that produced a colorful complex measurable by spectrophotometer in 420 Nm. Reactive mixture comprised of 0/2 mL EDTA (0/8 mM), 0/1 mL sodium azid (10 Nm), 0/1 mL hydrogen peroxide 2.5 mM and 0/2 mL homogenate that incubated in 37°C for 10 min. The reaction was stopped by adding 0/5 mL of acid trichloroacetic then all tubes were centrifuged with 2000 rpm for 15 min and 3 mL hydrogen disodium (0/8) and 0/1 mL of 4% DTNB were added to the supernatant solution then shortly produced color was measured in 420 nm. Glutathione peroxidase activity was expressed in micromole glutathione oxide per minute per milligram.

**Measurement of glutathione reductase activity:** Glutathione reductase activity was measured by method of Mohandas *et al.* (1984) according to following formula:

$$NADPH + H^+ + GSSG \rightarrow NADP^+ + 2GSH$$

Glutathione oxidate is being reduced in the presence of glutathione reductase at the same time NADPH is being oxidized to $NADP^+$. Enzymatic activity was measured with spectrophotometer at room temperature by the rate of NADPH disappearance per minute in 340 nm.

**Microscopic studies:** Samples were taken from diaphragmatic aspect of left hepatic lobe and fixated in 10% buffered formalin for histopathologic evaluation. Taken samples using current tissue passaging techniques and pathologic sectioning, cut into 5 micron thickness and prepared with H&E staining method. Pathologic slides were evaluated as double blind with semi-quantitative criteria.

Histopathologic changes based on the severity of injury were graded from 0-3 grades, zero represents normal state grade 1 represents slight hydropic degeneration, loss of Kupffer cells proliferation and loss of necrosis while grade 2 represents the presence of moderate hydropic degeneration, Kupffer cells proliferation and loss of necrosis or slight necrosis. Grade 3, represent severs hydropic degeneration, Kupffer cells proliferation and necrosis (Kart *et al.*, 2010). Evaluation and grading were randomly done in five microscopic fields with x100 magnification by means of light microscope. Nikon branded (ECLIPSE E200) made in Japan.

**Statistical analysis:** Data analysis was made by SPSS Software Ver. 13. Acquired quantitative data were presented as mean±standard deviation and significancy of differences between groups evaluated by ANOVA and post hoc tukey test. Significance level was set to $p<0/05$.

## RESULTS AND DISCUSSION

Consumption of the extract in healthy rat in compare with control group did not made a significant alteration in evaluated parameters. Serum levels of enzymes showing hepatic damage (ALT-AST-ALP -Total bilirubin) in mice of group 2 (Received isoniazid), in compare to healthy control group has a significant increase ($p<0/001$) and total Protein and serum albumin has significantly decreased. Also in mice treated with isoniazid, concentration of super oxid dismutase, catalase, glutathione peroxidase and glutathione reductase in compare to control group had a significant decrease ($p<0/001$) and malondialdehyde had a significant increase ($p<0/001$). Treating with 1/5% green tea extract result in significant decrease in hepatic damage enzymes and total serum bilirubin due to isoniazid administration. However, their concentration were not reached to the normal levels. But green tea administration could not induce any significant increase in reduced total serum protein and albumin when rifampin administrated (Table 1). In group four, treating with 1.5% green tea extract significantly increased levels of mentioned antioxidative enzymes, serum total protein and albumin that had decreased due to isoniazid administration ($p<0/05$). On the other hand, treating with green tea extract significantly lowered the increased levels of malondialdehyde, hepatic damage markers and bilirubin in isoniazid administered mice. Anyway, the mentioned dose of green tea extract never could normalize the mentioned altered parameters in treated rats (Table 2). All histopathologic changes in all groups of experiment has classified and compared. Study

Table 1: Comparison of the effect of green tea extract on serum markers of liver tissue injury among the experimental groups (mean±SEM)

| | | Biochemical parameters (mean±SEM) | | | | | |
|---|---|---|---|---|---|---|---|
| Groups | Treatments | ALT (IU $L^{-1}$) | AST (IU $L^{-1}$) | ALP (IU $L^{-1}$) | Total Bilirubin (mg $dL^{-1}$) | Albumin (g $dL^{-1}$) | Total protein (g $dL^{-1}$) |
| 1 | Normal healthy control rats | 54.82±2.36 | 68.9±2.31 | 194.87±9 | 0.81±0.03 | 4.38±0.32 | 8.28±0.58 |
| 2 | Healthy rats receiving GTE | 55.9±2.75 | 67.21±2.84 | 203.55±8.8 | 0.87±0.06 | 4.30±0.56 | 8.16±0.47 |
| 3 | Toxicant control | 76.45±3.95[a] | 101.19±3.96[a] | 270.2±10.5[a] | 1.21±0.08[a] | 3.17±0.25[a] | 5.15±0.48[a] |
| 4 | Toxicant drug+GTE | 51.3±2.64[b] | 70.19±3.11[b] | 214.73±9.9[b] | 0.89±0.04[b] | 4.29±0.41[b] | 7.21±0.52[b] |
| | ANOVA | p = 0.00 | p = 0.00 | p = 0.00 | p = 0.00 | p = 0.00 | p = 0.00 |

Table 2: Comparison of the effect of green tea extract on liver MDA content and antioxidant enzymes activities among the experimental groups (mean±SEM)

| | | Biochemical parameters (mean±SEM) | | | | |
|---|---|---|---|---|---|---|
| Groups | Treatments | MDA (nmol $g^{-1}$ protein) | SOD (U $mg^{-1}$ protein) | CAT (U $mg^{-1}$ protein) | GPX (U $mg^{-1}$ protein) | GR (U $mg^{-1}$ protein) |
| 1 | Normal healthy control rats | 3.14±0.14 | 16.53±0.97 | 68.42±4.4 | 12.28±0.92 | 110.83±7.89 |
| 2 | Healthy rats receiving GTE | 3.25±0.15 | 15.12±0.88 | 63.14±3.45 | 11.98±0.79 | 103.03±5.64 |
| 3 | Toxicant control | 4.33±0.25[a] | 10.59±0.59[a] | 42.45±3.19[a] | 6.34±0.76[a] | 71.17±4.75[a] |
| 4 | Toxicant drug+GTE | 3.72±0.16[b] | 13.55±0.64[a] | 55.77±3.13[a] | 9.45±0.7[a] | 96.58±5.92[a] |
| | ANOVA | p = 0.00 | p = 0.00 | p = 0.00 | p = 0.00 | p = 0.00 |

[a]Significantly different from group 1; [b]Significantly different from group 3

Table 3: Effect of green tea extract on hepatic injuries of experimental rats (mean±SEM)

| Groups | Treatments | Degree of injury |
|---|---|---|
| 1 | Normal healthy control rats | 0.0±0.000 |
| 2 | Healthy rats receiving GTE | 0.0±0.000 |
| 3 | Toxicant control | 2.65±0.17[a] |
| 4 | Toxicant drug+GTE | 0.25±0.14[b] |

[a]Significantly different from group 1; [b]Significantly different from group 3

of hepatic samples from healthy control and healthy treatment groups treated with green tea extract were absolutely normal (Fig. 1a and b). In microscopy of tissue samples from isoniazid administered group showed moderate to severe hydropic degeneration extended from central lobular to portal region. In severe cases there was hepatic cellular necrosis around central venules. There was a sinusoidal hyperemia and dominant increase of scattered Kupffer cells population in some of portal spaces along with mononuclear infiltration (Fig. 1c). In tissue samples of group receiving isoniazid and green tea extrac, in some cases there was tissue damage only as hydropic degeneration and slight hyperemia (Fig. 1d) that statistically there was no significant difference between them (Table 3).

Present research is the first study on the protective effects of green tea against Hepato toxicity isoniazid. In this research intraperitoneal administration of 50 mg $kg^{-1}$ isoniazid during 8 weeks resulted in severe hepatic damage that in compare with healthy control group caused a significant increase of serum ALT-AST-ALP-Total bilirubin and a significant decrease of total protein and albumin that is in agreement with earlier findings of Santhosh *et al.* (2007) and Yang *et al.* (1999). Also, isoniazid administration caused notable increase of malondialdehyde and decrease of some enzymatic activities (superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase) in hepatic tissue.

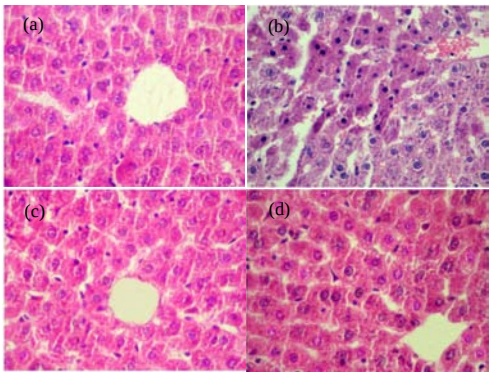


Fig. 1: a-d) Healthy control and healthy treatment groups treated with green tea extract were absolutely normal

These results were agreed with Prabakan *et al.* (2000), Tasduq *et al.* (2005) and Santhosh *et al.* (2007); (Prabakan *et al.*, 2000; Santhosh *et al.*, 2007; Tasduq *et al.*, 2005). Also, isoniazid administration caused notable increase in malondialdehyde levels and decrease in superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase levels in the liver. Isoniazid is a potent P450 cytochrome system stimulant that acts as drugs toxic metabolites producer and attachment site of covalents to hepatic macromolecules (Powell-Jackson *et al.*, 1982). In other words, biotransformation of isoniazid to active metabolites that are able to attach macro molecules causes hepatic damage (Georgieva *et al.*, 2004). Researchers have shown that due to inducing toxic effects in rats liver oxidative stress is the main mechanism that is being used by isoniazid (Attri *et al.*, 2000). Present findings verifies above mentioned pattern so that hepatic tissue of isoniazid

receiving rats (group 3) had a significant increase in lipid peroxidation that was along with significant decrease in mentioned antioxidative enzymes. It seems that in this research reaction of free radicals resulted from isoniazid metabolites with oxygen or reacting superoxide radicals with hydrogen peroxide causes peroxidation of membrane lipid and unsaturated fatty acids of endoplasmic reticulum that gives rise to lipid peroxides production (malondialdehyde). Malondialdehyde increasing, in rats treated with isoniazid is a marker for increase of peroxidation reactions that leads to antioxidative mechanisms weakness that makes impossible to prevent from exaggerated free radicals production (Naik, 2003). Superoxide dismutase, catalase and glutathione peroxidase are antioxidative enzymes that make a defense mechanism against reactive oxygen species (Ji *et al*., 1988). Decrease in superoxide dismutase activity is a sensitive criterion for hepatic damage evaluation. This enzyme is one of the most important factors in enzymatic antioxidative defense system. Superoxide dismutase purges the superoxide anion through its conversion to hydrogen peroxide and so decreases its toxic effects (Curtis *et al*., 1972). In the present study due to abundant production of superoxide anions, in rats treated with isoniazid superoxide dismutase levels significantly decreased. Also, hydrogen peroxide eliminator enzymes (catalase and glutathione peroxidase) significantly decreased. It seems that superoxide dismutase deactivation by increased superoxide anion levels causes inactivation of catalase and glutathione peroxidase. In this study, green tea consumption altered the isoniazid induced of high hepatic damage parameters to normal ranges. Also, using the extract prevented from decreasing superoxide dismutase, catalase and glutathione peroxidase that might be due to free radical scavenging that resulted in protection and surviving these enzymes. Normally in hepatic damage some enzymes like ALS, AST and ALP is being evaluated. Any necrosis or cell membrane damage causes releasing of mentioned enzyme into blood circulation. Increased AST levels show viral hepatic damage, cardiac infarction and muscular trauma. This study there were a wide range of degenerative changes ALT that catalyzestheconversion of alanine to glutamate and pyruvate is a liver specific enzyme and more appropriate parameter for hepatic damage diagnosis. The increased serum level of mentioned enzymes is indicative of cellular leakage and structural damage and hepatic cell membrane dysfunctioning (Drotman and Lawhorn, 1978). On the other hand, serum levels of ALP, bilirubin, albumin and total protein is related to the way of hepatic cellular function. High serum levels of ALP are due to increasing biliary pressure (Muriel *et al*., 1992). Normalizing the increased serum enzyme levels by green tea extract after isoniazid induced hepatic damage may be due to retained membrane stability and integrity or damaged cell repopulation that prevents from intracellular enzyme leakage (Zhao *et al*., 1989). Efficient control of ALP, total protein and bilirubin levels are representative of early functional and secretory mechanisms. In this study, there were a widespread degenerative changes and central lobular necrosis induced by isoniazid. Degenerative changes and pericentral venular necrosis may take place in toxic conditions (Cullen, 2007). Therefore, histopathologic findings of liver, in this research reflect the direct and sensible effects of isoniazid that is agreed with Tandon *et al*. (2008) and Tasduq *et al*. (2005). By administration of green tea results along with isoniazid, there were only slight degenerative changes and no evidence of necrosis that shows green tea protective properties against isoniazid induced hepatotoxicity. Mentioned effects may be attributed to the presence of antioxidant effects and decreasing the oxidative stress found in the extract (Crespy and Williamson, 2004). Anyhow histopathologic findings in this research were accordant with biochemical results. Catalase is an antioxidant enzyme that is highly spread in animal tissue and has maximal activity in the liver and RBCs. Catalase degrades the hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals (Chance *et al*., 1952). Therefore, catalase activity reduction will lead to some destructive effects of superoxide and hydrogen peroxide. Glutathione reductase is a hepatic cytosolic enzyme that is involved in glutathione oxide (GSSG) reduction (GSSG is considered as final product of glutathione peroxidase) (Naik and Panda, 2008). In the present study, after treatment with isoniazid there were remarkable reductions in glutathione peroxidase levels that gave rise to glutathione reductase access to the substrate and have finally lowered glutathione reductase activity.

Polyphenols especially catechins are of the main water soluble compartments found in green tea. The most important catechins found in green tea are: Epicatechin (EC) Epigallocatechin (EGC), Epicatechin Gallate, epigallocatechin gallate. In recent decades *in vitro* and *in vivo* studies have shown that green tea and its polyphenols have potent anti-oxidative effects (Guo *et al*., 1996, 1999; Shen *et al*., 1993; Zhao *et al*., 1989). Green tea catechins are powerful scavengers of superoxide, hydrogen peroxide, hydroxyl radicals and nitric oxide produced from different chemical materials. Catechins also attaches to the metals due to catechol structure and prevent from their free radical production (Rice-Evans and

Miller, 1997). In addition green tea catechins have the same anti oxidative role that urate, beta carotene, Vit. C and Vit. E have in protecting cells (Pietta *et al*., 1998). There are many studies about green tea protective effects against different chemicals. Jiao *et al*. (2003) showed the protective effects of green tea polyphenols against toxic effects of fenofibrate (a blood cholesterol lowering agent) on human HepG2 cells (Jiao *et al*., 2003). Guo *et al*. (2005) reported a protective effect of green tea polyphenols against SH-SY5Y cells apoptosis induced with hydroxydopamine an antiparkinson agent (Guo *et al*., 2005). After repetitive oral administration of 2-nitropropan Sai *et al*. (1998) showed green tea protective effects against its hepatotoxicity, DNA oxidative damage and cell proliferation in rats (Sai *et al*., 1998). Studies of Mehana *et al*. (2012) showed that green tea extract can protect rats liver against lead poisoning (Mehana *et al*., 2012). Xu *et al*. (2007) reported the protective effects of polyphenols in rat liver against Microcystin-R induced hepatotoxicity (Xu *et al*., 2007). Dobrzynska *et al*. (2005) identified the protective effects of green tea on RBCs membrane integrity in rats of different ages poisoned with ethanol (Dobrzynska *et al*., 2005). Hong *et al*. (2001) showed protective effects of green tea on ischemic reperfusion injuries in Mongolian gerbils brain (Hong *et al*., 2001). The studies results verify all results mentioned earlier concerning green tea antioxidative and free radicals scavenging properties.

## CONCLUSION

Results of present research shows the pharmacologic effects of green tea extract against isoniazid that can be used in human drug formulary or as a complementary or additive diet component to prevent from oxidative stress induced hepatic damages due to isoniazid therapy. However, exact dose determination and exact site and molecular cellular mechanism or mechanisms of action involved its pharmacologic action need to be more investigated.

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