ORIGINAL ARTICLE

Hepatomodulatory Action of *Camellia sinensis* Aqueous Extract against Isoniazid-Rifampicin Combination Induced Oxidative Stress in Rat

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ABSTRACT

Tuberculosis (TB) continues to be a serious disease worldwide and Hepatotoxicity is one of the most serious adverse effects of main anti tuberculosis drugs. The health benefits of green tea due to its catechin content for a wide variety of ailments, including different types of cancer, heart disease, and liver disease, were reported. So this study was conducted to evaluate the role of Green Tea Extract (GTE) to protect against Isoniazid-Rifampicin (INH-RIF) - induced liver injury in rats.

For this purpose, male Wistar rats weighing between 180 – 210 g were used. The animals group-housed 10 for each in wire mesh polypropylene cages fed ad libitum and divided into 3 groups: control (received normal saline), and two experimental groups as exp. 1 which received INH plus RIF at the dose of 50 mg/kg/day for each and exp. 2 received beside of INH–RIF, green tea 1.5% with dose 100 mg/kg/day as oral. INH-RIF were administered for 28 days to induce certain major hepatotoxicity. In addition to evaluate hepatoprotective effect, GTE administration started 3 days before INH-RIF induced intoxication and continued during the period of INH-RIF administration. After the experimental period, the animals were sacrificed then blood samples collected to biochemical assessment and liver pieces were prepared for light microscopic examination.

The results depicted the rats subjected INH-RIF, showed remarkable oxidative stress-induced liver injury with significant elevation in AST, ALT, ALP, LDH and Bilirubin (p<0.01) concomitant with decreasing of Total Protein (p<0.05). Also antioxidant activity of liver declined significantly in this situation (p<0.01 for SOD, p<0.05 for Catalase). Pretreatment with GTE in INH-RIF intoxicated rats could alter hepatoxicity due to INH-RIF so we can see remarkable reduction AST, ALT, ALP, LDH and Bilirubin. Pathologic slides confirmed biochemical findings.

The results suggested the aqueous extract of Green tea exhibit significant hepatoprotective properties in liver injuries due to Isoniazid and Rifampicin co-administration and so GTE may be able act as effective supplement in patient whom treated with serious hepatotoxic anti tuberculosis agents.

Key words: Isoniazid, Rifampicin, Green Tea, hepatoprotection, oxidative stress, rat

INTRODUCTION

Tuberculosis (TB) is one of the major causes of death from a curable infectious disease. About 9 million new TB cases occurred in 2004 and 1.7 million people died from TB that year. Sub-Saharan Africa has the highest incidence and mortality rates, mainly due to HIV/AIDS, whereas the South-East Asian region has the largest number of both new cases and deaths from TB [1]. Recommended standard treatment for adult respiratory TB is a regimen of isoniazid, rifampicin, and pyrazinamide for 2 months, followed by 4 months of isoniazid and Rifampicin [2]. The most frequent adverse effects of antituberculosis treatment are hepatotoxicity, skin reactions, gastrointestinal and neurological disorders. Hepatotoxicity is the most serious one and is the focus of the present review [3]. The severity of drug induced liver injury varies from minor non-specific changes to fulminant hepatic failure [4]. Antituberculosis drug-induced hepatotoxicity (ATDH) causes substantial morbidity and mortality and diminishes treatment effectiveness. Asymptomatic transaminase elevations are common during antituberculosis treatment, but hepatotoxicity can be fatal when not recognized early and when therapy is not interrupted in time. Adverse effects diminish treatment effectiveness, because they eventually contributing to treatment failure, relapse or the emergence of drug-resistance [5, 6]. Isoniazid, Rifampicin and pyrazinamide are potentially hepatotoxic drugs [7]. These drugs are metabolized by the liver. Metabolism is crucial in ATDH and toxic metabolites play a central role. There are various studies in experimental animal model to understand the disease process better. Different routes of drug administration have been in used in these animal models so that the most effective method could be established using different doses of INH–RIF [8, 9]. The fixed dose of RIF [10] with different doses of INH was taken

to produce the hepatotoxic model in rats. Epidemiological studies have strongly suggested that diet plays an important role in the prevention of chronic diseases [11]. Polyphenolics, commonly found in fruits, vegetables and grains, provide chemoprotective effects to combat oxidative stress in the body and maintain balance between oxidants and antioxidants to improve human health [12]. An imbalance caused by oxidants excess leads to oxidative stress, resulting in damage to DNA and protein and increases the risk of degenerative diseases such as cancer [13, 14]. Since ancient times, green tea has been an indispensable part of Japanese traditional food culture. Green tea is prepared from tea plant Camellia sinensis. These are rich in flavonoids, and in green tea most important polyphenolic compound. Nowadays, scientists recognize the effect of green tea on health enhancement and disease prevention is increasingly attracting attention [15, 16]. Studies have shown that tea possesses diverse pharmacological properties which include anti-inflammatory [17], anti-mutagenic [18], antiangiogenic [19], antiaging effects [20], and preventive effects against cancers as well as exerting protective effects against several diseases [21]. Green tea extract represents the richest source of natural polyphenols including catchins, theaflavins and the arubigins [22, 23]. Polyphenols found in green tea show 20 times more powerful antioxidant activity than vitamin C. Green tea polyphenols (GTP) has demonstrated a protective effect against a spectrum of offensive oxidants, like superoxide and peroxynitrite radicals [24]. It was found that green tea intake increases the activity of liver antioxidant enzymes as glutathione peroxidase (GPx) and oxidized glutathione (GSSG), as well as reduced glutathione (GSH) and improves the total antioxidant activity (TAA) [25]. It was postulated that, the supplementation of green tea attenuated the cyclosporineA and tamoxifen-induced oxidative stress and protected against the liver injury in rats [26, 27]. It was obvious that, some herbal extracts and their chemical constituents can significantly inhibit and protect hepatocytes against hepatic injury [28, 29].

In this study, we aimed to evaluate the role of GTE intake to isoniazid/rifampicin-intoxicated rats via monitoring the liver histopathological changes and the go insight the changes of different biochemical parameters such as serum alanine transaminase (sALT), aspartate transaminase (sAST), alkaline phosphatase (sALP), lactate dehydrogenase (sLDH) and total proteins; endogenous hepatic antioxidants e.g. Catalase, Superoxide Dismutase, lipid peroxides expressed as malondialdehyde(MDA) Moreover, liver protein carbonyl content was measured.

MATERIALS AND METHODS

Preparation of green tea extract
Green tea was extracted using the method described elsewhere [30] and modified in our laboratory. The plant was identified and authenticated by Department of Pharmacognosy, medical Sciences University of Tabriz, Iran. Briefly, the plant samples weighing about 50 g were individually extracted in 1 L water at 40°C for 60 min. After filtration, the extract was kept at 4°C.

Chemicals
A commercial formulation of Isoniazid and Rifampicin (darou pakhsh, Iran) was purchased from local pharmacy of Tabriz, Iran and was used in this study. All Kits were obtained from Human GmbH, Germany. Chemicals were of analytical reagent grade and chemicals required for all biochemical assays were obtained from Merck (Darmstadt, Germany) and Sigma-Aldrich Chemicals Company (St. Louis, Mo, USA).

Animals and treatment groups
Adult male Wistar rats weighing approximately 180 – 210 g were obtained from the Central animal house of Islamic azad university of Tabriz, Iran. The animals were kept in polypropylene wire-floored cages under standard laboratory conditions of 12 h light/dark cycles at 24 ± 4°C with 55% to 60% humidity and were provided with animal feed and water ad libitum and were acclimatized for 1 week before starting the experiments.

All animals were humanely treated in accordance with the World Health Organization's guideline for animal care, and the animal study design was approved by the Local Bioethical Committee at the Department of pharmacology and Physiology of medical sciences faculty of Tabriz Islamic Azad University. After 7 days of adaptation to laboratory conditions, the experimental animals were randomly divided into 3 groups of 10 each. There was no difference in body weight among the groups at the beginning of the experiment. Totally the numbers of animals were 30. Treatment groups include:

Group 1) as control group received normal saline.

Group 2) received combination of Isoniazid plus Rifampicin (INH plus RIF) at the dose of 50 mg/kg/day [31] for each of INH–RIF as intragastrically for a period of 28 days (i.e. minimum dose with maximum hepatotoxicity).

Group 3) beside of INH–RIF received green tea 1.5%, 100 mg/kg/day as oral [32]. This group has received green tea 3 days before administration of the INH–RIF and also all over 28 days of INH–RIF treatment, administration of GTE was continued.

After 28 days, rats were sacrificed and their liver was achieved to pathologic studies. Also, the blood was collected into clean test tubes for biochemical analysis. Blood samples were centrifuged at 3000 rpm to separate the serum and the following biochemical parameters were estimated: ALP, ALT and AST by Reitman method [33], LDH by Tietz method [34], total protein by Lowry method [35] and bilirubin by Mallory method [36].

For asses effect on body weight gain and relative organ weight, Body weight of all rats was recorded at the beginning and during of the experiment in days 14, 21 and 28. After blood collection, the rats were sacrificed by cervical dislocation; their livers were separated and weighted individually. Then, the relative liver weight was calculated.

For histopathological studies, the livers were excised quickly and washed with distilled water and removed extra muscle attach to it. After that liver was weighted and minced into small pieces. The liver tissue is homogenized by tissue homogenizer at 3000rpm for 10 min. in phosphate buffer with ratio of 1:10 of liver & phosphate buffer by weight. After that homogenized tissue was centrifuged at 12000rpm for 45min. at 4^{oC} and supernatant was used for study of Lipid peroxidation (Esterbauver et al. method) [37], Catalase (Aebi method) [38], Superoxide Dismutase (Marklund method) [39] and total protein (Lowry et al. method) [35].

Liver histological examination

The liver tissues were removed, plotted with normal saline between filter paper and fixed in 10% neutral buffered formalin and subsequently embedded in paraffin and sliced into slices of 5µm thickness followed by staining with hematoxylin and eosin and examined under light microscope (Olympus BX-200, Philippines).

Statistical analysis

The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 13.0, was used for statistical analysis. All data are presented as mean ± sem. Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Biochemical findings

In this study the hepatotoxicity was induced by INH–RIF. The biochemical parameter of different groups is shown in table 1. In the INH–RIF treated group, the levels of various parameters are ALP (143.5 ± 3.88), ALT (51.3 ± 0.5), AST (134.84 ± 4.73), LDH (212.33±11.2), Total protein (4.1±0.122) and Total bilirubin (1.66 ±0.07).

In contrast, the group treated with green tea extract (GTE) at dose of (100 mg/kg/day) once daily for 31 days (3 days before administration of INH–RIF) prevented the hepatotoxicity, thus the level of various parameters are ALP (111.27±2.97), ALT (38.42±2.67), AST (101.3±2.87), LDH (153.56±13.1), Total protein (5.3±0.368) and Total bilirubin (1.28±0.03) in a dose dependent manner.

The levels of homogenized lever tissue total protein & activities of SOD, Catalase & MDA In normal condition, INH–RIF received rats and animals treated with combination of INH–RIF plus GTE has been represented in table 2. As seen the levels of SOD, Catalase and MDA significantly decreased in hepatotoxic rats in compare with control group as SOD level reached from 5.88 ± 0.27 to 3.70 ± 0.19 in hepatotoxic condition. In this regard, Catalase level reached from 42.71 ± 2.55 to 24.59 ± 1.66 and also MDA level reached from 137.3 ± 19.6 to 385.3 ± 41.7.

Oral treatment with green tea extract (GTE) at dose of (100 mg/kg/day) once daily for 31 days (3 days before administration of INH–RIF) significantly prevented these alteration & maintained

enzyme levels at near normal levels as values of SOD, Catalase and MDA was 5.25 ± 0.45, 39.48 ± 1.55, 7.07 ± 1.56 respectively in presence of GTE beside of hepatotoxic agents.

Table 1. Serum AST, ALT, ALP, LDH, Total Protein and Total Bilirubin values in treatment groups at the end of the experiment. Results are expressed as Mean±SD. **P<0. 01, *P<0.05.

Group	AST (SF/100 ml)	ALT (SF/100 ml)	ALP (IU/L)	LDH (IU/L)	Total Protein (mg/ml)	Total Bilirubin (mg/dl)
Control	89.21 ± 1.67	32.0 ± 2.55	101.3 ± 2.89	151.33±16.0	5.8 ± 0.173	0.57 ± 0.03
INH–RIF only	134.84 ± 4.73**	51.3 ± 0.5**	143.5 ± 3.88**	212.33±11.2**	4.1 ± 0.122*	1.66 ±0.07**
INH–RIF plus GTE	101.3 ± 2.87*	38.42 ± 2.67*	111.27 ±2.97*	153.56±13.1	5.3 ± 0.368	1.28 ±0.03*

ALP: alkaline phosphatase, ***ALT***: alanine transaminase, ***AST***: aspartate transaminase, ***LDH***: lactate dehydrogenase

Table 2: serum SOD, Catalase, MDA and Total Protein values of homogenized liver tissue in treatment groups at the end of the experiment. Results are expressed as Mean±SD. ***P<0.001, **P<0.01, *P<0.05 in compare with control group. ※※※P<0.001, ※※P<0.01, ※P<0.05 in compare with group INH–RIF

Group	SOD (unit/min/mg protein)	Catalase (mole of H_2O_2 decomposed /min./mg protein)	MDA (n mol MDA/100mg protein)	Total Protein (mg/ml)
Control	5.88 ± 0.27	42.71 ± 2.55	137.3 ± 19.6	5.66 ± 0.46
INH–RIF only	3.70 ± 0.19**	24.59 ± 1.66*	385.3 ± 41.7***	13.22 ± 4.77
INH–RIF plus GTE	※※5.25 ± 0.45	※39.48 ± 1.55	※※※151.1 ± 25.4	7.07 ± 1.56

SOD: Superoxide Dismutase, ***MDA:*** malondialdehyde

In the present study, following chronic INH–RIF treatment intragastrically in all three groups, food and water intake pattern of all rats were noted. It was observed that food and water intake was reduced in INH–RIF only group. Data of final body weights and relative liver weights of male rats subjected to different treatments (saline normal, INH–RIF only and INH–RIF plus GTE) are shown in table 3. It was observed that INH–RIF-treated rats achieved significant decreases (P<0.01) in body weights and relative liver weights compared to control and other treatments. Co-treatment of INH–RIF exposed rats with GTE showed body weights and relative liver weights of no significant differences than those of control group. Also According to table No mortality occurred during the experimental period.

Table 3: Relative liver weight, mortality rate of animals at the end of study and body weights (g) of rats in days 0, 14, 21, 28 in various mentioned groups. Values represented as Mean ± SD.

Group	0 day	14 days	21 days	28 days	Relative liver weight (g)	Mortality (dead/total)
Control	182.33±7.21	196.89±4.73*	203.11±6.13*	213.58±6.21*	4.59±0.371	0/10
INH–RIF only	186.43±5.54	175.71±5.63	※168.63±4.45	※※157.47±5.29	2.05±0.218◈	0/10
INH–RIF plus GTE	185.57±6.18	199.12±5.25*	208.38±5.69*	217.31±6.05*	❖4.62±0.253	0/10

* P < 0.05 denotes significant difference between 0 day and 14, 21 and 28 days. ※※ P < 0.01 symbolizes significant difference between 0 day and 21 & 28 days. ◈ P < 0.05 denotes significant reduction in compare with other groups.
❖ Not significantly different from mean of control rats.

Microscopic findings:
Microscopical sections obtained from untreated rats (normal control):
As seen in figure 1, liver texture is normal and hepatocytes shows no symptoms of necrosis or degeneration. Structural units of liver, lobules, are normal and obvious. A lobule consists of specialized epithelial cells knows as Hepatocytes which arranged in irregular branching interconnected plates around the central vein.
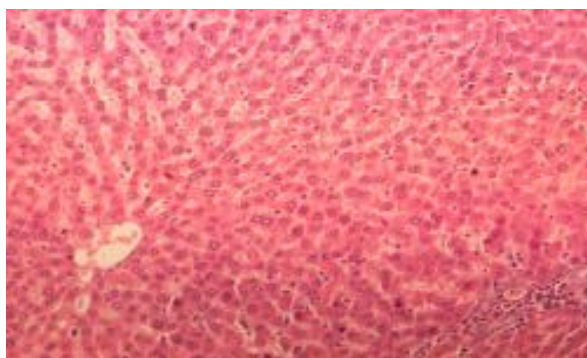
Figure 1. Photomicrograph of control group, H&E X100.

Microscopical sections obtained from INH–RIF treated rats:
As shown in figure 2, it reveals that hepatocytes underwent remarkable steatosis and hydropic degeneration and distended portal vein accompanied with periportal fibrosis are obvious. Also, necrosis, cellular degeneration surrounds the central vein, fatty acid degeneration and hepatocellular necrosis is seen. The normal entity of liver is completely damaged.
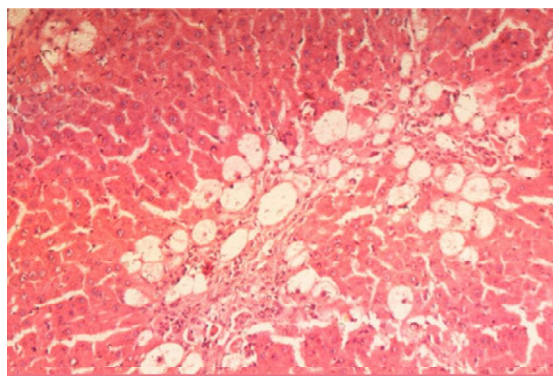

Figure 2. Photomicrograph of rat livers' section with INH–RIF treatment. H & E X100.

Microscopical sections obtained from INH–RIF plus GTE treated rats:
As presented in figure 3, which shows rat liver section of INH–RIF + GTE, indicates remarkable reduction in necrosis and fatty changes with pyknotic nuclei and cytoplasmic clearing, moderately brought central vein, hepatic cell with preserved cytoplasm and prominent nucleus. Hepatic cells are mostly normal so it shows that GTE shows the hepatoprotective activity.
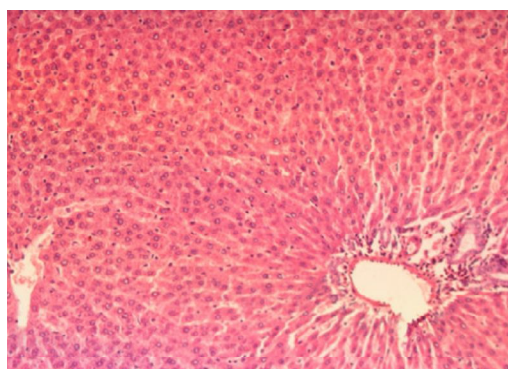

Figure 3. Photomicrograph of rat liver section of INH–RIF + GTE. H&E X100.

The liver can be injured by many chemicals & drugs. One of the most serious and frequent adverse effects of antituberculosis treatment is hepatotoxicity [3] and is the focus of the present review. The incidence of Antituberculosis drug-induced hepatotoxicity (ATDH) during standard multidrug

TB treatment has been variably reported as between 2% and 28% [40, 41]. This rate depends on the investigators' definition of hepatotoxicity as well as the population studied. Active TB is usually treated is with multiple drugs. Therefore, there are limited data on toxicity rates of antituberculosis drugs individually, except for isoniazid, which has been widely used as prophylactic monotherapy for latent TB infections [42].

Both animal and human case studies show that isoniazid-induced hepatotoxicity manifests mainly as hepatocellular steatosis and necrosis, and it has been suggested that toxic isoniazid metabolites bind covalently to cell macromolecules [43-45]. Hydrazine is the proposed toxic metabolite of Isoniazid and animal studies have shown that hydrazine causes steatosis, hepatocyte vacuolation and glutathione depletion. Lipid vacuoles and mitochondrial swelling is found in periportal and midzonal hepatocytes [46-48].

Rifampicin may cause transient hyperbilirubinemia, which is not a toxic effect but is due to interference with bilirubin excretion [49]. Rifampicin can cause hepatic lesions characterized by hepatocellular changes, with centrilobular necrosis, possibly associated with cholestasis. Histopathological findings range from spotty to diffuse necrosis with more or less complete cholestasis [50]. Bridging necrosis, lymphocytic infiltration, focal cholestasis, increased fibrosis, and micronodular cirrhosis were observed in the liver of a patient who died of rifampicin- and pyrazinamide-induced hepatotoxicity.

Among various doses of INH and RIF, 50 mg/kg/day of INH–RIF each was selected as hepatotoxic dose as animals administered with this dose showed maximum hepatotoxicity with minimum amount. Rats were chosen as hepatotoxic model in our study because a number of reports document that rats are more susceptible to injury [51].

Some authors have studied INH toxicity in other animals like dog [52]. Attri *et al.* have also studied INH–RIF induced liver injury in wistar rats [53], but their route for administration of drug was intraperitoneal. The intra gastric route for administration of INH–RIF in the present study was chosen because this is the most frequent route of drug administration in human beings [54-56].

INH was used as first line drug in the treatment and chemoprophylaxis of TB. It can cause moderate abnormalities in serum transaminases leading to hepatotoxicity. Hence, the measurement of serum transaminases is often advocated during INH administration to asses the extent of INH induced hepatotoxicity[45, 57]. Sub acute or chronic treatment with INH has been reported to induce hepatotoxicity in man [45], rats [58] and guinea pigs [59], which manifested as enhancement in activities of serum transaminases and alkaline phosphatase.

The chronic liver disease occurs due to accumulation of lipids & proteins in hepatocytes [60] with an impaired protein secretion by hepatocytes [61]. Serum enzymes including ALP, ALT, AST and LDH are mainly used in the evaluation of hepatic damage. During hepatic damage cellular enzymes ALP, ALT, AST & LDH present in the liver cells leak into the serum, resulting in increased concentration [62] and so cell damage exhibited good correlation with the enzyme leakage [63]. Also, the increase in serum LDH activity may be due to the hepatocellular necrosis leading to leakage of the enzyme into the blood stream [64].

INH–RIF administration for 28 days significantly increases all these serum enzymes and bilirubin while total protein is decreased. Also, these results are consistent with the damage to the hepatic tissues in the INH–RIF-treated rats seen by light microscopy.

The aqueous extract of green tea presented animals had significantly reduced ALP, AST, ALT, LDH & total bilirubin whereas, total protein concentration is increased, and indicating its hepatoprotective effect against INH–RIF induced liver damage.

About oxidative stress induced by INH–RIF and liver antioxidant activity induced by GTE it is worth mentioning that the results of this study have confirmed that chronic INH–RIF intoxication imbalance between antioxidant/oxidant proportion in liver so INH–RIF combination in mentioned dose and duration of administration induced the decrease of SOD, Catalase activity and the increase of MDA rate. The defence against long-term INH–RIF exposure consists of both antioxidants synthesized in the tissues and exogenous antioxidants supplied e.g. with diet. The present paper revealed that in animals intoxicated with INH–RIF, green tea partially prevents liver dysfunction and alterations of antioxidative parameters induced by INH–RIF. The protective effect of green tea

is connected with its components that possess scavenging free radicals. Indeed, tea extract enhances the expression of intracellular endogenous antioxidants such as SOD, Catalase and GPX by maintaining their activities higher compared to the intoxicated group and other antioxidants enzymes such as glutathione, glutathione reductase, glutathione-Sreductase, and quinone reductase [65, 66]. In fact, a direct genomic effect of tea flavonoids with estrogens responsive elements caused an induction of the enzymes antioxidants expression [67]. Green tea is a natural antioxidant that have been used in the most enduring of food cultures-Chinese and Japanese tea and to the safety concerns about nutrient supplements such as vitamin C., Vit. E and β-carotenes. Although these concerns still exist, consumption of naturally derived antioxidants such as green tea beverages and extract may a safer alternative and effective means of increasing the intake of antioxidants [68]. In addition, tea flavonoids might protect against toxicity in liver through the inhibition of oxidative damage. The oxidation of DNA is likely to be important causes of mutations that can potentially be reduced by dietary flavonoids which are one-electron donors. They serve as derivatives of conjugated ring structures and hydroxyl groups that have the potential to function as in vitro antioxidants by scavenging superoxide anion [69], singlet oxygen [70], lipid peroxyradicals [71], and/or stabilizing free radicals involved in oxidative processes through hydrogenation or complexing with oxidizing species [72]. Another mechanism proposed for protection against cancer by dietary flavonoids may include the induction of Phase II detoxification enzymes in cells. Modification of cellular detoxification enzymes could be a major mechanism for protection against the toxic effects [73].

On the other hand in toxicological studies, organ and relative organ weights are important criteria for evaluation of toxicity [74]. In the present study, the body weight and relative liver weights of rats treated with INH-RIF were significantly (P<0.01) lower than those of control group. The final body weights were decreased after INH-RIF administration. This may be attributed to decrease food intake (anorexia or food avoidance) or poor food palatability due to treatment related toxicity. Furthermore, INH-RIF may induce oxidative stress leading to generation of free radicals and alterations in antioxidant status or reactive oxygen species (ROS) which cause metabolic disorder and weight loss. For this reason, treatment with antioxidants and free radical scavengers such as GTE can decrease the oxidative stress and improve metabolic process of INH-RIF treated rats, so improve rat food palatability, food intake and consequently their body weight and liver/body weight ratio.

However it must be remember that green tea remedies not only does not always guarantee safety but also can cause serious troublesome in some situations.

Several in vivo studies, suggested that administration of high doses of the tea catechin, resulted in hepatotoxicity [75, 76], so disagreeing with the finding of [77-79] who considered green tea is safe in a wide range of doses, but hepatotxicity was related to consumption of high does of tea-based dietary supplements.

Our findings showed that administration of green tea could partly prevent hepatotoxicity due to tuberculosis essential treatments such as Isoniazid and Rifampicin through antioxidative and anti-inflammatory mechanisms. Despite it was determined that polyphenols of GTE are exhibit antioxidant and free radical scavenging properties, but signaling mechanisms associated with protection against the liver damage and oxidative stress status induced by INH-RIF via intake of GTE still need merit further investigations. In addition it will be advised to green tea supplement by one or two cups daily to be beneficially for human.

REFERENCES

1. World Health Organization (2006). Tuberculosis. WHO Fact Sheet No. 104. Revised. March 2006.
2. World Health Organization Global Tuberculosis Programme (2003).Treatment of Tuberculosis: Guidelines for National Programmes, 3rd edn. (WHO/CDS/TB/2003.13). Geneva: World Health Organization.
3. T.R. Frieden, T.R. Sterling, S.S. Munsiff, C.J. Watt and C. Dye (2003). Tuberculosis. Lancet. 362: 887–99.
4. Z. Hussawi, P. Kar and S.A. Husain (2003). Antituberculosis drug induced hepatitis: Risk factors, prevention and management. *Indian J of Exp Biol*. 41: 1226–1232.
5. F.A. Kaona, M. Tuba, S. Siziya and L. Sikaona (2004). An assessment of factors contributing to treatment adherence and knowledge of TB transmission among patients on TB treatment. B*MC Public Health*. 29;4:68.
6. D.F. Wares, S. Singh, A.K. Acharya and R. Dangi (2003). Non-adherence to tuberculosis treatment in the eastern Tarai of Nepal. *Int J Tuberc Lung Dis.* 7(4):327-35.

7. D.J. Girling (1978). The hepatic toxicity of antituberculosis regimens containing isoniazid, rifampicin and pyrazinamide. *Tubercle.* 59(1):13-32.
8. D.C. Quenelle, J.K. Staas, G.A.A. Winchester, E.L.W. Barrow and W.W. Barrow (1999). Efficacy of microencapsulated rifampicin in mycobacterium tuberculosis infected Mice. *Antimicrob Agents Chemother.* 43(5):1144-51.
9. D.C. Quenelle, G.A. Winchester, J.K. Staas and L.W. Barrlow (2001). Treatment of tuberculosis using a combination of sustained released rifampicin loaded microspheres and oral dosing with isoniazid. *Antimicrob Agents Chemother.* 45(6):1637-44.
10. N.P. Skakun and I.u.I. Slivka (1991). Effectiveness of tocopherol and anti-hypoxic agents in liver damage caused by antitubercular agents. *Probl Tuberk.* (3):57-9.
11. R. Niggeweg, A.J. Michael and C. Martin (2004). Engineering plants with increased rates of the antioxidant chlorogenic acid. *Nat Biotechnol.* 22(6):746-54.
12. K.K. Adom and H Liu (2002). Antioxidant Activity of Grains. *J Agric Food Chem,* 9;50(21):6182-7.
13. P. Moller, S. Loft (2004). Interventions with antioxidants and nutrients in relation to oxidative DNA damage and repair. *Mutat Res.* 13;551(1-2):79-89.
14. C.S. Sander, H. Chang, F. Hamm, P. Elsner and J.J. Thiele (2004). Role of oxidative stress and the antioxidant network in cutaneous carcinogenesis. *Int J Dermatol.* 43(5):326-35.
15. C. Cabrera , R. Artacho and R. Giménez (2006). Beneficial effects of green tea. *J Am Coll Nutr.* 25(2):79-99.
16. N. Khan and H. Mukhtar (2007). Tea polyphenols for health promotion. *Life Sci.* 81(7):519-33.
17. M. Mutoh, M. Takashi, K. Fukuda, H. Komatsu, T. Enya, Y. Masushima Hibiya, H. Mutoh, T. Sugimura and K. Wakabayashi (2000). Suppression by flavonoids of cyclooxygenase-2 promoter-dependent transcriptional activity in colon cancer cells: structure–activity relationship. *Jpn J Cancer Res.* 91(7):686-91.
18. V.E. Steele, G.J. Kelloff, D. Balentine, C.W. Boone, R. Mehta, D. Bagheri, C.C. Sigman, S. Zhu and S. Sharma (2000). Comparative chemopreventive mechanisms of green tea, black tea and selected polyphenol extracts measured by in vitro bioassays. *Carcinogenesis.* 21(1):63-7.
19. Y.D. Jung and L.M. Ellis (2001). Inhibition of tumour invasion and angiogenesis by epigallocatechin gallate (EGCG), a major component of green tea. *International Journal of Experimental Pathology.* Dec 82 (6), 309–316.
20. E. Esposito, D. Rotilio, V. Di Matteo, C. Di Giulio, M. Cacchio, S. Algeri (2002). A review of specific dietary antioxidants and the effects on biochemical mechanisms related to neurodegenerative processes. *Neurobiology of Aging.* 23 (5), 719–735.
21. L. Bravo (1998). Polyphenols: chemistry, dietary sources, metabolism and nutritional significance, Nutr Rev. 56(11):317-33.
22. E. Skrzydlewska , J. Ostrowska , R. Farbiszewski and K. Michalak (2002). Protective effect of green tea against lipid peroxidation in the rat liver, blood serum and the brain, Phytomedicine. 9(3):232-8.
23. B. Frei and J.V. Higdon (2003). Antioxidant activity of tea polyphenols in vivo: evidence from animal studies, *J Nutr.* 133(10):3275S-84S.
24. S.P. Pillai , L.A. Mitscher , S.R. Menon , C.A. Pillai and D.M. Shankel (1999). Antimutagenic/antioxidant activity of green tea components and related compounds, J Environ Pathol Toxicol Oncol. 18(3):147-58.
25. M.H. Siess , J.P. Mas , M.C. Canivenc-Lavier and M. Suschetet (1996). Time course of induction of the hepatic drug metabolizing enzyme activities following dietary administration of flavonoids, *J Toxicol Environ Health.* 6;49(5):481-96.
26. H.A. El-Beshbishy (2005). Hepatoprotective effect of green tea (Camellia sinensis) extract against tamoxifen-induced liver injury in rats*, J Biochem Mol Biol.* 30;38(5):563-70.
27. Mohamadin AM, El-Beshbishy HA, El-Mahdy MA (2005). Green tea extract attenuates cyclosporine A-induced oxidative stress in rats, *Pharmacol Res.* 51(1):51-7.
28. T.Y. Lee , L.M. Mai , G.J. Wang , J.H. Chiu , Y.L. Lin and H.C. Lin (2003). Protective mechanism of salvia miltiorrhiza on carbon tetrachloride-induced acute hepatotoxicity in rats, *J Pharmacol Sci.* 91(3):202-10.
29. F. Stickel and D. Schuppan (2007). Herbal medicine in the treatment of liver diseases, *Dig Liver Dis.* 39(4):293-304.
30. A. ITHARAT, P.J. HOUGHTON, E. ENO-AMOOQUAYE, P.J. BURKE, J.H. SAMPSON and A. RAMAN (2004). In vitro cytotoxic activity of Thai medicinal plants used traditionally to treat cáncer. *J Ethnopharmacol.* 90: 33-8.
31. S.V. Rana , R. Pal , K. Vaiphie and K. Singh (2006). Effect of different oral doses of isoniazid-rifampici n in rats, *Mol Cell Biochem.* 289(1-2):39-47.
32. M.C. Sabu, K. Smitha and R. Kuttan (2002). Anti-diabetic activity of green tea polyphenols and their role in reducing oxidative stress in experimental diabetes. *J Ethnopharmacol.* 83:109-116.
33. S. REITMAN and S. FRANKEL (1957). A calorimetric method for determination of serum glutamic oxalloacetic acid, glutamic pyruvic transaminase. *Am J Clin Pathol.* 28(1):56-63.
34. N.W. Tietz, E.L. Pruden and O. Siggard-Andersen (1995). Clinical Guide to Laboratory Tests, 3rd edition, W. B. Saunders. Company: Philadelphia.
35. O.H. LOWRY, N.J. ROSEBROUGH , A.L. FARR and R.J. RANDALL (1951). Protein measurement with the Folin- Phenol reagents, *J Biol Chem.* 193(1):265-75.
36. H.T. Mallory, E.A. Evelyn (1937). The determination of bilirubin with phosphoelectric calorimeter, *J Biol Chem.* 119, 481-485.
37. H. Esterbauer, K.H. Cheeseman , M.U. Dianzani, G. Poli and T.F. Slater (1982). Separation and characterization of the aldehydic products of lipid peroxidation stimulated by ADP-Fe2+ in rat liver microsomes, *Biochem J.* 15;208(1):129-40.
38. H. Aebi (1984). Catalase in vitro, Methods in Enzymology, 105, 121-126.
39. S. Marklund and G. Marklund (1974). Involvement of superoxide anion radical in the antioxidation of pyrogallol and a convenient assay for superoxide dismutase*, Eur J Biochem.* 16;47(3):469-74.

40. M. Dossing, J.T. Wilcke, D.S. Askgaard and B. Nybo (1996). Liver injury during antituberculosis treatment: an 11-year study. Tuber. Lung Dis. 77: 335–40.

41. J.N. Pande, S.P. Singh, G.C. Khilnani, S. Khilnani and R.K. Tandon (1996). Risk factors for hepatotoxicity from antituberculosis drugs: a case-control study. *Thorax.* 51: 132–6.

42. A. Tostmann, M.J. Boeree , R.E. Aarnoutse , W.C. de Lange, A.J. van der Ven and R. Dekhuijzen (2008). Antituberculosis drug-induced hepatotoxicity: concise up-to-date review. *J Gastroenterol Hepatol.* 23(2):192-202.

43. T.C. Sarich, T. Zhou, S.P. Adams, A.I. Bain, R.A. Wall and J.M. Wright (1995). A model of isoniazid-induced hepatotoxicity in rabbits. J. Pharmacol. *Toxicol. Methods.* 34: 109–16.

44. M. Black, J.R. Mitchell, H.J. Zimmerman, K.G. Ishak and G.R. Epler (1975). Isoniazid-associated hepatitis in 114 patients. *Gastroenterology.* 69: 289–302.

45. J.R. Mitchell, H.J. Zimmerman, K.G. Ishak and U.P. Thorgierson (1976). Isoniazid liver injury: Clinical spectrum pathology and probable pathogenesis. *Ann Intern Med.* 84: 181–192.

46. M.D. Scales and J.A. Timbrell (1982). Studies on hydrazine hepatotoxicity. 1. Pathological findings. *J. Toxicol. Environ. Health.* 10:941–53.

47. Timbrell JA, Scales MD, Streeter AJ (1982). Studies on hydrazine hepatotoxicity. 2. Biochemical findings. *J. Toxicol. Environ. Health*, 10: 955–68.

48. T.C. Sarich, M. Youssefi, T. Zhou, S.P. Adams, R.A. Wall and J.M. Wright (1996). Role of hydrazine in the mechanism of isoniazid hepatotoxicity in rabbits. *Arch. Toxicol.* 70: 835–40.

49. Capelle P, Dhumeaux D, Mora M, Feldmann G, Berthelot P (1972). Effect of rifampicin on liver function in man. Gut; 13: 366–71.

50. J.F. Westphal, D. Vetter and J.M. Brogard (1994). Hepatic side-effects of antibiotics. *J. Antimicrob.* Chemother. 33: 387- 401.

51. J.S. Brown, W.E. Wilson and L.D. Grant (2005). Dosimetric comparisons of particle deposition and retention in rats and humans. *Inhal Toxicol.* 17: 355–385.

52. I. Frank, D. Lahav and I. Aroch (2002). Myocardial necrosis and severe metabolic acidosis associated with isoniazid poisoning in dog. *Vet Reco.* 151: 638–639

53. S. Attri, S.V. Rana, K. Vaiphei, C.P. Sodi, R. Katyal, R.C. Goel, C.K. Nain and K. Singh (2000). Isoniazid–rifampicin induced oxidative hepatic injury protection by N–acetylcysteine. Human & Exp Toxico. 19: 517–522.

54. O.J. Kasilo, C.F.B. Nihach and M. Nahlet (1999). Isoniazid–International programme on chemical toxicity. Poisons information monograph . *Pharmaceutical.* 288–291.

55. H.J. Cro, B.M. Mood and J. Magarey (1980). Rifampicin IARC Summary and Evaluation: 24–26.

56. Rifandin (Hoechst Marian Roussel) (2000). In: PDR Phsycian desk reference. 54th ed 2000 oradell. N J Medical Economics Data. 1379–1382.

57. S. Karthikryan (1995). Hepatotoxicity of isoniazid: A study in the activity of marker enzymes of liver toxicity in serum and liver of rabbits. *Ind J Pharmaco.* 36:247–249.

58. W. Snodgross, W. Potter, J.A. Timbrell and D.I. Jollow (1974). Possible mechanism of isoniazid related hepatic injury. *Clin Res.* 22: 323–326.

59. G.B. Heiserg, W.C. Hughes and C.M. Rozmaiorkherh. (1980). The guinea pigs as model for isoniazid induced reactions. *Lab Animal Sci.* 30: 42–50.

60. L. Ashakumary and P.L. Vijayammal (1993). Additive effect of alcohol & nicotine on lipid metabolism in rats, *Indian J Exp Biol.* 31, 270-274.

61. H. Vilstrup and N. Tygstrup (1985). Glucoregulation in acute liver failure. *Eur J clin Invest.* 16, 193-197.

62. A.C. Deb (1998). Fundamentals of Biochemistry, 7th ed, New Central Book Agency: Kolkata.

63. M.E. Awad, M.S. Abdel-Rahman and S.A. Hassan (1998). Acrylamide toxicity in isolated rat hepatocytes. Toxicology. 12: 699-704.

64. X. Wang and W. Zhai (1988). Cellular and biochemical in bronchoalvelar lavage fluids of rats exposed to fenvalerate. Zhongguo Yaolixue YuDulixue Zoghi. 2: 271-276.

65. S.G. Khan, S.K. Katiyar, R. Agarwal and H. Mukhtar (1992). Enhancement of antioxidant and phase II enzymes by oral feeding of green tea polyphenols in drinking water to SKH-1 hairless mice: possible role in cancer chemoprevention. *Cancer Res.* 52: 4050– 4052.

66. J. L.G. Valerio, J.K. Kepa, G.V. Pickwell and L.C. Quattrochi (2001). Induction of human NAD(P)H:quinone oxidoreductase (NQOI) gene expression by the flavonol quercetin. *Toxicol. Lett* 119: 49– 57.

67. J. Vina, C. Borras, J. Gambini, J. Sastre and F.V. Pallardo (2005). Why females longer than males? Inportance of the upregulation of longevityassociated genes by oestrogenic compounds. FEBS lett 579: 2541-2545.

68. M. Luo, K. Kannar, M.L. Wahlqvist and R.C.O. Brien (1997). Inhibition of LDL oxidation by green tea extract. *THE LANCET.* 349: 360-631.

69. J. Robak and R.J. Gryglewski (1988). Flavonoids are scavengers of superoxide anion. *Biochem Pharmacol. 3*7: 83– 88.

70. S.R. Husain, J. Cillard and P. Cillard (1987). Hydroxyl radical scavenging activity of flavonoids. *Phytochemistry* 26: 2489– 2491.

71. J. Torel, J. Cillard and P. Cillard (1986). Antioxidant activity of flavonoids and reactivity with peroxy radical. *Phytochemistry.* 25: 383– 385.

72. F. Shahidi, P.K. Janitha and P.D. Wanasundara (1992). Phenolic antioxidants. *Crit Rev Food Sci Nutr.* 32: 67– 103.

73. P. Talalay, J.W. Fahey, W.D. Holtzclaw, T. Prestera and Y. Zhang (1995). Chemoprotection against cancer by phase 2 enzyme induction. *Toxicol. Lett.* 82: 173-179.

74. J.W. Crissman, D.G. Goodman, P.K. Hildebrandt, R.R. Maronpot, D.A. Prater, J.H. Riley, W.J. Seaman and D.C. Thake (2004). Best practice guideline: toxicologic histopathology. *Toxicol. Pathol.*, 32: 126-131.

75. R.A. Isbrucker, J.A. Edwards, E. Wolz, A. Davidovich and J. Bausch (2006). Safety studies on *Epigallocatechin gallate* (EGCG) preparations. Part 2: dermal, acute and short-term toxicity studies. *Food Chem. Toxicol.* 44: 636 – 650.
76. G. Galati, A. Lin, A.M. Sultan and P.J. O'Brien (2006). Cellular and in vivo hepatotoxicity caused by green tea phenolic acids and catechins. *Free Radic. Biol. Med.* 40: 570–580.
77. H.L. Bonkovsky (2006). Hepatotoxicity associated with supplements containing Chinese green tea (Camellia sinensis). Ann Intern Med. 144: 68–71.
78. A. Federico, A. Tiso and C. Loguercio (2007). A case of hepatotoxicity caused by green tea. *Free Radic. Biol. Med. 4*3: 474.
79. D.N. Sarma, M.L. Barrett, M.L. Chavez, P. Gardiner, R. Ko, G.B. Mahady, R.J. Marles, L.S. Pellicore, G.I. Giancaspro and T. Low Dog (2008). Safety of green tea extracts: a systematic review by the US Pharmacopeia. *Drug Saf*. 31: 469 – 484.