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Alterations in Antioxidant Defense System and Oxidative Damage in Experimental Hepatorenal Toxicity Induced by Isoniazid and Rifampicin in Rats: Effect of N-Acetyl Cysteine and White Tea Extract

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Abstract: ***Tuberculosis is a dangerous disease and its death toll is increasing year by year. Intake of isoniazid and rifampicin lead to fatal hepatorenal toxicity. Male albino rats were treated orally for induction of hepatorenal damage with (200 mg/kg body weight) of isoniazid and rifampicin daily, each for 45 d. For the protective studies, 200 mg/kg/day of N-acetyl cysteine and white tea starting from the 30th day were administered orally for 15 days. Our results revealed that serum liver enzymes activities, liver and kidney thiobarbituric acid reactive substance and nitric oxide levels, serum lipid profile and DNA fragmentation which exhibited by comet assay were significantly increased by isoniazid and rifampicin treatment associated with marked reduction of serum levels of albumin, total protein and albumin/globulin ratio, as well as liver and kidney glutathione contents and antioxidant enzymes activities, catalase and superoxide dismutase, as compared to normal controls. The present results showed that N-acetyl cysteine and white tea extract exerted their protective activity by different extents on liver and kidney by inhibiting the production of free radicals through induction of antioxidant enzymes and improving non-enzymatic thiol antioxidant glutathione and returning back the tested parameters towards near the normal controls. Furthermore, liver and kidney functions and their DNA fragmentation were improved greatly by N-acetyl cysteine followed by white tea extract. Based on this study, it might be concluded that N-acetyl cysteine and white tea possess a potent antioxidant properties and may be hopeful therapeutic regimens against antitubercular drugs-induced hepatorenal toxicity much better with N-acetyl cysteine than white tea extract.***

Keywords: Hepatorenal toxicity; isoniazid; rifampicin; N-acetyl cysteine; white tea.

1. Introduction

Even though a vaccine and numerous effective antituberculosis treatments are available for *Mycobacterium Tuberculosis* (TB) infection, several million people die from this devastating disease each year. Over one third of the worlds' population is estimated to be infected with TB and over 2 million people a year die from the disease **(Santhosh *et al*., 2007; Eminzade *et al*., 2008)**. In 2007, the estimated number of prevalent TB cases in Eastern Mediterranean region was 772,039 with a rate of 139 per 100,000 populations, 136,000 of which die each year. This means that every 5 minutes a person die of the disease and every minute a person will be infected **(WHO, 2007)**. Two treatment phases was recommended by **WHO report (2010)** for successful treatment of TB. Firstly, an intensive phase which is a daily treatment with isoniazid (INH), rifampicin (RIF), pyrazinamide and ethambutol for 2 months. Secondly, a continuation phase of 4-6 months of INH and RIF. However, this regimen often causes serious adverse drug reactions, which may result in discontinuing the scheduled treatment. Liver and kidney dysfunction possess a major problem for effective completion of the course of anti-TB chemotherapy **(WHO, 2010; Kumar *et al*., 2010)**.

Detoxification of xenobiotics is a specific task for the liver. By virtue of its unique vascular and metabolic features, liver is exposed to absorbed drugs and xenobiotics in concentrated form. Drug-metabolizing enzymes detoxify many xenobiotics but bioactivate or increase the toxicity of others. In case of bioactivation, the liver is the first organ exposed to the damaging effects of the newly formed toxic substance **(Mintra *et al*., 1998)**.

Nephropathy is one of the important microvascular complications of anti-TB therapy, especially RIF. RIF-induced acute renal failure is sometimes encountered in the treatment of TB. RIF often elicits a liver and renal toxicity response. A severe response to RIF typically occurs upon readministration of the drug **(Covic *et al*., 1998)**.

Isoniazid (isonicotinic acid hydrazide), introduced in 1950s, is still the most active agent against TB, and is used for both treatment and prophylaxis **(Girling, 1978)**. INH and RIF combination are widely used for the treatment of TB; since 1968; they kill more than 99% of tubercular bacilli within two months of initiation of therapy, leading to reducing the treatment course from 18 months to 6 months **(Saad *et al*., 2010)**.

The most frequent and most serious adverse effect of anti-TB treatments is hepatotoxicity. The incidence of hepatotoxicity during standard multidrug TB treatment has been variably reported as between 2% and 28%. The administration of INH-RIF combination produces many

metabolic and morphological aberrations in the liver due to the fact that the liver is the main detoxifying site for these anti-TB drugs **(Santhosh *et al*., 2007; Saad *et al*., 2010)**.

The bioactive metabolites of INH generated by the drug-metabolizing enzymes, in particular cytochrome P_{450} 2E1 (CYP2E1), have been implicated in INH-induced hepatotoxicity in humans **(Huang *et al*., 2002)** and rats **(Yue *et al*., 2004)**. RIF is a potent inducer of the hepatic CYP_{450} system in the liver and intestine, thereby increasing metabolism of many other compounds **(Girling, 1978)**. Furthermore, DNA fragmentation into oligonucleosomal fragments forming ladder pattern following gel electrophoresis, is a feature of apoptosis that was induced by INH and RIF **(Chen *et al*., 2011)**.

Dietary components, such as extracts of herbs, particularly tea (*Camellia Sinensis*), have been studied for their antioxidant properties *in vitro* and *in vivo*. The main consumed types are black and green tea but recently white tea (WT) has become more available to consumers in the East. Black, green, and WT mainly differ in their degrees of processing. White tea represents the least processed type of teas in that it goes through steaming and drying immediately after picking without a prior withering stage to prevent oxidation, giving it a light, delicate taste **(Gondoin *et al*., 2010)**. The antioxidant and free radical-scavenging activities of the major phenolics present in teas are the flavan-3-ols and the flavonols of green tea has been assessed by several researchers **(Koutelidakis*et al*., 2009; Nörnberg *et al*., 2012)**.

N-acetyl cysteine (NAC), an acetylated form of the amino acid cysteine, is an excellent source of sulfhydryl (SH) group acting as a precursor of glutathione (GSH). NAC is naturally formed in Allium plants such as garlic and onion. The primary role of NAC being replacement of intracellular stores of hepatic reduced GSH **(Maksimchik *et al*., 2008)**. Earlier in 1989, **Aruoma** and coworkers demonstrated that NAC was a potent scavenger of hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$) **(Aruoma *et al*., 1989)**. NAC was shown to penetrate the cell membrane easily, enhancing intracellular GSH biosynthesis both *in vitro* and *in vivo*, and promote an uptake of cystine from the culture medium for cellular GSH biosynthesis**(Attri *et al*., 2000; Maksimchik *et al*., 2008)**.

In light of the reported toxic effects of the anti-TB drugs, INH and RIF, this work was proposed to experimentally study; firstly the potential protective effects of both N-acetyl cysteine and white tea extract against the hazardous hepatorenal toxic effect of INH and RIF combination. Secondly to assess the interplay between the cellular antioxidant defense mechanism, nitric oxide, lipid profile parameters and INH and RIF-induced toxicity in rats.

2. Materials and Methods

Drugs and Chemicals

Drugs: INH and RIF were obtained from Sigma Aldrich for chemical industries; NAC from Sedico Company for pharmaceutical industries; while, WT purchased from the Twinnings, London. Chemicals and standards used in this work were obtained from Sigma Aldrich for chemical industries, and diagnostic kits were purchased from GPL Company, Spain except that of alaninetransaminase (ALT) and aspartatetransaminase (AST) were purchased from QCA, Spain.

Preparation of white tea extract

The100 gm powdered material was defatted with hexane using soxhlet apparatus. The defatted marc was successively extracted with increasing polarity of solvents like hexane, ethyl acetate, methanol and water. The filtrate was evaporated to dryness at 40°C in vacuum. Preliminary phytochemical analysis revealed high content of polyphenols in methanolic extract of white tea which represents 590 mg of Gallic Acid Equivalent per one gram dried weight of the sample **(Singleton *et al*. 1999)**. Total flavonoid content was determined by colorimetric method **(Chang *et al*, 2002)**, where the total flavonoids content of the sample was expressed in milligram quercetin equivalents 330 mg quercetin/g dried weight of sample.

Animals

Male white albino rats, weighing (200 ± 20g) were obtained from the animal house of the National Organization for Drug Control and Research (NODCAR) Egypt after approval from (NODCAR) animal ethical committee. Animals were housed in groups of four/per /plastic cage for two weeks before starting the experiment for adaptation to the new environments. The animals were maintained under standard condition of temperature (23±2°C) and relative humidity (55±10%) with 12 h each of dark and light cycle. Rats were fed with standard pellet and tap water *Ad libitum*.

Experimental protocol

Thirty two adult male albino rats, weighing 200±20 grams, were randomized into four groups each containing eight rats:
Group (I): Control: animals receiving a daily intragastric (i.g.) intubation of distilled deionized water for 45 days.
Group (II): Hepatotoxicity **(HT)** group: animals receiving a daily i.g. injection of INH (200mg/kg body weight) and RIF (200mg/kg body wt.) for 45 days, both are dissolved in distilled deionized water.
Group (III): NAC group **(HT + NAC)**: animals receiving INH and RIF for 45 days, and orally received NAC 200 mg/kg body weight i.g. starting from the 30th day for 15 days.
Group (IV): White Tea group **(HT + WT)**: animals receiving INH and RIF for 45 days, and white tea extract (200 mg/ kg body wt / day) orally, starting from the 30th day for 15 days.

Blood sampling

After 24 hours of the last dose, blood samples were obtained from the Retro-orbital venous plexus (by means of fine capillary glass tube), in clean and dry centrifuge tubes. The blood was allowed to clot at room temperature for 30 minutes. Serum was then separated by centrifugation at 5000 revolution per minute (rpm) for 10 minutes at 4°C. The separated serum was collected and divided into aliquots, then stored at -20°C for further determination of several parameters, ALT, AST, cholesterol, triglycerides, total lipids, albumin, total protein, urea and creatinine. Rats were dissected for isolation of liver and kidneys.

Preparation of liver and kidney homogenates
The separated liver and kidneys were rinsed with ice-cold saline, dried by blotting between filter papers, and then divided into many parts 1 gram each for the liver and 0.25 gram for the kidneys, where each part was homogenized in ice cold 1.15 % KCl. The homogenates produced were processed according to the procedure of each parameter. One part of liver and kidneys were used for DNA fragmentation by comet assay.

Biochemical analysis:

Assessment of liver functions
Serum alanine aminotransferase (AST) and aspartate aminotransferase (ALT) activities were determined using the method of **Reitman and Frankel (1957)**, Total protein concentration was determined by the method of **Lowry *et al.* (1951)** using bovine serum albumin as the standard and Serum albumin level estimated using the method of **Doumas *et al.* (1971**).

Assessment of kidney functions
Blood urea was assayed according to the colorimetric method described by **Patton and Crouch, (1977)**. Serum creatinine was determined by the method of **Henery *et al*. (1974)**.

Determination of serum (cholesterol, triglycerides and total lipids)
Serum cholesterol and triglycerides were measured enzymatically as described by **Allain *et al.* (1974)** and **Buccolo and David, (1973)**, respectively. Unsaturated lipids were detected by the method described by **Frings *et al.* (1972)**.

Assay of liver and kidney lipid peroxidation markers
Lipid peroxides concentration is determined by analyzing the thiobarbituric acid-reactive substances (TBARS) as malondialdehyde (MDA) by the method of **Uchiyama and Mihara, (1978)**.

Assay of liver and kidney non-enzymatic and enzymatic antioxidants
Total nitric oxide (NO) concentration was assayed in tissue according to the method of **Miranda *et al.* (2001)** and reduced GSH content was determined according to the method of **Beutler*et al.* (1963)**.The activities of superoxide dismutase (SOD;E.C.1.15.1.1)were carried out kinetically according to the method of **Marklund and Marklund, (1974)**, while catalase (CAT; E.C.1.11.1.6)activity was determined kinetically according to **Beers and Sizer (1952)**. Protein was assayed by the method of **Lowry *et al*. (1951)**.

Determination of liver and kidney DNA fragmentation (Comet assay)
DNA fragmentation was evaluated quantitatively according to **Singh *et al.*(1988)**, in which 1 gram of crushed samples were transferred to 1 ml phosphate buffered saline (PBS), then stirred for 5 min and filtered. Cell suspension (100 µl) was mixed with 600 µl of low-melting agarose (0.8 % in PBS). 100 µl of this mixture was spread on pre-coated slides. The coated slides were immersed in lyses buffer (0.045 M TBE, PH 8.4, containing 2.5 % SDS) for 15 min. the slides were placed in electrophoresis chamber containing the same TBE buffer, but devoid of SDS. The electrophoresis conditions were 2 V/cm for 2 min and 100 mA and staining with ethidium bromide 20µg/ml at 4^{o}C. The observation was with the samples still humid, the DNA fragment migration patterns of 100 cells for each dose level were evaluated with a fluorescence microscope (with excitation filter 420 nm-490 nm). The comets tails lengths were measured from the middle of the nucleus to the end of the tail with 40 x increase for the count and measure the size of the comet. For visualization of DNA damage, observations are made of EtBr- stained DNA using a 40 x objective on a fluorescent microscope.

Statistical analysis
Statistics were performed using SPSS program for Windows version 18.0 (SPSS Inc, Chicago, IL). The data were subjected to one-way ANOVA. LSD or James-Howell multiple comparison post hoc tests were performed to evaluate the significance of difference in means between various treatments groups according to the test for homogeneity. Values were presented as means ± SEM and a P value <0.05 were considered significant.

3. Results

Serum ALT and AST enzyme activities.
Figure (1) shows that INH and RIF combination resulted in significant elevation of serum activities of ALT and AST (P<0.05) compared to normal control group. About two folds increase with ALT and three folds increase with AST levels. Simultaneous administration of HT animals for 2 weeks with either NAC or WT resulted in significant reduction in ALT and AST activities compared to HT group.

Serum albumin and total protein level and A/G ratio.
Table 1, depicts the changes in serum proteins levels in the studied groups. The hepatotoxic group showed remarkable decrease (P<0.05) in serum albumin, total protein and (A/G) ratio compared with normal control group and amounting to 42%, 66% and 34% of their normal values, respectively. Co-treatment of HT animals with either NAC or WT groups showed significant elevation (P<0.05) for their serum proteins levels compared to HT group (43%, 18% and 66%, respectively) for NAC and (35%, 9% and 62%) for WT groups.

Serum urea and creatinine levels.
Table (2) demonstrates the renal function tests in the current study. INH and RIF treated animals showed a significant decline (P<0.05) in serum creatinine associated with significant elevation of urea levels compared to normal control values. However, co-treatment of HT animals with either NAC or WT reverse the alteration of both serum creatinine and urea levels to near normal control values. The later results reflect gradual restoration of the renal functions to near-normal values.

Hepatic TBARS, NO and GSH levels and SOD and CAT activities.
As indicated in Figure 2, administration of anti-TB combination to rats showed a remarkable elevation of liver TBARS and NO levels (P<0.05) associated with significant

decrease in GSH levels and SOD and CAT activities compared to the normal control group. Concomitant treatment of HT rats with NAC showed a significant reduction of the elevated levels of TBARS and NO ($P<0.05$) when compared with HT group. In contrast, no significant changes were observed between WT treatment and HT groups in liver TBARS levels. This was accompanied with significant reduction of the elevated levels of NO when compared to HT group. Co-treatment with either NAC or WT failed to modify the altered serum level of liver GSH content to any extent ($P<0.05$). However, concurrent administration with WT significantly elevated CAT activity while, no changes was observed in case of SOD activity ($P<0.05$) compared to HT group. On the other hand, NAC succeeded to significantly elevate SOD and CAT activities at $P<0.05$ compared to HT group. These results reflect restoration of the antioxidant defense systems to near-normal values.

Renal TBARS, NO and GSH levels and SOD and CAT activities.

Significant ($p < 0.05$) elevated levels of TBARS and NO and reduced levels of GSH levels and SOD, CAT activities were observed in the HT rats compared with normal control rats. Co-treatment of HT rats with NAC showed significant ($p < 0.05$) reduction of elevated levels of TBARS and NO and insignificant changes of GSH levels compared with HT group. However, co-treatment of NAC to HT animals revealed a significant elevation of SOD and CAT activities compared to HT group. On the other hand, no statistical difference observed in TBARS and NO levels between WT co-treatment and HT groups at $P<0.05$. However, co-treatment with WT efficiently normalized GSH level compared with HT group at $P<0.05$. Both NAC and WT succeeded to significantly increase SOD and CAT activities compared with HT group which reflects restoration of the antioxidant enzyme systems to near-normal values (Fig. 3).

Serum total cholesterol, triglycerides and total lipid levels.

Rats exposed to INH and RIF treatment showed significant increase ($P<0.05$) of serum cholesterol, triglyceride and total lipid levels (Figure 4) as compared to normal control rats. Concomitant administration of HT rats with either NAC or WT, resulted in a significant decrease ($P<0.05$) in the elevated levels of serum total cholesterol, triglyceride and total lipid compared with HT group.

Liver and Kidney DNA fragmentation (Comet assay).

As indicated in Figure (5 and 6), a marked elevation of DNA fragmentation ($P<0.05$) in both liver and kidney tissues of rats treated with (INH–RIF) compared with normal control, as indicated by increased tail moments which amounts (547% and1191% of their normal values, respectively). Co-treatments of HT animals with either NAC or WT succeeded to significantly decrease DNA fragmentation levels in both liver and kidney ($P<0.05$) compared to HT group.

4. Discussion

The transient abnormalities in liver and kidney functions are common during the early stages of anti-TB therapy, but sometimes hepatotoxicity may be more serious and require a change of treatment **(Kumar *et al.*, 2010)**. Therefore, we have investigated the effect of either NAC or WT on biomarkers of oxidative stress, and lipid peroxidation (LPO) in tissues of INH-RIF -induced hepatorenal damage in rats.

In the current study, we observed significant increase in the activities of liver marker enzymes ALT and AST in (INH and RIF) group, associated with a significant reduction of serum albumin, total protein and their ratio as compared with their normal control groups(figure, 1and table,1). This was in accordance with earlier reports **(Santhosh *et al.*, 2007; Kumar *et al.*, 2010; Dhamal *et al.*, 2012)**. Aminotransferases are originally present in high concentrations in the cytoplasm. Upon injury, these enzymes leak into the blood stream and manifest significantly elevated serum levels. Previous report in rats **(Jiang *et al.*, 2004)**, suggested that the hydrazine is involved in the development of INH-induced hepatotoxicity. The toxic metabolite hydrazine is produced, which further binds covalently to the intracellular macromolecules and causes peroxidative degradation of lipid membrane of the hepatocytes leading to disturbance in the transport function of the hepatocytes which in turn results in the leakage of these enzymes into the blood stream**(Kumar *et al.*, 2010; Dhamal *et al.*, 2012)**.Co-administeration of either NAC or WT to HT rats showed significant decrease of their elevated levels of aminotransferases activities, which coincides with **Attri *et al.*(2000)**and **El-Beshbishy *et al.*(2011)**, respectively. NAC has antioxidant, anticytotoxic and antiapoptotic properties and might therefore be useful in counteracting damaging events of INH and RIF-induced hepatitis through stabilizing membrane lipid bilayers**(Kelly, 1998)**. On the other hand, WT extract effectmay be attributed to its polyphenolic content of catechins **(El-Beshbishy *et al.*, 2011)** which had liver injury-protective effects and preserved the structural integrity of the hepatocytes membrane from the toxic effect of INH and RIF. The hepatoprotective effect of WT polyphenols was confirmed against azathioprine-induced liver damage **(El-Beshbishy *et al.*, 2011)** and chlorpyriphos in rats **(Khan and Kour, 2007)**. The polyphenolic catechins react with peroxyl radicals in phospholipids bilayers via a single electron transfer followed by deprotonation **(Javanovic *et al.*, 1996)**.

Nephropathy is one of the important microvascular complications of anti-TB therapy **(Rekha *et al.*, 2005)**. The elevated serum creatinine levels in HT animals indicate a significant degree of glomerular dysfunction (Table 2). These results are in consistence with the previous study of **Rekha *et al.* (2005)**. This renal injury is usually reversible if detected early and treated appropriately **(Rekha *et al.*, 2005)**. **Nyarko *et al.* (1997)** suggested that oxidative hepatic biotransformations are involved in the induction of kidney damage. This suggestion was supported by our results which revealed significant increase in kidney and liver tissue levels of TBARS and NO radicals, and depletion of antioxidant defense parameters, either non enzymatic (GSH) or enzymatic (SOD and CAT). Rats treated with NAC showed a slight reduction of serum level of creatinine as compared to INH and RIF-treated group, which coincides with the report of **Kheradpezhouh *et al.* (2010)**, during studying the therapeutic benefits of NAC on acute acetaminophen-induced hepatorenal damage. Indeed, several studies suggest

that NAC may ameliorate different etiologies of renal injury such as cisplatin **(Sheikh-Hamad *et al.*, 1997)** and cyclosporine **(Tariq *et al.*, 1999)**. Upon treatment with WT extract, serum creatinine level was nearly normalized. This was in harmony with **Elhalwagy *et al.* (2008)**. The results obtained confirm the antioxidant properties attributed to WT. This action could be attributed to the presence of catechins, the major component of white tea, which acts as direct or indirect antioxidant. Directly, catechins remove reactive oxygen and nitrogen species and chelates transition metal ions. Indirectly, they can inhibit redox-sensitive transcription factors, inhibit the synthesis of pro-oxidant enzymes, or activate the synthesis of antioxidant enzymes **(Elhalwagy *et al.*, 2008)**.

Our findings showed that administration of INH and RIF to rats significantly reduced the serum levels of urea compared to normal control group, which was in agreement with **Santhosh *et al.* (2007)**. Liver is the key organ in the maintenance of blood ammonia levels through the urea cycle. Formation of urea is the mode of disposal of nitrogen. In hepatotoxic condition, due to the failure of the liver in conversion of amino acids and ammonia to urea, a significant decrease in urea was observed. There is an increased catabolism of proteins, which confirmed in the current study by the decrease of serum levels of albumin and total protein, coupled with the diminished ability of kidneys to excrete the nitrogenous waste. These could be possibly the reasons for the lowered level of urea which shoulders with an early report of **Eule *et al.* (1986)**.

In the present study, liver and kidney LPO levels (measured as TBARS) were significantly increased associated with a significant reduction in non-enzymatic free radical scavenger (GSH) and enzymatic (SOD and CAT) activities in INH–RIF-treated rats. Reactive oxygen species (ROS)-induced oxidative damage has been implicated in the pathogenesis of several disorders, including anti-TB therapy. Oxidative stress is the imbalance between production and removal of ROS. Increased oxidative stress, which contributes substantially to the pathogenesis of anti-TB therapy complications, is the consequence of either enhanced ROS production or attenuated ROS-scavenging capacity. Moreover, administration of either NAC or WT to animals showed comparable significant reductions in the levels of liver and kidney peroxidative markers together with concomitant improvement in hepatic and renal antioxidative defense system, therefore, ameliorating the injury cascade induced by INH-RIF administration. These results were consistent with previous reports **(Attri *et al.*, 2000; Santhosh *et al.*, 2007; Chandane *et al.*, 2013& Jyothi *et al.*, 2013)**. In our hepatotoxicity model, free radicals formed could be attributed to either by the reaction of metabolites of INH and RIF (especially hydrazine and acetylhydrazine) with oxygen or by the interaction of superoxide radicals ($O_2^{\bullet-}$) with H_2O_2 producing hydroxyl radical ($HO^{\bullet}$), seem to initiate peroxidative degradation of membrane lipids and endoplasmic reticulum rich in poly unsaturated fatty acids (PUFAs). This leads to formation of lipid peroxides which in turn give products like TBARS that cause loss of integrity of cell membrane and damage to liver and kidney tissues**(Kumar *et al.*, 2010)**. NAC treatment significantly ameliorated LPO in both liver and kidney tissues after INH and RIF intoxication. **Attri *et al.* (2001)** and **Rana*et al.* (2006)** reported that NAC had the ability to ameliorate TBARS in RIF-induced liver damage in rats through its free radical scavenging abilities. NAC was believed to act as an effective scavenger of a harmful oxidative agent, hypochlorous acid (HOCl) preventing HOCl-induced oxidative damage. The free radical scavenging properties of NAC were close to those of GSH or methionine, and were higher than those of the known antioxidant melatonin **(Maksimchik *et al.*, 2008)**. On the other hand, WT resulted in returning back the liver and kidney TBARS content to near normal levels. It was reported early that green tea catechins protects against alcohol-induced liver and serum lipid peroxidation **(Skrzydlewska *et al.*, 2002)**. Also, it was found that green tea increases the activity of liver antioxidant enzymes glutathione peroxidase GSH-Px and the content of GSH as well as improvement of the total antioxidant status **(El-Beshbishy *et al.*, 2011)**. The antioxidant properties of WT could be attributed to its protective effects on GSH levels via increasing cysteine uptake, which is the rate limiting step of GSH synthesis**(Khan and Kour, 2007)**.Enzymatic antioxidants (SOD, CAT,) form the first line of the antioxidant defence mechanism to protect the organism from ROS-mediated oxidative damage **(Nonaka *et al.*, 1991)**. Our results lend credibility to these observations.

In the present study, liver and kidney NO levels were significantly elevated following INH and RIF administration, favoring the possibility of the involvement of overproduction of NO in the pathogenesis of combination-induced hepatorenal damage. This result was in accordance with **Saad *et al.* (2010)**. NO overproduction was previously linked to drug-induced hepatotoxicity such as; CCl_4 and cadmium **(Harstad & Klaassen, 2002)**. The reactive nature of NO with reactive oxygen species (ROS) suggests several biological pathways through which NO promote oxidative stress-induced cell injury. The released NO scavenges ($O_2^{\bullet-}$) to produce peroxynitrite anion ($ONOO^-$), a potent prooxidant and cytotoxic intermediate that is capable of damaging proteins, lipids and DNA **(Yue *et al.*, 2009; Saad *et al.*, 2010)**. ($ONOO^-$) is protonated in a short time, as in many other ROS with short half-life, and this reaction generates highly toxic ($HO^{\bullet}$), which may explain the cytotoxicity associated with the elevated level of NO **(Saad *et al.*, 2010)**. NAC previously demonstrated to have protective effect on the hepatic oxidative damage induced by CCl_4 **(Maksimchik *et al.*, 2008)**, through the scavenging of both liver and serum NO radicals. Furthermore, NAC supplementation was previously demonstrated to inhibit alloxan-induced iNOS expression and to decrease NO concentration in the pancreas of alloxan-induced diabetic mice**(Ho *et al.*, 1999)**. On the other hand, **Khan and Kour, (2007)** reported that WT polyphenol constituents possess potent antioxidant action, although to various degrees and are considered as potent scavengers of ROS such as ($O_2^{\bullet-}$), (H_2O_2), ($HO^{\bullet}$) and NO produced by various chemicals.

Our study revealed that, 45 days oral administration of INH and RIF to rats resulted in significant decrease in the levels of the non-enzymatic free radical scavenger GSH in both liver and kidney, especially hepatic GSH which was aggressively depleted (27%), as the liver is the main site of

drugs' detoxification. Our findings are similar with several *in vivo* previous studies**(Attri *et al.*, 2000; Santhosh *et al.*, 2007; Saad *et al.*, 2010; Dhamal *et al.*, 2012; Palanisamy and Manian, 2012)** and *in vitro***(Heidari*et al.*, 2013)**. On the other hand, our results are in conflict with **Rana *et al.* (2006)** probably because they used only RIF in a lower dose (50 mg/kg IP). Depletion of GSH is known to result in enhanced LPO and excessive LPO can cause increased GSH consumption **(Palanisamy and Manian, 2012)**. Another possible mechanism for GSH depletion could be illustrated by the decreased activities of GSTs, which are essential for GSH scavenging activities and recycling. GSTs' activities were decreased during INH treatment **(Yue *et al.*, 2009; Palanisamy and Manian, 2012)**, indicating a systemic reduction in free radical scavenging ability of GSH in response to INH and RIF treatment. NAC failed to elevate the depleted GSH levels in both liver and kidney. This result was in consistence with **Rana *et al.* (2006)** and **Heidari *et al.* (2013)**, who found that supplementation of NAC also failed to increase the threshold levels of GSH in RIF- or INH-intoxicated rats, respectively. In our model there was an extra elevation in both liver and kidney TBARS. Knowing that NAC act as GSH provider, we assumed that even the formed GSH from NAC was engaged in detoxification and scavenging of the extreme toxic radicals produced, either TBARS or NO in both kidney and liver tissues. WT was able to normalize the elevated kidney GSH level, but failed to elevate liver GSH content compared with HT group. **Skrzydlewska *et al.* (2002)** postulated that green tea treatment may either replenish the levels of antioxidant directly or spare the endogenous pool of GSH from being exhausted by the generated free radical. These findings are in agreement with our results of WT supplementation that enhanced the level of kidney GSH, while renal TBARS and NO levels showed no changes compared with HT group.

It is well established that SOD and CAT constitutes a mutually supportive team of antioxidant enzymes which provides a defense system against ROS**(Saad *et al.*, 2010)**. Our study revealed that liver and kidney activities of SOD and CAT were significantly depressed in HT rats. These results are in accordance with several reports discussing the role of enzymatic antioxidant defense mechanisms against anti-TB drugs-induced liver injury **(Santhosh *et al.*, 2007; Kumar *et al.*, 2010; Palanisamy and Manian, 2012;Chandane *et al.*, 2013)**. SOD and CAT activities were significantly decreased due to the abundant production of ($O_2^{\bullet-}$) and other free radicals in rats treated with INH and RIF. Moreover, it has been reported that ($O_2^{\bullet-}$) in addition to singlet oxygen and peroxyl radicals have been shown to directly inhibit the activity of CAT. These observations manifest and explain the significant inhibition of CAT activity in the INH and RIF administered group of rats. Another mechanism that might be involved in the reduced activities of SOD and CAT was through their inactivation by the ROS/RNS production. At high concentration of NO, the ROS and NO react to form ($ONOO^-$) which may react fast with Mn-center of SOD enzyme and inactivate the enzyme involving the nitration of Tyr^{34} amino acid, which is critical for SOD enzyme activity and is the most prone to ($ONOO^-$) mediated nitration **(Radi *et al.*, 2002)** This assumption was ascertained by the elevated NO levels in accordance with the decreased SOD activities. Co-administration of NAC with INH and RIF partially prevented decrease in SOD and CAT activities, which might be due to incomplete scavenging of radicals by NAC, resulting in partial protection of these enzymes. As NAC reacts with scavenging radicals slowly, residual superoxide radicals might interact with H_2O_2 resulting in the formation of hydroxyl radicals. This was supported by the results of **Rana *et al.* (2006)**. Treatment of intoxicated rats with WT resulted in significant increase in kidney SOD and CAT, and hepatic CAT activities, compared with HT group. The increase in hepatic and renal CAT activities according to the co-treatment of WT is consistent with that of **El-Beshbishy*et al.* (2011)** and (**Khan & Kour, (2007)**. It has been reported that WT exert its biological effects on the basis of the redox state of a particular cell/tissue and according to the level of green tea polyphenols accumulated in the tissues. Tea polyphenols can penetrate the lipid bilayer, decreasing free radicals concentration or influencing antioxidant capability in biomembranes. Also, they could reduce the mobility of free radicals into the lipid bilayers as well. Moreover, tea polyphenols can interact with phospholipid head groups, particularly with those containing hydroxyl groups, so they could decrease the fluidity in the polar surface of phospholipid bilayer **(Skrzydlewska *et al.*, 2002)**. So that, the results of this study made a speculation that white tea administration can modulate the susceptibility to lipid peroxidation coupled with up-regulation of the antioxidant status in liver and kidney of rats treated with INH and RIF.

In the present study, INH and RIF induced a significant elevation of serum levels of cholesterol, triglycerides and total lipids (Figure, 2). Our results coincide with different studies**(Kumar *et al.*, 2010; Saad *et al.*, 2010; Jyothi *et al.*, 2013)**. One of the major disorders encountered in anti-TB drugs-induced hepatotoxicity is fatty accumulation in the liver, which develops either due to excessive supply of lipids to the liver or interference with lipid deposition mechanisms **(Santhosh *et al.*, 2007; Saad *et al.*, 2010)**. Strong association between hypercholesterolemia and increased free radical production was previously documented **(Schaffer, 2003)**. ROS produced during oxidative stress react with lipoproteins to produce oxidation states, diminishing the cellular uptake of lipids from the blood **(Lin and Yin, 2008)**. The abnormal cholesterol deposition is favored by the dangerous tendency of cholesterol to undergo passive exchange between the plasma lipoproteins and the cell membranes. Hence, protective HDL-cholesterol levels were previously reported to be reduced in the rats treated with INH and RIF **(Kumar *et al.*, 2010)**. NAC treated rats showed significantly reduced levels of serum cholesterol, triglycerides and total lipids. NAC has been shown to reduce cholesterol levels in plasma and liver in mice consuming a high saturated fat diet **(Lin and Yin, 2008)**. Putative mechanisms accounting for the lipid-lowering effects of NAC might be related to its antioxidant properties. NAC in the present study led to reduced liver and kidney lipid peroxide content (TBARS), which is responsible for impairment of the normal structure of lipoprotein receptors, hence, improving the cellular uptake of serum lipids from the blood. Furthermore, **Lin and Yin, (2008)** reported that the hypolipidemic effect of NAC might be attributed partially to the suppression of mRNA expression of three lipogenic-related enzymes (malic enzyme, fatty acid

synthase and 3-hydroxy-3-methylglutaryl coenzyme A reductase) **(Lin and Yin, 2008)**. WT extract supplementation significantly reduced serum cholesterol, triglycerides associated with no changes observed in serum total lipids compared with HT group. Similar results were obtained by **Richard *et al*. (2009)** supplementing green tea to obese mice, observing increased excretion of lipids after ingestion of tea. Moreover, **Nörnberg *et al*. (2012)** postulated that an increase in energy present in the feces occurs with an increase in catechin doses ingested. Furthermore, it has been reported that catechins regulate the expression of hepatic LDL receptors modulating the lipid synthesis, excretion, and intracellular processing **(Nörnberg *et al*., 2012)**. Since a higher concentration of lipids was found in the cecal content of mice consuming white tea as reported by **Richard *et al*. (2009)**, it is suggested that the decrease in serum triglycerides may be due to decreased absorption rather than reduction in hepatic production or uptake of endogenous lipids.

DNA fragmentation has been recognized as the onset of many diseases including cancer and could be a useful indicator for the oxidative status and antioxidant defense system of organism **(Chen *et al*., 2011)**. The data obtained from the present study showed that INH and RIF induced a highly significant DNA damage, indicated by the increased tail moment in comet assay, in liver and kidney tissues (Figures 6A and 6B). These results are in accordance with previous studies **(Yue *et al*., 2009; Chen *et al*., 2011)**. A previous study showed that hydrazine and therapeutic agents with hydrazine functionality, including phenelzine and hydralazine, cause time- and concentration-dependent strand scission of DNA in human hemolysate **(Runge-Morris *et al*., 1994)**. DNA fragmentation into oligonucleosomal fragments forming ladder pattern following gel electrophoresis, is a feature of apoptosis that was induced by INH and RIF **(Chen *et al*., 2011)**. Administration of NAC showed a marked remodeling of INH and RIF-induced DNA damage in both liver and kidney tissues. This result synchronized with **Joshi *et al*. (2010)** where NAC significantly reduced dimethylmercury-induced DNA damage detected by comet assay. Furthermore, thiopronin, which is NAC analogue, markedly inhibited the generation of DNA adducts induced by INH in precision-cut rat liver slices, which suggests that thiopronin, and consequently NAC, prevents DNA damage caused by free radicals, which suggested that hepatoprotection by NAC is likely due free radical scavenging capacity. WT resulted in significant relieve of the toxic DNA damage in both liver and kidney. The administration of green tea to azathioprine-intoxicated rats resulted in normalization of caspase-3 levels, which suggested a potential protective effect of tea polyphenols against cell death and apoptosis under oxidative stress conditions **(El-Beshbishy *et al*., 2011)**.

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Tables

Table 1: Changes in serum levels of albumin, total protein and albumin/globulin ratio in 30 days isoniazid and rifampicin-induced hepatotoxic rats (HT), treated with either N-acetyl cysteine (HT + NAC), white tea (HT + WT) or rat bone marrow mesenchymal stem cells (HT + MSC).

	Albumin(mg/dl)		T. Protein(mg/dl)		(A/G) ratio	
	Mean ± SEM *(Range)*	*% of NC*	**Mean ± SEM** Range	*% of NC*	**Mean ± SEM** Range	*% of NC*
Control	**3.89±0.13**[(b)] *(3.45-4.34)*	*100%*	**7.34±0.17**[(b)] *(6.86-8.43)*	*100%*	**1.28±0.09**[(b)] *(0.89-1.73)*	*100%*
HT	**1.65±0.14**[(a)] *(0.99-2.2)*	*42%*	**4.84±0.24**[(a)] *(3.69-5.65)*	*66%*	**0.43±0.01**[(a)] *(0.21-1.04)*	*34%*
HT + NAC	**3.33±0.25**[(a,b)] *(2.75-4.92)*	*85%*	**6.13±0.89**[(a,b)] *(5.83-6.67)*	*84%*	**1.28±0.12**[(b)] *(0.95-1.97)*	*100%*
HT + WT	**2.99±0.19**[(a,b)] *(2.34-3.93)*	*77%*	**5.52±0.32**[(a,b)] *(3.58-6.57)*	*75%*	**1.23±0.13**[(b)] *(0.70-1.74)*	*96%*
HT + MSC	**3.63±0.90**[(b)] *(3.28-3.92)*	*93%*	**6.77±0.15**[(b)] *(6.13-7.62)*	*92%*	**1.36±0.22**[(b)] *(0.79-2.8)*	*106%*

Data are presented in mean ± SEM (n=8).
(a) Significantly different from normal control group (p<0.05).
(b) Significantly different from hepatotoxicity group (p<0.05).

Table 2: Changes in serum levels of urea and creatinine in 30 days isoniazid and rifampicin-induced hepatotoxic rats (HT), treated with either N-acetyl cysteine (HT + NAC), white tea (HT + WT) or rat bone marrow mesenchymal stem cells (HT + MSC).

	UREA		CREATININE	
	Mean ± SEM *(Range)*	*% of NC*	**Mean ± SEM** *(Range)*	*% of NC*
Control	**39.4±1.8**[(b)] *(33.2-46.8)*	*100%*	**0.65±0.05** [(b)] *(0.43-0.91)*	*100%*
HT	**14.7±1.0**[(a)] *(11.4-18.7)*	*37%*	**1.12±0.04**[(a)] *(1.00-1.30)*	*172%*
HT + NAC	**17.2±1.3**[(b)] *(10.7-22.1)*	*44%*	**0.85±0.03**[(a,b)] *(0.73-0.98)*	*131%*
HT + WT	**23.4±0.9**[(a,b)] *(20.4-27.1)*	*59%*	**0.75±0.03** [(a,b)] *(0.61-0.84)*	*115%*
HT + MSC	**31.1±1.5**[(a,b)] *(25.7-37.1)*	*79%*	**0.66±0.03**[(b)] *(0.43-1.30)*	*101%*

Data are presented in mean ± SEM (n=8).
(a) Significantly different from normal control group (p<0.05).
(b) Significantly different from hepatotoxicity group (p<0.05).

Figures

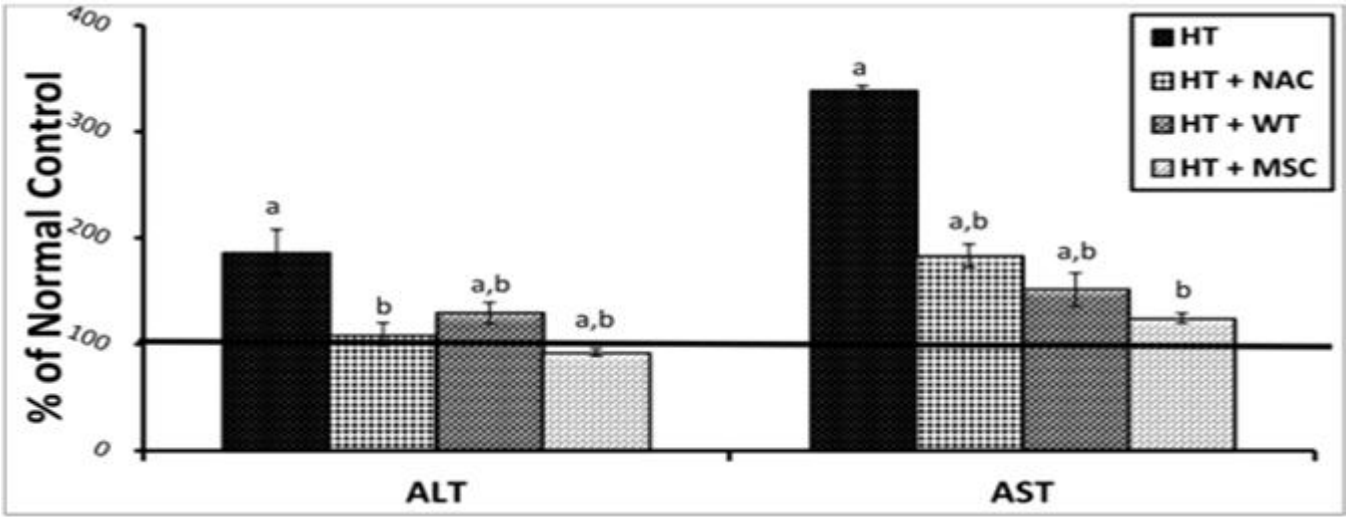

Figure 1: Changes in the activities of serum alanine transaminase and aspartate transaminase in 30 days isoniazid and rifampicin-induced hepatotoxicity in rats (HT), treated with either N-acetyl cysteine (HT + NAC), white tea (HT + WT) or rat bone marrow mesenchymal stem cells (HT + MSC).

Data are presented in percentage of normal control ± SEM (n=8).
(a) Significantly different from normal control group (p<0.05).
(b) Significantly different from hepatotoxicity group (p<0.05).

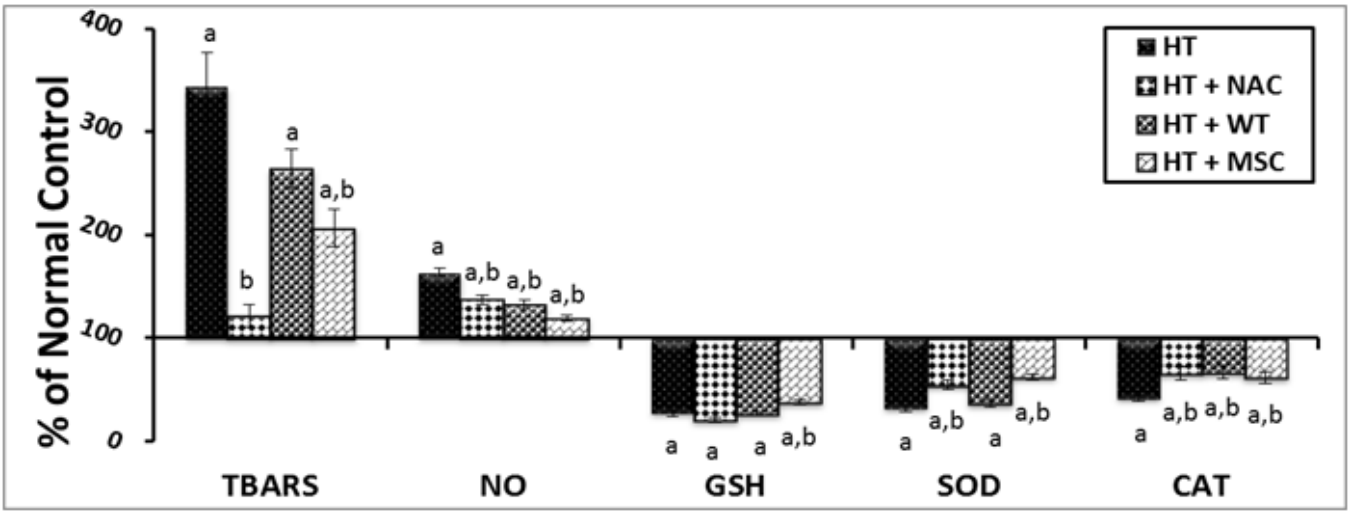

Figure 2: Changes of liver oxidative stress parameters, thiobarbituric acid reactive substance and nitric oxide, reduced glutathione content and the enzymatic activities of superoxide dismutase and catalase in 30 days isoniazid and rifampicin-induced hepatotoxic rats (HT), treated with either N-acetyl cysteine (HT + NAC), white tea (HT + WT) or rat bone marrow mesenchymal stem cells (HT + MSC).

Data are presented in percentage of normal control ± SEM (n=8).
(a) Significantly different from normal control group (p<0.05).
(b) Significantly different from hepatotoxicity group (p<0.05).

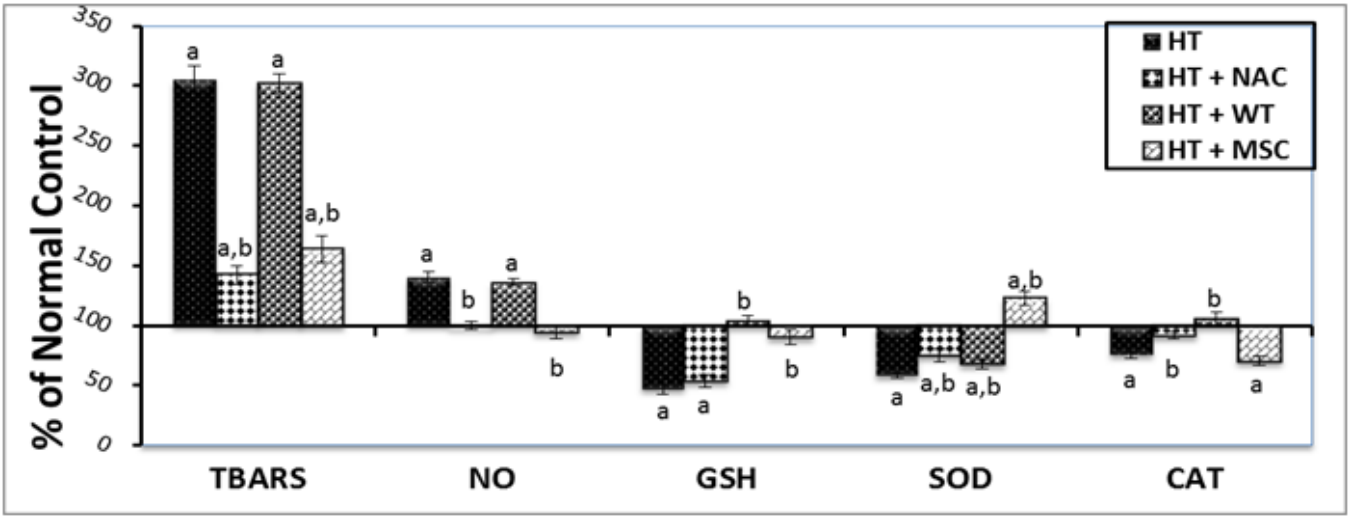

Figure 3: Changes of kidney oxidative stress parameters, thiobarbituric acid reactive substance and nitric oxide, reduced glutathione content and the enzymatic activities of superoxide dismutase and catalase in 30 days isoniazid and rifampicin-induced hepatotoxic rats (HT), treated with either N-acetyl cysteine (HT + NAC), white tea (HT + WT) or rat bone marrow mesenchymal stem cells (HT + MSC).

Data are presented in percentage of normal control ± SEM (n=8).
(a) Significantly different from normal control group (p<0.05).
(b) Significantly different from hepatotoxicity group (p<0.05).

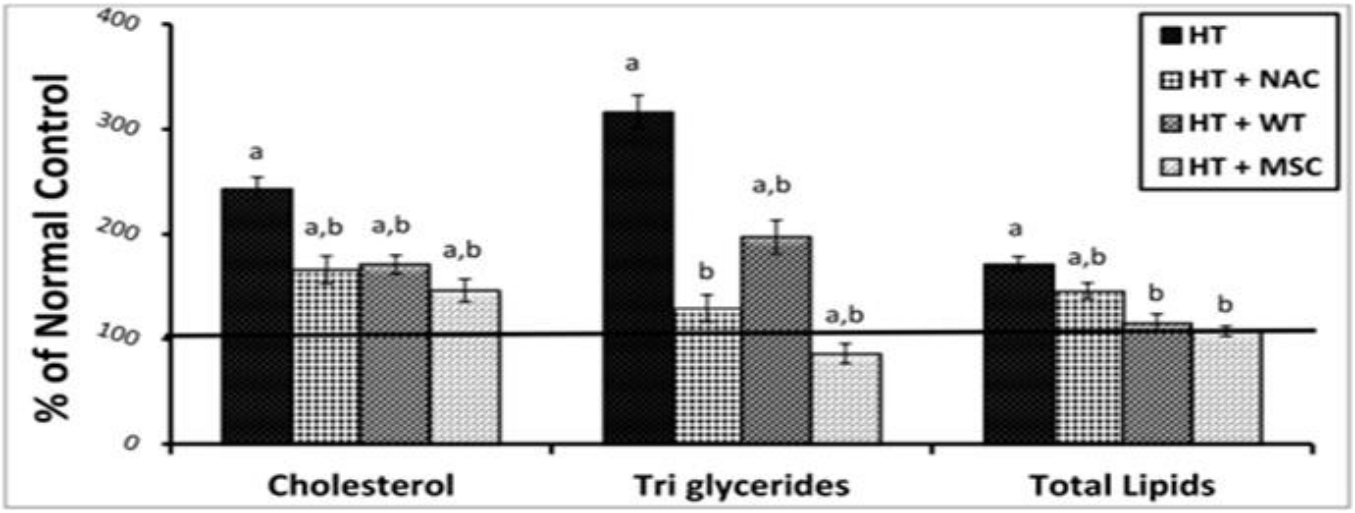

Figure 4: Changes in levels of serum cholesterol, triglycerides and total lipids in 30 days isoniazid and rifampicin-induced hepatotoxicity in rats (HT), treated with either N-acetyl cysteine (HT + NAC), white tea (HT + WT) or rat bone marrow mesenchymal stem cells (HT + MSC).

Data are presented in percentage of normal control ± SEM (n=8).
(a) Significantly different from normal control group (p<0.05).
(b) Significantly different from hepatotoxicity group (p<0.05).

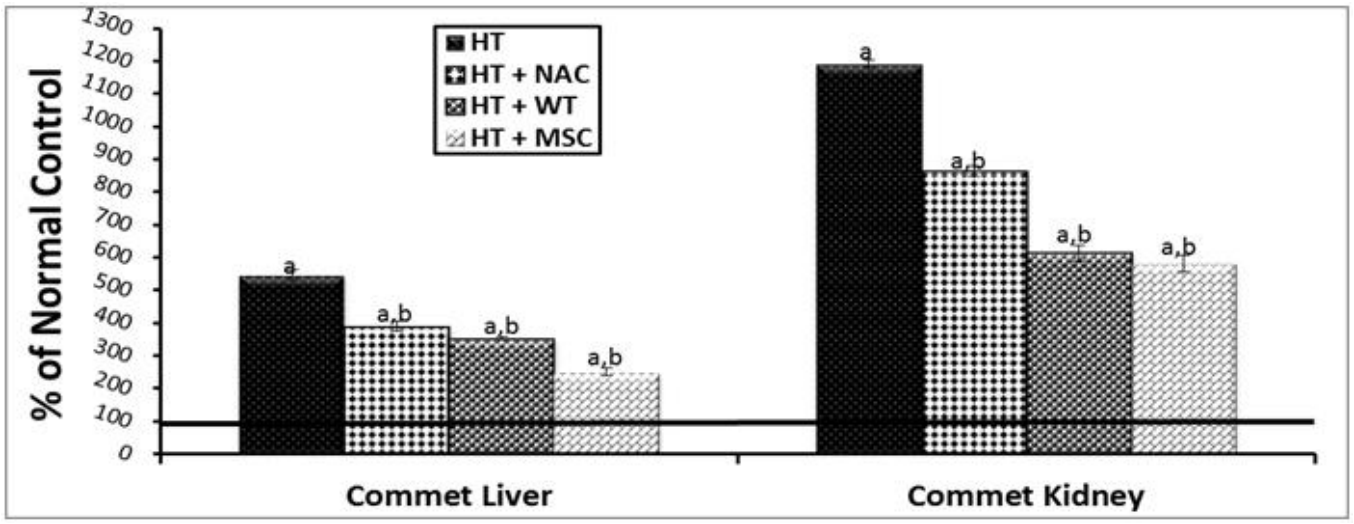

Figure 5: Changes of liver and kidney DNA fragmentation indicated by comet assay in 30 days isoniazid and rifampicin-induced hepatotoxic rats (HT), treated with either N-acetyl cysteine (HT + NAC), white tea (HT + WT) or rat bone marrow mesenchymal stem cells (HT + MSC).

Data are presented in percentage of normal control ± SEM (n=8).

(a) Significantly different from normal control group ($p<0.05$).

(b) Significantly different from hepatotoxicity group ($p<0.05$).

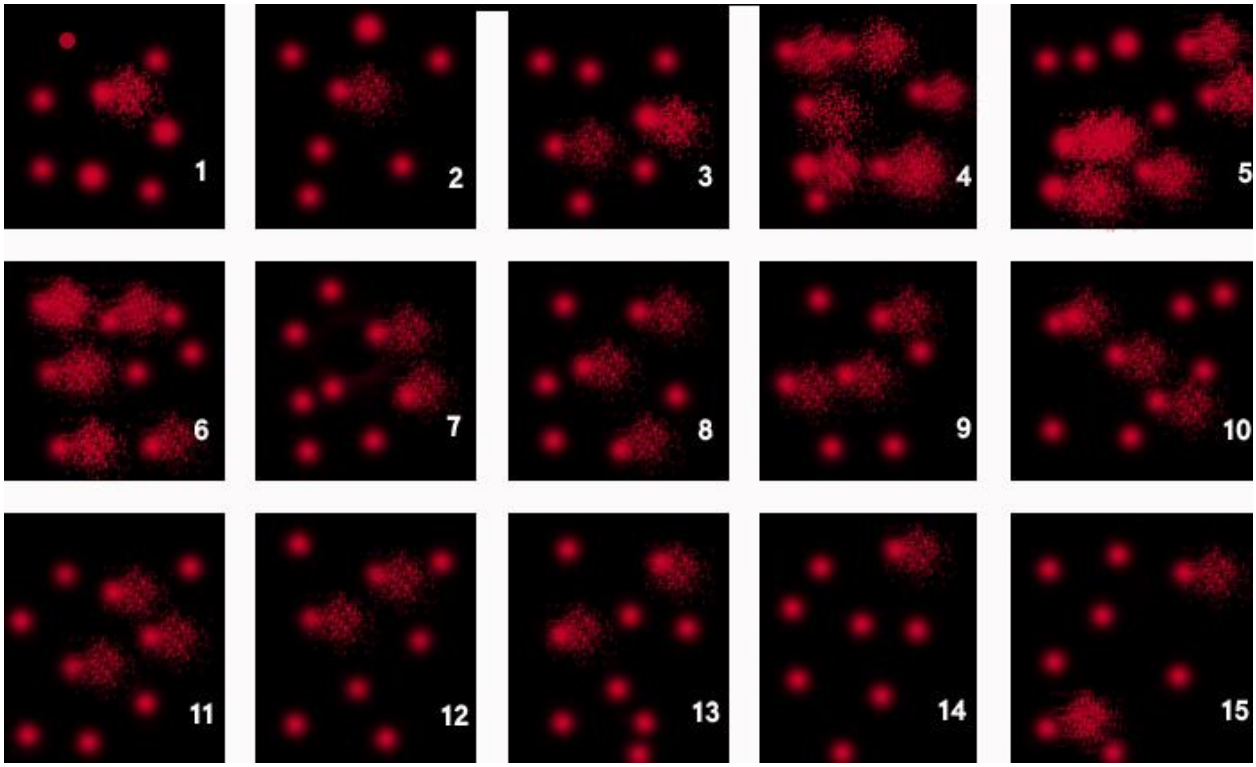

Figure 6A: Representative comet images of Liver tissuefor groups: Control (1-3), hepatotoxicity (4-6), N-acetyl cysteine (7-9), white tea (10-12) and mesenchymal stem cells (13-15)

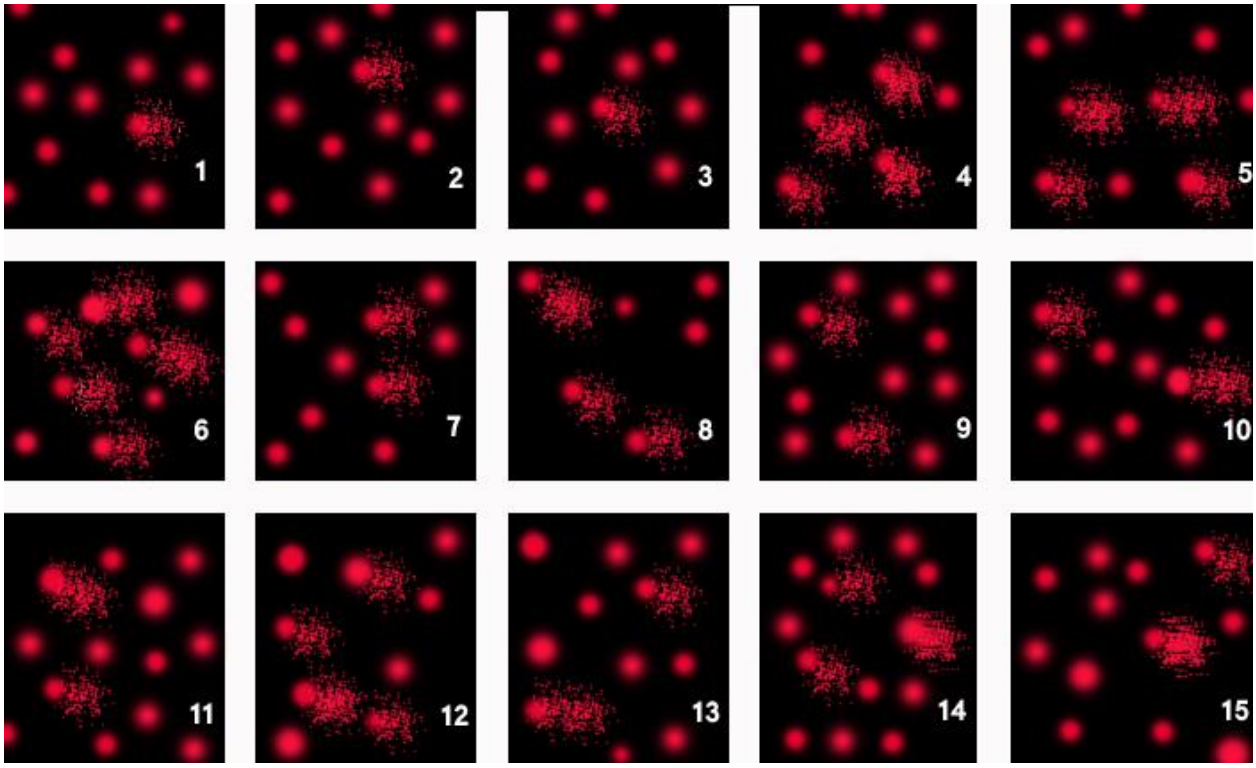

Figure 6B: Representative comet images of Kidney tissuefor groups: Control (1-3), hepatotoxicity (4-6), N-acetyl cysteine (7-9), white tea (10-12) and mesenchymal stem cells (13-15)