International Journal of Pharmacology

ISSN 1811-7775

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OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2017.516.528

Research Article
Hepatoprotective Effect of Rutin Against Oxidative Stress of Isoniazid in Albino Rats

[1]Osama Abdel-Ghaffar, [2]Salwa Thabet Mahmoud, [3]Azza Ali Said and [2]Fatma Abdel-Azeem Youssef Sanad

[1]Division of Physiology, Department of Zoology, Faculty of Science, Cairo University, Giza, Egypt
[2]National Organization for Drug Control and Research (NODCAR), Agouza, Giza, Egypt
[3]Division of Physiology, Department of Zoology, Faculty of Science, Fayoum University, Fayoum, Egypt

Abstract

Background and Objective: Isoniazid (INH) still represents a first-line drug for the treatment and prophylaxis of tuberculosis but different adverse reactions frequently develop in patients administered this drug. Rutin is a common dietary flavonoid consumed in fruits, vegetables and plant-derived beverages. Rutin showed in different reports anti-inflammatory and antioxidative properties. The aim of this study was to evaluate the antioxidative activity of rutin against the INH-induced hepatic toxicity using certain physiological criteria corroborated with a histopathological study of the liver. **Materials and Methods:** Eighty male albino rats were randomly divided into 4 groups: Control, INH-treated, (rutin+INH)-treated and rutin-treated groups. The INH and rutin were orally administered at dose levels of 54 and 40 mg kg^{-1} b.wt., respectively, daily for 4 weeks. Liver function markers were determined in serum in addition to the hepatic malondialdehyde (MDA) and glutathione (GSH) concentrations and superoxide dismutase (SOD) activity. These were supported with a histopathological study of the liver. Statistical analysis was carried out using t-test and analysis of variance (ANOVA). **Results:** Serum albumin concentration was reduced in INH-treated rats while the level of serum globulin was increased. So, serum albumin/globulin(A/G) ratio was significantly reduced ($p<0.05$). The activities of serum aspartate and alanine aminotransferases (ASAT and ALAT) and alkaline phosphatase (ALP) were significantly elevated($p<0.05$) in association with INH administration being indicative to the liver affection. The endogenous antioxidant system showed a decrease in the hepatic GSH content and SOD activity concurrent with elevated liver MDA concentration. The administration of rutin 1 h prior to the INH treatment resulted in the amelioration of these alterations suggesting a hepatoprotective role of rutin. The administration of rutin alone did not alter the studied parameters. The histopathological part supported the biochemical study by ensuring alteration of liver structure and infiltration of leukocytes to the hepatic tissue in INH-treated rats and the amelioration of these alterations because of the pretreatment with rutin. **Conclusion:** This study revealed that INH at a high therapeutic dose level could induce a hepatic injury but the co-administration of rutin could ameliorate this effect via its antioxidative activity.

Key words: Anti-TB drugs, isoniazid-induced hepatotoxicity, oxidative stress, reactive metabolites, flavonoid benefits, rutin antioxidative effect

Received: March 16, 2017 Accepted: May 17, 2017 Published: July 15, 2017

Citation: Osama Abdel-Ghaffar, Salwa Thabet Mahmoud, Azza Ali Said and Fatma Abdel-Azeem Youssef Sanad, 2017. Hepatoprotective effect of rutin against oxidative stress of isoniazid in albino rats. Int. J. Pharmacol., 13: 516-528.

Corresponding Author: Azza Ali Said, Division of Physiology, Department of Zoology, Faculty of Science, Fayoum University, Fayoum, Egypt
Tel: +201142821987

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

During the last few decades, attention of scientists has been directed towards the attribution of different diseases[1,2] and xenobiotic-induced toxicity or adverse reactions[3,4] to oxidative stress induced by reactive intermediates. Reactive intermediates may be free radicals or act as radical generators[5]. The free radical is a molecule having an unshared electron, in its outer orbit, which is usually designated by a dot[6]. Reactive Oxygen Species (ROS) can play physiological roles in signal transduction but in excess can contribute to the mechanisms of diseases[7].

Anti-tuberculosis (anti-TB) drugs have been reported to be associated with a significant number of adverse reactions that can cause significant morbidity, prolonged hospital stay and even death[8]. Among these adverse reactions is hepatotoxicity[9,10]. Although isoniazid (isonicotinic acid hydrazide, INH) is considered one of the most important first-line anti-TB drugs[11,12], it unfortunately was associated with different adverse reactions[13]. The INH-induced side effects include pneumonitis[14], neuropathy[15] and systemic lupus erythematosus[16]. Yet, the most serious adverse reaction of isoniazid was hepatotoxicity[17]. The INH metabolites that are capable of generating free radicals[18] were found to account for the INH-induced hepatotoxicity[19,20]. So as the INH-induced adverse reactions seemed as a consequence of oxidative stress, it may be necessary to suggest an antioxidative agent to abolish or at least ameliorate this effect.

Rutin is a common dietary flavonoid consumed in fruits, vegetables and plant-derived beverages[21]. It belongs to a class of flavonoids called flavonols[22]. It has been reported to have anti-inflammatory[23,24] and antioxidative activities[25,26].

In view of the aforementioned reports about the adverse reactions of INH induced by the oxidative stress and the anti-inflammatory and antioxidative activities of rutin, the present work was suggested. Actually, it is not necessary that any antioxidative agent could succeed in prevention of the adverse reactions of a certain drug. Yet as far as we are aware, the present study, when was suggested, could be preliminary specifically in determination of the INH-rutin couple interaction. The objective of this study was to assess the possible protective role of rutin against the adverse reactions or oxidative stress induced by INH at a dose level, equivalent to a high therapeutic dose used for humans, throughout four weeks of daily administration to rats. Furthermore, the possibility of the occurrence of any adverse reactions which could be induced by rutin administration was taken into account by evaluating its administration alone. In order to achieve these objectives, some biochemical parameters related to liver function, lipid peroxidation and endogenous antioxidant system were suggested to be measured in male albino rats. In addition, a histopathological study on liver was suggested to confirm the biochemical study.

MATERIALS AND METHODS

This study was carried out, 2011-2012, in the labs of the National Organization for Drug Control and Research NODCAR, Agouza, Giza, Egypt.

Experimental animals: The animals chosen for the present study were male albino rats of the Sprague-Dawley strain (raised in the National Organization for Drug Control and Research, NODCAR, Agouza, Giza, Egypt). Their weights ranged between 170 and 220 g. They were housed in wire cages in a temperature-controlled room (22-25°C) and they were supplied *ad libitum* with rodent basal diet and water.

Chemicals: Isoniazid was the drug chosen in the present study as an anti-TB drug to study its adverse reactions. It was obtained as a highly purified powder from Sigma Chemical Co. (St. Louis, MO, USA) and it was suspended in 1% carboxymethyl cellulose (CMC). The INH was orally administered to rats daily for 4 weeks at a dose level of 54 mg kg^{-1} b.wt., which is equivalent to a high therapeutic dose (600 mg day^{-1}) used in certain cases of patients suffering from TB[27] or TB plus Human Immunodeficiency Virus (HIV)[28]. This high dose is the double of the therapeutic dose (300 mg day^{-1}) recommended by WHO[29] for the treatment regimen of TB with other anti-TB drugs. The equivalent dose was calculated according to the body surface area ratio between rat and man[30]. Rutin was obtained as a pure powder from the Sigma Chemical Co. (St. Louis, MO, USA). It was suspended in 1% CMC and administered to the animals orally at a dose level of 40 mg kg^{-1} b.wt., a dose tested in rats by other studies[31,32].

Experimental design: Animals were randomly divided into 4 groups:

- **Control group:** Representing animals orally administered the vehicle (1% CMC), daily for 4 weeks

- **INH-treated group:** Representing animals orally administered isoniazid at dose level of 54 mg kg^{-1} b.wt., daily for 4 weeks
- **(Rutin+INH)-treated group:** Representing animals administered rutin (40 mg kg^{-1} b.wt.) 1 h prior to the administration with INH (54 mg kg^{-1} b.wt.), daily for 4 weeks
- **Rutin-treated group:** Representing animals orally administered rutin alone at dose level of 40 mg kg^{-1} b.wt., daily for 4 weeks

Preparation of samples: Five animals were weekly taken out from each of the 4 groups and blood was drawn from the retro-orbital plexus of animals using capillary tubes[33,34]. Blood samples were collected and centrifuged at 3000 rpm for 20 min, where sera were separated and kept in vials to be used for the biochemical study. Liver samples were separated from the sacrificed animals to determine the concentrations of malondialdehyde (MDA) and reduced glutathione (GSH) and the activity of superoxide dismutase (SOD). Other liver pieces were fixed in 10% formalin solution in small glass bottles to be used for the histopathological study.

Biochemical assays: The concentrations of serum Total Protein (TP), albumin and globulin were determined using commercial reagent kits according to the methods adopted by Doumas *et al.*[35,36] and Oser[37], respectively, using reagent kits. Aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) activities were determined in the serum according to the methods described by Reitman and Frankel[38]. The activity of the serum alkaline phosphatase (ALP) was determined according to the kinetic method proposed by Hausamen *et al.*[39]. The concentrations of MDA and GSH and the activity of SOD were determined in the liver depending on the methods adopted by Uchiyama and Mihara[40], Beutler *et al.*[41] and Marklund and Marklund[42], respectively. Perkin-Elmer Lambda spectrophotometer was used for the determination of sample absorbance.

Histopathological study: The liver pieces were fixed in 10% formalin solution. They were embedded in paraffin wax and then sectioned. Five sections (5 µm thick) were taken from each tissue, each section being at a distance of at least 900 µm from the other one. Liver sections were stained with hematoxylin and eosin.

Statistical analysis: To reveal the effect of INH treatment, the data of INH-treated group were compared with those of vehicle-treated control group. To clarify the ameliorative effect of rutin, the data of the animal group treated with rutin prior to INH administration were compared with those of INH-treated group. To show the side effects of rutin, the data of rutin-treated group were compared with those of vehicle-treated control group. Data are presented as Mean±Standard Error (M±SEM) of 5 animals. The levels of statistical significance ($p<0.05$, $p<0.01$ and $p<0.001$) of results were determined using *t*-test and one-way ANOVA according to the data analysis software of Microsoft Excel (version 14.0) and Campbell[43].

RESULTS

Biochemical study: As regards the serum Total Protein (TP) (Fig. 1a), the mean values of its concentration did not significantly change in response to the treatment with INH or rutin, prior to INH or alone, throughout the 4 weeks experimental period. Yet, the mean values of the serum albumin concentration decreased significantly in INH-treated animal group at the 3rd ($p<0.01$) and 4th ($p<0.05$) weeks of treatment period (Fig. 1b). On the other hand, the mean values of serum globulin concentration (Fig. 1c) in INH-treated rats were significantly ($p<0.05$) higher than the corresponding values of the control group particularly at the 3rd and 4th weeks of experimental period. Regarding the albumin/globulin (A/G) ratio (Fig. 1d), the mean values recorded for the INH-treated rats were significantly lower than the corresponding values of controls at the 1st ($p<0.05$), 3rd and 4th ($p<0.001$) weeks of treatment period. In the animal group treated with rutin prior to INH, no significant change was recorded in the mean values of serum TP but the INH-induced decrease in serum albumin level and A/G ratio was significantly ameliorated at the 3rd and 4th weeks of experimentation. ANOVA revealed significant differences among animal groups in serum albumin and A/G ratio due to INH administration ($F_{1,38}$= 15.369 and 11.462, respectively) and pretreatment with rutin before INH ($F_{1,38}$= 13.294 and 6.324, respectively).

Concerning liver function assays, data obtained for INH-treated rats showed significant elevations in the activity mean values of ASAT ($p<0.01$) and ALAT ($p<0.05$) at the last time interval and at the 3rd ($p< 0.001$) and 4th ($p<0.01$) weeks in case of ALP (Fig. 2a, b, c, respectively). At the same time intervals, the INH-induced elevations in these enzymes were significantly alleviated ($p<0.05$) in animal group treated with rutin + INH. ANOVA test disclosed significant differences among animal groups in the activities of ASAT, ALAT and ALP

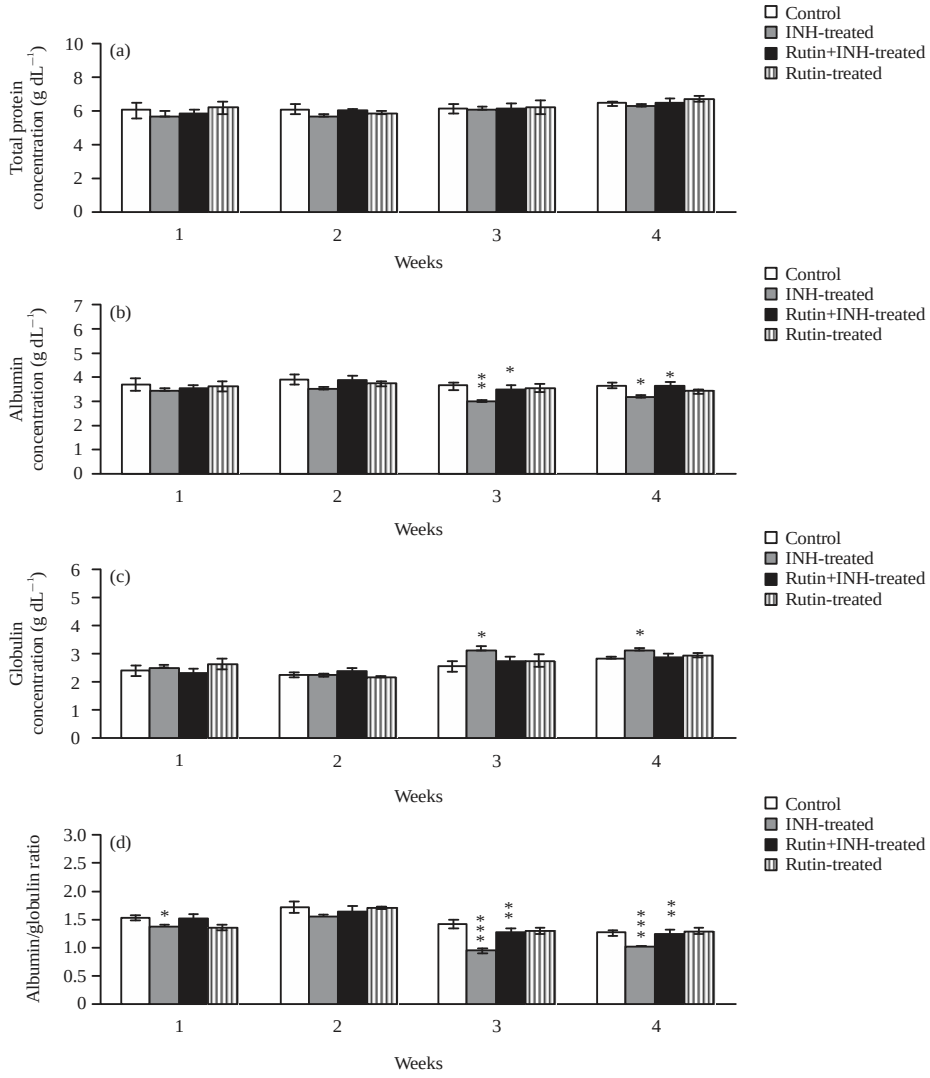

Fig. 1(a-d): Effect of daily oral administration of isoniazid (INH) (54 mg kg^{-1} b.wt.) and rutin (40 mg kg^{-1} b.wt.), 1 h prior to INH administration and alone, on the levels of (a) Serum total protein, (b) Albumin (c) Globulin and (d) Albumin/globulin ratio of male albino rats

Data are illustrated as mean±standard error (M±SEM) of five animals in each group. INH-treated and rutin-treated groups are compared with the control group while (rutin+INH)-treated group is compared with INH-treated group. *Significant (p<0.05), **Highly significant (p<0.01) and ***Very highly significant (p<0.001)

due to INH administration ($F_{1,38}$ = 10.436, 4.722 and 7.922, respectively) and rutin treatment prior to INH ($F_{1,38}$ = 4.351, 9.086 and 7.050, respectively).

With respect to the liver MDA (Fig. 3a), the mean values of its concentration were significantly elevated in the animal group treated with INH at the 1st (p<0.05), 2nd, 3rd and 4th (p<0.01) weeks of the treatment period. Yet, by the administration of rutin one hour prior to INH, the mean values of the liver MDA concentration were significantly (p<0.05) lower than the corresponding values of the INH-treated group at all time-intervals of the treatment duration. According to ANOVA, very highly significant variations (p<0.001) were recorded among animal groups as a result of the INH treatment ($F_{1,38}$ = 53.282) and the pretreatment with rutin before INH ($F_{1,38}$ = 21.217).

In the INH-treated rats, the mean values of hepatic GSH concentration showed a very highly significant reduction (p<0.001) at the 3rd and 4th weeks of treatment period but this reduction was significantly (p<0.01) ameliorated in rats treated with rutin prior to INH at the same time intervals (Fig. 3b). Also, the activity of SOD (Fig. 3c) showed a significant reduction in its mean values in INH-treated rats at the

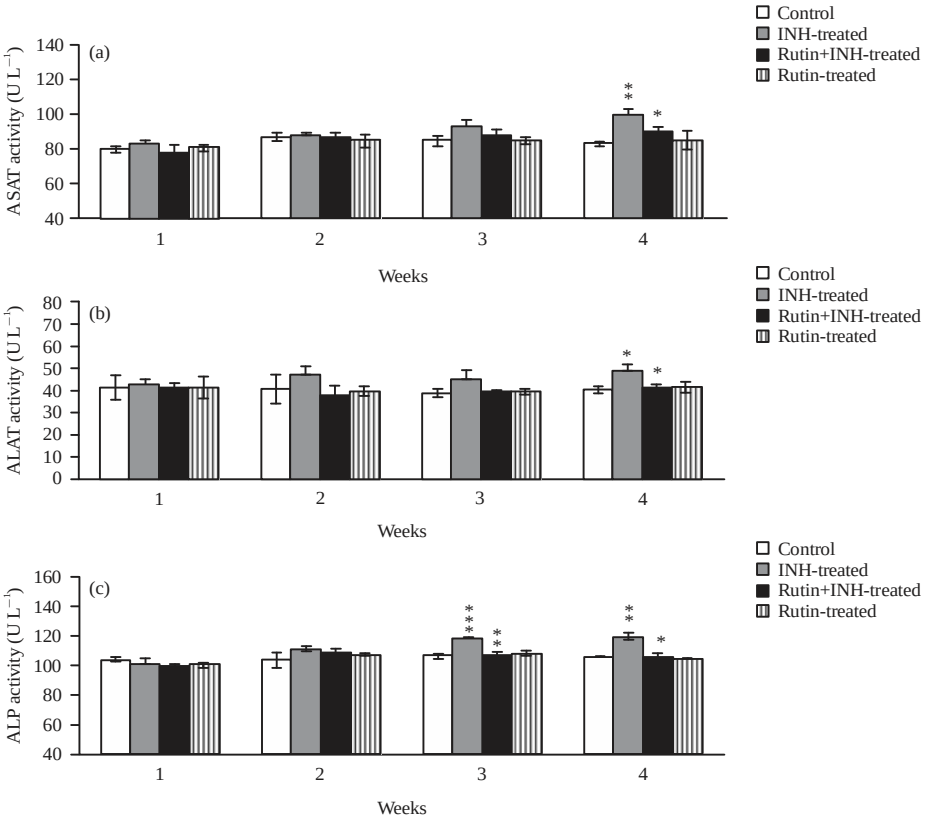

Fig. 2(a-c): Effect of daily oral administration of isoniazid (INH) (54 mg kg^{-1} b.wt.) and rutin (40 mg kg^{-1} b.wt.), 1 h prior to INH administration and alone, on the activities of (a) Serum aspartate aminotransferase (ASAT), (b) Alanine aminotransferase (ALAT) and (c) Alkaline phosphatase (ALP) of male albino rats

Data are illustrated as mean±standard error (M±SEM) of five animals in each group. INH-treated and rutin-treated groups are compared with the control group while (rutin+INH)-treated group is compared with INH-treated group. *Significant ($p<0.05$), **Highly significant ($p<0.01$) and ***Very highly significant ($p<0.001$)

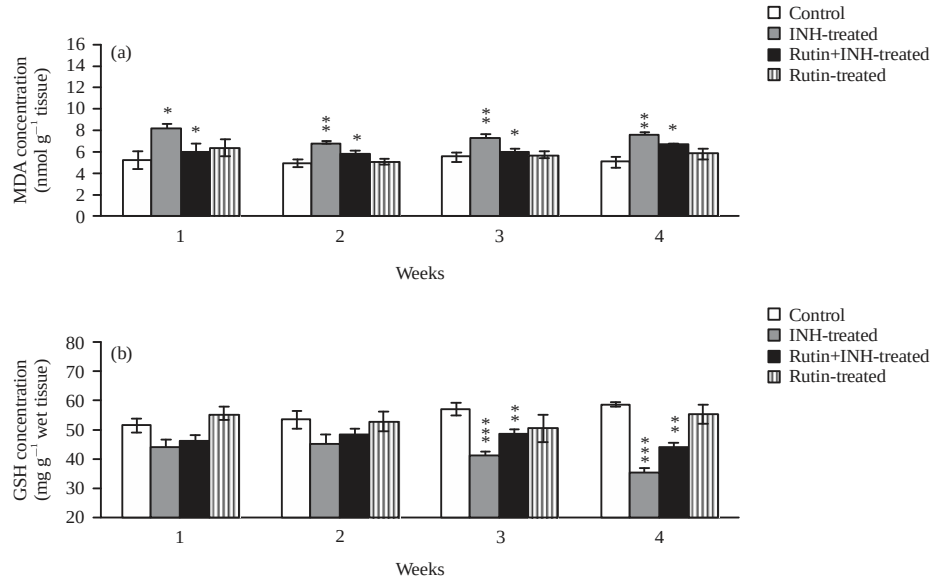

Fig. 3(a-c): Continue

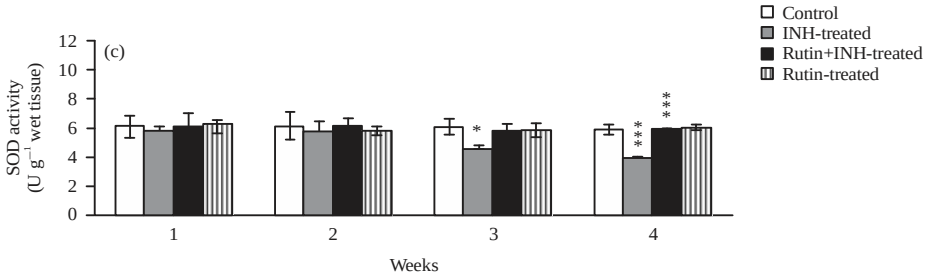

Fig. 3(a-c): Effect of daily oral administration of isoniazid (INH) (54 mg kg^{-1} b.wt.) and rutin (40 mg kg^{-1} b.wt.), 1 h prior to INH administration and alone, on the concentrations of (a) Liver malondialdehyde (MDA), (b) Glutathione (GSH) and (c) Activity of superoxide dismutase (SOD) of male albino rats

Data are illustrated as mean±standard error (M±SEM) of five animals in each group. INH-treated and rutin-treated groups are compared with the control group while (rutin+INH)-treated group is compared with INH-treated group. *Significant ($p<0.05$), **Highly significant ($p<0.01$) and ***Very highly significant ($p<0.001$)

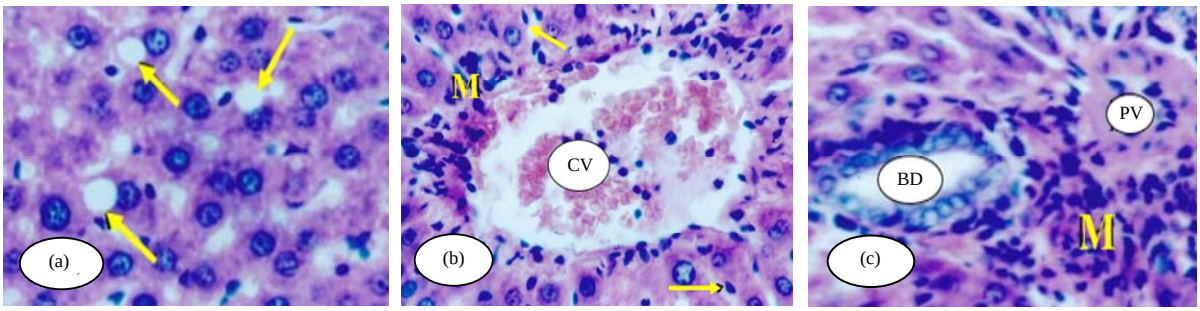

Fig. 4(a-c): Liver sections of rats administered isoniazid (54 mg kg^{-1} b. wt.) daily for one week showing (a) Fat vacuoles (arrows) in the cytoplasm of hepatocytes, (b) Infiltration of inflammatory mononuclear leukocytes (M) surrounding the congested central vein (CV) and (c) In between the bile duct (BD) and portal vein (PV) (Hematoxylin and eosin, Hx and E)

3rd ($p<0.05$) and 4th ($p<0.001$) weeks of administration. Nevertheless, in rats treated with rutin prior to the administration of INH, the mean values of SOD activity were significantly ($p<0.001$) higher than those of the animal group treated with INH alone at the 4th week of treatment period. ANOVA exhibited significant differences among animal groups in liver GSH concentration and SOD activity due to INH administration ($F_{1,38}$ = 54.519 and 7.118, respectively) and rutin treatment before INH ($F_{1,38}$ =10.038 and 6.429, respectively). In the animal group treated with rutin alone, no significant alteration was observed in any of the biochemical parameters studied.

Histopathological study: Regarding the INH-treated group, the liver sections obtained after one week of daily INH administration revealed a noticeable fatty change in the hepatocytes as intracytoplasmic vacuoles (Fig. 4a). This change was associated with infiltration of mononuclear leukocytic inflammatory cells, surrounding the dilated and congested central vein (Fig. 4b). In the portal area, infiltration of a massive number of leukocytic inflammatory cells was detected (Fig. 4c). After 2 weeks of daily administration of isoniazid, severe dilatation and congestion were observed in the central vein as well as in the portal vein with a massive infiltration of inflammatory cells in the portal area (Fig. 5a, b). Three weeks after daily administration of INH, the fatty change was observed in the hepatocytes (Fig. 6a), while infiltration of mononuclear leukocytic inflammatory cells was noticed in the portal area (Fig. 6b) and in between the hepatocytes (Fig. 6c). After 4 weeks of INH administration, the fatty change was ascertained in the hepatocytes (Fig. 7a) in addition to a focal aggregation of mononuclear inflammatory cells (M) with diffuse Kupffer cell proliferation (Fig. 7b). Moreover, a hyperplasia was noticed

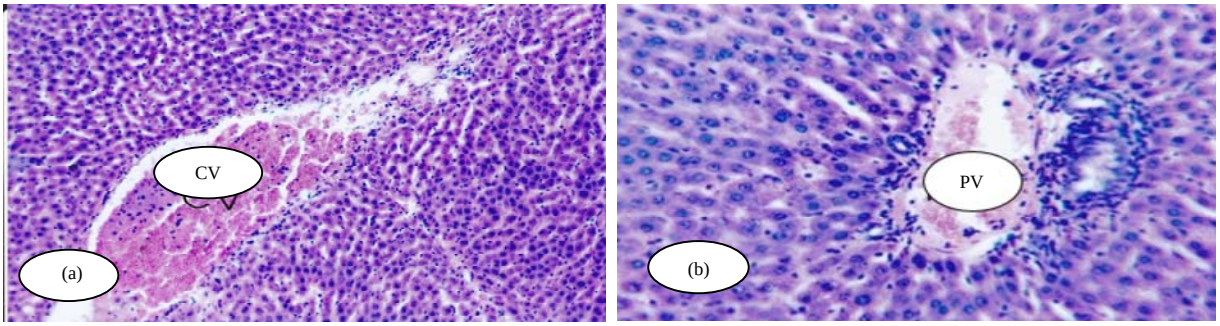

Fig. 5(a-b): Liver sections of rats administered isoniazid (54 mg kg^{-1} b. wt.) daily for 2 weeks showing (a) Severe dilatation and congestion of the central vein (CV) and (b) Infiltration of a massive number of inflammatory cells surrounding the congested portal vein (PV) in the portal area (Hx and E)

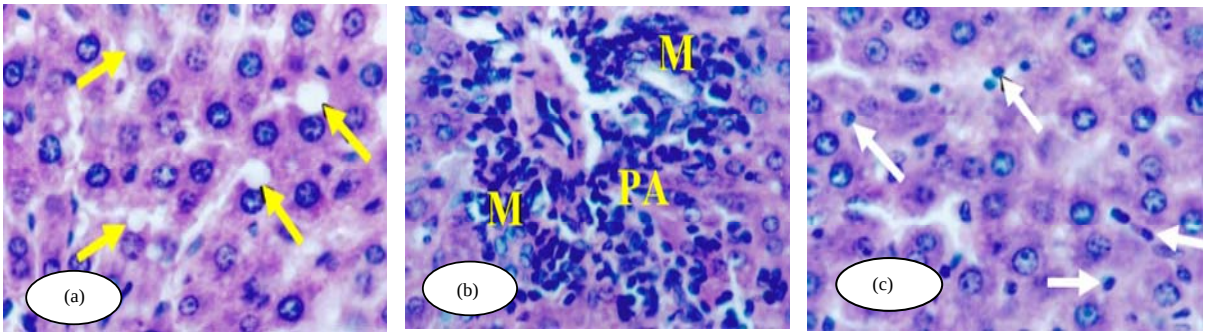

Fig. 6(a-c): Liver sections of rats administered isoniazid (54 mg kg^{-1} b.wt.) daily for 3 weeks showing (a) Fatty change (yellow arrows) in the hepatocytes, (b) Infiltration of a massive number of inflammatory mononuclear leukocytes (M) in the portal area (PA) and (c) In between the hepatocytes (white arrows) (Hx and E)

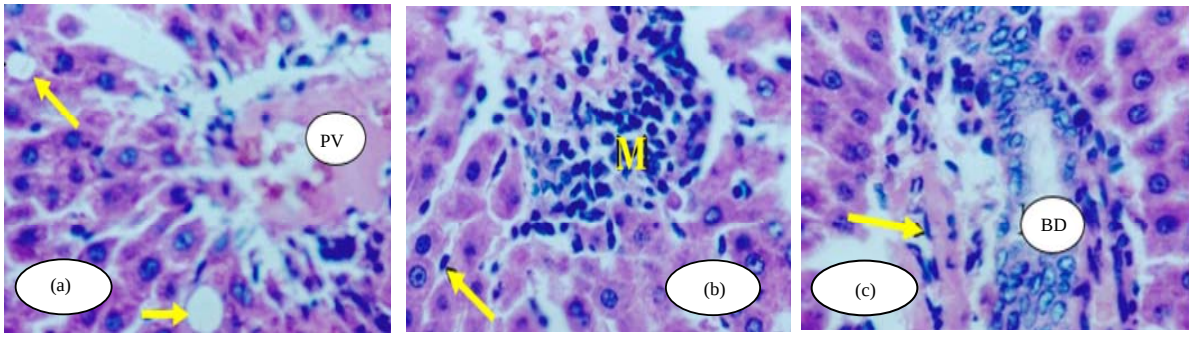

Fig. 7(a-c): Liver sections of rats administered isoniazid (54 mg kg^{-1} b.wt.) daily for 4 weeks showing (a) Severe dilatation of the portal vein (PV) with a fatty change in hepatocytes (arrows) , (b) Focal aggregation of mononuclear inflammatory cells (M) with diffuse Kupffer cell proliferation (arrow) and (c) Hyperplasia of the epithelial cells lining the bile duct (BD) with fibroblastic cell proliferation (arrow) in the portal area (Hx and E)

in the epithelial cells lining the Bile Duct (BD) concurrent with a fibroblastic cell proliferation (Fig. 7c).

The liver sections obtained from the animal group treated with rutin (40 mg kg^{-1} b.wt.) 1 h prior to isoniazid

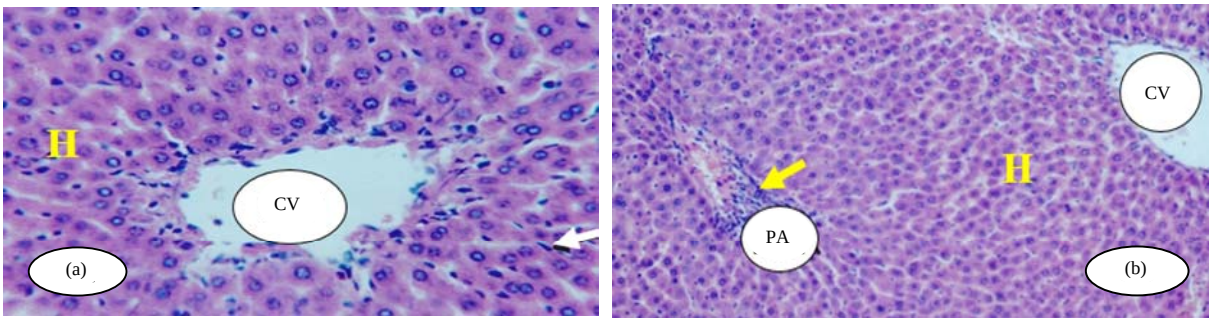

Fig. 8(a-b): Liver sections of rats administered rutin (40 mg kg^{-1} b.wt.) 1 h prior to isoniazid (54 mg kg^{-1} b.wt.) daily for (a) One week and (b) Two weeks, showing intact hepatocytes (H) without fatty changes Kupffer cells (white arrows) moderately appearing among hepatocytes and moderate infiltration of inflammatory cells (yellow arrow) in the portal area (PA) (Hx and E)

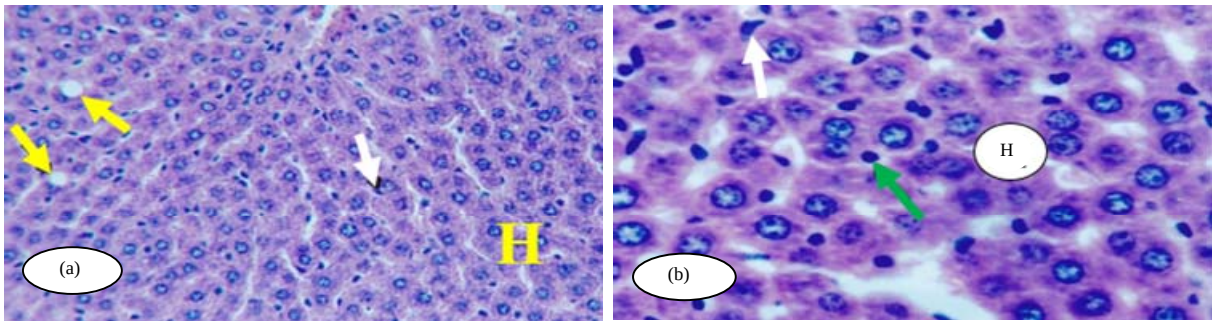

Fig. 9(a-b): Liver sections of rats administered rutin (40 mg kg^{-1} b.wt.) 1 h prior to isoniazid (54 mg kg^{-1} b.wt.) daily for (a) Three weeks and (b) Four weeks, showing a fatty change in some individual hepatocytes (yellow arrows) and diffuse Kupffer cells (white arrows) and moderate infiltration of inflammatory cells (green arrows) in between hepatocytes (H) (Hx and E)

(54 mg kg^{-1} b.wt.) daily for 1 week showed intact hepatocytes without fatty changes and Kupffer cells moderately appear among hepatocytes (Fig. 8a). After 2 weeks of rutin+INH treatment, hepatocytes (H) appeared intact without fatty changes and the portal area showed a moderate infiltration of mononuclear leukocytic inflammatory cells (Fig. 8b). Three weeks of pretreatment with rutin before INH administration, a reduced fatty change was observed in a few individual hepatocytes (Fig. 9a). After 4 weeks of co-administration of rutin and INH, liver sections showed a moderate inflammatory cell infiltration with diffuse Kupffer cell proliferation in between hepatocytes (Fig. 9b). As regards the animal group treated with rutin alone, the liver sections obtained throughout the 4 week-trial period showed intact architecture of the hepatic tissue, with a minimum diffuse of Kupffer cells (Fig. 10-11).

DISCUSSION

In the present study, serum albumin concentration markedly decreased while that of serum globulin noticeably increased in INH-treated rats. So, serum TP level was not significantly changed while albumin/globulin (A/G) ratio showed remarkable decrements in its mean values. Similarly, hypoalbuminemia has been recorded in rats[44] and patients[45] treated with INH with other anti-TB drugs. The decrease in the level of serum albumin herein recorded in INH-treated rats could be attributed to the impaired synthesis of albumin in the affected hepatic tissue under the oxidative stress of INH or one of its metabolites. The hepatotoxic effect of INH, in association with lowered serum albumin, was also reported in other studies of rats[44] and patients[46]. The increase in serum globulin herein observed in INH-treated rats could be related to the formation of antibodies against hapten-protein

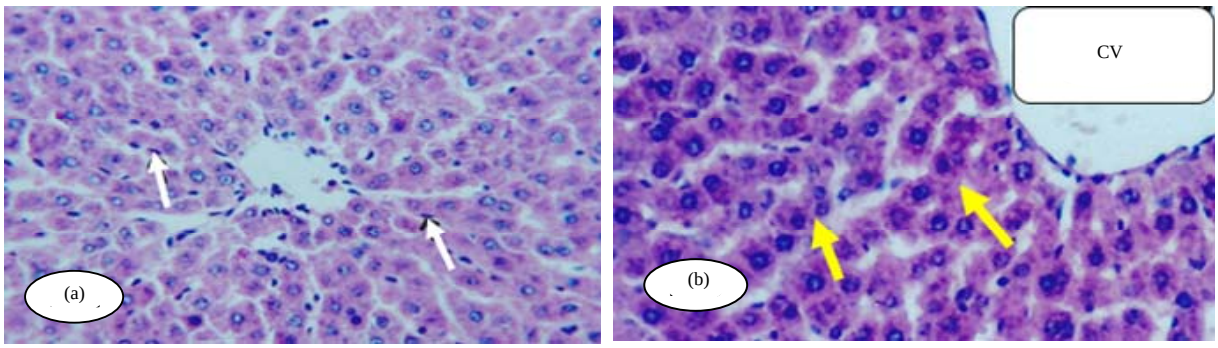

Fig. 10(a-b): Liver sections of rats administered rutin (40 mg kg^{-1} b.wt.) daily for (a) One week and (b) Two weeks, showing normal hepatic architecture with diffuse Kupffer cell proliferation (white arrows) and intact strands (yellow arrows) (Hx and E)

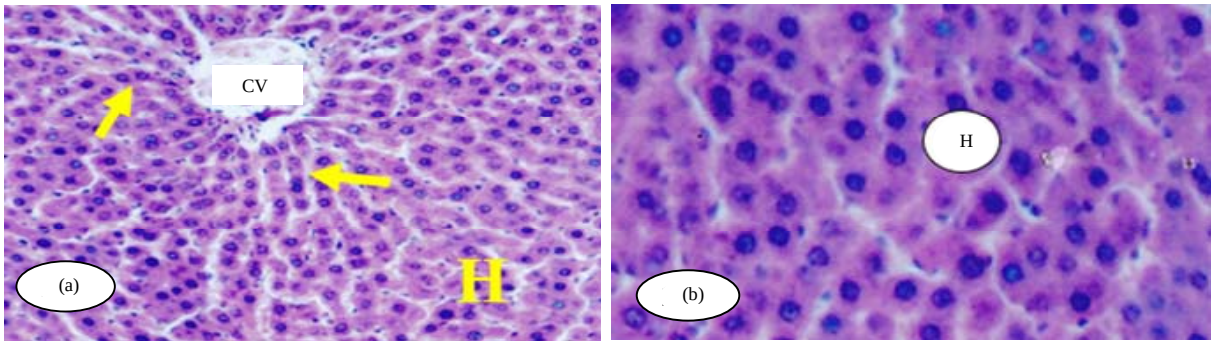

Fig. 11(a-b): Liver sections of rats administered rutin (40 mg kg^{-1} b.wt.) daily for (a) Three weeks and (b) Four weeks, showing intact hepatic strands (arrows) around central vein (CV) and intact hepatocytes (Hx and E)

complex, where INH represents the hapten. In this connection, Faulkner *et al.*[47] indicated that immune activation can occur when drugs or haptens bind covalently to proteins and then act as antigens. Also, anti-isoniazid and anti-cytochrome P450 antibodies were detected in patients with isoniazid-induced liver failure[48].

In rats treated with rutin 1 h prior to INH administration, serum TP and globulin levels did not significantly change while the INH-induced hypoalbuminemia was markedly alleviated. The unchanged serum globulin level may support the probability of autoimmune response against protein-hapten complex, which was not affected by rutin pretreatment. Yet, the improvement in serum albumin concentration could indicate the protecting role of rutin upon hepatic tissue maintaining albumin synthesis near normal. In rats suffering from oxidative stress due to exposure to CCl_4, it was found that the co-administration of rutin led to increased levels of serum TP and albumin[49].

As regards the enzymatic biomarkers of the hepatic function, the results obtained in the present study revealed that the activities of serum ASAT, ALAT and ALP were remarkably elevated in INH-treated rats particularly at the last week of treatment period. In the light of this elevation in serum enzyme activities and the significant decrease ($p<0.05$) in serum albumin level discussed, the hepatic tissue seemed to be affected to a certain extent. In accordance with these results, different reports have indicated the increased activities of serum ASAT, ALAT and ALP in association with the INH treatment, either alone or in combination with other anti-TB drugs, in patients[50], guinea pigs[51] and rats[52,53].

The elevation in the activities of serum ASAT, ALAT and ALP observed in INH-treated rats was found to be significantly reduced ($p<0.05$) in the animal group treated with rutin one hour before INH administration. This could confirm the hepatoprotective role of rutin via suppression of oxidative stress induced by INH. Using D-galactose to induce oxidative stress-mediated hepatic injury in mice, Zhang *et al.*[54] recorded

tissue architecture changes and serum ASAT and ALAT increases but these changes were suppressed by troxerutin (a derivative of rutin).

In the present study, the hepatic MDA level was remarkably elevated in animal group treated with INH alone reflecting the INH-induced LPO. On the other hand, both hepatic GSH level and SOD activity were decreased in INH-treated rats indicating consumption of GSH and inhibition of SOD activity by the oxidizing effect of INH or its metabolites. In accordance with these results, different studies reported increased level of MDA and decreased GSH concentration and SOD activity in liver of INH-treated rats[52] and mice[55].

The pretreatment of rutin prior to INH administration reversed the alterations observed in hepatic MDA and GSH levels and SOD activity ensuring the antioxidative effect of rutin against INH hepatotoxicity. Similarly, in a study of ethanol-induced hepatotoxicity in rats, the pretreatment with rutin protected the hepatic tissue by decreasing the LPO and increasing GSH content and SOD activity[56]. Moreover, in a recent *in vitro* study, rutin was found to attenuate H_2O_2-induced oxidation damage and apoptosis in Leydig cells, where MDA level was decreased while GSH level and SOD activity were increased[57].

Regarding the histopathological part of this study, liver sections obtained from INH-treated rats revealed different alterations in the hepatic structure. Hepatocytes showed fatty vacuoles in their cytoplasm and central vein was dilated and congested with blood cells. A massive infiltration of leukocytic inflammatory cells was observed in between the hepatocytes as well as in the portal area, exhibiting a focal manner in different areas. Bile duct epithelial cells showed hyperplasia and fibrosis. In this connection, in a study of 208 patients treated with INH for TB prophylaxis, liver showed portal and periportal lymphocytic infiltration with lesser numbers of plasma cells, neutrophils and eosinophils[58]. In Guinea pigs treated with INH plus RIF and PZA, hepatic changes were exhibited as focal necrosis, portal triaditis and steatosis[51]. In their review, Wang *et al.*[17] indicated that cytochrome P450 isoenzymes were proposed to be involved in the oxidization of INH metabolites, hydrazine (Hz) and acetyl hydrazine (AcHz), to reactive metabolites which are thought to be involved in INH hepatotoxicity. In addition, certain studies reported that INH itself can bind to liver proteins and cause immune-mediated hepatotoxicity[48,59].

The liver sections obtained in the present work from the animal group treated with rutin+INH showed amelioration of all the histological changes induced by INH. In addition, rutin alone did not alter the hepatic architecture. In another study, rutin at dose levels of 25, 50 and 100 mg kg^{-1} b.wt., showed a hepatoprotective effect against the histopathological changes induced in rats by ethanol, the higher dose being the most effective dose[56].

This study highlights the importance of finding out a natural supplement to reduce serious adverse reactions of INH and related drugs. The work might be limited by the relative short duration of experiment (4 weeks) which was suitable for rats but for patients, they may suffer from the adverse reactions of drugs for a long period due to the long duration of the treatment regimen. The study recommend that physicians and health professionals should be aware of the hepatotoxic effects of anti-TB agents and continuously monitor the status of patients by periodical analysis of blood and liver function during the treatment regimen. Researchers should search for new natural antioxidants and use new techniques and new scopes to clarify the exact etiological factors standing behind the drug-induced adverse reactions and the counter action of natural antioxidants. Further studies are required using a wide variety of parameters, different tissues, different animal species and human trials.

CONCLUSION

The administration of INH to rats, at a dose level equivalent to a high human therapeutic dose, resulted in remarkable alterations in the majority of the biochemical and histopathological criteria indicating hepatic damage. These INH-induced alterations were mainly attributed to the oxidative stress, LPO and suppression of endogenous antioxidant system. However, the pretreatment of rutin before INH administration prevented or at least ameliorated the INH-induced alterations. Moreover, rutin alone at its chosen dose did not significantly alter any of the parameters studied. This study suggests carrying out further studies to evaluate the co-administration of rutin with other chemotherapeutic agents, which are known to induce severe side effects caused mainly by oxidative stress.

SIGNIFICANCE STATEMENT

This study will help the researchers to uncover the mechanisms accounting for rutin ameliorating effect. Thus, a new trend on the drug-flavonoid combination and possibly other combinations, may be followed and developed using various recent techniques in different scientific scopes.

ACKNOWLEDGMENT

The authors would like to thank the colleagues in the Faculty of Science, Cairo University and NODCAR in Agouza, Giza, for their efforts and facilities to complete this study.

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