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Research Article
Ameliorative Effect of Rutin Against Isoniazid-induced Alterations in Certain Hematological and Biochemical Parameters of Albino Rats

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Abstract

Background and Objective: Isoniazid (INH) represents one of the first-line drugs recommended for the treatment and prophylaxis of tuberculosis. Yet, different reports indicated that INH treatment was associated with different adverse reactions in patients and experimental animals. Rutin belongs to a class of flavonoids called flavonols and it showed in different studies anti-inflammatory and antioxidative activities. The aim of this study was to evaluate the antioxidative activity of rutin against the INH-induced alterations in hematological parameters, serum glucose and lipids and renal function. **Materials and Methods:** Eighty male albino rats were randomly divided into four groups: control, INH-treated, (rutin+INH)-treated and rutin-treated groups. INH and rutin were orally administered at dose levels of 54 and 40 mg kg^{-1} b.wt., respectively, daily for four weeks. Statistical analysis was performed using t-test and one-way analysis of variance (ANOVA). **Results:** The administration of INH alone daily for four weeks resulted in normocytic normochromic anemia associated with leukopenia. Leukopenia was a direct consequence of the reduction in neutrophil, eosinophil and lymphocyte counts. A marked hypoglycemia was observed associated with significant increments ($p<0.001$) in serum levels of total cholesterol and triglycerides in rats treated with INH. Renal function of INH-treated animals was not affected as serum urea and creatinine concentrations were not significantly altered, but serum uric acid was markedly increased. The administration of rutin 1 h prior to the INH treatment resulted in the amelioration of above-mentioned alterations. Moreover, the administration of rutin alone did not significantly alter the studied parameters. **Conclusion:** Keeping in view, the importance of INH in treatment of tuberculosis, it seems necessary to co-administer a flavonoid such as rutin in order to ameliorate the INH-induced adverse reactions.

Key words: Anti-TB drugs, INH-induced adverse reactions, reactive metabolites, oxidative stress, flavonoids benefits, rutin antioxidative activity

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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

Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. It typically affects the lungs (pulmonary TB) but can affect other sites as well (extrapulmonary TB)[1]. The currently recommended treatment for new cases of drug-susceptible TB is a six-month regimen of four first-line drugs: isoniazid (isonicotinic acid hydrazide, INH), rifampicin (RIF), ethambutol (EMB) and pyrazinamide (PZA)[2].Among these drugs, INH was associated with different adverse reactions including systemic lupus erythematosus[3], pneumonitis[4], motor-dominant neuropathy[5] and anemia[6,7]. Nevertheless, the most serious adverse reaction of isoniazid was hepatotoxicity[8,9]. This hepatotoxicity has been ascribed to hydrazine (HZ)[10] and other HZ metabolites that are capable of generating free radicals[11].

Rutin (quercetin-3-O-rutinoside) is a natural flavonoid[12], consumed in fruits, vegetables and plant-derived beverages[13]. It belongs to a class of flavonoids called flavonols[14]. It has been reported to have anti-inflammatory[15] and antioxidative activities[16].

In the light of the afore-cited reports about the adverse reactions of INH induced by the oxidative stress and the antioxidative and anti-inflammatory activities of rutin, the present study was suggested to extend and complete our previous study[17], in which the effects of INH and rutin on liver and certain endogenous antioxidants were investigated. The objective of this study was to assess the possible protective role of rutin against the INH-induced adverse effects on certain hematological parameters, glucose, lipids and renal function 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.

MATERIALS AND METHODS

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

As the current work is an extension of the previous paper[17], the animals, chemicals (INH and rutin) doses (54 and 40 mg kg^{-1}, respectively) and design of experiment are described in details in that paper.

Preparation of samples: Every week, blood samples were collected from five rats of each group using the retro-orbital plexus technique[18]. A part of the blood was collected in vials containing EDTA (1.50±0.25 mg mL^{-1} blood) for the hematological assays, while the other part was centrifuged (3000 rpm for 20 min) to obtain sera which were kept in deep freezer (-70°C) till use in the biochemical study.

Hematological parameters: Red and white blood cell (RBC and WBC) counts, hematocrit (Hct) or Packed Cell Volume (PCV) and hemoglobin (Hb) content in addition to the red cell indices were determined according to Bain *et al.*[19].The red cell indices measured were the Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC).

Biochemical parameters: The concentrations of serum glucose, total cholesterol (Tch) and triglycerides (TG) were determined using commercial reagent kits which depend on methods adopted by Trinder[20], Siedel *et al.*[21] and McGowan *et al.*[22], respectively. The reagent kits were also used to estimate the concentrations of serum urea, uric acid and creatinine depending on the methods adopted by Fawcett and Scott[23], Caraway[24] and Larsen[25], respectively.

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 of mean (M±SEM) of five 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).

RESULTS

Hematological study: The results of the hematological parameters studied in the present work are graphically depicted in the Fig. 1-4. The data obtained for the RBC count (Fig. 1a) showed a significant reduction in its mean values recorded for the INH-treated rats at the 3rd ($p<0.05$) and 4th ($p<0.01$) weeks of treatment duration as compared with the control group. Yet, in the animal group treated with rutin1h prior to INH administration, the mean values of the RBC count were significantly ($p<0.05$) higher than those of the INH-treated rats particularly at the 3rd and 4th weeks.

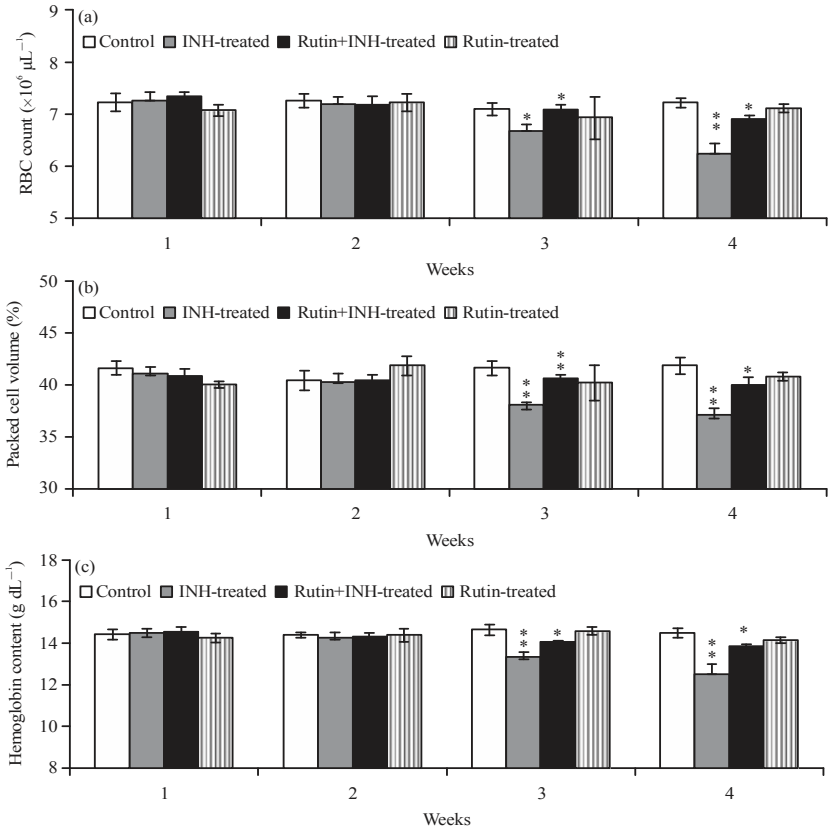

Fig. 1(a-c): Effect of daily oral administration of isoniazid (INH) (54 mg kg^{-1} b.wt.) and rutin (40 mg kg^{-1} b.wt.), 1 h prior to INH administration and alone, on (a) Red blood cell (RBC) count, (b) Packed cell volume and (c) Hemoglobin content of male albino rats

Data are illustrated as Mean±standard error of mean (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 ($p<0.05$) and **highly significant ($p<0.01$)

Relevant to the reduction in RBC count in response to INH treatment, the mean values of Hct or PCV significantly ($p<0.01$) decreased at the 3rd and 4th weeks of the INH treatment period (Fig. 1b). On the other hand, in the animal group treated with rutin prior to INH, the mean values of PCV were significantly higher than the corresponding values of animal group treated with INH alone at the 3rd ($p<0.01$) and 4th ($p<0.05$) weeks of treatment period.

The data obtained for the Hb content (Fig. 1c) revealed a highly significant reduction ($p<0.01$) in its mean values recorded for the INH-treated rats specially after elapsing of three and four weeks of treatment period. However, the mean values of the Hb content in (INH+rutin)-treated rats were significantly ($p<0.05$) higher than those recorded for rats treated merely with INH at the 3rd and 4th weeks.

ANOVA revealed significant differences among animal groups in RBC count ($F_{1,38} = 7.541$, $p<0.01$), PCV ($F_{1,38} = 14.008$, $p<0.001$) and Hb content ($F_{1,38} = 11.119$, $p<0.01$) due to INH administration. Also, significant variations ($p<0.05$) were observed in RBC count ($F_{1,38} = 4.434$) PCV ($F_{1,38} = 5.909$) and Hb content ($F_{1,38} = 4.805$) due to pretreatment with rutin before INH. No significant differences were recorded in any of the three parameters due to the administration of rutin alone.

Concerning the red cell indices, vis. MCV (Fig. 2a), MCH (Fig. 2b) and MCHC (Fig. 2c), data obtained did not show any significant changes in their mean values recorded for any of the three animal groups treated with INH alone, rutin plus INH or rutin alone throughout the four-week period of treatment. These results were further ensured by the ANOVA test, where there were no significant differences among the animal groups due to the different treatments.

The mean values of the Total Leukocyte Count (TLC) of the INH-treated rats were significantly ($p<0.05$) lower than the corresponding values of the control group particularly at the 3rd and 4th weeks of treatment period (Fig. 3a). Nevertheless, in the animal group treated with rutin, 1 h prior to INH, the

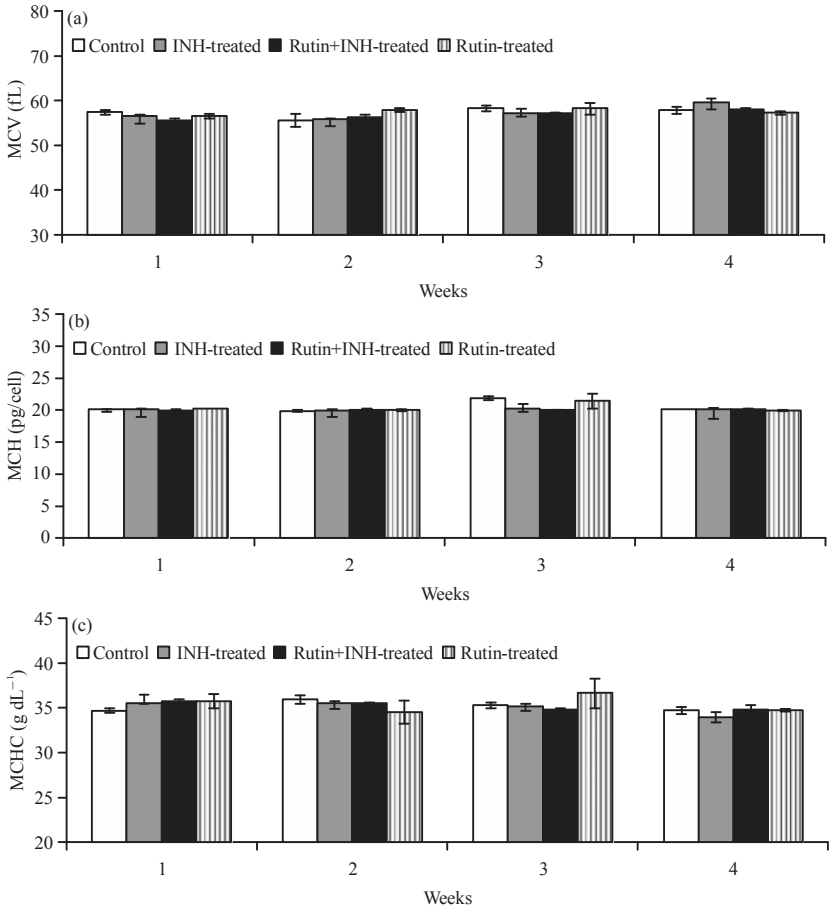

Fig. 2(a-c): Effect of daily oral administration of isoniazid (INH) (54 mg kg^{-1} b.wt.) and rutin (40 mg kg^{-1} b.wt.), 1 h prior to INH administration and alone, on red blood cell indices, (a) Mean corpuscular volume (MCV), (b) Mean corpuscular hemoglobin (MCH) and (c) Mean corpuscular hemoglobin concentration (MCHC) of male albino rats

Data are illustrated as Mean±standard error of mean (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. No significant variation was observed (p>0.01)

mean values of TLC were significantly higher than the corresponding values of INH-treated rats particularly at the 3rd ($p<0.01$) and 4th ($p<0.05$) weeks of treatment period. ANOVA test showed highly significant ($p<0.01$) variations in TLC between the animal groups due to the administration of INH alone ($F_{1, 38} = 8.688$) and rutin prior to INH ($F_{1, 38} = 10.425$).

The results of neutrophil count (Fig. 3b) revealed very highly significant decrements ($p<0.001$) in its mean values recorded for the INH-treated rats at the 3rd and 4th weeks of treatment duration. However, in the animal group treated with rutin prior to INH, the mean values of the neutrophil count were significantly ($p<0.001$) higher than the corresponding values of INH-treated rats also at the 3rd and 4th weeks. According to ANOVA test, the neutrophil count disclosed very highly significant differences ($p<0.001$) between animal groups due to the INH treatment ($F_{1, 38} = 18.918$) and the pretreatment with rutin ($F_{1, 38} = 17.861$).

The data obtained for the eosinophil count (Fig. 3c) revealed significant decrements in its mean values recorded for INH-treated rats at the 3rd ($p<0.05$) and 4th ($p<0.01$) weeks of treatment period. In animals treated with rutin before INH, eosinophil counts were significantly higher than those of INH-treated animals at the 1st ($p<0.05$) and 4th ($p<0.01$) weeks of experiment. ANOVA test displayed very highly significant variations ($p<0.001$) between animal groups due to the administration of INH alone ($F_{1, 38} = 14.645$) and due to the pretreatment with rutin before INH ($F_{1, 38} = 13.088$).

As regards the lymphocyte count (Fig. 4a), the results showed a significant reduction ($p<0.05$) in its mean values

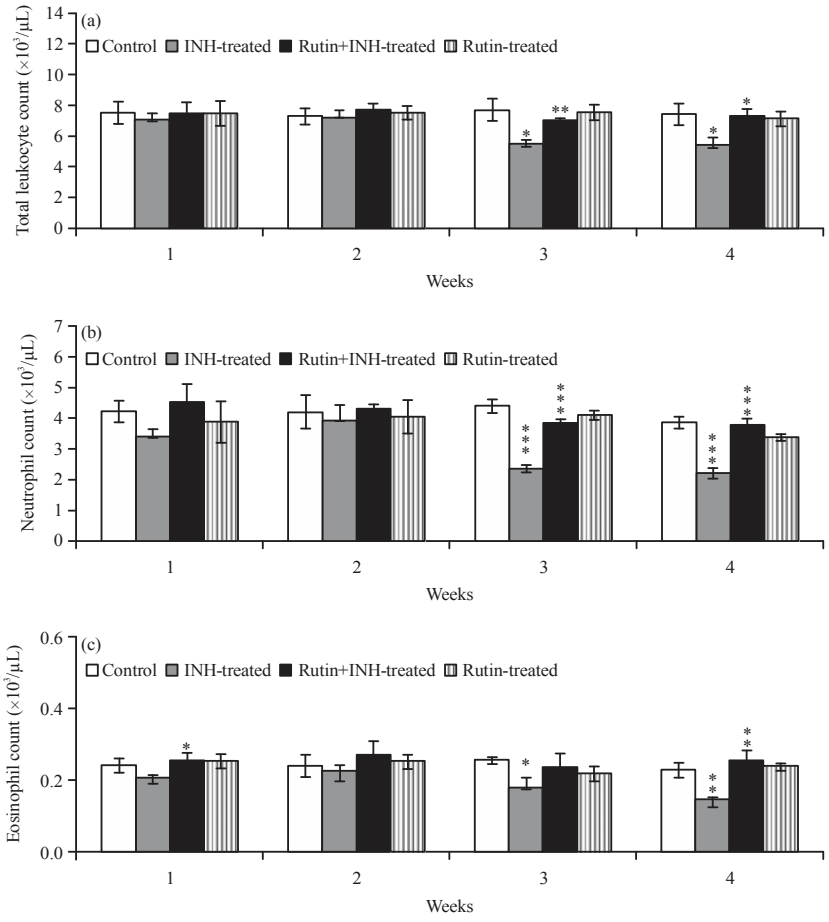

Fig. 3(a-c): Effect of daily oral administration of isoniazid (INH) (54 mg kg^{-1} b.wt.) and rutin (40 mg kg^{-1} b.wt.), 1 h prior to INH administration and alone, on (a) Total leukocyte count and absolute counts of (b) Neutrophils and (c) Eosinophils of male albino rats

Data are illustrated as Mean±standard error of mean (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 ($p<0.001$)

recorded for the INH-treated group at the 3rd and 4th weeks of treatment period. However, in the animal group treated with rutin prior to INH administration, the mean value of lymphocyte count recorded at the 4th week was significantly ($p<0.01$) higher than that of the animal group treated with INH alone. The results obtained for monocyte count (Fig. 4b) did not show any significant changes in its mean values due to the administration of INH alone or rutin before INH. In general, neither TLC nor differential leukocyte counts were altered in rats administered rutin alone.

Biochemical study: The results of the biochemical parameters investigated in the present study are graphically presented in the Fig. 5-6. Regarding serum glucose concentration (Fig. 5a), the data obtained revealed significant decrements in its mean values recorded for the INH-treated rats at the 3rd ($p<0.05$) and 4th ($p<0.01$) weeks of drug administration period. On the other hand, the mean values of serum glucose concentration of the animal group treated with rutin prior to INH treatment were significantly higher than the corresponding values of INH-treated rats at the 3rd ($p<0.05$) and 4th ($p<0.01$) weeks of experimental period. According to ANOVA test, very highly significant variations ($p<0.001$) were observed in serum glucose concentration between the animal groups owing to the treatment with INH ($F_{1, 38} = 19.368$) and the pretreatment with rutin before INH administration ($F_{1, 38} = 14.171$).

With respect to the serum TCh concentration (Fig. 5b), the results showed a significant elevation in its mean value recorded for the INH-treated group at the 4th ($p<0.05$) week of the trial period. Still, in the animal group treated with rutin

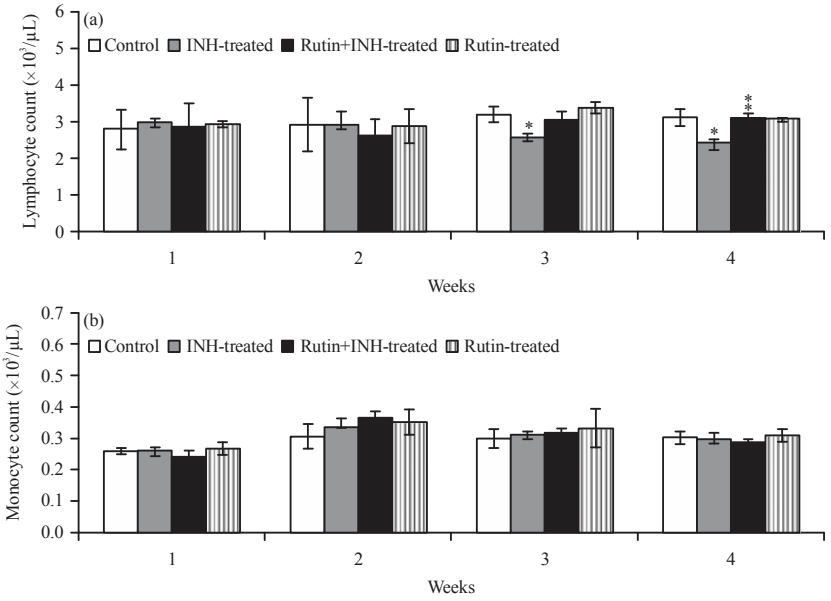

Fig. 4(a-b): Effect of daily oral administration of isoniazid (INH) (54 mg kg^{-1} b.wt.) and rutin (40 mg kg^{-1} b.wt.), 1 h prior to INH administration and alone, on absolute counts of (a) Lymphocytes and (b) Monocytes of male albino rats

Data are illustrated as Mean±standard error of mean (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) and **highly significant (p<0.01)

prior to INH, the mean value of serum TCh concentration, recorded at the last time interval of the treatment period, was significantly (p<0.01) lower than the corresponding value of the animal group treated with INH alone. ANOVA test disclosed significant variations in serum TCh concentration between animal groups due to the daily administration of INH alone ($F_{1,38}$ = 18.613, p<0.001) and the treatment with rutin1h prior to INH administration ($F_{1,38}$ = 11.217, p<0.01).

The data presented in Fig. 5c revealed that the mean values of serum TG concentration were significantly (p<0.05) increased in the INH-treated group at the 2nd, 3rd and 4th weeks of experimental period. In the animal group treated with rutin before INH, the mean values of serum TG level were significantly (p<0.05) lower than the corresponding values of INH-treated rats at the 3th and 4th weeks of experimental period. By applying the ANOVA test, significant differences were recorded in serum TG concentration between the animal groups owing to the INH administration ($F_{1,38}$ = 15.124, p<0.001) and the pretreatment with rutin prior to INH ($F_{1,38}$ = 6.481, p<0.05). The rats administered rutin alone did not show any significant variation in the concentrations of glucose, TCh or TG.

With respect to the serum urea and creatinine concentrations, the results obtained did not show any significant changes (p>0.05) in their mean values in response to the administration of the INH alone or rutin prior to INH or alone at any of the time intervals of treatment (Fig. 6a and b). These results were ensured by applying the ANOVA test.

The mean values of serum uric acid concentration were significantly increased in the INH-treated rats at the 2nd (p<0.001), 3rd (p<0.01) and 4th (p<0.001) weeks of the trial period as compared with the control values (Fig. 6c). Yet, in rats treated with rutin prior to INH, the mean values of serum uric acid were significantly lower than those of INH-treated rats at the 2nd (p<0.001), 3rd (p<0.05) and 4th (p<0.01) weeks of treatment period. ANOVA test exhibited significant differences in serum uric acid concentration between animal groups because of the treatment with INH alone ($F_{1,38}$ = 48.596, p<0.001) and rutin prior to INH ($F_{1,38}$ = 23.629, p<0.001). In the animal group administered rutin, no significant variation was recorded in serum uric acid concentration through the experimental period.

DISCUSSION

Data obtained in the present investigation revealed that the daily administration of isoniazid to the male Sprague-Dawley rats, at a dose equivalent to a high human therapeutic dose caused a normocytic normochromic anemia as RBC count, Hct and Hb content were decreased without changes

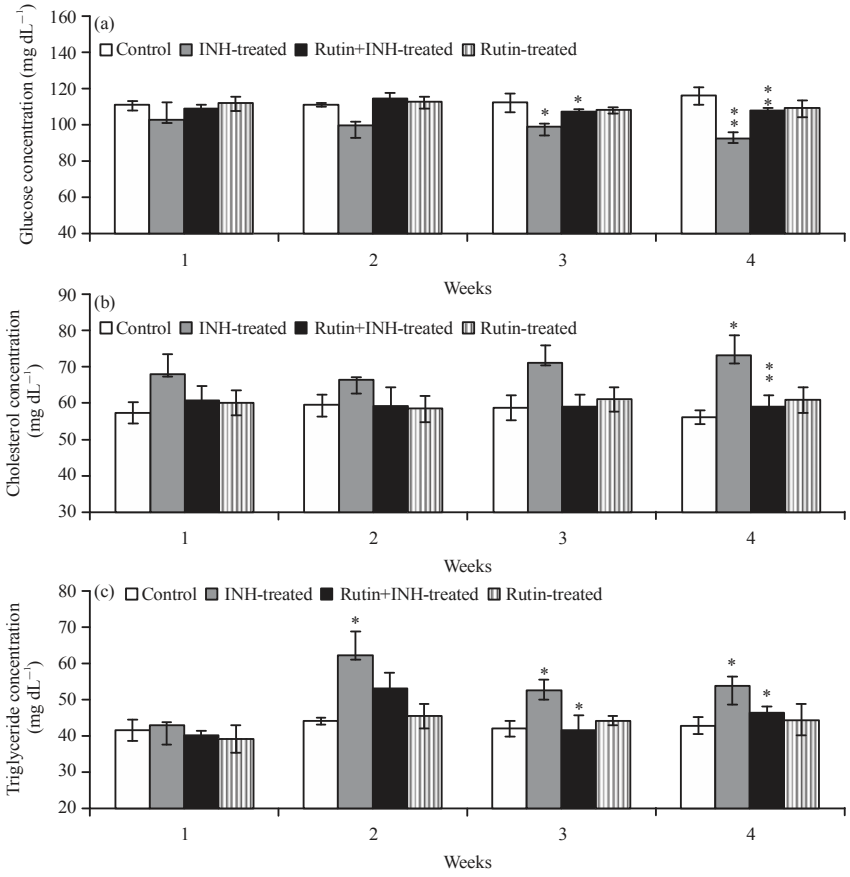

Fig. 5(a-c): Effect of daily oral administration of isoniazid (INH) (54 mg kg^{-1} b.wt.) and rutin (40 mg kg^{-1} b.wt.), 1 h prior to INH administration and alone, on the concentrations of (a) Serum glucose, (b) Total cholesterol and (c) Triglycerides of male albino rats

Data are illustrated as Mean±standard error of mean (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 ($p<0.05$) and **highly significant ($p<0.01$)

in MCV, MCH and MCHC. Etiologically, two major forms of anemia have been reported as adverse reactions of the isoniazid administration, pure red cell aplasia (PRCA)[6,26] and sideroblastic anemia[7,27]. PRCA is a syndrome characterized by a normocytic normochromic anemia with severe reticulocytopenia and marked reduction or absence of erythroid precursors from the bone marrow[28] and sideroblastic anemia is characterized by deficient heme synthesis and an increase of ring sideroblasts in bone marrow[7]. It has been reported that isoniazid-induced PRCA is usually reversible after the discontinuation of the drug[26]. In addition, sideroblastic anemia was rapidly resolved after cessation of isoniazid[7].

Regarding the INH-induced PRCA, Loulergue *et al.*[6] indicated that the exact mechanism of INH-induced PRCA remains unclear, but the demonstration of antibodies reacting with nucleated RBCs in about 50% of cases suggests an induction of autoimmunity. Also, INH-induced sideroblastic anemia was attributed to the inhibition of pyridoxine which acts as a co-factor in the synthesis of δ-aminolevulinate[7]. Recently, Fratz-Berilla *et al.*[27] indicated that INH can cause sideroblastic anemia by limiting pyridoxal 5'-phosphate (PLP) availability to human erythroid 5-aminolevulinate synthase (hALAS2), via inhibition of pyridoxal kinase or reaction with pyridoxal to form pyridoxal isonicotinoyl hydrazone. In addition, they hypothesized that INH also binds and directly inhibits hALAS2.

In the present study, the daily administration of rutin1h prior to INH administration for four weeks remarkably ameliorated the magnitude of anemic status observed in animal group treated with INH alone. This appeared in the improvement of RBC count, Hb content and PCV values of rutin+INH-treated rats as compared with those values of INH-treated animals. It seems likely that rutin protected the mature erythrocytes as well as the erythropoietic tissues and factors

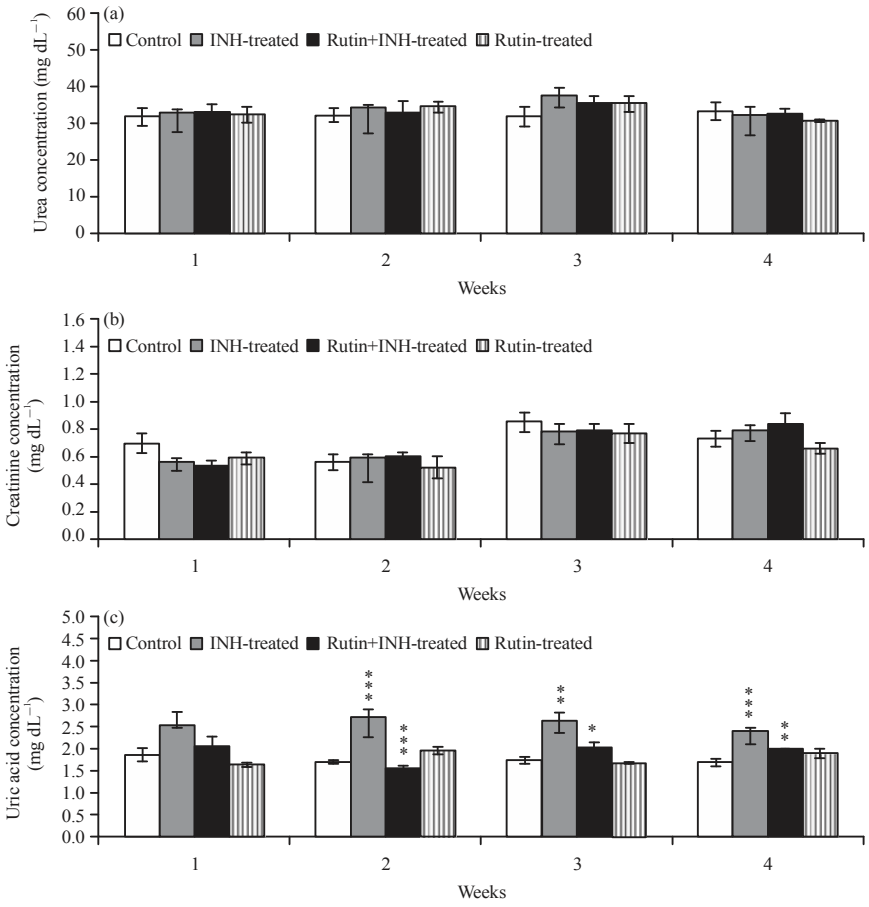

Fig. 6(a-c): Effect of daily oral administration of isoniazid (INH) (54 mg kg^{-1} b.wt.) and rutin (40 mg kg^{-1} b.wt.), 1 h prior to INH administration and alone, on the concentrations of (a) Serum urea, (b) Creatinine and (c) Uric acid of male albino rats

Data are illustrated as Mean±standard error of mean (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 ($p<0.001$)

against the oxidative stress induced by isoniazid or its metabolites. In different *in vitro* studies, rutin was found to protect erythrocytes against oxidative damage and hemolysis induced by free radical initiators[29,30]. Moreover, the hemolysis induced by the addition of Fe^{2+} to human RBCs was prevented by the pretreatment with rutin, being acting as a radical scavenger[31].

The results obtained in the present work displayed a noticeable reduction in the mean values of the WBC count of INH-treated animals throughout the period of INH administration. This INH-induced leukopenia was a direct consequence of the decrease in the absolute counts of neutrophils, eosinophils and lymphocytes herein observed in INH-treated rats. Yet, the monocyte count did not significantly change. In the same connection, leukopenia has been recorded in patients treated with anti-TB chemotherapy including RIF and INH[32,33]. Moreover, in patients suffering from TB and treated with anti-TB drugs including INH, neutropenia has been detected[34,35].

The acute inflammatory response is consistently marked by microvascular leakage and the accumulation of neutrophils and the chronic inflammation is characterized by the recruitment of mononuclear cells including lymphocytes, monocytes and plasma cells[36].It has been reported that INH can cause hepatotoxicity in 20% of patients that is usually associated with an inflammatory response[10].In view of the massive infiltration of leukocytic inflammatory cells, observed in the histopathological part of our previous study[17], in between the hepatocytes as well as in the portal area of the INH-treated rats, the infiltration of leukocytes to the inflamed tissue seems the most causative factor standing behind the decrease in leukocyte count in the circulation. In this

connection, it has been reported that the administration of INH plus RIF and PZA to rats was associated with inflammatory infiltrations in the liver[37]. In INH-treated patients, bronchoalveolar lavage fluid revealed a high proportion of neutrophils, lymphocytes[38] and eosinophils[4] and histological examination of biopsied lung tissue showed lymphocyte and eosinophil infiltrations, respectively. Similarly, in a patient treated with anti-TB drugs including INH, hypersensitivity pneumonia developed, but the drug-lymphocyte stimulation test was only positive for INH indicating that pneumonia was induced by INH[39].

In the present work, the results showed that the INH-induced decrements in TLC as well as in the neutrophil, eosinophil and lymphocyte counts were significantly ameliorated ($p<0.05$) in animal group treated with rutin prior to INH administration. This finding could probably reflect the antichemotactic and anti-inflammatory roles of rutin against the inflammatory effect of INH upon tissues and leukocytes. In this respect, different studies ensured the anti-inflammatory properties of rutin[40,41]. The results of Jung *et al.*[42] indicated that rutin could be useful in the treatment of immediate phase response and late phase response in asthma via inhibition of histamine, release of phospholipase A2 and peroxidase and reduced recruitment of neutrophils and eosinophils into the lung. Using a chemotactic stimulant in an *in vitro* study, Selloum *et al.*[40] reported that rutin reduced significantly polymorphonuclear (PMN) neutrophils chemotaxis to the stimulant. Also, Suyenaga *et al.*[43] used the same chemotactic stimulant to evaluate 25 flavonoids and they found that ten flavonoids, including rutin, significantly retarded the migration of PMNs.

Most of the biochemical parameters studied in the present work were remarkably altered in the animal group treated with INH alone daily for four weeks. With respect to the serum glucose concentration, data obtained revealed a significant reduction ($p<0.05$) in its mean values recorded for the INH-administered animals. In accordance with this result, hypoglycemia has been recorded in patients treated with isoniazid alone[44] or in combination with RIF and propranolol[45] or with glimepiride in a patient with type 2 diabetes mellitus[46]. Interestingly, severe hypoglycemia was recorded in a premature infant born to a woman with active tuberculosis and treated with isoniazid[47].

The mechanism of INH-induced hypoglycemia is not exactly known, but hyperinsulinemia and/or inhibited activity of certain enzymes in carbohydrate metabolism could probably account for INH-induced hypoglycemia. In this connection, Villaume *et al.*[44] recorded clinical symptoms of hypoglycemia in an INH-treated woman, but after the cessation of anti-TB medication, a moderate hypoglycemia, without clinical manifestations, concurrent with a noticeable hyperinsulinemia and normal C-peptide values was recognized. This hyperinsulinemia was attributed to a deficient catabolism of insulin. In another point of view, Boglou *et al.*[46] ascribed hypoglycemia induced after administration of INH to glimepiride-treated diabetic patient to INH-induced increase in serum glimepiride concentration resulting in hyperinsulinemia. Regarding the second probable causative factor standing behind INH-induced hypoglycemia, Saraswathy and Devi[48] found that the activity of hepatic glucose-6-phosphatase significantly decreased in rats treated with INH with RIF and PZA, for six weeks. Moreover, in an *in vitro* study, Xu and Purcell[49] reported that the exposure of hepatic spheroid culture to INH remarkably decreased the release of glucose.

In the animal group treated with rutin before INH, the level of serum glucose was within the normal range being higher than that of INH-treated rats. This may indicate the beneficial protective effect of rutin against the INH-induced alteration in glucose metabolism particularly in hepatic tissue. In fact, most studies concentrated on the anti-hyperglycemic effect of rutin, but they attributed this effect to the antioxidative and protective role of rutin against the diabetogenic effect of streptozotocin (STZ)[50,51]. So, as rutin plays a good role in the protection of tissues against oxidative damage, it could improve the glucose homeostasis by improving the alteration induced in its release or uptake. In this respect, Prince and Kamalakkannan[50] indicated that rutin improves glucose homeostasis in STZ diabetic rats by protection of pancreatic tissue and restoration of plasma glucose and insulin levels, liver and muscle glycogen content and activities of carbohydrate metabolic enzymes.

The results obtained in the present study exhibited a significant elevation ($p<0.05$) in the concentrations of serum TCh and TG. The rise in TCh level started at the fourth week while TG level increased significantly ($p<0.05$) from the second week of INH administration period. In rats orally administered INH and RIF, the levels of serum and liver cholesterol, TG and free fatty acids (FFA) were significantly increased[52]. In addition, in Wistar rats administered INH alone or with other anti-TB drugs (RIF or RIF+PZA), hyperlipidemia and hypercholesterolemia were evidenced[53]. The hyperlipidemic effect of INH herein observed seems probably a consequence of the oxidative stress and lipid peroxidation (LPO) in the hepatic tissue. The INH-induced LPO reflected by increased liver MDA level could be evidenced in our previous study[17] and in other studies[54,55]. Metabolism of INH leads to the production of HZ via both direct and indirect pathways, in which the

activity of an amidase is required to hydrolyze an amide bond[56]. So, the role of HZ in lipid alterations and hepatotoxicity has been proved by the study of Sarich *et al.*[56], who treated rabbits with the amidase inhibitor bis-p-nitrophenyl phosphate 30 min before injection of INH and they found that the formation of INH-derived HZ was inhibited and the hepatic TG accumulation and the hypercholesterolemia were decreased.

The data obtained in the present study showed that the administration of rutin1h before INH administration ameliorated the INH-induced hypercholesterolemia and hypertriglyceridemia. Yet, neither serum TCh level nor TG concentration significantly change in animal group treated with rutin alone. These results could indicate the beneficial antioxidative role of rutin against the INH-induced dyslipidemia. In the study of Da Silva *et al.*[57], the hypocholesterolemic effect of rutin was ensured where rutin reduced the levels of TCh, low density lipoprotein cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C) and TG. By the use of cupper-mediated oxidation, Milde *et al.*[58] found that rutin alone protected LDL against oxidation. Using the high fat diet (HFD)-induced dyslipidemia, Hsu *et al.*[59] evidenced that supplementation of HFD with rutin resulted in a significant decrease in hepatic triacylglycerol (TAG) and cholesterol levels and they concluded that rutin can be beneficial for the suppression of HFD-induced dyslipidemia, hepatosteatosis and oxidative stress. In addition, it has been reported that rutin had a lowering effect on plasma TCh and LDL-C of hypercholesterolemic rats[60] and plasma TG levels of hypercholesterolemic Golden Syrian hamsters[61]. Moreover, the STZ-induced dyslipidemia in rats was markedly improved by the administration of rutin[50,51]. Prince and Kamalakkannan[50] attributed the beneficial effects of rutin on lipids, lipoproteins, lipid metabolizing enzymes and glycoproteins to the antioxidant property.

With respect to the biochemical parameters related to the renal function, the results recorded in the present study revealed that neither serum urea concentration nor serum creatinine level was altered in animal group daily treated with INH for four weeks. This could indicate that INH at its chosen dose and duration of treatment did not alter the renal function in male albino rats. However, the data of serum uric acid recorded in INH-treated rats showed a remarkable elevation in its mean values. Coincident with the present results, Sud *et al.*[62] found that with the administration of INH (concomitant with cyclosporine) to seven renal transplant recipients, the renal function remained constant up to 4 weeks. In eleven newly diagnosed TB-patients and treated with INH-containing regimen for 14 days, renal function was normal before and during the investigation[63]. On the other hand, the increased serum uric acid concentration or hyperuricemia has been observed in TB patients treated with anti-TB drugs including isoniazid[64-66].

In view of the unaltered renal function in INH-treated rats, the INH-induced hyperuricemia could be related to an alteration in the activity of certain enzymes in purine metabolism. Xanthine oxidoreductase (XOR) is a ubiquitous complex cytosolic molybdenum flavoprotein which controls the rate-limiting step of purine catabolism by converting xanthine to uric acid[67]. So, it could be conceivable that Yilmaz *et al.*[68] reported increased activity of erythrocytic xanthine oxidase (XO) of rats injected with INH. Moreover, when Wakabayashi *et al.*[69] treated rats with HZ, the activity of hepatic XO was increased.

The hyperuricemia herein recorded in INH-treated rats was markedly alleviated in animal group treated with rutin prior to INH administration. Yet, no significant change was observed in rats treated with rutin alone. In accordance with this finding, rutin has been found to elicit an anti-hyperuricemic effect in oxonate-induced hyperuricemic mice[70] as well as in fructose-induced hyperuricemic rats[71]. It seems possible that the anti-hyperuricemic effect of rutin is due to its inhibitory effect upon INH-induced activation of XO enzyme. In this connection, Zhu *et al.*[70] tested rutin on mice liver homogenates and they found significant inhibitory action on xanthine dehydrogenase/xanthine oxidase (XDH/XO) activities. In the *in vitro* part of the study of Chen *et al.*[72], caffeic acid, resveratrol, rutin and oxyresveratrol isolated from ethyl acetate fraction of *Similax china* L. showed different inhibitory activities on XO. So, it has been reported that the antioxidant activity and the inhibitory activity of several substances including rutin were promising to continue *in vivo* hypouricemic studies[73].

CONCLUSION AND FUTURE RECOMMENDATION

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 hematological and biochemical criteria. However, the pretreatment of rutin before INH administration prevented or at least ameliorated the INH-induced alterations. In addition, the administration of rutin alone did not alter any of the parameters studied.

This study suggests further studies to evaluate the co-administration of rutin with other medications known with oxidative stress-induced side effects. In addition, recent techniques and sophisticated methods are required, from different points of view, to uncover the exact mechanisms of oxidant-antioxidant interaction.

SIGNIFICANCE STATEMENT

This study will help the researchers to uncover the mechanisms accounting for rutin ameliorating effect against drugs-induced hematological and metabolic alterations. 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.

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