Effect of Morin on Isoniazid and Rifampicin Induced Hepatotoxicity in Rats

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Abstract

The first line drugs used for tuberculosis therapy are isonizid and rifampicin (INH &RIF) and they are associated with hepatotoxicity, for this reason we aimed to study the protective effect of morin on the hepatotoxicity induced by the tow antituberculosis agents given together.

To reach study object 24 female Wistar albino rats 220-250 g were divided into four groups, each group consisted of six animals received standard diet and tab water along the 21 days which is the experimental period. The control groupleaved without treatments while the morin group, rats were treated with 30mg/kg/day morin via gavage along the days of the experiment. INF-RIF group, rats were treated with 100 mg/kg/day INH-RIF by intraperitoneal injection method for 21 days. INF-RIF and morin group, rats were treated with 100 mg/kg/day INH-RIF plus morin with a dose of 30 mg/kg /day via gavage along the experimental period.

The results had a significant increase in the activity of alanine aminotransferase, aspartate aminotransferase and malondialdehyde level and significant decrease in the activity of superoxide dismutase, glutathione peroxidase, glutathione s-transferase and catalase in animal groups treated with INH and RIF as compared to control groups. However, supplementation of INH-RIF intoxicated rats with morin ameliorated the antituberculosis drugs adverse effects as evidenced by a significant decrease in alanine aminotransferase, aspartate aminotransferase activity and malondialdehyde level and significant increase in superoxide dismutase, glutathione peroxidase, glutathione s-transferase and catalase. We concluded that morin have a potential to protect the liver from INH and RIF toxicity.

اثر المورين على السمية الكبدية الناتجة من الايزونيزايد والرفامبيسين في الجرذان

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الملخص

يعتبر الايزونيزايد و الرفامبسين من المضادات الحيوية التي تستخدم بشكل اساسي في علاج السل الرئوي ولكن هذه المضادات تكون مصاحبة لسمية في الكبد لذلك خطط لهذه التجربة لدراسة اثر المورين على الكبد كعنصر واقي لسمية هذه المضادات. في هذه التجربه كانت لدينا اربعة مجاميع احتوت كل مجموعة على ستة جرذان من نوع الوستر الابيض بوزن 220 الى 250 غرام. المجموعة الضابطة استلمت الماء و الغذاء المعياري فقط لمدة 21 يوم، المجموعة الثانية اعطيت المورين عن طريق الانبوب المعوي بجرعة 30 ملغ/ كغم من وزن الجرذ بالاضافة الى الغذاء المعياري و الماء على طول ايام التجربة ، المجموعة الثالثة حقنت بخليط المضاد الحيوي بجرعة 100ملغ/كغم من وزن الجرذ و اعطية المورين عن طريق الانبوب المعوي بجرعة 30 ملغ/ كغم من وزن الجرذ بالاضافة الى الغذاء المعياري و الماء لكل ايام التجربة الواحد و العشرين . اظهرت النتائج على ارتفاع مستوى فعالية انزيمات ALT و AST و تركيز MDA و انخفاض معنوي في فاعلية كل من الانزيمات المضادة للاكسدة التالية CAT , GST , $GSH\text{-}_{PX}$, SOD للمجموعة التي استلمت خليط المضادات الحيوية بالمقارنة مع المجموعة الضابطة، بينما تميزت المجموعة المعطاة لمادة المورين بانخفاض اثر المضادين الحيويين السلبي على الكبد حيث انخفضت فعالية الانزيمين ALT و AST و كذلك انخفض تركيز MDA و ارتفع كل من انزيمات CAT , GST , $SOD,GSH\text{-}_{PX}$, مما يدل على ان مادة المورين تملك خواص وقائية لسمية الكبد الناتجة من المضادين الحيويين.

Introduction

Tuberculosis (TB) is one of the most common infectious disease[1]. Caused by infection with *Mycobacterium tuberculosis*, about 2 million people kills each year by tuberculosis[2,3] .The first line drugs used for tuberculosis therapy are isoniazid (INH) and rifampisin (RIF) and associated with hepatotoxicity. Adverse effects of antitubercular therapy are sometimes potentiated by multiple drug regimens. Thus, though INH, RIF each in itself are potentially hepatotoxic, when given in combination their toxic effect is enhanced. The conversion of monoacetyl hydrazine, a metabolite of INH, to a toxic metabolite via cytochrome P450 leads to hepatotoxicity. Patients on concurrent rifampicin therapy have an increased incident of hepatitis. This has been postulated to be due to rifampicin-induced cytochrome P450 enzyme-induction, causing an increased production of toxic metabolites from acetyl hydrazine (AcHz) [4-9]. Oxidative stress as one of the mechanisms for INH-RIF induced hepatic injury[10].

Flavonoids are a group of polyphenolic compounds, which are widely distributed through-out the plant kingdom. There are about more than 3000 varieties of flavonoids are known.Flavonoids exhibit several biological effects such as anti-inflammatory, hepatotoxic and anti-ulcer actions. Scavenging of free radicals seems to play a considerable part in the antioxidant activity of flavonoid compound. Many have anti-allergic, antiviral actions and some of them provide protection against cardiovascular mortality. They have been shown to inhibit the growth of various cancer cell lines in vitro, and reduce tumour development in experimental animals[11-19].

Morin (3, 5, 7, 2', 4'- pentahydroxyflavone; a yellowish pigment) is a type of flavonoid belonging to the group of flavonols. Morin have antioxidant,

antimutagenesis, cardioprotective, antimutagenesis, xanthine oxidase inhibitor, protein kinase C inhibitor and cell proliferation inhibitor, cytoprotection, anticarcinogenesis, antiinflammatory, effects[20-24]. It was found that morin could modulate the activities of the metabolic enzymes, including cytochrome P450[25] . And it is also an antioxidant that protects various human cells, like endothelial cells, hepatocytes and erythrocytes, against oxidative damages [26,27]. Moreover, that morin could exert a significant chemopreventive effect on colon carcinogenesis induced by 1, 2-dimethylhydrazine[28]. Morin increases the bioavailability of tamoxifen and its main metabolite, 4-hydroxytamoxifen in rats [29].

The aim of the present work was to study the protective effect of morin on the hepatotoxicity produced by the tow antitubercular agents given together.

Materials and Methods

Drugs and Chemicals

Isoniazid and rifampicin were purchased from local pharmacy and prepared separately in sterile distilled water. Rats were treated with isoniazid (100 mg/kg, ip) and co-administered with rifampicin (100 mg/kg, ip), for 21 days [30 ,31]. Morin was purchased from Sigma Chemical Company, St. Louis, MO, USA. and was given orally by intra gastric tube (gavage) at a dose of 30 mg/kg as described[32].

Animals

In the experiment, we used 24 Wistar albino 220-250 g female rats which were housed in wire bottom cages, all rats housed in an air conditioned room at 30±3°C, and maintained on a tab water and standard diet *ad libitum* with a 12 h light/ dark cycle for 21 days. The animals were divided into 4 groups. Each group consisted of six animals.

Group 1 (CONTROL): control animals leaved without treatment for 21 days.

Group 2 (MORIN): rats were treated with 30mg/kg/day morin via gavage along the days of the experiment.

Group 3 (INF-RIF): rats were treated with 100 mg/kg/day INH-RIF via intraperitoneal method for 21 days.

Group 4 (INH-RIF & MORIN): Rats were treated with intraperitoneal 100 mg/kg INH-RIF plus morin 30mg/kg/day dose via gavage.

At the end of the treatment, the animals were fasted 24 h and sacrificed by decapitation, blood samples were collected, serum was separated from coagulant blood by centrifugation at 860g for 20 min, and then frozen at -20°C for biochemical analysis. The liver was dissected out, washed with chilled physiological saline, weighted, homogenized in 0.1M Tris HC1 buffer (pH 7.4) at 4ºC in potter Elvejem homogenizer, and then used for the evaluation.

Histopathological studies

The livers were excised and fixed in 10% formalin and stained with haemotoxylin and eosin and then observed under microscope for histopathological changes.

Biochemical Estimation

Alanine aminotrasferase (ALT) and aspartate aminotransferase (AST) activities were estimated according to Reitman and Frankel methods[33]. Malondialdehyde (MDA) was estimated according to Van methods[34]. Superoxide Dismutase (SOD) activities were estimated according to Durak *et al* methods[35] .Whereas glutathione peroxidase was estimated according to Paglia methods[36]. Catalase was estimated as described by Aebi[37]. Glutathione S transferases activities was measured as previously reported by Habig [38].

Statistical analysis

The data was analyzed using the Statistical Package for Social Science program (SPSS 12). For comparison between different experimental rat groups, one way analysis of variance (ANOVA) was used followed by Tukey's test. The results were expressed as means ± MSE and $P < 0.05$ was considered to be statistically significant.

Results

There was a significant increase in the activity of ALT and AST in animal groups treated with INH-RIF as compared to control groups. However, supplementation of INH-RIF intoxicated rats with morin ameliorated the antitubercular drugs adverse effects as evidenced by a significant decrease in ALT and AST activity as shown in Table 1.

As shown in table 2, administration of antitubercular drugs INH-RIF,100 mg / kg / day for 21 days, caused a significant increase in the level of MDA with significant decrease in the activities of antioxidant enzymes (GPX, GST,CAT and SOD) in liver of experimental groups of rats as compared to those normal control animals. Oral administration of morin 30 mg/kg/day for 21 days significantly decreased these antituberculosis drugs-induced adverse effects and maintained the rats at near normal status.

Animals treated with INH-RIF (100 mg/kg i.p.) for 21 days showed necrosis, and fatty degeneration in liver (Figure 3). This effect was relatively decreased in animals pretreated with morin (Figure 4). However histopathological changes were not observed on animals treated with morin (Figure 2) when compared with the control group (Figure 1) .

Table 1: The serum aminotransferase of the animal groups treated with antitubarcalosis drugs (isoniazid INH, rifampicin RIF), morin and antitubarcalosis co administrated with morin.

Parameters	CONTROL	INH & RIF 100mg/kg	MORIN 30mg/kg	INH-RIF 100mg/kg & MORIN 30mg/kg
ALT IU / L	20.83 ± 0.60^{a}	60.33 ± 1.45^{c}	21.17 ± 0.48^{a}	27.83 ± 2.57^{b}
AST IU / L	47.33 ± 1.31^{a}	242.00 ± 2.79^{c}	47.33 ± 1.15^{a}	93.67 ± 2.16^{b}

Values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, $P<0.05$

Table 2: the MDA and antioxidant enzymes parameters in hepatic tissue of the animal groups treated with antitubarcalosis drugs (isoniazid INH, rifampicin RIF), morin and antitubarcalosis co-administrated with morin.

Parameters	CONTROL	INH & RIF	MORIN	INH & RIF & MORIN
MDA nmol / mg P	11.66 ± 0.84^{ab}	21.62 ± 0.93^{c}	9.44 ± 0.50^{a}	13.65 ± 1.22^{b}
SOD U / mg P	2.42 ± 0.30^{b}	0.80 ± 0.04^{a}	2.54 ± 0.28^{b}	2.23 ± 0.12^{a}
CAT IU / mg P	5342.33 ± 187.23^{b}	4034.00 ± 18.43^{a}	5351.33 ± 36.60^{b}	5041.50 ± 69.96^{b}
GPX IU / mg P	1.008 ± 0.052^{b}	0.215 ± 0.009^{a}	0.977 ± 0.044^{b}	0.868 ± 0.027^{b}
GST IU / mg P	0.863 ± 0.023^{b}	0.292 ± 0.040^{a}	0.895 ± 0.047^{b}	0.810 ± 0.059^{b}

Values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, $P<0.05$

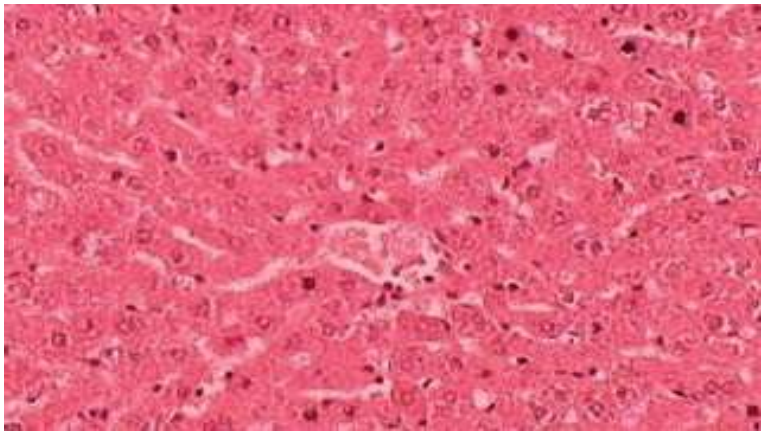

Figure (1) Liver section from a control rat showing normal morphology.

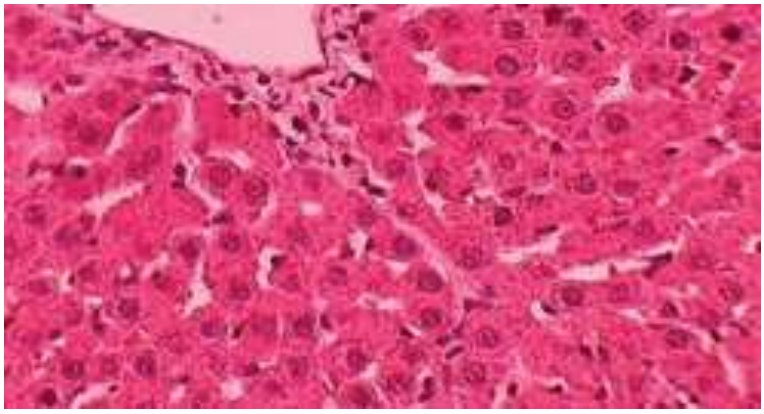

Figure (2) Liver section from rat treated with morin (30mg/kg/ day for 21 days) showing normal morphology when compared with control rat.

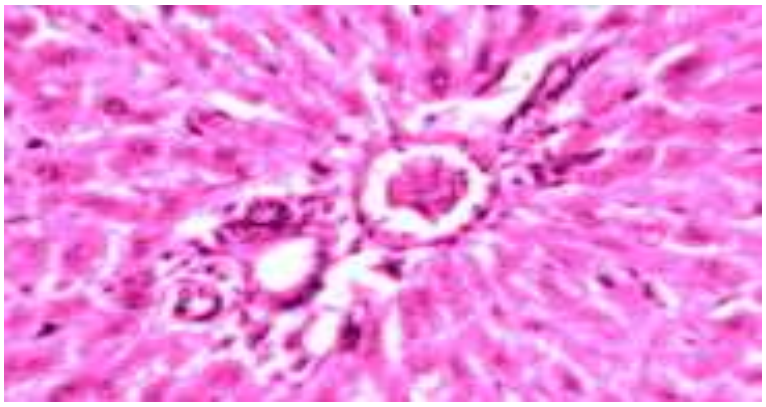

Figure (3) Liver section from rat treated with antitubarcalosis drugs (isoniazid INH, rifampicin RIF) 100mg/kg/ day for 21 days) showing necrosis and fatty degeneration.

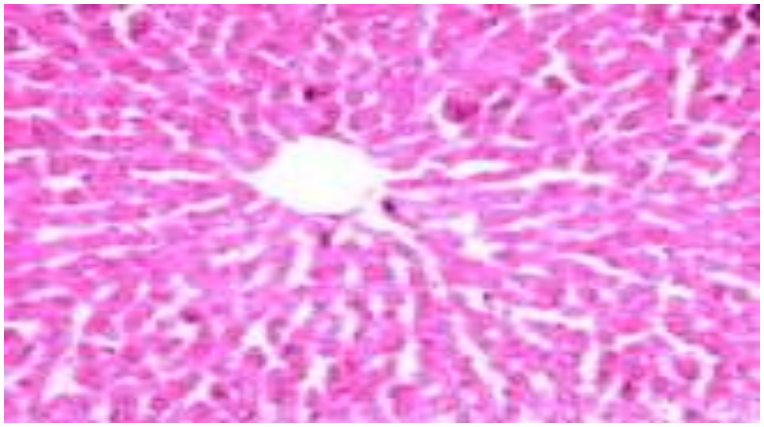

Figure (4) Liver section from rat treated with morin (30mg/kg/ day for 21 days) prior to and during treatment antitubarcalosis drugs (isoniazid INH, rifampicin RIF) 100mg/kg/ day for 21 days) shown reduction of necrosis and fatty degeneration when compared with animals treated with INH, RIF alone.

Discussion

This study aimed to evaluate the protective effects of morin against INF and RIF-induced hepatotoxicity in rats. INF and RIF treatment causes significant increase in the serum activity of liver function markers such as ALT and AST as compared to control indicating hepatic dysfunction. These injuries may be due to the production of free radicals and involvement of oxidative stress to hepatic toxicity caused by INF and RIF treatment.

The results from this study confirmed that INH and RIF at a dose of 100 mg/kg given i.p produces significant hepatotoxicity as evidenced by increase in serum AST and ALT. This elevation AST and ALT serum because of their cytoplasmic nature and are thus released in blood by changing in the permeability of hepatocyte membranes[39].This results obtained in this study are agree with other reports[40]. However, administration of morin along with INF and RIF caused significant decrease in AST and ALT suggested the protective effects of morin.

The present study shows that MDA levels in the liver were significantly higher in the INH and RIF-induced group when compared to the control group. This increase due to oxidative stress and lipid peroxidation (LPO). MDA is used commonly as an indicator of lipid peroxidation [41]. Lipid peroxidation refers to a process that causes polyunsaturated fatty acid to turn rancid and is related to many pathological processes which lead to cancer, degenerative disease, and other diseases . Lipid peroxidation initiators are reactive oxygen species (ROS) such as hydroxyl (OH•) and peroxyl radicals (ROO•) and the superoxide anion radicals (O2•-), which are formed by exogenous chemicals factors and endogenous metabolic processes in the human body or in food systems[42]. In recent years, a number of studies have showed that INH and RIF administration increases lipid peroxidation in the rat liver[9,40]. However, adminstration of morin along with INH and RIF caused significant decrease in MDA levels suggested the protective effects of morin.

Morin has been shown to exert a potent scavenging action on super oxide anion and hydroxyl anion *in vitro,* as well as the protective effect against lipid peroxidation , polyphenols compounds have the ability to protect cell from oxidative stress, it have both antioxidant and prooxidant properties, depending on the concentration and free radical source[26,27]. This results agree with Sivaramakrishnan et al,[22] who reported the administration of morin significantly reduced the levels of MDA in nitrosodiethylamine (NDEA) induced rats.

The endogenous antioxidant system may counteract the reactive oxygen species (ROS) and reduce the oxidative stress with the enzymic antioxidant Superoxide Dismutase (SOD), Glutathion S-Transferase (GST), Catalase (CAT), Glutathione Peroxidase (GSH-px). SOD accelerates the conversion of superoxide radical (O-2) to hydrogen peroxide while CAT and GSH-px converts H_2O_2 to H_2O. In the present study showed a significant decrease in SOD, GST, CAT and GSH-px activity in rats treated with INH- RIF. This is due to INH - RIF generate free radicals, disturbing the antioxidant status and ultimately leading to oxidative stress. This results agree with others [9,40]. On the other hand, there was a significant increase in SOD, GST, GSH-px and CAT activities in groups treated with morin. It has been reported that administration of morin significantly decreased lipid peroxidation and increased endogenous antioxidants, such as SOD, CAT, GSH-$_{PX}$ and GST[22]. Oxidative stress-induced tissue damage can be prevented or ameliorated by favoring the balance towards a lower oxidative stress status. It appears that the protective effect of morin involves the maintenance of antioxidant capacity in protecting the hepatic tissue against oxidative stress.

Conclusion

Our study shows that treatment with morin improved the activities of SOD, CAT, GSH-$_{PX}$ and GST, and decrease the level of MDA. This improvement may have resulted from changing the tissue redox system by scavenging the free radicals and improving the antioxidant status in the liver during INH and RIF hepatotoxicity.

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