Protective Effect of Some Natural Extracts against Isoniazid Induced Hepatotoxicity in Adult Male Rats

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ABSTRACT

Isoniazid (INH), being the first line drug used as antituberculous chemotherapy, is known to be associated with hepatotoxicity. The objective of this study was to determine the protective effect of rosemary and parsley aqueous extracts against isoniazid-induced hepatotoxicity. Adult male Wistar albino rats (120-150g) were randomly divided into six groups (10 animals each) as follows: group (1) rats administrated with saline and served as control, group (2) animals orally administrated with rosemary extract (440mg/kg b.wt./day), group (3) animals administrated with parsley extract (250 mg/kg b.wt./day), group (4) rats received isoniazid alone (50mg/kg b.wt./day), group (5) rats daily received isoniazid in combination with rosemary extract, and group (6) rats daily received isoniazid in combination with parsley extract. After eight weeks, the results revealed that administration of rosemary or parsley extract in combination with isoniazid ameliorated the isoniazid-induced liver deterioration. This was evidenced by the significant reduction of serum ALAT, ASAT, GGT, ALP, bilirubin, cholesterol and triglycerides as well as hepatic MDA coupled with an improvement in the levels of serum albumin as well as hepatic GSH, nitric oxide and Na/K ATPase. Moreover, the histopathological findings showed a potential protective effects of both rosemary and parsley extracts as they prevented isoniazid-induced degenerations those were in the form of pyknosis, karyolysis, swelling of some hepatocyte, foci of necrosis, edematous, fibrosis. In conclusion, either rosemary or parsley extract could play a beneficial role for prevention of isoniazid-hepatotoxicity via its anti-oxidative and anti-nitrosative voltage.

Key words: Tuberculosis, Isoniazid (INH), Hepatotoxicity, Rosemary, Parsley, Rats.

Introduction

The liver is the most important organ in the body. It plays a pivotal role in regulating various physiological processes. It helps in the maintenance of performance and regulating homeostasis of the body. It is involved in almost all the biochemical pathways to growth, fighting against disease, nutrient supply, energy provision and reproduction. In addition, it aids metabolism of carbohydrate, protein and fat, detoxification, secretion of bile and storage of vitamins (Ahsan et al., 2009). Hepatotoxicity has a considerable impact on health because many of the hepatic reactions induced by pharmaceutical preparations can be very severe (Balakrishna et al., 2014).

The splanchnic circulation carries ingested drugs directly into the liver, a phenomenon known as the “first pass” through the liver. Metabolic enzymes convert these chemicals through phase 1 pathways of oxidation, reduction, or hydrolysis, which are carried out principally by the cytochrome P450 class of enzymes. Phase 2 pathways include glucuronidation, sulfation, acetylation, and glutathione conjugation to form compounds that are readily excreted from the body. Other subsequent steps include deacetylation and deamination (Lee and Boyer, 2000).

Isoniazid or Isonicotinic acid hydrazide (INH), being the first line drug used as antituberculous chemotherapy, is known to be associated with hepatotoxicity (Tasduq et al., 2005). It is among the most common causes of drug-induced toxicities. The primary means for INH metabolism in humans is through acetylation by N-acetyltransferase (NAT-2) in the liver gene rating acetylsioniazid. Acetylsioniazid can undergo hydrolysis to form acetylhydrazine (and nontoxic isonicotinic acid). Polymorphisms of NAT-2 have been identified in the population that relegates humans to be either “rapid” or “slow” acetylators. Slow acetylators shunt some INH to a secondary metabolic pathway of oxidation via Cytochrome P450, producing hydrazine (and nontoxic isonicotinic acid also). It appears that both acetylhydrazine and hydrazine, generated by the rapid and slow acetylators respectively, are capable of participating in reactions that generate oxidative stress. Hydrazine may

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induce cytochrome P450 (CYP2E1), increasing production of additional toxic metabolite. Thus, hepatotoxicity may occur in both rapid and slow acetylators, though for slightly different reasons (Gurumurthy, 1984).  

Oxidative stress produced from INH causes a hepatic injury (Sodhi et al., 1998). The majority of normally formed free radicals are removed by the action of reduced glutathione; frequency of hepatotoxicity increases when excess free radical produced by drug is used in combination, this causing the initiation of lipid peroxidation (LPO) resulting in tissue injury (Shankar et al., 2010).

Natural components from plants and other organisms, behave and including different functional activities, for instance, antioxidant activity (Plaza et al., 2009), antimicrobial activity (Tiwari et al., 2009), anti-hypertensive (Yeo et al., 2015), anti-cancer (Woyengo et al., 2009), or neurodegenerative diseases prevention (Zhao and Mol, 2005; Yoo et al., 2008 and Maganha et al., 2010).

Rosemary (Rosmarinus officinalis) is one of the most appreciated natural sources for this kind of compounds. This plant has been widely studied due to the potent antioxidant activities associated to some of its components; among them, phenolic diterpenes have attracted more attention (Thorsen et al., 2003 and Wellwood & Cole, 2004). Parsley (Petroselinum crispum) leaf is used for treatment of constipation, flatulence, jaundice, colic, edema, rheumatism, diseases of prostate and liver. It has also been used as an aphrodisiac (Anonymous, 2000). Parsley is a good source of iron, calcium, phosphorous and antioxidants like luteolin, vitamin C, vitamin A and zinc, which may likely account for its hepatoprotective effect (Nehal and Belal, 2011). As a large number of herbs has been traditionally used to treat or reduce drug-induced complications, therefore the main objective of the present study was to explore the protective battery of both rosemary and parsley aqueous extract against isoniazid-induced hepatic injury in a trial to enhance the drug efficacy and tolerance as well as improve the chance of recovery of tuberculosis patients.

Materials and Methods

Herb extraction:

The used herbs were obtained from stores of a local supplier, Abd El-Rahman Harraz (Bab El-Khalk zone, Cairo, Egypt), identified and authenticated by scientific botanists at Botany department, Faculty of Science Al-Azhar University. The aqueous extraction process of the dry herb leaves was carried out according to the method of Gulcin et al. (2006). In brief, 100 g of the powdered herb leaves were placed in a 1000 ml round-bottom quick fit flask, and 400 ml distilled water were added; the mixture was left for 24 hours at 8 °C, and filtered through qualitative No.1 Whatman filter paper. In Aroma and Flavoring Department, National Research Center, the filtrate was subjected to lypholization process through freeze drier (Snijders Scientific-tiburg, Holland) under pressure, 0.1 to 0.5 mbar and temperature -35 to -41°C conditions. The dry extract was stored at -20°C until used. The yield, total phenolic content and radical scavenging activity of the obtained extract were investigated.

Determination of total phenolic content (TPC):

The concentration of total phenolic content in both herb extracts was determined using the method of Jayaprakasha et al. (2003) and the results were expressed as catechin equivalents (CE). 5 mg of the extract was dissolved in a 10 ml of acetone/water mixture (6:4 v/v); samples of 0.2 ml of that solution (50% w/v) was mixed with 1.0 ml of Folin-Ciocalteu (10-folds diluted) reagent and 0.8 ml of sodium carbonate solution (7.5%); after 30 minutes at room temperature, the absorbance was measured at 765 nm using UV–160 1PC UV-visible spectrophotometer. Estimation of phenolic compounds as catechin equivalents (CE) was carried out using standard curve of catechin.

Determination of Radical Scavenging Activity (RSA) by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay:

The capacity of antioxidants to quench DPPH radical was determined according to Nogala-Kalucka et al. (2005) method and calculated according to the equation:

\[
\text{RSA} \, (\%) = \left( \frac{A_{\text{control sample}} - A_{\text{sample extract}}}{A_{\text{control sample}}} \right) \times 100
\]

Certain of crude extract were dissolved in methanol to obtain a concentration of 200 ppm; then 0.2 ml of this solution was completed to 4 ml by methanol and 1 ml of DPPH (6.09 x 10⁻⁵ mol/L) solution in the same solvent was then added. The absorbance was measured after 10 min at 516nm against reference blank which was 1ml of DPPH solution and 4 ml methanol.

Animal and Experimental Design:

Adult male Wistar albino rats (Rattus norvegicus) weighting 120-150g were obtained from Animal House, National Research Centre, Dokki, Egypt. The animals were housed in suitable plastic cages for one week for acclimation with the new room conditions. Fresh tap water and standard rodent food pellets (Agricultural-Industrial Integration Company, Giza, Egypt) were always available. All animals were received human care in compliance with the standard insitutions criteria for the care and use of experimental animals. After animals
being acclimatized with the experimental room conditions, they were randomly divided into six groups (10 animals each); group (I) normal rats daily administrated (0.4 ml/kg b.w) saline by oral intubation for eight weeks and act as control, group (II) animals subjected to daily oral administration of rosemary aqueous extract (440mg/kg b.w) for eight weeks according to the dose of Amin and Hamza (2005), group (III) animals subjected to daily oral administration of parsley aqueous extract (250mg/kg b.w) for eight weeks according to Poursh et al. (2011), group (IV) animals subjected to daily oral administrated with INH (50mg/kg b.w) for eight weeks (Jehangir et al., 2010), group (V) animals subjected to daily oral administration of INH in combination with rosemary aqueous extract for eight weeks and finally group (VI) animals subjected to daily oral administration of INH in combination with parsley aqueous extract for a similar period.

**Blood sampling:**
At the end of the study period, animals were fasted overnight and following diethyl ether anesthesia and using heparinized capillary tubes, blood specimens were collected from the retro-orbital plexus into vacutainer collecting tubes and left 20 minutes to clot, then centrifuged at 3000 rpm for 10 minutes using cooling centrifuge (IEC centra-4R, International Equipment Co., USA). The sera were separated, divided into aliquots and stored at -70°C until biochemical measurements could be carried out as soon as possible.

**Tissue sampling:**
After blood collection, the animals were rapidly sacrificed and the livers' left lobe of each animal was dissected out, washed with saline, dried, rolled in a piece of aluminum foil and stored at -70°C until homogenization and biochemical determinations. The other liver portions were preserved in a formalin-saline solution (10%); immediately sectioned, stained and prepared for microscopic examination for histological changes.

**Biochemical measurements:**
The activity of serum aminotransferases (ALAT and ASAT) was determined according to the kinetic method described by Schumann and Klauke (2003) using reagent kits purchased from Human Gesell Schaff für Biochemical und Diagnostec mbH, Germany. Serum GGT activity was measured according to the kinetic method described by IFCC (1983) using reagent kits purchased from Bio Systems S.A. Costa Brava 30, Barcelona, Spain. Serum alkaline phosphatase (ALP) activity was assayed using Moss and Henderson (1999) method according to the instruction manual of DiaSys reagent kits purchased from DiaSys Diagnostic systems GmbH Germany. Serum total proteins and albumin concentrations were evaluated according to the photometric systems of Johnson et al. (1999) using reagent kits purchased from DiaSys Diagnostic systems GmbH Germany. Serum bilirubin level was measured colorimetrically (Young, 2001) with the reagent kits purchased from Diamond Diagnostics MDSS GmbH Schiffraben 41 30175 Hannover, Germany. Serum total cholesterol and triglycerides levels were determined according to Artiss and Zak (1997) and Cole et al. (1997) respectively, using DiaSys reagent kits purchased from DiaSys Diagnostic System GmbH, Germany. Serum HDL-cholesterol and LDL-cholesterol levels were determined according to Lopes-Virella et al. (1977) and Wieland and Seidel (1983) respectively, using DiaSys reagent kits purchased from DiaSys Diagnostic System GmbH, Germany.

Malondialdehyde (MDA) is the most abundant individual aldehyde resulting from lipid peroxidation breakdown in biological systems and used as an indirect index for lipid peroxidation (Draper and Hadley, 1990). Hepatic lipid peroxidation end product (MDA) level was determined chemically according to the method described by Ruiz- Lareena et al. (1994) on the base of MDA reaction with thioobarbituric acid (TBA) which forms a pink complex that can be measured photometrically. In this method 0.5 ml liver homogenate supernatant (1g liver tissue was homogenated in 10 ml phosphate buffer pH 7.4 and centrifuged at 5000 rpm for 10 minutes) was added to 4.5 ml working reagent (0.8 g TBA dissolved in 100 ml percloric acid 10%, and mixed with trichloroacetic acid (20%) in a ratio 1 to 3 v/v, respectively). In a boiling and shaking water bath, the sample-reagent mixture was left for 20 minutes, then carried to cool at room temperature and centrifuged for 5 minutes at 3000 rpm. Immediately, the absorbance of the clear pink supernatant was measured photometrically at 535nm against reagent blank (0.5 ml distilled water + 4.5 ml TBA working reagent).

Hepatic nitric oxide level was determined according to the method of Montgomery and Dymock (1961) using the reagent kits purchased from Biodiagnostic, Dokki, Giza, Egypt. Glutathione reduced (GSH) in liver homogenate was estimated spectrophotometrically according to Koracevic et al. (2001) with the reagent kit obtained from Biodiagnostic, Dokki, Giza, Egypt. Finally, Na+/K+ ATPase activity was measured according to the chemical modified method of Tsakiris et al. (2004).

**Histopathology:**
Paraffin sections of 5μm thick were stained with haematoxlin and eosin (Drury and Wallington, 1980) and investigated by light microscope.
Statistical analysis:

The obtained data were subjected to ANOVA-Tukey test using statistical analysis system (SAS) program software; copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA. The significance between the means was tested at p<0.05 (Steel and Torrie, 1980).

Results

With regard to the in vitro investigations; the mean values of yield, radical scavenging activity (RSA) and total phenolics content (TPC) of the aqueous extracts of tested natural herbs, rosemary (Rosmarinus officinalis) and parsley (Petroselinum crispum), are illustrated in Figure (1). The obtained data revealed that rosemary extract (RE) possesses values of yield, RSA (one of the antioxidant mechanism) and TPC higher than those of parsley extract.

![Graph](image)

Fig.1. Mean values of yield (g extract/100 crude herb), radical scavenging activity (RSA %) and total phenolics content (TPC, mg/g extract) of both rosemary and parsley extracts.

The data of this study illustrated that animals those were treated orally with the aqueous extract of ether rosemary or parsley (440 & 250 mg/kg/day respectively) for eight weeks showed a non significant changes in serum ALAT, ASAT, GGT and ALP activities. In contrast, animals group that received isoniazid only (50mg/kg/day) for a similar period revealed a significant increase in the same liver enzymes when both were compared to normal animals. In comparison with animals group that received isoniazid only, animals groups those were treated with rosemary or parsley extract combined with isoniazid showed a significant decrease in serum ALAT, ASAT, GGT and ALP. Parsley extract recorded the highest degree of improvement (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALAT (IU/L)</th>
<th>ASAT (IU/L)</th>
<th>GGT (IU/L)</th>
<th>ALP (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30.4±1.7c</td>
<td>33.4±2.3c</td>
<td>5.5±0.2b</td>
<td>99.6±16.2e</td>
</tr>
<tr>
<td>RE</td>
<td>31.4±1.8b</td>
<td>35.6±2.1c</td>
<td>4.6±0.8bcd</td>
<td>97±10.2bc</td>
</tr>
<tr>
<td>PE</td>
<td>29.2±1.7c</td>
<td>31.0±3.4c</td>
<td>3.7±0.3cd</td>
<td>98.7±19.6b</td>
</tr>
<tr>
<td>INH</td>
<td>89.7±5.0a</td>
<td>95.2±2.6a</td>
<td>9.0±0.4d</td>
<td>477±31.8a</td>
</tr>
<tr>
<td>INH+RE</td>
<td>42.2±1.0b</td>
<td>44.0±1.3c</td>
<td>5.1±0.3bc</td>
<td>221±19.4b</td>
</tr>
<tr>
<td>INH+PE</td>
<td>33.8±1.9bc</td>
<td>33.5±2.5bc</td>
<td>3.5±0.3d</td>
<td>155±19.3bc</td>
</tr>
</tbody>
</table>

Data are presented as mean ±standard error. Within each column; means with different superscript letters are significantly different at p< 0.05 using one way ANOVA, Tukey test.

Also, administration of rosemary and parsley extracts for eight weeks did not affect the serum bilirubin (total or direct), total protein and albumin levels, while animals those were treated with isoniazid showed a significant elevation in the serum level of total and direct bilirubin matched with a significant reduction in both total proteins and albumin when all were compared with control group.

Moreover, rats those were treated with either rosemary or parsley extract in combination with isoniazid resulted in a significant decline in serum total and direct bilirubin level in comparison to isoniazid-treated group, while levels of serum total proteins and albumin recorded a significant enhancement toward the corresponding levels of control group (Table 2).

In addition, the obtained data of this study showed that administration of 440 and 250 mg/kg/day of rosemary and parsley extracts respectively for eight weeks resulted in slight lowering potential in the level of serum total cholesterol, triglycerides and LDL-cholesterol matched with a small rise in serum HDL-cholesterol level. On the other side, isoniazid-treated animals group showed a significant elevation in serum total cholesterol, triglycerides and LDL-cholesterol levels, coupled with slight reduction in serum HDL-cholesterol level when all were compared to control group. With respect to group of animals treated with isoniazid only, animals groups
those received isoniazid combined with administration of either rosemary or parsley extract showed a significant decrease in serum total cholesterol, triglycerides and LDL-cholesterol levels coupled with a slight increase in HDL-cholesterol level (Table 3).

Table 2: Mean values of serum total bilirubin, direct bilirubin, total proteins and albumin levels of treated and control rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total bilirubin (mg/dl)</th>
<th>Direct bilirubin (mg/dl)</th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.33±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.14±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.7±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>RE</td>
<td>0.57±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.19±0.01&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>8.2±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.1±0.07&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>PE</td>
<td>0.38±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.15±0.003&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.04±0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.4±0.1&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>INH</td>
<td>1.4±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.39±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.2±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.2±0.07&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>INH+RE</td>
<td>0.61±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.2±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.3±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.3±0.08&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>INH+PE</td>
<td>0.61±0.05&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.22±0.01&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>8.3±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are presented as mean ±standard error. Within each column; means with different superscript letters are significantly different at p<0.05 using one way ANOVA, Tukey test.

Table 3: Mean values of serum total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol levels of treated and control rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>76±7.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>124±9.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.5±1.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>32.8±2.6&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>RE</td>
<td>89±3.4&lt;sup&gt;ad&lt;/sup&gt;</td>
<td>141±13.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.2±1.5&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>21.2±3.6&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>PE</td>
<td>106±9.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>121±4.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.9±2.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.5±2.3&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>INH</td>
<td>215±7.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>277±31.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.1±3.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100.3±11.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>INH+RE</td>
<td>119±8.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>134±15.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.1±3.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.02±8.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>INH+PE</td>
<td>130±8.2&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>149±15.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.9±2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>69.9±8.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are presented as mean ±standard error. Within each column; means with different superscript letters are significantly different at p<0.05 using one way ANOVA, Tukey test.

Comparing with control animals, administration of either rosemary or parsley extracts never adverse the oxidative stress battery represented herein in hepatic MDA, NO and GSH levels as well as Na<sup>+</sup>/K<sup>+</sup> ATPase activity, however isoniazid ingestion significantly lowered liver GSH level and ATPase activity, and increased both MDA and nitric oxide levels. With regard to animal groups treated with either rosemary or parsley extract besides to isoniazid for a similar period a significant decrease in hepatic MDA and NO levels matched with a significant increase in liver GSH level and Na<sup>+</sup>/K<sup>+</sup> ATPase activity was observed in compare to isoniazid-treated animals group (Table 4).

Table 4: Mean values of hepatic malodialdehde (MDA), nitric oxide (NO) and reduced glutathione (GSH) levels and Na<sup>+</sup>/K<sup>+</sup> ATPase activity of treated and control male Wistar albino rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (mg tissue)</th>
<th>NO (µmol/g tissue)</th>
<th>MDA (µmol/g tissue)</th>
<th>Na&lt;sup&gt;+&lt;/sup&gt;/K&lt;sup&gt;+&lt;/sup&gt; ATPase (µmol Pi/hr/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.5±0.43&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.7±2.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>694±54.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.4±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>RE</td>
<td>8.1±0.43&lt;sup&gt;c&lt;/sup&gt;</td>
<td>33.9±3.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>584±38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.3±0.16&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>PE</td>
<td>8.9±0.55&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>30.9±3.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>466±77.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.4±0.25&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>INH</td>
<td>3.9±0.24&lt;sup&gt;d&lt;/sup&gt;</td>
<td>50.4±4.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1306±167.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.6±0.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>INH+RE</td>
<td>8.8±0.72&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40.3±1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>562±41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.4±0.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>INH+PE</td>
<td>8.5±0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25±3.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>570±46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.2±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are presented as mean ±standard error. Within each column; means with different superscript letters are significantly different at p<0.05 using one way ANOVA, Tukey test.

The microscopic examination of liver sections of rats administrated with either rosemary or parsley extracts for eight weeks showed normal liver characteristic architecture and histological structures of hepatic central vein as well as control group as shown in Figure (2A&B) and Figure (3A&B).

In contrast, liver sections of rats treated with INH only showed much histopathological deteriorations those were in the form of dilated, congested, vacuolated portal vein; also, edematous, fibrosis and cellular infiltration around portal vein were seen. In addition dilated and congested central vein and thickening in the lining were observed. Furthermore, sings of degeneration in the form of pyknosis, karyolysis, swelling of some hepatocyte and foci of necrosis were seen as illustrated in Figures (4&5).

In the case of rat treated with INH along rosemary for eight weeks, most of hepatocytes appeared normal although congestion of small area of blood sinusoid was seen as shown in Figure (6); similarly, the liver sections of rats treated with INH along with parsley extract showed a marked improvement in spite of dilated and congested blood sinusoid and pyknosis of some hepatocyte still noticed as shown in Figure (7).
**Fig. (2A&B):** Sections of the livers of control rats showing normal histological structure of hepatic lobules and central vein (Star), (H&E 100 and 400).

**Fig. (3A&B):** Sections of the livers of rats administrated with rosemary (A) or parsley (B) for eight weeks showing normal histological structure of hepatic architecture (H&E400).
Fig. 4: Sections of the livers of rats treated with INH only (50 mg/Kg b.w) for eight weeks showing dilated, congested, vacuolated portal vein (star). Edema (short arrow-right), fibrosis (long arrow-left) and cellular infiltration around portal vein (short arrow-left)(H&E100).

Fig. 5: Another filed of the liver sections of a rat treated with INH only for eight weeks showing dilated and congested central vein (star) and thickening in the lining (curved arrows). Sings of degeneration in the form of pyknosis (red arrow), karyolysis (yellow arrow), swelling of some hepatocyte (wight arrow) and foci of necrosis are seen (H&E400).
Fig. 6: Section of the liver of a rat treated with INH (50 mg/Kg b.w) for eight weeks along with rosemary showing most of hepatocytes appeared normal with a congestion of small area of blood sinusoid. (H&E 200)

Fig. 7: Section of the liver of rat treated with INH (50 mg/kg) for eight weeks along with parsley showing an improvement of pathological changes, otherwise dilated and congested blood sinusoid (star), pyknosis of some hepatocyte (red arrow) and swelling of some hepatocyte were still seen (Hx&Ex400).
Discussion

Tuberculosis is a leading public health problem worldwide, particularly in developing countries. About one third of world's population has latent tuberculosis and approximately 9 million cases of active tuberculosis emerge annually resulting in 2–3 million deaths (Adhvaryu et al., 2007). Hepatotoxicity of anti-tuberculosis drugs is a serious problem because it is causing a significant morbidity and mortality that requires modification of the drug regimen (Dutt et al., 1984 and Sharma et al., 2002). The increased risk of hepatotoxicity with INH has been attributed to its metabolism; the plasma half life of acetyl-isoniazid (metabolite of INH) is quickly converted into its active metabolites, those are related to the higher incidence of liver necrosis caused by INH (Hussain et al., 2003). Herbs and especially herbal extracts, which contain different classes of polyphenols, are very attractive not only in the modern phytotherapy but also for the food industry. There is no suitable drug for treating hepatotoxicity caused by INH; in regard of that, herbs are implicated as potential hepatoprotective agents; therefore, the present study attempts to investigate the hepatoprotective and antioxidant potential of Rosmarinus officinalis and Petroselinum crispum against anti-tuberculosis drug-induced hepatotoxicity in male wistar rats. To achieve this objective, biochemical measurements of hepatotoxicity and oxidative stress were analyzed in serum and liver homogenates as well as histopathological examination was also carried out. The disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells due INH induced peroxidative damage or altered permeability of membrane (Yadav and Dixit, 2003). Increased protein catabolism and urea formation, which are seen in anti-tubercular drugs-induced hepatocellular injury and necrotic lesions in the hepatocytes, may also be responsible for the increase of these amino tranferases activities in liver (Amr et al., 2005).

Administration of either rosemary or parsley extracts didn't disturb serum ALAT, ASAT, ALP, GGT, bilirubin, proteins, cholesterol, triglycerides, LDL and HDL or hepatic GSH, NO, MDA and Na+/K+ ATPase values as well as the histological structures, all were similar to those of control group reflecting their safety. These findings are concomitant with Abdel-Wahhab et al. (2011), Al- Daraji et al. (2012), Virk et al. (2013) and Mahmoud and Bahr (2015).

The marked increase in ALAT, ASAT, ALP and GGT activities herein as a consequence of INH treatment goes in line with the finding of Yue et al. (2004 and 2009) and Bais and Saiju (2014); while oral administration of either rosemary or parsley extracts along with INH significantly ameliorated these changes. INH toxicity is the most common cause of hepatic failure requiring liver transplantation (Nolan et al., 1999). It was stated that toxicity begins with change in the endoplasmic reticulum, in its turn leads to loss of metabolic enzymes located in the intracellular structures (Avijheet et al., 2008). ALP activity on endothelial cell surfaces is responsible for the conversion of adenosine nucleotides to adenosine, a potent vasodilator and anti-inflammatory mediator that results from injury; so following injury, accumulation of interleukin-6 can lead to production of adenosine by alkaline phosphatase and subsequent protection against ischemic injury. This may be the reason for the increment in ALP in intoxicated rats due to liver toxicities (Sethumadhavan et al., 2007).

The abnormal cholesterol deposition is favored by the dangerous tendency of cholesterol to undergo passive exchange between the plasma lipoproteins and the cell membranes (Gibson et al., 1995). Following to INH administration, the serum levels of total cholesterol, triglycerides and LDL-1 were significately elevated matched with marked reduction in HDL level indicating the disturbance in lipids metabolism. These findings are in accordance with Dhamal et al. (2012) and Jyothi et al. (2013).

Rosemary and parsley extracts administration down regulated the levels of total cholesterol triglycerides and LDL those were increased as a consequence to INH treatment; also HDL level was prominently improved towards control levels. The probable mechanism of this potential may be due to the decrease in the biosynthesis of cholesterol in the liver or inhibition of the enzymes responsible for the synthesis of cholesterol, by the chemical constituents of rosemary and parsley. This effect may also be responsible for an improvement in the serum HDL-levels (Gibson et al., 1995).

In addition, serum bilirubin represents an index for the assessment of hepatic function and any abnormal increase in the level of bilirubin indicates hepatobiliary disease and severe disturbance of hepatocellular function (Vilwanathan et al., 2005). The disaggregation of poly ribosomal profiles induced by toxins is also associated with the inhibition of protein synthesis, which may be partially responsible for the fatty liver, probably not necrosis although it contributes to disabling of the cell (Sethumadhavan et al., 2007). Hence, decrease in protein levels, herein and increase in total bilirubin was observed in the animals treated with INH. Otherwise, oral administration of rosemary and parsley extracts in combination with INH up regulated serum proteins level and down regulated the total bilirubin level.

Based on in vivo and in vitro results, it is suggested that the underlying mechanism for hepatotoxicity is related to: 1) drug bioactivation by Cytochrome P450 2E1, which is one of the liver enzymes, is responsible for damage of hepatic cells (Vishal et al., 2008), 2) generation of oxygen free radicals and reactive metabolites of drugs, 3) imbalance in the oxidant/antioxidant defense and 4) eventual peroxidation of membrane lipids that leads
to the loss of hepatocellular integrity and failure of liver function. The available literature showed that the extracts obtained from several plants have hepatoprotective activities against the toxicity induced by xenobiotics, including those that are used in the treatment of tuberculosis (Morazzoni et al., 1993; Sodhi et al., 1997; Tasduq et al., 2005; Santhosh et al., 2006; Tasduq et al., 2006 and Pal et al., 2008).

The histopathological examinations of our study supported the biochemical findings as it explained both INH hepatotoxicity as well as hepatoprotective potential of rosemary and parsley extracts. INH induced hepatotoxicity could be due to the biotransformation to reactive metabolites that are capable of binding to cellular macromolecules (Georgieva et al., 2004). The role of oxidative stress in the mechanism of INH induced hepatitis has been reported by Attiri et al. (2000). In this study, free radicals formed by the reaction of metabolites of INH with oxygen or by the interaction of superoxide radicals with H₂O₂, seem to initiate peroxidative degradation of membrane lipids and endoplasmic reticulum rich in poly unsaturated fatty acids. This leads to formation of lipid peroxides which in turn give products like MDA (increased significantly), cause loss of integrity of cell membrane and damage to hepatic tissue. Reduced glutathione (GSH) is one of the most abundant non-enzymatic biological antioxidant present in the liver which scavenges reactive toxic metabolites of antitubercular drugs; liver injury has been observed when GSH stores were markedly depleted (Suresh et al., 2008), In our study, similar decrease in GSH matched with marked rise in MDA was observed as a consequence of INH treatment. This finding is in accordance with the finding of Kehinde & Adaramye (2015).

Kupffer cells are the phagocytic macrophages of the liver. When activated, kupffer cells release numerous signaling molecules, including hydrolytic enzymes, eicosanoids, NO and superoxide (Jaeschke et al., 2002; James et al., 2003). They may also release a number of inflammatory cytokines, including TNF-α, interleukins, prostaglandins and oxygen radicals are released in liver toxicity (Marín et al., 1992).

The present study demonstrated that both rosemary and parsley extracts along with INH significantly decreased hepatic NO level close to the control level; therefore, it seems in our study RE and PE can improve hepatotoxicity by decreasing NO to its preferable level Jassim, (2013) and Rasoolijazi et al. (2015).

Physiological process that interferes with the production of ATP may interfere with sodium pump activity, which in the turn results in decreased hepatocellular function. It has been hypothesized that oxidative damage of the membrane bound ATPase activity is crucial for mitochondrial membrane damage (Ramesh et al., 2013). A significant depletion was found in the enzymatic activity of ATPase after anti-tubercular drug intoxication in experimental animals which was responsible for impaired function of the respiratory chain and ATP metabolism and damage of the cellular membrane due to lipid peroxidation also lead to decrease in the activity of endoplasmic reticulum membrane bound enzyme (Ramesh et al., 2013). Our studies showed that exposure to INH drug significantly decreased ATPase activities in liver which might fragility and permeability of the organs; this finding is in agreement with Issabeagloo and Taghizadeh (2012).

Administration of rosemary or parsley extracts in combination with INH significantly restored the metabolic enzyme activities which indicated the improvement of the physiological function in liver tissues (Ramesh et al., 2013). Rosemary is one of the plants rich in different phytochemical derivatives such as triterpenes, flavonoids or polyphenols. Its extracts are able to donate electrons to reactive radicals, converting them to more stable and nonreactive species, therefore preventing them from reaching biomolecules, such as lipoproteins, poly unsaturated fatty acids, DNA, amino acids, proteins and sugars, in susceptible biological systems (Abdel-Wahhab et al., 2011). Also, it was concluded that rosemary extracts have a high scavenging capacity of different types of reactive oxygen and nitrogen species, mostly free radicals, is thought to be one of the main mechanisms of the antioxidant action exhibited by phenolic phytochemicals (Haraguchi et al., 1995). In addition, water soluble extracts of rosemary was reported to induce xenobiotic detoxification enzymes (XME) in rat liver, produce a significant increase in all enzyme activities of phase I [ethoxyresorufin O-deethylase (EROD), methoxyresorufin O-demethylase (MROD), pentoxyresorufin O-dealkylase (PROD), P-nitrophenol hydroxylase (PNPH) and nitric oxide (NO)] and phase II [quinnonereductase (QR), GST and UDP-glucuronosyltransferase (UGT)] (Sotélo-Félix et al., 2002), enhance both cytochrome P (CYP) and detoxifying enzymes (Debersac et al., 2001) and attenuate the depletion in hepatic GSH and catalase (CAT) (Fahim et al., 1999).

Parsley contains flavonoids (Fejes et al., 2000), carotenoids (Francis and Isaksen, 1989), ascorbic acid (Davey et al., 1996), tocopherol and coumarines (Fejes et al., 2000). These phytochemicals improve total antioxidant capacity, suppress destructive oxygen free radicals and prevents oxidative stress damage (Nguyen et al., 2005). Our study also showed that parsley (Petroselinum crispum) has a higher potential to increase serum total antioxidant capacity (Fatemeh et al., 2011).

Conclusion

Both rosemary and parsley aqueous extracts showed an antioxidative stress as well as hepatoprotective effects; the potential of rosemary was higher than that of parsley. This effect could be attributed to the antioxidant activity of their major constituents.
References


