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Anti-nephrotoxic and Antioxidant Efficiency of *Rosmarinus Officinalis* Extract Against Isoniazid®-Induced Nephropathy in Adult Male Albino Rats.

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INTRODUCTION

Tuberculosis accounted as a serious disease throughout the world, and nephrotoxicity is one of the most serious side effects of main anti-tuberculosis drugs. The objective of this study was to explore the nephroprotective potential of rosemary aqueous extract against Isoniazid®-induced nephrotoxicity. Adult male Wistar albino rats (150-170g) were randomly divided into four groups: 1) normal rats, 2) rats administrated with rosemary extract (440mg/kg/day), 3) rats received Isoniazid® (50mg/kg/day), and 4) rats treated with Isoniazid® in combination with rosemary extract. After eight weeks, the results revealed that rosemary extract along with Isoniazid® minimized the Isoniazid®-induced renal deterioration; this was evidenced by the significant reduction in serum levels of urea, creatinine, uric acid, TNF-α, IL-1β and Na⁺ as well as kidney MDA, nitric oxide and DNA fragmentation. This was matched with a marked enhancement in calcium and K⁺ serum levels, and so kidney GSH, and Na⁺/K⁺ ATPase activity. Moreover, the histopathological findings showed a potential protection as the extract succeeded in prevention of Isoniazid®-induced tissue degenerations. In conclusion, rosemary extract could play a beneficial role for the prevention of Isoniazid®-nephrotoxicity via its anti-oxidative and anti-nitrosative voltage.

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**ABSTRACT**

Tuberculosis is a main cause of death among curable infectious diseases, as 1.7 million people died from TB by the year 2004. A regimen of isoniazid, rifampicin, and pyrazinamide for 2 months, followed by 4 months of isoniazid and rifampicin was recommended as a standard treatment for adult respiratory disorders (Issabeagloo and Taghizadieh, 2012). Acute kidney failure is a clinical syndrome that characterized by a fast reduction in kidneys’ ability to eliminate waste products, acid-base balance disturbance, water homeostasis and fast decline in glomerular filtration rate (Bagshaw and Bellomo, 2005).

Nephrotoxicity is the commonest complication of many therapeutic drugs; as many cases of acute renal damage have been reported to be increased over the last 20 years and cause mortality and morbidity among patients (Schetz *et al*., 2005).
Recommended standard treatment for adult respiratory TB is a regimen of isoniazid, rifampicin, and pyrazinamide for 2 months, followed by 4 months of isoniazid and Rifampicin (WHO, 2004).

Isoniazid (INH), large lipid soluble semisynthetic macrocyclic antibiotic, produced from Streptomyces *mediterranei*. It is the early drug which mostly used in combination with rifampicin, ethambutol and pyrazinamide for treatment of all tuberculosis forms resulted from organisms with known or presumed sensitivity to it; as it has efficacy against organisms that divide rapidly (early bactericidal activity) and against semi-dormant bacterial populations, this was taken in consideration due to its sterilizing activity (Verma *et al.*, 2015).

It was referred to INH as a safe drug, but it is found associated with unfavorable reactions like nephrotoxicity which sometimes results in acute renal failure (Jover-Saenz *et al.*, 2006). In spite of renal function disturbance which associated with acute interstitial nephritis and/or acute tubular necrosis, typically occurs in patients treated with intermittent isoniazid therapy, some researchers have also suggested cases appearing during continuous isoniazid and rifampicin doses (Lee and Boelsterli, 2014). Many studies evidenced that isoniazid therapy is accompanied by distortion in renal histological structure characterized by distortion glomerulonephritis, interstitial nephritis and/or acute tubular necrosis (Salih *et al.*, 2008).

Usage of plants in medicine is an age-long practice in various parts of the globe for both preventive and curative. Today, it is estimated that about 80% of the world population relies on botanical preparations as medicine to meet their health needs (Ogbera *et al.*, 2010).

Since most medical treatments perform side effects post their long-term administration and high financial burdens, increased tendency towards alternative and traditional treatments is noticed. Many types of research have been conducted on herbs to ameliorate disorders resulted from acute renal failure.

Herbal medicine (either whale herb, herb parts, water or solvent extract, exudates, essential oils, resins, gums or other forms of advanced products) is used therapeutically to provide proactive enhancement of different physiological sets; in more conventional medical sense, it is used to cure, treat or inhibit a disease in either humans or animals (Weiss and Fintelmann, 2000). Approximately, 70–80% of the populations worldwide (particularly in the developing countries), rely on non-conventional medicine in their basic healthcare as stated by the WHO (Akerele, 1993).

The use of botanical medicines and nutraceuticals or phytonutrients continues to distribute rapidly worldwide with many people now forwarding to these products for the treatment of different health challenges in varies national healthcare settings (WHO, 2003). This aspect in plant-origin drugs be attributed to many reasons. Conventional medicine can be abusive, inefficient (e.g. side effects and ineffective therapy) and/or incorrect use of synthetic drugs as it results in many side effects and other problems, a large portion of the population worldwide does not possess access to conventional pharmacological treatment; as folk medicine and ecological awareness suggests that natural products are harmless.

Rosemary (*Rosmarinus officinalis* family: *Lamiaceae*) was found containing four main categories of compounds include phenols, flavonoids, terpenoids and volatile oil (Barnes *et al.*, 2007). This plant also possesses antioxidant properties due to various compounds
such as rosmarinic acids, rosmanol, epirosmenol, carnosic, and carnosol (Haraguchi et al., 1995). Due to its dilatory properties, several studies had illustrated that rosemary plant can increase blood flow as well as its external use possesses vaso-dilatory potential on the skin (Frishman et al., 2004). In addition, this plant performs antispasmodic or acetylcholine-antagonist properties a consequence to its alpha- and beta-pinenes (Taddei et al., 1988; Hosseinzadeh and Nourbakhsh, 1989). It was stated that rosemary leaves possess many different bioactivities, including antitumor, anti-inflammatory, antioxidant, anti-headaches and anti-HIV properties (Altinier et al., 2007). As tuberculosis is chronic and requires a long duration or treatment with antituberculosis drugs that accompanied by many complications, the objective herein of our study was to explore the protecting efficiency of rosemary extract towards the deteriorations that accompany the use of antituberculosis drug (INH) in a trial to enhance the drug efficacy and potentiate its use for a long term.

MATERIALS AND METHODS

Herbs and Extraction:

Rosemary (Rosmarinus officinalis) family Lamiaceae, was obtained from 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 and it was found to have a taxonomic serial number 32677. The aqueous extraction process of the dry herb leaves was carried out according to the method described before (Gulcin et al., 2006). In brief, 100 g of the powdered 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; then the filtrate was lypholyzed using freeze drier (Snijders Scientific-Tilburg, 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 determined.

Yield Percentage:

The yield was calculated (g % i.e. per 100 grams of crude powdered herb) according to the following equation:

\[
\text{Extract yield (g %)} = \frac{W_2 - W_3}{W_1} * 100
\]

Where,

- \(W_1\) is the weight of the crude powdered herb in grams used in the extraction process.
- \(W_2\) is the weight of clear and dry quick fit flask (grams).
- \(W_3\) is the weight of the flask with the extract after lypholization (grams).
- \(W_1\) is the weight of clear, dry and empty flask.

Total Phenolic Content (TPC):

Total phenolic content of the herbal extract was determined as catechin equivalents (CE) using the method described previously Jayaprakasha et al. (2003) as 5 mg of each extract was dissolved in 10 ml of acetone/water mixture (6:4 v/v); then 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 IPC UV-visible spectrophotometer, then total phenolic content as catechin equivalents (CE) was calculated from the standard curve of catechin.

Radical Scavenging Activity (RSA) by 1,1-diphenyl-2-picrylhydrazyl (DPPH):
The capacity of antioxidants to quench DPPH radical was determined according to Nogala-Kalucka et al. (2005) method and calculated according to the equation below. In this method, certain of the crude extract was 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 $\times$ 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.

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

**Animals and Experimental Design:**

Adult male Wistar albino rats (*Rattus norvegicus*) weighing 150-170g were obtained from Animal Colony, National Research Centre, Cairo, Egypt. The animals were housed in suitable plastic cages one week for acclimation. Fresh tap water and standard rodent food pellets [20.3% protein (20% casein and 0.3% DL-Methionine), 5% fat (corn oil), 5% fibers, 3.7% salt mixture and 1% vitamin mixture, obtained from Meladco Company, El-Obour City, Cairo, Egypt] were always available. All animals received human care in compliance with the standard institutional criteria for the care and use of experimental animals as cited by animal ethical committee number FWA00014747, National Research Centre. After animals being acclimatized with the experimental conditions, they were randomly divided into four groups (10 animals each); group 1) normal rats orally administrated with saline (0.4 ml/kg/day) and act as control, group 2) animals orally administrated with rosemary aqueous extract (440mg/kg/day) (Amin and Hamza, 2005), group 3) animals orally administrated with rosemary aqueous extract (440mg/kg/day) (Amin and Hamza, 2005), group 3) animals orally administrated with INH (50mg/kg/day) (Jehangir et al., 2010) and group 4) animals orally administrated with INH in combination with rosemary aqueous extract s.

**Blood and Tissue Sampling:**

After eight weeks of administration, animals have 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 -80°C. After blood collection, the animals were rapidly sacrificed and the right kidney of each animal was dissected out, washed with saline, dried, rolled in a piece of aluminum foil and stored at -80°C for homogenization and DNA fragmentation and biochemical determinations. The other kidney was soaked in a formalin-saline (10%) buffer; immediately sectioned, stained and prepared for microscopic examination.

**Biochemical Measurements:**

The level of serum albumin was determined according to colorimetric method described by Johnston& Morris (1996) and using reagent kits purchased from DiaSys Diagnostic systems GmbH Germany. Serum creatinine, urea, uric acid and total calcium levels were determined spectrophotometrically according to the kinetic methods described before (Chaney et al., 1960; Husdan and Ruppor, 1969; Trinder, 1969 and Tietz, 1976) using reagent kits purchased from Diamond Diagnostic MDSS GmbH Schiffgraben 41 30175 Hannover, Germany. The serum level of sodium and potassium was estimated using MEDICA Easylyte Na$^+$/K$^+$ ANALYZER (USA) and reagent kits purchased from Easylyte USA, according
Anti-nephrotoxic and antioxidant efficacy of R. officinalis extract against Isoniazid

Serum TNFα and IL1β levels were determined using rat ELISA reagent kits purchased from Assay pro, Charles, MO 63301-4046, USA.

**Kidney Tissue Biochemistry:**

Kidney nitric oxide (NO) and reduced glutathione (GSH) levels were determined according to the methods of Montgomery and Dymock (1961) and Koracevic et al. (2001) using the reagent kits obtained from Biodiagnostic, Dokki, Giza, Egypt. Kidney lipid peroxidation end product malondialdehyde (MDA) level was determined chemically according to the method described by Ruiz-Larrea, (1994); In this method 0.5 ml of supernatant homogenate (1g kidney tissue was homogenized 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 perchloric acid (10%) mixed with trichloroacetic acid (TCA, 20%) in a ratio 1 to 3 v/v, respectively]. In a boiling- 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). MDA level was calculated in nmol/g tissue according to the following formula: MDA (nmol/g tissue) = \[ \frac{[A_{535}\times10^9}{(1.56 \times 10^3 \times 10^3)} \times AD \times 10. \]

Where, 1.56x10^5 M⁻¹L⁻¹cm⁻¹ = extinction coefficient of MDA, AD = Assay dilution (10) [0.5 ml homogenate + 4.5 ml working reagent]. Na⁺/K⁺ ATPase activity was measured according to the modified chemical method of Tsakiris, 2004. 50μl kidney clear homogenate (in Tris Hcl buffer) was added to 2.5 ml of reaction mixture [1.97 M TCA, 1.372 M KCl, 0.0575 M MgCl₂, 20.537 M Sucrose, 0.0925 M EDTA 0.4133 M Adenosine- 5'- triphosphate disodium salt]. The mixture was incubated for 10 min. at 37°C in a shaking water bath, then 0.5 ml ice cold TCA (35%) was added before centrifugation at 3000 rpm for 15 min, 1 ml of the supernatant was added to 0.5 ml of TCA (10%), 0.25 ml of ammonium molybdate (1%) and 0.25 ml ascorbic acid (0.2%). Finally, the absorbencies were read against standard [prepared by adding 0.25 ml standard phosphorus to 1.25 ml of 10% TCA, 0.25 ml of 1% ammonium molybdate and 0.25 ml of 0.2% ascorbic acid], at wavelength 640 nm by using V-530 UV/Vis spectrophotometer. Na⁺/K⁺ ATPase activity was calculated using the formula below.

\[
\text{Ampoule} \times \frac{0.64 \times 10^{-10}}{A_{\text{standard}}} \times 10^{-10}
\]

**DNA Fragmentation:**

The degree of DNA fragmentation was determined by separating the cleaved DNA from the intact chromatin by centrifugation and measuring the amount of DNA present in the supernatant and pellet using the diphenylamine assay according to the quantitative method used for grading the DNA damage (Perandones, 1993). The degree of DNA fragmentation refers to the ratio of DNA in the supernatant to the total DNA in both supernatant and pellet. The kidney tissue was lysed in 0.5 ml of hypotonic lysis buffer containing 10 mM Tris–HCl (pH 8), 1mM EDTA and 0.2% Triton X-100, and centrifuged at 14,000 ×g for 20min at 4 °C. The pellets were resuspended in hypotonic lyses buffer. To the resuspended pellets and the supernatants, 0.5ml TCA (10%) was added. The samples were cool (4°C) centrifuged for 20 min at 10,000 ×g, and the pellets were suspended in 500μl TCA (5%). Subsequently, each sample was treated with a double volume of diphenylamine (DPA) solution [200mg DPA in 10 ml glacial acetic acid, 150 μl of sulfuric acid and 60μl acetaldehyde] and incubated at 4°C for 48h. The
proportion of fragmented DNA was calculated from the absorbance reading at 578nm using the equation below.

\[
\text{DNA fragmentation %} = \left(\frac{A_{\text{supernatant}}}{A_{\text{supernatant}} + A_{\text{pellets}}}\right) \times 100
\]

**Histopathology:**

The kidneys that soaked in formalin-saline (10%) buffer were processed as 5μm thick paraffin sections were stained with hematoxylin and eosin (Drury and Wallington, 1980) and investigated by light microscope.

**Statistical Analysis:**

The obtained data were subjected to one way ANOVA followed by post hoc test (Tukey) 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 \( p \leq 0.05 \) (Steel and Torrie, 1960).

**RESULTS**

Results of three replicates of *in vitro* estimation revealed that rosemary aqueous extract (RAE) possesses high values of yield (g/100g crude herb), RSA (%), and TPC (mg/100g crude herb) as displaced in figure 1.

In comparison to control group, animals treated with RAE didn't show any unfavorable changes in serum creatinine, urea, uric acid and albumin levels; while those administered with Isoniazid® showed a significant increase in serum creatinine, urea and uric acid, coupled with the reduction in serum albumin. Also, animals those were treated with RAE combined with Isoniazid® showed a significant decrease creatinine, urea and uric acid with a marked increase albumin in compare to animals group that received Isoniazid® (Table 1).

![Fig. 1](image-url) "Fig. 1: illustrates the mean values (of three replicates) of the yield (g extract/100 crude herb), RSA (%) and TPC (mg/g extract) of RAE."

**Table 1:** Serum creatinine, urea uric acid and serum albumin levels of treated and control rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Creatinine (mg/dl)</th>
<th>Urea (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
<th>Albumin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.24±0.07a</td>
<td>38.9±1.5b</td>
<td>4.9±0.5c</td>
<td>3.7±0.11a</td>
</tr>
<tr>
<td>RAE</td>
<td>1.26±0.09a</td>
<td>37.4±1.8b</td>
<td>4.7±0.9c</td>
<td>3.6±0.07a</td>
</tr>
<tr>
<td>INH</td>
<td>2.12±0.06b</td>
<td>81.5±2.1a</td>
<td>7.1±0.6a</td>
<td>2.8±0.07c</td>
</tr>
<tr>
<td>INH+RAE</td>
<td>1.30±0.05c</td>
<td>40.7±2.3b</td>
<td>5.8±0.7b</td>
<td>3.3±0.08b</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard error; data subjected to one-way ANOVA followed by post hoc (Tukey) test within each column; means with different superscript letters are significantly different at \( p \leq 0.05 \); (RAE) rosemary aqueous extract, INH (Isoniazid®).
Table 2 shows the effect of RAE on the level of calcium, sodium and potassium levels; the obtained data declared that administration of RAE also didn’t disturb the level of serum total calcium, Na⁺ and K⁺, while administration of INH induced a significant elevation in serum Na⁺, coupled with slight reduction in serum total calcium and K⁺ levels when all were compared to control group. With respect to group of animals treated with INH only, animals those received INH combined with RAE showed a significant reduction in serum Na⁺ level with a marked increase total calcium and K⁺ levels.

Table 2: Shows total Calcium, Sodium and Potassium levels of both treated and control rat’s groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Calcium (mmol/l)</th>
<th>Sodium (mmol/l)</th>
<th>Potassium (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.51±0.66ᵃ</td>
<td>152±1.32ᵃ</td>
<td>6.61±0.32ᵇ</td>
</tr>
<tr>
<td>RAE</td>
<td>3.48±0.81ᵃ</td>
<td>151±1.91ᵃ</td>
<td>6.41±0.21ᵇ</td>
</tr>
<tr>
<td>INH</td>
<td>2.18±0.39ᵇ</td>
<td>178±1.56ᵇ</td>
<td>4.14±0.42ᵇ</td>
</tr>
<tr>
<td>INH+RAE</td>
<td>2.91±1.19ᵇ</td>
<td>157±1.62ᵇ</td>
<td>5.81±0.62ᵇ</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard error; data subjected to one-way ANOVA followed by post hoc (Tukey) test within each column; means with different superscript letters are significantly different at p ≤ 0.05; (RAE) rosemary aqueous extract, INH (Isoniazid®).

Comparing with the control group, administration of RAE never adverse the kidney oxidative stress battery that was achieved from the normal levels of MDA, NO, GSH, Na⁺/K⁺ ATPase; however, INH-treatment significantly depleted GSH level and ATPase activity, and raised both MDA and nitric oxide levels. On the other side and compare to INH-treated group, animals group which treated with RAE in besides to INH showed marked improvement in the kidney oxidative status monitored from the significant decrease MDA and NO levels matched with a significant restore of GSH level and Na⁺/K⁺ ATPase activity (Table 3).

Table 3: Illustrates kidney MDA, NO and GSH levels as well as Na⁺/K⁺ ATP-ase activity of both treated and control rats groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (mg/g tissue)</th>
<th>NO (μmol/g tissue)</th>
<th>MDA (μmol/g tissue)</th>
<th>Na⁺/K⁺ ATPase (μmol pi/hr/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.31±0.65ᵃ</td>
<td>18.8±4.1ᵇ</td>
<td>490±36ᵈ</td>
<td>7.5±0.51ᵇ</td>
</tr>
<tr>
<td>RAE</td>
<td>6.78±0.89ᵇ</td>
<td>17.7±3.9ᵇ</td>
<td>399±28ᵇ</td>
<td>7.3±0.29ᵇ</td>
</tr>
<tr>
<td>INH</td>
<td>3.14±0.91ᵇ</td>
<td>41.5±5.1ᵇ</td>
<td>992±92ᵇ</td>
<td>3.9±0.58ᵇ</td>
</tr>
<tr>
<td>INH+RAE</td>
<td>5.93±0.77ᵇ</td>
<td>28.4±3.2ᵇ</td>
<td>587±32ᵇ</td>
<td>6.2±0.81ᵇ</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard error; data subjected to one-way ANOVA followed by post hoc (Tukey) test within each column; means with different superscript letters are significantly different at p ≤ 0.05; (RAE) rosemary aqueous extract, INH (Isoniazid®).

The results of serum cytokines came parallel to the other measurements as RAE-administration didn’t deteriorate serum TNFα and IL1β levels; however, treatment of rats with RAE succeeded significantly (when administrated in line with INH) to restore both cytokines those were deteriorated as a consequence to INH administration (Figure 2).
Results of DNA fragmentation percentage declared that RAE didn’t cause DNA fragmentation; while INH ingestion resulted in a significant raise in DNA fragmentation when compare both groups with control one. Fortunately, co-administration of rats with RAE along with INH resulted in a significant reduction in DNA fragmentation percent near that of control (Figure 3).

The microscopic examination of sections of control rats kidney illustrated a normal histological structure of renal tubules and glomerulus (Figure 4); while kidney sections of rats group treated with Isoniazid® showed vacuolar degenerations in most renal tubules, hyaline cast in lumen of most tubules and hemorrhage in either interstitial tissue or/and glomeruli. Also, cellular infiltration in interstitial tissue and glomerular degeneration were observed (Figure 5a & b).

DISCUSSION

The Kidney is the major organ that plays a crucial potential in maintaining body homeostasis; otherwise it is affected by many drugs and chemicals (Kumar et al., 2004). Dysfunction and injury of kidneys, as a consequence of medications use, can present as subtle damage and/or overt renal failure as some drugs distort renal perfusion and reduce its filtration capacity, while others directly damage vascular, tubular, glomerular and interstitial cells (Choudhury and Ahmed, 2006). Tuberculosis is a leading public health
problem worldwide, particularly in developing countries. About one-third of the 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). It was suggested that isoniazid is associated with unfavorable side effects such as nephrotoxicity that sometimes resulting in acute renal failure (Jover-Saenz et al., 2006). Although renal dysfunction associated with acute tubule-interstitial nephritis and/or acute tubular necrosis as result of receiving consecutive isoniazid therapy, some authors have also stated cases occurring during continuous isoniazid and rifampicin combined therapy (Lee and Boelsterli, 2014). Also, previous studies showed that isoniazid is associated with structural and morphological histopathological changes presented in glomerular distortion, interstitial nephritis, glomerulonephritis and/or severe tubular necrosis (Salih et al., 2008). As patients on anti-tuberculosis treatment may develop acute kidney injury but little is known about the renal outcome and prognostic factors, and there isn't drug efficient enough to cure or minimize INH-induced nephrotoxicities; therefore, the present study attempted to explore the renal improving ameliorating potential of the nutritive rosemary aqueous extract in rats treated with Isoniazid®. Firstly, administration of rosemary extract didn't disturb either serum levels of creatinine, urea, uric acid, total calcium, sodium and potassium or renal level of redox markers and activity of Na\(^+\)/K\(^+\) ATPase. Also, it didn't distort the histological structures and DNA fragmentation value reflecting its safety. These findings are concomitant with many previous studies (Zohrabi et al., 2012; Gad et al., 2015; El-Sherif and Issa, 2015; Abdel-Azeem et al., 2016 and Bayomy et al., 2017).

The marked increase in serum creatinine, urea and uric acid herein as a consequence of INH treatment goes in line with the finding of Hussein et al. (2015) and Adaramoye et al. (2016); fortunately, rosemary extract along with INH significantly ameliorated these changes. INH toxicity is the most common cause of renal failure (Tavakkolia et al., 2017). ROS and oxidative stress have been implicated in the pathogenesis of drug-induced renal damage (Mahmoud et al., 2015). Long chain polyunsaturated fatty acids are abundant in the composition of renal lipids, and this makes the kidney vulnerable to damage by ROS (Lopez-Novoa et al., 2011). ROS have the ability to induce lipid peroxidation, protein damage, cellular injury, DNA fragmentation and alter the antioxidant defense system (Ozbek, 2012). In the present study, INH administration significantly reduced renal GSH level and ATPase activity coupled with increased the MDA and NO levels and DNA fragmentation percent as well as distorted the kidney anatomical structure, revealing a serious damage to kidney tissue. These results were in accordance with Martin and Sabina (2016).

Many explanations for toxicities, produced by anti-tuberculosis drug, have been postulated: firstly, free radical’s formation which disturbs mitochondrial metabolism and causes a direct toxic effect on renal tissue; however, this mechanism was the most accepted one (Laurent et al., 2000). Thus, cellular basement membranes and membranes (that depend on the integration of non-oxidized lipids to maintain their managing architecture) may be deranged, a process through which glomerular proteinuria could occurs; this because it affects the capillary basement membrane, the main factor in glomerular filtration barrier; secondly, generation of ROS leading to damage of mitochondrial DNA, and respiratory chain dysfunction as a consequence (Lebrecht et al., 2004), resulting in disturbance in the minute
balance between production of ROS and antioxidant defenses, leading to oxidative insult and causing tissue injury, and eventually cell apoptosis (Alfaro-Lira et al., 2012). The direct severe INH-induced cytotoxicity is suggested to be secondary to DNA-intercalation, cross-linking or binding, free radical production with subsequent initiation of DNA damage and death of cells through apoptosis or/and necrosis (Patrakka and Tryggvason, 2010). This kind of cytotoxicity affects the tubular epithelial cells, which are particularly susceptible to toxic injuries (Thirunavukkarasu and Sakthisekaran, 2003); consequently, they performed structural alterations, as it confirmed herein through the microscopic examination.

DNA fragmentation has been recognized as the onset of many diseases and could be a useful indicator for the oxidative status and antioxidant defense system (Chen et al., 2011). The present study showed significant DNA damage as a result of INH treatment. This result is in accordance with previous studies (Yue et al., 2011 & Zhang et al., 2011). Various inflammatory cytokines produced during drug induced kidney injury have been reported to be involved in promoting tissue damage (Ishida et al., 2002). Isoniazid®-administration induced a significant increase in values of both pro-inflammatory cytokines (TNF-α and IL-1β) which represent important mediators of inflammatory tissue injury. Previous studies evidenced that nephrotoxicants could stimulate an inflammatory response towards organ injury (Araujo et al., 2012 & Mahmoud et al., 2014). The elevated TNF-α reflects a degree of inflammation; in this context, our study herein demonstrated a marked increase in serum pro-inflammatory cytokines post INH ingestion, and this result is in consistence with previous studies conducted on rats (Mahmoud et al., 2014 & Mahmoud et al., 2015). Moreover, IL-1β elicits potent pro-inflammatory actions through binding with its receptor and subsequently activates the transcription factors of NF-κB family (Dinarello, 2011). The elevated serum level of the inflammatory mediators in Isoniazid®-treated rats may be attributed to up regulation of kidney NF-κB by ROS (Czaja, 2007) and NO as suggested before (Matata and M Galinanes, 2002). Favorably, administration of rosemary extract resulted in pronounced reduction in serum TNF-α and IL-1β, indicating its anti-inflammatory behaviors. This finding is in agreement with previous studies that reported the ability of rosemary to decrease circulating levels of TNF-α in hyperammonemic rats (Lopez-Novoa et al., 2012; Ozbek, 2012; Bekker et al., 2016 and Ben Khedher et al., 2018).

The study pointed that rosemary water extract alleviated the INH-induced renal toxicity; this was manifested by the appearance of kidney tissues and decreased levels of creatinine, blood urea and uric acid that are close to the corresponding values of normal or control group. In parallel, Stohs et al. (2002) suggested that rosemary prevented both renal and cardiac histopathological structural changes and oxidative stress induced by an anticancer drug in mice. Further, rosemary was also found to possess a therapeutic efficiency for treating or preventing inflammatory nephrotoxicity (Raškovic et al., 2014).

The biological effects of rosemary water extracts are mostly attributed to its high content of phenolic compounds such as rosmarinic acid, rosmanol and epirosmanol that are known as non-synthetic natural antioxidants (Sakr and Lamfon, 2012). Also, rosemary showed in vitro bioactivities as antitumor, anti-inflammatory and chemo-preventive potentials which exhibited via antioxidant mechanisms (Mulinacci et al., 2011 & Al Sheyab et al., 2012). Moreover, the rosemary constituents of
water extract are also able to donate electrons to the liberated reactive radicals and stabilize them, therefore inhibit them from reaching molecules of the biostructures, such as amino acids, lipoproteins, DNA, polyunsaturated fatty acids, proteins and sugars, in susceptible biological tissues (Marzieh et al., 2012). Additionally, rosemary extract possesses a great scavenging capacity of various kinds of free radicals and reactive oxygen and nitrogen species; which is considered one of the major antioxidant-mechanisms exhibited by phenolic phyto-constituents present in rosemary (Bozin et al., 2007). The histopathological findings of this study supported the biochemical findings as they declared both nephrotoxicity of INH and nephro-protective potential of rosemary extract. The mechanisms that induce renal injury could be attributed to deposition of an immune complex in the blood vessels or interstitium which may have resulted in glomerular endotheliosis and eventually causing tubular injury (Rasoulian et al., 2008). Interestingly, the marked elevation in serum levels of urea, creatinine and uric acid as a consequence to INH herein are positively correlated with histopathological changes observed in the kidney. Administration of rosemary extract along with INH significantly decreased kidney NO to level close to that of control; therefore, it seems in our study rosemary can cure nephrotoxicity by decreasing NO as evidenced previously (Muthukumar et al., 2002 & Ashtiyani et al., 2013). The physiological process that interferes with the production of ATP may interfere with Na\(^+\)/K\(^+\) pump (Na\(^+\)/K\(^+\) ATPase) activity, which in turn results in decreased renal function. It has been hypothesized that oxidative damage of the membrane bound Na\(^+\)/K\(^+\) ATPase activity is crucial for mitochondrial membrane damage.

A significant depletion was found in the activity of ATPase after antitubercular drug intoxication in experimental animals (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 significantly decreased kidney ATPase activity; this finding is in agreement with Mahmoud et al. (2017). Occasionally, co-administration of rosemary extract with anti-tuberculosis drug significantly restored the metabolic enzyme activities this monitored from the improvement of the physiological function of kidney tissues and confirmed by Mahmoud et al. (2017). In addition, it was reported that rosemary extract which dissolve in water activate xenobiotic detoxification enzymes in rat kidney, produce a significant increase in all enzyme activities of phase I [ethoxyresorufin O-deethylase, methoxyresorufin O-demethylase, pentoxyresorufin O-dealkylase, P-nitrophenol hydroxylase and nitric oxide] and phase II [quinone reductase, GST and UDP-glucuronosyltransferase] enhance both cytochrome-P and detoxifying enzymes, and attenuate the depletion in kidney GSH and catalase (Fahim et al., 1999).

**IN CONCLUSION**

The present research confers new information on the protective mechanism of *Rosmarinus officinalis* extracts against INH-induced nephrotoxicity. This renal-protective effect could be attributed to the ability of rosemary to attenuate inflammation, inhibit lipid peroxidation, prevent GSH decline and improve the enzymatic antioxidant battery.
Fig. 4: Section of the kidney of control rat showing normal appearance of glomerulus and renal tubules (A). High power field showing normal appearance of glomerulus and renal tubules (B) (Hx & Ex400).

Fig. 5: Section of the kidney of a rat treated with Isoniazid® only showing a vacuolar degeneration in most of renal tubules (red arrow), hyaline cast in lumen of most tubules (star), hemorrhage (yellow arrow) in interstitial tissue (A). Another filed showing degeneration of glomeruli (yellow arrow), interglomerular and interstitial tissue hemorrhage (star), cellular infiltration (black arrow). Vacuolar degeneration in some tubular epithelial cells (red arrow) and pyknosis in some tubular cells (green light arrow). (B) (Hx & Ex400).

Fig. 6: Section of the kidney of a rat treated with rosemary for eight weeks, showing normal appearance of renal tubules and glomeruli. (HX & Ex400).

Fig. 7: Section of the kidney of a rat treated with Isoniazid® along with rosemary showing glomerular lobulation (black curved arrow) and others degeneration with wide urinary space (yellow arrow) and cell debris in the lumen of some tubules (blue curved arrow), few of interstitial hemorrhage (star). (Hx & Ex400)
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