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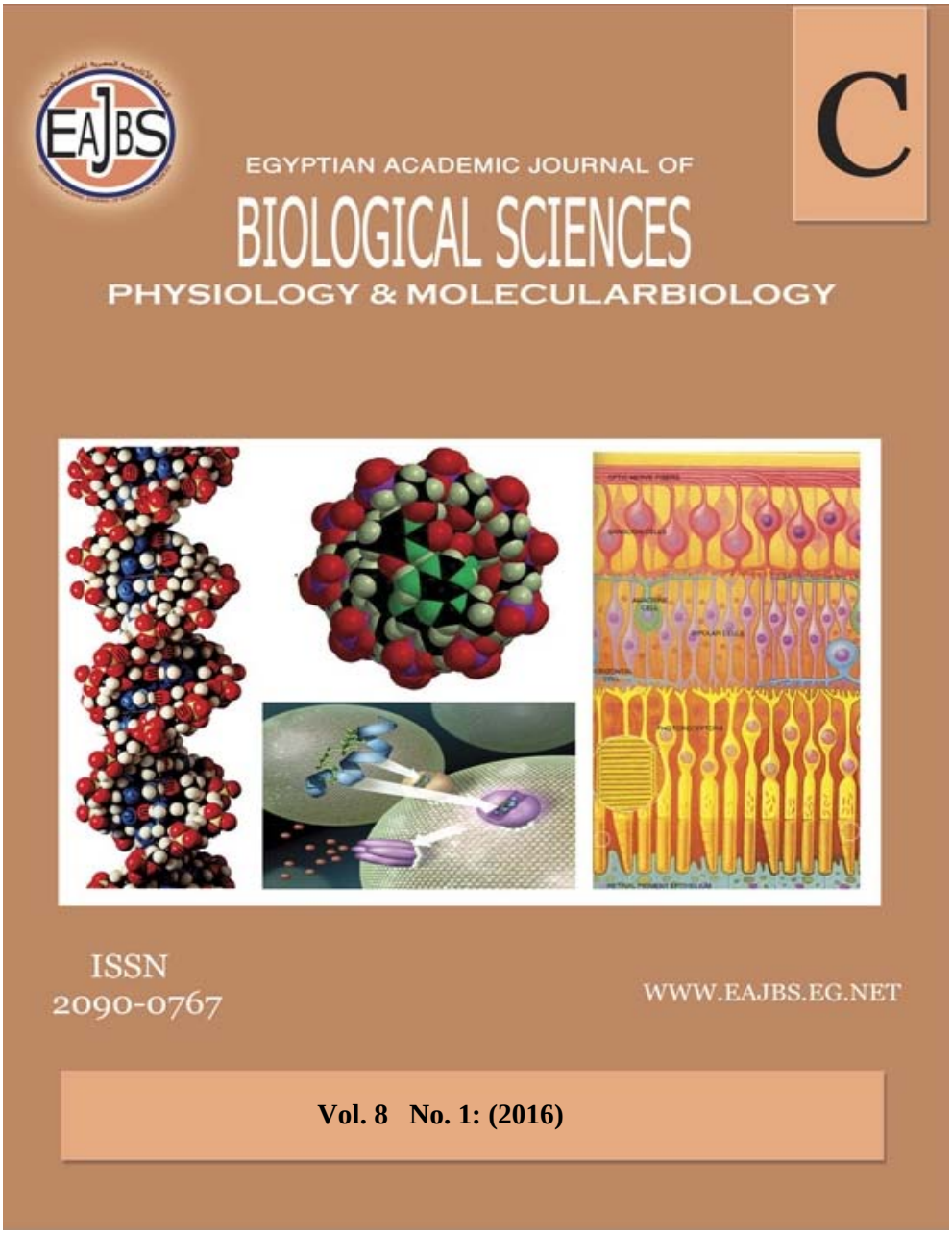

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Possible Protective Effect of Natural Extracts of Rosemary and Parsley Against Isoniazid-induced Nephrotoxicity in Adult Male Albino Rats

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ABSTRACT

The treatment of tuberculosis (TB) has been reported to induce nephrotoxicity arised in association with hepatotoxicity. Isoniazid (INH), being the first line drug used as anti-tuberculosis drugs, is known to induce renal toxicity associated with hepatotoxicity leading to termination of therapy in patients during the intensive phase. The present study aims to determine the protective effect of rosemary (*Rosmarinus officinalis*) and parsley (*Petroselinum crispum*) aqueous extracts against Isoniazid-induced nephrotoxicity. To achieve this purpose, two main experiments were conducted; short-term study for 4 weeks and long-term one for 8 weeks. Adult male albino rats (120-150g) were randomly divided into 6 groups for each experiment (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 body weight./day), group (3) animals administrated with parsley extract (250 mg/kg body weight./day), group (4) rats received Isoniazid alone (50 mg/kg body weight./day), group (5) rats daily received Isoniazid in combination with rosemary extract, and group (6) rats daily received Isoniazid in combination with parsley extract. The administration of natural extracts or Isoniazid was orally and daily for four weeks for the short-term experiment and for eight weeks for the long-term one. At the end of each experiment, all rats were weighed then sacrificed and the biochemical investigations indicative of kidney function as serum creatinine, urea and uric acid were assayed. In addition, serum gamma glutamy ltransferase (GGT) activity and the levels of serum inorganic ions (Na^+, K^+ and total Ca^{++}) were determined. Also, the change in the body weight gain was recorded. The results revealed that administration of rosemary or parsley extract in combination with isoniazid ameliorated the Isoniazid-induced nephrotoxicity. This was evidenced by the marked improvement in kidney function as monitored through the significant decrement in the elevated serum levels of creatinine, urea and uric acid in addition to the remarkable amelioration of GGT activity. Also, the administration of parsley or rosemary extracts in combination with Isoniazid normalized to some extent, the body weight gain and the other biochemical parameters in rats compared with those intoxicated with Isoniazid only. The improvement observed in the biochemical parameters was more pronounced in long-term study compared to the short-term one.

In conclusion, either rosemary or parsley extract could play an evidenced beneficial role for prevention of Isoniazid-induced nephrotoxic effects. This protective effect could be attributed to the antioxidant activity of their major constituents.

INTRODUCTION

Kidneys play an important role in the maintenance of endocrine and acid-base balance, blood pressure, erythropoiesis etc. Therefore, the kidney becomes critical when renal functions decline, induced by diseases which seem to have no direct relation to renal pathophysiology. Nephrotoxicity is a poisonous effect of some substances, both toxic chemicals and medication, on the kidneys.

There are various forms of toxicity. There are some medications having a predominantly renal excretion and need adjusting their dose for the impaired renal function. Reactions to drugs are relatively common and commonly associated with renal dysfunction. Nephrotoxicity arises through several mechanisms, including general and local vascular effects, direct effect on renal tubules, tubular obstruction and acute interstitial nephritis. Acute glomerulonephritis can also occur although this is less common (Parmar, 2010).

Kidney failure is nowadays increasing at an alarming rate, therefore it is of concern to show how kidney can be protected especially in conditions like diabetes mellitus and persons under long drug therapy. Nephropathy is one of the most important complications of diabetes mellitus and drug-induced toxicity. Nephrotoxicity is mostly related to oxidative stress and nowadays much attention has been made towards the possible kidney protective properties of medicinal plants and hence, flavonoids now have been come under light as they found to have protective effect in kidney. Nephrotoxins can be categorized according to intrinsic structural or functional characteristics of the xenobiotic. Xenobiotic causing kidney injury can be also classified into one of the following five mechanistic categories: directly perturbing cellular or subcellular organelle function (Parmar, 2010), causing injury via reactive intermediates or peroxidative stress (Kaufman *et al.,* 1991), perturbing levels of cellular (Nash *et al.,* 2002), interstitial or lumen substrate, perturbing renal hemodynamics (Bellomo, 2006), or eliciting immune-mediated injury (Kohli, 2000).

Traditional markers of kidney toxicity are blood urea nitrogen (BUN) and serum creatinine measurements. Urea and creatinine are nitrogenous end products of metabolism. Creatinine is the product of muscle creatine catabolism. Both are distribute throughout total body water. Also, urinary enzymes are used to determine damage along the nephron and to define subcellular involvement where the proximal tubule is the portion of the nephron with greater sensitivity to nephrotoxic effects of chemicals, and it is the site of several metabolic activities (Price*,* 1982). The lysosomal enzyme Nacetylglucosaminidase (NAG) and the brush border enzyme γ-glutamyltranspeptidase (GGT) have also been used to assess renal toxicity. GGT is an enzyme which is found in liver, kidney and pancreatic tissues, the enzyme concentration being low in liver as compared to kidney (Ozer *et al.,* 2008) where the enzyme is primarily located in the brush border of the proximal convoluted tubules of the kidney (Ward, 1975). It catalyzes transfer of *γ*-glutamyl groups to amino acids and short peptides. When the tubules are damaged, NAG and GGT are released and increased in the serum, then excreted into the lumen of the tubules which can be detected in the urine (Naidu and Lee, 1994). Moreover, urinary levels of specific enzymes have been proposed as specific markers of renal damage (Price*,* 1982; Kilty *et al.,* 1998).

Kidney injury is a severe complication that can disturb treatment and cause permanent kidney damage (DeVriese *et al.,* 1998). It has been reported that nephropathy is an important micro vascular complication of anti-tuberculosis therapy (Rekha *et al.,* 2005). The administration of Isoniazid (INH) has induced renal injury and glomerular dysfunction as evident by the elevated serum urea, creatinine and uric acid levels. These parameters are often regarded as reliable markers of renal damage. In addition, serum creatinine has been used to estimate glomerular

function and its elevation is an indicator of renal failure (Adeneye and Benebo, 2008).

Even at the 21th century, tuberculosis (TB) still remains an important public health problem worldwide despite global attention to eradicate the disease. Tuberculosis (TB) is an airborne disease caused by the bacterium Mycobacterium tuberculosis (*M. tuberculosis*) affecting one-third of the world's population and causes about 2 million deaths annually. Acute kidney injury arised in patients receiving anti-tuberculosis drugs make identifying risk and prognostic factors of acute kidney injury very important in the management of tuberculosis (Chang *et al*., 2014; WHO, 2014).

Drugs used for the treatment of tuberculosis have been reported to cause major adverse reaction and significant morbidity leading to a compromised treatment regimen. Side-effects such as hepatitis, dyspepsia, exanthema, arthralgia and nephrotoxicity were responsible for termination of therapy in up to 23% of patients during the intensive phase (Schaberg *et al.*, 1996). Treatment of TB is through using antibiotics. Effective TB treatment is difficult, due to the unusual structure and chemical composition of the mycobacterial cell wall, which hinders the entry of drugs and makes many antibiotics ineffective (Shimizu *et al*., 2007). The two antibiotics most commonly used are Isoniazid and rifampicin, and treatments can be prolonged, taking several months. Latent TB treatment usually employs a single antibiotic, while active TB disease is best treated with combinations of several antibiotics to reduce the risk of the bacteria developing antibiotic resistance (WHO, 2006).

Isoniazid (INH), the first line anti-tuberculosis drug; is a synthesized hydrazide derivative of isonicotinic acid. It has been used for the treatment of tuberculosis. The central nervous system, liver, kidney and hematologic system are the main targets of INH toxicity. Several studies had reported that leukopenia, eosinophilia, hemolytic anemia along with hepatotoxicity, fatigue, dizziness, headache, nephron-toxicity and dyspnoea, occurred after Isoniazid administration (Yakar *et al.,* 2013).

Natural components from plants and other organisms including different functional activities, behave 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; Maganha *et al*., 2010).

Rosemary is a medicinal herb rich in phytochemical derivatives such as triterpenes, flavonoids or polyphenols. Many studies reported that the preventive effects of rosemary and its extracts are attributed to its antioxidant activity. It was reported that carnosol, rosmanol and epirosmanol phenolic diterpenes of rosemary inhibit lipid peroxidation (Schwarz *et al*., 1992; Zengand Wang, 2001). Ursolic acid, a constant constituent of *Rosmarinus officinalis* extracts, has been shown to have antioxidant and anticarcinogenic properties (Huang *et al.,* 1994). Rosmarinic acid exhibits antioxidant, anti-inflammatory, hepatoprotective, nephroprotective and hematoprotective effects (Halliwell, 1996). Rosemary extracts are able to donate electrons to reactive radicals, converting them to more stable and non reactive species, therefore preventing them from reaching biomolecules. Also, it was concluded that rosemary extracts have a high scavenging capacity that are thought to be one of the main mechanisms of the antioxidant action exhibited by phenolic phytochemicals (Moreno *et al.,* 2006). Thus, Rosemary could be considered as 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 that have attracted more attention (Thorsen *et al*., 2003; Wellwood and Cole, 2004).

Parsley (*Petroselinum crispum)* 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 and hematoprotective effects, thus, it is used for treatment of many hematological disorders and diseases (Nehal and Belal, 2011; Abd El-Reheem *et al*. 2015). 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 evaluate the possible protective role of the two natural aqueous extracts of rosemary and parsley against Isoniazid-induced nephrotoxicityas a trial to enhance the drug efficacy and to improve the recovery chance of patients from tuberculosis.

MATERIALS AND METHODS

Isoniazid (Anti-tuberculosis Drug)

Isoniazid drug was obtained from Al-Hakim Pharmaceuticals, Egypt. Isoniazid solution was prepared separately in sterile distilled water and administrated to the experimental animals at daily oral doses of 50 mg/kg animal body weight for four weeks for the short-term experiment and for eight weeks for the long-term one according to Jehangir *et al*. (2010).

Herbs and Herb Aqueous Extracation

The used herbs; rosemary (*Rosmarinus officinalis*) and parsley (*Petroselinum crispum*) were 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. The aqueous extraction process of the dry herb leaves was carried out according to the method of Gulcin *et al*. (2006) where 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 What man filter paper. In Aroma and Flavoring Department, National Research Center, the filtrate was subjected to lypholyzation process through 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 (TPC) and radical scavenging activity (RSA) of the obtained extract were investigated.

This study dealt with the aqueous extract of the concerned herbs, rather than that of organic solvents; this was due to the possible conformational and configurationally effects of the organic solvents on the extract components.

Determination of Total Phenolic Content (TPC)

The concentration of total phenolic content (TPC) in both herb extracts was determined using the method of Jayaprakasha *et al.* (2003) and 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)

The capacity of the antioxidants to quench1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined according to Nogala-Kalucka *et al.*(2005) method. TheRadical Scavenging Activity (RSA) was calculated according to the equation:

$$RSA\ (\%) = \frac{A_{control\ sample} - A_{sample\ extract}}{A_{control\ sample}} \times 100$$

Where A is the Absorbance A 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. A reference blank was then prepared by dissolving 1 ml of DPPH (6.09 x 10^{-5} mol./L) solution in the same solvent. The absorbance was measured after 10 min at 516nm against the reference blank.

Experimental Design and Animal Groups

Adult male Wister albino rats (*Rattusnorvegicus*) weighting 120-150g were used as experimental animals throughout the study. The Animals were obtained from Animal House, National Research Centre, Dokki, Egypt. They were housed in especially designed cages and fed on a standard diet *ad libitum* with free access of water. All animals were maintained in the laboratory for one week for acclimation before experimentation.

The present study was conducted to determine the protective effect of rosemary (*Rosmarinus officinalis*) and parsley (*Petroselinum crispum*) aqueous extracts against isoniazid-induced nephrotoxicity arising in association with INH-induced hepatotoxicity. To achieve this purpose, two main experiments were conducted; short-term study for 4 weeks and long-term one for 8 weeks.

After acclimatization, adult male Wister albino rats (120-150g) were randomly divided into 6 groups for each experiment (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 body weight./day), group (3) animals administrated with parsley extract (250 mg/kg body weight./day), group (4) rats received isoniazid alone (50 mg/kg body weight./day), group (5) rats daily received isoniazid in combination with rosemary extract, and group (6) rats daily received isoniazid in combination with parsley extract .The administration of natural extracts or isoniazid was orally and daily for four weeks for the short-term experiment and for eight weeks for the long-term one. At the end of each experiment, all rats were weighed then sacrificed and the biochemical investigations indicative of kidney function were assayed.

Body Weight Gain

In all groups of the, the animals were weighed at the beginning and at the end of each experiment and their weights were recorded. The changes in the body weight (Body Weight Gain) were calculated according to the following formula:

$$\text{Body Weight Gain (\%)} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

Blood Sampling and Serum Preparation

One day after the last treatment, animals of all groups in the two experiment were weighed and sacrificed. Blood samples were collected into non-heparinized collecting tubes and left for 20 minutes to clot. After clotting, the non-heparinized blood samples were centrifuged at 3000 rpm for 10 minutes using cooling centrifuge (IEC centra-4R, International Equipment Co., USA). The

sera were separated at once by micro pipette, divided into aliquots and stored at -70°C until subsequent biochemical analysis.

Biochemical Assays

Serum creatinine, urea and uric acid were estimated using the methods of Chaney *et al*. (1960), Husdan and Rupoport (1969) and Trinder (1969), respectively. Also, the serum electrolyte level of sodium and potassium was measured according to the method of Tietz (1976). In addition, total calcium was determined according to the method described by Endres and Rude (1999). In addition, the enzymatic activity of serum GGT activity was measured using the method described by IFCC (1983).

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 $p<0.05$ where a, is non-significant compared with control; b, significantly increased compared with control; c, significantly decreased compared with control; d, significantly increased compared with Isoniazid and e, significantly decreased compared with Isoniazid (Steel and Torrie, 1980).

RESULTS

In the current study, the yield values, radical scavenging activity (RSA) and total phenolic content (TPC) of the aqueous extracts of rosemary (*Rosmarinus officinalis*) and parsley *(Petroselinum crispum*), are presented in Table (1) and illustrated in Figure (1) revealed that rosemary extract (RE) possesses values of yield, RSA and TPC higher than those of parsley extract.

Table 1: Mean values of yield, TPC and RSA of aqueous extracts ofrosemary (RE) andparsley (PE) aqueous extracts.

Extracts Parameters	**Parsley** (*Petroselinum crispum*)	**Rosemary** (*Rosmarinus officinalis*)
Yield (g %)	7.4±0.2	8.2±0.2
TPC (mg/g)	0.949±0.14	1.523±0.04
RSA (%)	47.8±2.1	79.8±1.8

All values are represented as means ± standard error (M ± SE).
TPC (total phenolic content), RSA (radical scavenging activity).

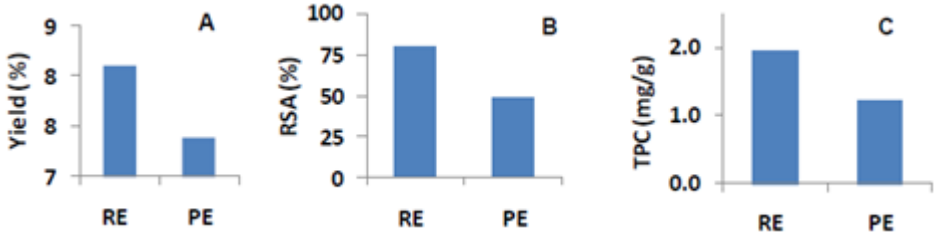

Fig. 1: Mean yield value (g extract/100 crude herb), radical scavenging activity (RSA %) and total phenolic content (TPC, mg/g extract) of both rosemary (RE) and parsley (PE) aqueous extracts.

Body Weight Gain

In the present study, the recorded results showed that rats INH treated animals in short or long-term studies showed significant decreases in their body weights; while rats treated with rosemary or parsley aqueous extract alone showed a significant increase inthe body weight comparing with the rats of control group. In INH treated rats, the administration of rosemary or parsley showed a significant increase in the body weight compared with the INH rats, reflecting the protective potential of both extracts (Table 2 & Fig. 2).

Table 2: Effect of Isoniazid (INH) alone and in combination with either rosemary (RE) or parsley (PE) aqueous extractson body weight gain (%) of malealbino rats.

Treatment(g/100 g body weight)	Control	INH	RE	PE	INH+RE	INH+PE
Four-week treatment	53.1±1.43	35.8±1.05 [c]	54.1±1.65 [a]	51.2±1.32 [a]	53.1±1.75 [d]	55.6±1.23 [d]
Eight-week treatment	65.6±1.43	20.7± 0.76 [c]	71.9±1.55 [a]	76.5±1.9 [a]	48.3±0.98 [d]	59.7±1.53 [d]

Data are presented as mean ±standard error (M±SE). . Means with different superscript letters are significantly different at $p< 0.05$ where: a (non-significant compared with control), c (significant decreased compared with control) and d (significant increased compared with Isoniazid group).

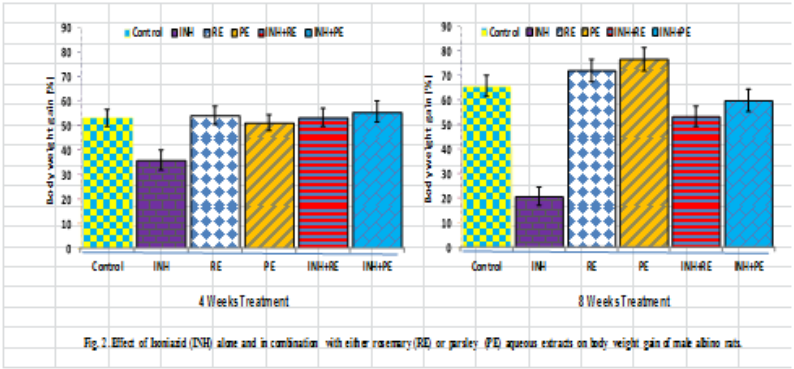

Fig. 2. Effect of Isoniazid (INH) alone and in combination with either rosemary (RE) or parsley (PE) aqueous extracts on body weight gain of male albino rats.

Gamma glutamyltransferase (GGT) Activity

The results of the present study revealed that the treatment of rats with Isoniazidfor either short (four weeks) or long (eight weeks) time resulted in a significant increase in GGT activity; while animals orally treated with the aqueous extract of either rosemary recorded non-significant changes when all groups were compared to the corresponding values of control ones.

The rats treated with INHin combination with either rosemary or parsley extract, showed significant decreases in the elevated GGT activity compared to INH treatedanimals. It was observed that the ameliorating potential was duration-dependent manner and evidenced from the greater improvement recorded in case of long-term study comparing with those of short-time study (Table 3 & Fig. 3).

Table 3: Affect of Isoniazid (INH) alone and in combination with either rosemary (RE) or parsley (PE) aqueous extractsonserum gamma glutamyltransferase (GGT) activityin male albino rats.

Treatment(U/L)	Control	INH	RE	PE	INH+RE	INH+PE
Four-week treatment	5.40±0.27	3.3±0.1 [b]	4.2±0.3 [a]	4.3±0.1 [a]	4.7±0.5 [e]	3.8±0.4 [e]
Eight-week treatment	5.5±0.29	0.0±0.4 [b]	3.8±0.8 [a]	3.5±0.3 [a]	5.1±0.3 [e]	3.8±0.3 [e]

Data are presented as mean ±standard error (M±SE). Means with different superscript letters are significantly different at $p< 0.05$ where: a (non-significant compared with control), b (significant increased compared with control) and e (significant decreased compared with isonazid group).

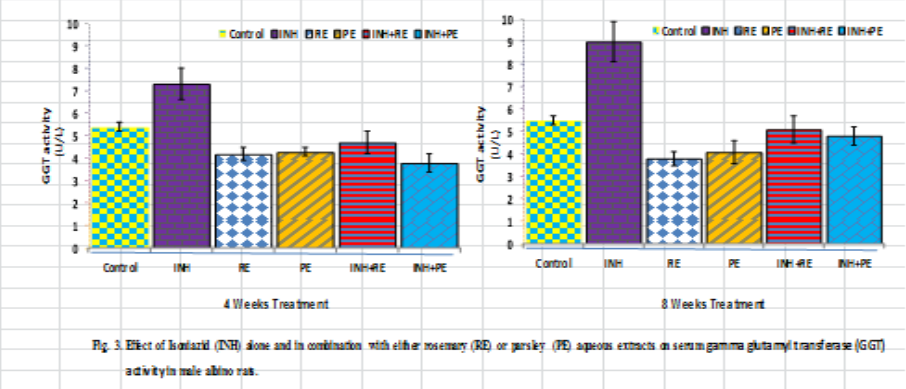

Fig. 3. Effect of Isoniazid (INH) alone and in combination with either rosemary (RE) or parsley (PE) aqueous extracts on serum gamma glutamyl transferase (GGT) activity in male albino rats.

Serum creatinine, urea and uric acid levels

Treating animals with INH caused significant elevation in creatinine in the sera. On the other hand, significant decrease was recorded after treatment with INH and rosemary or parsley aqueous extracts. Similarly, serum urea and uric acid exhibited a significant increase in the INH treated animals. Co-administration of rosemary or parsley aqueous extracts lead to a decrease of serum urea and uric acid. No significant change was recorded in values of creatinine and urea between rosemary or parsley and control group. Also, the present results revealed that the administration of the aqueous extracts of rosemary (RE) or parsley in rats treated with INH for short or long periods induced a pronounced amelioration in serum urea, creatinine and uric acid levels. It was noticed that the ameliorating potential of both extracts was duration-dependent manner (Table 4 & Figs.4-6).

Table 4: Effect of Isoniazid (INH) alone and in combination with either rosemary (RE) or parsley (PE) aqueous extracts on serumcreatinine, urea and uric acid levels inmalealbino rats.

Treatment	Short-term Study			Long-term Study		
	Creatinine mg/dl	**Urea** mg/dl	**Uric acid** mg/dl	**Creatinine** mg/dl	**Urea** mg/dl	**Uric acid** mg/dl
Control	0.83±0.05	30.4±2.2	3.9±0.1	1.04±0.08	35.8±3.1	3.9±0.1
INH	1.38±0.09^{b}	52.1±5.5^{b}	4.7±0.1^{b}	1.82±0.14^{b}	76.5±6.3^{b}	5.9±0.06^{b}
RE	0.87±0.05^{a}	33.8±3.3^{a}	4.1±0.2^{a}	1.2±0.12^{a}	34 5±3.5^{a}	4.4±0.2^{a}
PE	0.96±0.03^{a}	32.2±2.5^{a}	4.04±0.1^{a}	0.95±0.1^{a}	38.2±3.6^{a}	4.01±0.3^{a}
INH+RE	0.96±0.05^{e}	28.2±2.1^{e}	3.2±0.2^{e}	1.03±0.09^{e}	41.6±4.1^{e}	4.6±0.2^{e}
INH+PE	0.91±0.6^{e}	32.8±3.7^{e}	3.5±0.2^{e}	1.07±0.11^{e}	43.2±3.4^{e}	4.8±0.2^{e}

Data are presented as mean ±standard error (M±SE). Means with different superscript letters are significantly different at p< 0.05 where: a (non-significant compared with control), b (significantincreased compared with control) and e (significant decreased compared with isonazid group).

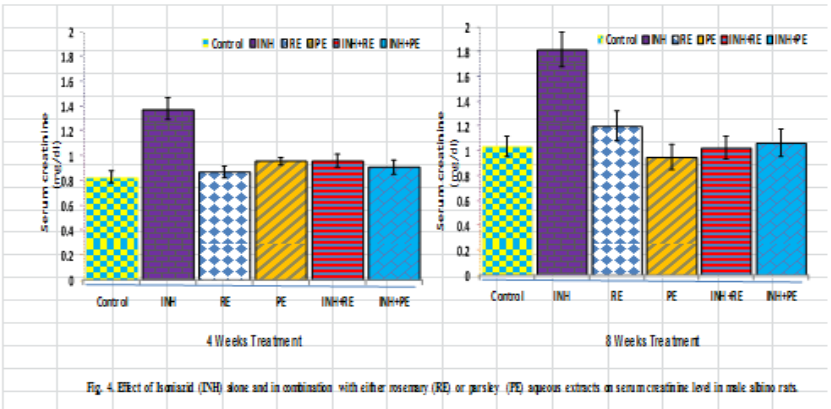

Fig. 4. Effect of Isoniazid (INH) alone and in combination with either rosemary (RE) or parsley (PE) aqueous extracts on serum creatinine level in male albino rats.

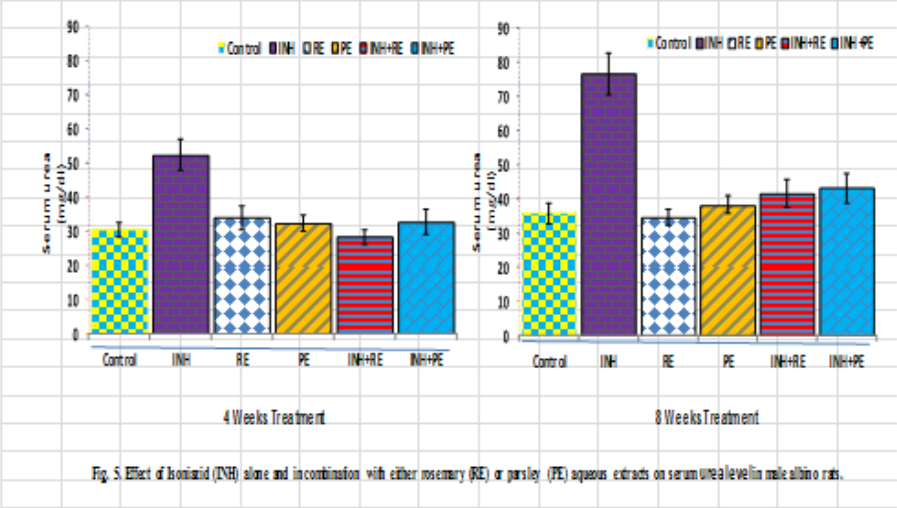

Fig. 5. Effect of Isoniazid (INH) alone and in combination with either rosemary (RE) or parsley (PE) aqueous extracts on serum urea level in male albino rats.

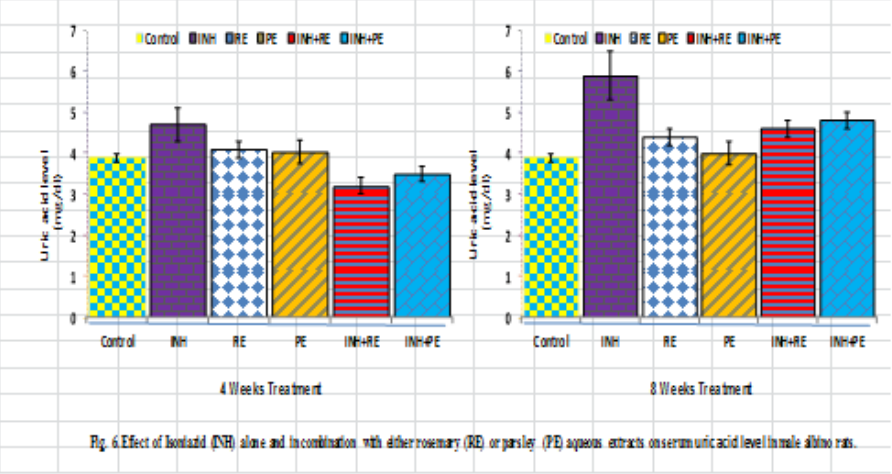

Fig. 6. Effect of Isoniazid (INH) alone and in combination with either rosemary (RE) or parsley (PE) aqueous extracts on serum uric acid level in male albino rats.

Serum inorganic ions; sodium (Na^+), potassium (K^+) and total calcium (Ca^{++})

In rats treated with INH, Na^+ and total Ca^{++} concentrations showed marked reductions while a highly significant increase in K^+ level was observed in serum of rats in both short and long-term study. Administration of RE or PE aqueous extract in rats treated with INH was found to induce a pronounced amelioration in the levels of the estimated electrolytes. A greater improvement was observed in the 8thweek-treated animals compared with the 4thweek treated ones suggesting that the ameliorating potential of RE and PE aqueous extracts is duration-dependent (Table 5 & Figs. 7-9).

Table 5: Effect of Isoniazid (INH) alone and in combination with either rosemary (RE) or parsley (PE) aqueous extract son serum sodium (Na^+), potassium (K^+) and total calcium (Ca^{++}) levels in male albino rats.

Treatment	Short-term Study			Long-term Study		
	Na^+ mmol/l	K^+ mmol/l	Total Ca mmol/l	Na^+ mmol/l	K^+ mmol/l	Total Ca mmol/l l
Control	148.5±10.8	4.8±0.1	2.37±0.3	149.2±11.0	4.6±0.1	2.40±0.5
INH	140.6±10.8^c	6.1±0. 4^b	1.97±0.2^c	137.2±11.8^c	7.7±0.6^b	1.77±0.4^c
RE	147.7±11. 3^a	4.4±0.2^a	2.52±0.4^a	148.2±11.5^a	4.3±0.1^a	2.37±0.6^a
PE	144.6±10.9^a	4.5±0.1^a	2.60±0.5^a	150.5±12.5^a	3.9±0.3^a	2.55±0.7^a
INH+RE	150.2±12.2^d	5.3±0.3^e	2.32±0.3^d	158.2±13.3^d	4.7±0.4^e	2.82±0.9^d
INH+PE	149.8±10.5^d	4.8±0.1^e	2.60±0.3^d	156.6±12.8^d	4.5±0.2^e	2.72±0.7^d

Data are presented as mean ±standard error (M±SE). Means with different superscript letters are significantly different at $p< 0.05$ where: a (non-significant compared with control), b (significant increased compared with control),c (significant decreased compared with control) ,d (significant increased compared with isonazid) and e (significant decreased compared with isonazid group).

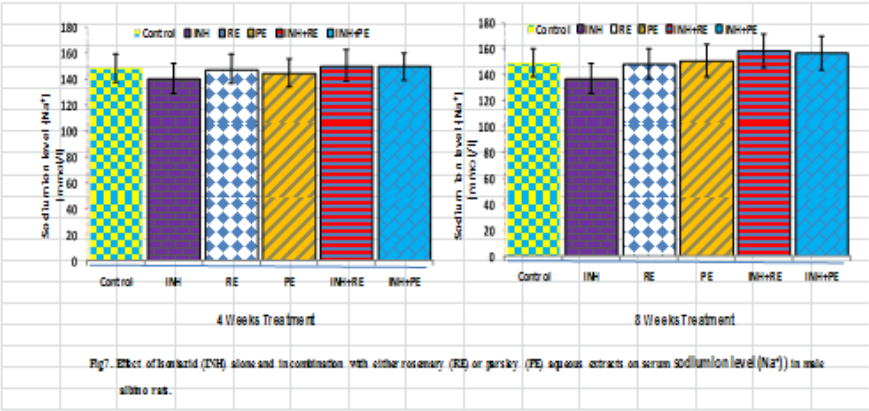

Fig7. Effect of Isoniazid (INH) alone and in combination with either rosemary (RE) or parsley (PE) aqueous extracts on serum sodium ion level (Na^+)) in male albino rats.

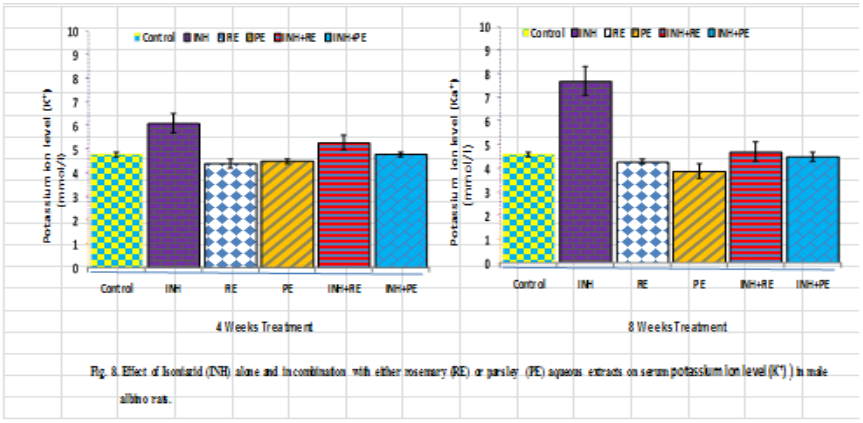

Fig. 8. Effect of Isoniazid (INH) alone and in combination with either rosemary (RE) or parsley (PE) aqueous extracts on serum potassium ion level (K^+)) in male albino rats.

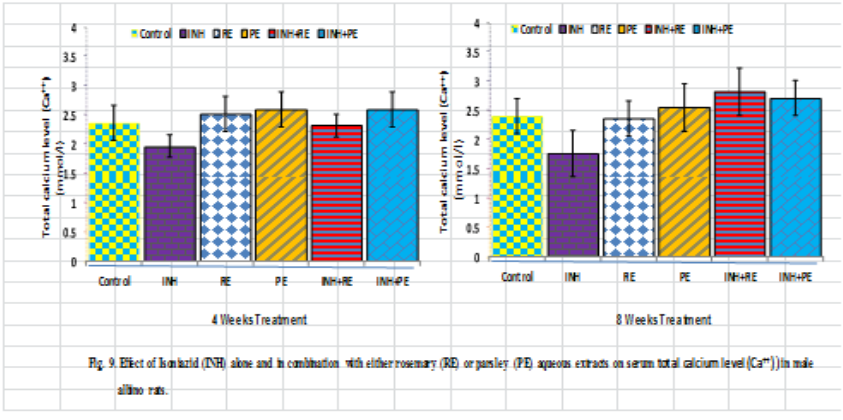

Fig. 9. Effect of Isoniazid (INH) alone and in combination with either rosemary (RE) or parsley (PE) aqueous extracts on serum total calcium level (Ca^{++})) in male albino rats.

DISCUSSION

The kidney is a common target of toxicity of therapeutic and environmental xenobiotics, because of its high blood flow, tubular transport processes and complex metabolic activities. The kidney is a chief regulator of all the body fluid and is primarily responsible for maintaining homeostasis or equilibrium of fluid and electrolytes of the body; any imbalance occurred might cause disorder of kidney functions rapidly. Kidney's main function includes urine formation, regulation of acid-base balance, excretion of waste products of metabolism and toxic substances, protein conservation, secretory functions and recovery of useful metabolites which filters through them. Kidney function disorder is resulted as an accumulation of toxic substances in the blood that should be removed from the body (Kumar *et al*., 2004; El-Sherif and Issa, 2015). Also, the kidney is affected by many chemicals and drugs where regulation of the internal environment of body cells is maintained mainly by glomerular filtration, selective reabsorption and secretion by renal tubules. Nephrotoxicity of Isoniazid which used as an anti-tuberculosis drug, is known to cause a number of biochemical dysfunction leading to a significant morbidity and mortality; thus a modification of the drug regimen is required (Sharma *et al.,* 2012; Abdel-Reheem *et al*., 2015; Kehinde and Adaramoye, 2015). The consumption of a variety of local herbs and vegetables by man is believed to contribute significantly to the improvement of human health in terms of prevention or cure of diseases because plants have long served as useful and rational sources of therapeutic agents (Yuan *et al*., 2016). Tuberculosis (TB) results in the release of a variety of chemicals which may damage body cells and tissues. The release of free radicals from macrophages, poor dietary intake of micronutrients and side effects of anti-TB drugs were factors contributing to oxidative stress. Availability of antioxidants to neutralise the harmful effects of the generated free radicals may be one of the factors determining severity of the disease (Ali *et al*., 2014; WHO, 2014).

Therefore, the present study attempts to study the possible protective effect of rosemary *(Rosmorinus officinalis*) and parsley (*Petroselinum crispum*) aqueous extracts rich in antioxidants against INH-induced nephrotoxicity. Biochemical parameters in addition to the change in the body weight gain were determined in male albino rats to assess the nephroprotective activity of both extracts. Findings of the present study clearly indicated that aqueous herbal extracts of both rosemary and parsley showed a significant protective activity against INH-induced renal injuries.

The obtained results of the present study showed that animals orally treated with Isoniazid (INH) showed a significant decrease in the body weight gain percentage compared to the normal group. This loss in the body weight may be due to the disturbance in the appetite of animals and reduction in food absorption and assimilation where the reduced food intake when these rats orally ingested some toxic substance; resulted in less digestion and absorption of the food which rats fed. Also, it may be due to the induced hepato-renal dysfunction that could be suggested through the disruption in the hepatic and renal physiological measurements, in this study. This finding is in agreement with the previous reports of kalra *et al*. (2007); Tayal *et al*. (2007); Noorani *et al.* (2010); Dhamal *et al.* (2012).

Antitubercular drugs mediated oxidative damage is generally attributed to the formation of free radicals, which

act as stimulator of lipid peroxidation and source for destruction and damage to the cell membrane. Isoniazid which is considered the main antitubercular drug; was found to exhibit certain toxic effects on both kidneys and liver, consequently, body weight loss which interfere with its therapeutic efficiency. This effect may be attributed to the gastrointestinal toxicity and the reduced ingestion of food as explained by Tayal *et al*. (2007); Dhamal *et al.* (2012).

On the other hand, the administration of either rosemary (RE) or parsley (PE) aqueous extract in male albino rats treated with INH for short or long time, resulted in a significant elevation in the decreased percentage of the body weight gain compared to the INH-treated rats which was close to the control group reflecting the ameliorative effect of both extracts.The higher body weight gain was observed in case of RE extract than PE one. The increase in the percentages of the body weight gain induced by RE or PE extract may be as a result of improved appetite exhibited by the INH-treated animals as an attempt to overcome the drug-induced stress. Similar findings were reported by Awe and Banjoko, (2013); Kehinde and Adaramoye, (2015). However, when rats fed on diet contain detoxifying antioxidant, an opposed effect against the toxic substance is occurred through the scavenging activity and enhance digestive system to digest and absorb food leading to restore the loss in body weight gain of these rats. Also, it was found that RE and PE have protective effects that were attributed to their antioxidant properties by inhibiting free radical generation (Afonso *et al.,* 2013).

In the current study, a marked increase in GGT activity was observed in serum of rats treated with INH. This increase may be due to the renal injury that leads to the leakage of the enzyme from cells due to peroxidative damage or altered permeability of membrane. Increased protein catabolism and urea formation, which are seen in anti-tubercular drugs induced renal injury, may also be responsible for the increase of the GGT activity in the kidney. The marked increase in GGT activity herein as a consequence of INH treatment goes in line with the finding of Yue *et al*., (2009); Bais and Saiju (2014). Administration of either RE or PE extracts didn't disturb serum GGT activity reflecting normalization of its level. This pronounced ameliorative effect may be due to the high antioxidant contents that play an important role in protecting the kidney from the free radicals and toxic metabolites of INH liberated in the body. These findings are concomitant with Abdel-Wahhab *et al.* (2011); Al-Daraji *et al*. (2012); Mahmoud and Bahr (2015).

In comparison with normal rats, the data showed that the administration of either RE or PE extract in male albino rats didn’t record any harm changes in the renal function monitored from serum levels of creatinine, urea, uric acid, sodium ions, potassium ions and ionized calcium in reflecting their safety. These observations are in agreement with many reports (Sakr and Lamfon, 2012; Zohrabi *et al*., 2012; Dahal and Mulukuri, 2015; El-Sherif and Issa, 2016). On the other hand, ingestion of INH alone led to kind of nephrotoxicity evidenced with the significant increase in serum levels of creatinine, urea, and uric acid. These results are in accordance with the studies reported by Kehinde and Adarmoye (2015). Nephrotoxicity is serious characterized by high blood urea and serum creatinine levels. Isoniazid induced lipid peroxidation and oxygen free radical generation in the kidney leading to its damage. Oxidative stress and ROS accumulation are among the main mechanisms of INH-induced nephrotoxicity (Kehinde and Adarmoye, 2015).

It was stated by Abd El Reheem and Zaahkcuk (2007) that elevated urea and creatinine levels as a consequence to toxins could be due to the decreased glomerular filtration rate, decreased renal blood flow, decreased number of furcating nephrons, obstruction of urinal flow and/or disordered protein metabolism.

It was reported that chloro-compounds in parsley often show significant biological activities, e.g. antibiotic, antitumor, antiviral, antibacterial, anti-inflammatory, antihepatotoxic, pesticidal, antioxidant activities and dissolves cholesterol within the veins which all reflected in enhancing the general health condition of the body (Holst and Engvild, 2000; Kery *et al.,* 2001; Al-Howiring *et al.,* 2003). From this point of view, parsley can improve bladder, kidney, liver, lung, stomach and thyroid function. Also, it helps in clearing of uric acid from the urinary tract; treatment of urinary tract infections, dissolve and expel gall stones and gravel; prevent kidney stone formation; acts as diuretic and increase urine volume; may also be used to treat edema and high blood pressure (Tipu *et al*., 2006).

It is well established that mitochondria is a critical target of Isoniazid toxicity and that the mitochondrial dysfunction may be a very early event in Isoniazid -induced nephrotoxicity. In addition, Ali *et al.* (2014) reported that Isoniazid-induced renal damage is associated with renal mitochondrial dysfunction and oxidative stress.

On the other hand, administration of either rosemary or parsley extracts in combination with Isoniazid restored the deteriorated function of the renal system. This anti-nephrotoxicity potential is evidenced by the significant reduction in the serum level of creatinine, urea and uric acid levels as compared to the Isoniazid-treated rats. These results are agonized by report of Gopi *et al.* (2010).

It was demonstrated that some thiol-containing compounds reducing the uptake of Isoniazid by the renal tubular cells and increasing the urinary excretion of the drug reflecting the cytoprotective mechanism (Zohrabi *et al*., 2012) It has been found that ROS may be involved in the impairment of glomerular filtration rate. Treatment with rosemary or parsley extracts was found to induce significant decreases in the urinary protein and glucose as well as serum creatinine and BUN in diabetic animals; also it reduces the activity of serum GGT. This effect may be related to the antioxidant properties of its constituents (Abdul-Rahim and Taha, 2011; Al-Sheyab, 2012).

The obtained data showed that the animal treated with INH only for four and eight weeks revealed a significant disturbance in the serum level of these elements, i.e reduction in glomerular filtration rate (GFR) by vasoconstriction of the afferent glomerular arterioles and impairment of tubular function with alterations in homeostasis of electrolytes leading to the elevation of kidney function tests. The elevation in creatinine, urea uric acid and the impairment of the electrolytes, recorded in this work indicated that INH caused renal toxicity in rats with impaired ability of the kidney to excrete these residual products in urine. This might be attributed to the renal tubular damage and consequently, reduction in the glomerular filtration rate (GFR), while the animals orally administered with the aqueous extract of either RE or PE in combination with INH showed non significant changes in the level of serum creatinine, urea, uric acid as well as Na+, K+, and Ca++ concentrations, demonstrating the functionality of the renal system which confirmed by the unchanged kidney function tests. Natural antioxidants strengthen the endogenous antioxidant

defenses against reactive oxygen species (ROS) and restore the optimal balance by neutralizing these reactive species. They are gaining immense importance by virtue of their critical role in disease prevention (Sakr and Lamfon, 2012), where the balance of electrolytes is essential for normal function of cells and organs, in addition, the electrolyte tests are commonly used to monitor treatment of certain health problems.

In conclusion, the current study showed that INH treatment in male albino rats resulted in significant elevations of serum urea, creatinine, uric acid as well as the marked increase in the serum GGT activity in addition to the serum electrolyte imbalance. Also, a significant reduction in the body weight gain was observed. Either RE or PE extracts could play an evidenced beneficial role for prevention of INH-nephrotoxicity. This protective effect may be due to the antioxidant activity of their major constituents. These parameters were reduced to normal or near normal when rats received treatment with RE or PE aqueous extracts. These findings revealed the protective effect of REand PE in rats with INH-induced nephrotoxicity. It was suggested that the amelioration of INH-induced nephrotoxicity by RE and PE aqueous extracts may be related to their antioxidant property and therefore, represents a potential therapeutic strategy for renal injury caused by Isoniazid treatment. Several clinically used therapeutic drugs are known to cause nephrotoxicity, which is similar to that of INH. Rosemary and parsley may be helpful in mitigating this particular side effect of such drugs. However, the effectiveness of the active components of the two herbs in acute renal failure and the mechanisms involved require further investigation. Finally, it can be concluded that, Isoniazid has adverse effects on the kidney. Different natural materials as rosemary and parsley were able to protect against these effects. So, the patients should be advised to take one of these materials while they are treated by Isoniazid.

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