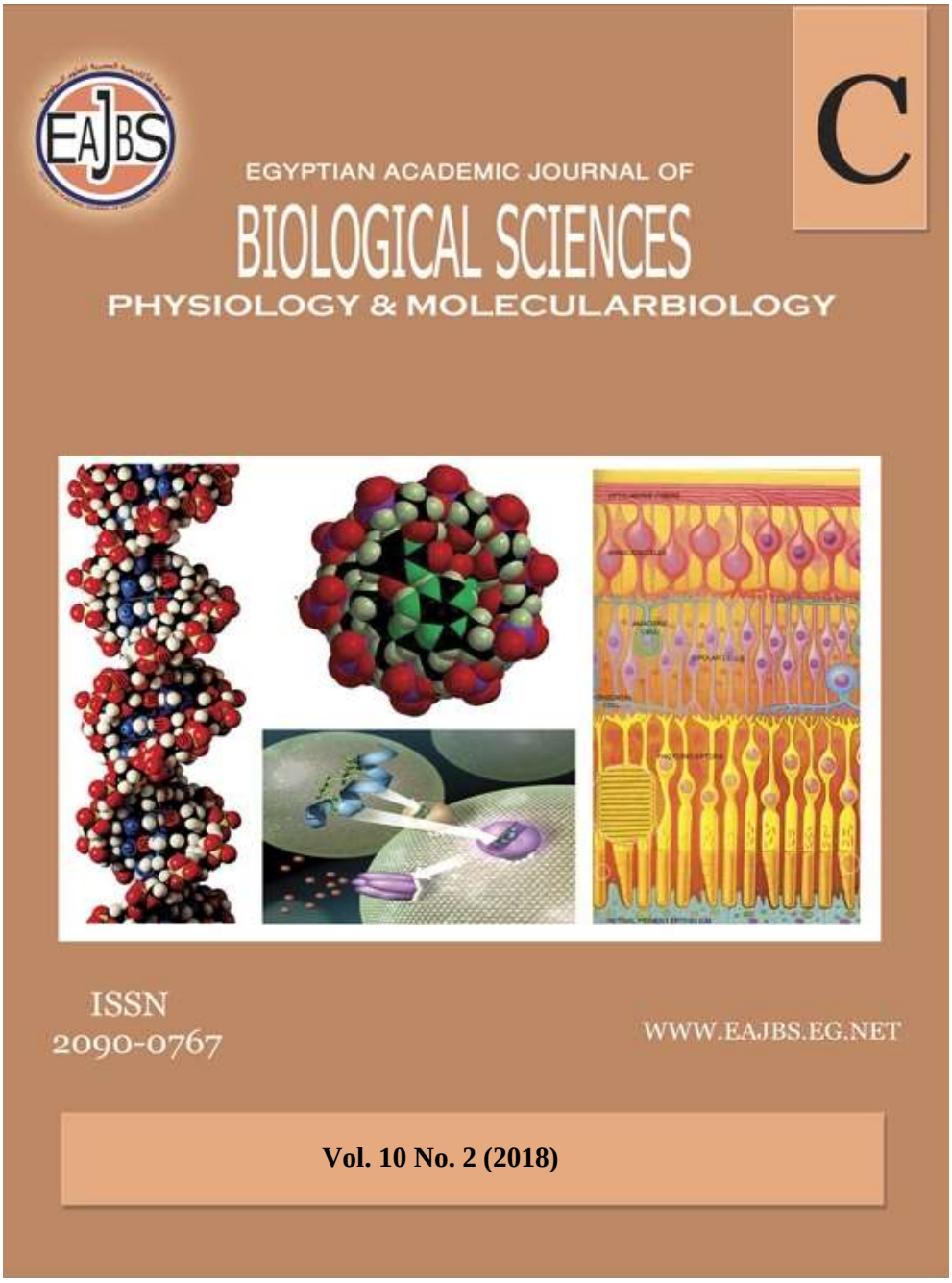


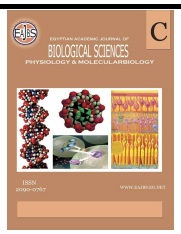
Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.

C. Physiology & Molecular Biology journal is one of the series issued twice by the Egyptian Academic Journal of Biological Sciences, and is devoted to publication of original papers that elucidate important biological, chemical, or physical mechanisms of broad physiological significance.

www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 10(2): 45- 61 (2018)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbs.eg.net



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

Mahmoud Ashry[1*], Mohamed A. Mustafa[2], Hagar H. Mourad[3], Mahitab I. EL-Kassaby[3], Fatma Adly Morsy[4], Sayed ON[5], Khaled G. Abdel-Wahhab[3]

1- Zoology Department, Faculty of Science, Al-Azhar University, Egypt
2-Basic Centre of Science, Misr University for Science and Technology, Egypt
3-Medical Physiology Department, National Research Centre, Egypt
4-Pathology Department, National Research Centre, Egypt
5-Chemistry Department, Fayoum University, Cairo, Egypt
Mail.: mahmoud_ashry20@yahoo.com

ARTICLE INFO

Article History
Received: 1/6/2018
Accepted: 2/7/2018

Keywords:
Tuberculosis
Isoniazid® (INH)
Nephrotoxicity
Rosemary
Rats

ABSTRACT

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 nephro-protective 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.

INTRODUCTION

Tuberculosis (TB) 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 tubule-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 anti-tuberculosis 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:

$$\text{Extract yield (g \%)} = \frac{W2-W3}{W1} * 100$$

Where,

W_1 is the weight of the crude powdered herb in grams used in the extraction process.

is the weight of clear and dry quick fit flask (grams).

W_2 is the weight of the flask with the extract after lypholization (grams).

W_3 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 1PC 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 $DPPH^{\bullet}$ (6.09×10^{-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 $DPPH^{\bullet}$ solution and 4 ml methanol.

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

Animals and Experimental Design:

Adult male Wistar albino rats (*Rattus norvegicus*) weighting 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 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 Rupopor, 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

to the method of Tietz, 1976. 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) = [{A_{535}× 10^9 / (1.56 × 10^5) × 10^3} x AD] × 10. Where, 1.56x105 $M^{-1}L^{-1}cm^{-1}$ = 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 $MgCL_2$, 20.537 M Sucrose, 0.0925 M EDTA 0.4133 M Adenosine- 5'- triphosphate disodium salt]. The mixture was incubated for 10 min. at 37^0C 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^+ ATP-ase activity was calculated using the formula below.

$$[\text{ATP ase activity } (\mu \text{ mol Pi/hr/g tissue}) = \frac{\text{A sample} * 0.64 \text{ x } 1.0 * 60.0}{\text{A standard} * 10 * 10}]$$

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.

$$\text{DNA fragmentation \%} = \frac{\text{A supernatant}}{\text{A supernatantA supernatant+A pellets}} * 100$$

Histopathology:

The kidneys that soaked in formalin-saline (10%) buffer were processed as 5um 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.

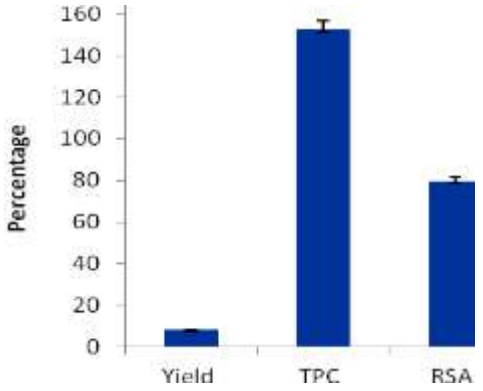


Fig. 1: illustrates the mean values (of three replicates) of the yield (g extract/100 crude herb), RSA (%) and TPC (mg/g extract) of RAE.

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).

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

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

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.

Groups	Total Calcium (mmol/l)	Sodium (mmol/l)	Potassium (mmol/l)
Control	3.51±0.66[a]	152±1.32 [a]	6.61±0.32 [b]
RAE	3.48±0.81[a]	151±1.91[a]	6.41±0.21[b]
INH	2.18±0.39[c]	178±1.56[b]	4.14±0.42 [a]
INH+RAE	2.91±1.19[b]	157±1.62 [a]	5.81±0.62 [c]

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®).

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^+ ATPaese 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.

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

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®).

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).

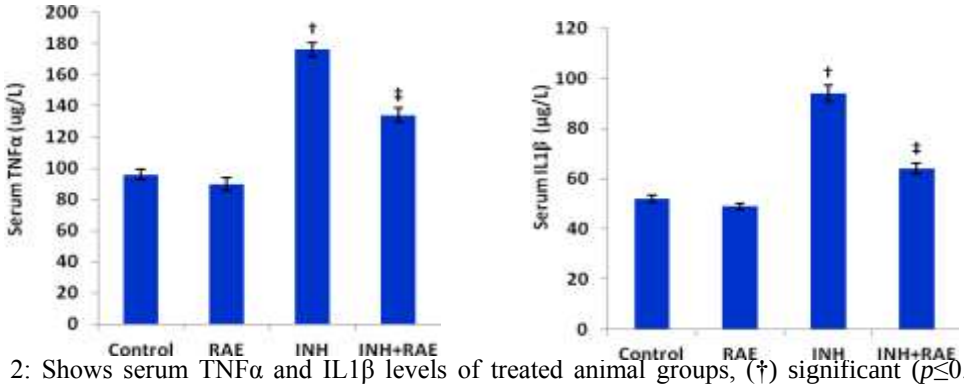


Fig. 2: Shows serum TNFα and IL1β levels of treated animal groups, (†) significant ($p \leq 0.05$) from control group, while (‡) is significant ($p \leq 0.05$) from INH group.

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).

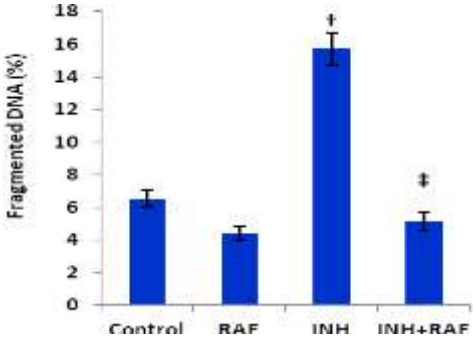


Fig. 3: Reveals DNA fragmentation (%) of treated animal groups as (†) significant ($p \leq 0.05$) from control group, while (‡) is significant ($p \leq 0.05$) from INH group

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).

The kidney of rats those treated with RAE showed the normal appearance of renal tubules and interstitial tissue (Figure 6). Favorably, kidneys of rats group supplemented with RAE along with INH administration revealed improvements in the kidney with minimal glomerular lobulation and reduction of the others degeneration with wide urinary space and few of interstitial tissue hemorrhage (Figure 7).

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 nephron-toxicants 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-kB family (Dinarello, 2011). The elevated serum level of the inflammatory mediators in Isoniazid®-treated rats may be attributed to up regulation of kidney NF-kB 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-Nova *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 anti-tubercular 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.

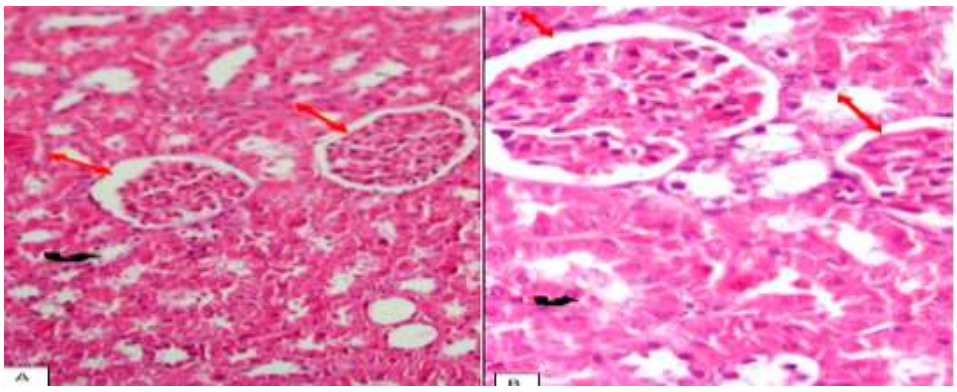


Fig. 4: Section of the kidney of control rat showing normal appearance of glomerulus and renal tubules (A). High power field showing normal appearance of glomerulus and renal tubules (B) (Hx & Ex400).

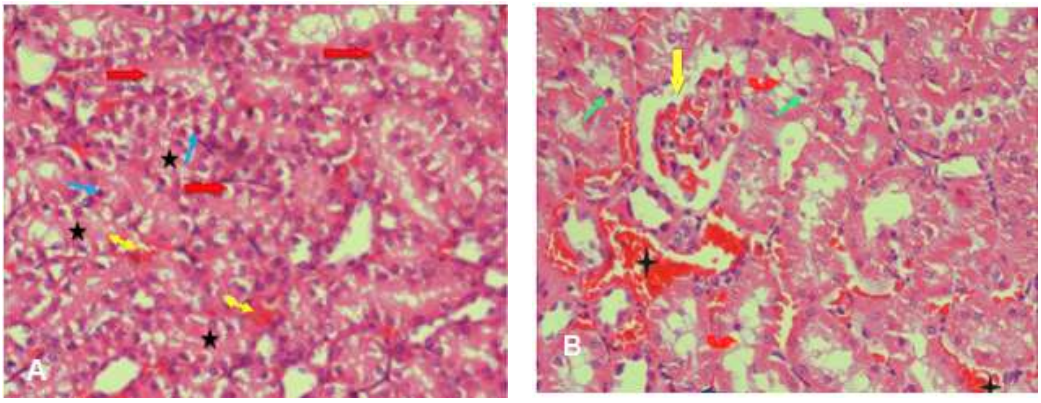


Fig. 5: Section of the kidney of a rat treated with Isoniazid® only showing a vacuolar degeneration in most of renal tubules (red arrow), hyaline cast in lumen of most tubules (star), hemorrhage (yellow arrow) in interstitial tissue (A). Another filed showing degeneration of glomeruli (yellow arrow),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

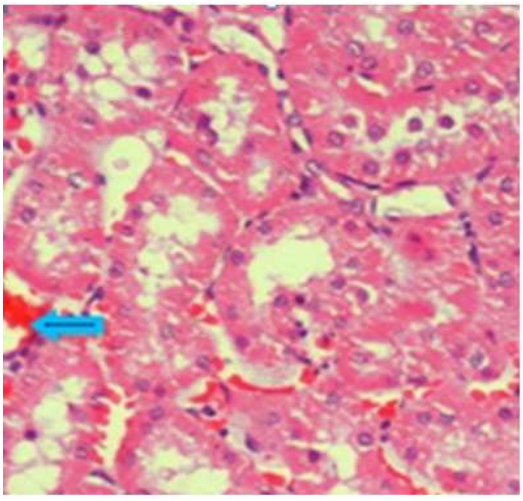


Fig. 6: Section of the kidney of a rat treated with rosemary for eight weeks, showing normal appearance of renal tubules and glomeruli. (HX & Ex400).

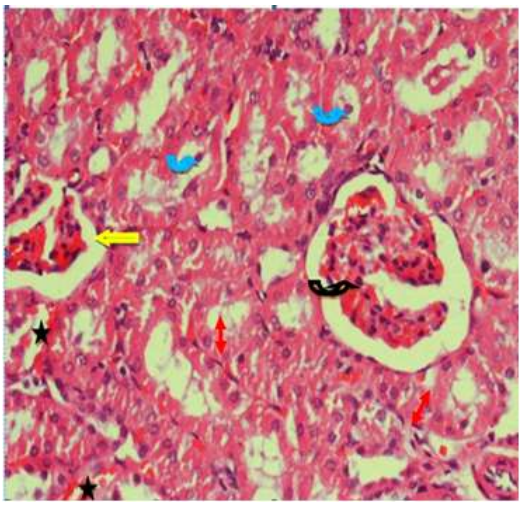


Fig. 7: Section of the kidney of a rat treated with Isoniazid® along with rosemary showing glomerular lobulation (black curved arrow) and others degeneration with wide urinary space (yellow arrow) and cell debris in the lumen of some tubules (blue curved arrow), few of interstitial hemorrhage (star).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

REFERENCES

Abdel-Azeem AS, Hegazy AM, Zeidan HM, Ibrahim KS, El-Sayed EM. Potential Renoprotective Effects of Rosemary and Thyme Against Gentamicin Toxicity in Rats, Journal of Dietary Supplements 2016; 14: 380-394

Adaramoye OA, Kehinde AO, Adefisan A, Adeyemi O, Oyinlola I, Akanni OO. Ameliorative Effects of Kolaviron, a Biflavonoid Fraction from Garcinia KolaSeed, on Hepato-renal Toxicity of Anti-tuberculosis Drugs in Wistar Rats. Tokai J Exp Clin Med, 2016; 41(1): 14-21.

Adhvaryu MR Reddy N, Parabia MH. Effects of four Indian Medicinal herbs on isoniazid and pyrazinamide induced hepatic injury and immunosuppression in guinea pigs. World Journal of Gasteroenterlogy, 2007; 13: 3199-3205.

Akerele O. Summary of WHO Guidelines for the Assessment of Herbal Medicines. Herbal Gram, 1993; 28, 13-19.

Al Sheyab F, Abuharfeil L, Awad D. The effect of rosemary (Rosmarinus officinalis. L) plant extracts on the immune response and lipid profile in mice. J Biol, 2012; 3:1-22.

Alfaro-Lira S, Pizarro-Ortiz M, Calaf GM. Malignant transformation of rat kidney induced by environmental substances and estrogen. Int J Environ Res Public Health, 2012; 9:1630-48

Altinier G, Sosa S, Aquino RP, Mencherini T, Della LR, Tubaro A. Characterization of topical antiinflammatory compounds in Rosmarinus officinalis L. J Agric Food Chem, 55:1718-23.

Amin A and Hamza AA. Hepatoprotective effects of hibicus, rosmarinus and salvia on azathiopreneinduced toxicity in rats. Life Sciences, 2005; 77: 299-278.

Araujo LP, Truzzi RR, Mendes GE, Luz MA, Burdmann EA .Annexin A1 protein attenuates cyclosporine-induced renal hemodynamics changes and macrophage infiltration in rats. Inflamm Res, 2012; 61: 189-196.

Ashtiyani SC, Najafi H, Jalalvandi S, Hosseinei F. Protective Effects of Rosa CaninaL Fruit Extracts on Renal Disturbances Induced by Reperfusion Injury in Rats, IJKD, 2013; 7: 290-298.

Bagshaw SM and Bellomo R. Acute renal failure. Surgery 2007; 25: 391-398.

Barnes J, Anderson LA, Phillipson JD. Textbook of HerbalMedicines, 3rd edn. Pharmaceutical Press, London, Chicago, 2007.

Bayomy MFF, Tousson E, Ahmed AA. Protective role of rosemary against anticancer drug Etoposide-induced testicular toxicity and oxidative stress in rats. ournal of Advanced Trends in Basic and Applied Science, 2017; 1, No.1:88-96.

Bekker Z, Walubo A, Du Plessis JB. Changes in IL-2 and IL-10 during Chronic Administration of Isoniazid, Nevirapine, and Paracetamol in Rats. Advances in Pharmacological Sciences, 2016; 12.

Ben Khedher MR, Hammami M, Arch JRS, Hislop D, Eze D, Wargent ET, Kpczyska MA, Zaibi MS. Preventive effects of *Rosmarinus officinalis* leaf extract on insulin resistance and inflammation in a model of high fat dietinduced obesity in mice that responds to rosiglitazone, Peer J, 2018; 18: 6-4166.

Bozin B, Mimica-Dukic N and Samojli K. Antimicrobial, antioxidant properties of rosemary and age. Essential oils. J Agric Food Chem, 2007; 57:879-7885.

Chaney AL, Marbach CP and Fowcett JK. A colorimetric method for

determination of blood urea concentration. J. Clin. Chem. 1960; 8: PP.130-135.

Chen X, Xu J, Zhang C, Yu T, Wang H, Zhao M. The protective effects of ursodeoxycholic acid on isoniazid plus rifampicin induced liver injury in mice. Eur J Pharmacol, 2011; 659: 53–60.

Choudhury D and Ahmed Z. Drug-associated renal dysfunction and injury, Nature Clinical Practice Nephrology, 2006; Vol 2 No 2, 80-91.

Czaja MJ. Cell signaling in oxidative stress-induced liver injury. Liver Dis, 2007; 27: 378- 389.

Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory disease. Blood, 2011; 117:3720- 3732.

Drury RAB and Wallington EA. Carletons histological technique 4th Ed.Oxford, NewYork, Toronto, Oxdford University Press, 1980.

El-Sherif NM and Issa NM.Protective Effect of Rosemary (Rosmarinus Officinalis) Extract on Naphthalene Induced Nephrotoxicity in Adult Male Albino Rat. J Interdiscipl Histopathol, 2015; 3(1): 24-32.

Endres DB and Rude RK. Mineral and bone metabolism. In: Burtis CA, Ashwood ER, editors. Tietz text book of clinical chemistry. 3rded. Philadelphia: W.B Saunders Company, 1999; pp. 1395 – 1457.

Fahim FA, Esmat AY, Fadel HM, Hassan KF. Allied studies on the effect of Rosmarinus officinalis. L. on experimental hepatotoxicity and mutagenesis. Int. J. Food Sci. and Nut, 1999; 50: 413-427.

Frishman WH, Sinatra ST and Moizuddin M. The use of herbs for treating cardiovascular disease. Semin. Integr. Med, 2004; 2: 23-35.

Gad AS and Sayd AF. Antioxidant Properties of Rosemary and Its Potential Uses as Natural Antioxidant in Dairy Products, Food and Nutrition Sciences, 2015; 6: 179-193.

Gulcin I, Elmastas M and Aboul-Enein HY. Determination of Antioxidant and Radical cavenging Activity of Basil (Ocimumbasilicum L. Family Lamiaceae) Assayed by Different ethodologies. Phytother Res, 2006; 21: 354-361.

Haraguchi H, Saito T, Okamura N and Yagi A. Inhibition of lipid peroxidation and superoxide generation by diterpenoids from Rosmarinus officinalis. Planta Med, 1995; 61: 333-336.

Hosseinzadeh H and Nourbakhsh M. Negative inotropic action of Rosemary oil, 1, 8-cineole and bornyl acetate. Planta Med, 1989; 55: 106-107.

Husdan H and Rupoport A. Estimation of creatinine by Jaffes reactions. Comparison of three method. Clin. Chem, 1969; 138: Pp459-470.

Hussein RM, AbdelAzim SA, Elgoly AHM and Rizk MR. Alterations in Antioxidant Defense System and Oxidative Damage in Experimental Hepatorenal Toxicity Induced by Isoniazid and Rifampicin inRats: Effect of N-Acetyl Cysteine and White Tea Extract. International Journal of Science and Research, (IJSR) ISSN (Online): 2015; 2319-7064.

Ishida YT, Kondo T, Ohshima H, Fujiwara, Iwakura MN. Apivotal involvement of IFN.Y. in the pathogenesis of acetaminophen-induced acute liver injury. FASEB.J, 2002; 16:1227-1236.

Issabeagloo E and Taghizadieh M. Hepatomodulatory Action of Camellia sinensis Aqueous Extract against Isoniazid-Rifampicin Combination Induced Oxidative Stress in Rat, Advances in Bioresearch, 2012; 3 (3): 18 – 27.

Jayaprakasha GK, Tamil S, Sakariah KK. Antibacterial and antioxidant activities

of grape (Vitisvinifera) seed extracts. Food Research International, 2003; 36: 117-122.

Jehangir A, Nagi A, Shahzad HM, Azamzia M. The Hepato-protective Effect of Cassia fistula (Amaltas) Leaves in Isoniazid and Rifampicin induced Hepatotoxicity in rodents. Biomedica, 2010; 26: 25-29.

Johnston JK and Morris DD. Alterations in blood proteins. In B. P. Smith (Ed.), International Animal Medicine (2nd Ed.). USA: Mosby Publishers, 1996.

Jover-Saenz A, Porcel-Perez JM, Madrona AB and Bielsa-Martin S. Acute nterstitial nephritis due to rifampicin. Enfermedades Infecciosasy Microbiology Clinica, 2006; 24:64.

Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. J ClinPathol. 2001; 54: 356-361.

Kumar V, Abbas A and Fausto N. Robbins & Cotran Pathologic Basisof Disease. 7thed. Philadelphia, PA: Elsevier Inc., Saunders, 2004; p. 955-1021.

Laurent A, Perdu-Durand E, Alary JDE, Brauwer L, Cravedi JP Metabolism of 4-hydroxynonenal, a cytotoxic product of lipid peroxidation in rat precision -cut liver slices. Toxicol Lett, 2000; 114:203-214.

Lebrecht D, Setzer B, Rohrbach R, Walker UA. Mitochondrial DNA and its respiratory chain products are defective in doxorubicin nephrosis. Nephrol Dial Transplant, 2004; 19:329-36.

Lee KK and Boelsterli UA. By passing the compromised mitochondrialel ectron transport with methylene blue alleviates favirenz/isoniazid induced oxidant stress and mitochondria-edited cell death in mouse hepatocytes. Redox Biology, 2014; 2: 596- 609

Lopez-Nova JM, Quiros Y, Vicente L. New insights into the mechanism of aminoglycoside nephrotoxicity an integrative point of view. Kidney Int, 2011; 79: 3- 45

Mahmoud A M, Germoush MO, Soliman AS. Berberine attenuates isoniazid-induced hepatotoxicity by modulating peroxisome proliferatoractivated receptor γ, oxidative stress and inflammation. Int J Pharmacol, 2014; 10: 451-460.

Mahmoud AM Morsy BM, Abdel-Hady DS, Samy RM. Prunus armeniacaLeaves Extract Protects against Isoniazid and Rifampicin Induced Nephrotoxicity through Modulation of Oxidative stress and Inflammation. Int J Food Nutr Sci, 2015; 2(2):100-105.

Mahmoud AM, Galaly SR, Ahmed OM. Thymoquinone and curcumin attenuate gentamicin-induced renal oxidative stress, inflammation and apoptosis in rats. EXCLI J, 2014; 13: 98-110.

Martin SJ and Sabina EP. Amelioration of anti-tuberculosis drug induced oxidative stress in kidneys by Spirulina fusiformis in a rat model. J RENAL FAILURE, 2016; VOL. 38, NO. 7, 1115–1121.

Marzieh Z, Saeed C, Saeed H and Akbar H. The study of 24 h post treatment effects of the aqueous extract of Rosmarinus officinalis after renal ischemia/reperfusion in rat. J Phys, 2012; 2:12-9.

Matata BM and Galinanes M. Peroxynitrite is an essential component of cytokines production mechanism in human monocytes through modulation of nuclear factor-kB DNA binding activity. J. Biol Chem, 2002; 277: 2330- 2335.

Montgomery HAC and Dymock JF.The determination of nitrite in water. Analyst, 1961; 86: 414-416.

Mulinacci N, Innocenti M, Bellumori M, Giaccherini C, Martini V, Michelozzi M. Storage method, drying processes and extraction procedures strongly

affect the phenolic fraction of rosemary leaves: An HPLC/DAD/MS study. Talanta, 2011; 85:167-76.

Muthukumar T, Jayakumar M, Fernando E, Muthusethupathi MA. Acute Renal Failure Due to Rifampicin. A study of 25 patients. American Journal of Kidney Disease, 2002; Vol. 40, 690-696.

Nogala-Kalucka M, Korczak J, Dratwia M, Lampart-Szczapa E, Siger A, Buchowski M.Changes in antioxidant activity and free radical scavenging potential of rosemary extract and tocopherols in isolated rapeseed oil triacylglycerols during accelerated tests. Food Chemis, 2005; 93: 227–235.

Ogbera AO, Dada O, Adeyeye F, Jewo PI. Complementary and alternative medicine use in diabetes mellitus. West Afr. J. Med, 2010; 29(3): 158-162.

Ozbek E. Induction of oxidative stress in kidney. Int J Nephrol, 2012; 465-897.

Patrakka J and Tryggvason K. Molecular make-up of the glomerular filtration barrier. Biochem Biophys Res Commun, 2010; 396:164-9.

Perandones CE, Illera VA, Peckham D, Stunz LL, Ashman RF: Regulation of apoptosis in vitro in mature murine spleen T cells. J Immunol, 1993; 151(7): 3521-9.

Ramesh KG, Ashok KG, Sudhansu RS, Vaishali1 GG, Saifuddin K, DidagiK KS, Rajnish KS. Anti-hepatotoxic and antioxidant influence of Ipomoea carneaagainst anti-tubercular drugs induced acute hepatopathy in experimental rodents. Journal of Coastal Life Medicine, 2013; 1:1359.

Raškovic A, Milanovic I, Pavlovic N, Cebovic T, Vukmirovic S, Mikov M. Antioxidant activity of rosemary (Rosmarinus officinalis L.) essential oil and its hepatoprotective potential. BMC Complement Altern Med, 2014; 14:225.

Rasoulian B, Jafari M, Noroozzadeh A, Mehrani H, Wahhab-Aghai H, Hashemi-Madani SM. Effects of ischemia-reperfusion on ratrenal tissue antioxidant systems and lipid peroxidation. Acta Med, 2008; 46:353-436.

Ruiz-Larrea MB, Leal AM, Liza M, Lacort M and HD. lipid peroxidation of rat liver microsomes. Steroids, 59: 383-388.

Sakr SA and Lamfon HA. Protective effect of rosemary (Rosmarinus officinalis) leaves extract on carbon tetrachloride-induced nephrotoxicity in albino rats. Life Sci J, 2012; 9:779-785.

Salih SB, Kharal M, Qahtani M, Dahneem L, Nohair S.Acute interstitial nephritis induced by intermittent use of rifampicin in patient with brucellosis. Saudi Journal of. Kidney Diseases. And Transplantation, 2008; 19(3):450-452.

Schetz M, Dasta J, Goldstein S, Golper T. Drug-induced acute kidney injury. Dec, 2005; 11(6):555-65.

Steel RG and Torrie GH. Principles and procedures of statistics and biometrical approach. 2nd ed. 1960; Pp: 71-117, McGraw-Hill Book Co., New York, Tronto and London.

Stohs SJ, Ohia S and Bagchi D. Naphthalene toxicity and antioxidant nutrients. Toxicology, 2002; 180:97-105.

Taddei I, Giachetti D, Taddei E, Mantovani P, Bianchi E. Spasmolytic activity of peppermint. Sage and Rosemary essences and their major onstituents. Fitoterapia, 1988; 59: 463-8.

Tavakkolia A, Ahmadi A, Razavi BM, Hosseinzadeh H. Black Seed (Nigella Sativa) and its Constituent Thymoquinone as an Antidote or a Protective Agent against Natural or Chemical Toxicities. Iranian Journal of Pharmaceutical Research, 16 (Special Issue), 2017; 2-23.

Thirunavukkarasu C and Sakthisekaran D. Sodium selenite, dietary micronutrient, prevents the lymphocyte DNA damage induced by Nnitrosodiethylamine and phenobarbital promoted experimental hepatocarcinogenesis. J Cell Biochem, 2003; 88: 578–588.

Tietz NW. Fundamentals of Clinical Chemistry, Saunders, Philadelphia, and Sec. Edit, 1976; 876.

Trinder P. Enzymatic colorimetric method of serum glucose. Ann.Clin.Biochem, 1969; 6:24.

Tsakiris S, Schulpis KH, Marinous K, Behrakis P. Protective effect of L-cysteine and glutathione on the modulated sukling rat brain Na^+/K^+-ATPase and Mg2+-ATPase activities induced by the in vitro gulactosaemia. Pharmacol Res, 2004; 49: 475-479.

Verma AK, Yadav A, Dewangan J, Singh SV, Mishra M, Singh PK, Rath SK. Isoniazid prevents Nrf2 ranslocation by inhibiting ERK1 phosphorylation and induces oxidative stress and apoptosis. Redox Biology, 2015; 6: 80-92.

Weiss RF and Fintelmann V. Herbal Medicine: 2nd English edition. New York: Thieme, 2000; 23-30.

WHO. WHO Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems, 2004; Geneva, Switzerland: World Health Organization

World Health Organization Global Tuberculosis Programme. Treatment of Tuberculosis: Guidelines for National Programmes, 3rd edn. (WHO/ CDS/TB/2003.13). Geneva: World Health Organization

Yue J, Dong G, He C, Chen J, Liu Y, Peng R. Protective effects of thiopronin against isoniazidinduced hepatotoxicity in rats. Toxicology, 2009; 264: 185–191.

Zhang B, Sun S, Shen L, Zu X, Chen Y, Hao J. DNA methylation in the rat livers induced by low dosage isoniazid treatment. Environmental toxicology and pharmacology, 2011; 32: 486–490.

Zohrabi M, Ashtiyani SC, Hajihashemi S, Hassanpoor A, Hossein N. The study of 24 h post treatment effects of the aqueous extract of Rosmarinus officinalis after renal ischemia/reperfusion in rat. Journal of Physiology and Pathophysiology, 2012; Vol. 3(2): 12-19.