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Egypt. J. Chem. **Vol. 64,** No. 4 pp. 1817 - 1829 (2021)

Egyptian Journal of Chemistry

Pathogenic Effects of Ethion Residues and the Expected Protective Role of the Ethanolic Extract of Rosemary (*Rosmarinus Officinalis* L.) Leaves in Male Rats

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Abstract

Organophosphate insecticides (OPI) poisoning remains a major cause of morbidity and mortality in the third world countries. The continuous use of these pesticides tends to leave residues of these pesticides in agricultural crops, which in turn may harm people. The present study aimed to investigate the pathogenic effects of the "ethion" residues and the expected protective role of the ethanolic extract of rosemary (Rosmarinus officinalis L.) (EER), in adult male rats. Feeding animals with Maize containing ethion residues (4 mg/Kg) caused elevation in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities ($P<0.01$, $P<0.001$ and $P<0.01$), respectively. Whereas total protein (T.P) and albumin (ALB) were significantly decreased ($P<0.01$ and $P<0.05$), respectively. As well as, an elevation in serum creatinine and urea levels ($P<0.01$). Also, a significant ($P<0.01$) increase in cholesterol, triglyceride and low density lipoprotein (LDL) and a significant ($P<0.001$) decrease in high density lipoprotein (HDL) content as compared to the control were obtained. In addition a decrease in plasma acetyl cholinesterase ($P<0.01$). Decrease in glutathione (GSH) ($P<0.01$) , Superoxide dismutase (SOD), ($P<0.05$), catalase (CAT) P ($P<0.01$) and an increase in Malondialdehyde (MDA) ($P<0.01$) were observed in liver , kidney and brain tissues, respectively as compared with controls. Supplementation with ethanolic extract of rosemary leaves (EER), effectively relieved most of the ethion-induced alterations. The histological investigation strongly confirmed the highly protective effect of the EER in the tissues of selected organs. These findings suggest that rosemary is effective in improving both the function and structure of the examined organs through their potent antioxidant effect.

Keywords: Ethion; maize; rosemary; hepatotoxicity; nephrotoxicity; lipid profile; oxidative stress; histopathology; rats

1. Introduction

The most commonly used class of insecticides is the organophosphorus compounds because they are highly effective and exhibit relatively non-persistent characteristics [1]. Previous studies have shown that OP, affected the functioning of various tissues/organs of animals and humans leading to manifestation of some pathological conditions [2-4]. Ethion *O,O,O,O*-tetraethyl *S,S*-methylene bis (phosphorodithioate) is a member of the organophosphate pesticide family that was first registered for use in the United States in 1965 [5]. It

was first developed as a nonsystemic insecticide and acaricide for use on fruit trees, including citrus and nut trees, cotton, seed and forage crops, and a wide variety of other fruits and vegetables [6]. Ethion used world widely causing public health concern [7].Very recently, ethion and other organophosphorus insecticides were removed from water using activated agricultural waste microstructure and metal organic framework adsorbents [8-11] Oxidative stress has been reported as one of the mechanisms of toxicity of ethion via the enhancement of oxidative stress markers and the disruption of antioxidant balance. In this context, there is the over accumulation of

reactive oxygen species (ROS) [12]. From past to present, herbs and spices have been used all over the world to improve the flavor of beverages and foods, as well as being employed as preservative agents [13]. Human has consumed local herbs and vegetables (medicinal plants) to improve health, and to prevent, protect from and cure diseases. Today, there has been increasing attention in research projects on probable advantages of herbal medications as alternative treatments for disease prevention or as antitoxic agents [14]. Extracts prepared from medicinal plants and other natural sources contain a variety of molecules with potent biological activities [15]. Herbs and plants are rich in phenolic compounds, enzymes, glutathione, vitamins E and C, with antioxidant properties [16]. Many plant extracts and their products have been shown to have significant antioxidant activity which may be an important property of medicinal plants associated with the treatment of several ill fated diseases including liver toxicity [17-19]. Rosemary (Rosmarinus officinalis) is one of household herbs that contains a number of phytochemicals, including rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid, and the antioxidants carnosic acid and it used in traditional medicine to treat a variety of disorders [20]. The most widely used medicinal herbs worldwide is a Rosemary (Rosmarinus officinalis), due to its good antioxidant activity [21]. The most significant feature of the antioxidant activity of Rosemary is the association between diterpenes and radical scavenging activity [22]. Furthermore, the protective effect of rosemary has been reported in several experimental models of liver injury [23] . Nowadays, humans, as well as animals, are exposed to different types of toxic agents directly or indirectly through various pathways including edibles, air, soil, and water [24]. Exposure to either natural or synthetic agents represents a worldwide public health problem. Of course, these toxic agents can produce hazardous toxicities including non-organ directed (carcinogenesis, endocrine disruption, and teratogenicity) or either single or multiple organ-directed noxiousness on liver, kidney, brain, heart, and reproductive system. Food consumption is the major source of pesticide exposure for the general population and dietary risk assessment studies are essential to identify exposure scenarios that could pose a potential health concern to humans [25]. Therefore, the aim of this study assessed the pathogenic effects of ethion residues and curative and protective potentials of ethanolic extract of rosemary using albino Wistar rats exposed to maize containing ethion residues. This was successfully carried out by using some biochemical parameters

including liver, kidney, and lipids profile and antioxidant status. Histopathological examination was also carried out to assess the level of damage caused by ethion residues as well as the protective effect of rosemary extract on damaged organs

2. MATERIALS AND METHODS

Chemicals
Pesticide preparation

Ethion was purified from commercial sources and compared with authentic sample. The Rf values of ethion in different solvent systems (Toluene: Xylene 20: 20; Dioxane: Xylene: Petroleum ether 10: 20: 20 and n-Hexane: Ethyl acetate 98: 2) are 0.75, 0.8 and 0.76, respectively. The absorbance of ethion was measured by UV spectrophotometer (JASCO) at λ=290 nm; the structure of ethion is shown in Figure 1.
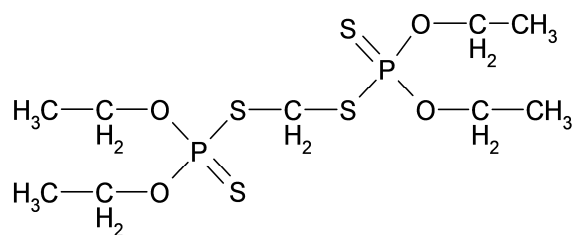

Fig.1.The chemical structure of ethion insecticide

Plant material and experimental design

The Seeds of maize containing a dose of 4 ppm of ethion insecticide as determined previously [26]. Maize plants were cultivated under normal field conditions in a field area as in practice. Shortly at blooming stage, leaves of plants were treated twice, 15 days apart, with ethion at the dose of 4 mg/plant. Maize cobs were collected at harvest time (30 days) after the second spray of ethion, plant seeds were dried, extracted and determination of its residues as described previously [26].

Preparation of rosemary ethanolic extract

Rosemary dry leaves were purchased from the local market in Cairo, Egypt. The leaves were scientifically defined by the herbarium of National Research Centre. The leaves grinded to a fine powder. The plant extract was prepared by addition of ethanol 70% to the fine powder in a firmly closed jar and let

for 3-5 days at room temperature with vigorous shaking twice a day. The mixture was filtered using filter paper, and a rotatory evaporator was used to evaporate the alcohol and obtain the pure extract. The residues were re-extracted by the same method to get the whole constituents of the plant. Ten grams of rosemary extracts were homogenized with 100 mL of distilled water and incorporated in 1 kg of control and Maize seeds were treated and mixed manually for at least 30 min. to ensure complete distribution.

Experimental Animals

Twenty Wistar male rats weighing 95-120g were obtained from the animal house of the National Research Centre, Dokki, Cairo. Rats were acclimatized to the experimental laboratory having temperature $20 \pm 1°C$, controlled humidity conditions (65%). Rats were housed in standard plastic cages, fed with standard diet, and water ad libitum. All experimental procedures were conducted in accordance with ethical guidelines of the Animal Care and Use Committee of National Research Centre.

Experimental protocol

The rats were randomly distributed into four groups of five rats each. The groups are:
Group I: Animals fed with maize seeds free from ethion residues or ethanolic extracts of rosemary (EER) (control).
Group II: Animals fed with Maize seeds free from ethion residues and containing ethanolic extracts of rosemary (EER) (1% w/w).
Group III: Animals fed with maize seeds containing ethion residues (4 ppm).
Group IV: Animals fed with maize seeds containing ethion residues (4 ppm) in addition to ethanolic extracts of rosemary (EER) (1% w/w).

Residue level

The residue levels of ethion insecticide in   liver, kidney, brain, fat and blood were estimated by the method of Marie et al. as modified by Mandal et al. [27, 28]. A colorimetric estimation is based on the extraction of maize seeds containing ethion residues, and   necessary clean-up, followed by hydrolysis to diethylphosphorodithioic   acid. The color complex then formed with copper sulfate is measured   at $\lambda=$ 418 nm. From the optical density the amount of ethion is determined   from a standard curve.

Sampling
Blood samples

Blood samples and organ tissue specimens were collected after overnight fasting from all animal groups at the end of experiment (one month). Blood samples collected from retro-orbital venous plexus of eyes in dry tubes and incubated for 20 min at room temperature to allow clotting for serum separation then centrifuged at 3000 rpm for 10 min, the clean clear serum aspirated by automatic micropipettes, received in dry tubes and kept in refrigerator (2-8 °C) until used for biochemical analysis. The animal was then killed with an overdose of ether, and samples from liver, kidney, brain, fat, and blood were collected and kept frozen till residue analysis

Tissue sample preparation for residual analysis

Tissues (4 g) were extracted for 10 min with acetonitrile (50 ml) and anhydrous sodium sulphate (1.0 g) using a homogenizer. The extract was filtered through anhydrous sodium sulphate (0.5 g) and the tissues were re-extracted twice with acetonitrile. The extract was clarified by centrifugation. The combined acetonitrile extracts were concentrated to 20 ml and partitioned with hexane. The hexane phases were discarded and the acetonitrile phase was evaporated to dryness using a rotary vacuum evaporator at 40 °C for colorimetric estimation.

Tissues samples for histopathological investigations

Selected tissues (liver, kidney and brain) were isolated and fixed in 10% neutral buffered formalin for 24 hours, rinsed with water, dehydrated in alcohols, cleared in xylene, and embedded in paraffin. Tissue blocks were sectioned at 4-5 micron thickness and routinely stained with Haematoxylin and Eosin (H&E) stain (Bancroft and Gamble, 2008) and examined by light microscopy.

Preparation of tissues homogenate

The remaining parts of the tissues were immediately washed in ice cold physiological saline and homogenized to render 10% homogenate in 50mM potassium phosphate buffer (pH 7.4). The homogenate was centrifuged at 4000 rpm for 15 min. at 4 °C. Aliquots of homogenates were used for CAT [29], SOD [30], GSH [31] and MDA [32] estimations.

Measurement of biochemical parameters

Cholinesterase activity was determined according to Ellman method [33] as modified by Gorun et al. [34]. The liver parameters [alanine aminotransferase

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(ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, and albumin], kidney parameters (urea and creatinine), lipid profile, [total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL)] and antioxidant parameters [malondialdehyde (MDA), glutathione (GSH)), catalase (CAT) and superoxide dismutase (SOD)] were purchased from Biodiagnostics (Egypt).

Statistical analysis

The obtained data from serum biochemical and enzymes analysis were statistically evaluated for the mean and standard error of the mean of each group. The significance of the changes between the tests and the control group was evaluated by the "t" test according to Parker method [35].

RESULTS AND DISCUSSIONS

Organophosphate insecticides (OPI) represent a major class of chemicals commonly applied in agricultural pest control [1]. It is efficiently absorbed and rapidly redistributed in various organs as part of their disposal mechanism. The residual analysis of the extracts of blood and different organs of treated rats revealed that, a considerable amount of the parent compound "ethion" was detected and amounted to, liver (0.22± 0.10), kidney (0.08 ± 0.038ppm), brain (0.06 ± 0.038) fat (0.28 ± 0.038ppm), and whole blood (0.14± 0.018 ppm) for animals fed with maize seeds containing ethion residues (4 ppm), respectively at the end the experimental period. (Table 1). Similar observations were reported from studies on the bioavailability of soybean bound residues of dichlorovos [36] and fenitrothion [37]. Also, the data obtained are in line with many other studies which indicate a moderate to high bioavailability of grain-bound 14C-pesticide residues in experimental animals [38, 39].

Table 1

Distribution of ethion residues in different organs of rats fed on maize seeds containing ethion residues   for 30 days

Organs	Ethion residue (ppm)*
Liver	0.22 ± 0.10
Kidney	0.08 ± 0.038
Brain	0.06 ± 0.038
Fat	0.28 ± 0.038
Blood	0.14 ± 0.018

*: Results are expressed as mean ±SD (n=5).

The OPI inhibit the enzyme acetylcholinesterase (AChE) in the central and peripheral (humans only) nervous systems, by binding to and phosphorylating

the AChE [40]. The primary molecular mechanism of action of the organophosphates is their ability to bind with acetylcholinesterase (AChE) enzyme, thus inhibiting AChE, which results in accumulation of acetylcholine [41]. The obtained data in (Table 2) revealed that feeding rats with maize containing ethion residues caused a significant inhibition in the plasma acetylcholinsterase (P<0.02) (Table 2). The inhibitory effect on acetylcholinsterase was expected, as the organophosphate compounds are anti cholinesterase agents for RBC, and plasma ChE, Such findings have been reported since the 1950's by many investigators [42, 43]. Meanwhile, significant inhibition (P< 0.01) was continued when EER supplemented to maize containing ethion   residues group. The result of the present study fully agree with that of Seham et al. (2010) who revealed that oral administration of rosmarinus officinalis (rosemary) extract with a dose of 582.4 mg/kg. (0.5 ml solution/rat) for 4 weeks resulted in a significant reduction of acetylcholine esterase activity in all tested brain areas of adult male albino rats [44]. In the other hand, our results in the present study is in contrast to that of Atwa et al. [45] who revealed that administration of rosmarinus officinalis (rosemary) extract   resulted in a high significant increase i.e. improvement in activity of   acetylcholinesteras enzyme in the brain tissue of rats treated with Acryl amide [45].

Table 2

Effect of ethanolic extract of rosemary on cholinesterase activities of rats treated with maize containing ethion   residues for 30 days

Groups	Plasma-Cholinesterase level (μ mole/ml)
	Means ^{a}±SD
Group I	1.376±0.065
Group II	1.276±0.065
Group III	1.188±0.064**
Group IV	1.15±0.032***

a: results are expressed as mean ± SD (n=5).
 **: Significance at P< 0.02
 ***: Significance at P< 0.01

The role of the liver is not limited to its physiological functions but extends to other important ways of protecting against the risks and side effects of drugs and various chemicals that enter the body of the organism. Liver has also a central role in the, transport and metabolism, xenobiotics clearance, and is therefore highly susceptible to chemical-induced toxicity. The obtained data demonstrated in Table (3) revealed that, feeding rats with   maize seeds containing ethion residues exhibited a significant

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increase in ALT (P<0.01), AST (P<0.001), ALK (P<0.001) activities and significant decrease (P<0.001) in total protein and albumin concentration (P<0.01) compared with control group. According to the data of our study, administration of maize containing ethion residues significantly enhanced the level of ALT, AST, ALP, and decrease of total protein and albumin in the serum which agrees with the context of liver injury. Diminution in protein content is a feature of liver damage and its subsequent fall in the capacity of the liver to synthesize proteins. In the present work, maize containing ethion residues induced a significant decline in the serum level of total protein and, albumin, which affirms liver damage. Herein, the release of markers of hepatic dysfunction into the blood stream might be due to several factors acting simultaneously like alterations in cell membrane integrity with cell damage, the impact of increased production of reactive oxygen species (ROS) and lipid peroxidation [46]. It has been shown that organophosphorus insecticides can elevate the enzymatic activities of ALP, ALT, AST, and LDH [47]. According to Ozer et al. [48] liver enzymes, AST, ALT and, ALK are the common liver damage biomarkers [49] because these enzymes are readily released into the extracellular space by the hepatocytes. Thus, the high levels of AST, ALT and, ALK observed in this study (Table 3) indicate that the organophosphate compound, ethion is hepatotoxic which is confirmed by the presence of ethion residues in liver tissues as mentioned before (Table 1). This hepatotoxic affinity of ethion to the liver and its enzymes has been earlier reported by Abdel-Gawad et al. [6, 43] who posited that ethion causes liver damage in rats. On the other hand, Rosemary administration to treated rats exhibited a significant decrease in serum ALT (P<0.01), AST (P<0.01), ALK (P<0.001), activities and significant increase in total protein (P<0.001), concentration and insignificant increase in albumin as compared with treated group. These results correlate with those found by many authors [50]. On the other hand, protection with rosemary ethanolic extract attenuated to a large extent the disturbance in liver function. These results are run in parallel with the results of Azab et al. [51] who found that, co-administration of nicotine and aqueous extract of rosemary significantly decreased the elevations in the serum ALT, AST, ALP, and GGT activities and increased the levels of serum total proteins and albumin a compared with nicotine treated group in Guinea pigs and also with the results of Abd El Kader et al.[52] who found that a significant improving effect of pre-treatment with rosemary on the altered activities of

serum ALT, AST, GGT and ALP induced by Pb-acetate intoxication. The observed decrease in these serum marker enzymes shows that rosemary preserves the structural integrity of liver against lead-induced damage[52]. Mannaa et al.[53] reported that, the hepatotoxic effects of AlCl3, as indicated by significant Zaugmentations of serum ALT and AST levels can be modified by rosemary supplementation in combination with AlCl3 [53]. These protective effects of rosemary may be attributed to its antioxidant and free radical scavenging activities due to its higher contents of polyphenolic compounds [53, 54]. This result agreed with Amin and Hamza who recorded that, rosemary administration with dose (50 mg/kg) exhibited a decrease in ALT, AST activities and increase albumin concentration [55].

Residual products (urea, creatinin, uric acid, etc.) formed as a result of metabolic reaction of any substance incoming the body is removed from the blood by kidneys which are natural treatment system of the body. The rationale for the use of creatinine or urea measurement to assess renal function is that plasma/serum levels of both reflect glomerular filtration rate (GFR), the parameter that defines kidney function for the clinician. Irrespective of its cause, kidney disease is associated with decrease in GFR, and the severity of kidney disease correlates closely but inversely with GFR [56]. Data presented in Table 4 showed that serum creatinine and urea, levels were significantly (P<0.001) increase in treated rats with maize containing ethion residues as compared to control group. Many pesticides can cause some toxic and adverse effects on the kidney tissues [57]. Kidney is one of the target organs of experimental animals attacked by OP compounds [58]. Pesticides can alter plasma urea, uric acid, and creatinine levels [58, 59]. EER supplementation to rats feed on maize containing ethion residues (as in G4) revealed a significant (P<0.001) decrease in creatinine and urea levels when compared with treated group (G3). The treatment of aspartame administered rats with rosemary extract induced a highly significant decrease in the levels of urea and creatinine when compared with corresponding groups [60]. Pre-administration of rosemary alleviates the harmful effects induced by lead acetate by improvement the kidney functions [61]. Mannaa et al. [53] reported that, renal dysfunctions of AlCl3, as indicated by significant augmentations of serum urea and creatinine levels, can be modified by rosemary supplementation in combination with AlCl3 [53]. The rosemary aqueous extract alleviates the toxicity induced by lead on the kidney through stimulation of endogenous antioxidant defense system [52].

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Table 3

Effect of ethanolic extract of rosemary on liver function of rats treated with treated with maize containing ethion   residues for 30 days

Groups	ALT(U/L)	AST(U/L)	(ALP)(U/L)	T.P (g/dL)	ALB(g/dL)
	Means[a]± SD	Means[a]± SD	Means[a]± SD	Means[a]± SD	Means[a]± SD
Group I	51.6± 10.01	112.4± 9.53	189.0± 11.79	4.33± 0.35	2.31± 0.43
Group II	40.4± 5.77	107.0± 7.42	184.0± 5.83	4.28± 0.192	2.08± 0.12
Group III	91.0± 4.38[***]	174.6± 12.95[****]	263.8± 12.05[****]	2.41± 0.31[****]	1.21± 0.22[***]
Group IV	55.4± 8.73[***]	119.0± 9.11[***]	203.2± 9.01[****]	4.0± 0.142[****]	1.97± 0.07

a: results are expressed as mean ± SD (n=5).
***: Significance at P< 0.01
****: Significance at P< 0.001

Table 4

Effect of ethanolic extract of rosemary on kidney function of rats treated with maize containing ethion residues for 30 days

Groups	Urea (mg %)	Creatinine (mg %)
	Means [a]±SD	Means [a]±SD
Group I	56.4±6.5	1.04±0.07
Group II	48.6±4.34	0.98±0.034
Group III	97±7[****]	2.06±0.07[****]
Group IV	57±5.83[****]	1.04±0.038[****]

a: results are expressed as mean ± SD (n=5).
****: Significance at P< 0.001

Hepatotoxicity is always associated with perturbations in the synthesis and metabolism of proteins and lipids. In our study, a significant increase was observed in the serum level of total cholesterol (P< 0.01), triglycerides (P< 0.001), LDL (P< 0.001) and a marked decrease in HDL (P< 0.001) after feeding rats with maize containing ethion residues (Table 5). This is in agreement with recent studies on bound ethion residue poisoning [6] and on CCl4 poisoning [62].   Several studies have demonstrated a significant increase in the serum-lipid constituents in the experimental animals, treated with different OPI [63, 64]. EER   supplementation also resulted in the significant attenuation in the level of serum, cholesterol (P< 0.01), triglycerides (P< 0.01), HDL (P< 0.001) and LDL (P< 0.001) in serum, toward the control level which again strengthens the hypolipidemic effect of this extract. The hypolipidemic effects of EER extract in our studies were previously reported by Ozkol et al. [64] and Labban et al. [65], respectively. Other reported

investigations are consistent with our results. Also, treatment rosemary oil in rats fed high fat diet modulated the elevation of lipids parameters [66].

Oxidative stress and antioxidant activity

Oxidative stress response can be detoxified by enzymatic defense systems such as GPx and CAT, or non-enzymatic systems by the scavenging action of GSH [67]. Organophosporous compounds have been shown to decrease the activity of antioxidant enzymes and increase ROS formation [68]. The concentrations of Malondialdehyde (MDA) (lipid peroxidation marker) and glutathione (GSH) (a non-enzymatic antioxidant) and the activities of Superoxide dismutase (SOD) and Catalase (CAT) (enzymatic antioxidants) in different studied organs were shown in Tables 6-8.

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Liver tissue

The level of MDA in the hepatic tissue of rats treated with maize containing ethion residues was significantly (P<0.01) elevated compared to the control group (Table 6). Administration of EER to treated rats markedly improve the level of MDA (P<0.01) when compared with treated group (G 3). Furthermore, the treatment with maize containing ethion residues decreased the activities of antioxidant enzymes with significance P<0.05 for SOD and P<0.01 for CAT, as well as GSH content as compared to the control animals. Administration of EER significantly elevated the activity of both SOD (P<0.05), CAT (P<0.01) as well as GSH content (P<0.05) in hepatic tissue towards their levels in the control group.

Kidney tissue

Treatment with maize containing ethion residues caused significant (P<0.01) elevation in MDA level in kidney homogenates as compared with the control group. Feeding with EER induced significant (P<0.01) decrease in MDA level when compared with maize containing ethion residues group (Table 7). Also treatment with maize containing ethion residues led to significant decrement (P<0.05) in SOD activity, CAT activity (P<0.01), and GSH content (P<0.01) in renal tissue as compared to the control group. However, Administration of EER improved the reduction of renal CAT activity (P<0.01); SOD activity (P<0.05) and GSH content (P<0.01) that induced by total ethion.

Brain tissues

In brain tissues, there was a significant (P<0.01) increase of MDA concentration in the maize containing ethion residues group as compared with control (Table 8). Administration of EER significantly (P<0.01) reduced the MDA level but its level is still higher than the control group. The activity of SOD which was observed to be lower in the total ethion group (P<0.05) as compared with control, was also attenuated by administration of EER (P<0.05). Treatment with maize containing ethion residues also produced a significant decrease in the activity of CAT (P<0.01) as compared to that of control group. Administration of EER significantly attenuated the CAT activity (P<0.01) as compared to control group. The GSH level showed markedreduction in the maize containing ethion residues (P<0.01) as compared to control group.

Similarly, Administration of EER reversed this effect (P< 0.01). Our results revealed that ethion residues treatment caused oxidative stress in the liver, kidney and brain of male rats, which is evident from the generation of lipid peroxidation (LPO). in the activity of CAT (P<0.01) as compared to that of control group. Administration of EER significantly attenuated the CAT activity (P<0.01) as compared to control group. The GSH level showed marked reduction in the maize containing ethion residues (P<0.01) as compared to control group. Similarly, Administration of EER reversed this effect (P< 0.01). Our results revealed that ethion residues treatment caused oxidative stress in the liver, kidney and brain of male rats, which is evident from the generation of lipid peroxidation (LPO). LPO is known to disturb the integrity of cellular membranes and implicated in the pathogenesis of various liver and kidney injuries [69, 70]. MDA is a major oxidation product of peroxidized polyunsatured fatty acids and increased MDA content is an important indicator of LPO. Xenobiotics such as pesticides cause increase of the MDA level in tissues [70, 71]. In the present study, also, the levels of SOD, CAT and GSH activity in rats reduced significantly in ethion residues treated group. The decrease in SOD, CAT and GSH could be in response to increased oxidative stress. The change in the activity of antioxidant enzymes have been reported to be an indicator of oxidative stress [72]. Many studies reported that pesticides e.g. chlorpyrifos, triazophos leads to decrease in SOD, CAT and GPx in liver and kidney of rats [58, 69, 70]. EER, with its antioxidant properties, modified antioxidant defense. Haraguchi et al.[73] confirmed that the rosemary is a good scavenger of peroxyl radicals and has ability to block the formation of hydroxyl radical in non-lipid systems as well [73]. It has been reported that many of rosemary constituents possess antioxidant activity [74]. As demonstrated in Tables 6-8, EER showed ability to prevent ethion residues-induced increase in MDA level, which suggests that EER can pre- serve cellular integrity and this may be the consequence of free radical scavenging activity.

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Table 5

Effect of ethanolic extract of rosemary on lipid profile of rats treated with maize containing ethion   residues for 30 days

Groups	Cholesterol (mg %)	Triglycerides (mg %)	HDL (mg %)	LDL (mg %)
	Means [a]± SD	Means [a]± SD	Means [a]± SD	Means [a]± SD
Group I	76±5.83	65.8±5.12	33.84±0.63	13.74±1.09
Group II	69±5.61	59.4±3.34	33.06±0.75	12.8±0.35
Group III	145.2±7.46[***]	125.8±9.12[****]	59.6±4.35[****]	36.4±4.52[****]
Group IV	84.8±4.35[***]	74±4.53[***]	35.98±0.90[****]	15.96±0.46[****]

a: results are expressed as mean ± SD (n=5).
***: Significance at P< 0.01
****: Significance at P< 0.001

Table 6

 Effect of ethanolic extract of rosemary on oxidative stress related parameters in liver tissues of rats treated with maize containing ethion residues for 30 days

Groups	MDA (nmol/g tissue)	GSH (mg/g tissue)	SOD (U/g tissue)	CAT (U/g tissue)
Group I	29.55±5.84	9.42±2.09	486.10±74.66	1.89±0.14
Group II	32.19±8.65	10.22±2.11	478.60.±87.29	1.84±0.17
Group III	133.83±13.39[**]	2.77±1.11[**]	260.34±76.72[*]	0.77±0.15[**]
Group IV	92.14±14.42[**]	5.34±1,56[*]	315.489±65.96[*]	1.15±0.17[**]

Results are expressed as mean ± SD (n=5).
* Significant at P<0.05
**Significant at p<0.01

Table 7

Effect of ethanolic extract of rosemary on oxidative stress related parameters in renal tissues of rats treated with maize containing ethion residues for 30 days

Groups	MDA (nmol/g tissue)	GSH (mg/g tissue)	SOD (U/g tissue)	CAT (U/g tissue)
Group I	26.57±6.04	22.82±1.86	449.01±56.86	1.86±0.10
Group II	29.72±4.29	20.30±2.66	425.19±72.14	1.68±0.15
Group III	99.62±10.55[**]	8.62±1.16[**]	274.42±70.46[*]	0.68±0.17[**]
Group IV	64.15±10.52[**]	15.16±3.25[**]	335.89±87.03*	1.17±0.17[**]

Results are expressed as mean ± SD (n=5).
* Significant at P<0.05
**Significant at p<0.01

Table 8

Effect of ethanolic extract of rosemary on oxidative stress related parameters in brain tissues of rats treated with maize containing ethion residues for 30 days

Groups	MDA (nmol/g tissue)	GSH (mg/g tissue)	SOD (U/g tissue)	CAT (U/g tissue
Group I	19.50±4.44	14.53±1.19	391.34±49.56	1.74±0.09
Group II	21.82±3.15	12.92±1.69	370.58±62.88	1.57±0.14
Group III	73.12±7.75[**]	5.49±0.74[**]	239.17±61.14[*]	0.63±0.16[**]
Group IV	47.08±7.73[**]	9.65±2.07[**]	292.75±75.85*	1.09±0.16[**]

Results are expressed as mean ± SD (n=5).
* Significant at P<0.05
**Significant at p<0.01

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Histopathological effect of ethion on selected tissues

The histopathological examination of control liver sections showed normal histological structure (Fig. 2. A). Examined sections exposed to maize containing ethion residues showed severe congestion of central veins and hepatic sinusoids (Fig. 2. B), associated with severe necrosis, vacuolar degeneration of hepatocytes and mononuclear inflammatory cells infiltration (Fig. 2. C).this pathologic picture was improved in animals treated with Rosemary (Fig. 2. D). The histopathological examination of liver revealed severe congestion of central veins and hepatic sinusoids, associated with severe necrosis, vacuolar degeneration of hepatocytes and mononuclear inflammatory cells infiltration due to pesticide treatment as documented previously [75]. The exposure to the pesticides impair the liver function and to cause injury to the hepatic tissues [76]. The drastic alterations observed in the liver tissues due to pesticide exposure suggested hepatotoxic effects as reported by Iseri et al.[77]. Control kidney sections showed normal histological features (Fig. 3. A). Male rats feeding with maize containing ethion residues showed severe interstitial nephritis, hemorrhage and edema (Fig. 3. B), associated with severe infiltration with inflammatory cells (Fig. 2. C). apparently improved with the use of Rosemary (Fig. 3. D). The most prominent histopathological alterations of maize containing ethion residues treated rats showed severe interstitial nephritis, hemorrhage and edema, associated with severe infiltration with inflammatory cells as previously concluded by Mamun et al.[78]. Sections from control and EER treated groups showed normal appearance of the cerebral cortex (Fig. 4. A) Exposure to maize containing ethion residues showed important degenerative changes in brain tissue in the form of congestion of cerebral blood vessels, edema vacuolations associated with diffuse gliosis (Fig. 4. B, C). The brain showed less congestion, degeneration and less edema in group received total Ethion and Rosemary extract (Fig. 4. D). Ethion showed important degenerative changes in brain tissue in the form of congestion of cerebral blood vessels, edema vacuolations as mentioned by Abdel-Salam et al. [79] who stated that treatment with only OPIs causes' neuronal damage in the cerebral cortex and degeneration of some Purkinje cells in the cerebellum. The oxidative stress increased in the brain tissue suggesting increased generation of free radicals leading to neuronal injury [68, 80, 81] as well as decreased antioxidant enzyme activities [68, 82].

The administration of EER has been reported to attenuate and ameliorate the alterations caused by ethion residues in male rat
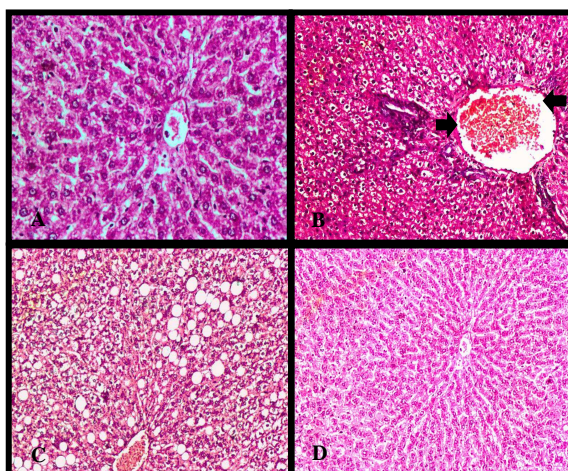
Fig. 2. Photomicrographs of H and E stained Liver sections. **Fig. A.** Control rat shows normal histologic structure of liver (X 200). **Fig. B.** shows severely congested central veins **(Arrows)** (X 100)**. Fig. C.**Shows severe necrosis and vacuolar degeneration of hepatocytes exposed to Total Ethion (X 100). **Fig. D.** Liver of rat treated with Total Ethion and Rose Mary showing apparent normal hepatocytes (X 100).
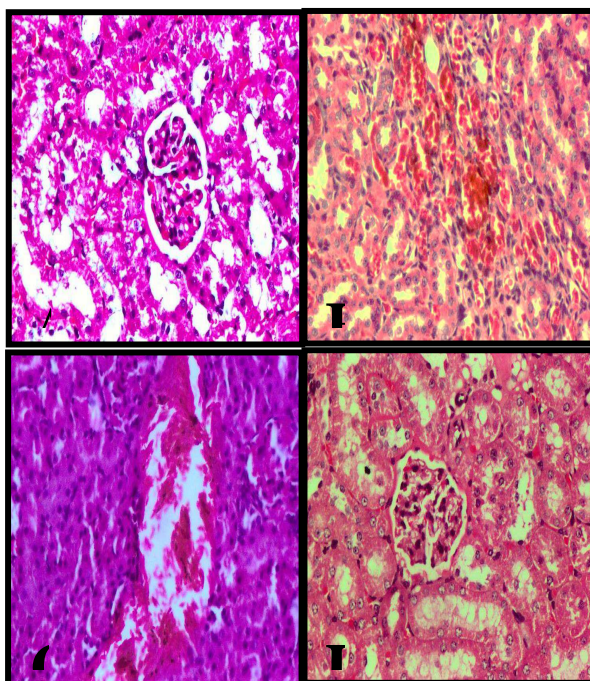
Fig. 3. Photomicrographs of H and E stained Kidney sections. **Fig. A.** Control rat shows normal histologic structure of kidney. **Fig. B.** shows severe hemorrhage**. Fig. C.**Shows severe interstitial nephritis of rats exposed to Total Ethion. **Fig. D.** Kidney of rat treated with Total Ethion and Rosemary showing apparent normal renal tissue (X 200).
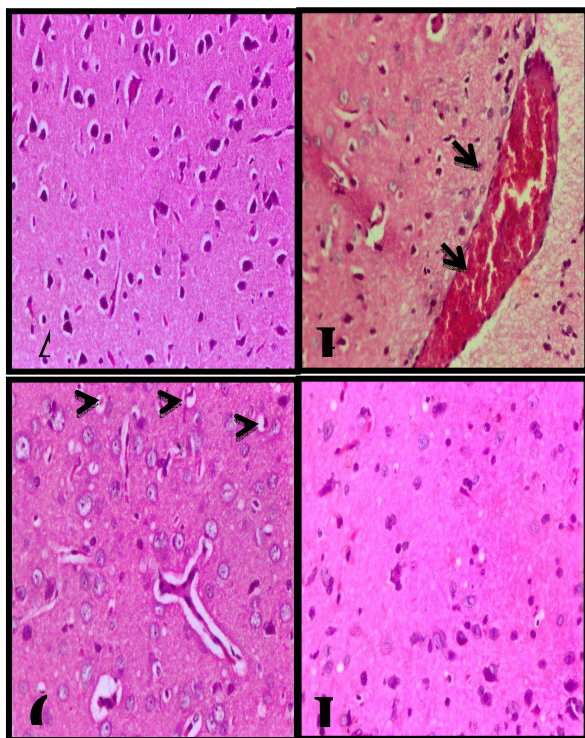
Fig. 4. Photomicrographs of H and E stained Brain sections. Fig. A. Control rat shows normal histological structure of brain. Fig. B. shows severe congestion and hemorrhage of cerebral blood vessels (Arrows). Fig. C. Shows vacuolar degeneration, necrosis and neuronal oedema in brain exposed to total Ethion. Fig. D. brain of rat treated with Total Ethion and Rosemary showing apparent normal histology (X 200).

3. Conclusions

The findings of present study concluded that exposure of rats to ethion residues induces a state of pathological changes of some biochemical parameters including liver, kidney, lipids profile as well as, antioxidant status, which results in increase ALT, AST, ALP, creatinine, urea, total cholesterol, triglycerides, LDL and MDL and decrease of total protein, albumin, HDL, AchE, GSH, SOD and CAT. This protective effect of rosemary may be attributed to the antioxidant and anti-inflammatory properties of one or more of its constituents. In addition, EER administration appears almost able to improve ethion residues-associated biochemical, antioxidant status and histopathological changes in rat.

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