Ahmed et al. Annals of Veterinary and Animal Science 2019
http://naturepub.org/navas
[Received: 13 Jun 19, Accepted: 23 Sep 19, Published: 30 Oct 19]

eISSN: 2313-5514
pISSN: 2312-9123
DOI:10.26609/avas.652

AVAS
Annals of Veterinary and Animal Science

RESEARCH ARTICLE V:6(5) OPEN ACCESS

Effects of rosemary oil against hemato-biochemical alterations and renal oxidative damage induced by Paracetamol in rats

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ABSTRACT

The present study was conducted to evaluate the reno-protective effect of rosemary oil (RO) against paracetamol (PCM) toxicity in rats by monitoring the hematological and biochemical parameters as well as renal oxidative stress parameters. Forty male albino rats were divided equally into 4 groups as follow, negative control group (Cont.), paracetamol-intoxicated group (PCM) (500 mg/kg BW), rosemary oil-treated group (RO) (250 mg/kg BW) and PCM+RO group treated with those previously mentioned doses. Rats were orally treated with their relevant doses daily for 6 weeks. Our result revealed that PCM treatment did not affect the erythrogram parameters meanwhile total leukocytic count was significantly elevated. Moreover, liver enzymes activities, glucose, cholesterol, and triglyceride levels were significantly increased in PCM intoxicated group. Also, PCM toxicity resulted in a significant elevation in the kidney function biomarkers and renal malondialdehyde meanwhile, the renal antioxidant system was significantly reduced (reduced glutathione, superoxide dismutase, and catalase). The rosemary oil improved the adverse effects induced by PCM on liver and kidney function biomarkers that were confirmed histopathologically. In conclusion, the treatment with natural antioxidants like rosemary oil may have a potential protective role against PCM-induced toxicity.

Keywords: Hepatorenal damage; Paracetamol; Oxidative stress; Rats; Rosemary oil.

INTRODUCTION

Acetaminophen (paracetamol PCM, N-acetyl p-aminophenol APAP), the most widely used analgesic and antipyretic drug in the world, is safe when used within the clinically recommended doses (Rosita et al., 2018). It exerts its analgesic effect by interacting with the cannabinoid receptors in the central nervous system (Dani et al., 2007). PCM traditionally not classified as an NSAID because of its week anti-inflammatory activity (Kadowaki et al., 2012).

Excessive use of paracetamol is commonly associated with hepatic and renal failure (Toklu et al., 2006; Stern et al., 2005), approximately, 1-2 % of the patient with an overdose of PCM exhibits renal failure which may occur even in the absence of liver injury (Prescott, 1993). Its toxicity is mediated by its toxic metabolite N-acetyl -P-benzoquinoneimine (NAPQI) which is detoxified by reduced glutathione (GSH). Therefore, an overdose of paracetamol leads to GSH depletion and accumulation of NAPQI that resulted in oxidative damage, cell death, and organ dysfunction (Zariyantey et al., 2012). These injuries are known as acetaminophen hepatoxicity and analgesic nephropathy in the liver and kidney (Lee, 2004).

Natural herbs are commonly used by humans daily, these natural products have many biological and pharmacological properties (Hosseinimeher, 2014) that can improve health, alleviate illness and protect against chronic diseases (Abdul-Rahim and Taha, 2011). Among these herbs is rosemary (*Romarinus Officinalis*), which has been widely consumed for different medicinal purposes. In traditional medicine, rosemary effective in curing headache, poor circulation, inflammation as well as physical and mental fatigue (Raskovic et al., 2014). Most pharmacological effects of rosemary are attributed to the potent antioxidant activity of its chemical constituents such as carnosol, carnosic acid, ursolic acid, rosmarinic acid, and caffeic acid (Ngo et al., 2011). According to the recommendation of European Medicine Agency (EMA) from 2010, the essential oils isolated from rosemary which include 1,8-cineole, camphor and α-pinene can be used as anti-spasmodic, anti-inflammatory, anti-depressant, cognition-enhancing, DNA protective and anticancer (Juhas et al., 2009;

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Cheung and Tai, 2007). Moreover, rosemary contains 19 chemicals with antibacterial activity and several volatile oils which reduces airway constriction caused by histamine **(**Hanan, 2012). Thus, this study aimed to investigate the protective effect of rosemary oil against the hematological, biochemical, antioxidant, and histopathological alterations in paracetamol-intoxicated rats.

MATERIALS AND METHODS

Chemicals

Rosemary oil: Pure essential oil produced by Sigma –Aldrich. CAS: 8000-25-7. Flp: 49 ºC. Paracetamol (Panadol tablet) was purchased from (GlaxoSmithKline) and dissolved in distilled water immediately before each use.

Experimental animal and design

Forty male albino rats of 1-2 month old (average body weight 120 g) were obtained from the Helwan farm of Laboratory Animals (Ministry of Public Health). The animals were acclimatized under standard laboratory conditions for 2 weeks before dosing and had free access to standard diet and water ad-libitum. Animals were handled following the animal welfare and Research Ethical Committee of the Faculty of Veterinary Medicine, Mansoura University, Egypt. Rats were randomly divided into four equal groups as follows, negative control (Cont.) take normal saline, paracetamol intoxicated group (PCM) treated with 500 mg/kg BW of PCM according to Pathan et al. (2013), Rosemary oil group (RO) treated with rosemary oil at a dose of 250 mg/kg BW (Rilson et al., 2014) and paracetamol plus rosemary oil group (PCM+RO) treated with PCM at a dose of 500 mg/kg BW & rosemary oil at a dose of 250 mg/kg BW. Rats were treated orally with their doses daily for six weeks.

Blood samples

At the end of the experiment, two blood samples were withdrawn from the medial canthus of the eye, the first sample collected in epipendorf tubes with EDTA for hematological examination and the second blood samples were collected in clean test tubes and allowed to clot, then centrifuged for ten minutes at 3000 rpm. The serum was separated and stored in epipendorf tubes at – 20ºC to be used for biochemical analysis.

Hematological parameters

Whole blood used for the determination of erythrocyte count, total leukocyte count (TLC), hemoglobin content, and PCV%.Then mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values were calculated (Feldman et al., 2000).

Biochemical parameters

Serum ALT and AST were determined using ready-made kits offered by Vitro Scient (Germany) according to Reitman and Frankel (1957). ALP was detected by using ELITech kits according to Young, (1997). γ-GT was estimated by the kinetic method using ready-made kits provided by Vitro Scient according to Szasz et al., (1979). Glucose, cholesterol, triglycerides, and uric acid were measured spectrophotometrically by using Spinract kits according to Young (2001), while total protein and albumin were estimated according to Dumas and Biggs (1972). Serum urea was estimated by using the Diamond kit according to Numann et al., (1977). Creatinine measured by using ready-made kits available by Human according to Henry et al., (1974). Serum sodium and potassium (Na^+ & K^+) were estimated by using kits presented by Spectrum according to Henry et al., (1974) and Tietz (1976) respectively.

Measurement of renal antioxidant and oxidative stress markers

The rats were euthanized by cervical dislocation, then one gram of renal tissues was collected from each rat then washed by chilled 0.85% NaCl solution and homogenized in 9 ml ice-cold PBS (PH7.5) using homogenizer instrument. The homogenate was cold centrifuged for 15 minutes at 3000 rpm and the supernatant (Ferdandez-Botran et al., 2002) then used

for the measurement of renal MDA, GSH, SOD, and CAT spectrophotometrically using commercial kits provided by Bio-diagnostic, Egypt, according to the methods of Ohkawa et al. (1979), Aebi (1984), Beutler et al. (1963) and Nishikimi et al. (1972) respectively.

Histopathological studies

Specimens from kidney and livers were fixed in 10% neutral buffered formalin. Section of 5-micron thickness was prepared and stained by hematoxylin and eosin (H&E) and examined microscopically according to Bancroft et al. (1990).

Statistical analysis

Data were analyzed using the statistical software program (SPSS for Windows, version 20, USA). Means and standard errors for each variable were estimated. Differences between means of different groups were carried out using one way ANOVA with Duncan multiple comparison tests. Dissimilar superscript letters in the same row show a significance ($P<0.05$). Graphs were drawn by using GraphPad Prism version 5 (GraphPad Software Inc.)

RESULTS

Hematological results

The RBCs count, Hb concentration, PCV as well as blood indices (MCV, MCH, and MCHC) were insignificantly changed in all investigated groups. Additionally, TLC was significantly increased in PCM intoxicated rats in comparison with the control group. Moreover, there were insignificant variations in the value of TLC in PCM+RO and RO treated groups compared either to the control or PCM group (Table 1).

Biochemical results

Liver function biomarkers

The serum activities of ALT, AST, ALP, and γ-GT as presented in Table (2) were significantly elevated in PCM intoxicated rats comparing with the control group. Additionally, treatment with rosemary oil in PCM+RO insignificantly affects the serum ALT and ALP activities as compared to the PCM group. Meanwhile, AST and γ-GT activities were significantly decreased but not return to their normal values. Our result showed that rosemary oil treatment alone (RO) did not alter the liver enzymes activities as compared with the control group.

Total protein, albumin, globulin, and A/G ratio were insignificantly changed in all groups except albumin level in PCM intoxicated rats were significantly reduced as compared to all examined groups (Table 2).

Cholesterol, triglyceride and glucose levels

As we have seen in Table (2) the serum glucose, cholesterol, and triglyceride levels were significantly increased in PCM intoxicated rats as compared to all investigated groups. Moreover, all the above-mentioned parameters were insignificantly changed in RO treated group as compared to the control one.

Kidney function markers

PCM intoxication induced a significant elevation in the serum urea, uric acid, and creatinine levels compared with all examined groups. Also, serum Na^+ level was significantly decreased meanwhile, serum K^+ level was significantly increased in PCM intoxicated group comparing with the control one. These alterations were significantly improved in PCM+RO treated rats but, not returned to its normal values. Moreover, all the above-mentioned renal biomarkers insignificantly differed in RO treated group comparing to the control one (Figure 1)

Oxidative stress and antioxidant markers in renal homogenate

A significant elevation in renal MDA level with a significant reduction in renal GSH and SOD activity was observed in PCM intoxicated group but, renal CAT activity was insignificantly differed as compared to the control rats. A slight improvement in MDA and GSH levels was recorded in PCM+RO treated group compared to the PCM intoxicated one but, SOD activity was significantly improved. Moreover, treatment

with rosemary alone in the RO group showed an insignificant effect on MDA, CAT, GSH, and SOD values as compared to the control (Figure 2).

Histopathological results

Kidney

Microscopically renal tissue in the PCM administrated group revealed a proliferation of the mesangial cells of renal glomeruli and necrosis of the renal tubular epithelium (Figure 3, B). Normal renal glomeruli and normal renal tubules lined by normal renal tubular epithelium were observed microscopically in control and RO as well as the PCM+RO group (Figure 3 A, C, D).

Liver

Histopathological studies of liver tissue in PCM intoxicated rats showed necrosis of hepatocytes (arrowhead) and fibroblastic proliferation in interstitial tissue (arrowhead) (Figure 4, B). Meanwhile, the liver of PCM+RO showed marked dilatation of the central vein and hepatic sinusoid (arrow) (Figure 4, C). Normal hepatocytes and normal portal areas with the normal portal vein (PV) were observed microscopically in the liver of rats treated with RO (Figure 4, D). Also the liver of the control group showed normal hepatocytes (arrow) and normal radial arrangement around the central vein (CV) (Figure 4, A).

DISCUSSION

Herbal medicine derived from plant extracts is being commonly used for treating a wide variety of chronic diseases due to their protective and antioxidant effects (Frei and Higdon, 2003). PCM is an effective analgesic and antipyretic drug that over-dose induces oxidative stress leading to sever hepatic and renal damage **(**Bajt et al., 2004). Our study evaluated the protective effect of rosemary oil in PCM intoxicated rats.

The erythrogram result in our study revealed that PCM treatment insignificantly affects RBCs count, Hb conc., PCV, MCV, MCH and MCHC at 6th week PT. In contrast, Saleh et al. (2018) recorded a significant decrease in the previously mentioned hematological parameter in paracetamol treated rats and explained that by the inability of damaged liver to produce erythropoietinogen **(**Yousef et al., 2010). Furthermore, paracetamol resulted in the destruction of mature erythrocytes due to oxidative damage and inhibit erythropoietin enzyme produced from the kidney (Oyedeji et al., 2013; Dwivedi et al., 2015). The difference may be attributed to the difference in PCM doses.

Leukocyte picture in our work represented that paracetamol intoxication induced leukocytosis, that may be a result of stress and inflammatory condition in body tissue

Table (1): Hematological results at the 6th week post-treatment with rosemary oil in paracetamol intoxicated rats.

Parameters	Treatment			
	Cont	PCM	RO	PCM+RO
RBCs 10^6/µL	6.21 ± 0.50[a]	7.03 ± 0.47 [a]	6.13 ± 0.24 [a]	7.07 ± 0.21 [a]
Hb g/dl	12.83 ± 0.43[a]	12.37 ± 0.55 [a]	12.99 ± 0.22[a]	12.46± 0.31[a]
PCV %	41.00 ± 0.55 [a]	45.80 ± 0.58 [a]	42.96 ± 0.47[a]	44.20±0.37 [a]
MCV fl	68.69 ±5.29[a]	64.98 ±3.13[a]	70.09 ±2.60 [a]	62.47 ± 3.21[a]
MCH pg	21.30±2.13[a]	17.77 ±0.90[a]	21.33 ±1.12 [a]	17.70 ±0.74 [a]
MCHC%	30.85±1.04[a]	27.43 ±1.12[a]	30.43 ± 1.09 [a]	28.55 ±1.50[a]
TLC 10^3/µL	16.14 ±0.75[b]	18.55±0.56[a]	17.06 ± 0.84[ab]	17.12±0.30[ab]

Cont(-ve control), **PCM** (paracetamol), **RO**(rosemary oil), **PCM+RO** (paracetamol and rosemary oil)
The different superscripts letters in the same row show a significant difference between groups (P<0.05) (Mean± SE).

responsible for toxic substance phagocytosis (Bhaumik and Sharma, 2002) or, attributed (Cytochrome P450) enzymes to NAPQI. NAPQI increase the formation of ROS

Table (2): Serum biochemical parameters at the 6th week post-treatment with rosemary oil in paracetamol intoxicated rats

Parameters	Treatment			
	Cont	PCM	RO	PCM+RO
ALT U/L	18.40 ± 0.97^{b}	30.40 ± 0.97^{a}	17.60 ± 0.97^{b}	27.60 ± 1.32^{a}
AST U/L	35.20 ± 2.93^{b}	47.20 ± 2.93^{a}	34.40 ± 2.71^{b}	37.20 ± 1.49^{b}
ALP U/L	436.14±17.29^{b}	552.14±45.44^{a}	387.36±5.81^{b}	523.90±17.85^{a}
γ-GT U/L	10.55± 1.26^{b}	19.30 ± 1.30^{a}	10.54± 1.01^{b}	14.40 ± 1.68^{b}
Total Protein g/dl	8.20 ± 0.18^{a}	7.65 ± 0.19^{a}	8.20 ± 0.32^{a}	8.30 ± 0.37^{a}
Albumin g/dl	3.83± 0.14^{a}	3.14 ± 0.16^{b}	3.82 ± 0.03^{a}	3.79 ± 0.09^{a}
Globulin g/dl	4.37 ± 0.17^{a}	4.51 ± 0.17^{a}	4.40 ± 0.07^{a}	4.51 ± 0.36^{a}
A/G ratio	0.88 ± 0.05^{a}	0.70 ± 0.05^{a}	0.89 ± 0.07^{a}	0.86 ± 0.08^{a}
Glucose mg/dl	88.39 ± 3.28^{b}	111.52±10.34^{a}	88.51±3.70^{b}	110.85±9.99^{ab}
Cholesterol mg/dl	75.23 ±1.24^{c}	107.42± 4.58^{a}	75.67 ±1.81^{c}	92.14 ± 4.77^{b}
Triglyceride mg/dl	154.24±11.95^{b}	199.06±7.52^{a}	146.70±11.15^{b}	167.86±7.40^{b}

Cont (-ve control), **PCM** (paracetamol), **RO**(rosemary oil), **PCM+RO**(paracetamol, and rosemary oil) *The different superscripts letters in the same row show a significant difference between groups (P<0.05) (Mean ± S.E).*

to the body defense mechanism that trying to protect the body against infections following the liver failure induced by PCM (Seriki et al., 2015). Similarliy, Hosny et al.(2019) reported leukocytosis in rats administrated with 500 mg/kg BW PCM orally for 5 days. In contrast, Dwivedi et al. (2015) clarified that rats intoxicated with a high dose of paracetamol had a significant decrease in the leukocytic count. Further, rosemary oil in PCM+RO improved the alteration induced by paracetamol in the leukogram that was in parallel with Mohamed et al. (2016) in lead intoxicated rabbits treated with rosemary ethanolic extract at dose 30 mg/kg for 4 weeks.

The biochemical analysis of liver function can provide avaluable assessment of liver condition, the elevation in serum ALT, AST and ALP activities reflected hepatocellular damage (Yen et al., 2007). The result of our study illustrated that PCM induced hepatotoxicity demonstrated by remarkable increase in ALT, AST, ALP, and γ-GT activities. This elevation may be attributed to the overdose of PCM which is metabolically activated by CYP (reactive oxygen species) and RNS (reactive nitrogen species) which can attack DNA, protein and phospholipids lead to lipid peroxidation and depletion of antioxidant enzymes results in oxidative stress (Hinson et al., 2004) as well as hepatic necrosis. Consequently, leakage of hepatic cytosolic enzymes (ALT, AST,and ALP) leading to their elevation in the serum. In the same line, Abdel-Zaher et al. (2007) , and Suchismita et al. (2015) reported that toxic dose of PCM lead to hepatic oxidative damage and subsequent increase serum ALT, AST,and ALP activities. The elevation of serum ALP and γ-GT activities in PCM intoxicated rats may be due to hepato-biliray damage due to intra-hepatic cholestasis and biliray cirrhosis **(**Olaleye et al., 2010; Monira and Naima, 2012). Our result agrees with what was previously stated by Sivakrishnan and Kottaimuthu (2014) and El-Shafey et al. (2015) in paracetamol intoxication.

The co-administration of rosemary oil in PCM+RO group failed to ameliorate the hepatotoxic effects of paracetamol that may be explained by the rapid absorption,

metabolism, and excretion of rosemary oil (Kohlert et al., 2000). But, it should be noted that γ-GT activity was significantly ameliorated in PCM+RO group compared with PCM intoxicated rats. Our result extract enhance antioxidant status and reduce lipid peroxidation in damaged membranes.

PCM significantly reduced serum total protein and albumin levels but, globulin

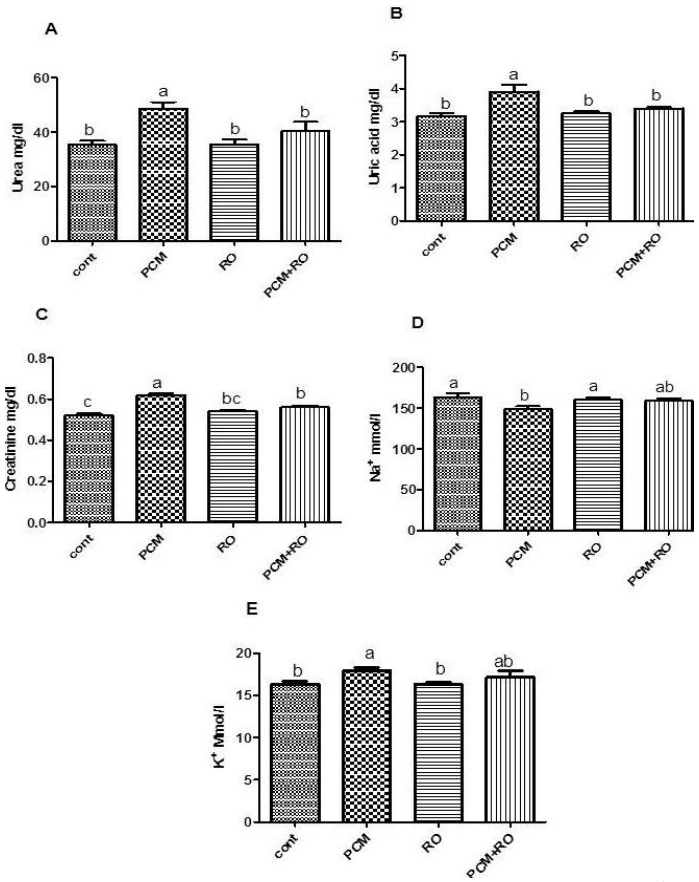

Fig. (1) : Renal function biomarkers in all experimental groups at the 6th week post-treatment with rosemary oil in PCM intoxicated rats.(**A)** Urea, (**B)** Uric acid, **(C)** Creatinine, (**D)** Na^+ **(E)** K^+ levels. Values with different superscripts letters within the same figure are significantly different (p <0.05)

partially agrees with Abd El-Ghany et al. (2012) who mentioned that treatment with rosemary powder in carbon tetrachloride (CCL_4) intoxicated rats significantly ameliorated the elevated serum activities ALT, AST, ALP and γ-GT . As rosemary preserve the structural integrity of liver cells (Monira et al., 2012), also rosemary essential oils contain a substance that direct the breakdown of peroxides into stable substances so that it delays the oxidation rate **(**Doha et al., 2010). Likewise, Labban et al. (2014) clarified that rosemary leaves

level insignificantly changed. This hypoproteinemia may be attributed to impaired protein synthesis by damaged liver tissue induced by PCM **(**Kumar et al. 2009; Monira and Naima, 2012), or may be referred to the nephrotoxic effect of PCM that induces albumin loss through the urine leading to decrease serum total protein level. Also, albumin depletion can be related to acute phase response (Iyanda and Adeniyi, 2011). Our result in accordance with Ekam et al. (2012), Dwivedi et al. (2015) and Ogah et al. (2016) in acetaminophen intoxicated

rats. Further, RO treatment in PCM+RO group induced a significant improvement in albumin level that get along with Selmi et al. (2017) who recorded that intraperitoneal injection of rosemary oils in diabetic rats induced asignificant elevation in albumin level.

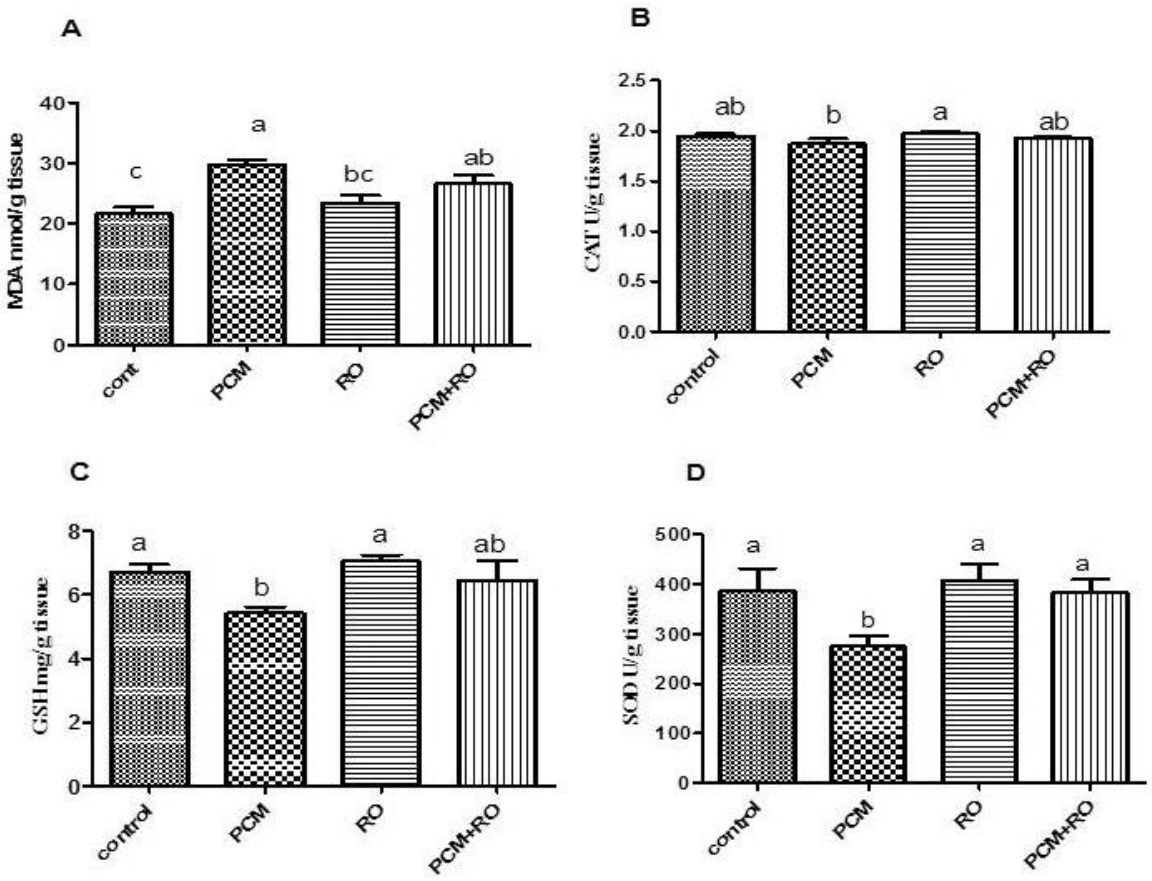

Fig. (2): Renal oxidative stress and antioxidant parameters in all experimental groups at the 6th week post-treatment with rosemary oil in PCM intoxicated rats. (**A)** MDA, **(B)** CAT, **(C)** GSH and **(D)** SOD activities. Values with different superscripts letters within the same figure are significantly different (p <0.05).

The serum glucose level was significantly increased in PCM intoxicated rats, this change may be related to liver dysfunction because liver is the main organ responsible for glucose metabolism (Saleem et al., 2010; Sharma and Rathore., 2010). This result is confirmed by Saleh et al. (2018) who reported a significant hyperglycemia in rats received 300 mg/kg B.WT PCM for 30 days. Administration of rosemary oil in PCM+RO group has corrective effect by reducing the elevated blood glucose level this may referred to antidiabetic properties of its flavonoids content which improves altered glucose and oxidative metabolism of diabetic cases (Suzuki et al., 2002). Also, Chauhan et al. (2008) and Mandal et al. (2008) stated that medicinal plant extract activate pancreatic β cell lead to increase secretion of insulin. Likewise, Naglaa, (2015) found a significant decline in blood glucose level after treatment with rosemary in diabetic and hypercholesterolemic rats.

Our data revealed that total cholesterol and triglycerides levels were significantly elevated in PCM intoxicated rats. That, indicating the ability of PCM to induce oxidative stress and formation of free radicals which disturb cholesterol catabolism

into bile acids (Abdel-Zaher et al., 2007; Ferreira et al., 2011). Also, PCM reduces lipase enzyme activity resulted in decrease triglyceride hydrolysis and distrubs lipid metabolism due to hepatic cell damage induced by PCM (Dwivedi et al., 2015).

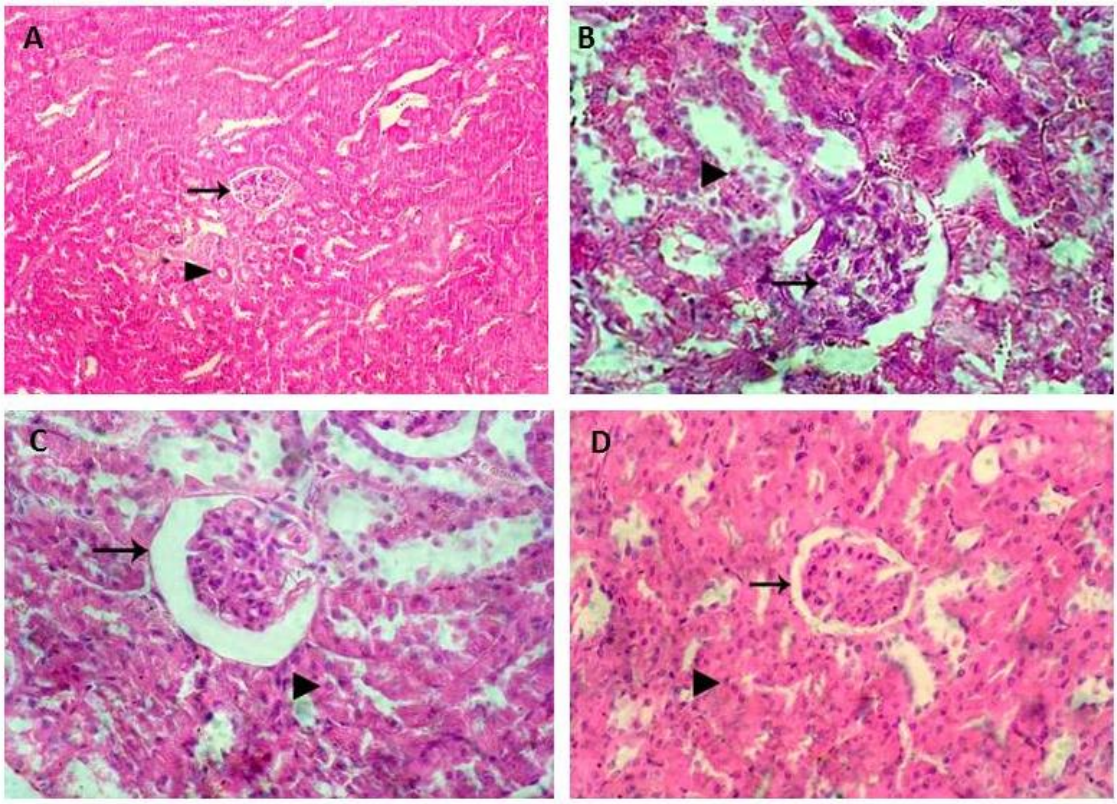

Fig. (3): Renal histopathology A) The micrograph of the control group shows normal renal glomeruli (arrow) and normal renal tubules lined by normal renal tubular epithelium (arrowhead). (HE, 100) **(B)** The micrograph of PCM treated group shows proliferation of the mesangial cells of the renal glomeruli (arrow) and necrosis of the renal tubular epithelium (arrowhead) (HE, 400) **(C)** The micrograph of PCM+RO treated group shows normal renal glomeruli (arrow) and normal renal tubules lined by renal tubular epithelium (arrowhead) (HE, 400) **(D)** The micrograph of RO treated group shows normal renal glomeruli (arrow) and normal renal tubules lined by normal renal tubular epithelium (arrowhead). (HE, 100).

Furthermore, PCM interacts with cell membrane permeability and loss of functional integrity of the kidney resulted in the elevation of the serum cholesterol level (Parameshappa et al., 2012).

Herein, RO in PCM+RO group ameliorated the bad effect of PCM on serum lipid profile, as explained by Wojdylo et al. (2007) who showed that rosemary had high content of flavonoids (quercetin, luteolin and kaempferol) and had high antioxidant activity which compensate oxidative stress and reduce ROS production. In addition, Hanan (2012) concluded that rosemary extract potentiate insulin secretion and consequently inhibit hormone sensitive lipase with concomitant decrease in plasma lipids. Also, rosemary aqueous extract could inhibit pancreatic lipase by aggregation of its protein which contributed to decline serum triglyceride levels (Hegazy et al., 2017). Furthermore, Yokozawa et al. (2002) discussed that polyphenols has the ability to increase fecal excretion of total cholesterol and bile acids consequently, enhanced need of cholesterol for synthesis of biliary juices and reduced absorption of cholesterol from the intestine lead to decreased plasma

cholesterol levels. Our results are in agreement with Naglaa (2015) who observed a significant reduction in the total cholesterol and triglycerides levels in hypercholesterolemic rats after administration of rosemary powder or rosemary essential oils.

Regarding renal function, our result clarified that serum urea, uric acid, and creatinine levels were significantly increased in the PCM group indicating deterioration of renal function as detected histopathologically. PCM nephrotoxicity resulted from its highly reactive and toxic metabolite NAPQI (Hart et al., 1994).This intermediates arylates protein in proximal renal tubules (Tran et al., 2001) which initiates apoptosis, necrosis and ultimately led to organ dysfunction (Cekmen et al., 2009). Moreover, PCM overdose caused glutathione depletion and consequent lipid peroxidation which considered the main reason for renal damage (Parameshappa et al. 2012) . Also, there is a relationship between oxidative stress and nephrotoxicity as the elevation of H_2O_2 and O_2 production alters the filtration surface area and interferes filtration coefficient of the glomeruli (Karadeniz et al., 2008; Ajami et al., 2010 ; Sivakrishnan and Kottaimuthu , 2014).

Rosemary oil has a nephroprotective effect as it significantly reduced the elevated levels of renal function biomarkers in PCM+ RO group.This is confirmed by previous data of Metwally et al. (2012) and Raskovic et al. (2014) in CCl_4 intoxicated rats after rosemary administration. The nephroprotective effect of rosemary may be

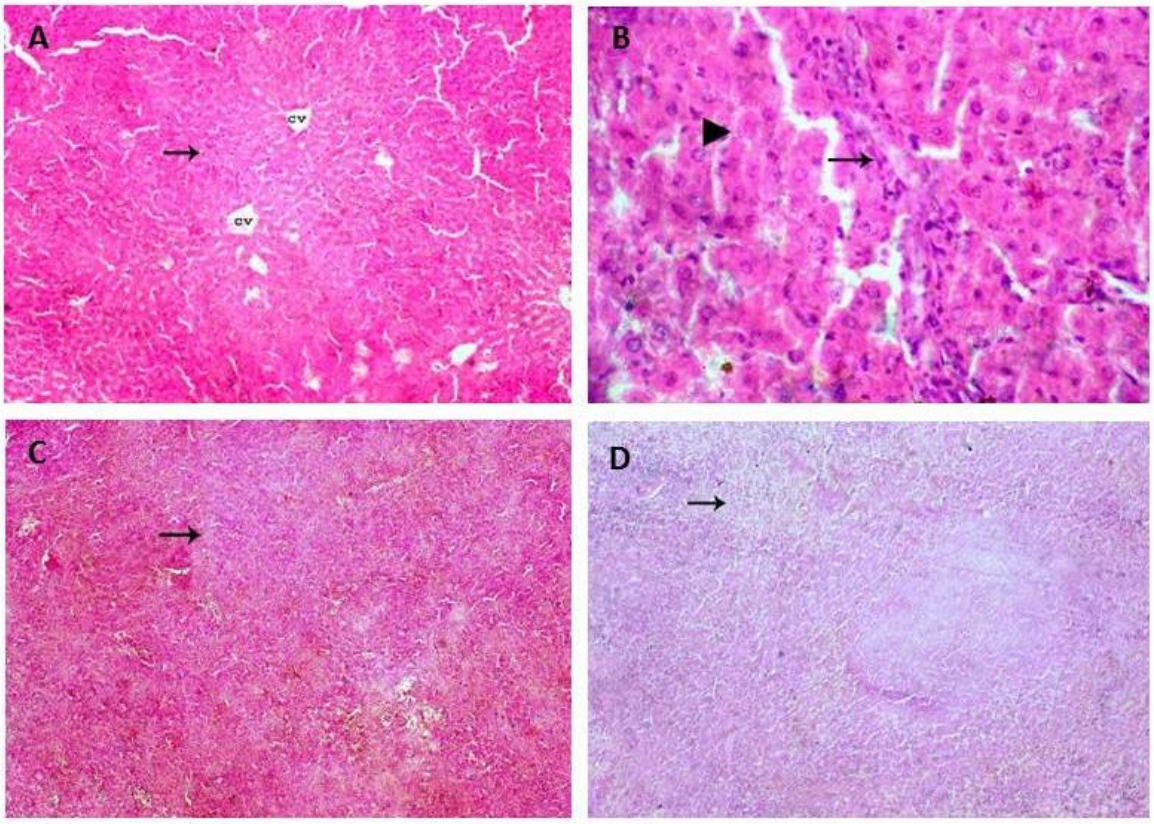

Fig. (4): Hepatic histopathology A) The micrograph of the control group shows normal hepatocytes (arrow) and normal radial arrangement around the central vein (CV) (HE, 100). **(B)** The micrograph of PCM treated group shows necrosis of hepatocytes (arrowhead) and fibroblastic proliferation in interstitial tissue (arrowhead) (HE, 400).**(C)** The micrograph of PCM+RO treated shows marked dilation of central vein and hepatic sinusoids (arrow) (HE,400). **(D)** The micrograph of RO treated group shows normal hepatocytes and normal

attributed to its high scavenging capacity of different types of reactive oxygen and nitrogen species due to its high content of polyphenolic compounds (Moreno et al., 2006; Tavafi and Ahmadvand, 2011). Also, Zohrabi et al. (2012) and Ashtiyani et al. (2013) reported that rosemary aqueous extract induced a significant increase in the renal blood flow which can be related to its vasodilatory, anti-spasmodic and anti-inflammatory properties.

Serum sodium and potassium levels are the most important parameters for the detection of electrolytes imbalance. In this study, PCM induced a significant decrease in serum Na^+ level along with significant elevation in the K^+ level which may be referred to kidney dysfunction and decrease glomerular filtration rate (Jones and Vale, 1993). This also documented by Saleh et al. (2018). Our findings partially agree with Oseni et al. (2017) who noted a significant increase in the serum sodium and potassium levels in rats orally intoxicated with 2 g/kg BW acetaminophen. The treatment with RO in the PCM+RO group resulted in a significant improvement in electrolyte imbalance induced by paracetamol. This also reported by Hozayen et al. (2014) in aspartame intoxicated rats. These findings confirmed the positive effect of rosemary on the damaged renal tubules resulted in decrease urinary excretion of sodium and increase urinary excretion of potassium (Ashtiyani et al., 2013).

Malondialdehyde (MDA) is the end product of lipid peroxidation and known as a second messenger of free radicals (Suchismita et al., 2015). In this work PCM induced a significant elevation in renal MDA concentration that was related to excessive generation of free radicals mediated oxidative stress and lipid peroxidation (Adekunle et al., 2012) as well as cell necrosis induced by bonding of its toxic metabolite NAPQI(N-acetyl -P-benzoquinoneimine) with sulfhydryl group of cell protein (Lin and Cheih, 1997). Our result is in accordance with Palani et al. (2009) and Kheradpezhouh et al. (2010) who clarified that renal MDA level significantly elevated in rats administrated with over doses of paracetamol.

Reduced glutathione (GSH) is the main cellular antioxidant which condiders the first line of defense in combating free radicals, maintain intracellular redox balance, eliminate xenobiotics and ROS and play a critical role in detoxification reaction (Myhrstad et al., 2002 ; Abdel-Zaher et al., 2007). Additionally, antioxidant defense mechanism in our body including SOD and CAT enzymes prevent oxidative stress by scavenging ROS and ameliorate the effect of oxygen metabolism (Pande and Flora, 2002; Ghosh et al., 2010). In the current study, the renal activities of SOD and CAT enzymes, as well as, GSH level were significantly decreased in PCM intoxicated group, this is attributed to enhanced lipid peroxidation and antioxidative enzymes inhibition (Palani et al., 2009). Also, Sener et al. (2005) and Kheradpezhouh et al. (2010) explained that paracetamol metabolism produces highly toxic metabolite (NAPQI) which initially detoxified by conjugation with GSH leading to its depletion up on toxic acetaminophen dose. Our result agree with Abbas (2014) and Suchismita et al. (2015) who recorded a significant suppression in antioxidant enzymes activities of SOD and CAT as well as GSH level in renal tissue in rats received a high dose of acetaminophen.

Using rosemary as a drug has an anti-lipoperoxidant activity that eliminates the generated free radicals and improves the antioxidant system and subsequently inhibits oxidative stress (Soyal et al., 2007; Zohrabi et al., 2012). Following this, the bad effect of paracetamol in the measured oxidant antioxidant parameters ameliorated by rosemary oil administration in the PCM+RO group as renal GSH content and SOD activity was significantly elevated but CAT activity and MDA level insignificantly changed. This effect is attributed to the presence of characteristic phenolic compounds in rosemary (Ahmad et al.,

2011), such as carnosic acid which has a strong ability to scavenge H_2O_2 (Aruoma et al.,1992). Our data partially agree with Hozayen et al. (2014) who recorded a significant decrease in renal MDA level along with a significant increase in renal GSH, CAT and SOD activity in rats treated with aspartame and rosemary extract.

The histopathological examination in our work exhibited that paracetamol intoxication induced marked dilatation of renal glomeruli with the proliferation of the mesangial cells and necrosis of the renal tubular epithelium that agree with Arash et al. (2015) . All these histopathological changes could be attributed to the accumulation of paracetamol toxic metabolite produced in the liver then excreted through the kidney inducing lipid peroxidation and tissue damage (Cekmen et al., 2009). Treatment with rosemary oil in PCM+RO group ameliorated the histopathological changes induced by paracetamol in the hepatic and renal tissues. This is accepted with Azab et al. (2014) who found that oral administration of 220 mg/kg rosemary for 10 days in gentamicin intoxicated guinea pigs significantly improved the histopathological changes induced in renal tissues. This improvement in renal tissues may result from the ability of rosemary for scavenging free radical and reactive oxygen and nitrogen species due to its high content of polyphenolic compounds (Moreno et al., 2006;Tavafi and Ahmadvand, 2011).

CONCLUSION

Based on our data, It was concluded that rosemary oil as a natural compound has a potential anti-oxidant effect, partially ameliorates biochemical and histopathological alterations induced by paracetamol toxicity in rats. Consequently, rosemary oil can be used in complementary and integrative medicine for the treatment of several diseases and to overcome the undesired effect of nephrotoxic drugs.

CONFLICT OF INTEREST

The authors have no conflict to declare.

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