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Hepatoprotective and Reno-protective Effects of Artichoke Leaf Extract and Rosemary Extract against Paracetamol Induced Toxicity in Albino Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors worked and contributed equally in all sections of the research and they read and approved the final manuscript in the research.

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ABSTRACT

Background: Paracetamol overdose is a predominant cause of hepatotoxicity and nephrotoxicity in both humans and experimental animals. There is an emerging focus on plant products to find a highly effective and reliable drug for the prevention of paracetamol –induced toxicity.
Objective: In this study, we investigated the Hepatoprotective and Reno-protective Effects of artichoke (*Cynara scolymus L.*) Leaf extract and rosemary (*Rosmarinus officinalis L.*) extract against paracetamol Induced toxicity in Albino Rats.
Materials and Methods: Rats were divided into five groups: Negative control, paracetamol (1000 mg/kg dose) PCT, artichoke leaf extract "ALE" (1.5 g/kg, orally + paracetamol for 30 d), rosemary extract "RE" (125 mg/kg + paracetamol for 30 days) and the last group was treated with PCT+ ALE+ RE for 30 days.
Results: Paracetamol caused marked liver damage as noted by significant increased activities of serum aminotransferases, alkaline phosphatase, gamma-glutamyl transferase and lactate dehydrogenase. Paracetamol also raised serum levels of urea, creatinine, and Cystatin-C. In addition, there was a significant decrease in serum total protein and albumin. Paracetamol caused

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an elevation in lipid peroxidation paralleled with significant decline in reduced glutathione (GSH) level and activities of glutathione-S- transferase (GST), glutathione (GPX) peroxidase, and superoxide dismutase (SOD) in the liver and kidney. These results are confirmed in the histological examination of the liver and kidney.

Conclusion: Treatment with artichoke leaf extract (ALE) and rosemary extract (RE) produced a potential protection of the liver and kidney against biochemical and histological alterations and oxidative stress induced by paracetamol.

Keywords: Paracetamol; artichoke leaf extract; rosemary extract; nephrotoxicity; hepatotoxicity; rats.

ABBREVIATIONS

ALP : Alkaline Phosphatase;
ALT : Alanine aminotransferase;
AST : Aspartate aminotransferase;
GGT : G-Glutamyl Transferase;
LDH : Lactate Dehydrogenase;
PCT : Paracetamol;
RE : Rosemary Extract,
ALE : Artichoke Leaf Extract;
GPx : Glutathione Peroxidase;
GSH : Reduced Glutathione;
GST : Glutathione-S-Transferase;
SOD : Superoxide Dismutase;
MDA : Malondialdehyde;
ANOVA: Analysis of variance.

1. INTRODUCTION

Paracetamol (PCT) used as a pain relief analgesic for arthritis, muscle aches, headache, fever and cold etc [1]. PCT is safe in therapeutic doses; however, overdose increase reactive oxygen species (ROS) production and worsen antioxidant defense [2,3].

Paracetamol has been repotted to cause acute kidney and liver injuries in experimental animal and humans [4,5].

Plant products are remarkably effective and reliable natural remedy for the prevention and cure of PCT -induced renal and hepatic-toxicity.

Rosemary (*Rosmarinus officinalis L.*), is a Mediterranean plant. It has antioxidant, anti-cancer, and anti-inflammatory activities [6].

Rosemary is rich in phytochemicals, including rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid, and the carnosic acid [7]. Extracts of rosemary leaves have a variety of antimicrobial [8] and antimutagenic properties [9].

Artichoke (*Cynara scolymus L.*) is rich in polyphenols and flavonoids. Artichoke leaf extract (ALE) significantly diminishes reactive oxygen species [10]. ALE decreases cardiac and hepatic oxidative stress in rats [11].

The researchers performed this study to investigate the hepatoprotective and Reno-protective effects of artichoke leaf extract and rosemary extract against PCT- induced toxicity in albino rats.

2. MATERIALS AND METHODS

2.1 Reagents and Chemicals

Chemicals and paracetamol were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2 Animals and Diet

40 male Wistar strain albino rats (weight, 180- ± 4 g) were bought from a breeding unit of Animal Laboratory -Medical Research Center (Faculty of Medicine - Ain Shams University).

Rats were housed under a controlled temperature of 25 ± 2°C and a relative humidity of 50-70%. balanced diet was prepared according to American Institute of Nutrition (AIN-93) and amended by Reeves et al. [12].

2.3 Hepatotoxicity and Nephrotoxicity Induction

Oral injection of [1000 / kg b.wt)] of Paracetamol was injected every other day to induce Hepatotoxicity and Nephrotoxicity [13,14].

2.4 Extraction of Plant Material

The Artichoke and Rosemary were collected on April, the identification and authentication of both plants were confirmed by the Botany Department of Ain Shams University.

2.4.1 Preparation of Artichoke leaves extract

2000 g of Fresh Artichoke leaves were blended mechanically with 2000 ml distilled water and

filtered. The residue was re-dissolved in 1000 ml distilled water. The first aqueous extract was added to the later one, then condensed in rotary evaporator under vacuum. The condensed extract stored at 4°C. The method of extraction was carried out according to [15].

2.4.2 Rosemary leaves extract preparation

The rosemary (*R. officinalis* L.) leaves were dried, powdered. The extract was prepared by refluxing leaves with distilled water for thirty-six hours. The liquid extract was evaporated until transformed to powder. The powder was redissolved in distilled water before use [16].

At the end of the experiment, serum and organs samples were collected, properly handeled and stored at -20°C for biochemical parameters.

2.5 Total Flavonoids and Total Phenols Content in ALE and RE

Total phenolic (TP) content in the extract of both plants was assessed according to the assay of Folin-Ciocalteu [18], where Folin-Ciocalteu was mixed with the extracts. Then Na_2CO_3 was added. The mixture left in dark for two hours. Then we eventually measured the optical density (O.D) against blank. (TP) content were expressed as mg GAE) /gm of the plant extract sample.

Flavonoid levels were calculated using a calibration curve prepared in parallel and in the same conditions as the samples obtained from a standard solution of quercetin.

2.6 Handling Liver and Kidney Specimens for Histopathological Examination

Liver and kidney sections were prepared for histological examination according to Luna [19] as follows:

Tissue sections fixed in 10% formalin were washed in distilled water for 5 minutes, followed by dehydration using ethanol, clearing using xylene, infiltration, embedding by paraffin wax blocks, sectioning to thin 6-7 micrometer then fixed in slides for staining using the (Hematoxlin/Eosine stain). Liver and kidney tissue sections were subjected to histopathological examination using hematoxylin and eosin stains according to [20,21].

2.7 Biochemical Measurement

2.7.1 Serum hepatic enzymes

Serum (ALT, AST) and ALP were evaluated according to Murray [7] and Belfield and Goldberg [22] respectively.

γ- Glutamyltransferase (γ–GT) activity was determined according to the method of Szasz [23].

2.7.2 Parameters of kidney function

Serum creatinine and urea were determined according [24] and Kaplan [25] respectively. Serum cystatin C was determined according to Pergande and Jung [26].

Total protein was evaluated according to Henry, Cannon, and Winkelman [27]. Serum Albumin was assessed according to Doumas et al. [28].

2.7.3 Parameters of Oxidative Stress

Hepatic and renal Lipid Peroxidation (LPO) were determined in terms of malondialdehyde (MDA) production according to the method described by Rehman [29].

Reduced Glutathione (GSH) in the kidney and liver were estimated by estimating free-SH groups using the method defined by Sedlak and Lindsay [30].

Table 1. The experimental protocol

Group	Treatment
Group 1 (C)	Standard diet and served as control negative group
Group 2 (PCT)	Paracetamol was given orally by gastric tube at a dose level of 1000 mg/kg/ every other day for 30 days and served as control positive group.
Group 3 (ALE)	(Administered Artichoke and paracetamol) was given artichoke at a dose level of 1.5 g/kg body weight and paracetamol for for 30 days. [17]
Group 4 (RE)	(Administered rosemary and paracetamol) was given rosemary at a dose level of 125 mg/kg body weight and paracetamol for for 30 days.
Group 5 (ALE+RE)	(Administered was given artichoke at a dose level of 1.5 g/kg body weight + rosemary at a dose level of 125 mg/kg body weight and paracetamol for 30 days.

Hepatic and renal Superoxide dismutase activities were determined as described by Madesh and Balasubramanian [31].

Glutathione peroxidase activities were estimated as described by Paglia and Valentine [32].

2.8 Statistical Analysis

Values are expressed as mean ± S.E. The difference in mean between the different experimental groups was evaluated by one-way analysis of variance (ANOVA), by SPSS software statistical package (version 27; SPSS, Chicago, IL) according to [33] was used for statistical analysis.

3. RESULTS AND DISCUSSION

Table 2 showed that ALE has more phenolic content than RE, however the total flavonoids of RE was higher than ALE.

3.1 Effects of Artichoke Leaf Extract and Rosemary Extract on Hepatic Biomarkers

Paracetamol caused marked liver damage as noted by significant increased activities of serum aminotransferases (ALT, AST) and alkaline phosphatase (Table 3). Treatment with ALE and RE diminished PCT-induced elevation in these parameters PCT significantly increased, gamma-glutamyl transferase (GGT) and lactate dehydrogenase (LDH) activities compared to the control. However, administration ALE and RE reversed paracetamol-induced elevation in GGT and LDH (Fig. 1 and Fig. 2).

The ALT, AST and ALP activities are used to assess liver function [34]. Paracetamol administration significantly increases hepatic transaminases (ALT), (AST) and ALP.

The observe significant increase in serum transaminases in PCT- intoxicated rats revealed diminished hepatocytes functional integrity, this elevation might attributed to leak of the hepatocytes plasma membrane which eventually led to elevation of AST and ALT.

(GGT) and (ALP) are membrane bound enzymes. GGT activity is considered one of the best delicate indicators of hepatic function [35].

LDH has a vital role in the formation of pyruvate from lactate via NAD+ as coenzyme of NAD [36]. The elevated LDH activity could be due to drip of LDH into the blood stream due to cellular damage because of PCT -intoxication.

Our findings agree with El Sayed et al., 2020 who reported that PCM increased hepatic liver enzymes, total bilirubin, (GGT) and (LDH) [37].

Paracetamol catalyzes formation of the reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI) via cytochrome P450. NAPQI directly induces oxidative stress, hepatocellular injury, and mitochondrial damage. Paracetamol has been reported to have traumatic impact on hepatic tissue [38,39].

Table 2. Total phenolic and total flavonoids content of artichoke leaf extract and rosemary leaf extract

Constituent	Total phenolic compounds (mg GAE/ acid/g extract)	Total flavonoids (mg of QE / g extract)
Artichoke leaf extract (ALE)	3900.91 ± 36.9	949.28 ± 39.04
Rosemary leaf extract (RE)	3367.240 ± 28.15	1329.03 ± 26.16

Values are expressed as mean of measurements (n=3) (GAE-Gallic acid equivalents, QE- Quercetin equivalents)

Table 3. Changes in liver enzyme activities in different experimental groups

Groups/parameters	ALT (mg/dL)	AST (mg/dL)	ALP (mg/dL)
Control (C)	27.33 ± 0.28^{a}	42.95 ± 0.54^{a}	119.65 ± 2.72^{a}
Paracetamol (PCT)	59.33 ± 0.62^{b}	95.93 ± 0.60^{b}	179.17 ± 3.13^{b}
Rosemary extract (RE)	52.21 ± 0.51^{c}	89.34 ± 0. 83^{c}	138.95 ± 1.79^{c}
Artichoke Leaf Extract (ALE)	46.92 ± 0.45^{d}	80.10 ± 0.93^{d}	129.89 ± 2.76^{d}
Artichoke leave extract (ALE)+ Rosemary extract (RE)	39.37 ± 0.49^{e}	59.38 ± 0.58^{e}	117.81 ± 3.65^{a,e}

Values are expressed as mean ± SE of 8 rats in each group

abcde-Mean values within a column not sharing the same superscript letters were significantly different, $P < 0.05$

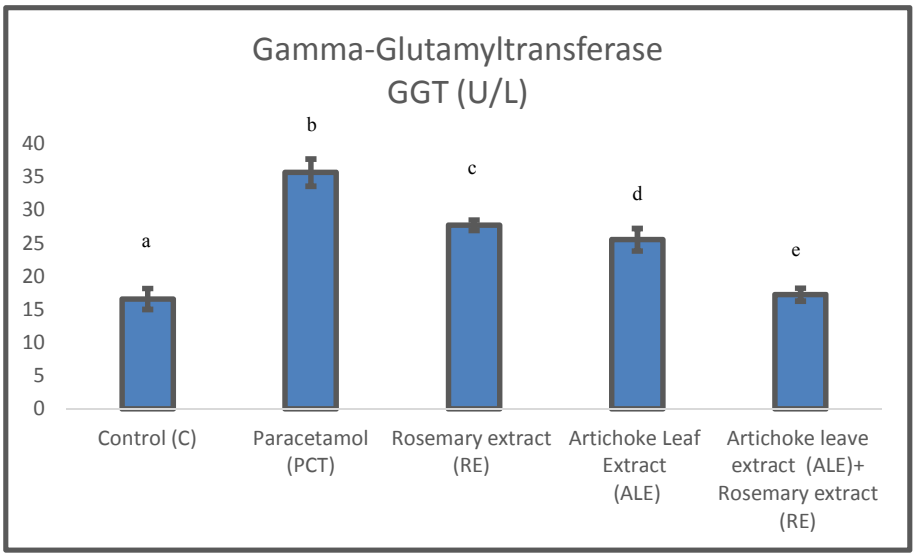

Fig. 1. Changes in serum GGT in different experimental groups
Values are expressed as mean ± SE of 8 rats in each group
abcde-Mean values not sharing the same superscript letters were significantly different, $P < 0.05$

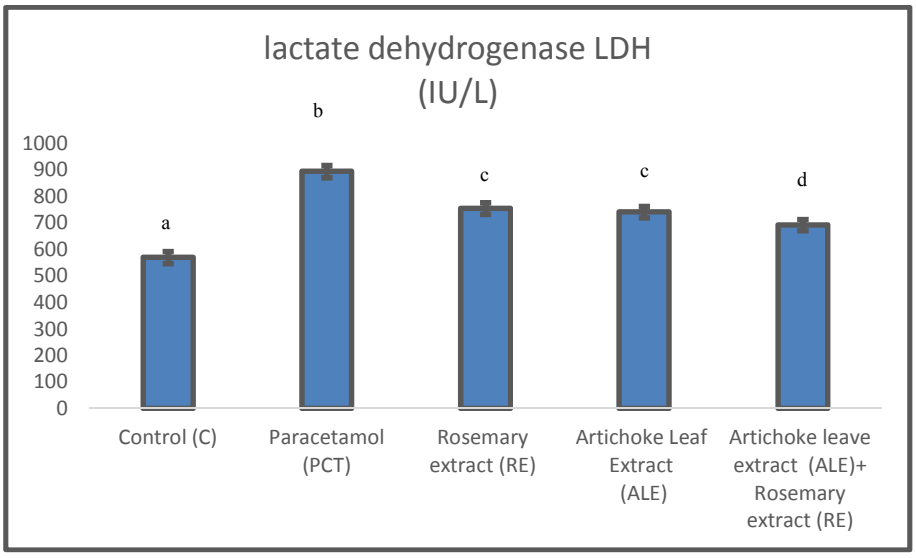

Fig. 2. The effect of different treatments on serum LDH
Values are expressed as mean ± SE of 8 rats in each group
abcde-Mean values not sharing the same superscript letters were significantly different, $P < 0.05$

However artichoke extract significantly reduced the elevated serum ALT, AST, ALP, LD and GGT levels which might be attributed to its precious phenolic content.

Artichoke extract remarkably reduced the elevated liver enzymes because of its capability to decrease free radical-induced oxidative damage in the liver (24), [40].

Domitrovic et al. [41] pointed out that Rosemary extract significantly inhibit CYP2E1 enzymatic activity in paracetamol- intoxicated rats. These results confirm our current findings of the inhibiting properties of RE.

Carnosol in the *R. officinalis* extract may contribute to RE hepatoprotective activity [42].

Treatment of paracetamol- intoxicated rats with either rosemary or artichoke extract can protect liver cells against damage, but the Co-treatment showed more ameliorating and promising effect as shown by the observed improvement in these biochemical parameters.

Cotreatment of ALE and RE improved hepatic biomarkers and showed obviously normal hepatocytes. This confirmed the protective effect of the ALE and RE or their combination and this effect might attribute to the diverse phytoconstituents and Flavonoids, which reveal protective influence on the liver. Furthermore, their precious antioxidant content may have a vital role in conserving hepatocellular membrane integrity.

These results are confirmed in the histological examination of the liver and kidney.

3.2 Effect of Artichoke Leaf Extract and Rosemary Extract on the Kidney Function

Paracetamol significantly raised serum urea, creatinine, and Cystatin-C as shown in (Table 4). On the other hand treatment of PCT- intoxicated rats with ALE, RE or both together significantly improved renal function via the significant reduction in serum urea, creatinine and cystatin-c.

The significant increase in serum urea in paracetamol – intoxicated rats might be attributed to the increased serum urea production, which exceeds urea clearance rate and, so tissue creatinine fragmentation which increases plasma creatinine levels [43,44].

High doses of paracetamol decrease glutathione levels and increase the production of toxic metabolites that are excreted through the kidney. These metabolites disrupt body homeostasis and may cause apoptosis and eventually resulted in renal dysfunction [45].

A systematic review about the relationship between paracetamol and renal impairment showed that PCT significantly increased the risk of renal impairment by (31%) in users without any history of renal disease [46].

The present study showed that the artichoke extracts attenuates induced elevation in serum creatinine and blood urea nitrogen (BUN) in rats.

Serum cystatin-C is an ideal marker of glomerular filtration [47,48]. It is considered one of the best markers for early diagnosis of renal dysfunction [49].

The improvement in renal functions upon ALE administration may be explained by the snoring content of artichoke, which accelerates urea metabolism and improves dieresis, which may initiate urea and creatinine excretion [50].

ALE may contribute in improving kidney functions via suppressing oxidative stress due to its phenolic content.

RE administration improved glomerular and renal function as shown by decreased Serum cystatin-C and serum creatinine which could be due to ROS-scavenging effect of RE.

Abd El-Ghany et al. [51] proved that aqueous extract of rosemary (RAE) inhibits free radical generation and lipid peroxidation and eventually recovers renal injury. The recovery effect of RE was due to its antioxidant constituents including rosmarinic acid, alpha tocopherol, carotenoids and diterpenoids.

The renal protective effect of *Rosmarinus officinalis* extract as manifested by the observed improvement in serum urea, creatinine and cystatin-C was proven by the normal appearance of kidney tissues.

Table 4. Changes in kidney function in different experimental groups

Groups/parameters	Urea (mg/dl)	Creatinine (mg/dl)	Cystatin-C (Pmg/ml)
Control (C)	25.45 ± 0.055[a]	0.46 ± 0.004 [a]	1.09 ± 0.012 [a]
Paracetamol (PCT)	44.76 ± 0.75 [b]	1.16 ± 0.007 [b]	4.32 ± 0.018 [b]
Rosemary extract (RE)	34.33 ± 0.42 [c]	0.75 ± 0.004 [c]	2.18 ± 0.024 [c]
Artichoke Leaf Extract (ALE)	36.06 ± 0.39 [d]	0.73 ± 0.009 [c]	2.24 ± 0.056 [c]
Artichoke leave extract (ALE)+ Rosemary extract (RE)	26.10 ± 0.38 [a,e]	0. 7 6± 0.006 [c]	1.05 ± 0.043[a,d]

Values are expressed as mean ± SE of 8 rats in each group.

abcde-Mean values within a column not sharing the same superscript letters were significantly different, $P < 0.05$

Fig. 3 shows that paracetamol significantly decreased serum total protein and albumin, however coadministration of ALE and RE with paracetamol reversed these changes.

The decreased in serum total protein and albumin upon paracetamol administration might be due to defective protein synthesis.

Administration and co-treatment ALE and RE resulted in a significant improvement in protein metabolism parameters, which could be explained by enhancement in protein synthesis.

3.3 Effect of Artichoke Leaf Extract and Rosemary Extract on Hepatic and Kidney Antioxidant Enzymes

The impact of different treatments on oxidative stress parameters in liver and kidney are presented in Tables 5&6 and Fig. 4a,b. The results implied that liver and kidney MDA were significantly increased (Fig. 4), while there was a significant decline in reduced glutathione (GSH) level and activities of glutathione-S- transferase (GST), glutathione (GPX) peroxidase, in the liver and kidney paralleled with significant decline in superoxide dismutase (SOD) in the liver and kidney in the paracetamol group. ALE and RE treatment reversed the alterations in these parameters Tables 5&6.

Paracetamol caused an elevation in lipid peroxidation paralleled with significant decline in reduced glutathione (GSH) level and activities of glutathione-S- transferase (GST), glutathione (GPX) peroxidase and superoxide dismutase (SOD) in the liver and kidney.

Acetaminophen is converted to NAPQI via cytochrome P450 2E1 (CYP2E1). In case of PCT overdose, the extra NAPQI diminishes GSH, lead to attachment of extra NAPQI to sulf- hydryl groups in mitochondrial proteins which induce mitochondrial dysfunction. This elevated superoxide free radicals and oxidative stress [52,53].

Paracetamol led to depletion of liver GSH which increased lipid peroxidation and lead to liver damage [54].

One of the potential mechanisms for reversing the oxidative stress may be through CYP2E1 activity in paracetamol- intoxicated rats.

Furthermore, there were no significant differences in CYP2E1 enzyme activity between Rosmary and Artichock extract -treated groups ($p<0.05$).

GST is integral constituent of the detoxification system [39]. Our current research revealed that Paracetamol significantly ($p<0.05$) reduced hepatic GSH and GST, while rosemary and artichoke extracts reserved these effects.

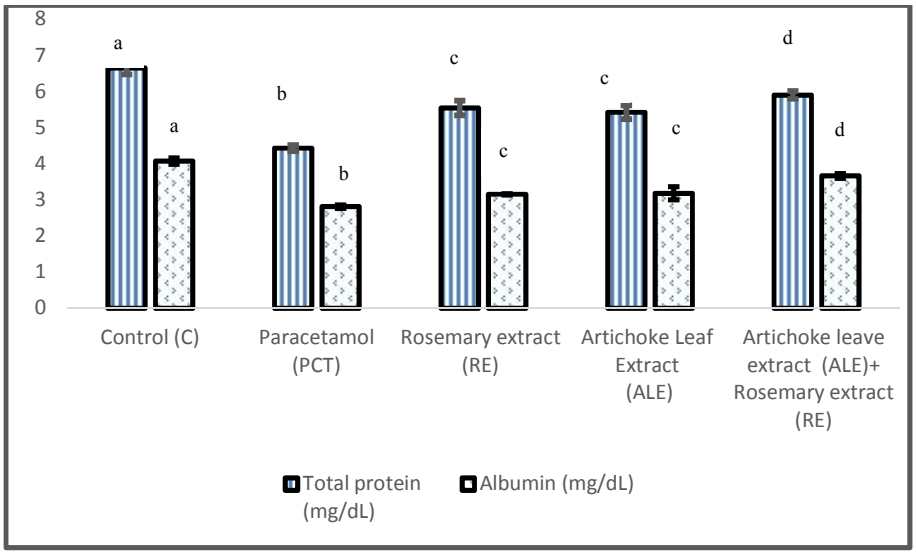

Fig. 3. Changes in serum total protein and albumin in different experimental groups

Values are expressed as mean ± SE of 8 rats in each group.

abcde-Mean values within a column not sharing the same superscript letters were significantly different, $P < 0.05$

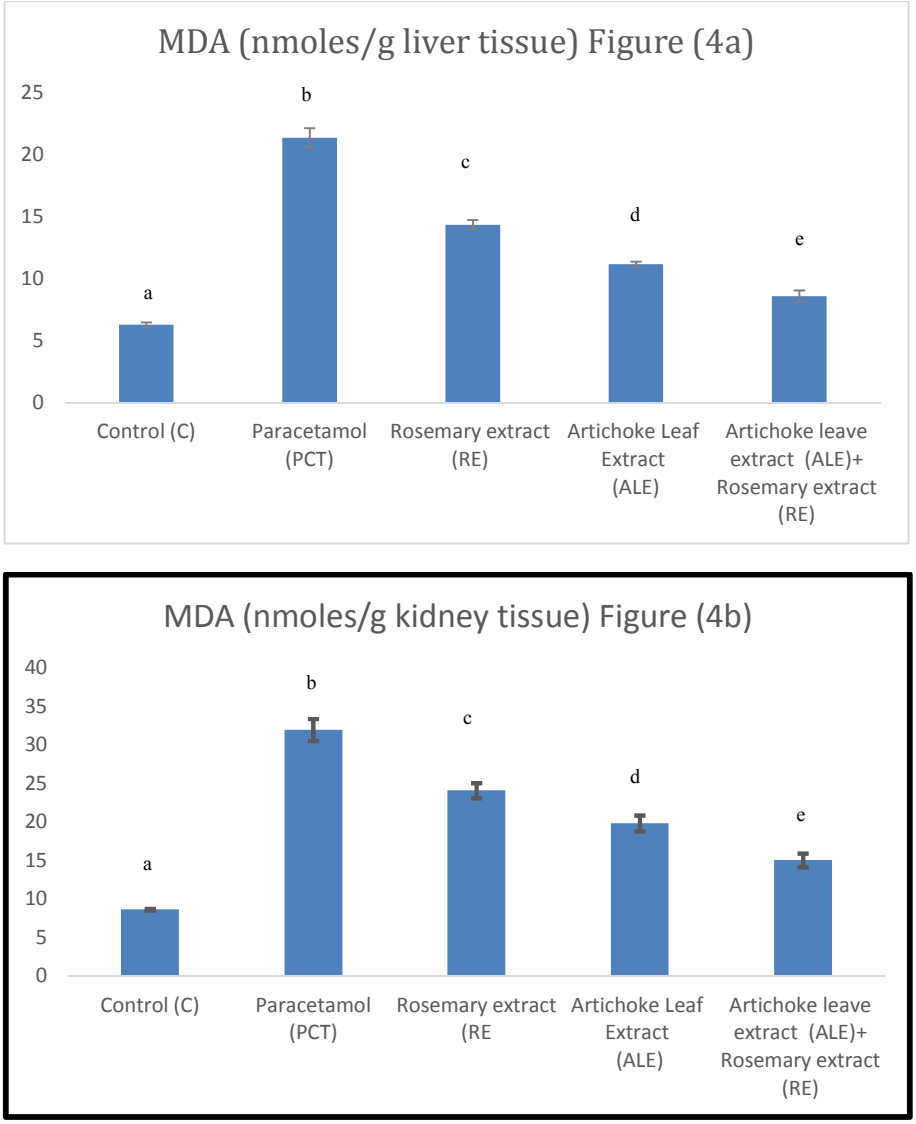

Fig. 4. Changes in hepatic and renal MDA of different experimental groups

Values are expressed as mean ± SE of 8 rats in each group.

abcde-Mean values within a column not sharing the same superscript letters were significantly different, $P < 0.05$

Table 5. Changes in antioxidant enzymes in liver of different experimental groups

Groups/parameters	GSH (nmoles/g tissue)	GPx (mU/mg protein)	GST (mU/mg protein)	SOD (U/mg protein)
Control (C)	9.25 ± 0.34 [a]	829.75 ± 19.26 [a]	583.03 ± 16.87 [a]	5.50 ± 0.040 [a]
Paracetamol (PCT)	5.24 ± 0.13 [b]	502.89 ± 14.70 [b]	356.95 ± 11.46 [b]	3.131 ± 0.007 [b]
Rosemary extract (RE)	7.05 ± 0.28 [c]	594.24 ± 12.49 [c]	403.33 ± 18.26 [c]	3.97 ± 0.013 [c]
Artichoke leaf extract (ALE)	7.31 ± 0.17 [c]	615.31 ± 13.08 [d]	422.36 ± 10.78 [d]	4.10 ± 0.006 [c]
Artichoke leave extract (ALE)+ Rosemary extract (RE)	8.96 ± 0.14 [a,d]	651.82 ± 11.21 [e]	467.58 ± 12.35 [e]	5.24 ± 0.023 [a,d]

Values are expressed as mean ± SE of 10 rats in each group.

abcd-Mean values within a column not sharing the same superscript letters were significantly different, $P < 0.05$

Table 6. Changes in antioxidant enzymes in kidney of different experimental groups

Groups/parameters	GSH (nmoles/g tissue)	GPx (mU/mg protein)	GST (mU/mg protein)	SOD (U/mg protein)
Control (C)	6.14 ± 0.10 [a]	816.86 ± 20.46 [a]	404.02 ± 21.25 [a]	1.031 ± 0.008 [a]
Paracetamol (PCT)	3.84 ± 0.32 [b]	545.66 ± 24.05 [b]	237.78 ± 17.68 [b]	0.55 ± 0.004 [b]
Rosemary extract (RE)	4.69 ± 0.32 [c]	599.02± 29.26 [c]	338.33 ± 15.56 [c]	0.67 ± 0.005 [c]
Artichoke leaf extract (ALE)	4.90 ± 0.20 [c]	605.81 ± 14.61 [d]	350.06 ± 19.07 [d]	0.79 ± 0.007 [d]
Artichoke leave extract (ALE)+ Rosemary extract (RE)	5.07 ± 0.12 [d]	646.42 ± 17.95 [e]	396.23 ± 13.64 [a,e]	0.97 ± 0.007 [e]

Values are expressed as mean ± SE of 10 rats in each group.
abcd-Mean values within a column not sharing the same superscript letters were significantly different, $P < 0.05$

Paracetamol administration increased lipid peroxidation as indicated by the significant increase ($p<0.05$) in liver and kidney MDA (Fig. 4).

In harmony with the results of the current study, increased hepatic lipid peroxidation during Paracetamol toxicity has been reported by other studies [55,56].

Our study showed that rosemary and artichoke extract decreased hepatic MDA which are in agreement with prior studies [57,58].

The decline in lipid peroxidation might be attributed to antioxidants constituents and cytoprotective properties of Rosemary extract [59]. RE owns ample biologically active antioxidants; include but not limited to rosmarinic acid, carnosic acid, betulinic acid, ursolic acid, rosmanol and rosmaridiphenol [60].

The role of ALE against oxidative stress are interrelated to its high flavonoids content which may express their effect through diminishing the ROS formation via inhibiting chelating trace elements or enzymes involved in the free radical production. These finding agreed with Mustafa et al., 2015 who reported that, ALE Flavonoids might exert these properties by scavenging ROS, and upregulate or protect the antioxidant defense [61].

ALE has been reported to increase glutathione peroxidase activity in liver and kidney.

3.4 Histopathology of Liver

Sections of livers in control group showed normal histological structure of hepatic lobule (Fig. 6A), Meanwhile, liver sections of paracetamol – intoxicated rats showed apoptosis of hepatocytes (small arrow), portal infiltration with mononuclear cells and proliferated oval cells (large arrow) (Fig. 6B1), in addition fibroplasia in the portal triad (small arrow) associated with mononuclear cells infiltration and oval cells proliferation (large arrow) were reported (Fig. 6B2), while, liver tissue sections of Paracetamol – intoxicated rats treated with artichoke extract showed mild improvement in hepatic histopathology (Fig. 6C) as proved by slight vacuolation of centrilobular hepatocytes (small arrow) and activation of Kupffer cells (large arrow) in addition, slight improvement in liver Histopathology was observed in rosemary extract treated group as shown slight vacuolation of some hepatocytes (small arrow), activation of Kupffer cells (large arrow) and binucleation of hepatocytes (arrow head) (Fig. 6D) however, the joint effect ALE and RE significantly improved liver histopathology as proven by activation of Kupffer cells (small arrow) and binucleation of hepatocytes (large arrow) (Fig. 6E).

The observed progress in liver histopathology upon artichoke and rosemary treatments might be due to the coincide decrease in the elevated transaminases and ALP, which previously attributed to the antioxidant defense mechanism of ALE and RE due to their precious antioxidant content as shown in Table 2.

3.5 Histopathology of Kidney

Renal histopathological examination of the control negative group showed normal histological structure (Fig. 5A).

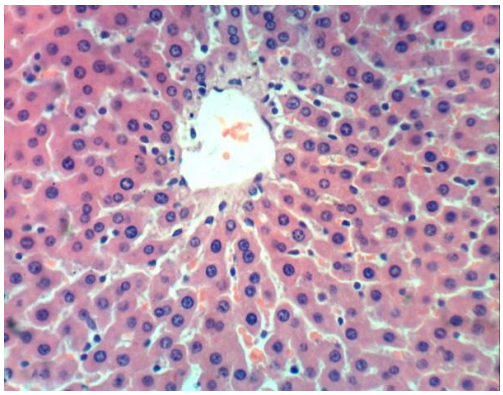

Fig. 5A.

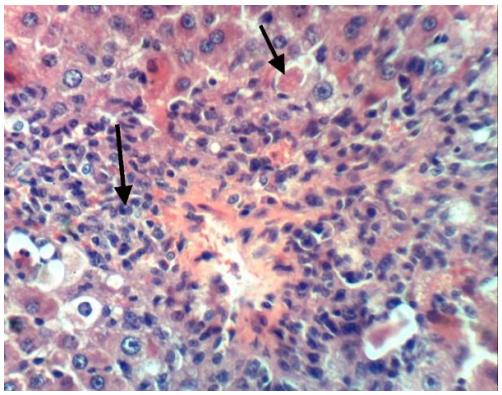

Fig. 5B1.

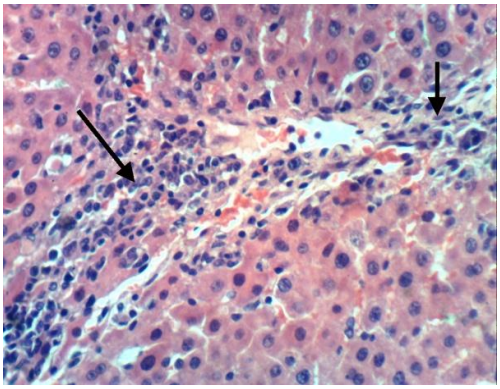

Fig. 5B2.

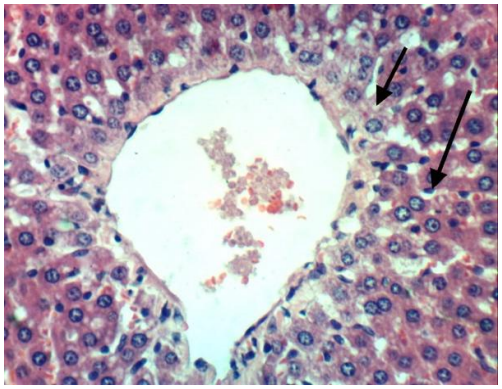

Fig. 5C.

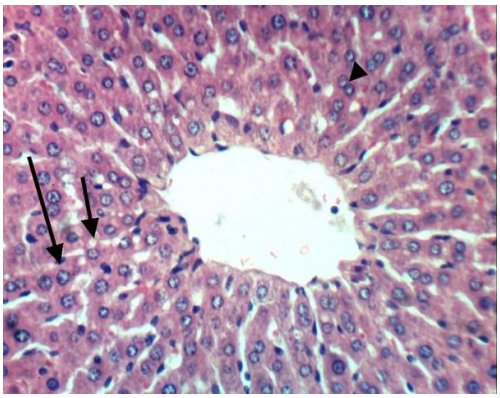

Fig. 5D.

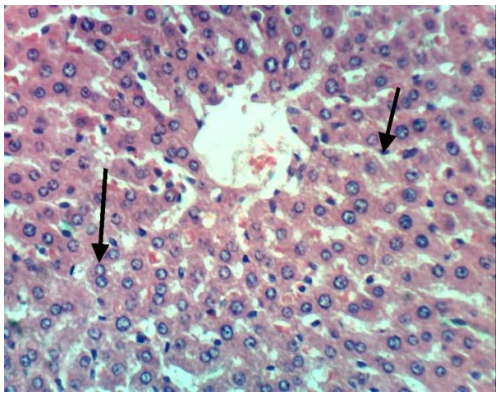

Fig. 5E.

Fig. 5A. Showing normal liver structure for the negative control group; Fig. 5B1,2. Showing apoptosis (small arrow) and portal infiltration (large arrow) for the positive control group intoxicated with PCT; Fig. 5C. ALE– Treated rats; Fig. 5D. RE- treated animals showing slight improvement and slight vacuolation of centrilobular hepatocytes (small arrow) and activation of Kupffer cells (large arrow) and finally Fig. 5E. Showing significant improvement in liver tissue structure which proves the improvement in liver enzyme activities

Histopathological examination of liver for the different experimental groups (H & E X 400)

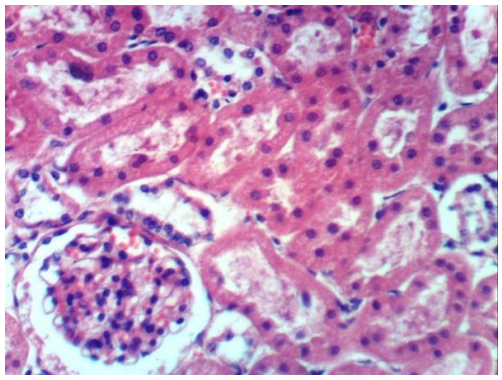

Fig. 6A.

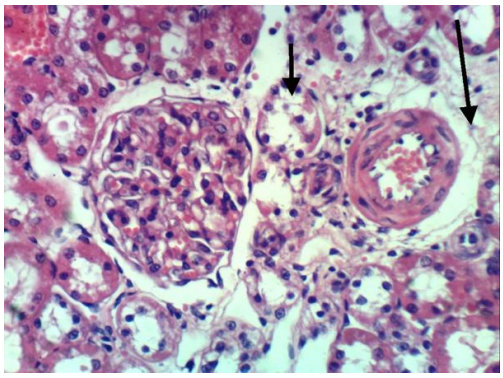

Fig. 6B1.

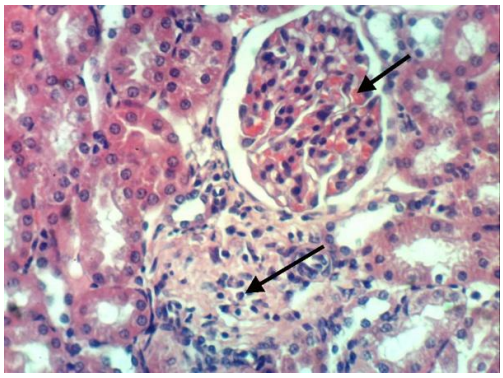

Fig. 6B2.

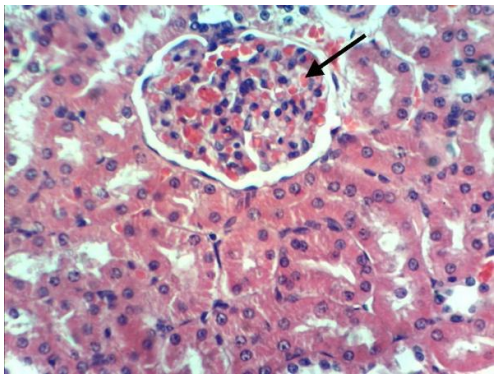

Fig. 6C.

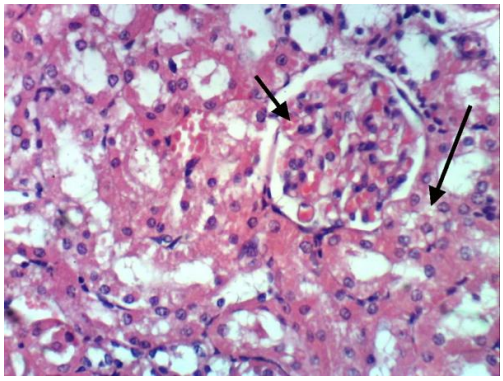

Fig. 6D.

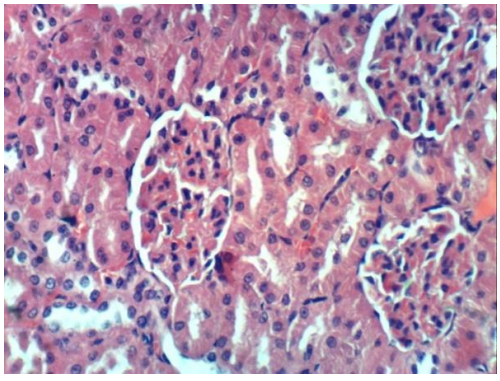

Fig. 6E.

Fig. 6A. Showing normal kidney structure for the negative control group; Fig. 6B1,2. It is for positive control intoxicated with PCT; Fig. 6C. ALE – treated rats; Fig. 6D. RE- treated animals showing slight improvement and finally; Fig. 6E. Showing significant improvement in kidney tissue structure which proves the improvemeent in kidney function biochemical parameters

Histopathological examination of kidney for the different experimental groups (H & E X 400)

With normal renal cortex, and medulla (figure), in control group as compared to paracetamol intoxicated group which showing vacuolation of epithelial lining renal tubules (small arrow) and perivascular oedema (large arrow) in addition to congestion of glomerular tuft (small arrow) and focal necrosis of renal tubules (Fig. 5B1,2).

However renal sections in experimental rats treated with ALE showing mild improvement in renal tissue with slight congestion of glomerular tuft (arrow) (Fig. 5C).

Treatment of paracetamol-intoxicated rats with RE moderately improved renal histologicay as confirmed by slight congestion of glomerular tuft (small arrow) and vacuolation of epithelial lining renal tubules (large arrow) (Fig. 5D).

The promising output from the current histopathological examination of kidney is the joint synergic effect of ALE & RE which observed clearly as improvement in kidney function as well as kidney histopathology where kidney of group 5 showed normal renal parenchyma (no histopathological changes) (Fig. 5E).

Current results are agreement with [59] who reported that rosemary prevented histopathological lesions and oxidative stress in liver, kidney.

The current improvement in renal structure might attributed to the simultaneous improvement in creatinine clearance, blood urea nitrogen and cystatin- c upon ALE and RE treatment and this improvement as mentioned earlier in the current research is due to the plentiful phenolic and flavonoid content of rosemary and artichoke extracts.

4. CONCLUSION

The current study proves that Artichoke Leaf Extract and Rosemary Extract are promising hepatoprotective and nephroprotective agents against Acetaminophen Induced toxicity in Albino Rats. ALE and RE restore urea, creatinine and Cystatin-C near to normal range, while coadministration of AE and RE has a significant antioxidant effect. Both Artichoke Leaf Extract and Rosemary Extract showed significant improvement in liver function. In conclusion Artichoke Leaf Extract and Rosemary Extract are highly advocated for paracetamol intoxication, to gain protection from liver and renal toxicity. Since each extract has its specific mechanisms in improving the tested parameters, a combination of both extracts may offer additional powerful effect for detoxification of liver and kidney.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Authors declare that they follow the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985). The proper ethics committee has approved all experiments and protocol - Ain Shams University.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Manchanda A, Cameron C, Robinson G. Beware of paracetamol use in alcohol abusers: A potential cause of acute liver injury. N Z Med J. 2013;126:80-4.
2. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities and impact of genetic variation. Pharmacol Ther. 2013;138:103–141.
3. Dadkhah A, Fatemi F, Ghaderi Z, Alipour M, Zolfaghari F, Razdan F. Protective effects of Iranian *Achillea wilhelmsii* essential oil on acetamino-phen-induced oxidative stress in rat liver. Pharm Biol. 2015;53:220–227.
4. McGill MR, Sharpe MR, Williams CD, Taha M, Curry SC, Jaeschke H. The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. J. Clin. Invest. 2012;12(5).

5. Karaali HF, Fahmi R, Borjac JM. Effect of *Ocimum basilicum* leaves extract on acetaminophen-induced nephrotoxicity in BALB/c mice. J. Complement. Integr. Med. 2018;16(2). DOI:10.1515/jcim-2018-01112(4):1574-83, 2012
6. Yang SY, Hong CO, Kim CT, Lee GP, Lee KW. The hepatoprotection of caffeic acid and rosmarinic acid, major compounds of *Perilla frutescens* against t-BHP-induced oxidative liver damage. Food Chem Toxicol. 2013;55:92–99.
7. Murray RL, Kaplan A, et al. Alanine aminotransferase. Clin Chem the C.V. Mosby Co, St Louis, Toronto, Princeton. 1984;1261–1266.
8. Bernardes WA, Lucarini R, Tozatti MG, Souza M, Silva MLA, Filho AAS, Martins CHG, Crotti AEM, Pauletti PM, Groppo M, Cunha WR. Antimicrobial activity of *Rosmarinus officinalis* against oral pathogens: Relevance of carnosic acid and carnosol. Chem. Biodivers. 2010;7(7): 1835-1840.
9. Furtado MA, De Almeida LCM, Furtado RH, Cunha WR, Tavares DC. Antimutagenicity of rosmarinic acid in Swiss mice evaluated by the micronucleus assay. Mutation Res. 2008;657:150-154.
10. Zapolska Downar D, Zapolski Downar A, Naruszewicz M, Siennickam A, Krasnodebska B, Koldziej B. Protective properties of artichoke (*Cynaras colymus L.*) against oxidative stress induced in cultured endothelial cells and monocytes. Life Sci. 2002;71(24):2897-2908.
11. Kucukgergin C, Aydin AF, Ozdemirler GO, Mehmetcik G, Kocar-Toker N, Uysal M. Effect of artichoke leaf extract on hepatic and cardiac oxidative stress in rats fed on high cholesterol diet. Biol. Trace. Elem. Res. 2010;135(1-3):264-274.
12. Reeves G, Nielsen FH, Fahey JG. AIN-1993 reformation of the AIN-76: Purified diet for laboratory rodents: final report of the American Institute of Nutrition, purified ad-hoc writing committee on the reformation of the AIN-76 a rodent diet. American Institute, J. Nutr. 1993;26:1939–1951.
13. Yousef MI, Omar SAM, El Guendi MI, Abdel Megid LA. Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat. Food Chem Toxicol. 2010;48:3246–3261.
14. Kiran PM, Raju AV, Rao BG. Investigation of hepatoprotective activity of *Cyathea gigantea* (Wall. ex. Hook.) leaves against paracetamol-induced hepatotoxicity in rats. Asian Pac J Trop Biomed. 2012;2:352–356.
15. Heidarian E, Rafieian Kopaei M. Protective effect of artichoke (*Cynara scolymus L.*) leaf extract against lead toxicity in rat. Pharm. Biol. 2013;51(9):1104–1109.
16. Abdella EM, Ahmed RR. Suppression of doxorubicin apoptotic, histopathologic, mutagenic and oxidative stress effects in mice bone marrow and testis tissues by aqueous rosemary leaves extract. Acta Biol. Par Curitiba. 2009;38(1-2):35-57.
17. Mehmetc IKG, Ozdemirler G, Koc ak Toker N. Effect of pretreatment with artichoke extract on carbon tetrachloride-induced liver injury and oxidative stress. Exp Toxicol Pathol. 2008;60:475–80.
18. Singleton VL, Orthofer RM, Lamuela Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Meth Enzymol. 1999;299:152–178.
19. Maia FJN, Ribeiro FWP, Rangel JHGD, Lomonaco D, Luna FMT, de Lima Neto P, Correia AN, Mazzetto SE. Evaluation of antioxidant action by electrochemical and accelerated oxidation experiments of phenolic compounds derived from cashew nut shell liquid. Indust. Crops and Prod. 2015;67:281–286.
20. Tousson E. Histopathological alterations after a growth promoter boldenone injection in rabbits. Toxicology and Industrial Health. 2016;32(2):299-305.
21. Tousson E, Ali EM, Ibrahim W, Ashraf RM. Histopathological and immuno-histochemical alterations in rat heart after thyroidectomy and the role of hemin and ketoconazole in treatment. Biomedicine & Pharmacotherapy. 2012;66(8):627-32.
22. Belfield A, Goldberg DM. Revised assay for serum phenyl phosphatase activity using 4- amino – antipyrine. Enzyme. 1971;12(5):561-573.
23. Szasz G. Reaction rate method for gamma-glutamyl transferase activity in serum. Clin. Chem. 1976;22:2051–2055.
24. Murray RL, Creatinine, Kaplan A, et al. Clin Chem the C.V. Mosby Co, St Louis, Toronto, Princeton. 1984;1261–1266.

25. Kaplan A. Urea, Kaplan A, et al. Clin Chem the CV Mosby Co St Louis. Toronto. Princeton. 1984;1257-1260:437-418.
26. Pergande M, Jung K, Sandwich. Enzyme immunoassay of Cystatin C in serum with commercially available antibodies. Clinical Chemistry. 1993;39(9):1885-1890.
27. Henry RJ, Cannon DC, Winkelman JW. Clinical chemistry principles and techniques. New York: Harper and Row. 1974;116–140.
28. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta. 1971;31(1):87–96.
29. Rehman SU. Lead-induced regional lipid peroxidation in brain. Toxicology Letters. 1984;21(3):333–337.
30. Sedlak J, Lindsay RH. Estimation of total, protein- bound and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Analytical Biochemistry C. 1968;25:192–205.
31. Madesh M, Balasubramanian KA. Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. Indian Journal of Biochemistry and Biophysics. 1998;35(3):184–188.
32. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione per-oxidase. The Journal of Laboratory and Clinical Medicine. 1967;70(1):158-169.
33. Levesque S. SPSS programming and data management; A guide for SPSS and SAS users, Fourth Edition. Spss Inc, Chicago; 2007.
34. Hao G, Yu Y, Gu B, Xing Y, Xue M. Protective effects of berberine against doxorubicin-induced cardiotoxicity in rats by inhibiting metabolism of doxorubicin. Xenobiotica. 2015;45(11):1024-1029.
35. El Faras AA, El Sawaf AL. Hepatoprotective activity of quercetin against paracetamol-induced liver toxicity in rats. Tanta Med J. 2017;45(2):92-8. Available:http://www.tdj.eg.net/text.asp?2017/45/2/92/216690 https://doi.org/10.4103/tmj.tmj_43_16
36. Burtis LA, Ashwood ER. Textbook for clinical chemistry. W.B. Saunders Company, Philadelphia, Pennsylvania; 1986.
37. Taha ME, Kamal AM, Ibrahim DR. Possible protective effect of olive leaves extract on paracetamol induced hepatotoxicity in male albino rats. Biosci. J. Uberlândia. 2020;36(1):245-255. (Received: 07/06/19) DOI:http://dx.doi.org/10.14393/BJ-v36n1a2020-49960
38. Omidi A, Riahinia N, Montazer-Torbati MB, Behdani MA. Hepatoprotective effect of *Crocus sativus* (saffron) petals extract against acetaminophen toxicity in male Wistar rats. Avicenna J Phytomed. 2014;4:330–336.
39. El-Morsy EM, Kamel R. Protective effect of artichoke leaf extract against paracetamol-induced hepatotoxicity in rats. Pharm Biol. 2015;53:167–173.
40. Zhang P, Tang Y, Li NG, Zhu Y, Duan JA. Bioactivity and chemical synthesis of caffeic acid phenethyl ester and its derivatives. Molecules. 2014;19(10): 16458–16476.
41. Domitrovic R, Potocnjak I, Crncevic Orlic Z, Skoda M. Nephroprotective activities of rosmarinic acid against cisplatin-induced kidney injury in mice. Food Chem Toxicol. 2014;66:321–328.
42. Sotelo Félix JI, Martinez Fong D, Muriel P. Protective effect of carnosol on CCl4-induced acute liver damage in rats. Eur. J. Gastroenterol. Hepat. 2002b;14:1001-1006.
43. Roy S, Pradhan S, Das K, Mandal A, Mandal S. Acetaminophen induced kidney failure in rats: A dose response study. Journal of Biological Sciences. 2015;15(4): 187-193.
44. Suh KS. Discovery of novel biomarkers for the development of personalized medicine. Translational Medicine. 2012;S1: e1-e2.
45. Gopi KS, Reddy AG, Jyothi K, Kumar BA. Acetaminophen-induced hepato and nephrotoxicity and amelioration by silymarin and *Terminalia chebula* in rats. Toxicol Int. 2010;17(2):64-66.
46. Kanchanasurakit S, Arsu A, Siriplabpla W. Acetaminophen use and risk of renal impairment: A systematic review and meta-analysis. Kidney Res Clin Pract. 2020;39:81-92.
47. Newman DJ, Thakkar H, Edwards RG, Wilkie M, White T. Serum cystatin C measured by automated immunoassay: A more sensitive marker of changes in GFR than serum creatinine. Kidney Int. 1995;47(1):312-318.
48. Knight EL, Verhave JC, Spiegelman D, Hillege HL, de-Zeeuw D. Factors

influencing serum cystatin C levels other than renal function and the impact on renal function measurement. Kidney Int. 2004;65:1416-1421.

49. Murty MSN, Sharma UK, Pandey VB, Kankare SB. Serum cystatin C as a marker of renal function in detection of early acute kidney injury. Indian J Nephrol. 2013;23(3): 180-183.
50. Stoev DS, Djuvinov D, Mirtcheva T, Pavlov D, Mantle P. Studies on some feed additives giving partial protection against ochratoxin A toxicity in chicks. Toxicol. Lett. 2002;135(1-2):33-50.
51. Abd-El-Ghany MA, Motawee MM, El-Kewawy. Biological effects of yoghurt with rosemary on injured liver rats. Aust J Basic Appl Sci. 2012;6(3):525–532.
52. Jiang J, Briedé JJ, Jennenetal DJG. Increased mitochondrial ROS formation by acetaminophen in human hepatic cells is associated with gene expression changes suggesting disruption of the mitochondrial electron transport chain. Toxicology Letters. 2015;234(2):139–150.
53. Zhao Z, Wei Q, Hua W, Liu Y, Liu X, Zhu Y. Hepato-protective effects of berberine on acetaminophen-induced hepatotoxicity in mice. Biomedicine & Pharmacotherapy. 2018;103:1319–1326.
54. Ciesielska E, Gwardys A, Metodiewa D. Anticancer, antiradical and antioxidative actions of novel Antoksyd S and its major components, baicalin and baicalein. Anticancer Research. 2002;22(5):2885–2892.
55. Dadkhah A, Fatemi F, Kazemnejad S, Rasmi Y, Ashrafi Helan J, Allameh A. Differential effects of acetaminophen on enzymatic and non-enzymatic antioxidant factors and plasma total antioxidant capacity in developing and adult rats. Mol Cell Biochem. 2006;281:145–152.
56. Dadkhah A, Fatemi F, Alipour M, Ghaderi Z, Zolfaghari F, Razdan F. Protective effects of Iranian *Achillea wilhelmsii* essential oil on acetaminophen-induced oxidative stress in rat liver. Pharm Biol. 2015;53:220–227.
57. Iuvone T, De-Filippis D, Esposito G, DAmico A, Izzo AA. The spice sage and its active ingredient rosmarinic acid protect PC12 cells from amyloid-beta peptide-induced neurotoxicity. J Pharmacol Exp Ther. 2006;317:1143–1149.
58. Ramalho LN, Pasta AA, Terra VA, Augusto M, Sanches SC, Souza-Neto FP, Cecchini R, Gulin F, Ramalho FS. Rosmarinic acid attenuates hepatic ischemia and reperfusion injury in rats. Food Chem Toxicol. 2014;74:270–278.
59. Munne Bosch S, Schwarz K, Alegre L. Enhanced formation of alpha-tecopherol and highly oxidized abietane diterpenes in water-stressed rosemary plants. Plant Physiol. 1999;121(3):1047–1052.
60. Sak SA, 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.
61. Najim SM, Numan IT, Hamad MN. The possible cardioprotective effects of different fractions of Artichoke extracts against 5-Fu induced cardiotoxicity in albino rats. International Journal of Pharmacy and Pharmaceutical Sciences. 2015;7(10):165-169.

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