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Original Article

Protective effects of *Zizyphus oxyphyla* on liver and kidney related serum biomarkers in (CCl_4) intoxicate rabbits

Efeitos protetores de *Zizyphus oxyphyla* em biomarcadores de soro relacionados ao fígado e rim em (CCl_4) intoxicam rabbtis

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Abstract

The study was aimed to evaluate the therapeutic effects of *Zizyphus oxyphy*la leaves methanolic (ZOX-LME), on serum liver, kidney and hematology along with other serum parameters in Carbon tetrachloride (CCl_4) intoxicated rabbits. Experimental animals were divided into five groups, six rabbits in each. These were: group NC (normal control), group, TC (toxic control) and group ST i.e. silymarine administered group at dose rate (50) mg/kg body weight (BW). Group ET1 and group ET2 treated with (ZOX-LME) at dose 200 mg/kg BW and 400 mg/kg BW. CCl_4 administration caused significant (P> 0.05) impairment in serum liver enzymes, blood factors and other serum indices. Treatment with (ZOX-LME) significantly (P<0.05) reduced and normalized the levels of serum alanine transaminase (ALT) aspartate transaminase (AST) and alkaline phosphatase (ALP) and hematological indices. Also significant (P< 0.05) reduction was observed in creatinine, urea, uric acid, blood urea nitrogen (BUN), and albumin and glucose concentrations. The altered levels of lipid profile and serum electrolytes (Ca, Mg, Cl, Na, K, and P) were significantly (P<0.05) change toward normal levels with (ZOX-LME) feeding. In addition (ZOX-LME) ingestion caused significant improvement in GSH, GST and CAT levels, while reducing the TBARS levels, exhibited antioxidant capacity. Also (ZOX-LME) showed increase inhibition against percent scavenging of 2, 2-diphenile-1-picrylehydrazyle (DPPH) free radical. Significant (P<0.05) normalizing effects were observed with high dose 400 mg/kg BW of (ZOX-LME and were equivalent to silymarine administered groups. The histological study of liver supported the hepatoprotective and renal curative activity of (ZOX-LME).

Keywords: methanol extract, hematological parameters, serum levels, 2, 2-diphenile-1-picrylehydrazyle (DPPH), hepatoprotective, (ZOX-LME).

Resumo

O estudo teve como objetivo avaliar os efeitos terapêuticos das folhas metanólicas de *Zizyphus oxyphyla* (ZOX-LME) no fígado, rim e hematologia séricos, juntamente com outros parâmetros séricos em coelhos intoxicados com tetracloreto de carbono (CCl_4). Os animais experimentais foram divididos em cinco grupos, seis coelhos em cada. Estes foram: grupo NC (controle normal), grupo TC (controle tóxico) e grupo ST, isto é, grupo administrado com silimarina na taxa de dose (50) mg / kg de peso corporal (PC). Grupo ET1 e grupo ET2 tratado com (ZOX-LME) na dose de 200 mg / kg de peso corporal e 400 mg / kg de peso corporal. A administração de CCl_4 causou prejuízo significativo (P > 0,05) nas enzimas hepáticas séricas, fatores sanguíneos e outros índices séricos. O tratamento com (ZOX-LME) reduziu significativamente (P < 0,05) e normalizou os níveis de alanina transaminase (ALT), aspartato transaminase (AST) e fosfatase alcalina (ALP) e os índices hematológicos. Também foi observada redução significativa (P < 0,05) nas concentrações de creatinina, ureia, ácido úrico, nitrogênio ureico no sangue (BUN), albumina e glicose. Os níveis alterados de perfil lipídico e eletrólitos séricos (Ca, Mg, Cl, Na, K e P) foram significativamente (P < 0,05) mudando em direção aos níveis normais com a alimentação (ZOX-LME). Além disso, a ingestão de (ZOX-LME) causou melhora significativa nos níveis de GSH, GST e CAT, enquanto reduzia os níveis de TBARS, exibindo capacidade antioxidante. Também (ZOX-LME) mostrou inibição aumentada contra a eliminação percentual do radical livre 2, 2-difenila-1-picrilehidrazila (DPPH). Efeitos de normalização significativos (P < 0,05) foram observados com

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altas doses de 400 mg / kg de peso corporal de (ZOX-LME) e foram equivalentes aos grupos administrados com silimarina. O estudo histológico do fígado confirmou a atividade hepatoprotetora e curativa renal de (ZOX-LME).

Palavras-chave: extrato de metanol, parâmetros hematológicos, níveis séricos, 2, 2-difenila-1-picrilehidrazila (DPPH), hepatoprotetor, (ZOX-LME).

1. Introduction

The liver is a vital organ responsible for various metabolic functions and clearance and transformation of drugs and toxins from the blood and regulate immune responses (Yan et al., 2014).

Kidney is also important homeostatic organ (Mitrakou, 2011). Thus both are exposed to toxic injury. Toxicity in animals may be either through the production of secondary metabolites or by the other organisms, i.e., microbes, plants or other animals hosted by them (Luckner, 2013). More than thousand drugs of the current pharmaceutical era have been shown to cause hepato and renal toxicity with diverse clinical appearances (Björnsson, 2016). Hepatic and renal injury is related always with cellular necrosis; decrease in tissue lipid peroxidation (Contreras-Zentella and Hernández-Muñoz, 2016). More over serum levels of many biochemical markers like alanine amino transaminase (ALT), aspartate amino transaminase (AST), serum glutamic oxalo acetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), total cholesterol, and total bilirubin (TB) are evaluated along with serum electrolyte, urea, uric acid and creatinine imbalances are assessed (Olaniyan and Ateni, 2018).

Different aspects of liver injury during hepatotoxicity may include hepatitis (Wang et al., 2013); granuloma (Kamal et al., 2012); vascular lesions (Rubbia-Brandt et al., 2010). The major effect of hepatotoxicity is jaundice which is caused due to bilirubin accumulation in the extra cellular fluid, causing weakness, severe fatigue, dark urine and light colored stool (Frenzel and Teschke, 2016).

A number of chemical toxicants have also been reported for toxicity in animals and humans. Carbon tetrachloride (CCl_4) is one of the toxic agent (Danladi et al., 2013) The level of tissue injury is related to the amount of dose and period of exposure to carbon tetrachloride (Abdel-Moneim et al., 2015). Its mechanism of toxicity is based on lipid membrane peroxidation and generation of trichlomethyl radical (•CCl3), causing severe cell injury (Tan et al., 2016).

Many investigators have given the reports about plants, that having phenolic compounds such as tannins, flavonoids, procyanidins, anthocyanins and phenolic acids have liver, heart and nephroprotective activities (Oliboni et al., 2011) Several studies have revealed that the plant extracts possessing antioxidant activity that defend carbon tetrachloride CCl_4 induced hepatotoxicity by preventing peroxidation of lipid and increasing antioxidant Enzyme activity (El-Haskoury et al., 2018; Mahmoodzadeh et al., 2017). In vitro anti-oxidant activates are carried out by using different free radicals including DPPH, Hydrogen peroxide, super oxide, Nitric oxide, trichloromethyl (CCl_3) (Leamklang, 2018) DPPH (2, 2-diphenyl-1- picrylhydrazyl) is a familiar "scavenger") for other radicals.(Adegoke et al., 2012). During the present study the *Zizyphus Oxyphyla* plant was selected. Commonly found in Pakistan, Africa, Australia, and tropical America (Kaleem, 2011). Traditionally in the rural areas the root of *Zizyphus oxyphyla* are dried, boiled in water, filtered, and used in the treatment of liver disorder, especially for jaundice (Ijaz et al., 2016). It has also been found that some species have antiulcer genic activity (Sharifi-Rad et al., 2018). Moreover the study was aimed to investigate the effect of methanolic extract of *Zizyphus oxyphyla* leave's (ZOX-LME) on liver and renal related serum parameters, hematological indices and lipid profile. In addition and serum electrolytes and serum antioxidant biomarkers like, glutathione (GSH), (GST) superoxide dismutase (SOD) and thiobarbituric acid reactive substances (TBARS) were studied. Furthermore in-vitro antioxidant capacity based on (DPPH) scavenging assay was performed and permanent slides of liver and kidney sections were prepared for histopathological findings.

2. Materials and Methods

2.1. Study area, plant material and extraction

The *Zizyphus oxyphyla* plant was collected from the Mountain of village Shamozai, District Swat. K.P.K Pakistan in the year 2019. Following authentication was made from a voucher specimen deposited in Herbarium, Botany Department University of Malakand. The shade dried leaves were ground with (ZK-115, Japan) and obtain powder of about 830 gram was soaked in 1500 mL of 100% methanol and crude extract @ (ZOX-LME) of red black color was obtained after processing.

2.2. Animals and study regime

Forty adult domestic male rabbits (*Oryctoagus cuniculus*), weighing round about 1000-1350 gram and 6-7 month old were procured from the local market. For acclimation, the animals were for 14 days, before start of the experimental work. All animal procedures are in accordance with the recommendations of the research animals committee for care and use of Griffin and Locke (2016). During the experimentation, a total of 40 adult male rabbits were divided into five groups, comprising of eight animals in each group.

Group NC: Normal control group (10 mL/kg body weight (BW) of normal saline orally;

Group TC: Toxic Control group (10 mL/kg body weight of CCl_4 (50% v/v in olive oil rally;

Group ST: Standard Control group (Silymarin @ 50 mg/kg BW dissolved in 10 mL mineral water each day oral after intoxication;

Group ET1: (ZOX-LME) @ 200 mg/kg BW, each day oral after intoxication;

Group ET2: (ZOX-LME) @ 400 mg/kg BW, each day oral after intoxication.

2.3. Acute toxicity test

The acute toxicity study was carried out for the (ZOX-LME) of different doses i.e. 1000, 1500 and 2000 in mg per kg body weight. Acute toxicity was determined according to the Organization for economic co-operation and development (OECD) guideline number, 420 (OECD, 2001). During this test, the (ZOX-LME) was assigned safe up to highest dose (2000 mg/kg BW) as no mortality was caused.

2.4. Hematological, biochemical and histopathological study

All animals were dissected on the last day of experimentation. Blood (3.5 mL) was collected in Ethylene Diamine Tetra-acetic Acid (EDTA)-coated tubes (K2-EDTA tubes) and into sterile coagulant tubes and were subjected for hematological and biochemical analysis i.e. alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP). Serum Glucose, urea, uric acid, creatinine, BUN and albumin were estimated, along with total serum cholesterol, HDL, LDL, VLDL and triglyceride were determined. Liver from each animal was rapidly excised after dissection. 0.5 g liver tissue was homogenized in 5 mL 0.9% Na Cl (10% w / v) using a Teflon homogenizer (Glass-Col, Terre Haute, USA).

2.5. Serum oxidative biomarkers assay

Serum catalase (CAT), glutathione-S-transferase (GST) and (GSH) also Thiobarbituric acid reactive substances (TBARS) were determined according to the manufacturer's instructions of assay kits (Biodiagnostic Company, Egypt).

2.6. DPPH radical scavenging activity

The *in vitro* antioxidant activities of (ZOX-LME) was evaluated on the basis of (DPPH) scavenging assay by using earlier procedures (Koksal et al., 2011). The concentration of solutions prepared for the activity were expressed as parts per million (ppm), equal to mg/L. A 25 mL stock solution of 500 ppm of (ZOX-LME) was prepared in methanol. From the stock solution; a 5mL solution each of 20, 40, 80,100 and 200 ppm was prepared in separate test tubes. Each concentration was taken in triplicate. The same procedure was repeated for ascorbic acid which was used as standard using 1700 Shimadzu Japan) at 517 nm for absorbance. The following Formula 1 was used:

$$Percent\ radical\ scavenging\ activity = \left(Ac - As\ /\ Ac\right) \times 100) \quad (1)$$

where 'Ac' is the absorbance of control, 'As' is the absorbance of sample.

2.7. Statistical analysis

The data (expressed as mean ± SE) were analyzed by one way ANOVA and "Tukey test" using SPSS software. Values of $p < 0.05$ were considered to be statistically significant.

3. Result

The results of acute oral toxicity showed no signs and symptoms of illness and mortality was seen with (ZOX-LME) administration up to 2000 mg/kg BW). The effect of (ZOX-LME) on the values of ALT, AST, ALP, RBCs, WBCs, platelets and haemoglobin (HB) are presented in Table 1. The results indicate that CCl_4 administration significantly ($P < 0.05$) increased the serum levels of ALT, AST and ALP as compare with control group A. After treatment with (ZOX-LME) at high dose (400 mg/kg BW), significantly ($P < 0.05$) lowered the concentration of ALT, AST and ALP (group = ET2) and caused a consequent normalization. The mean values of RBCs WBCs, Platelets and hemoglobin (Hb) showed in Table 1. A significant ($P < 0.05$) reduction in the activities of RBC, WBC, Platelets and hemoglobin (Hb) were observed in rabbits that received CCl_4 alone (group TC), when compared to normal control rabbits. The administration of (ZOX-LME) at 200 mg/kg BW, caused no-significant ($p>0.05$) reduction in the concentration of heamatological parameters while at dose rate 400 mg/kg body weight significantly reduced the elevated level of

Table 1. Shows liver function enzymes and heamatological indices in the experimental animal groups treated with CCl_4, silymarine and (ZOX-RBM), extract.

Parameters	Experimental groups				
	NC	TC	ST	ET1	ET2
Serum ALT (IU/L)	32.08 ± 2.8a	88.61± 6.7b	33.85 ± 4.2a	68.84 ± 3.8c	37.65 ±3.6a
Serum AST (IU/L)	44.55 ± 1.6a	102.7 ± 6.5b	48.15 ± 1.4a	88.89 ± 3.7c	49.25 ± 1.0a
Serum ALP (IU/L)	58.27 ± 2.4a	134.8 ± 2.2b	59.44± 6.7a	113.7 ± 3.8c	63.20 ± 2.9a
RBC $\times 10^6/\mu L$	5.7 ± 0.09a	3.2 ± 0.09b	5.4 ± 0.04a	4.2 ± 0.13c	5.4 ± 0.04ac
WBC $\times 10^3/\mu L$	6.2 ± 0.34 a	3.97 ± 0.08b	5.96 ± 0.25a	4.02 ± 0.11c	5.73 ± 0.0a
Platelets $\times 10^3/\mu L$	139 ± 5.64 a	241 ± 5.49 b	147 ± 1.19a	198 ± 3.8c	154 ± 3.85a
Hemoglobin g/dL	11.47 ± 0.45a	7.87 ± 0.63b	11.22 ± 0.51a	9.98 ± 0.27c	10 ± 1.41ac

NC = Normal Control; TC = Toxic Control; ST = Silymarine Control; ET1 = Extract (ZOX-LME) 200 mg/kg BW; ET2 = Extract (ZOX-LME) 400 mg/kg BW. Same alphabets in a row shows no significant difference ($P<0.05$) while the different alphabets in the same row show significant difference ($P<0.05$) among the mean of the parameters.

hemoglobin, RBC,WBC and platelets when compared to toxic control animals. Animals feed with silymarine significantly (p>0.05) normalized the values of liver enzymes and hemoglobin, RBC and Platelets respectively.

A statistically significantly (P < 0.05) elevation was observed in the creatinine, urea, uric acid, blood urea nitrogen, albumin and glucose levels, when the animals were exposed to CCl_4 (Table 2). Likewise serum electrolytes such as Ca, Mg, Cl, Na, K and P concentration were elevated. No significant reduction had occurred with the feeding of (ZOX-LME) at 200 mg/ kg BW. Though a highly significant (P < 0.05), reduction in the levels of urea, uric acid, creatinine, blood urea nitrogen, albumin, glucose and serum electrolytes were noted with the intake of (ZOX-LME) (400 mg/ kg.BW) and silymarine (50 mg/kg BW) respectively. The levels of total lipid, cholesterol, triglycerides, LDL and VLDL were significantly (P < 0.05) elevated and HDL was reduced in the in animals feed with CCl_4 shown in Table 3.

The ingestion of (ZOX-LME) at dose (400 mg/kg BW) showed significant (P < 0.05) therapeutic effect while, 200 mg/kg BW, (ZOX-LME) revealed non-significant effect on the levels of lipid profile when compared to toxic control animals.

Table 4 revealed that carbon tetrachloride CCl_4 induced hepatic and renal toxicity, as significant (p < 0.05) reduction was observed in serum glutathione GSH, GST and SOD, while (TBARS) levels was elevated when compared with control animals. The (ZOX-LME) (200 mg/kg BW) showed a mild curative effect but not significant effect on serum antioxidant biomarkers, while the (ZOX-LME) at dose rate 400 mg /kg BW, significantly (p < 0.05) improved the GSH, GST and SOD levels and reduced the (TBARS) toward normal value when compared to normal control and toxic control animals respectively. The results of high dose extract animals were comparable with silymarine administered animals. The results of high dose (ZOX-LME)) were comparable with that of silymarin (50 mg/

Table 2. Shows Kidney function enzymes, glucose and serum electrolytes level in the experimental animal groups treated with CCl_4, silymarine and (ZOX-LME), extract.

Parameters (mg/dl)	Experimental groups				
	NC	TC	ST	ET1	ET2
Creatinine	0.28 ± 0.08a	0.97 ± 0.10b	0.37 ± 0.8a	0.67 ± 0.04c	0.39 ± 0.09a
Urea	27.08 ± 1.2a	56.76 ± 0.7b	30.31 ± 0.65a	46.48 ± 2.6c	32.98 ± 1.7a
Uric acid	0.44 ± 0.02a	0.83 ± 0.09b	0.47 ± 0.02a	0.66 ± 0.00c	0.54 ± 0.02ac
BUN	14.09 ± 0.60a	26.81 ± 1.30b	16.45 ± 1.75a	20.95 ± 0.98c	17.23 ± 0.73a
Albumin	2.74 ± 0.19a	5.51 ± 0.21b	2.93 ± 0.06a	4.11 ± 0.25c	2.83 ± 0.41a
Glucose	95 ± 3.60a	137 ± 4.62b	104 ± 4.66a	113 ± 4.33c	106 ± 3.36ac
Ca (mmol/L)	4.97 ± 0.25a	9.06 ± 0.35b	4.90 ± 0.23a	6.19 ± 0.14c	5.16 ± 0.30ac
Mg (mmol/L)	0.42 ± 0.01a	0.72 ±0.04b	0.46 ± 0.01a	0.54 ± 0.01c	0.48 ± 0.02a
Cl (mmol/L)	99.4 ± 2.84a	120.6 ± 1.8b	101.7 ± 2.3a	109.9 ± 2.3c	105.4 ± 1.6ac
Na (mmol/L)	134.4 ± 1.5a	159.7 ± 2.7b	136.1 ± 0.75a	145.1 ± 1.16c	137.5 ± 0.36a
K (mmol/L)	5.30 ± 0.18a	7.62 ± 0.34b	5.56 ± 0.15a	6.33 ± 0.24c	5.58 ± 0.38a
P (mmol/L)	1.24 ± 0.01a	1.59 ± 0.04b	1.27 ± 0.02a	1.38 ± 0.00c	1.29 ± 0.00a

NC = Normal Control; TC = Toxic Control; ST = Silymarine Control; ET1 = Extract (ZOX-LME) 200 mg/kg BW; ET2 = Extract (ZOX-LME) 400 mg/kg BW. Same alphabets in a row shows no significant difference (P<0.05) while the different alphabets in the same row show significant difference (P<0.05) among the mean of the parameters.

Table 3. Shows (M ± SD) values of lipid profile of the experimental animal groups treated with CCl_4, silymarine and (ZOX-LME), extract.

Lipids (mg/dl)	Experimental groups				
	NC	TC	ST	ET1	ET2
TL	287.1 ± 7.5a	373.1 ± 1.8b	300.7 ± 8.4a	354.1 ± 7.7c	314.6 ± 6.08a
Cholesterol	93.83 ± 4.9a	166.5 ± 10.4b	103.8 ± 4.0a	135.8 ± 1.8c	110.4 ± 7.5a
HDL	27.05 ± 1.2a	19.13 ± 0.7b	25.26 ± 1.3a	21.23 ± 0.8c	24.50 ± 0.7ac
LDL	42.74 ± 2.0a	72.62 ± 2.8b	46.52 ± 1.0a	62.85 ± 3.42c	47.93 ± 1.81a
VLDL	22.62 ± 1.0a	48.11 ± 2.9b	25.38 ± 0.7a	30.86 ± 0.9c	26.00 ± 1.5a
TG	68.20 ± 1.5a	85.28 ± 3.6b	72.06 ± 2.3a	78.04 ± 2.5c	70.11 ± 1.9a

NC = Normal Control; TC = Toxic Control; ST = Silymarine Control; ET1 = Extract (ZOX-LME) 200 mg/kg BW; ET2 = Extract (ZOX-LME) 400 mg/kg BW. Same alphabets in a row shows no significant difference (P<0.05) while the different alphabets in the same row show significant difference (P<0.05) among the mean of the parameters.

kg BW) administered rabbits. Since it is clear that that (ZOX-LME) treatment is dose dependent i.e. high dose seems to be better in the recovery of hepatic and renal injury when compared with the results of toxic control animals that received CCl_4 alone confirmed the healing effects of (ZOX-LME).

3.1. Histological examinations

Photomicrographs taken from liver and kidney sections of all experimental animals and were shown in Figure 1 (A1, A2, A3, A4 and A5) and Figure 2 (B1, B2, B3, B4, B4 and B5) respectively. Liver sections from control rabbit showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus, nucleolus, central vein and compact arrangement of hepatocytes (Figure 1A1). In contrast to this, CCl_4 caused hydropic alteration and necrosis in centrilobular hepatocytes (Figure 1A2). Sinusoids and central vein Congestion clarified acute inflammatory cells infiltrating. In animals treated with silymarin, showed histoarchitecture with mild infiltration of inflamed cells (Figure 1A3). Animals treatment with (ZOX-LME) at dose of 200 mg/kg BW, sinusoids and central vein congestion and acute inflammatory cells infiltrating were of less extent (Figure 1A4). No tissue damage and necrosis was observed at hepatocyte cords in the liver of (ZOX-LME) ingestion at dose rate 400 mg/kg BW (Figure 1A5). In the Figure 2B1, B2, B3, B4 and B5, the kidney sections of the different groups have been shown. In Figure 2B1, there was normal lung having no lesions as compared to the normal control group. The group that was infected but not medicated (Figure 2B2) had tissue damage and necrosis with cellular congestion. The silymarine medicated group (Figure 2B3) had a normal kidney appearance. Kidney section of animals treated with 200 mg/kg BW of (ZOX-LME) has acute inflammatory cells infiltrating were and mild haemomoraghic status (Figure 1A4). Normal histoarchitecture with mild inflammation was seen in the kidney of animals ingested (ZOX-LME) at dose rate 400 mg/kg BW (Figure 1A5). The scoring of histological damage is displayed in Table 5.

3.2. DPPH activity

The results for antioxidant activity against DPPH of (ZOX-LME) at various concentrations are shown in Table 6. The percent inhibition values were 32.66%, 51.96%, 59.71%, 67.70% and 81.55% respectively. Various concentrations of

Table 4. Shows serum antioxidant enzyme activities (M ± SD) of the experimental animal groups treated with CCl_4, silymarine and (ZOX-LME), extract.

Antioxidant biomarkers	Experimental groups				
	NC	TC	ST	ET2	ET1
TBARS U/mL	3.5 ± 0.2a	5.2 ± 0.6b	3.4 ± 0.3a	4.6 ± 0.3bc	4.3 ± 0.4a
GSH U/mL	0.22 ± 0.01a	0.16 ± 0.01b	0.21 ± 0.00a	0.17 ± 0.00b	0.20 ± 0.01a
GST U/mL	0.84 ± 0.01a	0.72 ± 0.02b	0.83 ± 0.01a	0.75 ± 0.02b	0.82 ± 0.00a
CAT U/mL	0.96 ± 0.02a	0.67 ± 0.01b	0.90 ± 0.01a	0.73 ± .06bc	0.88 ± 0.03a

NC = Normal Control; TC = Toxic Control; ST = Silymarine Control; ET1 = Extract (ZOX-LME) 200 mg/kg BW; ET2 = Extract (ZOX-LME) 400 mg/kg BW. Same alphabets in a row shows no significant difference ($P<0.05$) while the different alphabets in the same row show significant difference ($P<0.05$) among the mean of the parameters.

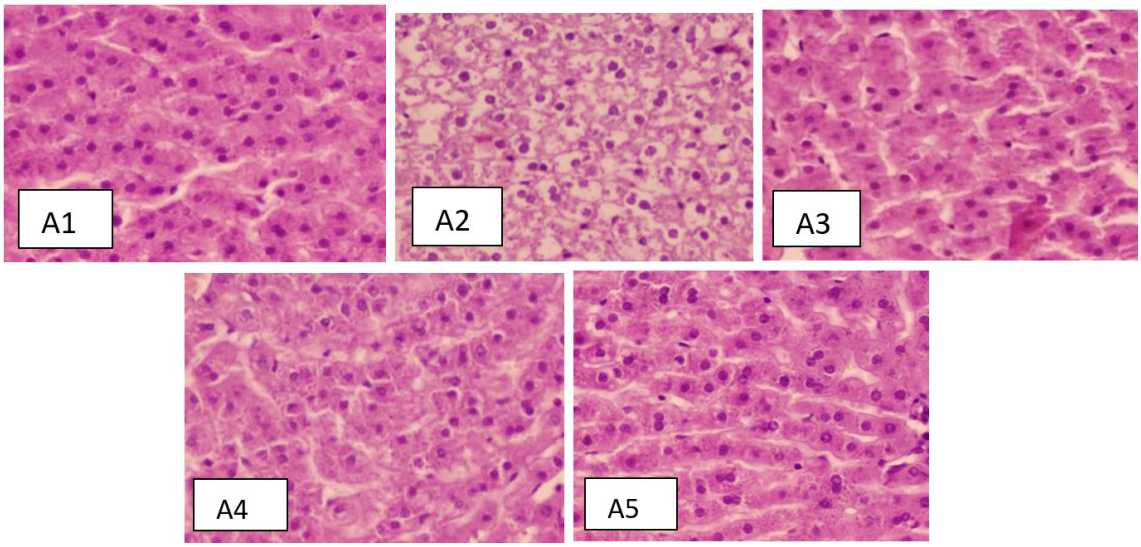

Figure 1. Photomicrographs of liver section from various experimental animal groups (A1, A2, A3, A4, and A5).

(ZOX-LME) showed percent inhibition in the following order 20 ppm < 60 ppm < 80 ppm < 100 ppm < 200 ppm. The increased in extract concentration caused an increase in percent inhibition showed the antioxidant potential of (ZOX-LME) (Table 6).

4. Discussion

Liver damage is a major health problem and is a serious challenge to public health in the world (Pimpin et al., 2018). The existing synthetic liver treatment drugs create

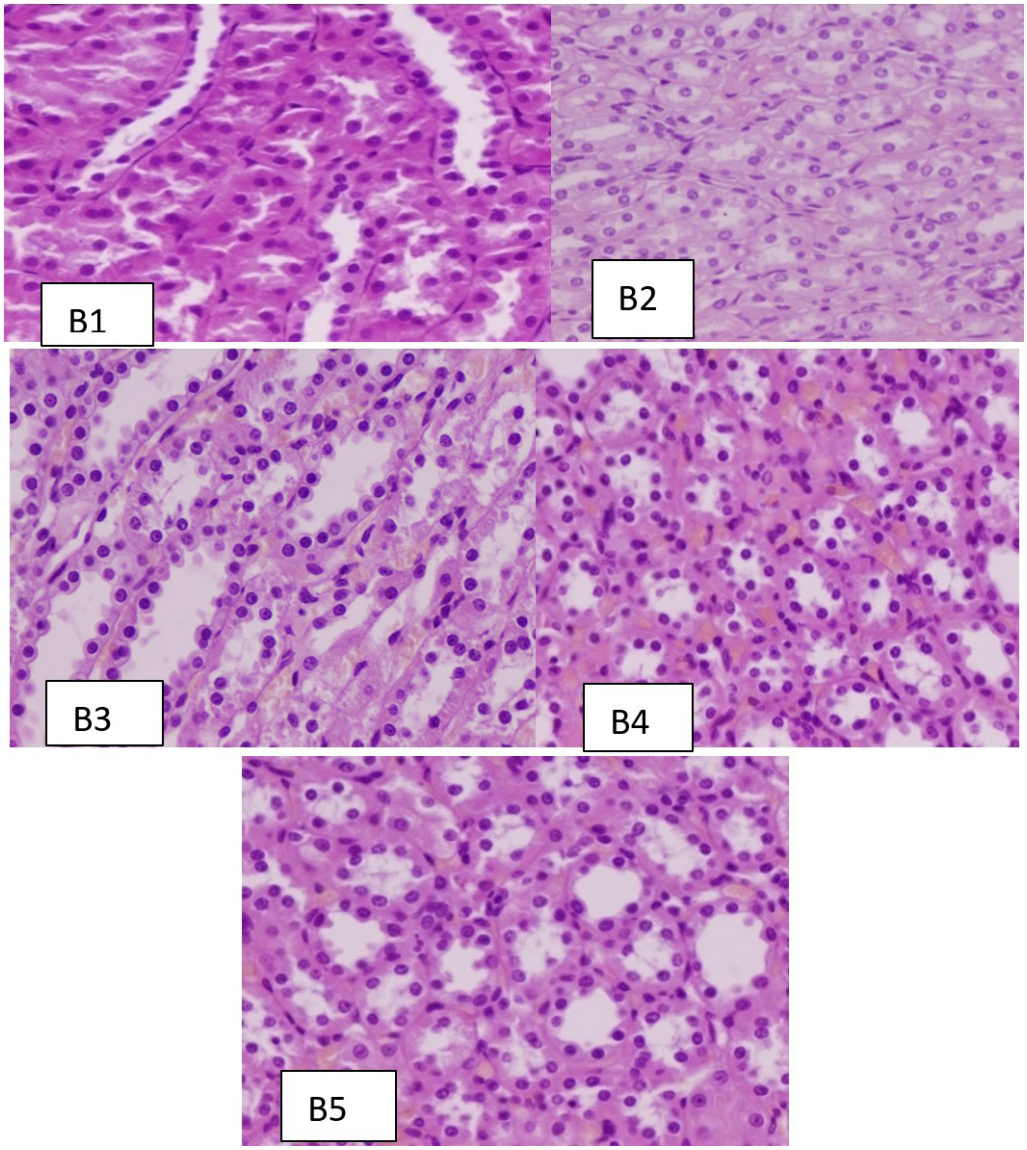

Figure 2. Photomicrographs of kidney section from various experimental animal groups (B1, B2, B3, B4 and B5).

Table 5. Semiquantitive score of histopathological findings.

Groups	Hydropic degeneration	Liver steatosis	Inflammatory cell infiltration	Necrosis
Group A	0	0	0	0
Group B	+++	++	+++	+++
Group C	+	0	0	+
Group D	++	0	+	+
Group E	+	0	0	+

Damage grade are as follow: 0 = no abnormality; + = mild injury; ++ = moderate injury and +++ = severe injury.

Table 6. Shows radical scavenging activity of (ZOX-LME), extract concentrations on the basis of percent inhibition of DPPH free radicals.

(ZOX-LME) extract Conc	% Inhibition	Mean ± SEM
20 ppm	32.66%	26.70 ± 4.38
60 ppm	51.96%	48.70 ± 3.49
80 ppm	59.71%	54.70 ± 4.20
100 ppm	67.70%	62.76± 1.21
200 ppm	81.55%	71.7 ± 0.89

further complications hence; herbal medicines have more demands and widespread use in hepatic disorders since long time (Rotman and Sanyal, 2017). The current study intended to reveals the hepato and renal protective and antioxidant effects of (ZOX-LME) against CCl_4-induced toxicity in rabbits. For the screening of hepatoprotective drugs, frequently CCl_4 is used as hepatotoxic agent in the laboratories. CCl_4 is break down to the trimethyl radical (CCl3) and a proxy trichlomethyl radical (OOCCl3) by cytochrome P-450 2EI enzyme (Jia et al., 2011), these radicals bind covalently to the macromolecules and probably caused lipid peroxidation by attacking membrane polyunsaturated fatty acids, there by disturbing membrane integrity and caused hepatic damage associated with oxidative stress (Dahiru et al., 2013). Liver damage is evaluated by assessing the concentration of discharged transaminases including ALT, AST and ALP in blood (van Deursen et al., 2014)

Results of the present study revealed increased in serum ALT, AST, ALP and RBCs, WBCs, platelets and haemoglobin levels in carbon tetra chloride (CCl_4) administered rabbits when compared with normal control rabbits ($P<0.05$).Immediate rise in serum transaminases showed, CCl_4 induced severe toxicity (Jannu et al., 2012). The statement was confirmed by necrosis and infiltration of inflammatory cells during histopathological examination of microphotographs of liver sections (Jiang et al., 2016), reported that several pharmaceutical drugs like rifampicin, isoniazid, paracetamol, etc. are used in medical therapy are hepatotoxic, producing free-radical that causes lipid membrane peroxidation resulting hepatocytes injury.

During the present research the rabbits intoxicated with carbon tetrachloride (CCl_4) were treated with a standard antioxidant drug, Silymarine and (ZOX-LME) of graded doses. Silymarine is a standard antioxidant drug and frequently used as a hepatoprotective medicine, derived from a plant, *Silybum marianum* (Tajmohammadi et al., 2018). In this study administration of (ZOX-LME) at dose 400 mg/kg BW to CCl_4 intoxicated rabbits reduced the elevated level of ALT, AST, ALP and blood factors toward normal. These results are nearly equivalent to the standard drug, Silymarine. The current study is in agreement with that of (Cordeiro and Kaliwal, 2013), who investigated the liver protective activity of the alcohol extract of *Capparis sepiaria* stem against CCl_4 intoxicated Albino rats. The kidney is a major homeostatic organ keeps the balance of body fluids by cleaning and secreting metabolites like urea, uric acid, creatinine, and minerals from the blood and eliminate the nitrogenous wastes together with water, as urine (Javaid et al., 2012). It retains the overall intracellular chemical composition by regulating the concentration of water, sodium chloride, potassium, phosphate and many other elements in the body.

An increase in creatinine, urea, uric acid and blood urea nitrogen along with serum ions concentrations (Ca, Mg, Cl, Na, K, and P) indicated a kidney dysfunction in a CCl_4 administered animals. However high dose of (ZOX-LME) feeding showed significant ($P>0.05$) normalizing effects. These finding are in agreement with previous studies (El Saied Azab et al., 2014) who demonstrated the nephro-preventive role of rosemary, curcumin and propolis against gentamicin induced toxicity in guinea pigs. Related findings were presented by (Muhammad et al., 2015), who investigate the effects of *Vitex doniana* aqueous bark extract on serum electrolytes levels in Albino rats. In a condition like hepatic or renal injury make the lipid profile disrupted. As evident from our study in which CCl_4 ingestion caused significant increase in total lipid (TL), cholesterol, LDL, VLDL and TG level and decrease HDL value. Moreover, administration of (ZOX-LME) extract regulated the serum lipid markers toward normal level. This is in agreement with (El-Baz et al., 2015), who investigate the effectiveness of sesame oil as antihypercholesterolemic substance in rats fed rich-fat diet.

In addition, carbon tetrachloride CCl_4 caused cellular toxicity and necrosis by reducing the efficiency of serum antioxidant enzymes i.e., catalase (CAT), glutathione peroxidase (GSH), glutathione (GST) and enhanced the production of TBARS (Alam, 2018). The cellular endogenous defense system consist of enzymatic and non-enzymatic antioxidants such as SOD, CAT, GSH and GST which controlled the oxidative stress generated as a result of tissues damage. In present study, the level of catalase (CAT), glutathione peroxidase (GSH), glutathione (GST), were significantly ($P> 0.05$) lowered in CCl_4 administered animals when compared to the control animals.

Treatment of rabbits with either (ZOX-LME) or silymarin ameliorated the toxic effects of CCl_4 and restored the levels of serum CAT, GSH, GST towards the control group in accordance with other findings (using *Oxalis corniculata* whole plant extract against carbon tetrachloride, toxicity in rats. They find a significant ameliorative effect on serum glutathione GSH, GST and CAT contents in rat. Similar findings were also presented by (Adesanoye and Farombi, 2010), who investigated the hepatoprotective effects of *Vernonia amygdalina* in carbon tetrachloride intoxicated rats. Investigative of TBARS serves as an indirect marker of lipid peroxidation of polyunsaturated fatty acids of hepatocyte membrane (Zargari and Sedighi, 2015). Rise in TBARS level by CCl_4 ingestion in this study shows the liver injury including series of chain reactions. Results of this study revealed that (ZOX-LME) ingestion at high dose reduced the TBARS levels toward normal value. This decreased in TBARS level may be due to active ingredients of (ZOX-LME) extract that may responsible for scavenging ROS and improving the antioxidant potentials of tissues. Our study is in agreement with (Quan et al., 2013), studied

the hepatoprotective of polysaccharides extracted from *Boschniakia rossica*, actin against carbon tetrachloride-induced toxicity in mice.

The antioxidant capacity of (ZOX-LME) was assessed through the DPPH radical scavenging assay. The present results showed that (ZOX-LME) exhibit good percent scavenging radical inhibition value even at lowest concentration and an increased was noted with increasing concentration of (ZOX-LME). The antioxidant activity of (ZOX-LME) may be due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers and some of the pharmacological effects might be due to these valuable compounds. These results are in accordance with findings of Jothy et al. (2011) and Enujiugha et al. (2012), whom explored African *Sphenostylis stenocarpa* phenolic extracts and *Cassia fistula* seeds extract for its scavenging capacity on the basis of DPPH radical scavenging assay.

The liver and kidney sections of rabbits treated with (ZOX-LME) after CCl_4 intoxication are revealed to have amended cellular membrane architecture or less damage to the hepatic cells as compared to rabbits treated with CCl_4. The improved histoarchitecture further verify the liver preventive potential of the (ZOX-LME) and support the results of biochemical parameters. Normalization of CCl_4 impaired liver and kidney architecture by therapeutic plants were described by many investigators like Nwaigwe et al. (2012) reported the regulation of hepatotoxicity by *Olax viridis*, *Tephrosia calophylla* and *Curcuma longa* respectively.

Finally it is evident from overall results and discussion that (ZOX-LME) ingestion can mitigate all the adverse effects of CCl_4 on liver transaminases, hematological indices, renal function biomarkers along with serum ion concentrations and the lipid profile by the virtue of its vital ingredients . In addition, the decreased level of (TBARS) and elevated activity of GSH, GST and CAT in CCl_4 intoxicated animals after giving (ZOX-LME) indicated that lipid peroxidation was inhibited. The effect of (ZOX-LME) similar to the effect of silymarine in comparison with toxic control animals. This action could be attributed to the high content of phytochemical constituents which are potent antioxidants can protect liver and kidney cells from injury. *Zizyphus oxyphyla* contain alkaloids, flavonoids and saponins and other active phytochemicals (Kaleem et al., 2014).

5. Conclusion

From the present study it was concluded that the (ZOX-LME) extract is very potent against liver and kidney damage. The (ZOX-LME) extract is also beneficial for most of hematological and lipid parameters. In addition, (ZOX-LME) extract has potent antioxidant capacity revealed form good healing ability by improving and regulating the impaired levels of serum glutathione GSH and GST and catalases CAT (cellular antioxidants). Therefore one can use this plant extract (ZOX-LME) traditionally, for many liver disorders. Further this plant extract can also be used scientifically for other fatal and chronic diseases. Hence this research recommends that the (ZOX-LME) possess the anti-oxidant property, which could be used as best source of advance medication. Thus further exploration of the plant is required.

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