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Effects of *Onopordum acanthium* L., seeds extracts on serum tumor necrosis factor-alpha and angiotensin-converting enzyme in hyperlipidemic and diabetic rats

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ABSTRACT

This study was designed to evaluate the role of local *Onopordum acanthium* L. (cotton thistle) seed extracts (200mg/kg) in diabetic and hyperlipidemic rats with the histopathological examination of liver, kidney and spleen tissues. Ninety adult male rats were randomly divided into nine equal groups. The first group was used as a control fed on a standard diet; the second group was treated with water extract and the third group was treated with the ethanolic extract. The fourth group was injected with streptozotocin (40mg/kg) as a diabetic group, while in the fifth group, diabetic rats were treated with water extract and in the sixth group, diabetic rats were treated with the alcoholic extract. The seventh group was fed on high cholesterol diet (hyperlipidemic group). In the eighth and ninth groups, hyperlipidemic rats were treated with water extract and alcoholic extracts, respectively. The fasting blood glucose (FBS), angiotensin-converting enzyme (ACE), tumor necrosis factor-alpha (TNF-α), lipid profile, liver and renal function parameters, CRP, WBCs, hematological parameters and body weight were almost improved when diabetic and hyperlipidemic rats were treated with water extract and ethanol extract. Histopathological changes in the liver, kidney and spleen in diabetic groups were improved in groups treated with both extracts. Conclusion: from the results of this study, it can be reported that extracts of local cotton thistle seeds (mostly water extract) were effective in controlling abnormal parameters and histopathological changes in diabetic and hyperlipidemic rats.

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INTRODUCTION

Diabetes mellitus and obesity are common metabolic disorders that are characterized by impaired metabolism of carbohydrates, lipids and proteins, resulting in the defects of insulin secretion and action affecting most systems of the body with fatal consequences when left uncontrolled (Mohamed *et al.*, 2016). Moreover, the role of inflammation in diabetes and dyslipidemia has been implicated in several studies that inflammatory reaction mostly mediated by the acute phase of proteins and cytokines, lead to the prevention or promotion of diabetes (Liu *et al.*, 2016). On the other hand, others reported that ACE might be altered or activated in diabetic patients.

Onopordum acanthium L. (family: Asteraceae) is distributed in different countries, traditionally used as a food and popularly in the medicine for anti-inflammatory agent against the tumor, skin ulcers

and healing of the respiratory system problems (Al-Snafi, 2020). Other investigations revealed its antioxidants, ACE inhibitor activities (Sharifi *et al.*, 2013). Moreover, the seeds of cotton thistle contain important compounds such as flavonoids, lignans, sesquiterpenes, triterpene and phenylpropanoids with multiple biological effects (Lajter *et al.*, 2015). However, the role of cotton thistle seeds on the serum level of TNF-α and ACE was poorly studied. Therefore, this study investigates the biological activities of the *Onopordum acanthium* L. seed extracts on diabetic and hyperlipidemic rats.

MATERIALS AND METHODS

Animal and housing

Rats were bred in the animal house at the Department of Biology / College of Science/University of Duhok, Iraq and placed in ventilated polypropylene cages ($30\times25\times17$cm), with free access to standard diet and water under ethical and standard laboratory conditions (12h light: 12h dark photoperiod, at 24 ± 2ºC). Animals were acclimated to the laboratory conditions for 10 days then mixed for breeding. Adult males weighting 160-180gm were used for the experimental study. Cages bedding were changed frequently, especially in diabetic groups due to polyuria.

Collection and identification of the plant species

Plant seeds were collected during ripening season (June 2019) in Duhok City and dry, ripped seeds were cleaned and stored in a dark glass container. The plant was authenticated and morphologically described by Professor Dr Saleem E. Shahbaz, Taxonomist at the College of Agriculture Engineering Science/ Department of Forestry / University of Duhok.

Preparation of water and alcoholic seed extracts

Seeds powder (100g) was suspended in 1liter of distilled water and another 100g of defatted seeds by Soxhlet (Jiangsu- china) added to 1liter of ethanol 80% (Scharlab S.L-Spain) under shaking for 24hrs. The mixtures were filtered by white cheese cloth and whatman filter papers 4.0μm. Filtrated solvents were evaporated to dryness by vacuum at 45C$^{\square}$ by Rotary Evaporator (Eyela-United Kingdom). The water extract was dissolved in water and ethanol extract in dimethyl sulfoxide. All extracts were purified weekly for obtaining fresh extract for experiment.

Induction of diabetes

Thirty male albino rats were fasted overnight and injected with a single intra-peritoneal of 40 mg/kg.bw of streptozotocin (STZ) (Sigma-Alorich-USA), which was dissolved in citrate buffer (PH = 4.5) and injected immediately. STZ injected animals, treated orally with 5% of D-Glucose (Scharlab S.L-Spain) after half an hour receiving STZ to prevent the potentially fatal hypoglycemic effects. Symptoms of diabetes were appeared within 24-72 hours after STZ injection; such as polyuria, polyphagia and polydipsia compared with the control group. The diagnosis of diabetes was further confirmed by testing blood sugar and glucose in urine by urine strips (Machinery-Nagel/ Germany).

Thus, animals with glycosuria, ketones in the urine and FBS more than 200mg/dl were considered diabetic rats (Margoni *et al.*, 2011).

Standard diet

Standard diet was prepared for each one kilogram (kg) as follows: wheat 665.5g, soya 256.2g, sunflower oil 43.5g, limestone 14.9g, $Ca_2(PO_4)$ 6.4, salt 6.3g, lysine 2.4g, methionine 1.5g, enzymes 0.8g, choline chloride 0.6g, vitamins 0.58g and trace elements 0.5g (National Research Council , 1995).

Preparation of high cholesterol diet

High fat diet that was prepared for each 1kg by adding 5g of cholesterol powder (Griffin-England), 1g of cholic acid (Rome-Italy) and 10ml of coconut oil (Emad-Iraq) to the standard diet (Margoni *et al.*, 2011).

Experimental Designs

Ninety albino rats were randomly divided into nine equal groups for 4 weeks as follows,

Group 1: Rats fed on a standard diet and served as a normal control group.

Group 2: Normal rats treated with water extract (200/kg.bw/day) only.

Group 3: Normal rats treated with ethanol extract (200/kg.bw/day) alone.

Group 4: STZ injected rats were fed on a standard diet and served as a diabetic group.

Group 5: Diabetic rats treated with water extract (200/kg.bw/day).

Group 6: Diabetic animals treated with the ethanolic extract (200/kg.bw/day).

Group 7: Rats fed on high cholesterol diet and served as a hyperlipidemic group.

Group 8: Hyperlipidemic rats treated with water extract (200/kg.bw/day).

Group 9: Hyperlipidemic rats treated with the ethanolic extract (200/kg.bw/day).

Estimation of hematological and biochemical parameters

At the end of the experiment, animals were deprived of food overnight with free access to water. Rats were anaesthetized with diethyl ether (Scharlab S.L-Spain) and blood samples were obtained directly by heart puncture in which 2 ml of blood were collected in heparinized tubes (Arzer Grande- Italy) for the determination of hematological parameters by the automated hematological analyzer (Horiba-France). Other blood were placed in gel tubes (Arzer Grande-Italy) for 30 minutes then centrifuged (at 4000 rpm for 15 minutes) for biochemical parameters such as FBS, serum lipids, renal and liver function parameters and ACE were estimated by Cobas 600(C501) automated chemistry analyzer (Roche/Germany). This apparatus estimates the tests depending on their regent's kits (Roche/Germany) that include: absorbance photometry (enzymes, substrates and specific proteins).

Estimation of Tumor Necrosis-Factor Alpha (TNF-α)

Serum level of TNF-α was determined by enzyme-linked immunosorbent assay (BioTeck-USA) followed by steps in a kit purchased from (Mybiosource-USA).

Histopathological study

After dissection of the animals, liver, kidney and spleen organs were immediately removed and washed with distilled water and fixed in 10% formalin, after preparing the paraffin block, thin sections (4 μM) were stained with Haematoxylin (H) and Eosin (E) stains by using Auto Staining Apparatus (Leica/Germany). The mounted slides were identified by a light microscope (Motic/China) for the detection of histological changes in each slide and the pointed field was taken by a specialized Camera (Anmo/Taiwan) connected with a microscope.

Ethics

The study was accepted by the scientific committee of the Biology Department/College of Science/Duhok University. All participants signed a consent form after the study proposal was explained through a seminar.

Statistical analysis

Data were analyzed using Microsoft Excel 2010 and GraphPad Prism 5 (California- USA) using analysis of variance (ANOVA) followed by Tukey test. Firstly, all groups were compared with the control group (pointed as *), then treated diabetic and hyperlipidemic groups were compared with diabetic and hyperlipidemic groups (pointed as capital letters; A, B, C and D). Results were expressed as mean $\pm$ standard errors and P-values <0.05 were considered a statistically significant value.

RESULTS

The results represent the effects of cotton thistle seed extracts on

The results illustrate the effects of local cotton thistle seed extracts in diabetic and hyperlidemic rats. The values represent mean $\pm$ standard errors. The results revealed; FBS, lipid profiles, liver enzymes, serum proteins, total serum bilirubin (TSB), urea, creatinine, ACE, WBCs, CRP, TNF-α, hematological parameters and histological examination of liver, kidney and spleen as follows

Blood glucose and serum lipids

As showed in (Tables 1 and 2), FBS was significantly (P<0.001) increased in the diabetic and hyperlipidemic groups (P<0.05) in comparison with the control group. In diabetic rats treated with water extract and ethanol extract, glucose level was significantly decreased (P<0.001, P<0.05) respectively in these groups compared to the diabetic group. In other groups, no obvious changes were observed in the FBS level (Figure 1A).

The level of serum lipids was significantly (P<0.001) higher in diabetic and hyperlipidemic groups than in the control group. In diabetic groups treated with both extracts, these parameters have almost become significantly lower. In hyperlipidemic groups treated with both extracts, no obvious statistical changes were noticed in serum lipids. Only LDL significantly decreased when treated with water extract (P<0.001) and ethanolic extract (P<0.05) in comparison with the hyperlipidemic group (Figure 1C).

TNF-α and ACE

TNF-α was significantly (P<0.001) increased in hyperlipidemic rats and improved significantly when treated with water extract (P<0.05) and ethanolic extract (P<0.01) (Table 4). In other groups, no obvious changes were noticed in the TNF-α level (Table 3 & Figure 1F).

Serum level of ACE in normal rats treated with extracts does not show obvious changes. ACE was significantly higher in diabetic (P<0.01) and hyperlipidemic group (P<0.05) after administration of seed extracts, diabetic and hyperlipidemic rats showed a significant (P<0.01) reduction in the ACE level observed in the group treated with water extract and ethanolic extract (P<0.05). In hyperlipidemic groups with both extracts, ACE

Table 1: Effects of cotton thistle extracts on FBS and lipid profile in diabetic groups

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Glucose (mg/dl)	112.1± 4.37	112.4± 3.18	107.7± 4.98	580.4± 15.13***A	273.4± 14.92***D	530.7± 16.86***B
Cholesterol (mg/dl)	57.50± 2.46	51.86± 2.79	55.70± 3.95	86.30± 2.87***A	63.20± 2.98 D	63.08± 2.63D
Triglyceride (mg/dl)	59.13± 3.04	57.21± 4.97	58.40± 2.91	114.± 4.33***A	70.0± 3.34 D	79.57± 4.83***A
HDL (mg/dl)	24.56± 1.18	27.59± 1.69	24.60± 1.80	23.70± 1.85 A	25.98± 1.68 A	23.92± 1.24 A
VLDL (mg/dl)	11.83± 0.61	11.44± 0.99	11.68± 0.59	22.82± 1.20***A	14.0± 1.07D	15.91± 3.61C
LDL (mg/dl)	15.17± 0.54	13.97± 0.66	13.54± 0.74	38.86± 3.12***A	21.32± 3.17 D	25.61± 2.66***D

Value expressed mean ± SE

Table 2: Effects of cotton thistle extracts on FBS and lipid profile in hyperlipidemic rats

Parameters	Group 1	Group 7	Group 8	Group 9
Glucose (mg/dl)	112.1±4.37	159.0±3.23*A	145.5±2.23A	146.8±2.49A
Cholesterol (mg/dl)	57.50±2.46	113.2±1.70***A	96.83±1.55***A	100.7±2.06***A
Triglyceride (mg/dl)	59.13±3.04	131.0± 3.98***A	116.8± 3.35***A	112.3± 2.92***A
HDL (mg/dl)	24.56±1.18	20.83±1.74A	24.17±0.79A	23.50±0.99A
VLDL (mg/dl)	11.83±0.61	26.20±0.79***A	23.37±1.01***A	22.47±1.01***A
LDL (mg/dl)	15.17±0.54	66.13±3.40***A	49.30±1.43***D	54.70±1.58***B

Value expressed mean ± SE

Table 3: Effects of cotton thistle extracts on TNF-α and ACE level in diabetic groups

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
TNF-α (pg/ml)	7.85±1.12	4.31±0.86	5.65±1.43	3.07±0.61A	7.09± 1.59 A	3.75±1.16A
ACE (U/L)	192.1±3.90	177.7±4.97	196.4±4.68	227.0±4.56***A	205.9±3.95 C	207.8±4.14B

Value expressed mean ± SE

Table 4: Effects of cotton thistle extracts on TNF-α and ACE level in hyperlipidemic groups

Parameters	Group 1	Group 7	Group 8	Group 9
TNF-α (pg/ml)	7.85±1.12	20.91± 1.87***A	13.59±1.79B	11.91± 2.59C
ACE (U/L)	192.1±3.90	214.0±3.56*A	199.1±4.49A	210.3±1.73A

Value expressed mean ± SE

level improved but statistically was non-significant in comparison with the hyperlipidemic group (Tables 3 and 4, Figure 1E).

Renal and liver function parameters

There was an increased level of serum urea (P<0.001) and creatinine (P<0.01) in diabetic rats. Serum urea was significantly (P<0.001) reduced in diabetic rats treated with both extracts (Figure 1D) and creatinine decreased significantly (P<0.01) in the diabetic group treated with water extract (Table 5). In other groups, no obvious changes were observed in the level of these parameters (Table 6).

AST and GGT in all groups showed no significant changes. ALT was significantly (P<0.001) reduced in diabetic rats treated with water extract in comparison with the untreated diabetic group. Serum ALP level in untreated diabetic and hyperlipidemic

Table 5: Effects of cotton thistle extracts on renal and liver function parameters in diabetic groups

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Urea (mg/dl)	35.8±0.9	31.3±2.5	31.3±3.2	136.5±3.6***A	75.4±5.26**D	90.9±5.3***D
Creatinine (mg/dl)	0.36±0.02	0.33±0.04	0.32±0.02	0.75±0.03**A	0.53±0.04**C	0.65±0.02**A
AST (GOT) IU/L	123.6±2.0	130.1±3.1	126.0±4.0	137.9±4.8 A	125.2±6.5A	132.8±5.A
ALT (GPT) (IU/L)	38.6±1.4	35.8± 2.5	36.3±1.2	66.4±2.9***A	46.7±3.9D	55.1±4.6**A
ALP (IU/L)	155.2±4.98	161.4±6.4	167.3±7.3	490.6±16.7***A	307.2±14.2**D	312.0±11.2***D
GGT (IU/L)	1.6±0.16	1.5±0.29	2.1±0.50	4.6±0.56A	2.5±0.34A	3.0±0.35A
TSB (mg/dl)	0.18±0.013	0.16±0.014	0.18±0.02	0.48±0.05***A	0.21±0.03C	0.32±0.06A
Total Protein (g/dl)	6.5±0.10	6.4±0.18	6.6±0.11	5.5±0.15**A	6.2±0.12C	5.8±0.09***A
Albumin (g/dl)	3.6±0.02	3.7±0.08	3.6±0.15	2.8±0.13***A	3.4±0.09D	3.1±0.11**A
Globulin (g/dl)	2.9±0.12	2.7±0.13	3.0±0.17	2.7±0.07A	2.8±0.08A	2.7±0.11A

Value expressed mean ± SE

Table 6: Effects of cotton thistle extracts on renal and liver function parameters

Parameters	Group 1	Group 7	Group 8	Group 9
Urea (mg/dl)	35.8±0.9	41.2±1.7A	37.3±0.9A	37.2±1.3A
Creatinine(mg/dl)	0.36±0.02	0.44±0.02A	0.43±0.01A	0.42±0.02A
AST (GOT) IU/L	123.6±2.0	140.7±4.7A	128.8±4.8A	137.7±4.9A
ALT (GPT) (IU/L)	38.6±14	45.5±4.29A	39.8±1.5A	40.3±1.4A
ALP (IU/L)	155.2±4.98	308.8±6.69***A	262.3±10.38***A	282.0±10.25***A
GGT (IU/L)	1.6±0.16	2.7±0.43A	2.0±0.52A	2.33±0.56A
TSB (mg/dl)	0.18±0.013	0.22±0.023A	0.20±0.025A	0.21±0.03A
Total Protein (g/dl)	6.5±0.10	6.13± 0.071A	6.5±0.15A	6.4±0.04A
Albumin (g/dl)	3.6±0.02	3.32±0.54A	3.52±0.54A	3.49±0.46A
Globulin (g/dl)	2.9±0.12	2.86±0.56A	2.96±0.14A	2.98±0.47A

Value expressed mean ± SE

groups was significantly increased (P<0.001) in comparison with a control group and decreased significantly (P<0.001) when diabetic rats were treated with both extracts.

TSB level was significantly (P<0.001) elevated in the diabetic group and reduced significantly (P<0.01) when treated with water extract.

Serum total proteins and albumin were significantly (P<0.001) lower in untreated diabetic and diabetic treated with ethanol extract (P<0.01) in comparison to the control group. In diabetic rats treated with water extract, total protein (P<0.01) and albumin (P<0.001) were significantly improved.

In other groups, no obvious changes were seen in the level of these parameters. Globulin remains with no significant changes in all groups compared to the control group (Tables 5 and 6).

CRP, WBCs and hematological parameters

CRP level in diabetic rats increased significantly (P<0.01) and it was decreased significantly (P<0.01) when treated with both seed extracts in comparison with the diabetic group. WBC significantly increased (P<0.05) in diabetic rats and improved almost near the control group with both extracts treatment (Table 7). Granulocyte% and lymphocyte% showed no obvious changes in all groups. Monocytes% increased significantly (P<0.001) in the positive diabetic group and the diabetic group treated with ethanol extract (P<0.01). In diabetic rats treated with water extract, monocytes% decreased significantly (P<0.05) when compared to diabetic rats. In other groups, no significant changes were found in the percentages of monocytes. Hematological parameters showed no significant alteration in all groups (Tables 7 and 8).

Table 7: Effects of cotton thistle extracts on CRP, WBCs and hematological parameters in diabetic groups

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
CRP (mg/l)	0.16±0.09	0.15±0.03	0.14±0.02	2.67± 0.22***A	0.88± 0.23*D	1.14± 0.16***D
WBC ($x10^6/mm^3$)	8.3±0.60	8.1±0.39	8.2±0.28	12.2± 1.03*A	8.5± 0.75A	9.5± 1.21A
Granulocyte (%)	7.8±0.58	8.08±1.16	7.36±0.51	10.96± 1.1A	7.08± 0.79A	10.8± 0.50A
Lymphocyte (%)	80.40±1.74	81.20±2.34	84.91±1.71	72.28± 4.15A	82.63± 2.62A	73.08± 1.46A
Monocytes (%)	9.97±0.78	9.60±0. 82	9.24±0.94	16.05± 1.75***A	10.54± 1.73B	15.11± 0.96**A
RBC ($x10^6/mm^3$)	6.41±0.52	6.23±0.15	6.11±0.13	6.23 ±0.07A	6.55 ±0.08A	6.34 ±0.08a
Hb (g/dL)	13.3±0.08	13.4±0.2	13.0±0.2	12.6± 0.1A	13.2± 0.3A	13.1± 0.3A
PCV (%)	41.4±0.5	40.5±0.7	40.1±7	38.3± 0.1A	41.9± 0.6A	41.1± 1.3A
MCV (fL)	57.1±1.8	60.2±2.1	55.8±1.6	54.6± 0.9A	59.80± 3.4A	58.5± 1.9A
MCH (pg)	18.7±0.8	21.7±1.1	18.4±0.8	16.1± 0.4A	17.9± 0.8A	17.5± 0.7A
MCHC (g/dL)	32.8±0.7	36.4±2.9	33.6±0.3	30.9± 0.1A	31.1± 0.8A	31.4± 0.1A
Platelets ($/mm^3$)	574.0±12.5	523.8±16.9	536.0±18.7	552.4± 11.1A	547.0± 17.8A	532.4± 23.5A

Value expressed mean ± SE

Table 8: Effects of cotton thistle extracts on CRP, WBCs and hematological parameters in hyperlipidemic groups

Parameters	Group 1	Group 7	Group 8	Group 9
CRP (mg/l)	0.16±0.09	0.17±0.07A	0.14±0.08A	0.13±0.02A
WBC ($x10^6/mm^3$)	8.3±0.60	10.8±0.47A	9.4±0.21A	9.5±0.17A
Granulocyte (%)	7.8±0.58	9.30±0.24A	9.17±1.39A	8.45±0.59A
Lymphocyte (%)	80.40±1.74	85.83±0.69A	82.42±2.95A	81.47±1.63A
Monocytes (%)	9.97±0.78	10.43±0.7A	9.41±0.69A	10.43±0.6A
RBC ($x10^6/mm^3$)	6.41±0.52	6.705±0.071A	6.55±0.17A	6.49±0.16A
Hb (g/dL)	13.3±0.08	13.7±0.01A	13.5±0.19A	13.3±0.3A
PCV (%)	41.4±0.5	41.5±0.6A	40.9±0.5A	41.1±0.6A
MCV (fL)	57.1±1.8	65.8±1.4A	62.3±2.7A	60.3±1.1A
MCH (pg)	18.7±0.8	23.8±0.6A	22.0±1.2A	20.0±1.1A
MCHC (g/dL)	32.8±0.7	36.7±0.7A	36.1±0.5A	34.4±0.5A
Platelets ($/mm^3$)	574.0±12.5	598.2±6.8A	573.8±17.2A	580.8±12.7A

Value expressed mean ± SE

Table 9: Effects of cotton thistle extracts on bodyweight in diabetic groups

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Initial Body weight (g)	166.3±2.3	166.2±1.8	165.4±1.5	175.5± 17A	173.4± 3.4A	176.1± 1.9A
Final Body weight (g)	304.3±2.1	277.7±2.1	280.1±2.8	159.9± 11.5***A	190.9± 5.4***B	179.9± 14.62***A
Body weight gain (g)	138.0	115.5	114.7	-15.6	17.5	3.8

Value expressed mean ± SE

Table 10: Effects of cotton thistle extracts on body weight in hyperlipidemic groups

Parameters	Group 1	Group 7	Group 8	Group 9
Initial Body weight (g)	166.3±2.3	163.2± 1.2A	162.7± 1.1A	163.8± 1.5A
Final Body weight (g)	304.3±2.1	349.2± 1.9***A	309.8± 1.5B	322.5± 3.8A
Body weight gain (g)	138.0	186.0	147.1	158.7

Value expressed mean ± SE

Bodyweight

In diabetic rats, final body weight (FBW) was reduced significantly (P<0.001), whereas in hyperlipidemic rats, FBW significantly (P<0.01) increased in rats in comparison with the control group. Animals get higher weight when diabetic rats were treated with ethanol extract and body weight gets further improvement with water extract treatment. In hyperlipidemic rats treated with water extract, the FBW was reduced (P<0.05) significantly in comparison with hyperlipidemic rats (Tables 9 and 10).

Histological examination of kidney, liver and spleen

Kidney

In the control group (Figure 2A), groups treated with extracts only (Figure 2B, C) and hyperlipidemic groups (not listed figures) showed almost normal cytoarchitecture appearance of glomeruli and renal tubules. In untreated diabetic rats (Figure 3D), kidney sections showed variable damages through degeneration in glomeruli, dilatation of Bowman's capsule, necrosis in renal tubules, congestion of blood vessels and hemorrhage. In diabetic rats treated with ethanolic extract (Figure 3F), structural improvement was seen in kidney tissues and further improved with water extract (Figure 3E) through a reduction in damages by decreasing congestion, Hemorrhage and necrosis in glomeruli and renal tubules.

Liver

In normal rats treated with onopordum extracts (Figure 4B, C) and hyperlipidemic groups (not listed figures), liver sections showed mostly no obvious structural changes in comparison with the normal histological appearance of the control group (Figure 4A). In the diabetic group, liver sections showed structural damages with variable congestion, hemorrhage, necrosis in the central vein, increased vacuolated hepatocytes, sinusoidal dilation and variable degrees of inflammatory cells (Figure 5D). In the diabetic rats treated with the ethanolic extract (Figure 5F), Liver sections showed fewer damages while in diabetic rats treated water extract; liver sections showed further reduction in the hemorrhage, congestion, necrosis and other complications of diabetes on liver damage (Figure 5E).

Spleen

Normal rats treated with seed extracts (Figure 6B, C) and hyperlipidemic groups (not listed figures), spleen sections showed the normal histological appearance of white pulps, red pulps, germinal center and central arteriole as compared to spleen structure of the control group (Figure 6A). In the diabetic group showed obvious changes in the structure of white and red pulp, a large number of macrophages were present (arrows), apoptotic changes in megakaryocyte with fragmented nuclei (Figure 7D). In diabetic groups treated with onopordum, extracts showed almost normal parenchyma consists of white pulp, red pulp, central and decreased apoptotic changes (Figure 7E).

DISCUSSION

Onopordum acanthium contains various compounds, including flavonoids and phenolic com-

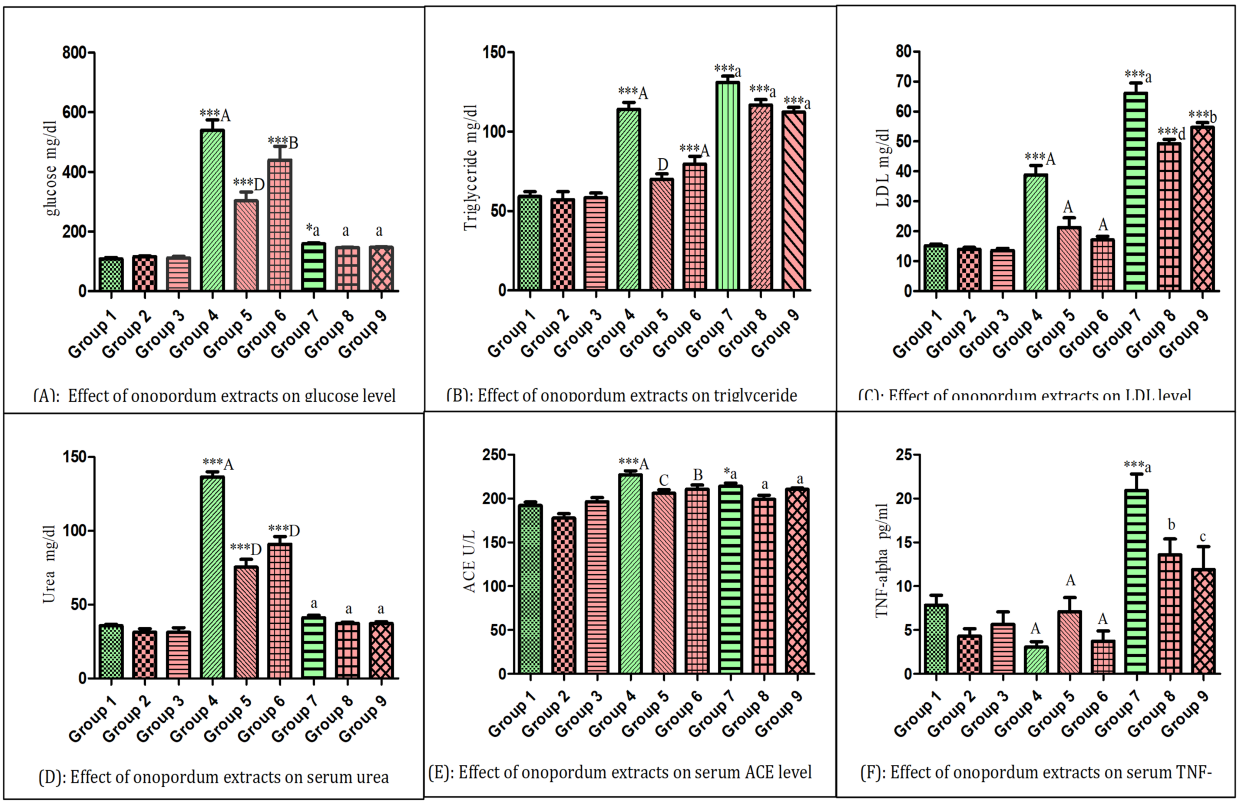

Figure 1: Effect of onopordum extracts on some parameters in which * = comparison of all groups with control group, capital letters = comparison between diabetic groups, small letters = comparison among hyperlipidemic groups

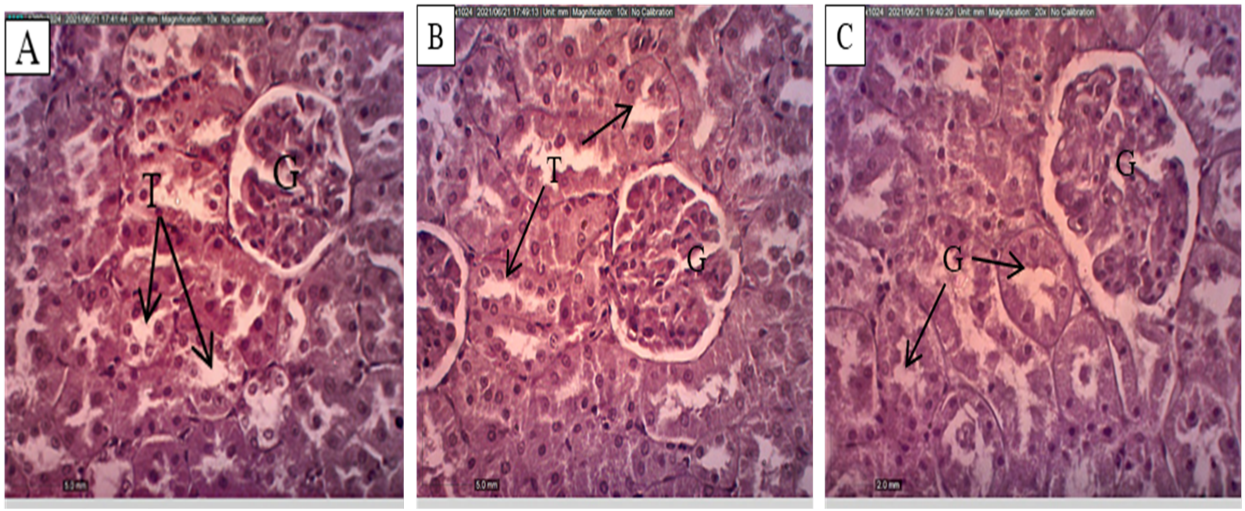

Figure 2: Cross sections of kidney at 20x magnifications(H&E): control group (A), group treated with water extract (B),and ethanolic extract (C) showing normal glomeruli (G) and renal tubules(T)

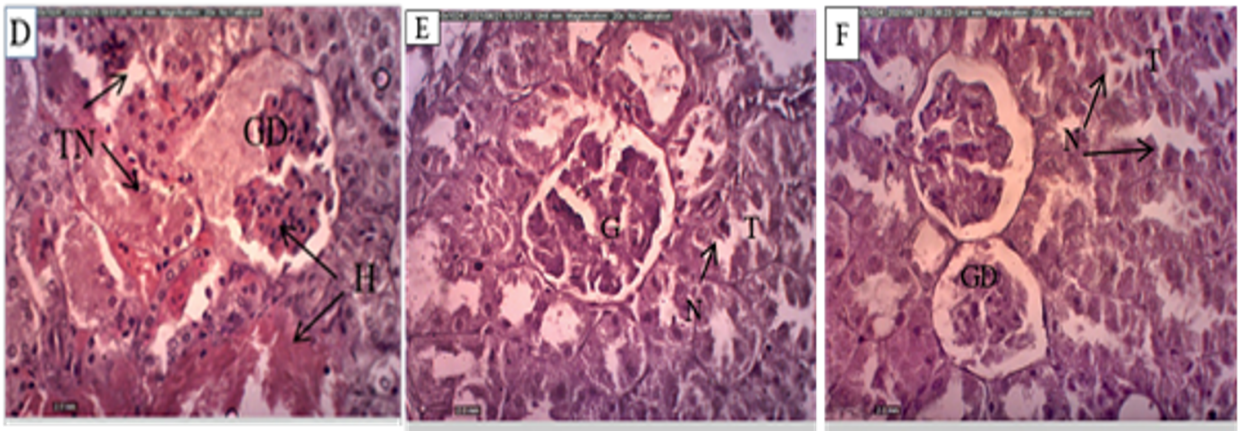

Figure 3: Kidney of diabetic group (D) showing glomerulus degeneration (GD), hemorrhages (H), and tubular necrosis (N). Diabetic rats treated with water (E) and ethanolic (F) extracts showing reduction in kidney damages

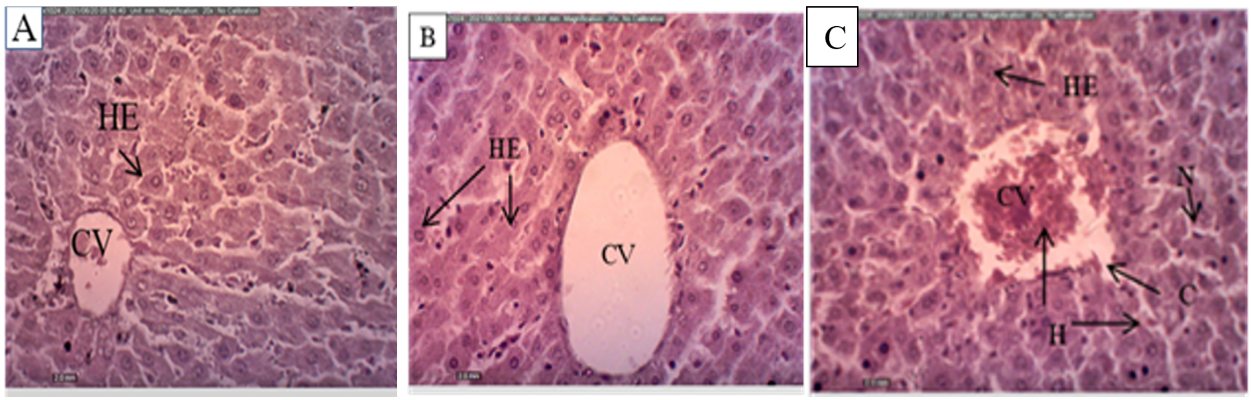

Figure 4: Cross sections of liver at 20x magnifications(H&E): in control group (A), rats treated with water extract (B)and ethanolic extract (C) showing normal structure of central vein (CV) and hepatocyte (HE)

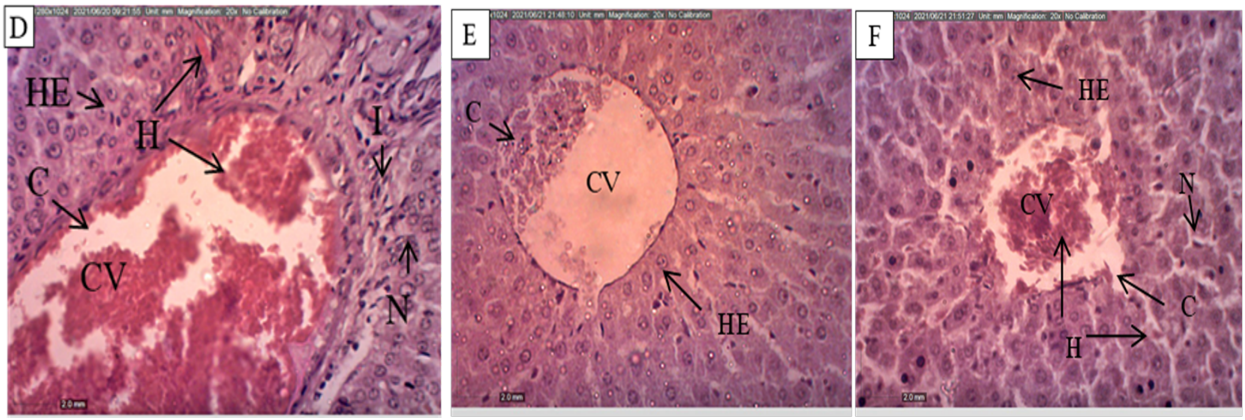

Figure 5: Liver sections in diabetic group (D)showing congestion (C), hemorrhage (H), necrosis (N) and inflammatory cells(I). Diabetic rats treated with water (E) and ethanolic extracts (F)with less liver damages

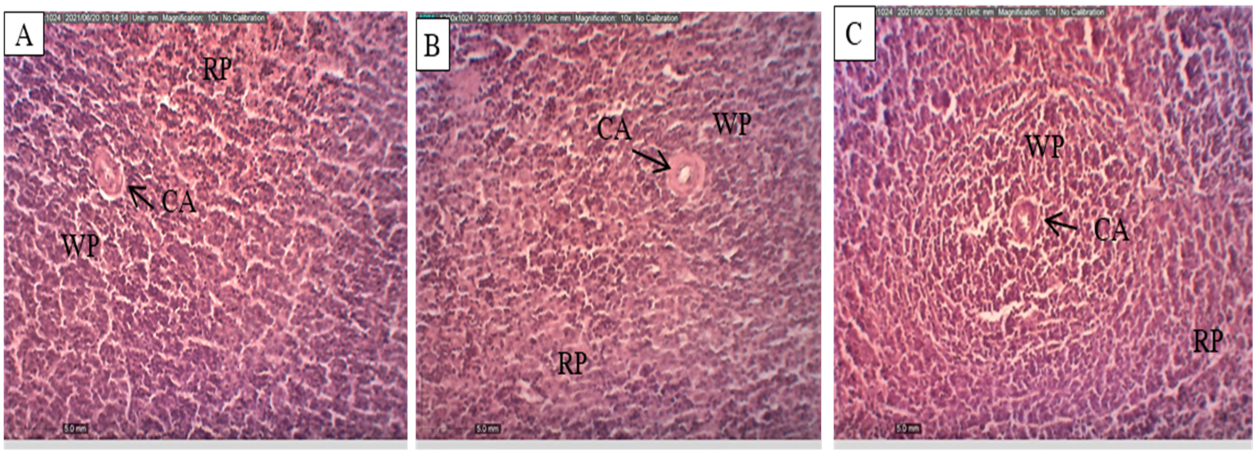

Figure 6: Cross section of spleen at 10x in control group (A), rats treated with water extract (B) and ethanolic extract (C) showing normal parenchyma architecture of white pulp (WP), red pulp (RP), central arteriole (CA)

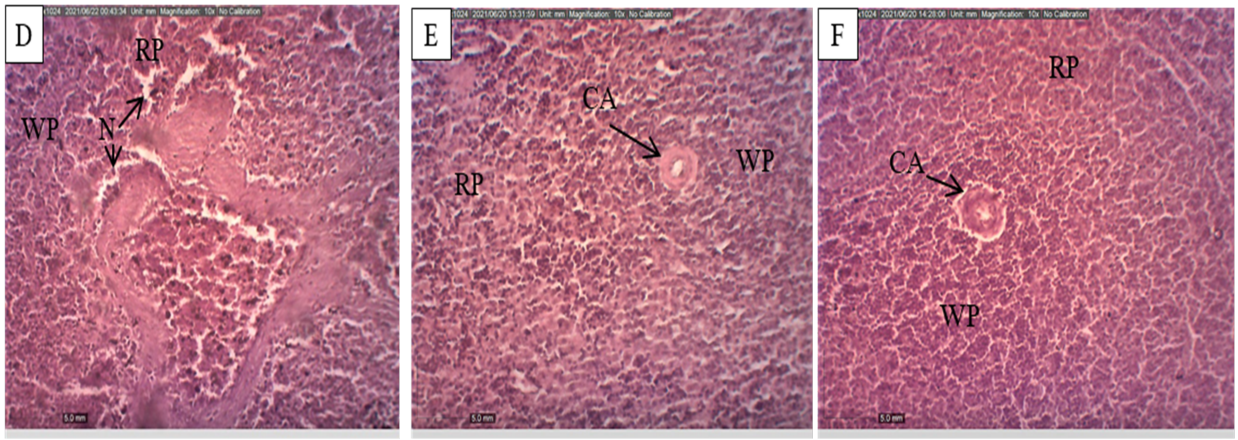

Figure 7: Spleen sections in diabetic rat (D) showing changes in the white and red pulps through scattered necrotic areas in the parenchyma (N). Diabetic rats treated with water (E) and ethanolic extracts (F) showing less damages

pounds such as sesquiterpenes, flavonolignans and phenylpropanoids. It has been proved that these compounds are responsible for various health-related activities and pharmacological properties (Strzelecka *et al.*, 2005; Al-Snafi, 2020).

The chemical composition of our plant has been planned to be investigated in collaboration with a university in Romania. Still, due to the spread of the Coronavirus and the closure of movement between countries for a long time, it was depended on the crude extract for this study. In future research, we plan to concentrate on the role of active components of the local cotton thistle on both types of diabetes.

It has been reported in other studies that the extracted trans-cinnamic acid, which is phenolic compounds from the defatted seeds of cotton thistle, has different biological characteristics and pharmacokinetic roles (Patra *et al.*, 2012). However, others showed that using the whole extract is more effective than using a single constituent of the plant (Yusri *et al.*, 2012). This indicates that the onopordum plant gains a new botanical source that could provide a useful therapeutic and preventive agent for the liver and oxidative stress-related diseases.

Effect of cotton thistle extracts on FBS and serum lipids

In diabetic and hyperlipidemic groups, FBS and serum lipid significantly increased as compared to the control group. These results were supported previously (Dokumacioglu *et al.*, 2018; Kraska *et al.*, 2021). In the current study, oral administration of onopordum seed extracts to diabetic rats and hyperlipidemic rats showed an effective role of seed extracts in the controlling of FBS and serum lipids.

Most glucose lowering plants can act directly or indirectly as a hypoglycemic agent through enhancement of insulin secretion or activating

the insulin-receptors and stimulate glucose transporters. These, in turn, facilitate the entering of glucose into the hepatic cells, adipocytes and skeletal muscles and increase glycogen storage (Aba and Asuzu, 2018). One of the important effects of onopordum extracts was speculated in the current study during fatal hyperglycemia; the mortality rate was decreased when diabetic rats were treated with the ethanolic extract. Most of the animals survived until the end of the experiment when treated with water extract. On the other hand, antioxidant substances such as tocopherols, flavonoids and phenolic acids have been confirmed in onopordum previously. These compounds are most popular among the phytochemicals, which can protect organs against tissue damages and improve glucose, lipid, hormones and enzymes metabolism that protect the body from obesity, diabetes and other related disorders (Angelov *et al.*, 2012).

Effect of cotton thistle extracts on the liver and renal function parameters

In diabetic rats, levels of liver enzymes, serum urea and creatinine were elevated significantly, whereas serum proteins levels and FBW were reduced significantly. Similarly, these results have been reported previously (Dokumacioglu *et al.*, 2018). These parameters were significantly improved when treated with plant extracts. It has been reported that hyperglycemia induced oxidative stress as a key factor of diabetic nephropathy and hepatopathy (Sharifi *et al.*, 2013). Others reported that increasing hepatic and renal parameters might be associated with diabetes by free radical generations caused glucose oxidation and protein glycation as well as defects in antioxidant defence systems, which play a critical role in the pathogenesis of diabetes and tissue damage (Kraska *et al.*, 2021).

The changes in the levels of liver enzymes and renal function markers before and after treatment of onopordum extracts in normal rats was not significantly altered. The antioxidant (Sharifi *et al.*, 2013) and hypoglycemic effects of cotton thistle extracts in this study could be possible reasons for decreasing the catabolic state and other complications of diabetes which help conserving of liver and renal tissues. These results have been further supported by improved histopathological examination of kidneys and liver in the present study.

Effect of Onopordum extracts on ACE

ACE level was increased significantly in diabetic and hyperlipidemic groups and became significantly reduced when treated with both extracts. It has been reported that ACE protein expression increased in the glomeruli of the diabetic rats by approximately six-folds in comparison with the normal group (Sharifi *et al.*, 2013).

The exact mechanisms underlying the effects of onopordum as an ACE inhibitor is not fully interpreted. However, other studies extracted and identified novels compound from *Onopordum acanthium* seeds named onopordia that were functionally effective as antioxidants, ACE inhibitors, hypotensive and diuretic agents (Ghods *et al.*, 2018). In the present study, the diabetic groups treated with plant extracts showed down expression of ACE and inflammatory markers (such as TNF-α, CRP and WBCs) with improved functional parameters and histopathological findings of liver and kidney in comparison with damaged untreated diabetic rats. This showed a prominent role of onopordum seed extracts in decreasing complications of diabetes and over secretion of ACE. The effects of onopordum extracts require more investigations to see the beneficial role of the extracts as an ACE inhibitor.

Effect of onopordum extracts on TNF-α, CRP and WBCs

In this research, the serum level of TNF-α was significantly elevated only in the hyperlipidemic group, while in diabetic groups was not changed obviously. Similar results reported that TNF-α level in diabetic rats was lower or almost similar to the control group and increased in obese rats receiving a high-fat diet compared to animals fed on a standard diet (Dokumacioglu *et al.*, 2018). The exact interpreted mechanism between diabetes and inflammation was not clarified yet, whether poor glycemic control leads to inflammation or inflammation leads to higher glucose levels or other factors that influence both. However, it has been reported that high levels of TNF-α in diabetic and hyperlipidemic patients indicate a strong correlation between serum lipids and TNF-α levels. Moreover, the role of TNF-α in obesity and diabetes also remains a subject of active research work (Margoni *et al.*, 2011; Liu *et al.*, 2016).

It has been reported that the *Onopordum acanthium* extracts contain important substances such as sesquiterpene lactones, triterpenoids, flavonoids, phenols, lignans, terpenes, etc., have anti-inflammatory, anti-proliferative and antihypertensive activities. These effects may be used in the treatment of chronic inflammation and to prevent various types of tumors (Garsiya *et al.*, 2019; Al-Snafi, 2020). Additionally, the water extract of cotton thistle has been reported as an inhibitor of endotoxin-induced realizing of pro-inflammatory markers (Talhouk *et al.*, 2009), while the hydro-alcoholic extracts reduced the secretion of TNF-α (Strzelecka *et al.*, 2005). The water extract

of cotton thistle was concluded by other researchers that reduced the elevated levels of inflammatory cytokines in cells exposed to the inflammation by lipopolysaccharide (Al-Bakheit *et al.*, 2019). Furthermore, the pretreatment of cells by cinnamic acid extracted from defatted seeds of cotton thistle resulted in a significant elevation in the levels of superoxide dismutase, glutathione S-transferase and catalase antioxidant enzymes (Patra *et al.*, 2012).

Alteration of the TNF-α level in hyperlipidemic and diabetic groups can be possibly attributed to the ability of the adipose tissue to produce and secrete this cytokine. In this study, we speculate when weight loss of diabetic rats and tissue damages improved by seed extracts (mostly water extract), it resulted in the recovering in the TNF-α level.

Effect of Onopordum extract on final body weight (FBW)

FBW of the diabetic group was decreased significantly, whereas it increased significantly in the hyperlipidemic group. After treatment of these groups with seed extracts, FBW was controlled. Similarly, these results were reported (Dokumacioglu *et al.*, 2018).

Weight loss during diabetes has been reported as a result of altering the level of insulin; glucagon, catecholamines and other hormones enhance lipolysis caused decreasing in adipocytes and continual loss of body weight. The effects of Onopordum extract were appeared more prominent in the current study during fatal hyperglycemia; the mortality rate was 35% in untreated diabetic rats with a severe reduction in the body weight, 9.0 % in diabetic rats treated with ethanolic extract and 5.0% of the diabetic rats treated with water extract. The mortality of diabetic rats could be due to that most of the insulin secretary beta cells of the pancreas destructed after induction of diabetes by STZ with continual body weight loss clearly observed in this study; as a result, only a few cells were survived might be stimulated by onopordum extracts caused increase in insulin secretion conserving body weight.

Effect of Onopordum extracts on the histology of kidney, liver and spleen

It has been reported that cells that express GLUT 2, such as hepatocytes and the renal tubular cells, are also susceptible to damage by STZ. Others recently mentioned that the toxic effect of STZ injection, in addition to the generation of reactive oxygen species (ROS), reduces glutathione concentration in the kidneys, liver and spleen, which is an important antioxidant balance in the cells (Kraska *et al.*, 2021). Histopathological structures of kidney, liver and spleen of diabetic rats treated with seed extracts showed almost improvement and less damaged tissues compared with severely damaged organs of diabetic rats.

Extracts of *Onopordum acanthium* seeds contain different active compounds such as flavonoids, phenolics, triterpene and flavanones aglycons glycoside forms of apigenin quercetin, and luteolin (Lajter *et al.*, 2015; Garsiya *et al.*, 2019). Some of these compounds have been proved in other plants that can effectively regulate insulin metabolism and act as an antioxidant that protect morphological alterations of organs in diabetic animals, which is mostly due to their antioxidant effect that has been previously reported (Angelov *et al.*, 2012; Sharifi *et al.*, 2013). Overall, the hypoglycemic and antioxidant roles of onopordum seed extracts can protect the liver, kidney and spleen damages in diabetic animals. This was further supported by improving renal and hepatic parameters, serum protein, ACE, TNF-α, CRP in diabetic rats treated with the local cotton thistle seed extracts.

CONCLUSIONS

The data obtained from the current study have established the hypoglycemic effect of the local *Onopordum acanthium* seed extracts by the improvement of the TNF-α, ACE, lipid profile, liver and renal function parameters levels in diabetic and hyperlipidemic rats. Furthermore, seeds of cotton thistle have ameliorating the tissues damages in the liver, kidney and spleen of diabetic rats.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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