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Original article

Effect of nano extracts of olea europaea leaves, ficus carica and liraglutide in lipidemic liver of type 2 diabetic rat model

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ABSTRACT

The study aimed to evaluate the impact of *Ficus carica* mixture and *Olea europaea* leaf nano extracts, and liraglutide, on liver tissue and serum lipids in type 2 diabetic male albino rat model. Forty rats were divided equally into 4 groups were used. Group 1 was the non-diabetic control group. The animals in Groups 2–4 was injected intraperitoneally with a single dose of 60 mg/kg b.w. Streptozotocin to induce a diabetic rat model. Group 2 served as a positive control for diabetes. 0.02 mg/kg b.w./day of Liraglutide gave to groups 3 and 4 and 4.8 ng/ml $\times$ 10^5 b.w./day of a mixture of the nano extracts, respectively. Eight weeks after treatment, the animals were sacrificed. Blood was collected for glucose analysis and serum low-density lipoprotein, high-density lipoprotein, total cholesterol, and triglycerides analysis, and the livers processed for histopathological examination. The elevated lipid profiles and blood glucose levels in diabetic group (Group 2) were significantly reduced ($p < 0.001$) following the administration of liraglutide and nano extracts in Groups 3 and 4. Progressive fatty acid changes were found in the liver sections, indicated by the deposition of various sizes of lipid droplets in most liver lobules, along with patchy hepatocyte necrosis. These pathological changes were ameliorated in the liraglutide- and nano-extract-treated rats. Treatment with the nano extracts resulted in significant power assays associated with recovery of hepatic histology and functional alterations, compared to liraglutide treatment.

1. Introduction

One of the most common complications of diabetes mellitus, particularly type 2 diabetes mellitus (T2DM), is liver damage. As a result, diabetes mellitus is linked to liver inflammation, cirrhosis, and apoptosis, leading to liver failure and necrosis (Safhi et al., 2019). Unfortunately, all the antidiabetic agents currently available are expensive and have many adverse effects; therefore, finding alternative antidiabetic regimens with fewer adverse effects and lower costs than traditional agents is a major challenge for researchers (Chaudhury et al., 2017). Among glucagon-like peptide 1 (GLP-1) agonists, liraglutide (LD) has been a popular option since its approval by the Food and Drug Administration in 2010. LD is an effective hypoglycemic agent used in T2DM treatment. Numerous studies have demonstrated that LD increases insulin secretion and inhibits glucagon secretion; however, the side effects are unclear (Mathiesen et al., 2019; Alatawi and Alshubaily 2021).

Currently, many people across the globe prefer traditional and indigenous medicine to other medicine (Thoker and Patel 2020). Fruits, roots, and leaves are used in traditional medicine to treat various complaints (Anwar et al., 2016). In addition, (Stephen Irudayaraj et al., 2017) concluded that *Ficus carica* leaves showed hypoglycemic and hypolipidemic activities in streptozotocin (STZ)-induced T2DM in rats and considerable effect on the enzymes of carbohydrate metabolism. (Nasir et al., 2020) stated that the methanolic extract of *F. carica* showed hepatoprotective activity against medicine-induced hepatotoxicity. *Olea europaea* is one of the most important and oldest cultivated plants; it is the sixth most important oil crop worldwide, olive oil has a high economic value and profitable nutritional characteristics (Reale et al., 2019). (Afify et al., 2018) suggested that olive leaves have potential chemopreventive benefits against toxicity and could be used to prevent T2DM tissue complications such as nephropathy and liver tissue damage by enhancing the glycemic state of the tissues. Moreover, the histopathological results of (Al-Attar and

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Alsalmi, 2019a, 2019b; Taha et al., 2020) illustrated that the treatment of diabetic rats with olive leaf extract led to a reduction in various physiological parameters and severe hepatic tissue alterations.

2. Materials and methods

2.1. Streptozotocin (STZ)

Streptozocin was purchased from SIGMA Aldrich.

2.2. Preparation of STZ solution and induction of T2DM

The experimental animals fasted overnight then a single intraperitoneal (i.p.) injection of (60 mg/kg body weight) of freshly prepared solution of STZ was used to induce diabetes (Yang and Kang 2018). In the present research, they were classified based on FBG level into diabetic (126 mg/dl and above), and non-diabetic (125 mg/dl and below) according to (Birgani et al., 2018).

2.3. Liraglutide drug

Liraglutide (Victoza, Novo Nordisk A/S, Bagsvaerd, Denmark) was marketed as Victoza was purchased from Al Nahdi Pharmacy.

2.4. Determination of appropriate doses of liraglutide

According to many previous data, including our present preliminary trials, which were performed with different doses of liraglutide indicated that the optimal dose is (0.02 mg/kg b.w./day) given as subcutaneous administration to the experimental animals (Duarte et al., 2020).

2.5. Plant materials collection and identification

Ficus carica and *Olea europaea* leaves were purchased from local grocery market in Jazan, and Al-Jouf Corner Consecutively. All the plant leaves were Collection and identified according to (Hummdi and Qahl 2020), Authenticated by a plant taxonomist at King Abdulaziz University's Department of Arid Land Agriculture, Faculty of Meteorology, Environment, and Arid Land Agriculture.

2.6. Methanolic extraction leaves procedure

The samples were finely grounded and placed in a porous bag made from a strong filter paper and placed in a solvent of methanol (80%), heated in a bottom flask to vaporize into the sample, condensed to drip back, then liquid contents were emptied into the bottom flask again and the process is continued. Herbal extract was dried then concentrated by a rotary evaporator (Eyela A-10005 China) for absolute dryness and transfered to a freeze dryer to complete dryness of methanolic extract (Labconco USA). Dried samples stored in air-tight containers for subsequent use (Lins et al., 2018). (Loha et al., 2019) recorded that 80% methanol was the ideal solvent for powdered leaves because hydroethanolic solvents are usually better in extracting most important chemical constituents of different plants (Monteiro et al., 2020).

2.7. Synthesis of herbal nanoparticles

Nanotechnology is a field that aims to develop dosage forms in range of 1 to 100 nm. The use of plant leaves extract for nanoparticles synthesis offers numerous benefits in a wide range of pharmaceutical, medical and biomedical applications (Kumar et al., 2014). Sharma and Hussain (2020) recommend application of nano-encapsulation of leaves extract as a suitable novel technique to improve antioxidant capacity of natural ingredients of leaves extract.

Initially, the mixture of methanol extracts was prepared according to (Lins et al., 2018). A mix grinder was used to grind the dried leaves and gathered as coarse powders, then they were milled into fine powders.

The obtained powders were equally (nearly 5 mg) milled again into nanoparticles ranging in size from (50–100 nm). The powders were then given orally after being dissolved in 1 ml of distilled water (Lins et al., 2018).

2.8. Determination and preparation of plant dried leaves extract doses

The preparation of *F. carica*, and *O. europaea* leaves extract was kept at a moderate temperature to prevent moisture and was prepared each day by mixing both dried leaves extracts (4.8 ng/m $\times 10^5$ b.w./day), then giving it to the rats in the treatment groups by gastric tubing (Orally administered) according to the (Zhou et al., 2020) method.

2.9. Experimental design

The forty experimental animals were divided randomly into four groups, each contains ten rats. Group 1 that served as negative Non-Diabetic control. Single dose of STZ (60 mg/kg) were injected to rest of rats according to (Zhang et al., 2018) method to induce STZ diabetes. Rats that exhibited blood glucose levels over than 126 mg/dL considered as diabetic type 2 model rats and were divided to three groups as follows: Group 2 served as positive control (STZ-DMT2). The next group, Group 3 had 10 STZ-induced diabetic rats that were subcutaneously (S.C.) injected with LD (0.02 mg/kg b.w./day) according to (Zhang et al., 2018) method. Finally, Group 4 had 10 STZ-induced diabetic rats were administered with (4.8 ng/1m $\times 10^5$ b.w./day), of nano extract mixture of *F. carica* and *O. europaea* leaves, according to (Hummdi and Qahl 2020) method. The experimental period lasted for two months and the animals were daily observed for signs of toxicity up to 48 h after each treatment in all groups.

2.10. Blood samples collection

At the end of each week, the rats in all the experimental groups fasted for 12 h overnight, blood samples were taken from the *retro*-orbital plexus right away by the retro orbital plexus technique using clean dried centrifuge capillary glass tubes. Pasteur pipettes were used to carefully separate clear serum samples, which were then frozen at −20 °C until biochemical analysis (Hassan et al., 2018).

2.11. Determination of serum liver lipid profile

All tests for serologic markers were performed according to the ELISA kits instructions. Total cholesterol (rat total cholesterol Quick Detect TM ELISA kit), low density lipoprotein cholesterol (LDL-C) (rat LDL ELISA kit), high density lipoprotein cholesterol (HDL-C) (rat HDL-C ELISA kit), and triglyceride (TG) (rat TG ELISA kit) were purchased from My BioSource, USA. Serum of lipid profile of the TG, TC, HDL-C and LDL-C estimated according to the methods described by (Hassan et al., 2018).

2.12. Histopathological procedures

At the end of the exprimental period (8 weeks), the treated and control rats were sacrificed and their livers were harvested,

weighted, immediately rinsed in normal saline and cut to small pieces 0.5 cm. Then immersed in neutral buffered formalin 10% for histopathologcal procedures for light microscopy include the following stages; tissue sampling, fixation, dehydration and clearing, embedding, sectioning, mounting and staining the sections (Zhanmu et al., 2020).

2.13. Statistical analysis

The Statistical Package for Social Sciences was used to conduct the statistical analysis (SPSS for windows, version 22.0). According to (Al-Attar and Alsalmi, 2019a, 2019b) the results was considered statistically significant if the *P*-values were <0.05.

3. Results

3.1. Absolute and relative liver weights

A decrease in in absolute liver weight was noted in G3 (STZ-DMT2 + LD) after 8 weeks compared with control**.** Meanwhile, relative liver weight was significantly increased in group 2 (STZ-DMT2) versus G1 (control), G3 (STZ-DMT2 + LD) and G4 (STZ-DMT2 + mixture of *F. carica* and *O. europaea* leaf extracts) groups (Table 1).

3.2. Biochemical analysis of liver lipid profile

The effects of LD and *F. carica* and *O. europaea* leaf nano extracts mixture on the lipid profile concentrations of TG, TC, LDL-C, HDL-C in non-diabetic control rats and STZ-DMT2 rats were shown in Tables 2–5. Serum lipid concentrations of TG, TC, and LDL-C were significantly higher in all treated groups (Groups 2, 3, and 4) after STZ administration, and they showed significantly lower HDL-C levels compared with negative control.

In contrast, serum levels of TG, TC, and LDL-C markedly decreased after 8 weeks in Groups 3 (STZ-DMT2 + LD) and 4 (STZ-DMT2 + mixture of *F. carica* and *O. europaea* leaf extracts) compared to diabetic model group (STZ-DMT2). The decline in TG, TC, and LDL-C was more obvious in Group 4 (STZ-DMT2 + mixture of *F. carica* and *O. europaea* leaf extracts) than in Group 3 (STZ-DMT2 + LD) but did not reach significant level. Meanwhile, the concentration of HDL-C was significantly elevated in Groups 3 and 4 versus Group 2 (STZ-DMT2). However, the differences in the HDL-C values between Groups 3 and 4 were statistically insignificant.

3.3. Histopathological results

In the current study, the histological examination of the liver of the negative control group showed a normal liver architecture with normally arranged classic hepatic lobules (Fig. 1). The histological results of the STZ-induced diabetic group (Group 2) liver is presented in Fig. 2 alterations that varied considerably from moderate histological changes to severe cytoplasmic degeneration. Various sizes of lipid droplets were visible in most liver lobules, with patchy hepatocyte necrosis (Fig. 2.3). There are two types of fatty acid changes that have been detected: microvesicular and macrovesicular. Mixed forms were detected frequently (Fig. 2.4).

The histological results of the livers of the STZ diabetic rats treated with LD (Group 3) are presented in Fig. 3. The first type was characterized by moderate degenerative changes, which include focal areas of necrosis (Fig. 3.6); the second type included marked dilatation and congestion of blood sinusoids, the distortion of central veins, and the swelling of hepatocytes with nuclear pleomorphism, all of which are associated with inflammatory cell infiltration (Fig. 3.5). The current study also yielded surprising results in the histology of the liver tissue of rats that had been given the mixture of *F. carica* and *O. europaea* leaf extracts (Group 4) Fig. 4. Hepatocyte regeneration features were observed in the form of enlarged nuclei and the presence of hepatocytes with two nuclei (Fig. 4.8). The natural shape of the central veins could be seen as well as a good composition of portal tracts, consisting of portal venules, hepatic arterioles, and bile ducts. No vascular congestion, necrosis, or inflammation was observed (Fig. 4.7).

4. Discussion

F. carica and *O. europaea* are important medicinal plants and rich sources of naturally occurring antioxidants, such as phenolics, flavonoids, phytosterols, sterols, anthocyanins, and volatile compounds, that play a vital role in preventing health disorders (Salma et al., 2020). Consistent with the findings of the current study, (Bawazeer and Qahl 2016) reported that hyperlipidemic rats treated with a mixture of *F. carica* and *O. europaea* extracts showed notable histopathological improvement of the liver tissue. According to the literature, *F. carica* and *O. europaea* leaves have positive effects on body organ tissues and on biochemical parameters related to diabetes, via many mechanisms (Acar-Tek and Ağagündüz 2020).

The measurement of relative liver weight provides a more accurate approach to demonstrating changes in liver size. In agreement with results of this study, (Sahar et al., 2014) reported an insignificant decrease in absolute liver weight in diabetic rats.

Table 1
Absolute liver weight (g) and relative liver weight (%) of the experimental groups.

Groups		(G1)	(G2)	(G3)	(G4)
End of Treatment	**Absolute Liver Weight**				
	Mean ± SD	11.63 ± 1.40	10.25 ± 1.55	9.91 ± 2.14	11.16 ± 1.56
	SE	0.44	0.49	0.68	0.49
	Sig.		P = 0.075	P = **0.028***	P = 0.540
				^{1}P = 0.655	^{1}P = 0.236
	Pre.		^{1}Pre = −11.87%	^{2}Pre = −3.32%	^{2}Pre = 8.88%
	Relative Liver Weight				
	Mean ± SD	3.82 ± 0.61	5.00 ± 0.99	4.17 ± 0.89	3.99 ± 0.91
	SE	0.19	0.31	0.28	0.29
	Sig.		P = **0.003****	P = 0.347	P = 0.654
				^{1}P = **0.032***	^{1}**P = 0.009****
	Pre.		^{1}Pre = 38.12%	^{2}Pre = −16.60%	^{2}Pre = −13.40%

Data are expressed as Mean ± SD, SE. **P:** significance versus Negative Control group, **^{1}P:** significance versus Positive Control group, using OneWay Anova test. Significant levels: **P > 0.05** not significant: **P < 0.05** 0significant. *: mild significance < 0.05; **: moderate significance < 0.010; ***: highly significance < 0.001. **Pre:** Percentage changes. **^{1}Pre:** percentage versus control; **^{2}Pre**: percentage versus diabetic.

Table 2
Serum levels of Triglyceride (TG) (mg/100 ml) of the experimental groups.

groups	(G1)	(G2)	(G3)	(G4)
Before STZ				
Mean ± SD	0.69 ± 0.08	0.73 ± 0.09	0.72 ± 0.08	0.70 ± 0.07
SE	0.02	0.03	0.03	0.02
Sig.		P = 0.336	P = 0.474 ^{1}P = 0.804	P = 0.843 ^{1}P = 0.444
Pre.		^{1}Pre = 5.80%	^{2}Pre = −1.37%	^{2}Pre = −4.11%
After STZ				
Mean ± SD	0.90 ± 0.02	1.17 ± 0.09	1.14 ± 0.08	1.14 ± 0.08
SE	0.01	0.03	0.02	0.04
Sig.		P = **0.001*****	P = **0.001***** ^{1}P = 0.361	P = **0.001***** ^{1}P = 0.361
Pre.		^{1}Pre = 30.00%	^{2}Pre = −2.56%	^{2}Pre = −2.56%
One-month treatment				
Mean ± SD	0.99 ± 0.01	1.28 ± 0.03	1.00 ± 0.004	0.95 ± 0.04
SE	0.002	0.01	0.001	0.01
Sig.		P = **0.001*****	P = 0.709; ^{1}P = **0.001*****	P = **0.001***** ^{1}P = **0.001*****
Pre.		^{1}Pre = 29.29%	^{2}Pre = −21.88%	^{2}Pre = −25.78%
Two months treatment				
Mean ± SD	1.07 ± 0.03	1.37 ± 0.06	0.82 ± 0.03	0.76 ± 0.01
SE	0.01	0.02	0.01	0.002
Sig.		**P = 0.001*****	**P = 0.001***** **^{1}P = 0.001*****	**P = 0.001***** **^{1}P = 0.001*****
Pre.		^{1}Pre = 28.04%	^{2}Pre = −40.15%	^{2}Pre = −44.53%

Data are expressed as Mean ± SD, SE. **P:** significance versus Negative Control group, **^{1}P:** significance versus Positive Control group, using OneWay Anova test. Significant levels: P > 0.050 insignificant: **P < 0.050** significant. *: mild significance < 0.050; **: moderate significance < 0.010; ***: highly significance < 0.001. **Pre:** Percentage changes. **^{1}Pre:** percentage versus control; **^{2}Pre**: percentage versus diabetic.

Table 3
Serum level total cholesterol (TC) (mg/100 ml) of the experimental groups.

groups	(G1)	(G2)	(G3)	(G4)
Before STZ				
Mean ± SD	1.36 ± 0.16	1.44 ± 0.19	1.37 ± 0.27	1.28 ± 0.17
SE	0.05	0.06	0.09	0.05
Sig.		P = 0.368	P = 0.934 ^{1}P = 0.413	P = 0.331 ^{1}P = 0.064
Pre.		^{1}Pre = 5.88%	^{2}Pre = −4.86%	^{2}Pre = −11.11%
After STZ				
Mean ± SD	1.27 ± 0.18	2.17 ± 0.10	2.16 ± 0.10	2.14 ± 0.05
SE	0.06	0.03	0.03	0.02
Sig.		P = **0.001*****	P = **0.001***** ^{1}P = 0.823	P = **0.001***** ^{1}P = 0.528
Pre.		^{1}Pre = 70.87%	^{2}Pre = −0.46%	^{2}Pre = −1.38%
One month treatment				
Mean ± SD	1.42 ± 0.22	2.15 ± 0.12	2.07 ± 0.03	1.95 ± 0.03
SE	0.07	0.04	0.01	0.01
Sig.		P = **0.001*****	P = **0.001***** ^{1}P = 0.254	P = **0.001***** ^{1}P = **0.005****
Pre.		^{1}Pre = 51.41%	^{2}Pre = −3.72%	^{2}Pre = −9.30%
Two months treatment				
Mean ± SD	1.29 ± 0.18	2.14 ± 0.07	1.85 ± 0.01	1.86 ± 0.02
SE	0.06	0.02	0.004	0.01
Sig.		**P = 0.001*****	**P = 0.001***** **^{1}P = 0.001*****	**P = 0.001***** **^{1}P = 0.001*****
Pre.		^{1}Pre = 65.89%	^{2}Pre = −12.55%	^{2}Pre = −13.08%

Data are expressed as Mean ± SD, SE. **P:** significance versus Negative Control group, **^{1}P:** significance versus Positive Control group, using OneWay Anova test. Significant levels: P > 0.050 insignificant: P < 0.050 significant. *: mild significance < 0.050; **: moderate significance < 0.010; ***: highly significance < 0.001. Pre: Percentage changes. ^{1}Pre: percentage versus control; ^{2}Pre: percentage versus diabetic.

However, again similar to results of this study, (Gond and Khadabadi 2008) showed a notable increase in the relative liver weight of diabetic rats. Further supporting our results, (Gao et al., 2015) pointed out that LD reduces body weight and absolute liver weight, and (Nugroho 2020) indicated that rats given *Ficus* leaf extract had a lower relative liver weight, in comparison to the control. Several previous studies proposed reduction in liver weight that could be due to reductions in collagen and fat deposition in rats fed olive leaf extracts, which have been associated with visceral fat thermogenesis upregulation and hepatic lipogenesis downregulation (Shen et al., 2014). In our study, relative liver weight was highly reduced by treatment with the *F. carica* and *O. europaea* leaf nano extract mixture, and several authors have previously suggested the benefits of polyphenols

Table 4
Serum levels of low-density lipoprotein cholesterol (LDL-C) (mg/100 ml) of the experimental groups.

groups	(G1)	(G2)	(G3)	(G4)
Before STZ				
Mean ± SD	0.26 ± 0.023	0.26 ± 0.027	0.25 ± 0.027	0.26 ± 0.021
SE	0.007	0.009	0.009	0.007
Sig.		P = 0.789	P = 0.533	P = 1.000
			^{1}P = 0.374	^{1}P = 0.789
Pre.		0	^{2}Pre = −3.85%	0
After STZ				
Mean ± SD	0.25 ± 0.03	0.48 ± 0.07	0.48 ± 0.05	0.49 ± 0.05
SE	0.01	0.02	0.02	0.01
Sig.		P = **0.001*****	P = **0.001*****	P = **0.001*****
			^{1}P = 0.861	^{1}P = 0.760
Pre.		^{1}Pre = 92.00%	0	^{2}Pre = 2.08%
One month treatment				
Mean ± SD	0.25 ± 0.023	0.48 ± 0.049	0.28 ± 0.015	0.27 ± 0.018
SE	0.007	0.015	0.005	0.006
Sig.		P = **0.001*****	P = **0.002****	P = 0.072
			^{1}P = **0.001*****	^{1}P = **0.001*****
Pre.		^{1}Pre = 92.00%	^{2}Pre = −41.67%	^{2}Pre = −43.75%
Two months treatment				
Mean ± SD	0.23 ± 0.027	0.48 ± 0.042	0.28 ± 0.005	0.25 ± 0.012
SE	0.008	0.013	0.002	0.004
Sig.		P = **0.001*****	P = **0.001*****	P = 0.123
			^{1}P = **0.001*****	^{1}P = **0.001*****
Pre.		^{1}Pre = 108.70%	^{2}Pre = −41.67%	^{2}Pre = −47.92%

Data are expressed as Mean ± SD, SE. **P:** significance versus Negative Control group, **^{1}P:** significance versus Positive Control group, using OneWay Anova test. Significant levels: P > 0.050 insignificant: P < 0.050 significant. *: mild significance < 0.050; **: moderate significance < 0.010; ***: highly significance < 0.001. Pre: Percentage changes. ^{1}Pre: percentage versus control; ^{2}Pre: percentage versus diabetic.

Table 5
Serum levels of high-density lipoprotein cholesterol (HDL-C) (mg/100 ml) of the experimental groups.

groups	(G1)	(G2)	(G3)	(G4)
Before STZ				
Mean ± SD	1.35 ± 0.025	1.34 ± 0.025	1.33 ± 0.024	1.33 ± 0.018
SE	0.008	0.008	0.008	0.006
Sig.		P = 0.182	P = 0.107	P = 0.128
			^{1}P = 0.773	^{1}P = 0.848
Pre.		^{1}Pre = −0.74%	^{2}Pre = −0.75 %	^{2}Pre = −0.75%
After STZ				
Mean ± SD	1.42 ± 0.011	1.26 ± 0.004	1.26 ± 0.005	1.26 ± 0.009
SE	0.003	0.001	0.002	0.003
Sig.		P = **0.001*****	P = **0.001*****	P = **0.001*****
			^{1}P = 0.571	^{1}P = 0.259
Pre.		^{1}Pre = −11.27%	0	0
One month treatment				
Mean ± SD	1.48 ± 0.020	1.22 ± 0.014	1.41 ± 0.019	1.40 ± 0.005
SE	0.007	0.004	0.004	0.002
Sig.		**P = 0.001*****	**P = 0.001*****; **^{1}P = 0.001*****	**P = 0.001*****; **^{1}P = 0.001*****
Pre.		^{1}Pre = −17.57%	^{2}Pre = 15.57%	^{2}Pre = 14.75%
Two months treatment				
Mean ± SD	1.56 ± 0.003	1.18 ± 0.014	1.42 ± 0.013	1.40 ± 0.005
SE	0.001	0.005	0.004	0.002
Sig.		**P = 0.001*****	**P = 0.001*****; **^{1}P = 0.001*****	**P = 0.001*****; **^{1}P = 0.001*****
Pre.		^{1}Pre = −24.36%	^{2}Pre = 20.34%	^{2}Pre = 18.64%

Data are expressed as Mean ± SD, SE. **P:** significance versus Negative Control group, **^{1}P:** significance versus Positive Control group, using OneWay Anova test. Significant levels: P > 0.050 insignificant: P < 0.050 significant. *: mild significance < 0.050; **: moderate significance < 0.010; ***: highly significance < 0.001. Pre: Percentage changes. ^{1}Pre: percentage versus control; ^{2}Pre: percentage versus diabetic.

in maintaining the body and liver weights of diabetic animals (Alcántara et al., 2020).

The changes in the lipid profiles observed in this research were characterized by the significant elevation of TG, TC, and LDL-C in the diabetic model rats. The marked increase in the LDL-C, TC, and TG levels of the diabetic rats was mainly caused by insulin as it supresses hormone-sensitive lipase, resulting in enhanced mobilization of free fatty acids from peripheral deposits (Movahedian et al., 2010). In the present study, the levels of TG, TC, and LDL-C in the rats treated with LD were significantly decreased, while their serum HDL-C levels increased compared with diabetic rats. Similar to our results, preventive treatment with of *F. carica* leaf extract has been demonstrated to significantly improve lipid profiles and decrease adipogenic risk factors in diabetic rats by increasing HDL-C levels and decreasing serum TC, LDL-C, and TG levels (El-Bushuty 2015).

In addition, (Vogel et al., 2015) reported the beneficial effects of the polyphenols in olive leaves (mainly oleuropein) on lipid pro-

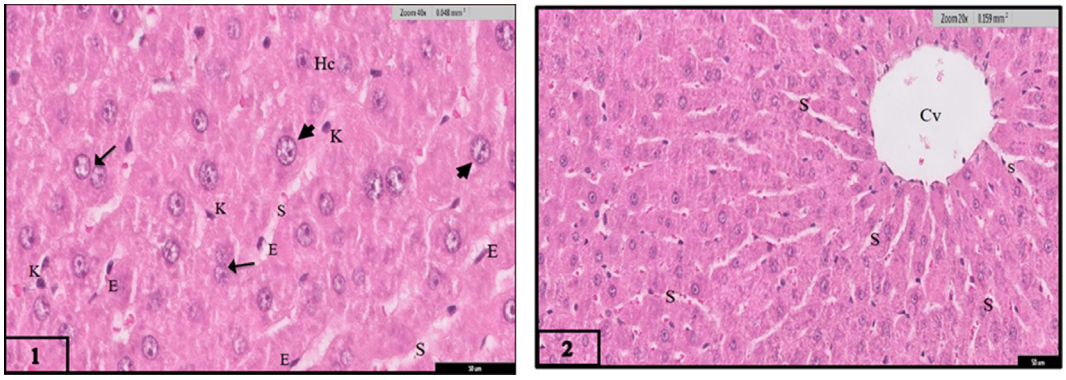

Fig. 1. Liver of G1:1. Showing radiating cords of polyhedral hepatocytes **(Hc)**; with acidophilic cytoplasm, large rounded centrally located nuclei **(arrowheads)**; binucleated cells **(arrows)**; blood sinusoids **(S)**; lined by flattened endothelial cells **(E)** and Kupffer cells with ovoid nuclei **(K)**, **(400x)0.**2. Showing normal structure of central vein **(Cv)**; which consist of radial plates of hepatocytes with narrow radiating blood sinusoids **(S)**, **(200x).**

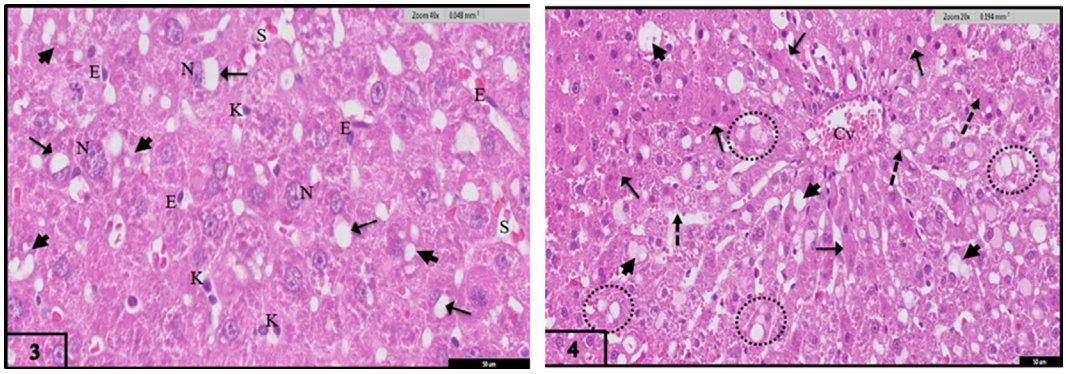

Fig. 2. Liver of G2. 3. Showing fatty liver steatosis, macrovascular infiltration of hepatocytes **(arrows)** with nuclear displacement; microvascular infiltration **(arrowheads)**; nuclear pleomorphism **(N)**; disruption of blood sinusoids **(S)**; hypertrophied or necrosis of endothelial **(E)** and Kupffer cells **(K)**, **(200x). 4.** Showing sever disorganization of hepatic plats: accumulation of macrovesicular fatty infiltrations **(arrowheads)**; and microvesicular fatty infiltrations **(circles)**; around of centrilobular **(Cv)** area; massive pericentral hepatocytes necrosis **(arrows);** and lysis **(dott arrows)**, **(400x)**.

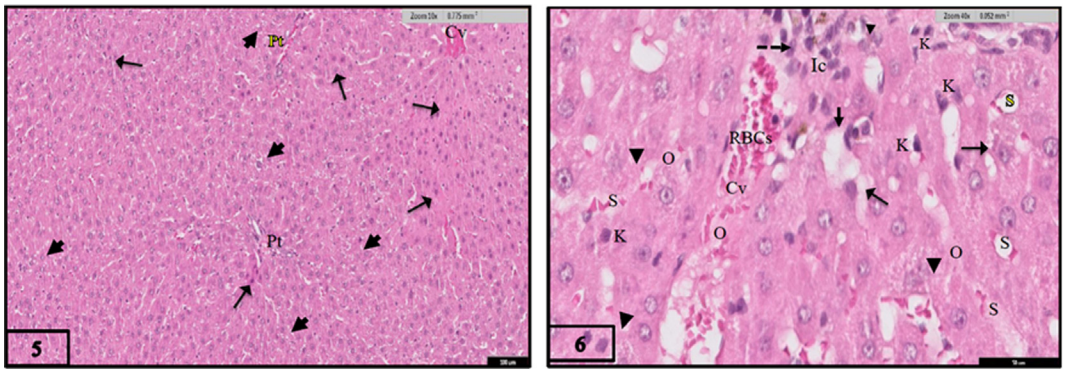

Fig. 3. Liver of G3. 5. Showing focal zones of hepatic cellular necrosis **(arrows)**; which outstretched around the central vein **(Cv)** and peri portal tract **(Pt);** some hepatocytes vacuolation **(arrowheads)**, **(200x). 6.** Showing moderate degenerative changes of the hepatocytes architecture apparently in hydropic cytoplasmic degeneration ‘‘deposition of lipid droplets” **(arrows)**; nuclear karyolysis **(arrowheads)**; oedema **(O)**; blood sinusoids dilated and congested **(S);** with endothelial cells loss**;** Kupffer cells **(K)** hypertrophy and proliferation; central vein **(Cv)** pooling with **(RBCs)**; and inflammatory cells aggregates **(Ic)**; contain scarce granules of hemosiderin pigment **(dott arrows)**, **(400x).**

files. In this respect, the beneficial properties of *O. europaea* leaves have been attributed to their phenolic compound, triterpenic acid, and flavonoid content, as these substances possess lipid metabolism management properties (Markhali et al., 2020).

The present study was in good agreement with several previous studies that reported similar histopathological changes following STZ injection-induced diabetes. However, (Sherif 2019) reported that hepatocyte ballooning is an adaptive (physiological) change of hepatocytes, while, in a related study on STZ-induced diabetic rats, (Yi et al., 2019) reported that it is a degenerative (pathological) change. Diabetes mellitus also has a negative effect on liver cell nuclei, as stated by (Wieczorek et al., 2017), causing duplicate hepatocyte nuclei and heterogeneity. These findings are consistent with our presented results. (Marchisello et al., 2019) considered fatty infiltration of the liver to be a precursor of fatty steatosis and pericentral fibrosis. Our data are in agreement with previous studies, which have also shown fatty acid changes in the centrilobular portions of the livers of diabetic animals (Greaves 2011).

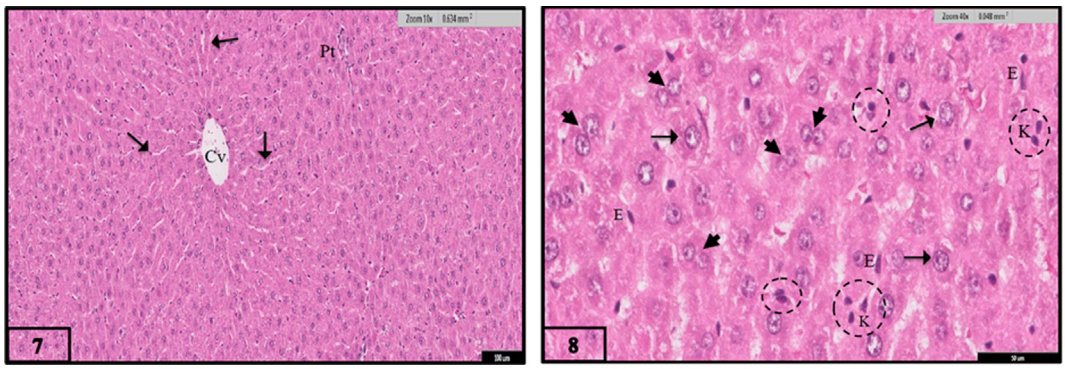

Fig. 4. Liver of G4. 7. Showing normal hepatic tissue pattern comprise of the of hepatocytes arranged in radiating cords with mild dilation of some blood sinusoids **(arrows)**; surrounding the normal central vein **(Cv)**; emergence of good composition portal tract **(Pt)**, **(100x)0.8.** Showing the liver cells plates; maintaining natural organization; the hepatocytes appeared polygonal in shape-containing rounded nuclei with acidophilic cytoplasm. Notice regeneration in the form of enlarged nuclei **(arrows)**; binucleate cells **(arrowheads)**; elongated endothelial cells that line blood sinusoids **(E)**; activated and proliferation Kupffer cells **(circle)**, **(400x)**.

Microvesicular lipidosis is an indication of more severe hepatic impairment, which is usually seen in broad range of injuries as a reaction, aslo considered to be physiological adaptation shown as an imbalance between the release of lipoproteins by hepatocytes and the absorption of lipids from the blood (Odze and Goldblum 2014).Similar to the present study, previous studies (e.g., (Tan et al., 2013)) have reported cellular infiltration around and between the central veins and portal tracts, and even between hepatocytes. This confirms that the lobular or acinar infiltration of lymphocytes and neutrophils is a diagnostic feature of diabetic liver disease.

5. Conclusions

In this study, *O. europaea* and *F. carica* leaf extracts were combined and evaluated to determine their potential effectiveness in STZ-induced diabetic rats. According to obtained results, an extract of this leaf mixture may be a feasible option in diabetes mellitus treatment, as it shows ameliorative activity on lipid levels. Its natural origins, minimal side effects, and low costs make it an attractive alternative which could be successfully utilized for the treatment of diabetes and related diseases owing to its hypoglycemic actions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Declaration of competing interest

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