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A Comparative Hepatoprotective Effect of Liraglutide and Nano Extracts of *Ficus carica* and *Olea europaea* Leaves on Diabetes-Induced in Laboratory Animals

[1]Laila A Hummdi*
[2]Safa H Qahl

Author's Affiliation:
[1,2]University of Jeddah, Department of Biology, Jeddah, Saudi Arabia, Box# 21959 Jeddah.

***Corresponding Author:**
Laila A Hummdi
University of Jeddah, Department of Biology, Jeddah, Saudi Arabia, Box # 21959 Jeddah.

E-mail: underrain21@hotmail.com

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Abstract:
Effects of Liraglutide and nano extracts of *ficus carica* and *Olea europaea* leaves (NEML) on liver were investigatedin type 2 diabetes mellitus (T2DM) rats' model. Forty male albino Wistar rats were used in this experimental study. Thirty rats were injected intraperitoneal (i.p.) with STZ single dose (60 mg/kg) to induce T2DM and assigned into groups (2, 3 & 4). Ten rats served as negative control (group 1, NDC). Group (2) rats served as positive control (DC), group (3) (LD) received Liraglutide subcutaneous injected (0.2 mg/kg/day) for 8 weeks; group (4) (NEML) received orally NEML ($45X10^7$ ng /250gb.w./day) for eight weeks. After eight weeks, rats were killed and blood collected for assessment of liver functions. Liver were harvested for histopathological examination. Results showed significantly elevated serum levels of alanine amino transferase (ALT), alkaline phosphatase (ALP), aspartate amino transferase (AST), and gamma glutamyl transpeptidase (GGT) in groups (2) indicative of hepatocellular damage. Histopathological liver examination revealed marked hepatic degeneration characterized by vascular congestion and degeneration of endothelial lining, cellular infiltration, sinusoidal dilatation, hydropic degeneration, focal necrosis, nuclear pleomorphism, and loss of kupffer and endothelial cells lining blood sinusoids, bile duct proliferation compared to control group. Alterations of liver functions and hepatocytes lesions were significantly reduced with Liraglutide (group 3) and nano extracts of NEML (group 4). In conclusion, treatment with Liraglutide and nano extract of NEML leaves restored altered parameters in liver enzymes towards normal, and nano extract of NEML leaves exhibited better hepatoprotective properties against diabetic-induced hepatocellular damage than Liraglutide.

Keywords: Liraglutide, diabetic ratsmodel, hepatoxicity, *ficuscarica*, *olea europaea*.

INTRODUCTION

Diabetes Mellitus (DM) is a complex disease that had reached epidemic proportions in the century. Diabetes is known to produce substantial changes in most tissues, including liver (Manna et al., 2010). DM is the most common cause of liver diseases which is an important reason of death in diabetic patients(Al-Attar and Alsalmi, 2019).Oxidative stress during diabetes mellitush as major role in occurrence of liver disorders (Adeyemi et al., 2014). Free radicals enhanced the occurrence of hepatic disorders by induction of hepatocyte apoptosis, hepatic inflammatory action and fibrogenesis (Goetz and Luch, 2008). Glucagon-like peptide-1 (GLP-1) receptor agonists (GLP-1RA) are new types of anti-diabetic therapy that has action like incretin hormones. The glucose-lowering actions of GLP-1 are glucose dependent, which decreased hypoglycemic risk (Chia and Egan, 2008). Several trials reported that liraglutide is well tolerated, corrects glycemic control with a low risk of hypoglycemia accompanied with decreased in weight (Garber et al., 2009, Nauck et al., 2009). Liraglutide improved liver functions associated with reduction in body weight among nonalcoholic fatty liver disorders (NAFLD) with type 2 diabetes mellitus (T2DM) (Ohki et al., 2012).(Iwao et al., 2015) stated that the liraglutide has limited information is currently available effects of on liver parameters. Other researches stated that patient treatment with liraglutide improved steatosis, hepatocyte ballooning, lobular inflammation and fibrosis associated with obesity and/or T2DM (Guss and Mohanty, 2016). Other beneficial actions of liraglutide drugs, including anti-inflammatory and anti-oxidant which are important for improvement of chronic liver diseases including deregulations of hepatic cells leading to sinusoidal disorders, architectural alterations of the liver parenchyma and vasculature (de Mesquita et al., 2017).

Medicinal plants are main source of oral hypoglycemic substances for the development of new pharmaceutical leads as well as dietary supplements to existing therapies (Kavishankar et al., 2011). There are many herbs and herbal formulation that used for treating hepatic disorders (Atif et al., 2015). Herbal medicine has significant efficacy, low side effects, low cost and relative safety (Hassan et al., 2015), while synthetic anti-diabetic agents can leads to serious side effects, as hypoglycemic coma and abnormalities of the liver and kidney actions (Bayramoglu et al., 2014). *Ficus carica* is member of Moraceae family and its health management characteristics have been mentioned in traditional therapy. It holds manysubstancesas phenolic compounds, minerals and vitamins that had role in disease cure (Rahmani and Aldebasi, 2017).Aqueous extract of *Ficus carica* has hypoglycemic effects in diabetic rats and diabetic patients by reducing oxidative stress (Zhang et al., 2019).Olive tree (*Olea europaea*) has been widely accepted as one of species with highest antioxidant action via its oil, leaves and fruits (Al-Attar and Alsalmi, 2019).The olive leaves have antioxidant molecules as oleuropein, hydroxytyrosol, oleuropein aglycone, and tyrosol (Jemai et al., 2008). Many studies pointed olive leaves ability for the therapy and improvement of various diseases (Kumral et al., 2015, Al-Attar et al., 2016, Al-Attar et al., 2017).

The present study was designed to investigate the therapeutic and hepatoprotective actions of liraglutide and nano extracts of NEML on liver of STZ-induced diabetic rats model.

MATERIAL AND METHODS

Chemicals and drugs

Streptozocin (STZ) also known as Zanosar, was get from SIGMA Aldrich (St. Louis, MO, USA). Liraglutide (Victoza- Novo Nordisk) purchased from Al Nahdi Medical Company (Jeddah, Saudi Arabia).

Plant Materials Collection and identification

Ficus carica and Olea europaea leaves were purchased from local grocery market in Jazan, and Al-Jouf Corner Consecutively, Kingdom of Saudi Arabia. All the plant leaves were identified and

authenticated by a plant taxonomist at the Department of Arid Land Agriculture, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia.

Synthesis of herbal nanoparticles

Initially, the mixture of methanol extracts was prepared according to (Lins et al., 2018)(Emmanuel et al., 2014). A mixer grinder was used to grind the dried leaves. The ground powders gathered as coarse powders. The obtained powders were milled to make fine powders. Then, the obtained powders were equally (nearly 5 mg) again milled to nanoparticles ranging in size from (10 - 19 nm). Mixer grinder was used to powder the dried specimens**.** The powder then given orally after dissolved in 1 ml of distilled water (Karthik et al., 2016)

Characterizations of herbal nanoparticles

The prepared herbal nanoparticles were subjected to scanning electron microscopy (SEM) coupled with energy-dispersive X-ray (SEM-EDX,) analysis to examine morphology and microstructure of prepared nanoparticle and tested by X-ray diffraction (XRD) method (Rautela et al., 2019)**.**

Animals

Forty adult male rats Wistar Rattus norvegicus 5-week- old weighing range from 190 and 200 g were used in this experimental research. The rats were getting from King Fahd Medical Research Center, and Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia. The rats were kept under a 12: 12 h light/dark cycle at a temperature of 25°C and relative humidity ranging from 60 to 70% during the experimental periods. Rats were housed in polypropylene cages with abundant pine bedding (5 rats in each cage). All rats had ad libitum access to regular water and food. The experimental procedures were conducted in strict compliance with the rules and regulations made by Research Ethics Committee at King Abdul-Aziz University after obtaining their ethical approval to pursue this research.

Experimental protocol

Rats were held to acclimatizate for approximately two weeks before the study began. The rats were randomly distributed into 4 groups of (10 rats each). Group I (NDC) negative group non-diabetic control feeding on a regular meal and drinking water and injected with vehicle. For induced diabetic rat model. Type 2 diabetes mellitus was induced in thirty rats by intraperitoneally injecting a single dose of streptozotocin (STZ) (60 mg/kg body weight) according to (Birgani et al., 2018). Then the rats were divided into (3) groups as follows: Group 2 (DC): The positive diabetic control. Group 3 (LD): Diabetic animals were treated with liraglutide drug(0.02 mg/kg b.w./day) for 8 weeks (Zhang et al., 2018a). Group 4 (NEML): Diabetic animals were treated orally with Nano extract of mixture of *Ficus carica* and *Olea europaea* leaves (1 ml/250 g b.w./day) for 8 weeks.

Liver enzymes assay

The treated and control rats were sacrificed by decapitation at the end of the experimental period of 8 weeks, and the blood samples were immediately gathered from the retro-orbital plexus capillary (Afify et al., 2018)into plain tubes. The bloods were spin at 3000 cycle per minute (cpm) and sera were collected and aliquot and stored at -20^{o}C for biochemical study. Alanine amino transferase (ALT), alkaline phosphatase (ALP), aspartate amino transferase (AST), and gamma glutamyl transpeptidase (GGT) in serum were determined usingELISA kits supplied by Sigma Aldrich (Inc., St. Louis, USA).

Histopathological examinations

After animals sacrificed, the livers of rats were removed and fixed in 10% neutral formalin then were embedded in paraffin and cut into 2 µmthickness. Sections were stained with haematoxylin and eosin for histopathological evaluation (Suvarna et al., 2018).

Statistical analysis

Data were analyzed by IBM SPSS Statistics program for Windows, version 23 (IBM SPSS, IBM Corp., Armonk, N.Y., USA). Data were expressed as mean +/- standard deviation (SD) and standard error (SE). Percentage changes of liver enzyme levels were calculated as group enzyme level minus control

enzyme level divided by control level then multiply by 100. Differences between groups were made using OneWay ANOVA test followed by least significant test (LSD). *P*- value <0.05 was significant.

RESULTS

Liver function tests

Data in Table (1) showed statistical comparison among serum of GGT, ALT, AST and ALP concentrations in control and experimental groups. In DC group, ALT, AST, ALP and GGT serum levels significantly increased by 23.9%, 80.66%, 121.62% and 150.00% versus NDC group. Meanwhile the levels of ALT, AST, ALP and GGT significantly decreased versus DC group by -7.13%, -20.87%, -41.54% and -6.57% in LD group and by -6.76%, -33.76%, -44.49% and -38.57% in NEML groups. But these declines were more in NEML than LD group.

Table 1: Comparison of liver enzymes levels indifferent groups at end the experiment.

groups **Enzyme**	**NDC** **(G1)**	**DC** **(G2)**	**LD** **(G3)**	**NEML** **(G4)**
ALT				
Mean ± SD SE	65.70±2.06 0.65	81.40±6.92 2.19	75.60±1.26 0.40	75.90±2.33 0.74
Sig.		**P =0.001*** **	**P =0.001*** [1] **P=0.001*** **	**P =0.001*** ** **[1]P=0.001*** **
Pre.		[1]Pre = 23.90%	[2] Pre = -7.13%	[2] Pre = -6.76%
AST				
Mean ± SD SE	61.00±9.38 2.97	110.20±8.13 2.57	87.20±4.44 1.40	73.00±1.41 0.45
Sig.		**P =0.001*** **	**P =0.001*** **; **[1]P=0.001*** **	**P =0.001*** ** **[1]P=0.001*** **
Pre.		[1] Pre = 80.66%	[2] Pre = -20.87%	[2] Pre = -33.76%
ALP				
Mean ± SD SE	64.30±4.50 1.42	142.50±26.45 8.36	83.30±13.94 4.41	79.10±14.65 4.63
Sig.		**P =0.001*** **	**P =0.013* ** **[1]P=0.001*** **	**P =0.051** **[1]P=0.001*** **
Pre.		[1] Pre = 121.62%	[2] Pre = -41.54%	[2] Pre = -44.49%
GGT				
Mean ± SD SE	1.40±0.52 0.16	3.50±0.53 0.17	3.27±0.16 0.05	2.15±0.53 0.17
Sig.		**P =0.001*** **	**P =0.001*** ** **[1]P=0.225**	**P =0.001*** **; **[1]P=0.001*** **
Pre.		[1]Pre = 150.00%	[2] Pre = -6.57%	[2] Pre = -38.57%

Data were expressed as mean +/- SD, SE. NDC: non diabetic control; DC: diabetic control; LD: diabetic animals were treated with liraglutide drug. NEML: Diabetic rats treated nano extract of mixture of *Ficus carica* and *Olea europaea* leaves.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 significant. *: mild significance <0.05; **: moderate significance <0.01; ***: highly significance < 0.001. Pre: Percentage changes. [1]Pre: percentage versus NDC; [2]Pre: percentage versus DC.

Histopathological results

Histological evaluation of liver sections of control rats (Group I) revealed the normal appearance of hepatic lobules, hepatocytes, central vines, blood sinusoids, portal areas, and bile ducts (Figures. 1d, 2d, 3d). Liver sections of induced diabetic animals (DC) Group (2) showed noticeable pathological

distortion, most of hepatocytes represented vacuolar cytoplasmic degeneration, while, some hepatocytes revealed lipid droplets accumulation, necrosis with duplicate pleomorphism nuclei, pyknotic, karyolysis and karyorrhexis were also observed. Stagnation of red blood cells in the central veins and blood sinusoids congested with dilation and disrupted of endothelial lining walls, decreased number of Kupffer and endothelial cells, or hypertrophy in some blood sinusoids (Figures. 1a, 2a). Dilated portal area spaces with edematous portal artery, stagnation of red blood cells in widened blood sinusoids around portal areas with heavy aggregates of inflammatory cells, bile duct proliferation surrounded by fibrous tissue were also noted (Figure 3a).

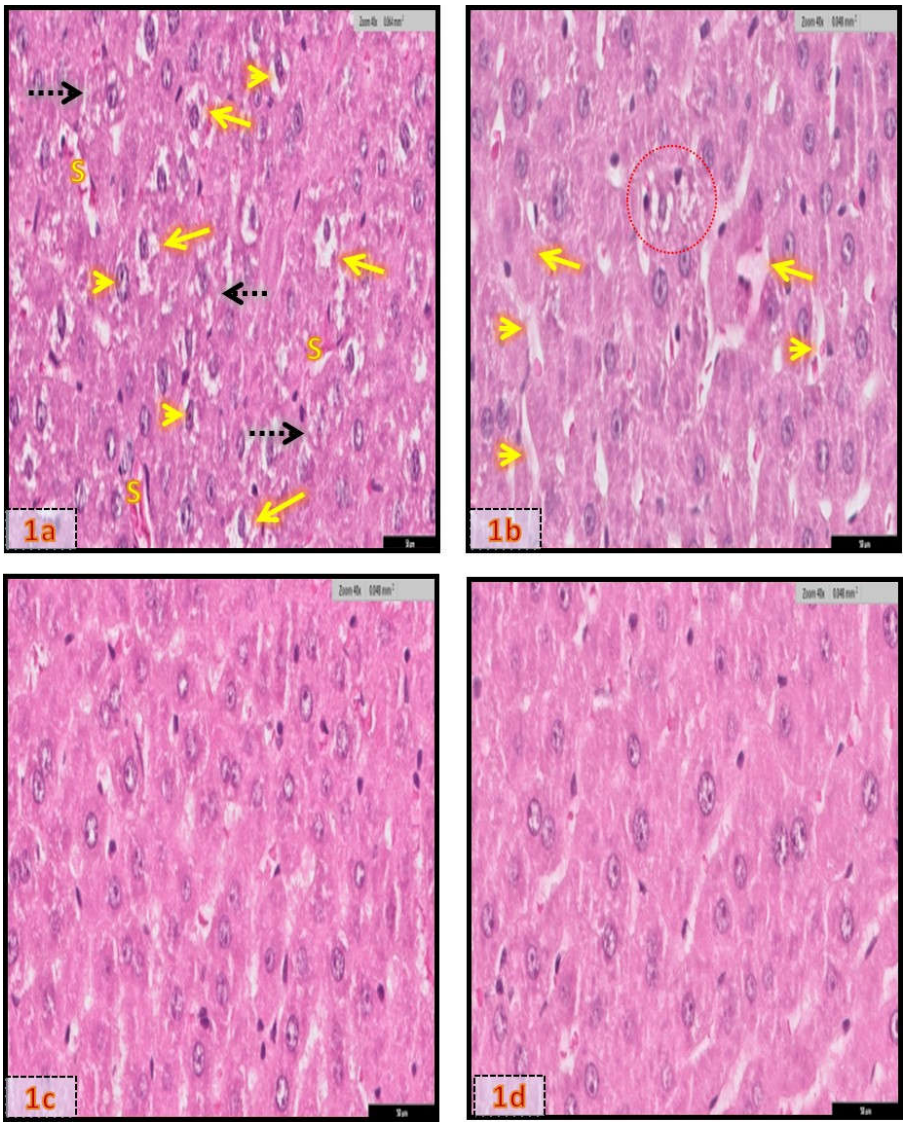

Figure 1 (a-d): **Micrographs images liver section: (a) from STZ- induced diabetic rats (DC); hepatocytes showing marked vacuolar cytoplasmic degeneration (arrows), fatty infiltrations (dot arrows) pleomorphic nuclei (head arrows), congestion and distortion of sinusoids (S),necrotic kupffer and endothelial cells. ; H. & E.; (400X). (b) hepatocytes from liraglutide treated rat (LD); showing mild level of toxicity in hepatocytes; focal vacuolated(circle), and necrosis(arrows) of some hepatocytes , blood sinusoids disrupted and edematous(head arrows).; H. & E.; (400X).(c) liver section from nano NEML leaves extracts treated rats; showing entirely normal histological features. ; H. & E.; (400X) (d) Section from control liver (NDC); showing normal liver histology; H. & E.; (400X).**

The administration of liraglutide to diabetic rats (LD) (Group III) illustrating some signs of mild alterations as disrupted and edematous congestion of central veins and blood sinusoids dilation, focal vacuolization and necrosis of some hepatocytes with pyknotic nuclei, Kupffer and endothelial cells hypertrophy (Figures1b, 2b). Portal areas exhibited mild inflammation of the mononuclear cells detected and hyperplasia of bile duct, disrupted of some blood sinusoids whilst the remaining liver cells appeared normal (Figure 3b). Sections concerning the liver of the animals received nano extracts of leaves (NEML) (Group 4) were at variant with those of the treatment Groups 2 & 3 (Figures 1c,2c,3c) with an almost normal appearance sections of the liver tissue and conformed to the basic histological features of the control liver (NDC) (Group 1) (Figures 1d, 2d,3d).

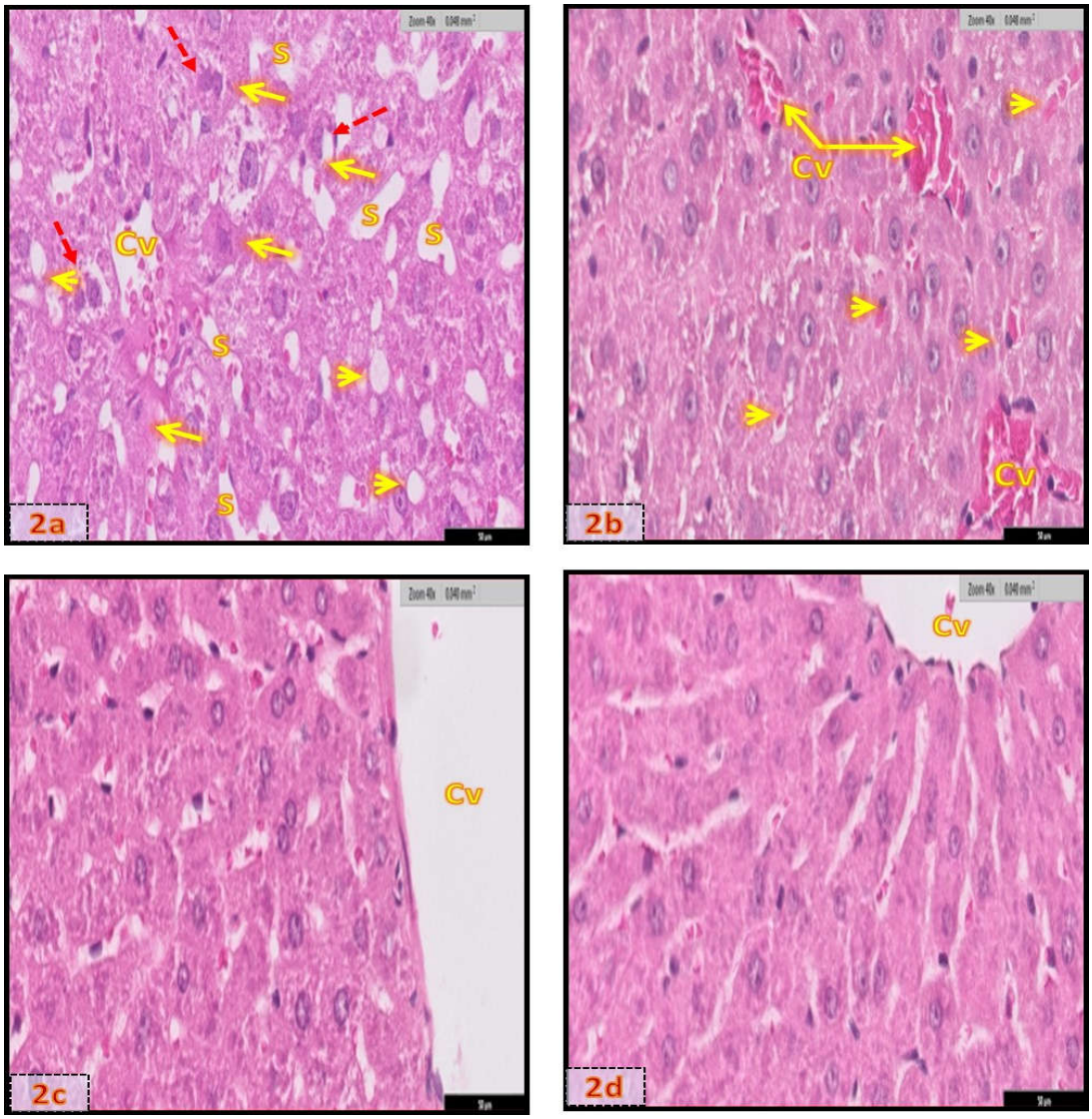

Figure 2 (a-d): **Micrographs images section of central veins areas; (a) STZ- induced diabetic rats(DC); showing central vein (CV) surrounded by extensive necrosis (arrows) of hepatocytes, clearly Lipid droplets (head arrows), sinusoidal dilatation (S), duplicate pleomorphism nuclei (dot arrows); H. & E.; (400X). (b) liraglutide treated rats (LD); exhibit congestion central veins (arrows) and blood Sinusoids (head arrows).; H. & E.; (400X).(c) nano NEML leaves extracts treated rats; showing normal central veins architecture.; H. & E.; (400X). (d) Section from control (NDC); showing normal central vein morphology; H. & E.; (400X).**

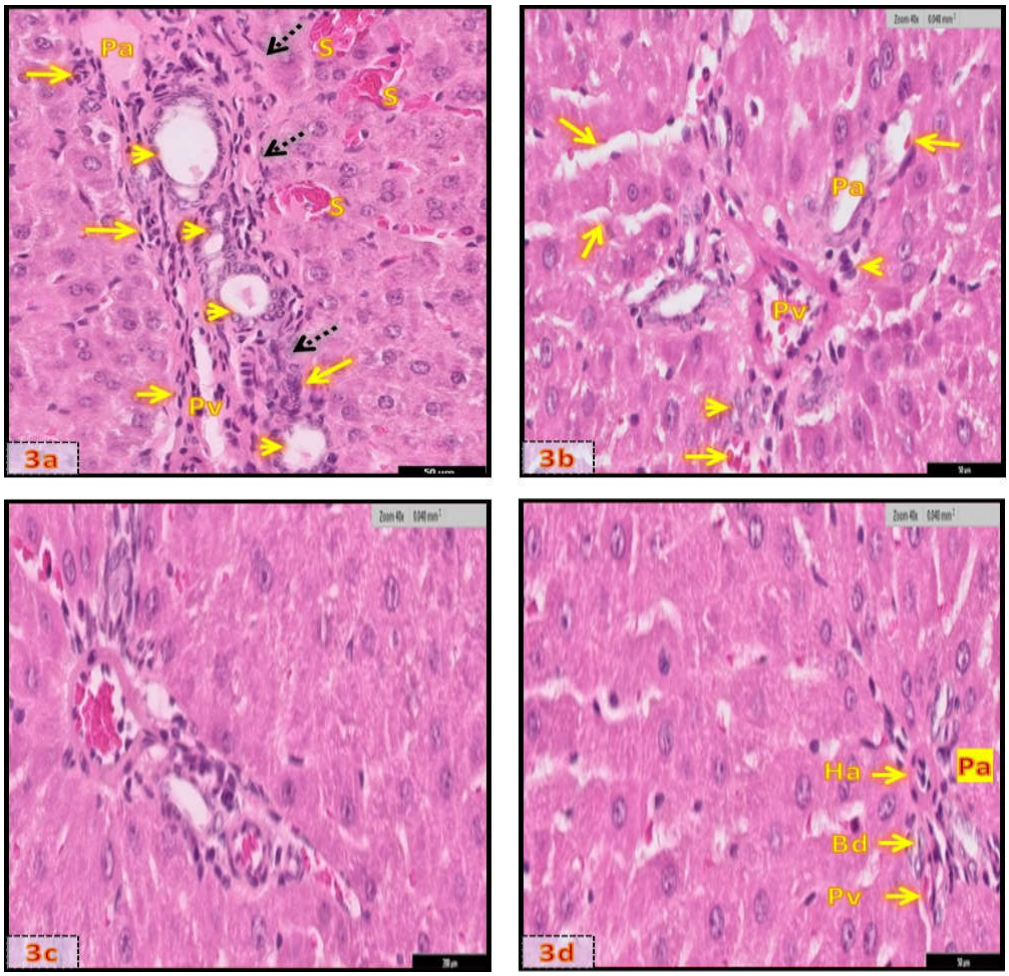

Figure 3 (a-d): **Micrographs images section of portal areas; (a) STZ- induced diabetic rats(DC); elongated portal area and edematous portal artery (Pa), mononuclear leukocyte and lymphocytes aggregates around portal area (arrows), fibrosis(dot arrows), bile duct proliferation (head arrows), stagnation of red blood cells in blood sinusoids(S) around portal areas; H. & E.; (400X). (b) liraglutide treated rats (LD); Portal areas exhibited mild inflammation (head arrows), distorted of some blood sinusoids (arrows).; H. & E.; (400X). (c) nano NEML leaves extracts treated rats; exhibited unremarkable changes in portal region.; H. & E.; (400X). (d) Section from control (NDC); showing normal portal tract structure; H. & E.; (400X).**

DISCUSION

Diabetes mellitus is correlating with many hepatic complications; the initial and most important indicators in assessing liver damage are levels of plasma ALT, AST, GGT and ALP. Elevation of serum levels of AST and ALT is common in T2DM patients (Trombetta et al., 2005). The present study stated percentage of serum levels of ALT, GGT, AST and ALP were significantly increased in DC group versus NDC; while were significantly decreased in LD and NEML groups versus DC. In agreement with current study, previous clinical data (Agarwal et al., 2006, Gowda et al., 2009)showed that type 2 diabetic patients had higher incidence of liver function test abnormalities compared to healthy subjects. (Tousson et al., 2014) reported that ALP is a membrane associated enzyme and an increased ALP activity means hepatic dysfunction. Plasma levels of ALT and AST were elevated after liver damage and cell membranes loss of the functional integrity. While, ALP and GGT levels increased in biliary tree obstruction (Lee et al., 2012).

Results of this study revealed that daily administration of liraglutide to STZ-induced diabetic rats (LD) decreased serum ALT, AST, ALP and GGT levels compared with DC. These results compatible with (Gao et al., 2015, Zhang et al., 2018b)who reported that liraglutide administration to diabetic patients can effectively improve blood glucose as well as liver function parameters. Decreased ALT, AST, and GGT activities while increased glutathione of diabetic patients' treatment with liraglutide was reported (Abdelsameea et al., 2017).

On other hand, administration of *ficus carica* and *olea europaea* leavesto diabetic rats in this study significantly decreased serum ALT, AST and ALP, GGT serum concentration in comparison to DC. The present findings come in agreement with several reports that documented that different parts of *ficus* carica and olea europaea leaves reverts AST and ALT enzyme activities near to normal status in diabetic rats (Saoudi and El Feki, 2012). Concord to this study, (Thapa and Walia, 2007, Mohamed et al., 2016); showed that GGT serum level increased markedly in case of liver diseases as acute viral hepatitis and cholestasis. Similarly, to the present study (Žukovec Topalović et al., 2015) showed that olive leaf extract had strong antioxidant actions, ameliorated the oxidative liver damage and led to decline in serum ALT and AST activities. (Ghouri et al., 2010) pointed that the diabetes mellitus has been reported to induce pathological changes in the liver which could led to hepatic fibrosis and cirrhosis.

Diabetes induced by STZ caused pathophysiological alterations in the liver of rats. These changes were similar to the modifications observed in human liver (Bilal et al., 2016).(Butler et al., 2018) reported several alterations in the liver of diabetic animals, two weeks after STZ-injection, including cloudy swelling andvacuolization of cytoplasm, mild infiltration of lymphocytes with hemorrhage, sever congestion, necrotic foci, hydropic changes, aggregation of lymphocytes around central and portal veins. (Bilal et al., 2016) showed the nuclei of binuclear hepatocytes of STZ-diabetic mice were not same in distribution pattern of chromatin granules, and frequently differ in size and showed irregular membrane. (Brancatelli et al., 2018) established that thehepatic sinusoidal dilatation caused by hepatic venous outflow obstruction, which resulted in vascular stasis and congestion of hepatic parenchyma. (Kume et al., 1994) observed that diabetic mice had acute bile duct hyperplasia and existence of various fat vacuoles in liver cells around the portal vessels which indicated hepatic fibrosis. Cellular infiltrations around and between central veins, portal tracts, and even between hepatocytes, lobular or acinar infiltration with lymphocytes and neutrophils confirm data observed by other investigators (Brunt, 2011, Tan et al., 2013)

.Inflammatory cell migration is driven by a complex interaction between inflammatory cells and their environment. In order to maintain health, inflammation needs to resolve, allowing the surrounding tissues to recover and heal (Elks et al., 2011). Kupffer cells and sinusoidal endothelial cells play a crucial rolein the innate immune response and liver homeostasis and in the pathogenesis of liver diseases (Thomson and Knolle, 2010). Kupffer cells can be protective in a number of situations, as drug-induced liver destruction and toxin-inducefibrosis (Ramachandran and Iredale, 2012).

However, the architecture of hepatocytes of STZ- diabetic rats treated with liraglutide showed a more or less normal pattern with a mild degree of vacuolations, fatty change, necrosis and lymphocyte infiltrations. Evidences recorded that elevated incidences of hepatotoxicity had been shown in patients with diabetes mellitus taking drug therapies. Present results were corroborated with (Dąbroś et al., 2006, Lucchesi et al., 2015); studies that showed administration of liraglutide and treatment of hyperglycemic condition, all of the ultra-structural abnormalities in the rats hepatocytes were restored. Formal studies revealed that liraglutide, glucagon-like peptide-1 (GLP-1) analogue, could regulate glucose homeostasis as good therapy for Type 2 diabetes mellitus (Lu et al., 2016). Glucagon-like peptide-1 analogs is a new class of antidiabetic therapy that mimic the actions of incretins and have actions in some organs (Milani et al., 2019).

Liraglutide is a long-acting synthetic analog of GLP-1 with 97% homology to human GLP-1, and was shown to inhibit oxidative stress and improve hepatic cell apoptosis in chronic hepatic diseases

models (Gao et al., 2015)**.** and hepatic glucolipotoxicity (Guo et al., 2018). Our findings ensure the beneficial actions of liraglutide therapy on hepatic histology**,** and reducing liver inflammation and fibrosis. These results also suggested by(Frias et al., 2019) who reported anti-inflammatory and anti-fibrotic effects of liraglutide that were not dependent upon lipid metabolism**.**

Studies based on *in vivo* and *in vitro* reported that figs fruits, stem, leaves, and latex had health management action through antioxidant, anti-inflammatory, antimicrobial, anticarcinogenic, and anti-inflammatory actions. Moreover, an inverse relation between figs utilization and disease development was found (Rahmani and Aldebasi, 2017)**.** In contrast, (Sankar et al., 2015) mentioned that rats treated with *Ficus carica leaves* extracts showed a more or less normal liver architecture with a moderate cytoplasmic changes, necrosis and lymphocyte infiltration. Other research reported, that animal group treated with *Ficus carica* leaf extracts had livers which were similar to normal rats (Khan et al., 2012). Olive leaves present a unique opportunity to study the effects of olive oil polyphenols on metabolic profile and hepatic structure and functions, because the leaf contains these polyphenols with only a little amount of oleic acid. Methanolic extracts of olive leaves had secoiridoids such as oleuropein, ligostroside, dimethyloleuropein, and oleoside (El and Karakaya, 2009); flavonoids, including apigenin, kaempferol, and luteolin; as well as phenolic compounds such as caffeic acid, tyrosol, and hydroxytyrosol (Chiou et al., 2007).

These findings proved by this study revealed that nano extracts of *olea europaea* and *ficus* leaves had a protective action versus hepatic damage in diabetic rats. Previously, (Rice-Evans et al., 1997) concluded that the olive leave extracts had ameliorative action against toxicity of diabetes in rats may be due to its phenolic substances. (Alsahli et al., 2019) suggested that polyphenols of olive leaves, mainly oleuropein, have interesting functions on the human body as antioxidant capability, antihypertensive, hypoglycemic, hypocholesterolemic factors. Moreover, the present results showed that nano leaves extracts NEML ameliorated liver injury via its polyphenolic substances that act as antioxidant factors, these findings are approved by (Alsahli et al., 2019).

COCLUSION

Based on above mentioned observations, existing research shows that NEML extracts have beneficial in decreasing blood glucose levels than liraglutide drug in experimental STZ-diabetic rats**,** and prevent the development of long-term liver complications without causing adverse effects. Evidences indicated the observations of this study can provide support to further studies on NEML extracts to obtain more explanations of its mechanism of action and to establish its therapeutic potential in treatment of diabetic-induced hepatocellular damage.

REFERENCES

1. Abdelsameea, A. A., Abbas, N. A. and Raouf, S. M. A. (2017). Liraglutide attenuates partial warm ischemia-reperfusion injury in rat livers. *Naunyn-Schmiedeberg's archives of pharmacology,* 390 **(**3): 311-319.
2. Adeyemi, D. O., Ukwenya, V. O., Obuotor, E. M. and Adewole, S. O. (2014). Anti-hepatotoxic activities of Hibiscus sabdariffa L. in animal model of streptozotocin diabetes-induced liver damage. *BMC complementary and alternative medicine,* 14 **(**1): 277.http://www.biomedcentral.com/1472-6882/14/277
3. Afify, A., El-Beltagi, H., Fayed, S. and El-Ansary, A. (2018). In vivo correlation of olive leaves extract on some oxidative stress markers in streptozotocin-induced diabetes mellitus in rats. *Grasas y Aceites,* 69 **(**1): 243.
4. Agarwal, M., Srivastava, V., Saxena, K. and Kumar, A. (2006). Hepatoprotective activity of Beta vulgaris against CCl4-induced hepatic injury in rats. *Fitoterapia,* 77 **(**2): 91-93.
5. Al-Attar, A. M., Alrobai, A. A. and Almalki, D. A. (2016). Effect of Olea oleaster and Juniperus procera leaves extracts on thioacetamide induced hepatic cirrhosis in male albino mice. *Saudi journal of biological sciences,* 23 **(**3): 363-371.

6. Al-Attar, A. M., Alrobai, A. A. and Almalki, D. A. (2017). Protective effect of olive and juniper leaves extracts on nephrotoxicity induced by thioacetamide in male mice. *Saudi journal of biological sciences,* 24 **(**1): 15-22.
7. Al-Attar, A. M. and Alsalmi, F. A. (2019). Effect of Olea europaea leaves extract on streptozotocin induced diabetes in male albino rats. *Saudi journal of biological sciences,* 26 **(**1): 118-128.
8. Alsahli, M. A., Almatroudi, A., Khan, A. A., Alhumaydhi, F. A., Alrumaihi, F. and Rahmani, A. H. (2019). Ficus carica (Fig) Fruit Extract Attenuates CCl 4-induced Hepatic Injury in Mice: A Histological and Immunohistochemical Study. *Pharmacology,* 15 **(**3): 370-376.
9. Atif, M., Rahman, S. A., Ghori, S. S., Ahmed, M. I., Mahmood, S. B. and Muqtadar, M. A. (2015). Hepatoprotective activity of ficus dalhousiae miq leaves ethanolic extract on carbon tetrachloride and paracetamol induced hepatotoxicity in wistar albino rats. *International Journal of Pharmaceutical Sciences and Research,* 6 **(**4): 1415.
10. Bayramoglu, G., Senturk, H., Bayramoglu, A., Uyanoglu, M., Colak, S., Ozmen, A. and Kolankaya, D. (2014). Carvacrol partially reverses symptoms of diabetes in STZ-induced diabetic rats. *Cytotechnology,* 66 **(**2): 251-257.
11. Bilal, H. M., Riaz, F., Munir, K., Saqib, A. and Sarwar, M. R. (2016). Histological changes in the liver of diabetic rats: A review of pathogenesis of nonalcoholic fatty liver disease in type 1 diabetes mellitus. *Cogent Medicine,* 3 **(**1): 1275415.
12. Birgani, G. A., Ahangarpour, A., Khorsandi, L. and Moghaddam, H. F. (2018). Anti-diabetic effect of betulinic acid on streptozotocin-nicotinamide induced diabetic male mouse model. *Brazilian Journal of Pharmaceutical Sciences,* 54 **(**2):e17171.
13. Brancatelli, G., Furlan, A., Calandra, A. and Burgio, M. D. (2018). Hepatic sinusoidal dilatation. *Abdominal Radiology,* 43 **(**8): 2011-2022.
14. Brunt, E. M. (2011). Non-alcoholic fatty liver disease: what's new under the microscope? *Gut,* 60 **(**8): 1152-1158.
15. Butler, D. C., Lewin, D. N. and Batalis, N. I. (2018). Differential Diagnosis of Hepatic Necrosis Encountered at Autopsy. *Academic forensic pathology,* 8 **(**2): 256-295.
16. Chia, C. W. and Egan, J. M. (2008). Incretin-based therapies in type 2 diabetes mellitus. *The Journal of Clinical Endocrinology & Metabolism,* 93 **(**10): 3703-3716.
17. Chiou, A., Salta, F., Kalogeropoulos, N., Mylona, A., Ntalla, I. and Andrikopoulos, N. (2007). Retention and distribution of polyphenols after pan□frying of French fries in oils enriched with olive leaf extract. *Journal of food science,* 72 **(**8): S574-S584.
18. DąBroś, W., Goc, A., Turyna, B. and Kordowiak, A. (2006). Sodium metavanadate affected control and streptozotocin-diabetic rat liver golgi complexes. *Polish journal of pathology: official journal of the Polish Society of Pathologists,* 57 **(**2): 91-97.
19. De Mesquita, F. C., Guixé-Muntet, S., Fernández-Iglesias, A., Maeso-Díaz, R., Vila, S., Hide, D., Ortega-Ribera, M., Rosa, J. L., García-Pagán, J. C. and Bosch, J. (2017). Liraglutide improves liver microvascular dysfunction in cirrhosis: Evidence from translational studies. *Scientific reports,* 7 **(**1): 1-10.
20. El, S. N. and Karakaya, S. (2009). Olive tree (Olea europaea) leaves: potential beneficial effects on human health. *Nutrition reviews,* 67 **(**11): 632-638.
21. Elks, P. M., Loynes, C. A. and Renshaw, S. A., 2011. Measuring inflammatory cell migration in the zebrafish. *Cell Migration*. Springer. Humana Press, pp. 261-275.
22. Emmanuel, S., Olajide, O., Abubakar, S., Idowu, I., Orishadipe, A. and Thomas, S. (2014). Phytochemical and antimicrobial studies of methanol, ethyl acetate, and aqueous extracts of Moringa oleifera seeds. *American Journal of Ethnomedicine,* 1 **(**5): 346-354.
23. Frias, J. P., Evenou, P., Dalton, D. and Dungan, K. M. (2019). 128-LB: insulin glargine 300 U/mL (Gla-300) vs. first-generation basal insulins (BI) in insulin-naïve type 2 diabetes (T2D) patients (Pts) with and without cardiovascular disease (CVD): outcomes of the randomized pragmatic real-life clinical trial ACHIEVE Control. Am Diabetes Assoc. https://doi.org/10.2337/db19-128-LB
24. Gao, H., Zeng, Z., Zhang, H., Zhou, X., Guan, L., Deng, W. and Xu, L. (2015). The glucagon-like peptide-1 analogue liraglutide inhibits oxidative stress and inflammatory response in the

liver of rats with diet-induced non-alcoholic fatty liver disease. *Biological and Pharmaceutical Bulletin,* 38 **(**5): 694-702.

25. Garber, A., Henry, R., Ratner, R., Garcia-Hernandez, P. A., Rodriguez-Pattzi, H., Olvera-Alvarez, I., Hale, P. M., Zdravkovic, M., Bode, B. and Group, L.-S. (2009). Liraglutide versus glimepiride monotherapy for type 2 diabetes (LEAD-3 Mono): a randomised, 52-week, phase III, double-blind, parallel-treatment trial. *The Lancet,* 373 **(**9662): 473-481.
26. Ghouri, N., Preiss, D. and Sattar, N. (2010). Liver enzymes, nonalcoholic fatty liver disease, and incident cardiovascular disease: a narrative review and clinical perspective of prospective data. *Hepatology,* 52 **(**3): 1156-1161.
27. Goetz, M. E. and Luch, A. (2008). Reactive species: a cell damaging rout assisting to chemical carcinogens. *Cancer letters,* 266 **(**1): 73-83.
28. Gowda, S., Desai, P. B., Hull, V. V., Math, A. a. K., Vernekar, S. N. and Kulkarni, S. S. (2009). A review on laboratory liver function tests. *The Pan african medical journal,* 3.
29. Guo, J., Li, C., Yang, C., Li, B., Wei, J., Lin, Y., Ye, P., Hu, G. and Li, J. (2018). Liraglutide reduces hepatic glucolipotoxicity-induced liver cell apoptosis through NRF2 signaling in Zucker diabetic fatty rats. *Molecular medicine reports,* 17 **(**6): 8316-8324.
30. Guss, D. A. and Mohanty, S. R. (2016). Liraglutide's use in treatment of non-alcoholic fatty liver: an evaluation of the non-alcoholic steatohepatitis study. *Hepatobiliary surgery and nutrition,* 5 **(**6): 515-518.
31. Hassan, S. K., El-Sammad, N. M., Mousa, A. M., Mohammed, M. H., Farrag, A. E. R. H., Hashim, A. N. E., Werner, V., Lindequist, U. and Nawwar, M. a. E.-M. (2015). Hypoglycemic and antioxidant activities of Caesalpinia ferrea Martius leaf extract in streptozotocin-induced diabetic rats. *Asian Pacific Journal of Tropical Biomedicine,* 5 **(**6): 462-471.
32. Iwao, T., Sakai, K. and Ando, E. (2015). Liraglutide With or Without Metformin Ameliorates Liver Function and Fatty Liver in Obese Patients with Type 2 Diabetes Mellitus. *JOURNAL OF DIABETES & METABOLISM,* 6 **(**9):1000598.
33. Jemai, H., Bouaziz, M., Fki, I., El Feki, A. and Sayadi, S. (2008). Hypolipidimic and antioxidant activities of oleuropein and its hydrolysis derivative-rich extracts from Chemlali olive leaves. *Chemico-Biological Interactions,* 176 **(**2-3): 88-98.
34. Karthik, S., Suriyaprabha, R., Balu, K. S., Manivasakan, P. and Rajendran, V. (2016). Influence of ball milling on the particle size and antimicrobial properties of Tridax procumbens leaf nanoparticles. *IET nanobiotechnology,* 11 **(**1): 12-17.
35. Kavishankar, G., Lakshmidevi, N., Murthy, S. M., Prakash, H. and Niranjana, S. (2011). Diabetes and medicinal plants-A review. *Int J Pharm Biomed Sci,* 2 **(**3): 65-80.
36. Khan, R., Khan, A. Q., Qamar, W., Lateef, A., Tahir, M., Rehman, M. U., Ali, F. and Sultana, S. (2012). Chrysin protects against cisplatin-induced colon. toxicity via amelioration of oxidative stress and apoptosis: probable role of p38MAPK and p53. *Toxicology and applied pharmacology,* 258 **(**3): 315-329.
37. Kume, E., Ohmachi, Y., Itagaki, S.-I., Tamura, K. and Doi, K. (1994). Hepatic changes of mice in the subacute phase of streptozotocin (SZ)-induced diabetes. *Experimental and Toxicologic Pathology,* 46 **(**4-5): 368-374.
38. Kumral, A., Soluk-Tekkeşin, M., Olgaç, V., Doğru-Abbasoğlu, S., Türkoğlu, Ü. and Uysal, M. (2015). Effect of olive leaf extract treatment on doxorubicin-induced cardiac, hepatic and renal toxicity in rats. *Pathophysiology,* 22 **(**2): 117-123.
39. Lee, T. H., Kim, W. R. and Poterucha, J. J. (2012). Evaluation of elevated liver enzymes. *Clinics in Liver Disease,* 16 **(**2): 183-198.
40. Lins, P. G., Pugine, S. M. P., Scatolini, A. M. and De Melo, M. P. (2018). In vitro antioxidant activity of olive leaf extract (Olea europaea L.) and its protective effect on oxidative damage in human erythrocytes. *Heliyon,* 4 **(**9): e00805.
41. Lu, J., Gu, Y., Guo, M., Chen, P., Wang, H. and Yu, X. (2016). Serum magnesium concentration is inversely associated with albuminuria and retinopathy among patients with diabetes. *Journal of diabetes research,* http://dx.doi.org/10.1155/2016/1260141.
42. Lucchesi, A. N., Cassettari, L. L. and Spadella, C. T. (2015). Alloxan-induced diabetes causes morphological and ultrastructural changes in rat liver that resemble the natural history of chronic fatty liver disease in humans. *Journal of diabetes research,* https://doi.org/10.1155/2015/494578.

43. Manna, P., Das, J., Ghosh, J. and Sil, P. C. (2010). Contribution of type 1 diabetes to rat liver dysfunction and cellular damage via activation of NOS, PARP, IκBα/NF-κB, MAPKs, and mitochondria-dependent pathways: prophylactic role of arjunolic acid. *Free Radical Biology and Medicine,* 48 **(**11): 1465-1484.
44. Milani, L., Galindo, C. M., De Oliveira, N. M. T., Corso, C. R., Adami, E. R., Stipp, M. C., Beltrame, O. C. and Acco, A. (2019). The GLP-1 analog liraglutide attenuates acute liver injury in mice. *Annals of hepatology,* 18 **(**6): 918-928.
45. Mohamed, J., Nafizah, A. N., Zariyantey, A. and Budin, S. B. (2016). Mechanisms of diabetes-induced liver damage: the role of oxidative stress and inflammation. *Sultan Qaboos University Medical Journal,* 16 **(**2): e132.
46. Nauck, M., Frid, A., Hermansen, K., Shah, N. S., Tankova, T., Mitha, I. H., Zdravkovic, M., Düring, M. and Matthews, D. R. (2009). Efficacy and safety comparison of liraglutide, glimepiride, and placebo, all in combination with metformin, in type 2 diabetes: the LEAD (liraglutide effect and action in diabetes)-2 study. *Diabetes care,* 32 **(**1): 84-90.
47. Ohki, T., Isogawa, A., Iwamoto, M., Ohsugi, M., Yoshida, H., Toda, N., Tagawa, K., Omata, M. and Koike, K. (2012). The effectiveness of liraglutide in nonalcoholic fatty liver disease patients with type 2 diabetes mellitus compared to sitagliptin and pioglitazone. *The Scientific World Journal,* 2012:496453.
48. Rahmani, A. H. and Aldebasi, Y. H. (2017). Ficus carica and its constituents role in management of diseases. *Asian J Pharm Clin Res,* 10 **(**6): 49-53.
49. Ramachandran, P. and Iredale, J. P. (2012). Macrophages: central regulators of hepatic fibrogenesis and fibrosis resolution. *Journal of hepatology,* 56 **(**6): 1417-1419.
50. Rautela, A., Rani, J. and Das, M. D. (2019). Green synthesis of silver nanoparticles from Tectona grandis seeds extract: characterization and mechanism of antimicrobial action on different microorganisms. *Journal of Analytical Science and Technology,* 10 **(**1): 1-10.
51. Rice-Evans, C. A., Sampson, J., Bramley, P. M. and Holloway, D. E. (1997). Why do we expect carotenoids to be antioxidants in vivo? *Free radical research,* 26 **(**4): 381-398.
52. Sankar, M., Rajkumar, J. and Devi, J. (2015). Hepatoprotective activity of hepatoplus on isonaizid and rifampicin induced hepatotoxicity in rats. *Pak J Pharm Sci,* 28 **(**3): 983-990.
53. Saoudi, M. and El Feki, A. (2012). Protective role of Ficus carica stem extract against hepatic oxidative damage induced by methanol in male Wistar rats. *Evidence-Based Complementary and Alternative Medicine,* 2012.doi:10.1155/2012/150458.
54. Suvarna, K. S., Layton, C. and Bancroft, J. D. (2018). *Bancroft's theory and practice of histological techniques E-Book*, Elsevier Health Sciences.
55. Tan, Y., Lao, W., Xiao, L., Wang, Z., Xiao, W., Kamal, M. A., Seale, J. P. and Qu, X. (2013). Managing the combination of nonalcoholic fatty liver disease and metabolic syndrome with Chinese herbal extracts in high-fat-diet fed rats. *Evidence-Based Complementary and Alternative Medicine,* 2013. http://dx.doi.org/10.1155/2013/306738.
56. Thapa, B. and Walia, A. (2007). Liver function tests and their interpretation. *The Indian Journal of Pediatrics,* 74 **(**7): 663-671.
57. Thomson, A. W. and Knolle, P. A. (2010). Antigen-presenting cell function in the tolerogenic liver environment. *Nature Reviews Immunology,* 10 **(**11): 753-766.
58. Tousson, E., Zaki, Z. T., Abu-Shaeir, W. A. and Hassan, H. (2014). Methotrexate-induced hepatic and renal toxicity: role of L-carnitine in treatment. *Biomed Biotechnol,* 2 **(**4): 85-92.
59. Trombetta, M., Spiazzi, G., Zoppini, G. and Muggeo, M. (2005). type 2 diabetes and chronic liver disease in the Verona diabetes study. *Alimentary pharmacology & therapeutics,* 2224-27.
60. Zhang, L., Zhang, S.-T., Yin, Y.-C., Xing, S., Li, W.-N. and Fu, X.-Q. (2018a). Hypoglycemic effect and mechanism of isoquercitrin as an inhibitor of dipeptidyl peptidase-4 in type 2 diabetic mice. *RSC advances,* 8 **(**27): 14967-14974.
61. Zhang, Y., Chen, J., Zeng, Y., Huang, D. and Xu, Q. (2019). Involvement of AMPK activation in the inhibition of hepatic gluconeogenesis by Ficus carica leaf extract in diabetic mice and HepG2 cells. *Biomedicine & Pharmacotherapy,* 109:188-194.
62. Zhang, Z., Qi, Y., Kong, W., Jin, Q., Wang, X., Dong, Y., Wang, Y. and Li, H. (2018b). Efficacy and Clinical Value of Liraglutide for Treatment of Diabetes Mellitus Complicated by Non-

Alcoholic Fatty Liver Disease. *Medical science monitor: international medical journal of experimental and clinical research,* 24:7399-7404.

63. Žukovec Topalović, D., Živković, L., Čabarkapa, A., Djelić, N., Bajić, V., Dekanski, D. and Spremo-Potparević, B. (2015). Dry olive leaf extract counteracts L-thyroxine-induced genotoxicity in human peripheral blood leukocytes in vitro. *Oxidative medicine and cellular longevity,* 2015.https://doi.org/10.1155/2015/762192.