**EFFECT OF EMPAGLIFLOZINE ALONE AND IN COMBINATION WITH METFORMIN ON CONTROLLING GLYCEMIC STATUS AND LIVER FUNCTIONS IN NA-STZ DIABETIC RATS**

**Abstract:**

**Background:** Non-alcoholic steatohepatitis is acondition of which diabetic fatty liver accounts for a large proportion. Metformin (met) is one of the most commonly used drugs to treat Diabetes Mellitus (DM) type II. Empagliflozin (Empa) is a sodium -glucose co-transporter 2 inhibitors , which has recently been shown to improve the prognosis in high-risk cardiovascular patients. **Aim of the study:** this work aimed at exploring the possible prophylactic effect of empa alone and in combination with met on NASH induced experimentally by high fat diet in rats with DM type II. **Material and methods:** 36Rats were divided into 5 groups after experimental inductuion of DM type II : Control normal group , Untreated NASH diabetic group (received a high fat diet for 12 weeks), Met pre-treated NASH diabetic group (treated with met 100 mg/kg/day orally for 12 weeks) , empa pre-treated NASH diabetic group (treated with empa 10 mg/kg/day orally for 12 weeks) and met and empa pre-treated diabetic group(treated with met100 mg/kg/day and empa 10 mg/kg/day orally for 12 weeks).**Results:**Met and empa produced reduction of liver enzymes , total cholesterol, triglycerides and LDL-c , fasting blood glucose , insulin level and insulin resistance index and significant elevation of HDL-c and serum adiponectin These results were supported by marked improvement of liver histopathology compared to non-treated NASH diabetic group, with the best results obtained by administration of met along with empa . **Conclusion:**It was found that met and /or empa affords hepatoprotective effects.

Key words:Non-alcoholic steatohepatitis, high fat diet , Metformin and Empagliflozin.

**Introduction**

NASH is a common chronic condition of which diabetic fatty liver accounts for a large proportion, with 50 to 75% of the subjects demonstrating fat in the liver on Ultrasound. As a result of epidemic increase in obesity, hyperlipidemia and diabetic patients ,the prevalence of NASH in the general population is increasing. NASH encompasses both simple steatosis and non-alcoholic setatohepatitis, which may ultimately lead to liver cirrhosis . NASH can lead to liver related death in 12-25%. It can develop into sub- acute liver failure and hepato cellular carcinoma ***.***1The progression of the disease makes insulin secretion unable to maintain glucose homeostasis, producing hyperglycaemia. Patients with DM type II are mostly characterized by being obese or having a higher body fat percentage, distributed predominantly in the abdominal region. In this condition, adipose tissue promotes insulin resistance (IR )through various inflammatory mechanisms, including increased free fatty acid (FFA) release and adipokine deregulation. 2 Metformin is a kind of general insulin sensitizer***.***3 Although its molecular mechanisms of action are complex and not completely understood, metformin has been shown to act via both adenosine monophosphate-activated protein kinase (AMPK)-dependent and AMPK-independent mechanisms.4

Empagliflozin is one of SGLT2 inhibitors which are novel class of anti-diabetic drugs that improve hyperglycemia by inhibiting urinary glucose reabsorption from the kidney. 5In addition to a glucose-lowering effect, SGLT2 inhibitors exert several cardio-renal and metabolic benefits, including weight loss.6

This work aimed at exploring the possible prophylactic effect of empagliflozin alone and in combination with metformin on NASH induced experimentally by high fat diet in rats with diabetes mellitus type II.

**MATERIALS AND METHODS**

**Animals:** 36 Adult male albino rats (brought from Vacsera-Cairo) weighing between 150-180 g (at the beginning of the study), were used for in-vivo experiments. They have acclimatized for one week and were caged in fully ventilated room (at temperature ranging from 25-30oc). Food and water were provided ad libitum.

***Type of study:*** prospective

***Duration:*** 10/2/2021 to 20/6/2021

***Place:*** pharmacology department-benha faculty of medicine

***Ethical considerations:***

Experimental rats were placed under complete healthy conditions all over the experiment in the form of:

-Clean environment.

-Good ventilation.

-Good nutrition.

-Number of rats in each cage was eight.

-The rats were under the care of a professional technician and a qualified researcher. The study was approved by the ethical committee of Faculty of medicine, Benha University, which adopt the guidelines for ethical conduct in the care and use of animals provided by American Association of Psychologists.

**Drugs and chemicals:**

**Empagliflozine powder** (Pharma biotechnology, India). **Cholesterol powder** : (Sigma chemical company). **Metformin powder** (Pharma biotechnology, India).

Metformin and empagliflozin were dissolved in distilled water**.**

**Induction of NASH on top of NA-STZ DM type II:**

DM type II was induced in rats by a single IP injection of streptozotocin (STZ )(65Mg /kg BW), 15minutes after IP administration of nicotinamide (NA) (110 mg/kg BW). STZ was dissolved in citrate buffer (0.1 M, pH 4.5) and NA was dissolved in normal saline. 7,8

NASH was induced by high fat diet formed of a laboratory chow diet in addition to 10% animal fat, 2% cholesterol, and 5% corn oil for 12 weeks***.*9**

**\* Animal groups:-**

36 adult male albino rats were divided into five groups: Group I (Control normal group): received a standard chow diet and tap water with no medication, Group II (Untreated NASH diabetic group): received a high fat diet for 12 weeks with no medication after induction of NA-STZ DM type II9 ***,***Group III (Metformin pre-treated NASH diabetic group): received a high fat diet with oral administration of metformin at a dose of (100 mg/kg/day) for 12 weeks after induction of NA-STZ DM type II 9,**10**and Group IV (Empagliflozin pre-treated NASH diabetic group)*:* received a high fat diet with oral administration of empagliflozin at a dose of (10 mg/kg/day) for 12 weeks after induction of NA-STZ DM type II. 9,**11** and Group V (Metformin and empagliflozin pre-treated NASH diabetic group)*:* received a high fat diet with oral administration of metformin at a dose of (100 mg/kg/day) and empagliflozin at a dose of (10 mg/kg/day) for 12 weeks after induction of NA-STZ DM type II. 9,**10,11**

**Sample collection**

At the end of the 12th week ,rats were overnight fasted for 8 hours,blood samples were taken from rats tails for assessment of fasting blood sugar ,then fasting was compeleted for 12 hours. rats were weighed for calculation of LWI (liver weight index), rats were anesthetized with urethane a dose of 0.6 ml/100 gm BWT of 25% fresh prepared solution**11**the blood sample was taken from the heart by syringe***.*** Samples for biochemical analysis of liver enzymes, lipid profile and insulin levels were incubated at 37oc until blood clotted and then centrifuged at 3000 revolution per minute (rpm) for 15 min for separation of serum and stored at – 20o C.**12**

**1-Determination of liver function tests:** were performed on samples by colorimetric methods (AST, ALT), by using ALT and AST kit (Human , Egypt),according to the method of Reitman and Frankel***.* 13**

**2-Determination of serum lipid profile:** Serum levels of total cholesterol (TC), triglyceride (TG) and high- density lipoprotein-cholesterol (HDL-C) were determined using colorimetric enzymatic kits (Bio Diagnostic, Dokki, Egypt) according to the manufacturer‟s instructions.**14***,***15,16** Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula [LDL- cholesterol= Total Cholesterol- (HDL-cholesterol+ TG/5) (mg/dl)]***.***  **17**

**3-Liver mass index (LMI):**

The liver was immediately removed and weighed after rinsing with ice cold saline . The liver weight index (%) was calculated as liver weight/body weight × 100.

**4-Measurement of fasting blood glucose:**

Fasting blood glucose was measured on a Beckman Glucose Analyzer II (Beckman, Fullerton, CA). **18**

**5-Measurement of fasting insulin level*:*** by rat insulin ELISA kit (SunLong Biotech Co., LTD) *.***19**

**6-Insulin resistance by HOMA-IR index:** Insulin resistance was measured using the homeostasis model assessment (HOMA-IR) defined by the following formula: **20**

**HOMA-IR=Fasting glucose level (mg/dl) x Fasting insulin level (μIU/mL) /405.**

**7- Measurement of serum adiponectin level:** using Adiponectin rat ELISA kits (Biovendor,Germany),This ELISA kit uses Sandwich ELISA as the method. **21**

**Histopathology of the liver:**

After functional studies were completed, the liver of rats were put into a buffered 4% formaline fixation solution and processed with paraffin wax for histopathological examination. Sections (5μm) were stained with hematoxylin and eosin. **22**

**Statistical analysis:** In the statistical comparison between the different groups, the significance of difference was tested using ANOVA test (F value):-Used to compare mean of more than two groups of quantitative data using multiple comparison post hoc test (LSD). *P* value <0.05 was considered statistically significant while >0.05 statistically insignificant P value <0.01 was considered highly significant.

**RESULTS**

Induction of NASH by high fat diet in diabetic rats resulted in significant elevation in liver enzymes (AST and ALT) (figure1,2), TC, TG, LDL-C (table2), fasting glucose and insulin levels and insulin sensitivity (table1) with significant reduction of HDL-C (table 2) and serum adiponectin level (figure4) in untreated NASH group when compared to control group.

Treatment with metformin and /or empagliflozin resulted in significant reduction of liver enzymes (AST and ALT) (figure1,2), TC, TG, LDL-C(table2), fasting glucose and insulin levels, with improvement of insulin sensitivity observed by lowering of HOMA-IR index(table1). The serum HDL-C (table 2) and adiponectin levels (figure4) were significantly elevated in treated groups when compared to untreated NASH group, with the best results in combination group.

Microscopic examination of liver sections of rats of control group displayed normal liver architecture (figure5). Rats of untreated-NASH diabetic group showed severe steatosis, hepatocyte ballooning and degeneration with multiple foci of inflammatory cell infiltration (figure 6). Whereas, hepatocyte ballooning and degeneration and the infiltration of inflammatory cells were markedly ameliorated in metformin pre-treated NASH diabetic group and empagliflozin pre-treated NASH diabetic group (figure 7,8). However combination therapy exhibited better improvement of liver architecture (figure 9).

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Parameters mean ± SD** | | |
| **HOMA IR** | **Plasma insulin( IU/L)** | **FBG(mg/dl)** | **Groups** |
| 1.35  ±0.105 | 6.95  ±0.10 | 84.93  ±5.71 | **Group I: Normal control**  **Group** |
| 17.33  ±0.82 a | 25.83  ±2.14 a | 198.5  ±9.89 a | **Group II: NASH diabetic non treated group group** |
| 13.17  ±0.75 ab | 17.17  ±1.17 ab | 177.17  ±9.85 ab | **Group III: Met pre- treated NASH diabetic group** |
| 9.0  ±2.37 abc | 13.83  ±0.75 abc | 155.17  ±8.38 abc | **Group IV: Emp pre-treated NASH diabetic group** |
| 3.88  ±0.23 abcd | 10.33  ±0.52 abcd | 113.17  ±2.48abcd | **Group V: Met +Emp pre-treated NASH diabetic group** |

**Table ( 1 ):** Effect of pre- treatment with metformin (100mg/kg/day orally) and /orempaglifozine (10 mg/kg/day orally) for 12 weeks in experimental induction NASH on top of NA-STZ DM type II in adult male albino rats on FBG ,fasting plasma insulin level and HOMA IR.

Data are presented as Mean ± SD

a: Significant difference versus control group<0.05.

b: Significant difference versus NASH diabetic non treated group at p<0.05.

c: Significant difference versus Met. treated NASH diabetic group at p<0.05.

d: Significant difference versus Empa .treated NASH diabetic group at p<0.05.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Parameters mean ± SD** | | | |
| **VLDL(mg/dl)** | **LDL(mg/dl)** | **HDL(mg/dl)** | **CH(mg/dl)** | **Groups** |
| 26.00  ±4.47 | 74.67  ±3.67 | 95.83  ±4.96 | 33.67  ±1.03 | **Group I: Normal control**  **Group** |
| 90.00  ±4.47 a | 112.33  ±3.88 a | 76.50  ±3.45 a | 76.17  ±2.14 a | **Group II: NASH diabetic non treated group group** |
| 64.17  ±2.64 ab | 95.83  ±1.84 ab | 95.33  ±4.18 ab | 59.50  ±1.22 ab | **Group III: Met pre- treated NASH diabetic group** |
| 52.83  ±3.87 abc | 87.67  ±1.51 abc | 107.17  ±3.66 abc | 49.83  ±1.17 abc | **Group IV: Emp pre-treated NASH diabetic group** |
| 43.33  ±1.03 abcd | 80.67  ±0.82 abcd | 130.17  ±3.76 abcd | 41.83  ±1.60abcd | **Group V: Met +Emp pre-treated NASH diabetic group** |

**Table (2):** Effect of pre -treatment with metformin (100mg/kg/day orally)and /or empaglifozine (10 mg/kg/day orally) for 12 weeks in experimental induction NASH on top of NA-STZ DM type II in adult male albino rats on lipid profile

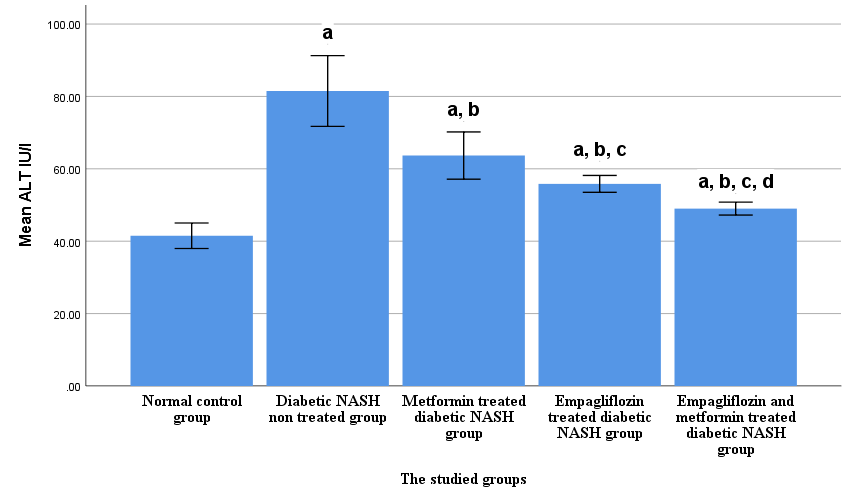
Data are presented as Mean ± SD

a: Significant difference versus control group<0.05.

b: Significant difference versus NASH diabetic non treated group at p<0.05.

c: Significant difference versus Met. treated NASH diabetic group at p<0.05.

d: Significant difference versus Empa .treated NASH diabetic group at p<0.05.

**Figure ( 1 ):** Effect of pre- treatment with metformin (100mg/kg/day orally)and /or empaglifozine (10 mg/kg/day orally) for 12 weeks in experimental induction NASH on top of NA-STZ DM type II in adult male albino rats on ALT:

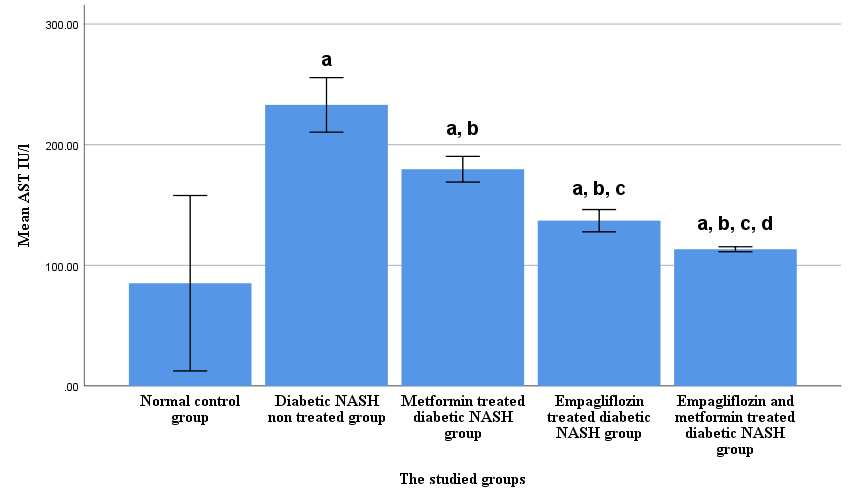
**N.B**

a: Significant difference versus control group at p<0.05.

b: Significant difference versus NASH diabetic non treated group at p<0.05.

c: Significant difference versus Met. treated NASH diabetic group at p<0.05.

d: Significant difference versus Empa .treated NASH diabetic group at p<0.05.

**Figure ( 2 ):** Effect of pre- treatment with metformin (100mg/kg/day orally)and /or empaglifozine (10 mg/kg/day orally) for 12 weeks in experimental induction NASH on top of NA-STZ DM type II in adult male albino rats on AST.

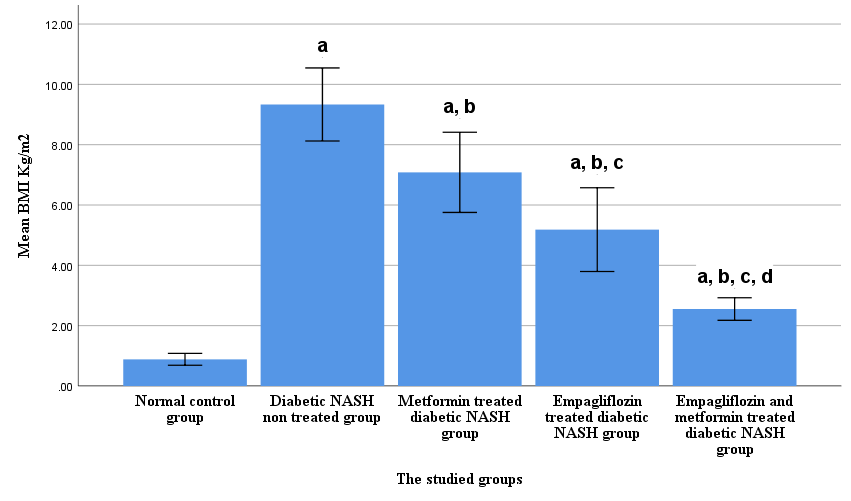
**N.B**

a: Significant difference versus control group at p<0.05.

b: Significant difference versus NASH diabetic non treated group at p<0.05.

c: Significant difference versus Met. treated NASH diabetic group at p<0.05.

d: Significant difference versus Empa .treated NASH diabetic group at p<0.05.



Mean liver weight index index

**Figure (3):** Effect of pre -treatment with metformin (100mg/kg/day orally)and /or empaglifozine (10 mg/kg/day orally) for 12 weeks in experimental induction NASH on top of NA-STZ DM type II in adult male albino rats on Liver weight index.

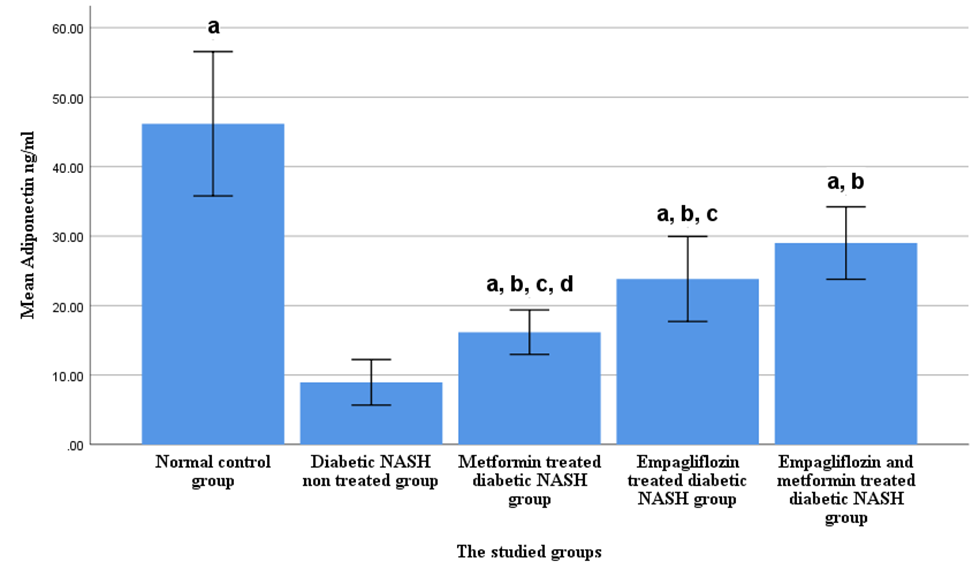
**NB**

a: Significant difference versus control group at p<0.05.

b: Significant difference versus NASH diabetic non treated group at p<0.05.

c: Significant difference versus Met. treated NASH diabetic group at p<0.05.

d: Significant difference versus Empa .treated NASH diabetic group at p<0.05.



**Figure (4):** Effect of pre- treatment with metformin (100mg/kg/day orally)and /or empaglifozine (10 mg/kg/day orally) for 12 weeks in experimental induction NASH on top of NA-STZ DM type II in adult male albino rats on adiponectin.

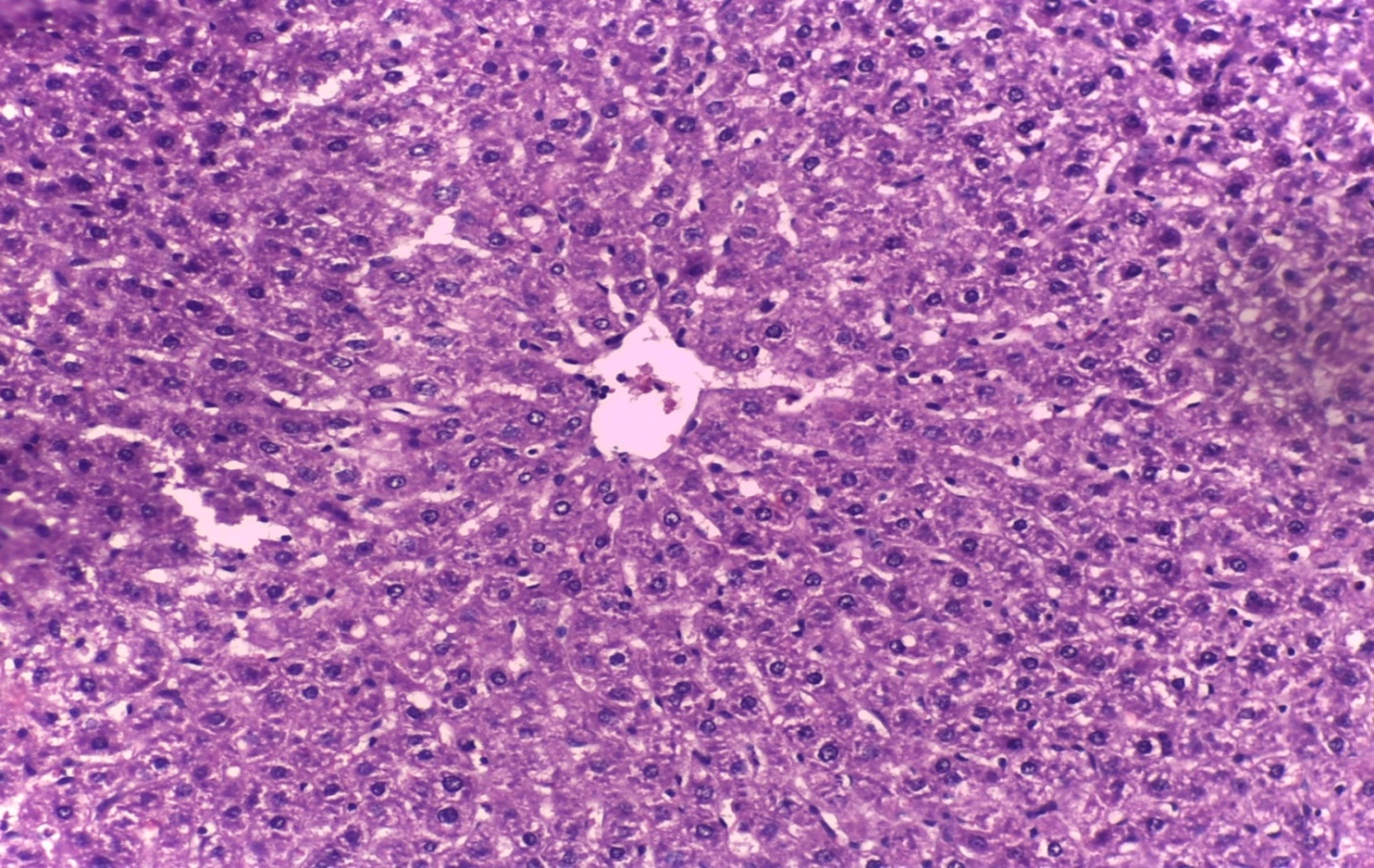
**N.B**

a: Significant difference versus control group at p<0.05.

b: Significant difference versus NASH diabetic non treated group at p<0.05.

c: Significant difference versus Met. treated NASH diabetic group at p<0.05.

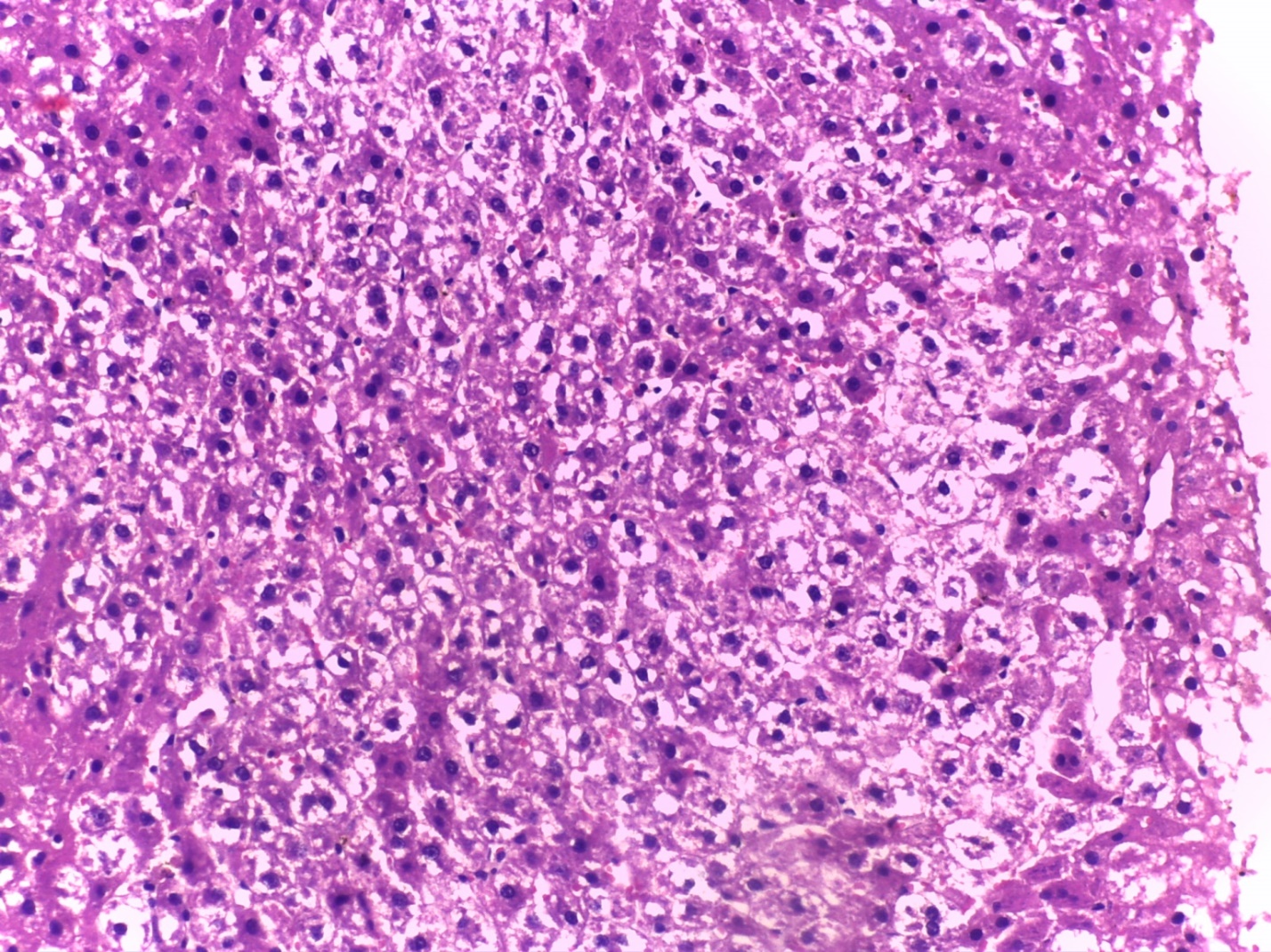
d: Significant difference versus Empa .treated NASH diabetic group at p<0.05.



B

A

**Figure(5):** A photomicrograph of a cut section in the liver of normal control rat showing normal liver architecture composed of hexagonadal or pentagonadal lobules with (a)central veins and peripheral hepatic triads or tetrads embedded in connective tissue.(B) Hepatocytes are arranged in trabecules running radiantly from the central vein and are separated by sinusoids (H x & E x 20).



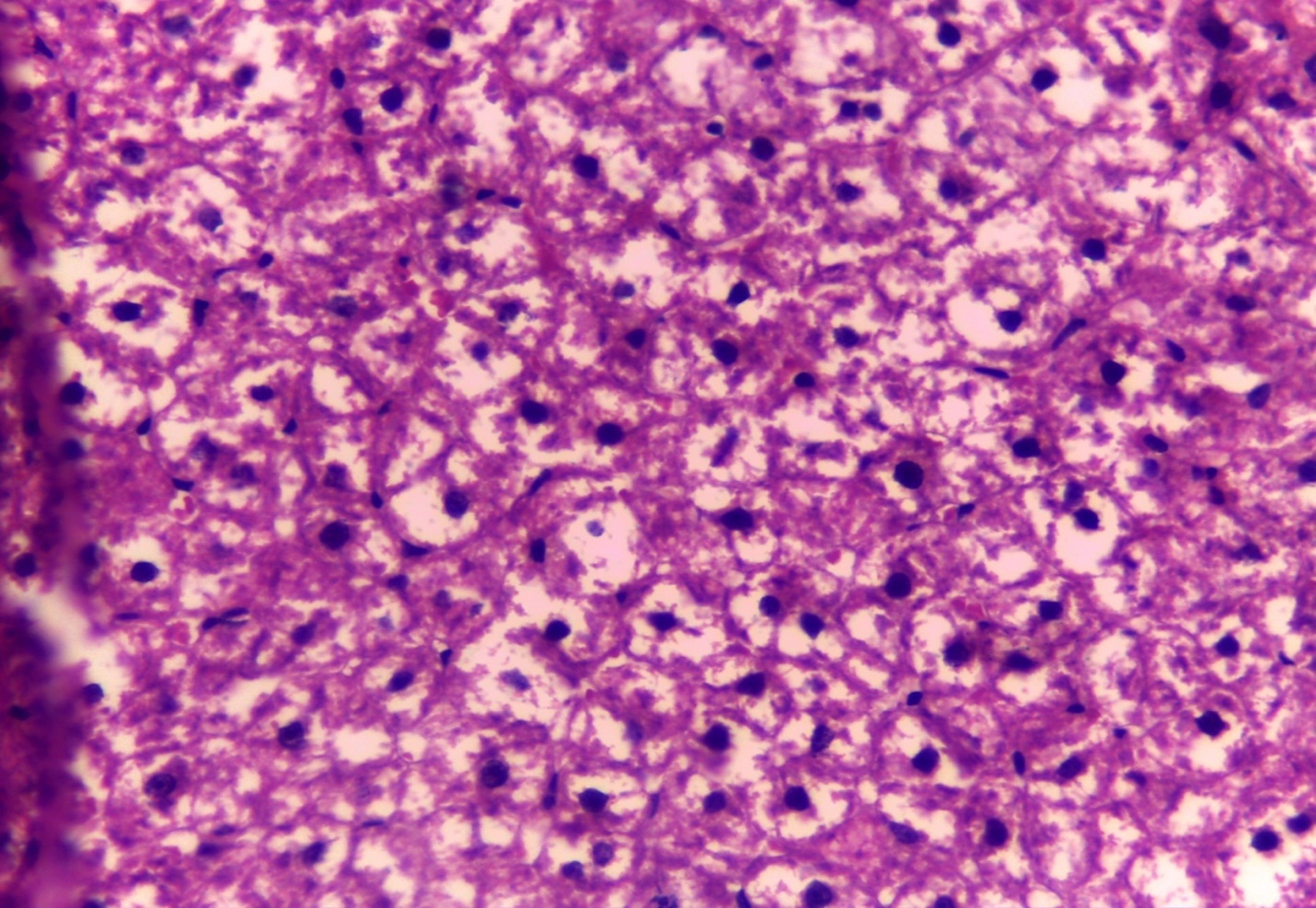
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A

Figure (6): A photomicrograph of a longitudinal section in the liver of NASHdiabetic non treated rats showing preserved liver architecture, (A)hepatocytes show marked steatosis (B) hepatocytes show marked hydropic changes, (C) some necro- inflammmatory foci in hepatic nodule & (D) portal tract inflammation(H x & E x 20).



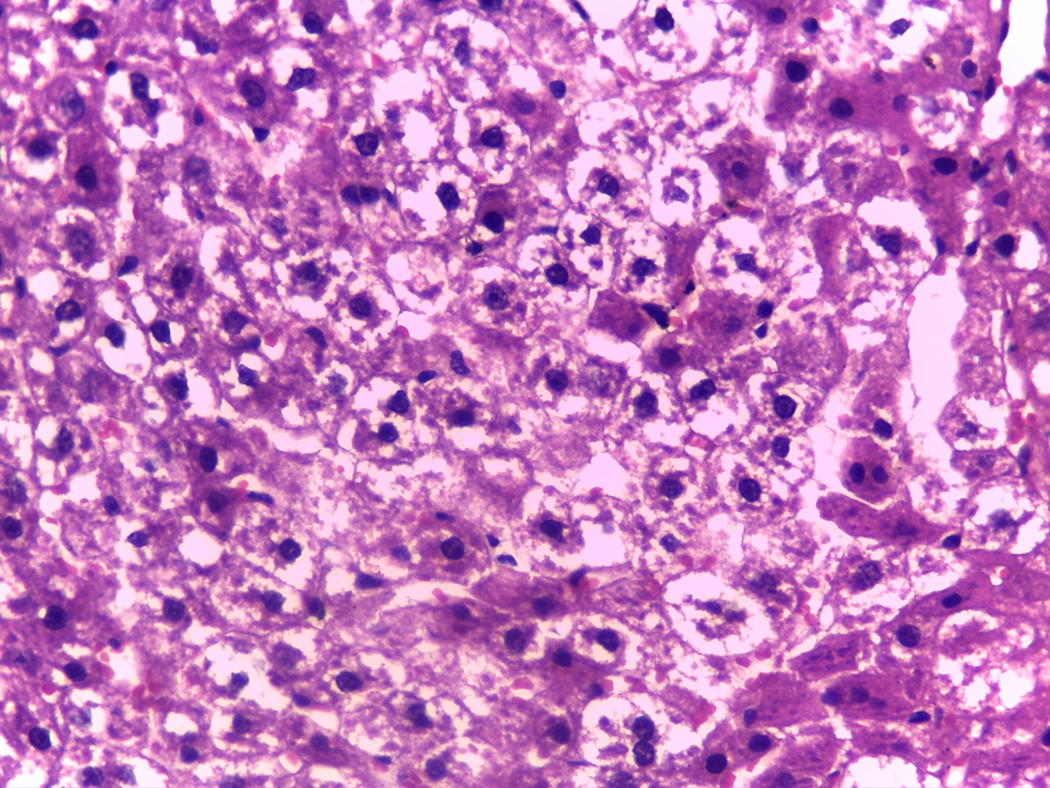
C

B

A

Figure (7): A photomicrograph of a longitudinal section in the liver of metformin treated NASHdiabetic rats showing preserved liver architecture, (A)hepatocytes show steatosis

(B) hepatocytes show hydropic changes, (C) some inflammmatory foci in hepatic nodule of lesser degree than diabetic NASH non treated rats (H x & E x 20).

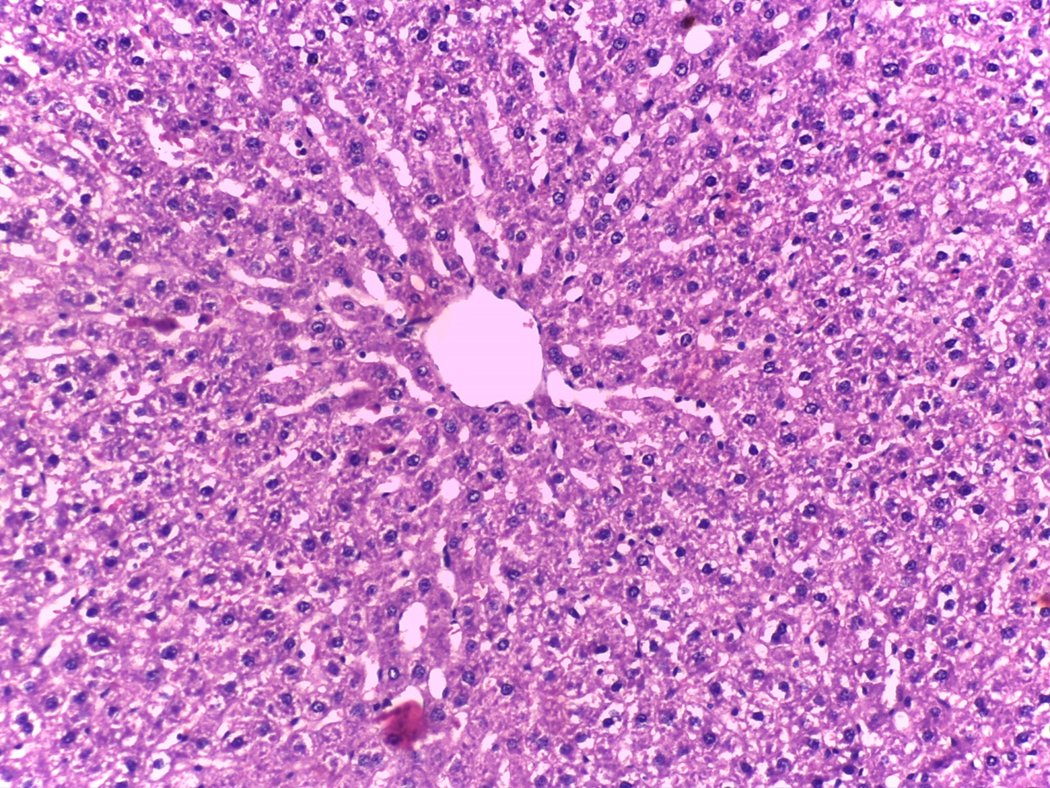


C

B

A

Figure (8): A photomicrograph of a longitudinal section in the liver of empagliflozin treated NASHdiabetic rats showing preserved liver architecture, (A) hepatocytes show steatosis (B) hepatocytes show hydropic changes, (C) some inflammmatory foci in hepatic nodule of lesser degree than diabetic NASH non treated rats (H x & E x 20).



B

A

Figure (9): A photomicrograph of a longitudinal section in the liver of combination( empagliflozin+ metformin)treated NASHdiabetic rats showing preserved liver architecture, (A)hepatocytes show steatosis and (B) hepatocytes show hydropic changes of lesser degree than monotherapy with vildagliptin and with metformin and in diabetic non treated rats (H x & E x 40).

**Discussion**

Diabetes mellitus (DM) is a severe global health problem and contributes to increased health care costs. It is estimated that more than 450 million people are affected by this disease, and this number will reach 700 million people by 2045.23

Nonalcoholic fatty liver disease (NAFLD) is associated with liver-related morbidity, including progression to nonalcoholic steato-hepatitis (NASH), advanced fibrosis, and hepatocellular carcinoma (HCC). NAFLD is the fastest growing cause of HCC in liver transplant candidates, 24 and it is projected that NAFLD is becoming the leading cause of liver transplantation***.*** 25

Metformin, a derivative of biguanide, is one of the most commonly used drugs to treat type 2 diabetes (T2D), Metformin inhibits mitochondrial complex I , which leads to AMPK activation ***.***26

The fundamental activity of SGLT2 is to inhibit the active reverse transport of glucose by SGLT2 in the luminal surface of the S1 segment of the proximal renal tubule, which is linked to Na+ transport maintained by Na+ active extrusion. 27

In this study diabetic nonalcoholic fatty liver disease was induced by by a single IP injection of STZ 65mg/ kg BW, 15 minutes after IP administration of NA110 mg/kg BW. STZ was dissolved in citrate buffer (0.1 M, pH 4.5) and NA was dissolved in normal saline. Diabetic rats were developed nonalcoholic fatty liver disease after 12 month of the study by high fat diet. 28

The data of this work revealed that induction of NASH on top of NA-STZ DM type II resulted in a significant elevation in FBG, fasting plasma insulin, HOMA IR, serum level of CH, TG, LDL-c, VLDL ,liver enzymes, liver mass index , adiponectin and significant decrease in HDL-c level.

Histopathological examination revealed hepatic alteration in the form of marked steatosis and marked hydropic changes of hepatocytes, some necro- inflammmatory foci in hepatic nodule and portal tract inflammation.

The feeding of high fat diet resulted in excess hepatic triglycerides accumulation due to increased synthesis and decreased secretion of triglycerides and increased de novo lipogenesis. 29

High cholesterol diet causes lipid peroxidation resulting in production of ROS, ROS react with protein to produce a variety of sulphur oxidation states, thus diminishing the cellular uptake of lipids from the blood and changing lipid constituents of LDL, inducing LDL-oxidation leading to oxidative stress in various organs such as the liver, heart, and aorta ***.***30,31

Results of this work showed that level of fasting blood glucose was significantly elevated in rats fed on HFD; this is consistent with(32) and (33) who reported that NAFLD is associated with hyperglycemia.

Results obtained showed that, liver weight index was significantly elevated in rats fed on HFD, this is consistent with( 34 )who reported that liver weight index is one of the anthropometric indexes that affected in NASH.

Higher activities of these enzymes in serum have been found in response to oxidative stress induced by high fat diets.  35,36

In present study these parameters were significantly enhanced by the high fat diet, suggesting that excessive fat intake might cause critical injury to the organ due to the over-production of free radicals and ROS, which exert harmful effects on liver, this is in line with(37)

Results obtained showed that, serum levels of triglycerides, total cholesterol, and LDL-c were significantly elevated while HDL-c was significantly reduced in rats fed on HFD, this is consistent with (38)

Adipose tissue dysfunction as a result of DM type II can results in an imbalance between pro-inflammatory and anti-inflammatory adipokines, and is one of the mechanisms of DM type II complications***.***39

Several studies indicate that adipokines are related to insulin resistance (IR), and can result in endothelial dysfunction, and pro-inflammatory and pro-atherogenic states.40

Adiponectin acts through ADIPOR1 (Adiponectin Receptor 1) and ADIPOR2 (Adiponectin Receptor 2) receptors and the peroxisome proliferator-activated receptor α (PPARα) pathway, leading to decreased hepatic gluconeogenesis, increased liver and skeletal muscle fatty acid oxidation, increased glucose uptake in skeletal muscle and white adipose tissue ***.***41

This is in agreement with(42 )who reported that nonalcoholic fatty liver diseases were associated significant increase in levels of serum total cholesterol, TG, LDL-c and VLDL and associated significant decrease in HDL-c.

Also, these results are in agreement with (43)who proved that NAFLD is associated with significant increase in liver enzymes and lipid profile.

In the present work, the obtained data revealed that daily oral administration of metformin to NASH diabetic rats just after inducion of NA-STZ type 2 DM for 12 weeks study in experimental induction of NASH resulted in significant decrease in FBG level, fasting plasma insulin,HOMA IR,liver enzymes,CH,TG,LDL-c and VLDL with significant increase in HDL-c level and adiponectin level compared with NASH diabetic non treated rats.

This is in agreement with(44) who proved that metformin improved fatty liver disease and reversed the hepatomegaly, steatosis, and alanine aminotransferase (ALT) level by decreasing the expression of tumor necrosis factor-alpha (TNF-α) that promote hepatic lipid accumulation and adenosine triphosphate depletion or autophagy activation.

Also, this is in agreement with ( 45) who reported that metformin can improve liver function, insulin resistance, and body mass index (BMI).

In the present work, the obtained data revealed that daily oral administration of empagliflozin to diabetic rats just after induction of of NA-STZ DM type II for 12 weeks study in experimental induction of NASH resulted in significant decrease in FBG level, insulin,HOMA IR,liver enzymes,CH,TG,LDL-c and VLDL with significant increase in HDL-c level and adiponectin level compared with diabetic non treated rats.

This is in agreement with(46 )who reported that empagliflozin improved liver steatosis and fibrosis.

This is disagree with (47)who proved that empagliflozin increases the plasma LDL-C level concomitantly with higher free fatty acids (FFAs) and total ketone body levels, suggesting that SGLT2 inhibition induces ketogenesis and increased lipid oxidation to compensate for the carbohydrate shortage.

This is in agreement with(48 ) and (49)who reported that empagliflozin improves pancreatic β-cell dysfunction in obese mice, resulting in the amelioration of glucose tolerance and insulin sensitivity.

SGLT2 inhibitor reverses High Glucose Induced Toll-like receptor-4(TLR4 ) Expression ,TLR4 is a ligand activated membrane bound receptor and is involved in nuclear factor kappa B (NF-κB) mediated inflammation. 50

GLT2 inhibitor reduces high Glucose Induced IL-6 Secretion, IL-6 is a secreted proinflammatory cytokine, SGLT2 inhibitor Reverses high Glucose induced activator protein 1AP-1 binding ,both NF-κB and AP-1 are key transcription factors mediating the fibrotic and inflammatory pathways in human kidney PTC line (HK2 cells) exposed to high glucose. 51

We found that daily oral administration of empagliflozin to diabetic rats just after induction of NA-STZ DM type II for 12 weeks study in experimental induction of NASH in diabetic rats resulted in significant increase in adiponectin, this result is in consistent with(52) who proved that there is a decreased expression of inﬂammatory markers, such as TNF-αand IL-6 with empagliﬂozin treatment.,The decrease in the expression of the inﬂammatory indices has been shown to reduce macrophage inﬁltration and lobar inﬂammation in liver leading to alleviation of hepatic steatosis and inﬂammation .

And, this is line with( 53) who reported that the reduction of lipogenesis enzymes following empagliﬂozin administration could explain the decreased steatosis and fatty droplets area observed with in hepatocytes.

Also, these results are in agreement with(54 ) and (55)who reported that inhibition of SGLT2 promoted catabolic pathways such as fatty acid oxidation by phosphorylation of AMP-activated protein kinase α and acetyl-CoA carboxylase in skeletal muscle, thus alleviating energy homeostasis.

Moreover, this is in agreement with(56) and (57) who reported that empagliflozin promoted alternative macrophage activation and increased plasma adiponectin levels, which may contribute to adipose tissue browning.

Also,Our results are in consistent with(58)who proved that the empagliflozin reduced hepatic steatosis and decreased collagen deposition and the down regulation of inflammatory cytokines in the liver.

In the present work ,the obtained data revealed that daily oral administration of metformin combined with empagliflozin to diabetic rats just after induction of NA-STZ DM type II for 12 weeks study in experimental induction of NASH resulted in a significant decrease in FBG level,fasting plasma insulin,HOMA IR,liver enzymes,CH,TG,LDL and VLDL more than metformin or empagliflozin alone with significant increase in HDL level and adiponectin level compared with NASH diabetic non treated rats and more than metformin or empagliflozin alone.

These results are in agreement with (57)who reported that combined treatment with empagliflozin and metformin in NASHcaused significant improvement of liver ezymes, lipid profile and ultrasound findings more than use of metformin alone.

Also, this is in line with(59)that reported that combined treatment with empagliflozin and metformin in NASH caused significant improvement of liver ezymes, lipid profile and ultrasound findings more than use of metformin alone.

Moreover, these results are in consistent with (60)who proved that addition of SGLT2 inhibitos to metformin monotherapy is associated with a significant body weight reduction.

**Conclusion:**

In our study , it was found that metformin and /or empagliflozin affords hepatoprotective effects as regard to liver enzymes, total cholesterol, triglycerides, LDL-C, HDL-C, fasting insulin and blood glucose, insulin resistance index (HOMA-IR), and serum adiponectin levels with improvement of histopathological changes of the liver.

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