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Evaluation of Ameliorative Effect of Taurine and Metformin in Streptozotocin-Induced Diabetes

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KEYWORDS:
Liver, Diabetes, Oxidative stress, Metformin, Taurine.

ABSTRACT
The aim of the study was to evaluate the anti-diabetic effect of Taurine (TAU), singly and in combination on streptozotocin (STZ) diabetic rats. For this purpose, male Albino wistar male, 200–250 g in weight, assigned to groups of five, were intraperitoneally (i.p.) treated with the diabetogen streptozotocin (STZ, 60 mg/kg, in citrate buffer pH 4.5) on day four, orally (p.o.) with either MET, TAU or MET-TAU (each at 2.4 mM/kg, in water). Control rats received only citrate buffer pH 4.5 (2 mL). After the study duration, the blood samples were withdrawn through retro-orbital plexus and blood glucose level (GLC) and insulin (INS) were measured. Further, various lipid parameters were also observed from blood. Immediately thereafter, the liver were surgically removed and a portion was used to prepare a homogenate in 0.1 M PBS pH 7.4, which was use for the determination of various parameters from liver. Diabetes group raised the plasma GLC level (497.1±9.3 mg/dL) but lowered that of the blood INS (27.7±3.5 µU/mL) compared to corresponding values from nondiabetic rats (GLC =88.5±3.2 mg/dL and INS=43.7±4.8 µU/mL) after STZ administration. In addition, it raised the liver malondialdehyde (MDA) level but lowered the reduced glutathione (GSH), and glutathione disulfide (GSSG) significantly (all at $p < 0.01$) was also observed in rats after administration STZ. After the treatment, with TAU and MET alone or in combination, the significant reduction of GLC and enhancement of INS was observed. Moreover, liver MDA level reduced but enhanced the GSH, and GSSG was also observed significantly (all at $p < 0.01$) in rats after administration of TAU alone or in combination with MET. The histopathological, significant alterations in the diabetic control rats livers were disorderly hepatocyte, cytoplasm dissolution, monocellular leukocytic infiltration, karyomegaly, hyperchromatic nuclei, nucleus karyolysis, and dilated congested portal vein were also observed. Finally, it was concluded that the effect of TAU alone on liver parameters is comparable to MET on rats but their combination exhibited a synergistic effect on, liver parameters, and on GLC as well as on INS.

Introduction

Diabetes is a disease that occurs when blood glucose (sugar) extended than the normal range, also called blood sugar, and is too high. Insulin is a hormone made by the pancreas that helps glucose get into your cells to be used for energy. In case of diabetes, body doesn't make enough or doesn't use insulin properly. [1]. Diabetes raises the risk for damage to the eyes, kidneys, nerves, and heart. [1]. Taking steps to prevent or manage diabetes may lower your risk of developing diabetes health problems.

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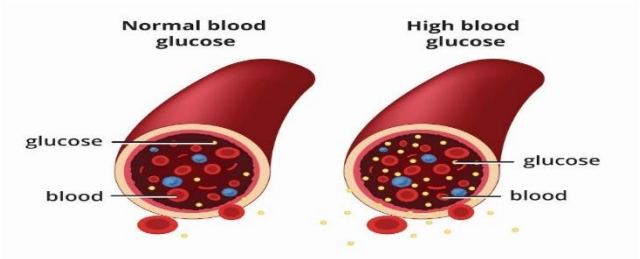

Fig. 1. Exploring the normal and high glucose level [1]

The most common types of diabetes are type 1, type 2, and gestational diabetes.

If a person has type 1 diabetes, body makes little or no insulin. Type 1 diabetes is usually diagnosed in children and young adults, although it can appear at age. People with type 1 diabetes need to take insulin every day to stay alive.

In case of type 2 diabetes, the cells in body don't use insulin properly. The pancreas may be making insulin but body cells became insensitive to utilize insulin to keep blood glucose level in the normal range. [3].

Gestational diabetes is a type of diabetes that develops during pregnancy [3].

Many synthetic drug have been reported and used to reduce the hyperglycaemic condition in the body. Synthetic drug metformin and naturally occurring amino acid taurine also used for this purpose. Taurine is a sulfonate-containing beta-amino acid isolated from bovine bile [8]. Taurine is widely distributed in various tissues and organs, especially in excitable tissues, where the content is more abundant, such as the liver, brain, heart and skeletal muscle [9]. As a naturally occurring amino acid, taurine has few side effects, and current studies have not found any genotoxic, carcinogenic, or teratogenic effects ([10]

Due to its good safety, taurine is widely used in functional drinks [11], infant formula [12] and other products. Meat, especially seafood products, are rich in taurine [13]. Taurine plays beneficial roles in a variety of metabolic and physiological processes, such as glucose and lipid regulation, energy metabolism, anti-inflammatory regulation and antioxidation [14]. Taurine has certain functions in cell development, nutrition and survival [15], the depletion of taurine leads to a wide range of pathological conditions, including severe cardiomyopathy [16], renal dysfunction [17], pancreatic β cell malfunction [18] and loss of retinal photoreceptors [19]. Taurine has been used as a potential energy enhancer to improve exercise performance. It is worth noticing that several factors such as taurine intake time, delivery mode and exercise program will affect the effect of taurine on exercise performance [20].Taurine has a wide range of anti-inflammatory effect [21].Taurine supplements are beneficial to epilepsy [22], heart disease [23], cystic fibrosis [24] and diabetes [25]. Taurine is a major antioxidant that scavenges reactive oxygen species and protects organs, including the brain [26], from oxidative stress. It has neuroprotective effects and has been shown in animal studies to prevent neurotoxic damage caused by alcohol, ammonia, lead and other substances. Taurine is considered to be a modulator of neuronal activity. It is structurally similar to the main inhibitory neurotransmitters in the brain γ-Aminobutyric acid [27].

Metformin (a biguanide derivative), by controlling blood glucose level decreases these complications. Metformin works by helping to restore the body's response to insulin. It decreases the amount of blood sugar that the liver produces and that the intestines or stomach absorb [31]. Metformin, other than hypoglycaemic activity, has been taken with diet and exercise changes to prevent diabetes in people who are at high risk for becoming diabetic. It is also used in women with polycystic ovarian syndrome. Metformin may make menstrual cycles more regular and increase fertility [32]. Theoretically, its use has been prohibited in a large group of patients with type 2 diabetes mellitus due to the risk of lactic acidosis. However, it has been shown that several diabetic patients who are considered to be at risk have received metformin with no increased risk of lactic acidosis [31-34]. Furthermore, recently some papers have been published indicating renoprotective properties for metformin [35-38].

In the facts of above said facts, out aimed here to evaluate the ameliorative effect of taurine and metformin in streptozotocin induced diabetes in experimental animals (rats). Several groups were developed and respective treatments were given to observe the anti-diabetic effect of taurine and metformin either alone or in combination to evaluate the synergistic effect against various aspects involved in diabetes management.

Materials

The Streptozotocin (STZ), Taurine, 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB, Ellman's reagent), 1-chloro-2, 4-dinitrobenzene (CDNB), reduced glutathione (GSH), superoxide dismutase (SOD) were purchased from Sigma, St. Louis, MO. Metformin was a gift sample from Amoli Organics, Mumbai. All solvents and chemicals used were of analytical grade, solvents for HPLC were of HPLC grade procured from Qualigens

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Fine Chemicals, India. Nanopure water from a Millipore Milli-Q system (Bangalore, India) was used for preparing the solutions and all the solutions were prepared fresh.

Methods

Animal grouping (IAEC ETHICS APPROVAL)

Thirty Albino Wistar male rats 7-8 weeks old and weighing 200-250g were used. The animals were obtained from the animal house, Subharti University, Meerut, UP, India. The animals were maintained under controlled conditions of temperature (23° ± 2°C), humidity (50% ± 5%) and 12h light-dark cycles. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water ad libitum. All the studies conducted were approved by the Institutional Ethical Committee, Subharti Medical College, Meerut, UP, India (vide letter 1204/PO/RE/S/CPCEA/22/02), according to the prescribed guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. We selected male animals for all our studies since females are shown to be protected from changes in lipid-induced insulin action [39].

EXPERIMENTAL DESIGN Animals Grouping

Total 5 groups were developed with 30 total animals as follows:

Group 1(G1): Control Group (citrate buffer pH 4.5 treated group).

Group 2 (G2): STZ induced diabetes group.

Group 3 (G3). STZ induced diabetes group treated with Taurine.

Group 4 (G4). STZ induced diabetes group treated with Metformin.

Group 5 (G5). STZ induced diabetes group treated with Taurine and Metformin combination.

Standardization of Streptozotocin Dose for Induction of Diabetes Mellitus. Doses of STZ (60 mg/kg) to select the appropriate dose of STZ for induction of diabetes. STZ injection (i.p., dissolved in 0.01 M citrate buffer, pH 4.5) was given to induce diabetes mellitus. After four days, blood glucose (BGL) levels were estimated by the enzymatic glucose oxidase method using a commercial glucometer (Accu-chek® Active, Roche Diagnostic, and Mannheim, Germany). Based on study results, it was found that 60 mg/kg STZ produced diabetes in experimental rats. Therefore, a single STZ injection (60 mg/kg body wt., i.p.) was standardized to induce diabetes mellitus.

Treatments and Samples

Diabetes was induced with a single 60 mg/kg intraperitoneal (i.p.) dose of STZ in citrate buffer pH 4.5. Starting on day 15 and continuing for the next 41 days, separate groups of diabetic rats received a 2.4 mM/kg, daily dose of either MET, TAU or MET plus TAU in distilled water by oral gavage. Nondiabetic (control) rats received only citrate buffer pH 4.5 (2 mL, i.p.) on day 1, and physiological saline (2 mL) by oral gavage from day 15 onwards. A diabetic group received no other treatment than one with STZ. All treatments were conducted for a total of 41 days.

Evaluation Parameters

Plasma Glucose (GLC)

BGL was estimated by the enzymatic glucose oxidase method using a commercial glucometer. The results were expressed in mg/dL.

Oral glucose tolerance test (OGTT)

At the end of dietary manipulation (i.e., two weeks of respective drug treatment), glucose (2g/kg) was administered to 12h fasted rats and blood samples were collected at 0 (immediately after glucose load), 30, 60 and 120min after glucose administration. BGL was estimated by the enzymatic glucose oxidase method using a commercial glucometer.

Anthropometric parameter: like Body weight (gm) were measured.

Estimation of Biochemical Parameters

After completion of OGTT, blood samples were collected from retro-orbital plexus. Serum was separated and analysed spectrophotometrically for triglyceride (STG), total cholesterol (STC), HDL-cholesterol (HDL-c), using diagnostic reagent kit (Nicholas Piramal, Mumbai). Serum insulin (SI) was estimated by radioimmunoassay method using a kit of Crytal Chem, Mumbai from Fedlife Lab. HOMA, TC/HDL-c, LDL-c/HDL-c, VLDL, cholesterol (VLDL-c) and LDL-cholesterol (LDL-c) in serum were calculated [41].

Endogenous antioxidant status

Animals were sacrificed by cervical dislocation; the liver was perfused with saline; the whole liver was dissected out. Ten percent homogenates of the livers of each rats of different group were prepared with ice cold saline-EDTA and protein content was determined. The homogenates were further subjected to the estimation of non-enzymatic (reduced glutathione and total thiols) and enzymatic antioxidants (catalase, GSH, and SOD) using standardized protocols of our laboratory quoted in our previous publication [42]. Lipid peroxidation which was determined by estimating the level of thiobarbituric acid reactive substances (TBARS) in the liver homogenates [43].

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Histological Investigation

After dissection, livers were rapidly removed and fixed in 10% neutral phosphate-buffered formalin for 24 hours. Following a thorough rinse in tap water, the samples were dehydrated using a series of ethyl alcohol dilutions (50%, 70%, 90%, 95%, and 100%) in a furnace set at 56°C for 24 hours and then the samples were cleaned with xylene before being submerged into paraffin wax. Sections of 5-μm thickness were made from paraffin wax tissue blocks with a sliding microtome. For a standard examination, the tissue sections were mounted on glass slides, dewaxed, and stained with haematoxylin and eosin (H&E) [42]. The examination was carried out using a light electric microscope.

Statistical evaluation

The data were expressed as mean ± SEM. Statistical comparisons were performed by one-way ANOVA followed by Tukey's post-test using GraphPad Prism version 4.0.

Result and Discussion

In STZ induced group significantly increased ($p < 0.001$) body weight of rats compared to control group. Further, STZ group also significantly elevated basal BGL, and SI at the end of the five weak study. At the end of three weeks, STZ (60 mg/kg, i.p.) significantly ($p < 0.001$) increased levels of BGL, and SI, indicating a higher level of insulin resistance in this group compared to animal control groups. (Table 2). Also, STZ diabetic rats developed symptoms of polyphagia, polydipsia and polyuria when compared to control group. However, STZ (60 mg/kg, i.p.) produced significantly higher ($p < 0.001$) BGL and drastic reduction ($p < 0.05$; $p < 0.01$) in body weights of rats. Further, STZ (60 mg/kg) diabetic rats showed significantly reduced ($p < 0.001$) SI levels and HOMA values (Table 2).

Therefore, a dose of 60 mg/kg of STZ was chosen as optimum for inducing type 2 like diabetic condition in rats. This dose was selected to screen the extracts/fractions.

Table 2. Effect of different doses of STZ on body weight and biochemical parameters in rats

Parameters	NPD	STZ (60mg/kg)	STZ (65mg/kg)	STZ (70mg/kg)
Body weight (g)	230.4±5.5	280.2±10.9[a]	250.2±8.3[d]	237.1±11.4[e]
GLC (mg/dL)	88.5±3.2	378.1±9.8 [c,f]	497.1±9.3 [c,f,i]	509.2±8.3 [c,f,i]
INS(µU/mL)	43.7±4.8	67.9±11.8[c]	27.7±3.5 [f,h]	ND

Each value represents mean ± SEM (n= 4, ND, not determined; BGL, blood glucose; SI, serum insulin; [a]p <0.05, [c]p <0.001 compared to NPD; [d]p <0.05, [e]p <0.01, [f]p <0.001 compared to HFD; [g]p <0.05, [h]p <0.01 compared to STZ.

Oral glucose tolerance test (OGTT)

Administration of glucose (2 g/kg, p.o.) did not produce any significant change in the BGL levels of control group rats. The STZ diabetic rats exhibited significant elevation in fasting BGL (at time zero) and showed significant impairment in glucose tolerance to exogenously administered glucose compared to control group rats.

Effects on Circulating GLC and INS Level

Diabetic animals showed much higher levels of plasma GLC than nondiabetic ones by the end of study as shown in table 3. A daily treatment of the diabetic rats with MET reduced this increase markedly 196.4 ± 5.0), an effect that was ~1.34-fold greater than one with TAU 263.3 ± 7.3). Treating the diabetic rats with MET plus TAU led only to a small increase in potency relative to MET alone 145.5 ± 4.7). On the other hand, a treatment with MET-TAU further reduced the inhibitory action of diabetes on INS secretion although the effect was not significantly different from that attained with MET alone. Neither TAU or MET were found to significantly affect the basal circulating levels of both GLC and INS. At the end of five weeks of dietary manipulation (i.e., after three weeks of STZ (60 mg/kg,) injection), MET-TAU-STZ diabetic rats exhibited significant ($p < 0.001$) hyperglycemia (BGL levels rose to between 190.43 ± 2.22mg/dL) and hyperinsulinemia (39.02 µU/mL)

Table 3: The effects of MET-TAU on the plasma GLC and blood INS levels of rats made diabetic with STZ [a,b]

Group	Normal Group (NPD)	
	Plasma GLC, mg/dL	Blood INS, µIU/mL
Control	88.5±3.2+++	43.7±4.8+++
STZ	328.2 ± 21.77***	17.7±4.8± 1.33***
MET-STZ	196.4 ± 5.0***,+++	28.3 ± 1.5**,+++
TAU-STZ	263.3 ± 7.3***,++	21.3 ± 1.8***,+++

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MET-TAU-STZ	145.5 ± 4.7$^{***,+++}$	36.02 ± 1.87$^{*,+++}$

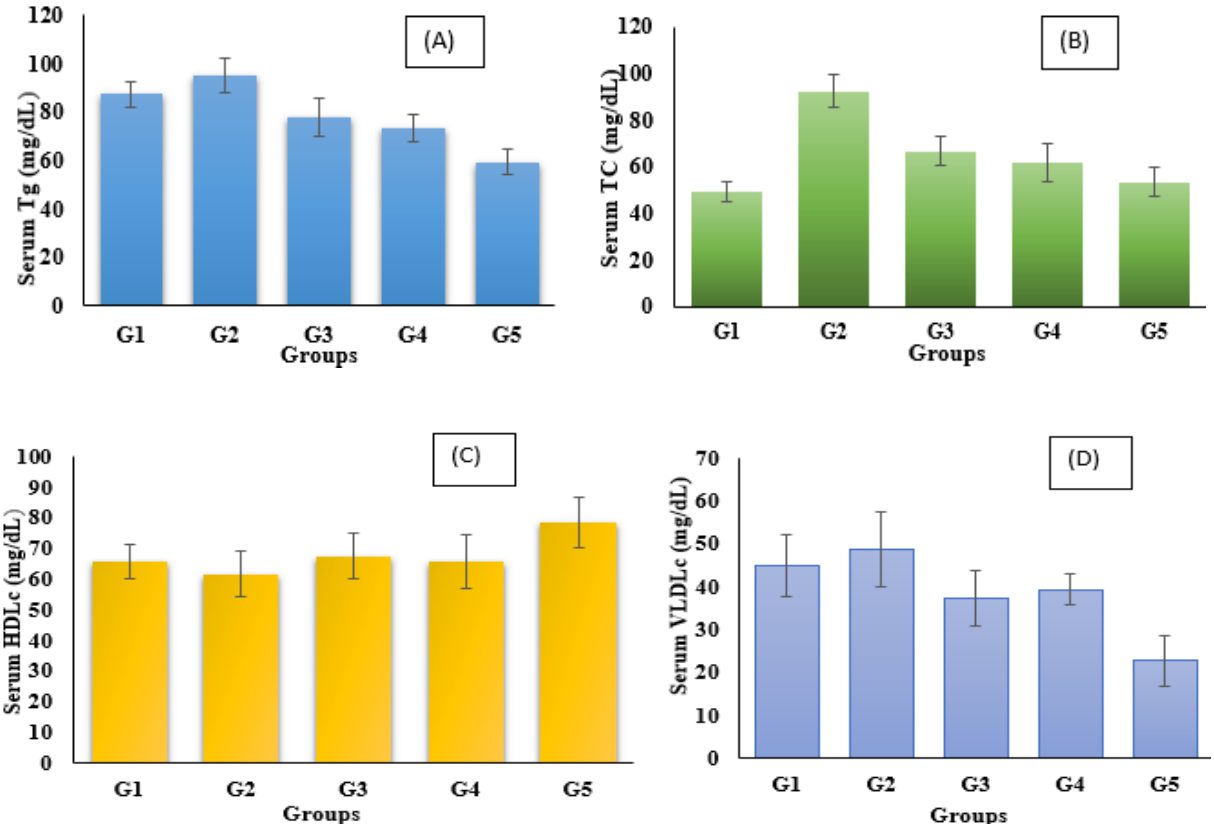
[a] Values are shown as the mean ± SEM for n = 4, [b]Statistical comparisons were significantly different from Control at *p < 0.05, **p < 0.01 and ***p < 0.001; and from STZ at ++p < 0.01 and +++p < 0.001

Effect on lipid parameters

All lipid parameters are depicted in Fig. 2 and in Table 4.STZ diabetic rats exhibited significantly (p <0.01) higher levels of STG, STC, VLDL-c and LDL-c, whereas lower levels of HDL-c compared to normal group rats. Treatment with TAU and MET showed significant (p <0.01) reduction in STG, STC, VLDL-c and LDL-c levels and increased HDL-c levels, as compared to STZ diabetic rats in both normal diet, respectively (Fig.2). Treatment also significantly reduced (p <0.01) markers of dyslipidemia (Fig. 2). Further, treatment with a combination of MET and TAU exhibited significantly diminished STG, STC, VLDL-c, and LDL-c levels in normal group (G1). Moreover, Metformin also significantly increased HDL-c levels and decreased levels of dyslipidemic markers (Fig.2).

Table 4: The effects of TAU, MET, and combination of MET-TAU on the lipid parameters

Groups	Serum TG (mg/dL)	Serum TC (mg/dL)	Serum HDLc (mg/dL)	Serum LDLc (mg/dL)	Serum VLDLc (mg/dL)
G1	87.56±5.28	49.47±4.26	65.83±5.52	52.53±6.35	44.83±7.32
G2	95.34±7.27	92.39±7.25	61.63±7.32	63.91±4.92	48.69±8.63
G3	77.91±8.18	66.62±6.42	67.52±7.53	57.62±3.63	37.43±3.62
G4	73.48±5.62	61.92±8.32	65.71±8.65	50.39±5.81	39.34±3.84
G5	59.35±5.21	53.32±6.34	78.32±8.29	38.13±4.72	22.73±6.32



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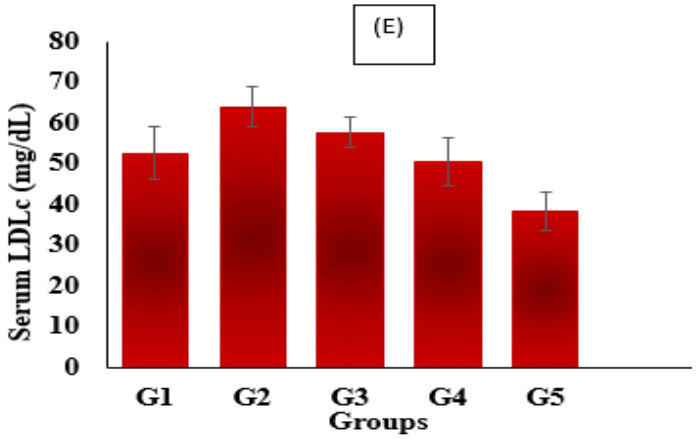

Fig. 2. Effect of TAU and MET alone and in combination on lipid parameters (A) serum TG, (B) serum TC, (C) serum HDLc levels (D) serum VLDLc and (E) serum LDLc in normal group with STZ, and a combination of TAU and MET in control group with STZ. Each bar represents the mean ± SD (n = 5). Comparisons were made at P<0.05 was considered significant and P<0.01 was considered highly significant.

Effect on endogenous antioxidant levels

The TBARS test, which uses thiobarbituric acid (TBA) as a reagent, detects TBARS as a consequence of lipid peroxidation (i.e. as fat degradation products). TBARS can be upregulated by a heart attack or a certain type of stroke, for example. The TBARS assay measures lipid peroxidation in cell and tissue extracts as well as biological fluids [47]. Normal diet with STZ rats showed basal TBARS levels of about 23.47 ±2.36 nmol/g in liver tissue. STZ diabetic rats showed significantly increased ($p < 0.01$) in TBARS levels (118.25±8.13 nmol/g of tissue). Treatment with TAU, MET, and a combination of MET-TAU significantly ($p < 0.05$; $p < 0.01$) stopped the increase in TBARS levels induced by STZ (Fig. 3A).

Effect on non-enzymatic antioxidants

The control group showed a GSH level of 15.36 ± 1.29 nmol/mg of protein. The normal diet with STZ rats displayed a GSH level of 5.38±1.35 nmol/mg and 3.47±0.56 nmol/mg, respectively which was significantly decreased ($p < 0.01$) as compared to the control group. Treatment with TAU, MET, and a combination of MET-TAU significantly ($p < 0.05$; $p < 0.01$) improved GSH levels in both normal diets with STZ rats. (Fig. 3B).The control group of rats showed a thiol level of 6.26± 0.98 µmol/mg. The normal diet with STZ rats displayed a thiol level of 1.98±0.67 µmol/mg and 1.65±0.42 µmol/mg, respectively which was significantly lowered ($p < 0.01$) as compared to the control group. Treatment with TAU, MET, and a combination of MET-TAU significantly ($p < 0.05$; $p < 0.01$) enhanced thiol levels in both normal diets with STZ rats. (Fig. 3C).

Effect on enzymatic antioxidants

The control group of rats showed a catalase level of 225.37 ± 7.26 U/mg. The level of catalase in normal diet with STZ rats was 75.28±11.26, respectively which was significantly ($p < 0.01$) less than that of the control group. After the treatment with TAU, MET, and a combination of MET-TAU in STZ induced rats group, the level of catalase was significantly ($p < 0.05$; $p < 0.01$) enhanced in STZ induced rats group (Fig. 3D). The action of MET-TAU is better than all the treatments. The control group of rats showed a GST level of 0.01± 0.0003 U/mg. The level of GST in normal diet with STZ rats was 0.002±0.0005 and, respectively which was significantly ($p < 0.01$) less than that of the control group. After the treatment with TAU, MET, and a combination of MET-TAU in STZ rats groups, the level of GST was significantly ($p < 0.05$; $p < 0.01$) enhanced in STZ rats group (Fig. 3E).

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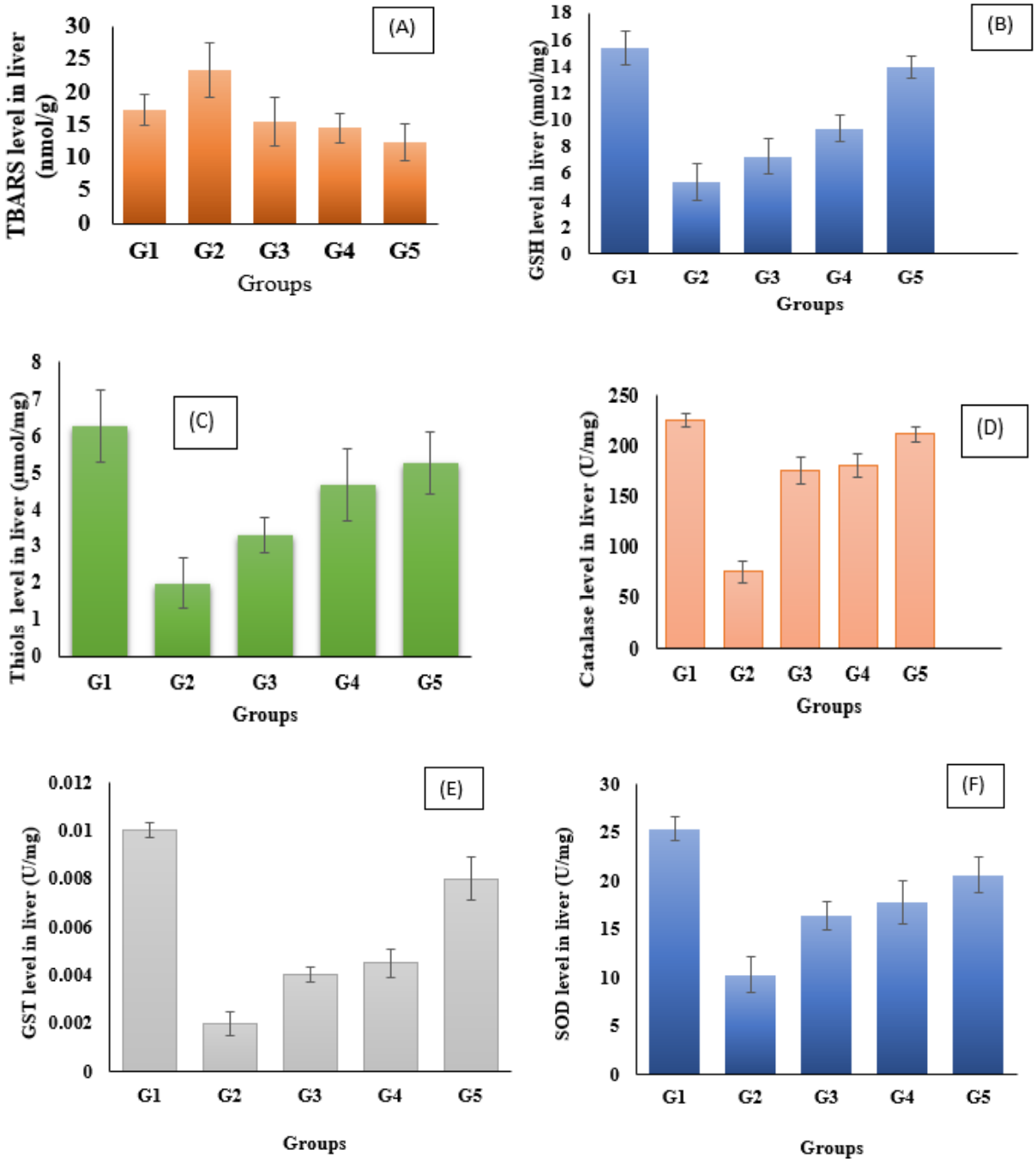

Fig. 3. Effect of TAU and MET alone and in combination on Anti-oxidants extracted from liver (A) liver TBARS, (B) Liver GSH, (C) liver thiol (D) liver catalase and (E) liver GST and (F) liver SOD in normal diet with STZ and a combination of TAU and MET with STZ. Each bar represents the mean ± SD (n = 5). Comparisons were made at P<0.05 was considered significant and P<0.01 was considered highly significant.

Similar results were obtained with SOD. The control group of rats showed a SOD level of 25.38± 1.37 U/mg. The level of SOD in STZ rats group was 10.27 ± 1.83 U/mg, respectively which was significantly ($p < 0.01$) less than that of the control group. After the treatment with TAU, MET, and a combination of MET-TAU in both normal diets with STZ rats groups, the level of SOD was significantly ($p < 0.05$; $p < 0.01$) enhanced in STZ rats group. (Fig. 3F).

Histopathology of Liver

Haematoxylin and Eosin (H&E) staining results obtained upon histological examination shown in Figure 3. The hepatocytes in the control group were

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distributed in an ordered fashion and displayed a typical hepatic architecture, including organized hepatocytic cords, normal hepatocyte morphology, and a portal vein with sinusoidal cords (Figures 3(a) and 3(b)). On the other hand, the most significant alterations in the diabetic control rats' livers were disorderly hepatocyte, cytoplasm dissolution, monocellular leukocytic infiltration, karyomegaly, hyperchromatic nuclei, nucleus karyolysis, and dilated congested portal vein. The proliferation of bile ducts and degenerative changes in the wall of some bile ducts were also observed. Dilated hyperemic sinusoids and thickened walls were also seen (Figures 3(c)–3(f)). Treated groups with TAU and MET showed amelioration of hepatocytes, sinusoid, and central vein (Figures 3(g) and 3(h)).

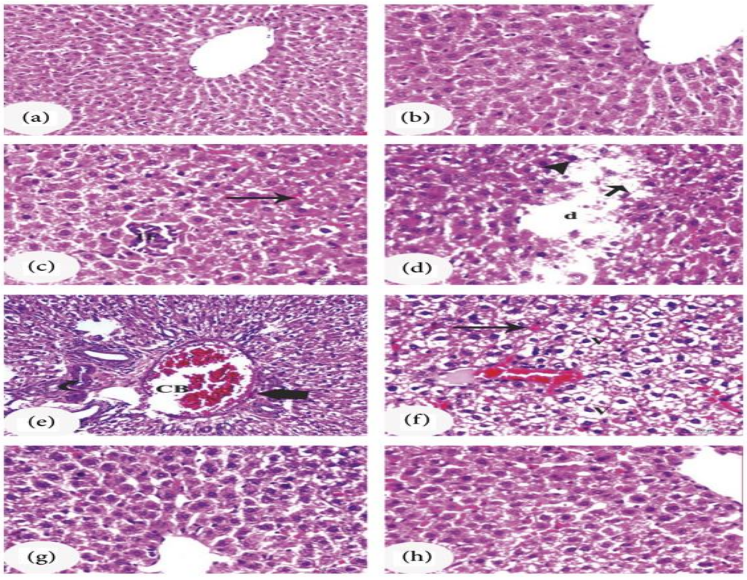

Figure 3

Photomicrographs of liver sections of rats of the 4 study groups. (a, b) Photomicrographs of the negative control group showed hepatocytes arranged in an orderly and normal hepatic architecture with normal hepatocyte morphology and organized hepatic cell cords radiating from the central vein. (b) is a higher magnification of (a). (c-f) Photomicrographs of the diabetic control group showed disordered hepatocyte, dissolution of cytoplasm (d), monocellular leukocytic infiltration (IF), hydropic degeneration and vacuolations (v), karyomegaly of the nucleus or hyperchromatic nucleus (arrowhead), karyolysis of the nucleus (short arrow), and dilated congested portal vein (CB). The proliferation of bile ducts and degenerative changes in the wall of some bile ducts are also observed (curved arrow). Dilated hyperemic sinusoids (long arrow) and thickened wall (thick arrow) are also seen. Diabetic groups treated with TAU (g) and MET (h) showed amelioration of hepatocytes' microscopical structure, sinusoid, and central vein. Scale bars of photomicrographs (a), (e), and (f) = 100 μm and scale bars of photomicrographs (b), (c), (d), (g), and (h) = 50 μm.

Conclusion

The aim of the study was to evaluate antidiabetic activity of TAU alone or in combination of MET to see the benefits of the combination. The antidiabetic activity of TAU less than MET but in combination a synergistic effect was observed on the both blood glucose level and insulin. The effect of TAU alone or in combination was observed on the lipid parameters as well as anti-oxidant components from Liver was observed. Favourable results were observed. Furthermore, TAU in combination with MET decreased Liver malondialdehyde levels while increased reduced glutathione and glutathione disulfide in rats. Following therapy with TAU and MET alone or in combination, there was a considerable reduction in GLC and an increase in insulin. Finally, it was shown that the effect of TAU alone on liver parameters is equivalent to that of MET group rats, but their combination displayed a synergistic effect on liver parameters, GLC, and INS.

List of abbreviations

CAT =Catalase,
GLC= Glucose,
GSH= Reduced glutathione,
GSSG= Glutathione disulfide,
GST =Glutathione S-transferase
LF = Leukocytic infiltration
INS= Insulin,
MDA= Malondialdehyde

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H&E = Haematoxylin and Eosin
MET =Metformin,
SOD= Superoxide dismutase,
TAU= Taurine

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