Journal of Advanced Zoology
ISSN: 0253-7214
Volume 44 Issue 5 Year 2023 Page 850:862

Evaluation Of Ameliorative Effect Of Taurine And Metformin In High Fat Diet-Induced Diabetes

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Article History

Received: 10/08/2023
Revised: 12/9/2023
Accepted: 16/10/2023

Abstract

The aim of the study was to evaluate the anti-diabetic effect of Taurine (TAU), singly and in combination with Metformin (MET) High Fat Diet (HFD) induced diabetic rats.For this purpose, male Albino wistar male, 200–250 g in weight, assigned to groups of five (5). The rats were fed on either normal pellet diet (NPD) (4.1% fat, 22.2% protein, and 12.1% carbohydrates, as a percentage of total kcal) or high fat diet (HFD) (58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal) ad libitum, for an initial period of three weeks. Either MET, TAU or MET-TAU (each at 2.4 mM/kg, in water). Control rats received only distilled water and normal diet. After the study duration, the blood samples were withdrawn through retro-orbital plexus and blood glucose level (GLC) and insulin (INS) were measured.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 used for the for the determination of various parameters from the liver. Diabetes group raised the plasma GLC level (497.1±9.3 mg/dL in HFD) 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). 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 the normal diet and HFD rats. After the treatment, with TAU and MET alone or in combination, the significant reduction of GLC and enhancement of INS was observed. Moreover, MDA level in liver reduced but enhanced the GSH, and GSSG was also observed significantly (all at $p < 0.01$) in the normal diet and HFD rats after administration of TAU alone or in combination with MET. Finally, it is was concluded that the effect of TAU alone on various tissue and liver parameters is comparable to MET on both normal diet and HFD rats but their combination exhibited a synergistic effect on tissue, liver parameters, and on GLC as well as on INS.

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

Introduction

Diabetes is a disease that occurs when blood glucose (sugar) extended than the normal range, also called blood sugar, is too high. Glucose is your body's main source of energy. Your body can make glucose, but glucose also comes from the food you eat.
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. Glucose then accumulated in blood and doesn't reach your cells [1].
Diabetes raises the risk for damage to the Liver, Eyes, kidneys, nerves, and heart. Diabetes is also linked to some types of cancer [1]. Taking steps to prevent or manage diabetes may lower your risk of developing diabetes health problems.
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. In this case immune system attacks and destroys the cells of pancreas that make insulin. Type 1 diabetes is usually diagnosed in children and young adults, although it can appear at any 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. Type 2 diabetes is the most common type of diabetes. Type 2 diabetes occurs in case of risk factors, such as overweight or obesity, and a family history of the disease. It can occur at any age, even during childhood. A person can help delay or prevent type 2 diabetes by knowing the risk factors and taking steps toward a healthier lifestyle, such as losing weight or preventing weight gain [3].

Gestational diabetes is a type of diabetes that develops during pregnancy. Most of the time, this type of diabetes goes away after the baby is born. However, if you've had gestational diabetes, you have a higher chance of developing type 2 diabetes later in life. Sometimes diabetes diagnosed during pregnancy is type 2 diabetes [3]. Many synthetic drug have been reported and used to reduce the hyperglycemic 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, 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 Liver & 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].

The protective effect of taurine on diabetes and its complications has been demonstrated in many animal model [28]. Multiple studies have found that plasma taurine concentration is inversely correlated with fasting blood sugar (FBS) and diabetic complications, suggesting that taurine has a protective role in the progression of diabetes [29]. A large number of animal experiments have shown that taurine can improve various diabetic complications, including endothelial dysfunction, diabetic nephropathy, diabetic retinopathy, diabetic cataract, diabetic neuropathy, diabetic cardiomyopathy, and so on [30]. Although a number of controlled clinical trials

have been conducted to study the effects of taurine supplementation in patients with DM (diabetes mellitus), the efficacy of taurine supplementation for DM in human studies has been inconsistent. Recognizing that individual studies might not be able to provide sufficient data on clinical practice, we sought to objectively assess the potential role of taurine in the management of diabetes [31].

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 hypoglycemic 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 HFD 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 and Methods

The feed ingredients such as casein sodium salt from bovine milk (Sigma, C8654), cholesterol and sodium cholate (Hi-Media Laboratories, Mumbai, India), sodium carboxymethylcellulose (Na-CMC), and DL methionine (both from Loba Chemie, Mumbai), vitamin and mineral mix (D protein from British Biologicals, Bangalore, India), and yeast powder (Eagle Products, Mumbai) were procured from commercial sources. Lard (fat) was collected from the local slaughter house. Bovine serum albumin (BSA), 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 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 five groups were developed with 30 total animals as follows:
Group (G1): Control Group (Distilled water treated group).
Group (G2). HFD induced diabetes group.
Group (G3). HFD induced diabetes group treated with Taurine.
Group (G4). HFD induced diabetes group treated with Metformin.
Group (G5). HFD induced diabetes group treated with Taurine and Metformin combination.

High fat diet induced diabetes

The rats were fed on either normal pellet diet (NPD) (4.1% fat, 22.2% protein, and 12.1% carbohydrates, as a percentage of total kcal) or high fat diet (HFD) (58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal) ad libitum, for an initial period of three weeks. The composition (Table 1) and preparation of the HFD has been described earlier [40]

Table 1: High fat diet composition

S. No.	Ingredients	Quantity desired (gm/100 HFD)
1.	Powdered NPD	36.5
2.	Lard	31.0
3.	Casein	25.0
4.	Cholesterol	1.0
5.	Sodium cholate	0.5
6.	Vitamin and mineral mix	6.0
7.	dl-Methionine	0.3
8.	Yeast powder	0.1
9.	Sodium chloride	0.1

Treatments and Samples

Diabetes was induced with a fed of HFD for continuation or till end of study and control group fed by normal diet. Starting on day 21 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 distilled water and normal diet 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 HFD. All treatments were conducted for a total duration of study.

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 from Bhabha Atomic Research Centre, Mumbai. 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].

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

High fat diet effect

High fat diet (HFD) significantly increased ($p < 0.001$) body weight of rats compared to NPD. Further, HFD also significantly elevated basal BGL, and SI at the end of the three weak study. At the end of three weeks, HFD significantly ($p < 0.001$) increased levels of BGL, and SI, indicating a higher level of insulin resistance in this group compared to animal groups receiving only HFD or NPD. Further produced significant reduction in body weights compared to HFD rats, which was still significantly higher ($p < 0.05$) than NPD rats (Table 2). Also, all HFD diabetic rats developed symptoms of polyphagia, polydipsia and polyuria when compared to NPD rats. However, HFD produced significantly higher ($p < 0.001$) BGL and drastic reduction ($p < 0.05$; $p < 0.01$) in body weights of HFD rats. Further, HFD diabetic rats showed significantly reduced ($p < 0.001$) SI levels and HOMA values (Table 2).

Table 2. Effect of high fat diet (HFD) at low to high dose fed, on body weight and biochemical parameters in rats

Parameters	NPD	HFD	HFD Dose (Low)	HFD Dose (Medium)	HFD Dose (High)
Body weight (g)	230.4±5.5	288.6±11.6^{c}	280.2±10.9^{a}	250.2±8.3^{d}	237.1±11.4^{e}
GLC (mg/dL)	88.5±3.2	150.2±7.8^{a}	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	96.8±11.4^{c}	67.9±11.8^{c}	27.7±3.5 ^{f,h}	ND

Each value represents mean ± SEM (n= 6, 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 HFD.

Oral glucose tolerance test (OGTT)

Administration of glucose (2 g/kg, p.o.) did not produce any significant change in the BGL levels of NPD rats. The HFD diabetic rats exhibited significant elevation in fasting BGL (at time zero) and showed significant impairment in glucose tolerance to exogenously administered glucose compared to NPD 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). Similar results were obtained in HFD groups but somewhat less benefits as compared to NPD. 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 HFD + MET-TAU diabetic rats exhibited significant ($p < 0.001$) hyperglycaemia (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 HFD [a,b]

Group	Normal diet (NPD)		HFD	
	Plasma GLC, mg/dL	Blood INS, µIU/mL	Plasma GLC, mg/dL	Blood INS, µIU/mL
Control	88.5±3.2+++	43.7±4.8+++	150.2±7.8	96.8±11.4+++
HFD	328.2 ± 21.77***	17.7±4.8± 1.33***	431.4 ± 13.4***	27.9±11.8± 2.49***
MET-HFD	196.4 ± 5.0***,+++	28.3 ± 1.5**,+++	237.4 ± 1.6***,+++	38.50 ± 2.35**,+++
TAU-HFD	263.3 ± 7.3***,++	21.3 ± 1.8***,+++	330.37 ± 5.8***,++	31.5 ± 1.3***,+++
MET-TAU-HFD	145.5 ± 4.7***,+++	36.02 ± 1.87*,+++	190.43 ± 2.22***,+++	39.02 ± 1.87*,+++

[a] Values are shown as the mean ± SEM for n = 6, [b]Statistical comparisons were significantly different from Control at *p < 0.05, **p < 0.01 and ***p < 0.001; and from HFD at ++p < 0.01 and +++p < 0.001

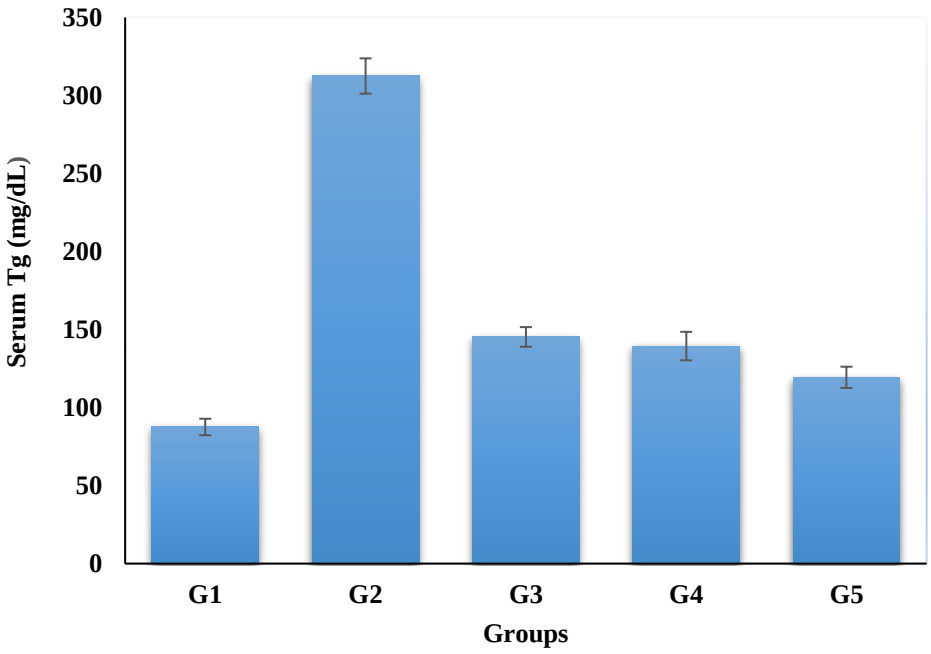
Effect on lipid parameters

All lipid parameters are depicted in Fig. 2 and in Table 4. HFD 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 NPD rats (G3). 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 HFD induced diabetic rat 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 both normal diets as well as HFD. Moreover, metformin also significantly increased HDL-c levels and decreased levels of dyslipidemic markers (Fig.2).

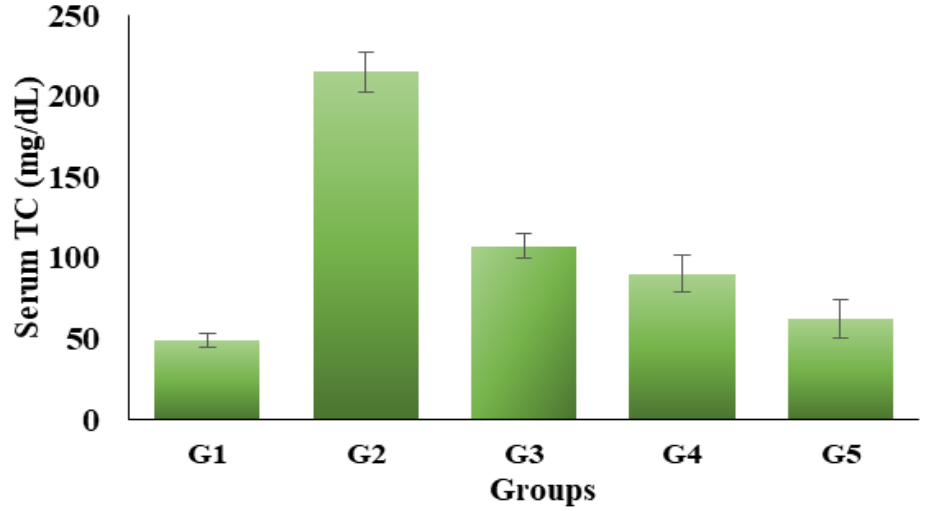
Table 4: The effects of TAU, MET, and combination of MET-TAU on the lipid parameters

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	312.47±11.31	215.19±12.36	42.96±9.23	168.72±7.24	92.52±6.45
G3	145.27±6.29	107.46±7.25	51.67±9.27	122.84±8.62	51.83±5.92
G4	139.41±9.13	90.26±11.56	53.92±5.52	128.83±6.32	48.32±5.21
G5	119.36±6.82	62.78±11.85	76.42±6.92	89.48±7.67	29.52±4.95

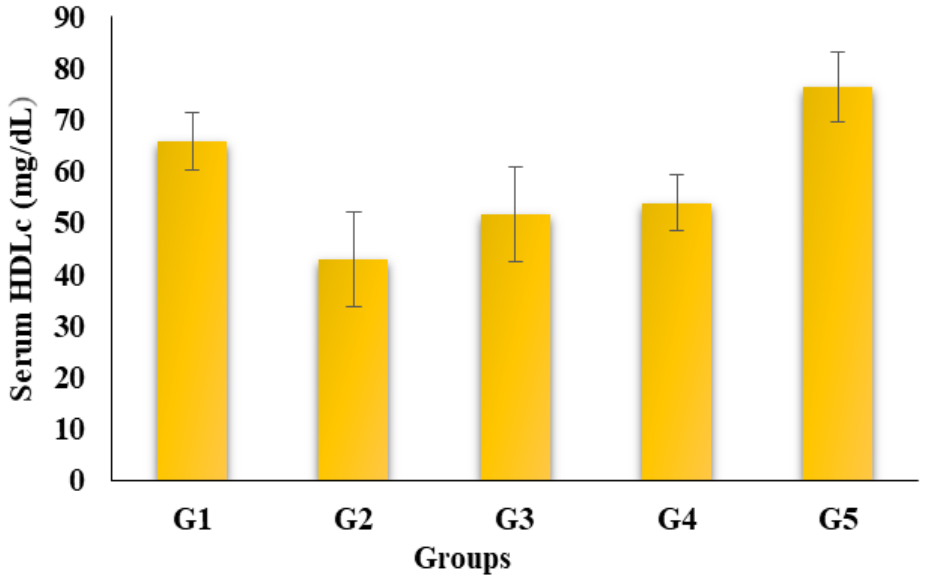
(A)



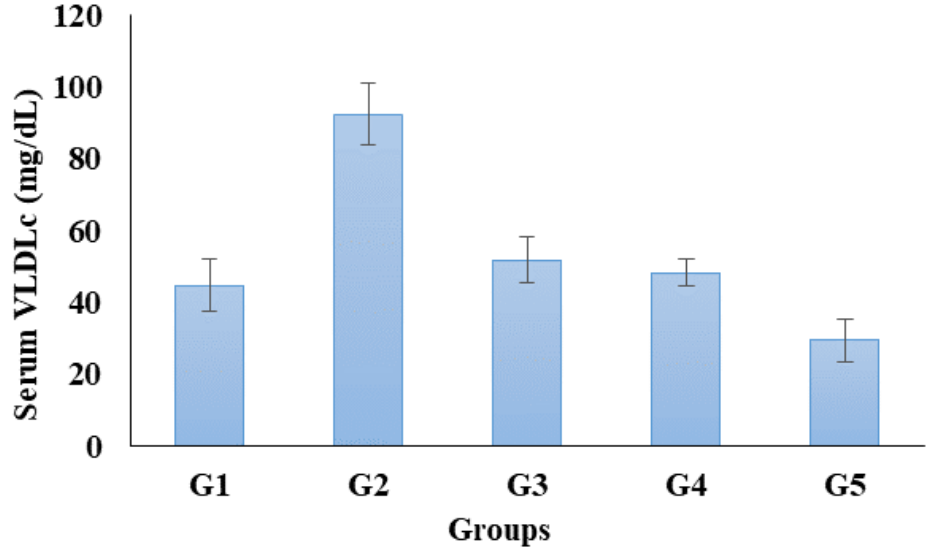
(B)


250
200
150
100
50
0
Serum TC (mg/dL)
G1
G2
G3
G4
G5
Groups

(C)


90
80
70
60
50
40
30
20
10
0
Serum HDLc (mg/dL)
G1
G2
G3
G4
G5
Groups

(D)


120
100
80
60
40
20
0
Serum VLDLc (mg/dL)
G1
G2
G3
G4
G5
Groups

(E)

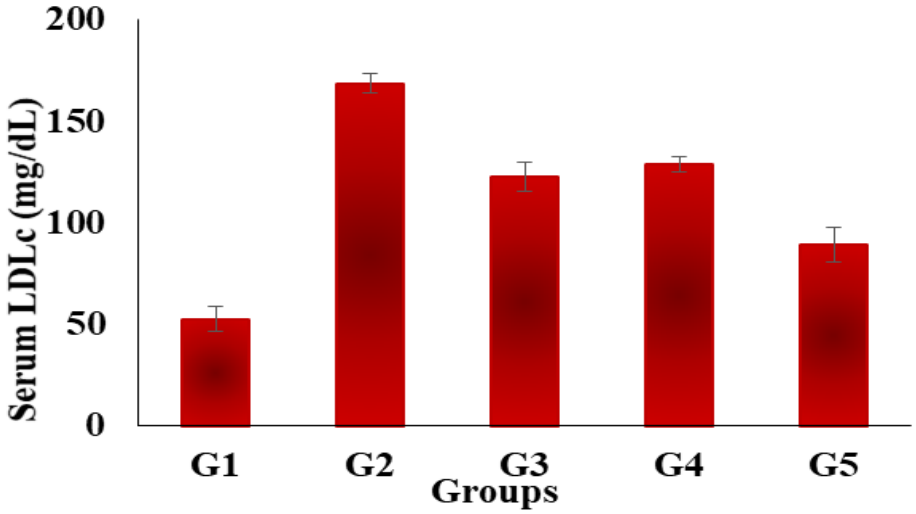


Fig. 2. Effect of TAU and MET alone and in combination on lipid parameters (A) serum TG, (B) serum TC, (C) serum HDLc levels (D) serum VLDLc and (E) serum LDLc in normal diet and with HFD, and a combination of TAU and MET in normal diet with HFD. Each bar represents the mean ± SD (n = 5). Comparisons 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 rats showed basal TBARS levels of about **23.47** ±2.36 nmol/g in liver tissue. HFD induced 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 HFD (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 and HFD 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 rats and HFD (Fig. 3B). The control group of rats showed a thiol level of 6.26± 0.98 µmol/mg. The normal diet rats and HFD 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 rats and HFD (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 rats and HFD rats was 75.28±11.26 and 60.26±9.95 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 diet rats and HFD rat groups, the level of catalase was significantly ($p < 0.05$; $p < 0.01$) enhanced in both normal diets rats and HFD (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 rats and HFD rats was 0.002±0.0005 and 0.0015±0.0006 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 diet rats and HFD rat groups, the level of GST was significantly ($p < 0.05$; $p < 0.01$) enhanced in both normal diets rats and HFD (Fig. 3E).

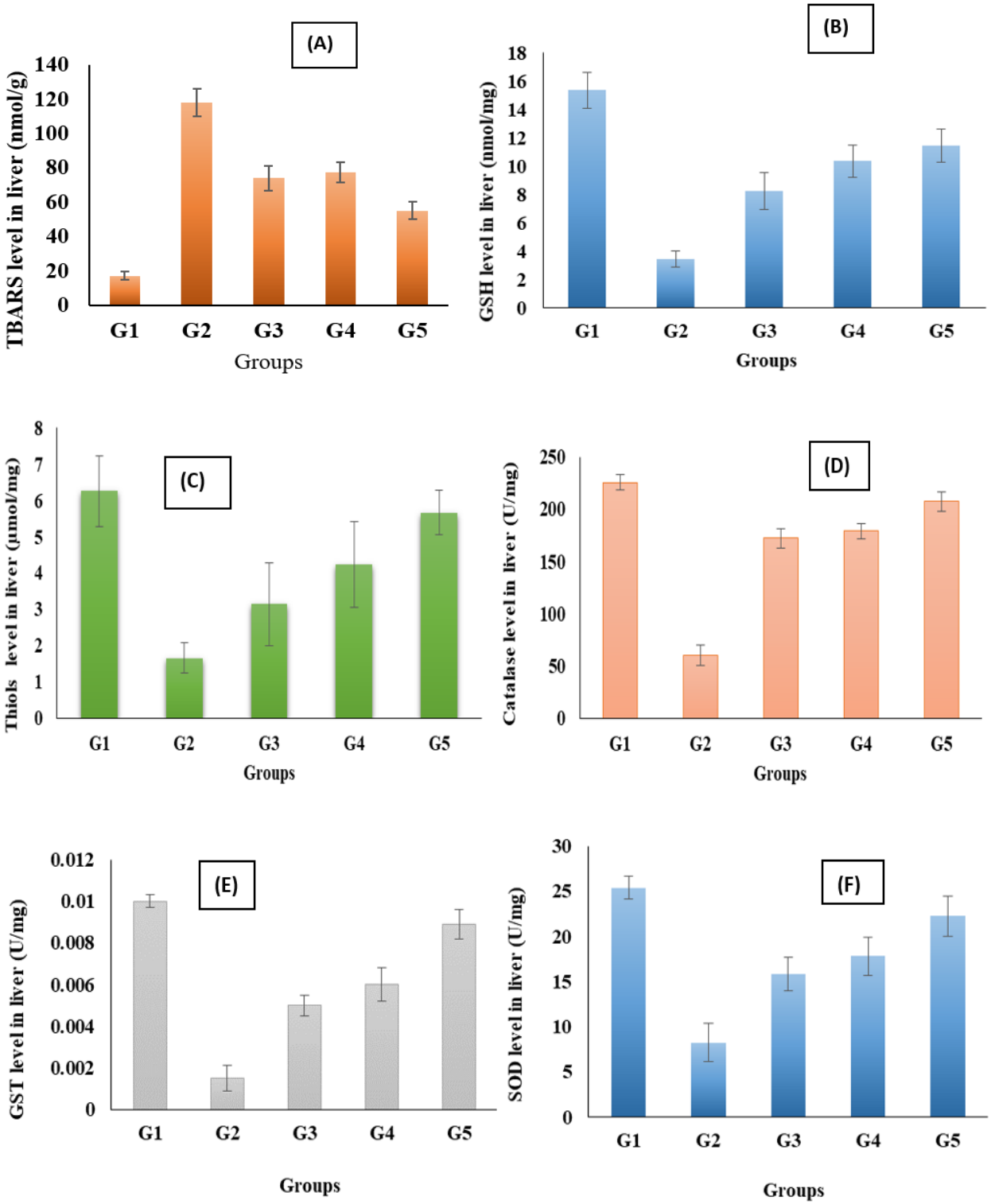


Fig. 3. Effect of TAU and MET alone and in combination on Anti-oxidants extracted from liver (A) liver TBARS, (B) Liver GSH, (C) liver thiol (D) liver catalase and (E) liver GST and (F) liver SOD in normal diet rats and HFD induced diabetes rats, and a combination of TAU and MET in normal diet and HFD diabetes induced rates. 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 normal diet rats and HFD rats was 10.27 ± 1.83 and 8.25±2.14 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 rats and HFD rat groups, the level of SOD was significantly ($p <0.05$; $p <0.01$) enhanced in both normal diets with rats and HFD (Fig. 3F).

Histopathology of Liver

Haematoxylin and Eosin (H&E) staining results obtained upon histological examination wn in Figure 4. The hepatocytes in the control group were 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 4(a) and 4(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 4(c)–4(f)). Treated groups with TAU and MET showed amelioration of hepatocytes, sinusoid, and central vein (Figures 4(g) and 4(h)).

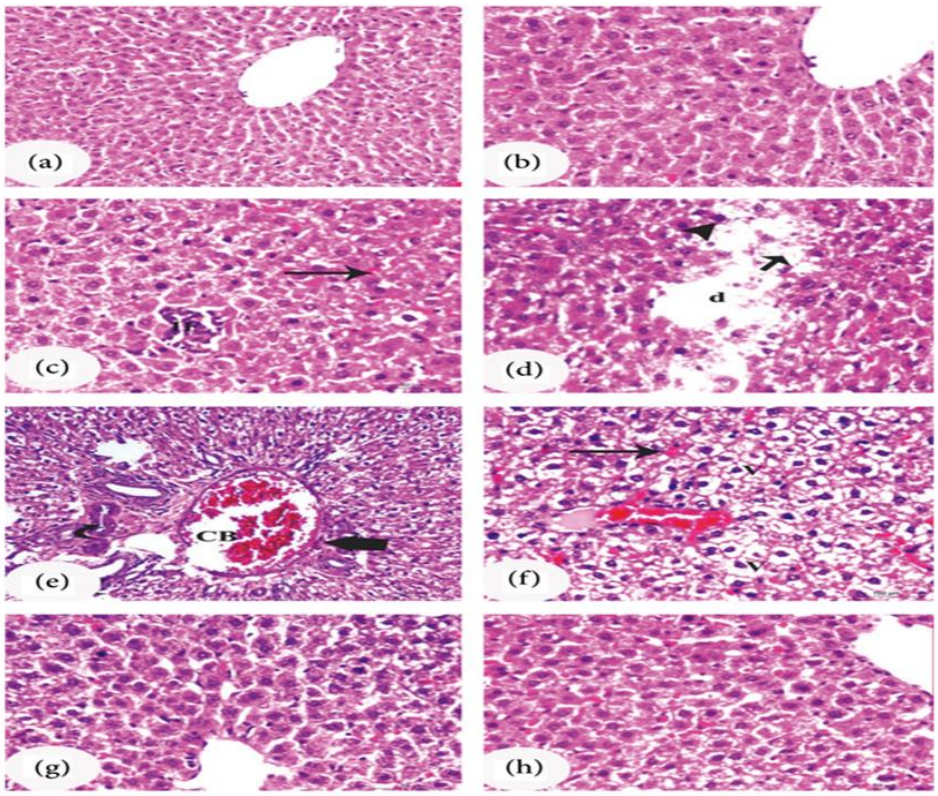


Figure 4 Photomicrographs of liver sections of rats of the 5 study groups. (a, b) Photomicrographs of the negative control group showed hepatocytes arranged in an orderly and normal hepatic architecture with normal hepatocyte morphology and organized hepatic cell cords radiating from the central vein. (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 and blood was also observed. Favourable results were observed in both cases. Furthermore, TAU in combination with MET decreased liver malondialdehyde levels while increased reduced glutathione and glutathione disulfide in regular

diet and HFD diabetic 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 several liver parameters is equivalent to that of MET in both normal diet and HFD 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, INS= Insulin, MDA= Malondialdehyde, MET =Metformin, SOD= Superoxide dismutase, TAU= Taurine

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