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Research Article

Effects of Combined Therapy of Ethanolic Extract of *Trigonella foenum graecum* or *Gymnema sylvestre* on the Hypoglycemic Activity of Metformin in the Regulation of Alloxan Induced Hyperglycemia and Associated Adverse Effects.

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ABSTRACT

The present study was designed to explore the combined effects of a conventional antidiabetic drug, metformin (MET) and *Gymnema sylvestre* (GS) or *Trigonella foenum graecum* (TFG) extract in alloxan (ALX, 150 mg / kg body weight, IP)-induced swiss albino mice. Other than serum glucose, changes in total serum cholesterol (TC), triglyceride (TG), urea, α-amylase and alkaline phosphatase (ALP) levels were examined. In addition alterations in tissue biochemical indices such as lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx), lipid hydroperoxide (LOOH) and advanced oxidation protein products (AOPP) were also studied in liver, kidney and heart. A daily administration of MET (50 mg/kg body weight) alone or their combination with the herbal extracts for 15 days showed a significant reduction in glucose, TC, TG, urea, ALP, LPO, LOOH and AOPP with a concomitant increase in SOD, CAT, GSH and GPx. However, when both MET and either of the plant extracts was administered simultaneously the beneficial effects were more pronounced as compared to their individual effects.

Keywords: lipid peroxidation, diabetes, alloxan, mice, *Trigonella foenum graecum*, *Gymnema sylvestre*, metformin.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders, characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both[1]. The chronic hyperglycemia or diabetes is associated with long term damage, dysfunction and failure of various organs especially the eyes, kidney, nerves, heart and blood vessels[2]. The abnormalities in carbohydrate, fat and protein metabolism during diabetes is due to the deficient action of insulin on target tissues[1]. It is also believed that the increased oxidative stress is the widely accepted factor in the development and progression of diabetes and its complications[3]. DM is usually accompanied by increased production of free radicals or an impaired antioxidant defense. Very high levels of free radicals cause damage to cellular proteins, membrane lipids and nucleic acids and eventually cell death[4,5].

One of the important sources of free radicals in diabetes is the interaction of glucose with proteins leading to the formation of Amadori product and then advanced glycation end products (AGEs). AGEs via their receptors inactivate antioxidative enzymes and alter their structures and functions, therefore speeding up the oxidative damage to cells[4].

The inhibition of this intracellular free radical formation would provide a therapeutic strategy to prevent oxidative stress and diabetes related complications. Antioxidants may act at different levels, inhibiting the formation of reactive oxygen species (ROS), scavenge free radicals or increase the antioxidants defense enzyme capabilities[6]. The natural plants, vegetables and fruits are enriched with antioxidants including polyphenols and flavonoids which are associated with certain health benefits. Several natural products are being used in India as well as in other parts of the world for the management of diabetes and oxidative stress. GS and TFG are reported to have their beneficial effects on diabetes and oxidative stress[7,8].

Trigonella foenum graecum (TFG) is a herb used for the treatment of DM in many parts of the world. TFG is found to be rich in polyphenols, flavonoids, fibres and saponins which are responsible for its antihyperglycemic and antioxidative activity. TFG seeds have the potential to slow enzymatic digestion of carbohydrate reduce gastrointestinal absorption of glucose and thus reduce post prandial glucose level[9]. Similarly, *Gymnema sylvestre* (GS) extract is also known as a traditional remedy for DM as it plays a major role in blood glucose homeostasis through regenerating β cells of pancreas[10,11].

Many synthetic drugs and insulin are currently available in the market for the treatment of DM but they are associated with several side effects[12]. One such drug is metformin (MET), that is widely used for the treatment of DM. It produces hypoglycemic effect primarily by decreasing hepatic glucose output. It also increases

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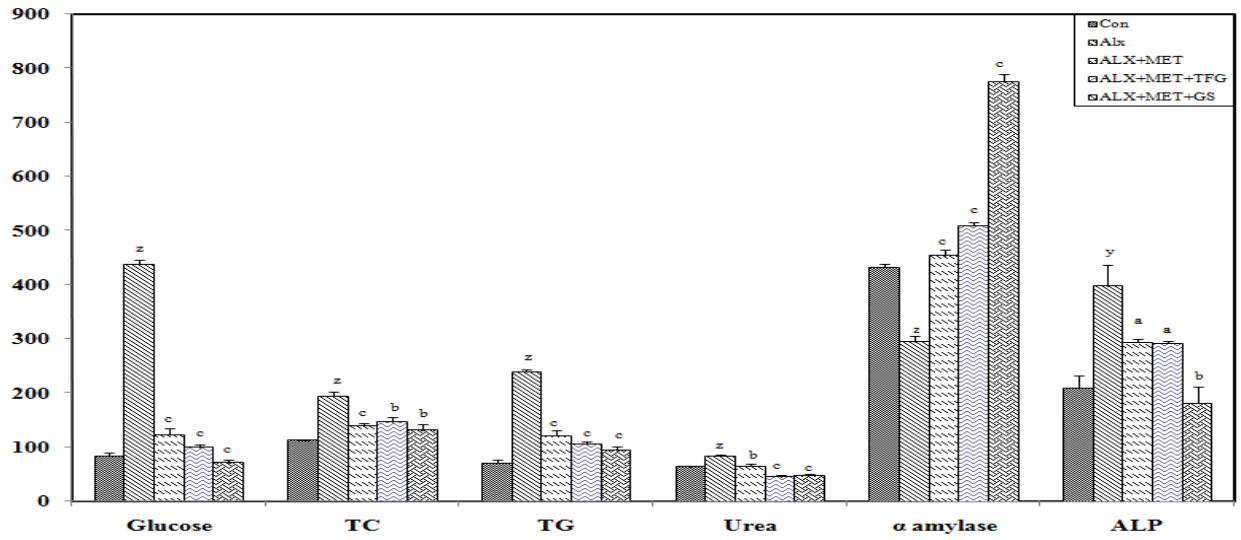

Fig.1: Effect on glucose, TC, TG, urea, α-amylase and ALP concentrations.

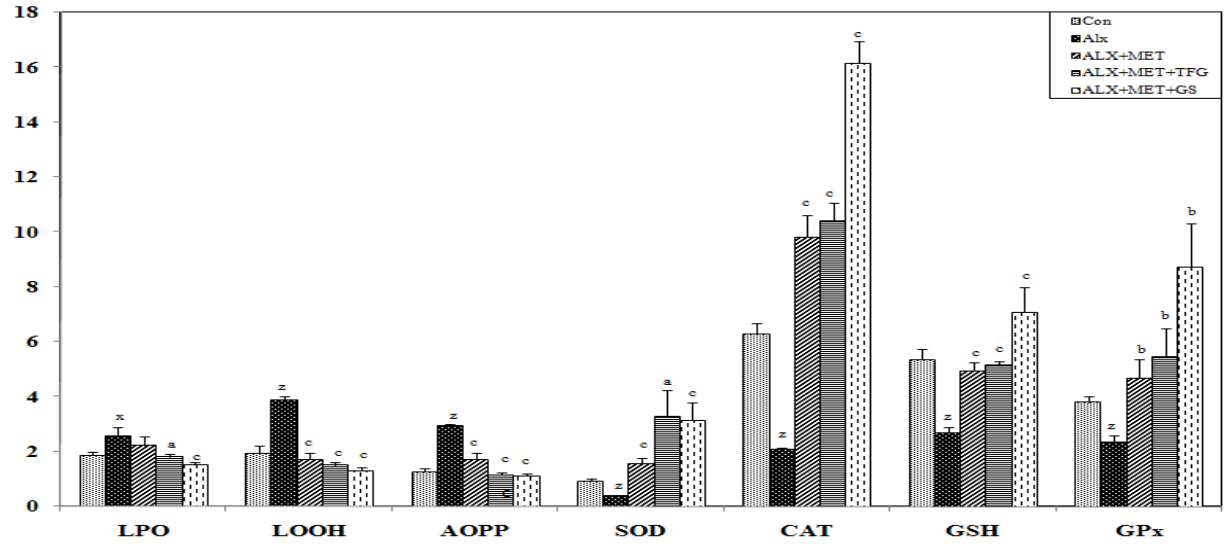

Fig.2: Effect on hepatic LPO, LOOH, AOPP, SOD, CAT, GSH and GPx

insulin mediated glucose utilization in peripheral tissues. As MET treatment alone is associated with some side effects[13], there is a need for safe, quick acting alternate therapy for the treatment of DM. Herbal drugs are found to be comparatively safe and allopathic medicines are known to act quickly. Therefore, present study was planned to explore the beneficial effects of combined treatments with TFG/GS and MET, if any in the alloxan-induced hyperglycemia and oxidative stress in diabetic mice.

MATERIALS AND METHODS

Material: The test drug metformin hydrochloride (Glycomet) was obtained from a registered local pharmacy shop. Thiobarbituric acid (TBA), sodium dodecyl sulphate (SDS), Ellman's reagent, tricholoro acetic acid (TCA), tris buffer were purchased from E merck Ltd, Mumbai, India, while thiobarbituric acid (TBA), ethylene diamine tetra acetic acid (EDTA), xylenol orange, ammonium ferrous sulphate and meta-phosphoric acid were purchased from Hi-media, Mumbai.

Preparation of the plant extract: For this methodology followed earlier by Gholap and Kar[14] & Sharma et al.[15] was considered. In brief the dried powder of GS leaf / TFG seed (5gm) was soaked in 200 ml of 70% ethanol and was allowed to stand for 24 h and then filtered. It was then evaporated at 37ºC. The dried powder so obtained was stored at -4ºC until use.

Experimental Animals: Swiss albino mice, weighing 30 ± 2 g were used. They were housed in polypropylene cages in a standard photoperiod (14 h light: 10h dark) and temperature (27±1ºC) controlled room with the provision of laboratory feed (Gold Mahur feed, Hindustan Lever Limited, Mumbai, India) and water ad libitum. Standard

ethical guidelines of the Committee for the Purpose of Control and Supervision on Experiments in Animals (CPCSEA), Ministry of Environment and Forest, Government of India, were followed. The approval of the departmental ethical committee for handling and maintenance for experimental animals was also obtained before starting the investigation.

Thirty five healthy male mice were divided into five groups of seven in each. Group I animals receiving simple drinking water (0.1ml DDW) served as control, whereas those of group II, III, IV and V received single dose of alloxan at 150 mg/kg body weight[16]. After rendering them diabetic, animals of group III received MET orally at 50 mg/kg body weight[17], group IV received MET along with TFG orally at 100 mg/kg body weight[18] and animals of group V were treated with MET along with GS orally at 600 mg/kg body weight[14]. Experiment was continued for 15 consecutive days.

The extract, drug and vehicle were administered between 11.00 to 12.00 hr of the day to avoid circadian variation, if any. On 16th day, all overnight fasted animals were sacrificed by cervical dislocation. Blood from each animal was collected and serum was isolated for the estimation of glucose, triglyceride and total cholesterol. Liver, kidney, heart, muscle, brain and testis were excised and processed for the estimation of different biochemical parameters.

Biochemical estimation: Overnight fasted animals were sacrificed under mild anesthesia, blood from each animal was collected and serum was separated for the estimation of different biochemical parameters including serum glucose, total cholesterol (TC), triglyceride (TG), urea, alpha amylase and alkaline phosphatase (ALP). After exsanguinations, liver, kidney and heart were removed quickly, washed and cleaned with phosphate buffered saline (PBS); tissue were homogenized in PBS (0.1M, pH 7.4), centrifuged at 15,000g for 30 min at 4ºC and the supernatant was used for the estimation of lipid peroxidation (LPO), lipid hydroperoxide (LOOH), advanced oxidation protein products (AOPP), super-oxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and glutathione peroxidase (GPx) activities.

LPO was determined by the method of Ohkawa et al[19], LOOH was estimated by the protocol of Griffiths et al.[20], for AOPP the protocol of Sarsat et al.[21] was followed, SOD was estimated by using the protocol of Marklund and Marklund[22], catalase activity by the method of Aebi[23], estimation of GSH content was done by the protocol of Ellman[24], while for the estimation of GPx protocol of Mohandas et al.[25], and for protein content method of Lowry et al.[26] was followed.

STASTICAL ANALYSIS

Data are expressed as mean ± SE. Statistical evaluation of the data was made using analysis of variance (ANOVA), followed by student's t-test. *P* values of 5 % and less were considered to be significant.

RESULTS

Effect on fasting serum glucose level (Fig 1)

A significant elevation in glucose level in alloxan-induced diabetic mice was found (P<0.001,as compared to the value of control mice). Glucose level was decreased significantly in the diabetic mice after the treatment of TFG /GS either alone or in combination with MET (P <0.001 for all). However, differences in percent decrease were found between the three groups. It was 72, 77 and 84 % for MET, MET+TFG and MET+GS respectively, as compared to the value of alloxan treated group.

Effect on α-amylase (Fig 1): Administration of alloxan significantly reduced the level of α – amylase (P <0.001), while after the administration of MET alone or in combination with TFG / GS a significant elevation was found (P <0.001 for all) with a percent increase of 54, 73 and 163 % for MET, MET+TFG and MET+GS respectively.

Effect on other serum parameters: With respect to TG, TC, urea and ALP, a significant elevation was found in alloxan treated animals (P<0.001 for TG, TC, urea and P<0.01 for ALP), which were significantly decreased after the administration of MET either alone (P<0.001, P<0.001, P<0.01 and P<0.05 for TC, TG, urea and ALP respectively) or in combination with TFG (P<0.01, P<0.001, P<0.001, P<0.05 for TC, TG, urea and ALP respectively) and GS (P<0.01, P<0.001, P<0.001 and P<0.01 for TC, TG, urea and ALP respectively). However, when both the drugs were administered simultaneously the protective effects were found to be more in terms of percent changes. When TFG was combined with MET, the percent decrease was found to be 56, 24, 46 and 27%; when combined with GS, it was 61, 32, 42 and 54% decrease for TG, TC, urea and ALP respectively.

Fig.1 Effects of administering metformin (MET) alone or in combination with *Trigonella foenum graecum* (TFG) seed extract or *Gymnema sylvestre* (GS) leaf extract in alloxan-induced diabetic mice on serum glucose (mg/dl), TC (mg/dl), TG (mg/dl), urea (mg/dl), α-amylase (IU/L) & ALP (IU/L) concentrations.

Data are in mean ± SEM (n = 7). ^{z}, P< 0.001, as compared to the respective control values. ^{a,} P<0.05, ^{b,} P<0.01 and ^{c,} P<0.001 as compared to the respective values in alloxan treated group.

Effect of hepatic LPO, LOOH, AOPP, SOD, CAT, GSH, GPx (Fig 2)

The levels of MDA, LOOH and AOPP were increased significantly (P<0.05, P<0.001 and P<0.001 respectively) in alloxan induced diabetic mice; which were decreased significantly after the administration of MET (P<0.001 for LOOH and AOPP) except for LPO. However, there was a marked and significant reduction in the above mentioned parameters with higher percent decreases when MET+TFG (P<0.05 for LPO, P<0.001 for LOOH and AOPP with percent decrease of 30, 61 and 61% respectively) and MET+GS (P<0.001 for all with percent decrease of 41, 62 and 66% respectively) were administered.

The levels of antioxidative enzymatic and non enzymatic parameters like SOD, CAT, GSH and GPx were reduced

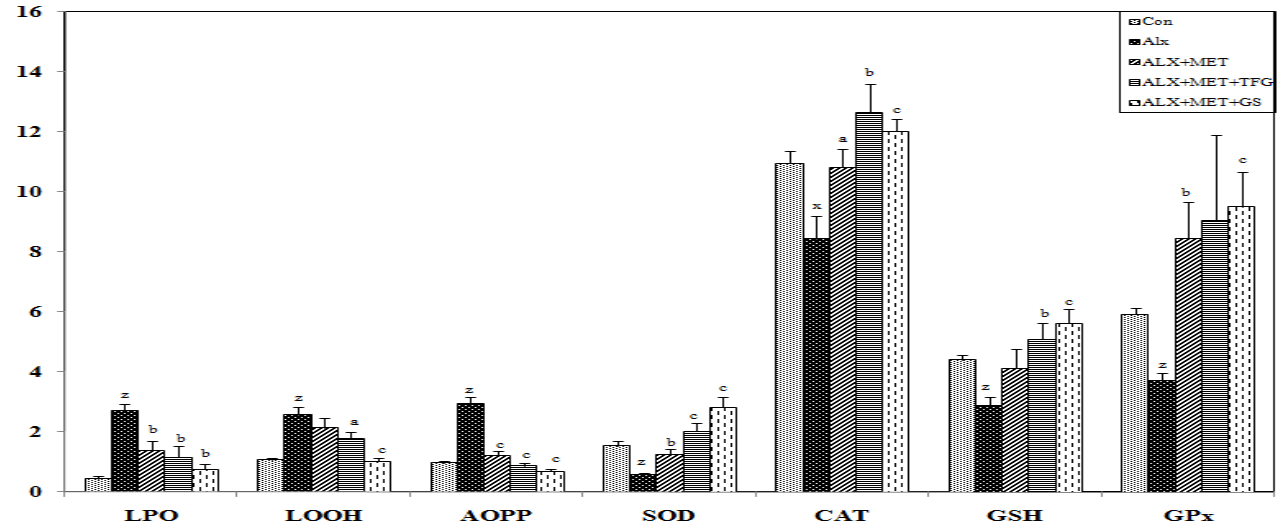

Fig. 3: Effect on renal LPO, LOOH, AOPP, SOD, CAT, GSH and GPx

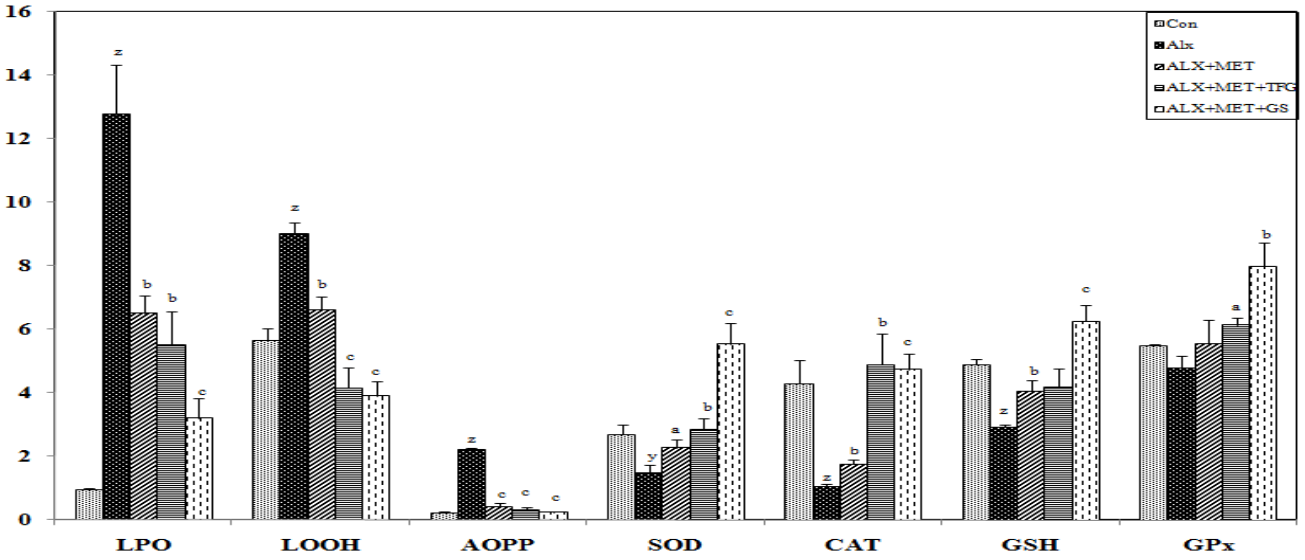

Fig. 4: Effect on cardiac LPO, LOOH, AOPP, SOD, CAT, GSH and GPx

significantly (P<0.001 for all) following the administration of alloxan. The administration of test drugs significantly reversed these changes exerted by alloxan.

MET administration increased the levels of SOD, CAT, GSH and GPx with 288, 370, 83 and 98 % respectively (P<0.001 for SOD, CAT, GSH; P<0.01 for GPx). Following the administration of MET+TFG, percent increase was 720, 399, 91 and 131% respectively (P<0.05, P<0.001, P<0.001 and P<0.01 for SOD, CAT, GSH and GPx). Similarly, following the treatment of MET+GS the percent increase was found to be 685, 674, 162 and 269% respectively (P<0.001 for SOD, CAT and GSH; P<0.01 for GPx).

Fig. 2 Effects of administering metformin (MET) alone or in combination with *Trigonella foenum graecum* (TFG) seed extract or *Gymnema sylvestre* (GS) leaf extract in alloxan-induced diabetic mice on liver LPO (nM MDA h^{-1} mg protein $^{-1}$), LOOH (nM lipid hydroperoxides mg protein^{-1}), AOPP (µg chloramines T equivalent mg protein^{-1}), SOD (units mg protein $^{-1}$), CAT (µM H_2O_2 decomposed min $^{-1}$ mg protein $^{-1}$), GSH (µM GSH mg protein^{-1}) and GPx (µM GSH mg protein^{-1}).

Data are in mean ± SEM (n = 7). ^{z}, P< 0.001, as compared to the respective control values. ^{a}, P<0.05, ^{b}, P<0.01 and ^{c}, P<0.001 as compared to the respective values in alloxan treated group.

Effect on renal LPO, LOOH, AOPP, SOD, CAT, GSH and GPx (Fig 3)

Here also a significant elevation (P<0.001) was observed in the levels of LPO, LOOH and AOPP following the administration of alloxan which were decreased significantly after the administration of MET alone (P<0.01 and P<0.001 for LPO and AOPP respectively)

or MET+TFG (P<0.01, P<0.001 and P<0.05 for LPO, LOOH and AOPP) and MET+GS (P<0.001 for all).

The combined treatment came out to be more protective in terms of percent decrease. In MET treated group, it was 49, 59 and 17 % for LPO, LOOH and AOPP ; while 58, 70 and 30% in MET+TFG treated animals and 72, 77 and 60 % respectively in animals treated with GS + MET treated animals.

While the levels of SOD, CAT, GSH and GPx were reduced significantly (P<0.001 for SOD, GSH and GPx; P<0.05 for CAT) after the administration of alloxan, an increase in these indices was noticed after the administration of drugs. MET increased the levels of SOD, CAT, GSH and GPx with a percent of 119, 28, 44 and 127% (P<0.01, P<0.05 and P<0.01 for SOD, CAT and GPx respectively). No significant increase was found in GSH levels after the administration of MET.

Following the administration of MET along with TFG the percent increase was 256, 50, 78 and 144 % for SOD, CAT, GSH and GPx respectively as compared to alloxan treated animals (P<0.001, P<0.01 and P<0.01 for SOD, CAT and GSH respectively). No significant increase was observed in GPx in this group. Similarly following the treatment of MET along with GS, the % increase was 396, 43, 96 and 156 % for SOD, CAT, GSH and GPx respectively as compared to the respective value of alloxan treated animals (P<0.001 for all).

Fig. 3 Effects of administering metformin (MET) alone or in combination with *Trigonella foenum graecum* (TFG) seed extract or *Gymnema sylvestre* (GS) leaf extract in alloxan-induced diabetic mice on kidney LPO (nM MDA h^{-1} mg protein $^{-1}$), LOOH (nM lipid hydroperoxides mg protein^{-1}), AOPP (µg chloramines T equivalent mg protein^{-1}), SOD (units mg protein $^{-1}$), CAT (µM H_2O_2 decomposed min $^{-1}$ mg protein $^{-1}$), GSH (µM GSH mg protein^{-1}) and GPx (µM GSH mg protein^{-1}).

Data are in mean ± SEM (n = 7). ^{z}, P< 0.001, as compared to the respective control values. ^{a,} P<0.05, ^{b,} P<0.01 and ^{c,} P<0.001 as compared to the respective values in alloxan treated group.

Effect on cardiac LPO, LOOH, AOPP, SOD, CAT, GSH and GPx (Fig 4)

A significant elevation (P<0.001) was found in the levels of LPO, LOOH and AOPP following the administration of alloxan which were reduced significantly after the administration of MET (P<0.001 for LOOH; P<0.01 for LPO and AOPP; 49, 81 and 27 % decrease for LPO, LOOH and AOPP respectively), co-administration of MET+TFG (P<0.01 for LPO; P<0.001 for LOOH and AOPP; 57, 85 and 54 % decrease for LPO, LOOH and AOPP respectively) and also the co-administration of MET+GS (P<0.001 for all; 75, 89 and 57 % decrease for LPO, LOOH and AOPP respectively).

The levels of SOD, CAT, GSH and GPx were reduced significantly following the administration of alloxan (P<0.001 for CAT, GSH; P<0.01 for SOD), which were elevated after the administration of test drugs. While MET alone increased the level of SOD, CAT, GSH and GPx with a percent of 53, 66, 38 and 16% respectively (P<0.001 for SOD; P<0.01 for CAT, GSH); in MET + TFG group the percent increase was 93, 365,42 and 28% for SOD, CAT, GSH and GPx respectively as compared to the respective values of alloxan treated group (P<0.001 for GPx; P<0.01 for SOD and CAT). No significant difference was observed in GSH level.

Similarly, following the administration of MET+GS the percent increase was 274, 351, 113 and 67 % for SOD, CAT, GSH and GPx respectively as compared to the respective values of alloxan treated group (P<0.001 for SOD, CAT and GSH; P<0.01 for GPx).

Fig. 4 Effects of administering metformin (MET) alone or in combination with *Trigonella foenum graecum* (TFG) seed extract or *Gymnema sylvestre* (GS) leaf extract in alloxan-induced diabetic mice on heart LPO (nM MDA h^{-1} mg protein $^{-1}$), LOOH (nM lipid hydroperoxides mg protein^{-1}), AOPP (µg chloramines T equivalent mg protein^{-1}), SOD (units mg protein $^{-1}$), CAT (µM H_2O_2 decomposed min $^{-1}$ mg protein $^{-1}$), GSH (µM GSH mg protein^{-1}) and GPx (µM GSH mg protein^{-1}).

Data are in mean ± SEM (n = 7). ^{z}, P< 0.001, as compared to the respective control values. ^{a,} P<0.05, ^{b,} P<0.01 and ^{c,} P<0.001 as compared to the respective values in alloxan treated group.

DISCUSSION

Results from our study clearly demonstrated that taking TFG/GS along with MET ameliorated alloxan induced hyperglycemia and oxidative damage in mice as evidenced by decrease in the levels of serum glucose, cholesterol, triglyceride, MDA, LOOH and AOPP and increase in other diabetes related antioxidative parameters such as SOD, CAT, GSH and GPx, in contrast to alloxan which led to hyperglycemia and increased oxidative stress as already reported earlier by other workers[27,28].

In addition to antioxidative effect of MET, for the first time we hypothesize that the combined therapy of TFG +MET or GS +MET might be more beneficial in ameliorating the adverse effects exerted by alloxan. To ascertain this hypothesis, we also measured the levels of serum lipids, urea, α-amylase and ALP as well as antioxidative enzymes like SOD, CAT, GSH and GPx in liver, kidney and heart which are commonly known as the biomarkers of oxidative stress.

Diabetes mellitus is a chronic disease characterized primarily by hyperglycemia, hypoinsulinemia, metabolic disorders, oxidative stress and its complications[29]. Free-radical generation have been implicated in the pathology of several human diseases, including diabetes mellitus[4]. In fact, free radicals and other reactive oxygen species (ROS) are constantly formed in the body. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance. These consequences of oxidative stress can promote the development of complications of diabetes mellitus. Peroxidation of polyunsaturated fatty acids (PUFA) leads to the formation of malondialdehyde and causes cellular injury that very often results in several pathological conditions[30]. In fact, the content of MDA and LOOH

reflects the degree of the peroxidation and cell damage[31], while SOD and GPx are the important enzymes of scavenging oxygen free radicals in organism, and protect the tissue against oxidative stress injury[32,33].

Alloxan is a toxic glucose analogue, which destroys insulin producing cells in the pancreas when administered to rodents[34]. It is selectively toxic to β cells because it is preferentially accumulated in β cells through uptake via glucose transporter, GLUT. Alloxan in the presence of intracellular thiols results in the formation of disulphide bonds and generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. Auto oxidation of dialuric acid involves the intermediate formation of alloxan radical establishing a redox cycle for the generation of ROS[34]. Therefore, the toxic action of alloxan is thought to be mediated by the generation of ROS. It is reported earlier that diabetes patients or diabetic animal models exhibit the depletion of antioxidant capacity and/or immune function, and then disturb the lipid metabolism[35,36]. In the present investigation, significant elevation in the levels of glucose, TC, TG, urea and ALP after alloxan administration was recorded as already reported earlier by other workers[28,37] which were reversed following the treatment of MET alone. Interestingly, the present study revealed that by the combined administration of TFG+MET and GS+MET the alloxan induced toxic effects were found to be reversed more effectively.

GSH is a well-known antioxidant that provides the major protection against cellular oxidative damages and maintains SH level in proteins**.** GSH forms reduced glutathione from oxidized glutathione, which in turn, reduces hydrogen peroxide, lipid peroxides, disulfides, ascorbate and free radicals[38,39]. In this experiment, GSH level decreased significantly in the alloxan treated mice, but the administration of any of the test drugs increased the level of GSH, suggesting that the test drugs have the potential to enhance the status of cellular antioxidant. Interestingly, the combined drug therapy (MET+ GS/TFG) ameliorated GSH status in a much better way as compared to MET alone.

GPx is a cytosolic enzyme that complements with CAT in order to detoxify hydrogen peroxide and hydroperoxide organic[40]. Reduction of hydroperoxide by glutathione in the presence of GPx has been shown to protect mammalian cells from oxidative damage[41]. In the present investigation a significant decrease in GPx activity in alloxan induced DM group was observed, but when combined with any of the test drugs, there was a significant increase in this enzyme level.

The serum α-amylase levels were also decreased significantly by the administration of alloxan , but increased significantly after the administration of MET, TFG+MET and GS+MET. This enzyme is produced in pancreas and saliva and is responsible for the digestion of carbohydrates. When damage occurs in pancreas the release of amylase in the blood takes place[42]. So in this study we measured the level of this enzyme as a marker of pancreatic damage by alloxan. We also observed a better response in the level of this enzyme, following the combined treatments of test plant extract and MET.

The mixture of TFG+MET and GS+MET not only succeeded in ameliorating the serum parameters but also lead to a marked and additional decrease in MDA, LOOH levels as well as in AOPP. AOPP are formed during oxidative stress by the action of chlorinated oxidants, mainly hypochlorous acid and chloramines (produced by myeloperoxidase in activated neutrophils). They are elevated in patients with renal insufficiency and diabetes mellitus[43]. Attack of ROS modifies the conformations of amino acids like lysine, arginine, proline and histidine residues forming cross linked protein products, which are known as AOPP. These AOPPs have been identified as an early marker of oxidative stress and are used as a measure of protein damage[44]. The co-administration of drugs was not only helpful in scavenging the free radicals but also beneficial in restoring the antioxidative enzyme levels.

TFG and GS are two very well known antidiabetic herbal plants. Studies on different experimental models have proved their strong antidiabetic properties[7,8]. Studies have confirmed the glucose and lipid-lowering ability of TFG [45]. The therapeutic potential of TFG is primarily due to the presence of many active components in it, which includes 4-hydroxyisoleucine, trigonelline, diosgenin, naringenin, quercetin, rutin, tricin, vitexin, kaempferol[15,46-49]. All these compounds are known to be antihyperglycemic in nature which act by enhancing insulin release, insulin sensitivity and glucose uptake in peripheral tissues[50].This could be a reason for the additional beneficial response following MET+TFG treatment in our study.

GS, on the other hand works by stimulating insulin secretion from the islets of Langerhans and thereby decreases glucose release in the blood. Pancreatic beta cells may be regenerated or repaired on GS supplementation; this is supported by the raised insulin levels in the serum of patients after supplementation[51,52]. Its active constituents include gymnemic acids, stigmasterol, coumarin, kaempferol, quercetin and catechin which are responsible for the antihyperglycemic effect exerted by GS extract[10,53,54].

MET hydrochloride is a biguanide hypoglycemic agent used in the treatment of diabetes. MET works by delaying the absorption of glucose from the gastrointestinal tract, increases the insulin sensitivity of cells, suppresses hepatic gluconeogenesis, and enhances glucose transport in fat and muscles[55]. MET also increases intestinal glucose utilization via non oxidative metabolism. Activation of the enzyme AMP activated protein kinase (AMPK) appears to be the mechanism by which MET lowers serum lipid and blood glucose concentration. AMPK dependent inhibitory phosphorylation of acetyl co-A carboxylase suppresses lipogenesis and lowers cellular fatty acid synthesis in liver and muscle which in turn improves insulin sensitivity and reduces blood glucose levels[56].

Since conventional medicines including MET are associated with various side effects[13], our results appear

to be useful in suggesting that for minimizing the side effects a conventional drug its therapeutic dose can be reduced and can be prescribed along with herbal drugs such as *Trigonella foenum graecum* or *Gymnema sylvestre* extract for relatively safe and appropriate antihyperglycemic effect. However, proper precaution and care should be taken to avoid severe hypoglycemia that may occur due to combination of these agents.

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