Chapter 5

COMBINED EFFECTS OF DIFFERENT TEST HERBAL EXTRACTS AND METFORMIN OR GLIBENCLAMIDE IN DEXAMETHASONE-INDUCED DIABETIC ANIMALS

This section deals with the effects of five different test plant extracts in combination (one at a time) with either of the conventional medicines, metformin (MET) or glibenclamide (GLB) in the amelioration of dexamethasone (DEX)-induced diabetes mellitus (DM) to find out the synergistic effects of the test drugs, if any.
5.1. Introduction

In the previous chapter combined effects of the test plant extracts and conventional medicines in the regulation of alloxan-induced DM were found to be encouraging. It was therefore thought to investigate the combined effects of the each test plant extract and a conventional medicine in DEX-induced type 2 DM.

Type 2 diabetes is mostly characterized by insulin resistance in the major target tissues, often coupled with insufficient insulin secretion ultimately leading to impaired uptake and metabolism of glucose. The evolvement of type 2 diabetes is considered to be a gradual process usually starting with insulin resistance. When there is a concomitant vulnerability in β cell capacity, a relative insulin deficiency eventually occurs upon elevated demand leading to the development of DM (Lin and Son, 2010; Olokoba et al., 2012).

Glucocorticoids are widely used as therapeutic agents for some diseases, particularly in the treatment for anti-inflammatory and immunomodulatory ailments. However, their excess use causes side effects including hyperglycemia, sometimes known as steroid diabetes. The diabetogenic action of glucocorticoids is well documented in the literature (Dalmazi et al., 2012; Rafacho et al., 2014). The name glucocorticoids itself suggest that they mainly influence glucose metabolism (Ganong, 1995). Excess of glucocorticoids in the circulation invariably increases serum glucose concentration and thus potentially induce DM in man as well as experimental animals (Crispin and Langslow, 1980; Thompson et al., 2000; Iwamoto et al., 2004). It is believed that the glucocorticoids increase
gluconeogenesis and decrease glucose uptake by tissue, inducing glucose tolerance and insulin resistance. As the degree of glucose intolerance is directly proportional to the dose and duration of glucocorticoids, excess use may ultimately lead to hyperglycemia and hyperinsulinemia or overt DM (Hwang and Wiess, 2014). They are known to oppose insulin action at the level of the liver by promoting gluconeogenesis and at the periphery, especially in muscle, by inhibiting glucose uptake. In addition, the glucocorticoids are believed to attenuate insulin secretion, particularly the extent of the anticipated hyperinsulinemic response to the insulin resistant state (Henrikson et al., 1997).

DEX is a potent synthetic member of the glucocorticoids class of steroid hormones (Okwuosa et al., 2011). Several studies have been conducted on the use of herbal extracts against DEX-induced diabetic animals (Shetty et al., 2010; Ghasias et al., 2011; Okwuosa et al., 2011; Bhujbal et al., 2012; Rajasekaran et al., 2013; Das et al., 2014), but with respect to the plant extracts considered for our study, reports are available only on Gymnema sylvestre (GS), that too from our laboratory only (Gholap and Kar, 2003). To the best of our knowledge, no other plant considered in our study has been evaluated for their antihyperglycemic activity against DEX-induced diabetes. However, conventional medicines have previously been shown to be effective against DEX-treated diabetic animals (Thomas et al., 1998; Jatwa and Kar, 2010; Shetty et al., 2010; Mohanraghupathy et al., 2013). An attempt has now been made to investigate the protective effects of combined drug treatment (herbal and conventional), if any, against DEX-induced diabetic mice model.
5.2. Experimental design

In this section five experiments were performed using different plant extracts taken in the previous chapter and the two oral hypoglycemic drugs, GLB and MET on DEX-induced diabetic mice.

In each experiment, all, except in the control groups, animals were first treated with DEX at 1.0 mg/kg body weight for 7 consecutive days, following which effects of each test drug was studied along with MET or GLB for 15 days. On the last day of each experiment, serum was isolated, tissues were cleaned and washed with phosphate buffered saline (PBS) and then processed further for biochemical estimations of lipid peroxidation (LPO), lipid hydroperoxides (LOOH), advanced glycation end products (AOPPs), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx) and liver glycogen content. In addition, serum glucose, insulin, total cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDL) were also estimated.

The referred/tested doses of five test plant extracts were tried in combination (one plant extract at a time) with either of the conventional medicines in type 2 diabetic animal models separately. MET and GLB were taken at concentration of 50 mg/kg and 500 μg/kg body weight respectively (Okine et al., 2005; Shetty et al., 2010). Following experiments were conducted.

Experiment 12: Combined effects of *Momordica charantia* extract and glibenclamide in dexamethasone-induced hyperglycemic animals.
In this experiment the combined efficacy of *Momordica charantia* (MC) extract and GLB either alone or in combination was tested in DEX-induced hyperglycemic mice. After 15 days of drugs treatment, the animals were sacrificed and all the parameters as mentioned earlier were studied.

**Experiment 13 and 14:** Combined effects of *Trigonella foenum graecum* or *Pterocarpus marsupium* and *Gymnema sylvestre* or *Syzygium cumini* extract along with glibenclamide in dexamethasone-induced hyperglycemic animals.

In these experiments the combined effects of either *Trigonella foenum graecum* (TFG) or *Pterocarpus marsupium* (PM) extract and *Gymnema sylvestre* (GS) or *Syzygium cumini* (SC) extract along with GLB was tested in DEX-induced hyperglycemic mice, considering all the parameters used in experiment 12.

**Experiment 15 and 16:** Combined effects of *Trigonella foenum graecum* or *Pterocarpus marsupium* or *Gymnema sylvestre* and of *Syzygium cumini* or *Momordica charantia* along with metformin in dexamethasone-induced hyperglycemic animals.

In these two experiments the combined effects of TFG/PM/GS and of SC/MC along with MET were studied for 15 days in DEX-induced hyperglycemic mice considering all the aforesaid parameters.

**5.3. Results**

**Experiment 12:** Combined effects of *Momordica charantia* extract and glibenclamide in dexamethasone-induced hyperglycemic animals.
**Glucose, Insulin and other serum parameters (Fig. 47)**

DEX-administration led to a significant rise in the levels of glucose, TC and TG with a parallel decrease in HDL level ($P<0.001$ for all). When MC extract was administered, a significant decrease was noticed in glucose, TC and TG ($P<0.001$ for all) with a concomitant increase in HDL level ($P<0.001$). Similarly, following GLB administration; glucose, TC and TG levels were lowered significantly ($P<0.001$, $P<0.001$ and $P<0.01$ respectively) with an increase in HDL level ($P<0.01$); while co-administration of both the drugs markedly brought significant decrease in glucose, TC and TG with an increase in HDL level ($P<0.001$ for all).

**Liver tissue (Figs. 48 & 49)**

Following DEX-administration, a marked increase in tissue LPO, LOOH and AOPP ($P<0.001$, $P<0.01$ and $P<0.001$ respectively) was observed indicating a peroxidative condition. While the test extract or GLB alone decreased these peroxidative parameters induced by DEX ($P<0.05$, $P<0.01$ and $P<0.001$ respectively for MC treated animals; $P<0.001$ both for LPO and AOPP for GLB alone treated group); with the simultaneous administration of MC extract and GLB all three indices LPO, LOOH and AOPP were markedly decreased ($P<0.001$ for LPO and AOPP; $P<0.01$ for LOOH).

With an increase in peroxidative parameters, a parallel decrease in antioxidative markers was found following administration of DEX ($P<0.001$ for SOD, GSH and GPx; $P<0.01$ for CAT). When MC extract or GLB was administered alone there was a significant increase in SOD, CAT and GSH ($P<0.01$ for the first two; $P<0.001$ for
latter in case of MC alone treated group; \( P<0.05 \) for SOD and CAT, \( P<0.001 \) for GSH in case of GLB treated group); but no significant increase was observed in the CAT levels. When both the drugs were administered simultaneously, a marked increase was observed in above mentioned all antioxidants (\( P<0.001 \) for SOD, CAT and GSH; \( P<0.05 \) for GPx).

With respect to liver glycogen content a significant decrease was noticed after DEX-administration (\( P<0.01 \)). However, following the treatment with MC either alone or along with GLB, a significant increase was observed (\( P<0.05 \) and \( P<0.001 \) respectively). However, the treatment of GLB alone had no significant effect.

**Kidney tissue (Figs. 50 & 51)**

In case of DEX-treated group there was a significant increase in peroxidative parameters such as LPO and LOOH (\( P<0.001 \)). Concomitantly, when drugs were administered either individually or simultaneously to diabetic animals, a significant decrease was noted in LPO and LOOH (\( P<0.001 \)). A significant decrease was also observed in the level of AOPP when the test drugs were co-treated (\( P<0.01 \)).

With an increase in peroxidative parameters there was a parallel decrease in antioxidants such as SOD, CAT, GSH and GPx following DEX-administration (\( P<0.001 \) for all). When the drugs were treated either alone or in combination, there was a significant increase in all three antioxidative parameters (\( P<0.001 \) for all).

When the percent changes in the glucose level and in tissue LPO were calculated, observed positive changes were maximum in groups where conventional and herbal drugs were treated together (Table 8).
Experiment 13: Combined effects of *Trigonella foenum graecum* or *Pterocarpus marsupium* extract with glibenclamide on dexamethasone-induced diabetic animals.

*Glucose, insulin and other serum parameters (Fig. 52)*

There was a significant increase in serum glucose, TC and TG levels with a parallel decrease in HDL following DEX-treatment \((P<0.001)\). When drugs were administered either alone or simultaneously, the glucose, TG and TC levels were decreased with a concomitant increase in HDL \((P<0.001\) for glucose, TG and HDL levels, while \(P<0.01\) for TC level for GLB treated groups; \(P<0.001\) for all parameters in combined drug treated groups).

*Liver tissue (Figs. 53 & 54)*

As observed earlier, DEX-treatment increased the levels of LPO, LOOH and AOPP significantly \((P<0.001\) for LPO and LOOH; \(P<0.01\) for AOPP), which were lowered following the drug administration either individually or simultaneously \((P<0.01, P<0.001\) and \(P<0.01\) for LPO, LOOH and AOPP in case of GLB alone treated group; \(P<0.001\) for LPO and LOOH, \(P<0.01\) for AOPP in case of groups treated with PM+GLB and TFG+GLB).

After the treatment of DEX, there was a significant reduction in the levels of enzymatic and non-enzymatic antioxidants such as SOD, CAT, GSH and GPx \((P<0.001\) for all). When GLB was administered alone there was a significant decrease in the levels of CAT and GPx \((P<0.001\) and \(P<0.01\) respectively). While
following the combined drug treatment, a significant decrease was noted in SOD, CAT and GSH levels ($P<0.001$ for all in case of PM+GLB treated animals; $P<0.01$, $P<0.001$ and $P<0.001$ respectively in case of TFG+GLB treated animals). No significant changes were observed in the level of GSH in any of the drug treated groups.

The hepatic glycogen content was significantly lowered in DEX-treated animals ($P<0.001$), while the administration of GLB alone or in combination with PM and TFG increased these changes induced by DEX significantly ($P<0.001$ for all).

**Kidney tissue (Figs. 55 & 56)**

DEX enhanced the peroxidative parameters such as LPO, LOOH and AOPP in kidney tissues also ($P<0.001$ for LPO and AOPP; $P<0.01$ for LOOH); while the administration of drugs either alone or in combination reversed these changes.

DEX led to a significant decrease in antioxidants like SOD, CAT and GSH ($P<0.01$, $P<0.001$ and $P<0.01$ respectively). On the administration of GLB alone and in combination with PM extract, significant elevation was found in SOD, CAT and GSH ($P<0.05$ for all three in GLB treated group; $P<0.001$ for SOD and CAT, while $P<0.01$ for GSH in case of group treated with PM and GLB both). When TFG was co-administered with GLB, a significant elevation was noticed in all four antioxidative parameters ($P<0.01$ for SOD, CAT and GSH; $P<0.05$ for GPx).
When the percent changes in the glucose level and in tissue LPO were calculated, best positive effects were observed in groups treated both with herbal and conventional drugs (Table 9).

**Experiment 14: Combined effects of *Syzygium cumini* or *Gymnema sylvestre* extract with glibenclamide on dexamethasone-induced hyperglycemic animals.**

**Glucose, insulin and serum parameters (Fig. 57)**

Following DEX-treatment the levels of glucose, TG and TC were increased ($P<0.001$); while after the treatment of drugs either individually or simultaneously, their levels were decreased significantly ($P<0.001$). The level of HDL was decreased following DEX-administration ($P<0.001$); while the test drugs were able to elevate its level significantly ($P<0.01$ in case of GLB treated group; $P<0.001$ in case of GS+GLB and SC+GLB treated groups).

**Liver tissue (Figs. 58 & 59)**

Following DEX-administration there was a significant elevation in the levels of LPO, LOOH and AOPP ($P<0.001$). After GLB treatment alone and its co-treatment with GS or SC, a significant reduction in these parameters was observed ($P<0.001$ for LPO and LOOH in case of all three groups; $P<0.05$, $P<0.001$ and $P<0.01$ for GLB, GS+GLB and SC+GLB treated groups respectively).

A significant reduction in antioxidative parameters such as SOD, CAT, GSH and GPx was observed in DEX-treated animals ($P<0.001$ for SOD and CAT; $P<0.05$ for GSH;
On administration of GLB a significant increase was noticed only in the levels of CAT and GPx ($P<0.001$ and $P<0.05$ respectively). Following the co-treatment of GS and GLB, significant elevation was seen in all 4 indices ($P<0.01$, $P<0.001$, $P<0.05$ and $P<0.001$ for SOD, CAT, GSH and GPx respectively); while after the co-administration of SC and GLB, significant increase was noted in SOD, CAT and GPx ($P<0.001$ for all). No significant change was observed in GSH level.

The glycogen content was reduced significantly after DEX administration, while the changes were reversed following drug treatment ($P<0.001$ for all).

**Kidney tissue (Figs. 60 & 61)**

Here also DEX-administration led to a significant elevation in antioxidative parameters ($P<0.001$ for LPO and AOPP; $P<0.01$ for LOOH). When GLB was administered alone a significant reduction was observed in all three parameters ($P<0.001$ for LPO and AOPP; $P<0.01$ for LOOH). However, after combined drug treatment, a marked reduction was observed in all ($P<0.001$).

Following DEX-treatment, a significant decrease in most antioxidative parameters was observed ($P<0.001$). These conditions were reversed when GLB was administered either alone or in combination with GS or SC extracts. No significant changes in any of the groups were noticed with respect to GPx level.

When the percent changes in the glucose level and in tissue LPO were calculated, best positive effects were observed in groups treated both with herbal and conventional drugs (Table 10).
Experiment 15: Effects of combined administration of *Trigonella foenum graecum* or *Pterocarpus marsupium* or *Gymnema sylvestre* extract with metformin on dexamethasone-induced hyperglycemic animals.

**Glucose, insulin and other serum parameters (Fig. 62)**

With respect to serum parameters a significant elevation was seen in glucose, TC and TG levels with a parallel decrease HDL level ($P<0.001$ for all) for DEX-treated animals. Following the treatment of drugs either alone or in combination there was a marked reduction in the levels of glucose, TC and TG levels ($P<0.001$ for all). When MET was administered to DEX-induced diabetic animals, HDL level was improved significantly ($P<0.001$). Following the combined treatment of drugs, the HDL level was markedly elevated ($P<0.001$ for HDL level in all combined drug treated groups).

**Liver tissue (Figs. 63 & 64)**

On administration of DEX to normal mice, there was a significant elevation in the levels of peroxidative parameters like LPO, LOOH and AOPP ($P<0.001$ for LPO and LOOH; $P<0.05$ for AOPP). On the contrary these changes were reversed following the treatment to MET either alone or in combination with TFG/PM/GS extract. MET alone treatment did not bring any significant reduction in the level of AOPP.

In case of antioxidative enzymatic and non-enzymatic parameters, marked reduction was observed after the treatment of DEX ($P<0.001$ for SOD, CAT and GSH; $P<0.05$ for GPx). As expected, their levels were increased significantly after the treatment of MET ($P<0.001$ for SOD, CAT and GSH), TFG+MET ($P<0.001$ for SOD, CAT and
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GSH; $P<0.05$ for GPx), PM+MET and GS+MET ($P<0.001$ for SOD, CAT and GSH; $P<0.01$ for GPx).

As observed earlier the hepatic glycogen content was lowered significantly after the treatment of DEX ($P<0.001$), while administration of drugs either individually or simultaneously significantly elevated it ($P<0.001$ for all).

**Kidney tissue (Figs. 65 & 66)**

Here also DEX led to a marked elevation in the levels of peroxidative markers ($P<0.001$, $P<0.05$ and $P<0.01$ for LPO, LOOH and AOPP respectively); while simultaneous administration of MET significantly lowered their levels ($P<0.001$, $P<0.05$ and $P<0.01$ for LPO, LOOH and AOPP respectively). However, an additional and marked reduction was observed when MET was combined with TFG extract ($P<0.001$ for all); PM and GS extracts ($P<0.001$ for LPO and AOPP; $P<0.01$ for LOOH).

With respect to antioxidative parameters, a significant reduction was noticed after DEX-treatment ($P<0.01$ for SOD; $P<0.001$ for CAT, GSH and GPx). When MET was administered to DEX-induced diabetic mice there was a significant elevation in the levels of SOD, CAT and GSH ($P<0.05$, $P<0.01$ and $P<0.001$ respectively). Co-treatment of MET with TFG, PM and GS extracts led to a significant elevation in all the antioxidative parameters ($P<0.001$ for all the three groups in SOD, CAT and GSH; $P<0.01$ for GPx level).
When the percent changes in the glucose level and in tissue LPO were calculated, best positive effects were observed in groups treated with both the herbal and conventional drugs (Table 11).

**Experiment 16: Combined effects of *Syzygium cumini* or *Momordica charantia* extract with metformin on dexamethasone-induced hyperglycemic animals.**

**Glucose, insulin and other serum parameters (Fig. 67)**

DEX markedly elevated the levels of serum glucose, TC and TG \( (P<0.001) \); while the administration of drugs either individually or in combination significantly decreased the levels of these parameters \( (P<0.001) \). HDL level was reduced significantly on DEX-treatment \( (P<0.001) \); while treatment with MET significantly elevated its level \( (P<0.05) \). However, a marked elevation was observed following the administration of MET along with SC or MC extract \( (P<0.001) \).

**Liver tissue (Figs. 68 & 69)**

With respect to hepatic peroxidative parameters, significant elevation was observed after DEX-administration \( (P<0.001) \) with a concomitant decrease following the administration of MET alone \( (P<0.001 \text{ for LPO and LOOH}; P<0.01 \text{ for AOPP}) \) and in combination with SC and MC \( (P<0.001) \).

In case of antioxidative parameters like SOD, CAT, GSH and GPx marked reduction was noticed following DEX-treatment \( (P<0.001) \). When MET was administered alone their levels were increased significantly \( (P<0.001 \text{ for SOD, CAT and GSH;}) \).
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$P<0.05$ for GPx). However, following the administration of MET along with SC or MC extract, a marked increase was noticed ($P<0.001$ for all).

With respect to hepatic glycogen content, DEX-administration reduced its level significantly ($P<0.001$). When MET alone was administered, glycogen level was increased significantly ($P<0.05$), while after combined drug administration, a striking increase was observed in the level of glycogen ($P<0.001$ for both).

Kidney tissue (Figs. 70 & 71)

With respect to renal LPO, LOOH and AOPP, a marked elevation was observed following DEX-treatment ($P<0.001$). When MET was administered to diabetic mice there was a significant reduction in LOOH and AOPP ($P<0.05$). No significant alteration was seen in the level of LPO in MET treated group. With the combined drug administration, reduction in all three peroxidative parameters was observed ($P<0.05$, $P<0.01$ and $P<0.001$ for LPO, LOOH and AOPP respectively in case of SC+MET treated group; $P<0.05$ for LPO and LOOH, $P<0.01$ for AOPP in case of MC+MET treated group).

Following the administration of DEX, a marked reduction was noticed in the levels of antioxidative parameters ($P<0.001$); while administration of MET either alone or in combination increased the levels of these parameters induced by DEX significantly ($P<0.05$ for SOD, and CAT; $P<0.001$ for GSH in case of MET alone treated group; $P<0.001$ for SOD, CAT and GSH in case of SC+MET treated group; $P<0.001$ for SOD, CAT and GSH, $P<0.05$ for GPx in MC+MET treated group). No significant
increase in the level of GPx was noticed when MET was treated either alone or in combination with SC extract.

When the percent changes in the glucose level and in tissue LPO were calculated, best positive effects were observed in groups treated both with herbal and conventional drugs (Table 12).

5.4. Discussion

Results of the aforesaid experiments clearly reveal that the antihyperglycemic effects of test drugs were best expressed when both herbal and conventional drugs were administered in combination. It was observed that the effects of individual drugs (plant extract or conventional drug) were more or less similar. The herbal drugs or conventional drug in combination, however, showed additive effects. This appears to be a novel finding as, for the first time the combined effects of herbal and conventional drugs have been reported. Although reports are available on the antihyperglycemic and antioxidative roles of the different herbal extracts and of few allopathic medicines (Thomas et al., 1998; Akhila et al., 2010; Shetty et al., 2010; Ghasias et al., 2011; Bhujbal et al., 2012; Gholap and Kar, 2003; Rajasekaran et al., 2013), none of them are on the combined effects of both types of drugs.

While an increase in serum glucose, TC and TG levels with a parallel decrease in HDL levels was observed following DEX-administration; all these changes exerted by DEX were found to exhibit a reversed pattern following the
administration of test drugs. Interestingly, when a herbal extract as well as a conventional drug (GLB/MET) were administered together, better effects were obtained suggesting a synergistic response of the drugs.

An increase in serum glucose with no significant changes in insulin level was observed following DEX-treatment. This was quite expected as glucocorticoids are known to induce insulin resistance and that their clinical use often exacerbates diabetes (Buren et al., 2002). In fact, insulin resistance plays a major role in the onset and progression of DM (Labovitz, 2006). Although many factors cause insulin resistance (e.g., obesity, high-fat diets, insufficient exercise, stress), glucocorticoids are the most common hormones causing insulin resistance. In the present investigation we did not observe significant change in the level of insulin, but an increase in the glucose level was seen following the administration of DEX, a common glucocorticoid. These results are in accordance with the earlier findings (Buren et al., 2002).

Glucocorticoid-induced hyperglycemia is also partially due to increased hepatic glucose production. Moreover, glucocorticoids are known to inhibit insulin secretion (Lambillotte et al., 1997). Glucocorticoids also have a direct inhibitory effect on glucose-induced insulin release in the β-cells. They also potentially inhibit glucose uptake at one or more steps along the signaling pathway through which insulin stimulates glucose transport (Sakoda et al., 2000). The glucose uptake in insulin sensitive cells like muscle and fat cells is to a great extent an
insulin regulated process, mediated primarily by the facilitative glucose transport (Ranta et al., 2006; Tayade et al., 2012).

To date, two possible mechanisms underlying DEX-induced insulin resistance have been suggested. One possibility is the down regulation of insulin receptor substrate (IRS)-1 expression by DEX (as has been observed in 3T3-L1 adipocytes) because IRS-1 plays a major role in the activation of phosphatidylinositol 3-kinase (PI3-K), which is essential for glucose transporter 4 (GLUT 4) translocation (Buren et al., 2002). On the other hand, in the liver, DEX-treatment reportedly decreased IRS-1 phosphorylation and IRS-1-associated PI3-K levels despite an increased IRS-1 protein content. Taking these reports into consideration, impaired PI3-K activation may be regarded as a cause of insulin resistance. The other possibility is that DEX impairs the GLUT4 translocation step independently of insulin signaling. This possibility may be supported by evidence that glucocorticoids inhibit not only insulin-induced but also hypoxia-induced GLUT4 translocation to the cell surface in skeletal muscle (Sakoda et al., 2000).

The change in serum glucose was also supported by alterations in tissue peroxidation and antioxidant system. An increase in the levels of peroxidative markers in both liver and kidney tissues was recorded following the administration of DEX as there was an increase in the levels of LPO, LOOH and AOPP. These observations are in accordance with the earlier reports on DEX-induced oxidative stress (Jatwa and Kar, 2010; Lingaiah et al., 2012). It was also
observed that the DEX-treatment markedly decreased the levels of endogenous antioxidants such as SOD, CAT, GSH and GPx. Decline in these endogenous antioxidants was also expected as glucocorticoids are well known to alter the thiobarbituric acid (TBA)-reactants and antioxidative enzymes in different tissues. (Lingaiah et al., 2012).

One of the most frequently used biomarkers to investigate the oxidative damage on lipids is the measurement of MDA (Tiwari et al., 2013), the major LPO product, that can react with the free amino group of proteins, phospholipids and nucleic acids leading to structural modification and commonly induces dysfunctioning of immune systems (Niki et al., 2008). A high level of LPO products can also be detected in cell degradation after cell injury or disease. LPO is a free-radical chain reaction which is accelerated by reactive oxygen species (ROS). Cell membranes are phospholipid bilayers with extrinsic proteins and are direct targets of lipid oxidation. Unstable carbon radicals from fatty acids can rearrange to short alkanes and conjugated dienes which react with oxygen further to peroxyl radicals and finally by hydrogen abstraction to result in lipid hydroperoxides (Ayala et al., 2014).

Proteins are likely to be major targets, as a result of their abundance in cells (proteins compose approximately 70% of the dry mass of most cells), plasma, and most tissues, and they rapidly react both with radicals and with other oxidants. ROS can lead to oxidation of amino acid residue side chains, formation of
protein-protein cross-linkages, and oxidation of the protein backbone resulting in protein fragmentation (Bandyopadhyay et al., 1999). Therefore, the measurement of the protein oxidation is a clinically important factor for the prediction of the diseased condition. Oxidation of proteins can lead to a whole variety of amino acid modifications. Action of chloraminated oxidants, mainly hypochlorous acid and chloramines, produced by myeloperoxidase in activated neutrophils, forms dityrosine containing cross-linked protein products known as AOPPs and are also considered as bio markers to estimate the degree of oxidative modifications of proteins (Tiwari et al., 2013). All the aforesaid facts might be the cause of enhanced peroxidation by DEX in this study.

An increase in the oxidative stress is expected to decrease the antioxidative defense system of the body (McColl et al., 1997; Ergüder et al., 2006; Shradha and Sisodia, 2010). In the present study also similar observations were made and the results obtained were in accordance to this i.e. by the increase in the levels of LPO, LOOH and AOPP; there was a decrease in the levels of SOD, CAT, GSH and GPx. In fact, several enzymes have evolved in aerobic cells to overcome the damaging effects of ROS. They are significantly used to maintain the redox balance during oxidative stress and are collectively called as endogenous antioxidative enzymes. SOD, GPx and CAT are the main endogenous enzymes of all aerobic cells (Birben et al., 2012; Noori, 2012). They give protection by directly scavenging superoxide radicals and hydrogen peroxide, converting them to less reactive species. SOD catalyzes the dismutation of superoxide radical (\(\bullet O_2^-\))
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to hydrogen peroxide (H$_2$O$_2$). Although H$_2$O$_2$ is not a radical, it is rapidly converted by fenton reaction into •OH radical which is very reactive (Bourg, 2005; Naso et al., 2011). Among various antioxidant mechanisms in the body, SOD is thought to be one of the major enzymes that protect cells from ROS. The liver, in particular, is very high in SOD. Cellular concentration of SOD relative to metabolic activity is a very good lifespan predictor of animal species (Pandey and Rizvi, 2010; Noori, 2012).

GSH is a major intracellular non-protein -SH compound and is accepted as the most important intracellular hydrophilic antioxidant. GSH has many biological functions, including nutrient metabolism, and regulation of cellular events such as gene expression, deoxy ribonucleic acid (DNA) and protein synthesis, cell proliferation and apoptosis, signal transduction, cytokine production and immune response, and protein glutathionylation. GSH also plays a role in maintenance of membrane protein -SH groups in the reduced form, the oxidation of which can otherwise cause altered cellular structure and function. Glutathione deficiency has been reported in many diseases such as Kwashiorkor, Alzheimer disease, Parkinson’s disease, liver disease, cystic fibrosis, sickle cell anemia, acquired immune deficiency syndrome (AIDS), cancer, heart attack, stroke and diabetes. Glutathione system is the most important endogenous defense system against oxidative stress in body. Under oxidative conditions GSH is reversibly oxidized to glutathione disulfide (GSSG). GSH plays a key role in protecting cells against electrophiles and free radicals (INOUE, 2001; Masella et al., 2005; Pandey and Rizvi, 2010).
GPx neutralizes hydrogen peroxide by taking hydrogens from two GSH molecules resulting in two water molecules (H₂O) and one GSSG. The enzyme glutathione reductase then regenerates GSH from GSSG with nicotinamide adenine dinucleotide phosphate (NADPH) as a source of hydrogen (Masella et al., 2005). Another important part of the enzymatic defense system is CAT. It is one of the most active catalysts produced by nature. It is largely, but not exclusively, localized in peroxisomes, wherein many H₂O₂ producing enzymes reside. Thus CAT, which exhibits a high Km (Michaelis constant, substrate concentration at half maximum velocity) for H₂O₂, can act upon H₂O₂ produced before it diffuses to other parts of the cell. CAT is a tetrameric heme containing enzyme that is found in all aerobic organisms. Because of its wide distribution, evolutionary conservation and capacity to rapidly degrade hydrogen peroxide, it has been proposed that CAT plays an important role in systems which have evolved to allow organisms to live in aerobic environments. (Pandey and Rizvi, 2010; Newsholme et al., 2012).

In this study, the treatment with test drugs indicated the protection from the oxidative stress induced by DEX. It was also revealed that the antihyperglycemic and anti-peroxidative effects of the drugs were best expressed in combination of two types of drugs as observed earlier (Kim et al., 2004; Gao and Hu, 2006). Although, the effects of individual drugs (conventional or herbal) were more or less similar, the beneficial responses in combination were additive as observed by few others (Ahmed et al., 1997; Sultana et al., 2008).
While a good number of reports are available on herbal formulations on the regulation of DM and LPO (Mutalik et al., 2005; Bhujbal et al., 2011; Dwivedi et al., 2013), on the combined effect of a herbal drug and a conventional drug, investigations are extremely limited (Bugudare et al., 2011; Vifhya Sagar et al., 2011). Practically not even a single one on corticosteroid-induced diabetes is there. Here, for the first time it is reported that the synergistic beneficial effect is possible when a herbal extract and a conventional drug are administered together for the treatment of type 2 diabetes.

The observations that the combined effects of the drugs were more beneficial as compared to individual drug treatment were also reflected by the percent changes. Administration of MC extract alone indicated a marked decrease in serum glucose level and in liver and kidney LPO with a percent of 33, 44 and 53% respectively. Similarly, when GLB was administered the percent decreases were 39, 62 and 47% respectively. Interestingly, when both the drugs were co-administered the beneficial effects were found to be more pronounced in terms of percent decrease i.e. 48, 80 and 71% for serum glucose and in liver and kidney LPO, clearly suggesting the better effects of combined treatment.

When TFG or PM extract was combined with GLB the percent decrease was more for serum glucose level and in liver and kidney LPO (i.e. 52, 58 and 81% respectively for PM+GLB treated group; 48, 56 and 66% respectively for TFG+GLB treated group), in contrast to GLB alone treated group where percent decrease was 43, 37 and 56% respectively.
The additional benefits were also noticed when GS or SC were co-treated with GLB. When GLB was administered alone the percent decreases in serum glucose level and in liver and kidney LPO were 43, 36 and 41% respectively. However, on treatment of GLB with GS or SC extracts, the percent decrease was more, it was 51, 58 and 67% respectively for GS+GLB treated group and 49, 57 and 58% respectively for SC+GLB treated group, clearly indicating the synergistic effects of drugs.

Similarly following the treatment of TFG+MET the percent decrease was 54, 84 and 51%; for PM+MET treated group, it was 53, 87 and 59%; for GS+MET, it was 49, 88 and 45% as against MET alone treated group; where the percent decrease was 43, 80 and 26%, for serum glucose level and for liver and kidney LPO respectively.

Almost similar effects were noticed when MC or SC extracts were administered along with MET. When MET was given to diabetic mice the percent decreases for serum glucose and for liver and kidney LPO were 41, 80 and 29% respectively; while it was 48, 90 and 44% respectively when SC was given along with MET and 48, 90 and 48% respectively when MC extract was given along with MET. Thus, test plant extracts along with MET had greater effect always.

In the present investigation, interestingly the combined administration of a herbal and conventional medicine showed synergistic action. This augmented activity may be through increasing the rate of absorption of allopathic drugs, suggesting that the combination may enhance the therapeutic value (Vidhya Sagar et al., 2011). Through the combined therapy, it may also be possible that the dose of conventional medicines
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can be reduced to maintain glucose homeostasis. In fact, it has already been reported that the co-administration of GLB with TFG increased the bioavailability of GLB, which may be one of the reasons responsible for the synergistic action in our study (Al-Ajmi, 2011).

Whatever may be the reason for the effects obtained in this study, it was clear that the co-treatment of herbal drugs with either of the allopathic medicines (GLB or MET) produced synergistic and additional benefits as compared to their individual treatment.

This may be emphasized that, despite a good number of reports available on herbal formulations on the regulation of DM and LPO; investigations are extremely limited on the combined effects of a herbal and conventional drugs. Practically not even a single one is there on corticosteroid-induced DM. For the first time it was observed that the co-treatment of a herbal extract and a conventional drug may produce synergistic beneficial effects against corticosteroid-induced DM (Sharma et al., 2014).

Further, when the results of the previous and present experiments were combined together, out of all five herbal extracts TFG and PM were found to be more effective. Keeping these observations in mind, it appears to be interesting to study the combined effects of plant with either of the conventional drugs against both ALX and DEX-induced diabetic mice.

Some of the findings have been published (Sharma et al, 2014b)
Table 8: Relative percent decrease in hepatic & renal lipid peroxidation (LPO) and serum glucose level as compared to the respective values of dexamethasone (DEX)-treated animals following the administration of glibenclamide (GLB) and *Momordica charantia* (MC) extract, either alone or in combination.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DEX+MC</th>
<th>DEX+GLB</th>
<th>DEX+MC+GLB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver LPO</td>
<td>44%</td>
<td>62%</td>
<td>80%</td>
</tr>
<tr>
<td>Kidney LPO</td>
<td>53%</td>
<td>47%</td>
<td>71%</td>
</tr>
<tr>
<td>Serum glucose</td>
<td>33%</td>
<td>39%</td>
<td>48%</td>
</tr>
</tbody>
</table>

Table 9: Relative percent decrease in hepatic & renal lipid peroxidation (LPO) and serum glucose level as compared to the respective values of dexamethasone (DEX)-treated animals following the administration of glibenclamide (GLB), either alone or in combination with *Pterocarpus marsupium* (PM)/*Trigonella foenum graecum* (TFG) extracts.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DEX+GLB</th>
<th>DEX+GLB+PM</th>
<th>DEX+GLB+TFG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver LPO</td>
<td>37%</td>
<td>58%</td>
<td>56%</td>
</tr>
<tr>
<td>Kidney LPO</td>
<td>56%</td>
<td>81%</td>
<td>66%</td>
</tr>
<tr>
<td>Serum glucose</td>
<td>43%</td>
<td>52%</td>
<td>48%</td>
</tr>
</tbody>
</table>

Table 10: Relative percent decrease in hepatic & renal lipid peroxidation (LPO) and serum glucose level as compared to the respective values of dexamethasone (DEX)-treated animals following the administration of glibenclamide (GLB), either alone or in combination with *Gymnema sylvestre* (GS)/*Syzygium cumini* (SC) extracts.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DEX+GLB</th>
<th>DEX+GLB+GS</th>
<th>DEX+GLB+SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver LPO</td>
<td>36%</td>
<td>58%</td>
<td>57%</td>
</tr>
<tr>
<td>Kidney LPO</td>
<td>41%</td>
<td>67%</td>
<td>58%</td>
</tr>
<tr>
<td>Serum glucose</td>
<td>43%</td>
<td>51%</td>
<td>49%</td>
</tr>
</tbody>
</table>
Table 11: Relative percent decrease in hepatic & renal lipid peroxidation (LPO) and serum glucose level as compared to the respective values of dexamethasone (DEX)-treated animals following the administration of metformin (MET), either alone or in combination with *Trigonella foenum graecum* (TFG)/*Pterocarpus marsupium* (PM)/*Gymnema sylvestre* (GS) extracts.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DEX+MET</th>
<th>DEX+MET+TFG</th>
<th>DEX+MET+PM</th>
<th>DEX+MET+GS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver LPO</td>
<td>80%</td>
<td>84%</td>
<td>87%</td>
<td>88%</td>
</tr>
<tr>
<td>Kidney LPO</td>
<td>26%</td>
<td>51%</td>
<td>59%</td>
<td>45%</td>
</tr>
<tr>
<td>Serum glucose</td>
<td>43%</td>
<td>54%</td>
<td>53%</td>
<td>49%</td>
</tr>
</tbody>
</table>

Table 12: Relative percent decrease in hepatic & renal lipid peroxidation (LPO) and serum glucose level as compared to the respective values of dexamethasone (DEX)-treated animals following the administration of metformin (MET), either alone or in combination with *Syzygium cumini* (SC)/*Momordica charantia* (MC) extracts.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DEX+MET</th>
<th>DEX+MET+SC</th>
<th>DEX+MET+MC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver LPO</td>
<td>80%</td>
<td>90%</td>
<td>90%</td>
</tr>
<tr>
<td>Kidney LPO</td>
<td>29%</td>
<td>44%</td>
<td>48%</td>
</tr>
<tr>
<td>Serum glucose</td>
<td>41%</td>
<td>48%</td>
<td>48%</td>
</tr>
</tbody>
</table>
Figure 47. Changes in the concentrations of serum glucose (mg/dl), insulin (µ IU/ml), triglyceride (TG, mg/dl), total cholesterol (TC, mg/dl) and high density lipoprotein cholesterol (HDL, mg/dl) following the administration of *Momordica charantia* (MC) extract and glibenclamide (GLB) alone or in combination on dexamethasone (DEX)-induced diabetic animals. Each bar represents the mean ±SE (n=7) \(^a\), \(P<0.001\) as compared to respective control values and \(^x\), \(P<0.001\), and \(^y\), \(P<0.01\) as compared to respective values of DEX-induced animals.

Figure 48. Changes in the hepatic lipid peroxidation (LPO, nm MDA formed/hr/mg protein), lipid hydroperoxide (LOOH, nm LOOH formed/mg protein) and advanced oxidation protein products (AOPPs, µm/L) following the administration of *Momordica charantia* (MC) extract and glibenclamide (GLB) alone or in combination on dexamethasone (DEX)-induced diabetic animals. Each bar represents the mean±SE (n=7) \(^a\), \(P<0.001\) and \(^b\), \(P<0.01\) as compared to respective control values and \(^x\), \(P<0.001\), \(^y\), \(P<0.01\) and \(^z\), \(P<0.05\) as compared to respective values of DEX-induced animals.
Figure 49. Changes in the hepatic superoxide dismutase (SOD, units/mg protein), catalase (CAT, μM H₂O₂ decomposed/min/mg protein), reduced glutathione (GSH, μM GSH/mg protein), glutathione peroxidase (GPx, μM GSH oxidized/mg protein) activities and glycogen content (mg/100gm tissue) following the administration of *Momordica charantia* (MC) extract and glibenclamide (GLB) alone or in combination on dexamethasone (DEX)-induced diabetic animals. Each bar represents the mean ± SE (n=7) a, P<0.001 and b, P<0.01 as compared to respective control values and x, P<0.001, y, P<0.01 and z, P<0.05 as compared to respective values of DEX-induced animals.

Figure 50. Changes in the renal lipid peroxidation (LPO, nm MDA formed/hr/mg protein), lipid hydroperoxide (LOOH, nm LOOH formed/mg protein) and advanced oxidation protein products (AOPPs, μM/L) following the administration of *Momordica charantia* (MC) extract and glibenclamide (GLB) alone or in combination on dexamethasone (DEX)-induced diabetic animals. Each bar represents the mean ± SE (n=7) a, P<0.001 as compared to respective control values and x, P<0.001 and y, P<0.01 as compared to respective values of DEX-induced animals.
**Figure 51.** Changes in the renal superoxide dismutase (SOD, units/mg protein), catalase (CAT, \( \mu \)M H\(_2\)O\(_2\) decomposed/min/mg protein), reduced glutathione (GSH, \( \mu \)m GSH/mg protein) and glutathione peroxidase (GPx, \( \mu \)m GSH oxidized/mg protein) activities following the administration of *Momordica charantia* (MC) extract and glibenclamide (GLB) alone or in combination on dexamethasone (DEX)-induced diabetic animals.

Each bar represents the mean±SE (n=7) \(^a\), \( P<0.001 \) as compared to respective control values and \(^x\), \( P<0.001 \) as compared to respective values of DEX-induced animals.

**Figure 52.** Changes in the concentrations of serum glucose (mg/dl), insulin (\( \mu \)IU/ml), triglyceride (TG, mg/dl), total cholesterol (TC, mg/dl) and high density lipoprotein cholesterol (HDL, mg/dl) following the administration of *Trigonella foenum graecum* (TFG)*/Pterocarpus marsupium* (PM) extract and glibenclamide (GLB) alone or in combination on dexamethasone (DEX)-induced diabetic animals.

Each bar represents the mean±SE (n=7) \(^a\), \( P<0.001 \) as compared to respective control values and \(^x\), \( P<0.001 \) as compared to respective values of DEX-induced animals.
Figure 53. Changes in the hepatic lipid peroxidation (LPO, nm MDA formed/hr/mg protein), lipid hydroperoxide (LOOH, nm LOOH formed/mg protein) and advanced oxidation protein products (AOPPs, μm/L) following the administration of *Trigonella foemum graecum* (TFG)/*Pterocarpus marsupium* (PM) extract and glibenclamide (GLB) alone or in combination on dexamethasone (DEX)-induced diabetic animals.

Each bar represents the mean±SE (n=7) \(^a\), \(P<0.001\) and \(^b\), \(P<0.01\) as compared to respective control values and \(^x\), \(P<0.001\) and \(^y\), \(P<0.01\) as compared to respective values of DEX-induced animals.

Figure 54. Changes in the hepatic superoxide dismutase (SOD, units/mg protein), catalase (CAT, μM \(H_2O_2\) decomposed/min/mg protein), reduced glutathione (GSH, μm GSH/mg protein), glutathione peroxidase (GPx, μm GSH oxidized/mg protein) activities and glycogen content (mg/100gm tissue) following the administration of *Trigonella foemum graecum* (TFG)/*Pterocarpus marsupium* (PM) extract and glibenclamide (GLB) alone or in combination on dexamethasone (DEX)-induced diabetic animals.

Each bar represents the mean±SE (n=7) \(^a\), \(P<0.001\) as compared to respective control values and \(^x\), \(P<0.001\) and \(^y\), \(P<0.01\) as compared to respective values of DEX-induced animals.
Figure 55. Changes in the renal lipid peroxidation (LPO, nm MDA formed/hr/mg protein), lipid hydroperoxide (LOOH, nm LOOH formed/mg protein) and advanced oxidation protein products (AOPPs, μM/L) following the administration of Trigonella foenum graecum (TFG)/Pterocarpus marsupium (PM) extract and glibenclamide (GLB) alone or in combination on dexamethasone (DEX)-induced diabetic animals.

Each bar represents the mean±SE (n=7) a, P<0.001 and b, P<0.01 as compared to respective control values and x, P<0.001 as compared to respective values of DEX-induced animals.

Figure 56. Changes in the renal superoxide dismutase (SOD, units/mg protein), catalase (CAT, μM H₂O₂ decomposed/min/mg protein), reduced glutathione (GSH, μg GSH/mg protein) and glutathione peroxidase (GPx, μg GSH oxidized/mg protein) activities following the administration of Trigonella foenum graecum (TFG)/Pterocarpus marsupium (PM) extract and glibenclamide (GLB) alone or in combination on dexamethasone (DEX)-induced diabetic animals.

Each bar represents the mean±SE (n=7) a, P<0.001 and b, P<0.01 as compared to respective control values and b, P<0.001, z, P<0.01 and z, P<0.05 as compared to respective values of DEX-induced animals.
Figure 57. Changes in the concentrations of serum glucose (mg/dl), insulin (µU/ml), triglyceride (TG, mg/dl), total cholesterol (TC, mg/dl) and high density lipoprotein cholesterol (HDL, mg/dl) following the administration of Gymnema sylvestre (GS)/Syzygium cumini (SC) extract and glibenclamide (GLB) alone or in combination on dexamethasone (DEX)-induced diabetic animals. Each bar represents the mean±SE (n=7) a, P<0.001 as compared to respective control values and x, P<0.001 and y, P<0.01 as compared to respective values of DEX-induced animals.

Figure 58. Changes in the hepatic lipid peroxidation (LPO, nm MDA formed/hr/mg protein), lipid hydroperoxide (LOOH, nm LOOH formed/mg protein) and advanced oxidation protein products (AOPPs, µm/L) following the administration of Gymnema sylvestre (GS)/Syzygium cumini (SC) extract and glibenclamide (GLB) alone or in combination on dexamethasone (DEX)-induced diabetic animals. Each bar represents the mean±SE (n=7) a, P<0.001 as compared to respective control values and x, P<0.001, y, P<0.01 and z, P<0.05 as compared to respective values of DEX-induced animals.
Figure 59. Changes in the hepatic superoxide dismutase (SOD, units/mg protein), catalase (CAT, μM H2O2 decomposed/min/mg protein), reduced glutathione (GSH, μM GSH/mg protein), glutathione peroxidase (GPx, μM GSH oxidized/mg protein) activities and glycogen content (mg/100gm tissue) following the administration of Gymnema sylvestre (GS)/Syzygium cumini (SC) extract and glibenclamide (GLB) alone or in combination on dexamethasone (DEX)-induced diabetic animals. Each bar represents the mean±SE (n=7) a, P<0.001, b, P<0.01 and c, P<0.05 as compared to respective control values and a, P<0.001, b, P<0.01 and c, P<0.05 as compared to respective values of DEX-induced animals.

Figure 60. Changes in the renal lipid peroxidation (LPO, nm MDA formed/hr/mg protein), lipid hydroperoxide (LOOH, nm LOOH formed/mg protein) and advanced oxidation protein products (AOPPs, μM/L) following the administration of Gymnema sylvestre (GS)/Syzygium cumini (SC) extract and glibenclamide (GLB) alone or in combination on dexamethasone (DEX)-induced diabetic animals. Each bar represents the mean±SE (n=7) a, P<0.001 and b, P<0.01 as compared to respective control values and a, P<0.001 and b, P<0.01 as compared to respective values of DEX-induced animals.
Figure 61. Changes in the renal superoxide dismutase (SOD, units/mg protein), catalase (CAT, μM H₂O₂ decomposed/min/mg protein), reduced glutathione (GSH, μm GSH/mg protein) and glutathione peroxidase (GPx, μm GSH oxidized/mg protein) activities following the administration of Gymnema sylvestre (GS)/Syzygium cumini (SC) extract and glipizide (GLB) alone or in combination on dexamethasone (DEX)-induced diabetic animals.

Each bar represents the mean±SE (n=7) a, P<0.001 as compared to respective control values and x, P<0.001 and y, P<0.01 as compared to respective values of DEX-induced animals.

Figure 62. Changes in the concentrations of serum glucose (mg/dl), insulin (μIU/ml), triglyceride (TG, mg/dl), total cholesterol (TC, mg/dl) and high density lipoprotein cholesterol (HDL, mg/dl) following the administration of Trigonella foenum graecum (TFG)/Pterocarpus marsupium (PM)/Gymnema sylvestre (GS) extract and metformin (MET) alone or in combination on dexamethasone (DEX)-induced diabetic animals.

Each bar represents the mean±SE (n=7) a, P<0.001 as compared to respective control values and x, P<0.001 as compared to respective values of DEX-induced animals.
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Figure 63. Changes in the hepatic lipid peroxidation (LPO, nm MDA formed/hr/mg protein), lipid hydroperoxide (LOOH, nm LOOH formed/mg protein) and advanced oxidation protein products (AOPPs, μm/L) following the administration of *Trigonella foenum graecum* (TFG)/*Pterocarpus marsupium* (PM)/*Gymnema sylvestre* (GS) extract and metformin (MET) alone or in combination on dexamethasone (DEX)-induced diabetic animals. Each bar represents the mean±SE (n=7) a, P<0.001 and c, P<0.05 as compared to respective control values and x, P<0.001, y, P<0.01 and z, P<0.05 as compared to respective values of DEX-induced animals.

Figure 64. Changes in the hepatic superoxide dismutase (SOD, units/mg protein), catalase (CAT, μM H₂O₂ decomposed/min/mg protein), reduced glutathione (GSH, μm GSH/mg protein), glutathione peroxidase (GPx, μm GSH oxidized/mg protein) activities and glycogen content (mg/100gm tissue) following the administration of *Trigonella foenum graecum* (TFG)/*Pterocarpus marsupium* (PM)/*Gymnema sylvestre* (GS) extract and metformin (MET) alone or in combination on dexamethasone (DEX)-induced diabetic animals. Each bar represents the mean±SE (n=7) a, P<0.001 and c, P<0.05 as compared to respective control values and x, P<0.001, y, P<0.01 and z, P<0.05 as compared to respective values of DEX-induced animals.
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**Figure 65.** Changes in the renal lipid peroxidation (LPO, nm MDA formed/hr/mg protein), lipid hydroperoxide (LOOH, nm LOOH formed/mg protein) and advanced oxidation protein products (AOPPs, μm/L) following the administration of *Trigonella foenum graecum* (TFG)/*Pterocarpus marsupium* (PM)/*Gymnema sylvestre* (GS) extract and metformin (MET) alone or in combination on dexamethasone (DEX)-induced diabetic animals.

Each bar represents the mean±SE (n=7) \(^a, P<0.001\), \(^b, P<0.01\) and \(^c, P<0.05\) as compared to respective control values and \(^x, P<0.001\), \(^y, P<0.01\) and \(^z, P<0.05\) as compared to respective values of DEX-induced animals.

**Figure 66.** Changes in the renal superoxide dismutase (SOD, units/mg protein), catalase (CAT, μM H₂O₂ decomposed/min/mg protein), reduced glutathione (GSH, μm GSH/mg protein) and glutathione peroxidase (GPx, μm GSH oxidized/mg protein) activities following the administration of *Trigonella foenum graecum* (TFG)/*Pterocarpus marsupium* (PM)/*Gymnema sylvestre* (GS) extract and metformin (MET) alone or in combination on dexamethasone (DEX)-induced diabetic animals.

Each bar represents the mean±SE (n=7) \(^a, P<0.001\) and \(^b, P<0.01\) as compared to respective control values and \(^x, P<0.001\), \(^y, P<0.01\) and \(^z, P<0.05\) as compared to respective values of DEX-induced animals.
Figure 67. Changes in the concentrations of serum glucose (mg/dl), insulin (μIU/ml), triglyceride (TG, mg/dl), total cholesterol (TC, mg/dl) and high density lipoprotein cholesterol (HDL, mg/dl) following the administration of Syzygium cumini (SC)/Momordica charantia (MC) extract and metformin (MET) alone or in combination on dexamethasone (DEX)-induced diabetic animals. Each bar represents the mean±SE (n=7) \( a, P<0.001 \) as compared to respective control values and \( x, P<0.05 \) as compared to respective values of DEX-induced animals.

Figure 68. Changes in the hepatic lipid peroxidation (LPO, nm MDA formed/hr/mg protein), lipid hydroperoxide (LOOH, nm LOOH formed/mg protein) and advanced oxidation protein products (AOPPs, μm/L) following the administration of Syzygium cumini (SC)/Momordica charantia (MC) extract and metformin (MET) alone or in combination on dexamethasone (DEX)-induced diabetic animals. Each bar represents the mean±SE (n=7) \( a, P<0.001 \) as compared to respective control values and \( x, P<0.001 \) as compared to respective values of DEX-induced animals.
Figure 69. Changes in the hepatic superoxide dismutase (SOD, units/mg protein), catalase (CAT, μM \( \text{H}_2\text{O}_2 \) decomposed/min/mg protein), reduced glutathione (GSH, μm GSH/mg protein), glutathione peroxidase (GPx, μm GSH oxidized/mg protein) activities and glycogen content (mg/100gm tissue) following the administration of Syzygium cumini (SC)/Momordica charantia (MC) extract and metformin (MET) alone or in combination on dexamethasone (DEX)-induced diabetic animals. Each bar represents the mean±SE (n=7) \( ^a, P<0.001 \) as compared to respective control values and \( ^x, P<0.001 \) and \( ^z, P<0.05 \) as compared to respective values of DEX-induced animals.

Figure 70. Changes in the renal lipid peroxidation (LPO, nm MDA formed/hr/mg protein), lipid hydroperoxide (LOOH, nm LOOH formed/mg protein) and advanced oxidation protein products (AOPPs, μm/L) following the administration of Syzygium cumini (SC)/Momordica charantia (MC) extract and metformin (MET) alone or in combination on dexamethasone (DEX)-induced diabetic animals. Each bar represents the mean±SE (n=7) \( ^x, P<0.001 \), \( ^y, P<0.01 \) and \( ^z, P<0.05 \) as compared to respective control values and \( ^x, P<0.001 \), \( ^y, P<0.01 \) and \( ^z, P<0.05 \) as compared to respective values of DEX-induced animals.
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Figure 71. Changes in the renal superoxide dismutase (SOD, units/mg protein), catalase (CAT, \( \mu \text{M } \text{H}_2\text{O}_2 \) decomposed/min/mg protein), reduced glutathione (GSH, \( \mu \text{m GSH/mg protein} \)) and glutathione peroxidase (GPx, \( \mu \text{m GSH oxidized/mg protein} \)) activities following the administration of *Syzygium cumini* (SC)/*Momordica charantia* (MC) extract and metformin (MET) alone or in combination on dexamethasone (DEX)-induced diabetic animals. Each bar represents the mean±SE (n=7) \(^a\), \( P<0.001 \) as compared to respective control values and \(^x\), \( P<0.001 \) and \(^z\), \( P<0.05 \) as compared to respective values of DEX-induced animals.