Chapter 8

SUMMARY, REFERENCES AND LIST OF PUBLICATIONS
8.1. Summary

Diabetes mellitus (DM) is a metabolic disorder characterized by increased blood glucose level (hyperglycemia). This is one of the most common health problems both in India and in abroad. It is considered as the five leading causes of deaths in the world. According to an estimate, 366 million people had DM in 2011 and by 2030 this would have risen to 552 million (Whiting et al., 2011). In 2013, according to International Diabetes Federation an estimated 381 million people had diabetes. WHO projects that diabetes will be the 7th leading cause of death in 2030. Thus, DM is one of the major diseases at present.

DM is usually the result of insufficient or inefficient insulin secretion / response. Sometimes it results because of insulin resistance. It can also be characterized by disturbances in carbohydrate, protein and fat metabolism (Bastaki, 2005).

With the increasing incidence of aforesaid disease throughout the world there is not only the urge for the development of alternative and safe drugs but for the development of suitable strategy for the regulation of DM. As existing treatment options are costly and have limited effects, there is a need of new medicines and new approach.

At present the treatment of DM includes use of insulin and many other hypoglycemic drugs. However, these are found to cause various side effects especially the development of resistance after a certain period of time (Stein et al., 2013). In fact, management of DM without any side effects is still a challenge to the medical system and this has led to an increasing demand for natural products with antidiabetic activity and fewer side effects.
Botanicals, conventional medicines and diabetes

(Modak et al., 2007; Khan et al., 2012). Now a days herbal therapies are preferred and are becoming increasingly popular in general patients because of their acceptability, safety and cost effectiveness. Numerous medicinal plants have been studied and some have been validated for their antihyperglycemic properties using diabetic animal models. Although many plant extracts are known to be hypoglycemic in nature (Arumugam et al., 2013; El-Abhar and Schaalan, 2014), most of them are not fast acting. Therefore, one of the approaches could be their combined effects with commonly available drugs to get a better hypoglycemic control.

Keeping this in mind, in the present dissertation an attempt has been made to evaluate the combined effects of some antidiabetic herbs and some conventional antidiabetic drugs, if any.

Five indigenous herbs that are commonly considered as antihyperglycemic were selected in the present study. These were: *Momordica charantia*, *Gymnema sylvestre*, *Syzigium cumini*, *Pterocarpus marsupium* and *Trigonella foenum graecum* (in Hindi, Karela, Gudmar, Jamun, Vijaysar and Methi respectively). Metformin and glibenclamide were the two antihyperglycemic drugs selected for the study which are very commonly prescribed against DM.

Swiss colony bred albino male mice weighing 28±2 were primarily used in most experiments. They were housed in a room maintained at 27±1°C and 10 h light; 14 h dark photo schedule with the provision of mice feed and water *ad libitum*. Animals were handled in accordance with guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Govt. of India, India and of the...
in institutional ethical committee (Reg. No 779) of Devi Ahilya University, Indore, India. Swiss colony bred albino male mice weighing 28±2 were housed in a room maintained at 27±1°C and 10 h light; 14 h dark photo schedule with the provision of mice feed and water ad libitum.

The entire dissertation work was carried out in six major sections:

**Section A: Revalidation of the test herbal extracts for their potential to regulate hyperglycemia in alloxan- and dexamethasone-induced diabetic animals**

Under this section the test extracts were revalidated for their antihyperglycemic activity.

Following are the concentrations of the test plant extracts which were reported earlier:

1. *Momordica charantia* (MC) – 150 mg/kg (Fernandes et al., 2007; Tripathi & Chandra, 2010)

2. *Gymnema sylvestre* (GS) – 600 mg/kg – (Gholap and Kar, 2003; Mall et al., 2009).

3. *Syzygium cumini* (SC) – 100 mg/kg – (Prince et al., 2004; Farswan et al., 2009).

4. *Pterocarpus marsupium* (PM) – 150 mg/kg (Dhanbadal et al., 2006; Maruthupandian & Mohan, 2011).

5. *Trigonella foenum graecum* (TFG) – 100 mg/kg (Eidi et al., 2004; Mowla et al., 2009).
Considering these doses following experiments were performed.

**Experiment 1: Effects of TFG, MC and GS on alloxan-induced diabetic animals**

Type I diabetes was induced by alloxan monohydrate at a single pre-standardized dose of 150 mg/kg body weight (Yan et al., 2009). In this experiment effects of three plant extracts such as TFG, MC and GS at the above mentioned doses were treated for 15 days in alloxan-induced hyperglycemic mice and at the end animals were sacrificed under mild anesthesia. Serum and liver were collected from each animal (section B). Serum glucose and lipid peroxidation (LPO) in major target organ, liver were studied just to validate their antihyperglycemic and safe nature.

**Experiment 2: Effects of SC and PM on alloxan-induced diabetic animals**

In this experiment remaining two plant extracts, i.e. SC and PM at the above mentioned doses were considered and the experiment was repeated to see the effects on the alterations in serum glucose and liver LPO in alloxan induced hyperglycemic mice.

**Experiment 3: Effects of TFG, MC and GS on dexamethasone-induced diabetic animals**

Type-II diabetes was induced by dexamethasone. Mice were rendered hyperglycemic by daily administration of dexamethasone (intramuscular, i.m.) at a pre-standardized dose of 1 mg/kg body weight for 7 days (Gholap & Kar, 2003). Then, after dividing animals in different groups, drugs such as TFG, MC and GS were administered for 15 days to test their anti-hyperglycemic efficacy in mice.
Experiment 4: Effects of SC and PM on dexamethasone-induced diabetic animals

In this experiment effect of remaining two plants, i.e. SC and PM were observed against dexamethasone induced hyperglycemia in mice, considering the same duration and parameters as in experiment 3.

Results: Alloxan/dexamethasone administration increased serum glucose concentration and tissue LPO. However, results of different experiments revealed varied potencies of different plant extracts on alloxan or dexamethasone-induced hyperglycemic animals. Although, all were found to be antidiabetic in nature, differences in the extent of their antihyperglycemic and antioxidative activities between these plant extracts were noticed. The order of the effects and exerted by different plant extracts in alloxan-induced animals was as follows.

PM > TFG > MC > GS > SC

Evaluation of the five herbal extracts for their ameliorating/preventive properties on the chemically induced animal models of type 2 diabetes was done using dexamethasone as hyperglycemic agent. The findings suggested that all five herbal extracts are effective in ameliorating corticosteroid-induced hyperglycemia as in alloxan induced diabetes. However, differential effects were found in these plant extracts and the order of the effects exerted by different plant extracts were as follows:

TFG > PM > MC > GS > SC
Section B: Combined effects of different test herbal extracts and conventional medicines in alloxan-induced diabetic animals.

In this section, combined effects of one of the test extracts and an established allopathic medicine (metformin or glibenclamide) were evaluated. Laboratory animals were rendered hyperglycemic and were treated with one plant extract along with a conventional antidiabetic drug. All the five test extracts were combined individually with either metformin or glibenclamide. Each experiment was continued for 15 days.

On the last day of experiment animals were sacrificed by cervical dislocation under mild anesthesia, blood was collected and centrifuged to get a clear serum. Organs were excised, cleaned and washed with phosphate buffered saline and then processed for further biochemical estimations. In serum concentration of glucose, insulin, total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL) were estimated; while in different tissues, lipid peroxidation (LPO), lipid hydroperoxide (LOOH), advanced oxidation protein products (AOPP) and different cellular antioxidants such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx), and hepatic glycogen content were estimated. Following experiments were conducted under this section.

Experiment 5: Combined effects of TFG and glibenclamide in alloxan-induced diabetic animals.

In this experiment mice were made hyperglycemic by injecting alloxan at a pre-standardized dose and then the effects of TFG (100 mg / kg, as mentioned earlier) or
glibenclamide (GLB) at 2 mg / kg (Ezeigbo, 2010) or 0.5 mg/kg (Ahmad et al., 2014) or TFG and GLB both together, were studied in different groups of animals for 15 days, following which alterations in different indices were studied.

On the last day of each experiment animals were sacrificed by cervical dislocation, blood was collected to get a clear serum and organs were excised, cleaned and washed with phosphate buffered saline and then processed for further biochemical estimations, including LPO, LOOH, AOPP, SOD, CAT, GSH, GPx and liver glycogen content. In addition serum glucose, insulin, TC, TG and HDL were also estimated.

**Experiment 6: Combined effects of PM and glibenclamide in alloxan-induced diabetic animals**

Here 5 groups of animals were established. While gr. 1 animals receiving only normal saline served as control, gr II to V were treated with single dose of alloxan at 150 mg/kg, following which, animals of gr. III, IV and V received PM with pre-standardized dose of 150 mg / kg, GLB at 500 µg / kg (the dose that was found to be more effective in expt. 5) or equivalent doses of PM+GLB respectively for 15 days. At the end, alterations in all the indices were studied as mentioned earlier.

**Experiment 7 to 9: Combined effects of GS or MC or SC with GLB in alloxan-induced diabetic animals**

Similar to expt. 6, three more experiments (# 7-9) were performed. However, instead of PM, GS or MC or SC were administered either alone or along with GLB to reveal the combined effects of each test plant extract and GLB, if any, in the regulation of serum
glucose, tissue lipid peroxidation, lipid hydroperoxides and other antioxidative enzyme activities against alloxan induced diabetic animals.

**Experiment 10: Combined effects of TFG or GS with metformin**

In this experiment, effects of combined therapy of extract of TFG or GS and metformin at a dose of 50 mg / kg (Meshram et al., 2013) were studied in the regulation of alloxan induced hyperglycemia and associated adverse effects.

**Experiment 11: Combined effects of SC or PM or MC with metformin**

Similar to the above experiment, effects of combined therapy of SC or PM or MC extract along with metformin were studied in the regulation of alloxan induced hyperglycemia and associated adverse effects.

**Results** of this section revealed that when individual test extracts were combined with either of the conventional drugs in alloxan induced diabetic animals; additional benefits were observed as compared to their individual effects, suggesting that the known antidiabetic herbal extract in combination with conventional medicine may provide better effectiveness in regulating diabetes mellitus (Sharma & Kar, 2014a,b; Sharma et al., 2014a;)

**Section C: Combined effects of different test herbal extract and glibenclamide or metformin in dexamethasone-induced diabetic mice**

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

**Experiment 12: Combined effects of *M. Charantia* extract and glibenclamide in dexamethasone-induced hyperglycemic mice**

In this experiment the combined efficacy of MC or GLB either alone or in combination was tested in dexamethasone induced hyperglycemic mice after 15 days treatment. All the parameters mentioned earlier were also considered in this study.

**Experiment 13 and 14: Combined effects of TFG/PM and GS/SC along with glibenclamide in dexamethasone-induced hyperglycemic mice**

In these experiments the combined effects of either TFG/PM and GS/SC along with glibenclamide was tested in dexamethasone induced hyperglycemic mice, considering all the parameters used in experiment 12.

**Experiment 15 and 16: Combined effects of TFG/PM/GS and of SC/MC along with Metformin on dexamethasone induced hyperglycemic mice.**

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

**Results** of this section revealed that when individual test extract was combined with either of the conventional drugs, additional benefits were observed even in dexamethasone-induced DM as compared to their individual effects, suggesting that the known
antidiabetic herbal extract in combination with conventional medicine may provide better
effectiveness in regulating diabetes mellitus (Sharma et al., 2014b).

Section D: Combined effects of two or more plant extracts with
glibenclamide or metformin in alloxan- or dexamethasone-induced
diabetic animals.

Here an attempt was made to evaluate the combined efficacy of the most effective test
extracts (as found in above studies) and the conventional medicines in the regulation of
diabetes mellitus.

The two herbal extracts that came out to be most effective from our earlier studies were
TFG and PM. Therefore, two herbal extracts were combined with the allopathic
medicines, metformin or glibenclamide in alloxan as well as dexamethasone-induced
diabetic animals. Experiments were continued for 15 days and the effects were studied
considering all the parameters used earlier.

Experiment 17 and 18: Combined effects TFG and PM along with glibenclamide or
metformin in alloxan-induced animals

While in experiment 17, the combined effects of TFG and PM along with glibenclamide
were investigated for their antidiabetic and antioxidative activities on alloxan induced
diabetic animals, in expt. 18 similar type of study was repeated considering metformin
instead of glibenclamide.
Experiment 19 and 20: Combined effects TFG and PM along with glibenclamide or metformin in dexamethasone-induced animals

While in experiment 19, the combined effects of TFG and PM along with glibenclamide were investigated for their antidiabetic and antioxidative activities on dexamethasone-induced diabetic animals, in expt. 20 similar type of study was repeated considering metformin instead of glibenclamide.

Results of these experiments indicated that no additional benefit was observed when the two herbal drugs were combined with either of the allopathic medicines (glibenclamide or metformin) as compared to the combination of one herbal and either of the allopathic drugs.

Section E: Combined effects of active compounds and the conventional medicine in alloxan- or dexamethasone-induced diabetic animals.

Assuming that the effects of the herbal extracts are due to the presence of active components, in this section investigations were made considering the active principles that are found to be most common in the five test plants along with metformin which appeared to be more effective as compared to glibenclamide (evident from the results of earlier experiments). This work was done in both the diabetic animal models.
Experiment 21 and 22: Combined effects of active compounds in alloxan-induced diabetic animals.

While in experiment 21, effects of quercetin at 10 mg/kg (Sanders et al., 2001) was combined with previously used dose of metformin (50 mg/kg) to find out their effects in alloxan-induced hyperglycemia; in expt. 22 similar protocol was repeated involving rutin/naringin at 50 mg/kg (Kamakakkan and Prince, 2006; Ahmad et al., 2012) instead of quercetin.

Experiment 23 and 24: Combined effects of active compounds in dexamethasone-induced diabetic animals

In experiment 23, effects of quercetin at a prestandardized dose of 10 mg/kg along with metformin were investigated in dexamethasone-induced hyperglycemia. However, in expt. 24, the active compounds used were rutin and naringin. Rest of the protocol was similar to the earlier one.

Results of this section (experiment 21-24) indicated that the combined treatment of quercetin/ rutin/ naringin with metformin could ameliorate the hyperglycemia in a better way as compared to their individual effects. When the effects of individual active compounds were compared, naringin was found to be highly effective as compared to rutin and quercetin.
Major findings:

1. The herbal drugs were found to exhibit varying effects in ameliorating hyperglycemia and the toxic effects induced by alloxan and dexamethasone.

2. Combined effects of a herbal test extract with conventional medicine (glibenclamide / metformin) brought a synergistic effects in controlling alloxan/dexamethasone induced hyperglycemic state

3. Out of the 05 herbal extracts taken in our study ie. *Momordica charantia*, *Gymnema sylvestre*, *Syzygium cumini*, *Pterocarpus marsupium* and *Trigonella foenum graecum*, the two plants extract *T. foenum graecum* and *P. marsupium* proved to be most effective against hyperglycemic state. Metformin was found to be more effective than glibenclamide.

4. No synergistic or additional benefits were noticed when two herbal extracts were combined with a conventional drug.

5. Co-treatment of active component (quercetin / rutin/ naringin) with metformin produced additional benefits as compared to their individual treatments, but only with the latter two. In fact, naringin appeared to be the most effective.
Common Active Principles
(Quercetin, Rutin & Naringin)

Most effective plants
*T. foenum graecum + P. marsupium*

Alloxan + Dexamethasone + Metformin

(Naringin - most effective)

(Similar effects as with one plant extract)