Abstract

Diabetes mellitus is a major heterogeneous endocrine and metabolic disorder, characterized by altered metabolism of carbohydrate, lipid and protein, which leads to hyperglycaemia and causes many physiological complications, such as hyperlipidemia, hypertension and atherosclerosis.

The global prevalence of diabetes among adults as per WHO, was estimated at 150 million in 1995, and this is projected to increase to 300 million by 2025. The oxidative stress, caused by free radicals induced by hyperglycemia, contributes to the development and progression of diabetes along with secondary complications. The plants containing natural antioxidants (tannins, flavonoids, vitamin C and E, etc.) that can preserve β-cell function and prevent diabetes induced ROS formation and many plant species are known in folk medicine of different cultures to be used for their hypoglycaemic properties and therefore used for treatment of DM (Abdel-Barry et al., 1997; Pushparaj et al., 2000). Despite this, few traditionally used antidiabetic plants have received proper scientific screening (Bakirel et al, 2008). The World Health Organization (WHO) has recommended that this area warrants further evaluation (WHO, 1980).

*Solanum nigrum* Linn. (Solanaceae) commonly known as Black Berried Nightshade found in disturbed habitats, distributed throughout India. The leaves are reported to contain several constituents e.g. flavonols like Quercetin, Hyperoside, Steroids and alkaloids etc. The Kondh tribes of Orissa, India use the hot aqueous extract of the fruits and leaves as a folk medicine for the treatment of diabetes mellitus.

*Mollugo pentaphylla* Linn. commonly known as carpet weed (English), Pitta saga (Oriya) is a perennial herb found throughout India. The plant is reported to contain flavones such as Apigenin and Mollupentin, Mollugogenol A, an antifungal triterpenoid, Mollugogenol B, Mollugogenol D, Oleanolic acid and a steroid – Beta Sitosterol.

Objective of the study

- Basing on the broad literature survey, objective of the study focus on scientific evaluation of antidiabetic/ hypoglycemic activity of extracts of *S. nigrum* and *M. pentaphylla* in different experimental models in support of the activity.

Experimental protocols undertaken in the study

- Identification, authentication, collection and processing of plant materials, leaves of *S. nigrum* and aerial parts of *M. pentaphylla* for successive extraction, using pet. Ether, chloroform, ethanol and water.
- Qualitative phytochemical analysis using standard chemical methods.
**Abstract**

- The potent extract of either plant were selected out from the acute alloxan induced diabetic model and used for the further study.
- LD$_{50}$ Determination of the selected extracts and dose level fixation for systemic antidiabetic study.
- **Antidiabetic / Hypoglycemic study:**
  - Acute and sub-acute effects of the extracts on blood sugar level in normoglycaemic, alloxan induced hyperglycemic rats were carried out in both single and multi dose treatment by oral route, along with glucose loaded hyperglycemic rats.
  - In-vitro study on glucose utilization by isolated rat hemidiaphragm.
  - The body weight variation study was also carried out in 30-days treated diabetic rats.
  - The biochemical parameters undertaken in the study are plasma-insulin, β-cell degranulation scores, serum lipid profile, serum ASAT, ALAT, ALP levels and Liver Glucose-6-Phosphatase, Hexokinase, HMG CoA reductase and Arginase levels of the treated animals using standard experimental procedure. The glycogen concentration in Liver and Kidney of the treated rats and various serum haematological parameters were estimated.
- **Antioxidant study:** Diphenylpicrylhydrazyl radical, peroxide radical, superoxide radical and nitric oxide radical scavenging activity with reference to standard antioxidant ascorbic acid along with total Phenolic and Flavonoid content, total antioxidant potential and reducing power; as in-vitro models. For in-vivo antioxidant study, the parameters undertaken are liver lipid peroxidation products and antioxidant enzymes, such as thiobarbituric acid reacting substances, hydroperoxides, malondialdehyde, conjugated dienes, reduced glutathione, glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase.
- **Isolation and Characterization** of new compound from the most potent extract from the plants.

**Results and Discussion**

- The preliminary phytochemical investigation revealed the presence of carbohydrates, polypeptides, saponins, tannins, alkaloids, flavonoids, coumarin, terpenoids, and steroids in the aqueous extract of leaves of S.nigrum (ALS N) and the aqueous extract of the aerial parts of M.pentaphylla (AAMP) showed the presence of carbohydrates, glycosides, polypeptides, saponins, tannins, alkaloids, flavonoids, terpenoids and steroids.
- Among all the four tested extract of each plant, the aqueous extract of both plants was found to possess comparatively maximum reduction in the blood sugar level. The LD$_{50}$
value was determined and based on the LD<sub>50</sub>, the dose levels were rest with, for ALSN as 50 and 100 mg/kg and for AAMP as 250 and 500 mg/kg b. w.

**Antidiabetic / Hypoglycemic study of ALSN and AAMP**

- The results embodied in the thesis, revealed that, both plant extracts at the tested dose levels, significantly reduce blood sugar in both normoglycemic and hyperglycemic rats, both on acute and sub-acute study, as well as in glucose loaded hyperglycemic rats. The glucose lowering activity of the extract may be due to the effect of the extract on pancreatic cells and/or on the extra pancreatic site. The extra pancreatic site of action of the extract is supported by glucose uptake study, in which both the extracts showed significant activity in the peripheral utilization of glucose, which is still evidenced by recovery of body weight of the animals in sub-acute test, with fact that increased utilization and decrease storage of protein responsible for reduction of body weight essentially by depletion of body proteins. The pancreatic action of the extracts is supported by the insulinotropic and beta cytotropic effect of ALSN & AAMP as per the study report of determination of plasma insulin levels and loss in granularity of beta cells. The test extracts also shown to affect the glycogen synthesis in a positively screwed manner which may be due to reactivation of the glycogen synthetase system, a mechanism resembling with the effect of insulin therapy. Furthermore, both the extracts affect Liver Glucose-6-phosphatase and Hexokinase, in an inverse way i.e. inhibition of G-6Pase and elevation of hexokinase which might be one of the reason in respect of reduction of blood glucose level.

- Both the extracts at both the dose levels showed a dose dependent and significant reduction in all tested lipid profile parameters, except HDL which showed an increase level, which reveals that the extract may enhance the transcription of lipoprotein lipase like that of insulin, which is further supported by the significant reduction in the levels of HMG CoA reductase, the rate controlling enzyme in the pathway of cholesterol synthesis. The effective reduction in the levels of serum enzymes like ASAT, ALAT and ALP, which are considered to be good indices of cell damage suggesting the protective effect of the extracts against cellular damage in diabetes.

- The test extract showed no significant alteration in the haematological parameters studied. The architecture of the liver and kidney tissues showed no significant pathological changes at the end of the study in treated animals.

**Anti-oxidant study**

- A marked amount of phenolics and flavonoids were found in aqueous extract of ALSN and AAMP. The extracts also showed marked total antioxidant activity and ferric reducing capabilities, supporting their antioxidant property. Furthermore the test extracts have
significant scavenging activity against the DPPH, Superoxide, Peroxide, NO radicals in a concentration dependent manner and provide protection against oxidative damage induced by the biomolecules. The antioxidant potential of the test extracts was further studied by taking in-vivo parameters and found that the liver lipid peroxidation products were decreased while antioxidant enzyme levels were increased in a significant extent, which evidenced the in-vivo antioxidant property of the extracts supporting their antidiabetic activity.

**Isolation and Characterization of a phytochemical from AAMP**

- Since the test report embodied in the thesis evidenced that AAMP shows comparatively better activity, hence it enforced us to isolate the new compound present in the extract, and found an Oleanolic acid glycoside derivative from the combined fractions of Ethyl acetate and n-Hexane, having molecular formula C_{40}H_{64}O_{13} with IUPAC name as 2, 2, 6a, 6b, 9, 9, 12a- Heptamethyl- 10-[4′, 5′, 6′-trihydroxy-3′-(3″, 4″, 5″, 6″-tetrahydroxy-tetrahydro-pyran-2-yloxy)-tetrahydro-pyran-2-yloxy]-1, 3, 4, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 12b, 13, 14b-octadecahydro-2H-picene-4a-carboxylic acid, based on the characteristics information of FTIR, ^{13}C-NMR, ^{1}H-NMR and LC-MS spectra.

**Conclusion**

The antidiabetic and antioxidant experimental results embodied in the thesis revealed that:

- Both the plant extracts ALSN and AAMP have glucose lowering activity in the normal and alloxan induced hyperglycemic animals in acute as well as sub-acute models.
- The in-vitro and in-vivo antioxidant experimental results shown that both the plant extracts significantly scavenge the toxic free radicals and possess potent antioxidant activity, which might contribute to their antidiabetic and hypoglycemic potential.
- The plant extracts are also in a good agreement with the safetyness in animal study as evidenced by sub-acute antidiabetic studies.
- The new compound isolated from the AAMP is 2, 2, 6a, 6b, 9, 9, 12a- Heptamethyl- 10-[4′, 5′, 6′-trihydroxy-3′-(3″, 4″, 5″, 6″-tetrahydroxy-tetrahydro-pyran-2-yloxy)-tetrahydro-pyran-2-yloxy]-1, 3, 4, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 12b, 13, 14b-octadecahydro-2H-picene-4a-carboxylic acid, an Oleanolic acid glycoside derivative.
- Hence, it is concluded that, both the plant extracts endowed with significant antidiabetogenic activity comparable to the clinical drug glibenclamide, out of which the *M. pentaphylla* extract was found to be more potent over *Solanum nigrum*.