Therapeutic Efficacy of *Mimosa Pudica*-*Aegle Marmelos* formulation on Diabetes Mellitus

Anitha V⁷, Rajarajeswari⁷, Bupesh G⁸, Vasanth S⁹, Tirumalai Vasan P¹⁰, Sahoo U¹¹, Pitambar Humane¹²

¹Department of Noinadal, Sri Sairam Siddha Medical College & Research Center, Chennai, Tamilnadu, India
²Sree Balaji Medical College and Hospital, R&D Wing, BIHER, Chennai, Tamilnadu, India
³Department of Forest Science, School of Science, Nagaland University (Central), Lumami, Zunheboto, Nagaland-798627, India
⁴Department of Biotechnology, Srimad Andavar Arts and Science College, Tiruvanaikaval, Tiruchirappalli, Tamilnadu, India
⁵Department of Forestry, Mizoram University, Aizawl, Mizoram -796004
⁶Department of Botany, Dharampeth M P Deo Memorial Science College, Nagpur (MS), India

**ABSTRACT**

In the present study, the antidiabetic formulation was evaluated for the *in vitro* antidiabetic, antioxidant and a pilot *in vivo* study was conducted with a mixture of herbal powders, i.e. *Mimosa pudica* L. and *Aegle marmelos* (L) Corr. The formulation was screened for its antioxidant activity using DPPH free radical assay and inhibitory effect against the α-glucosidase and lipase compared with the standard. Assays were carried out at the concentration varying from 50 – 500 μg/ml (antioxidant activity) and 20 – 100 μg/ml (antidiabetic). Results of the present study have clearly indicated that the formulation possesses significant α-glucosidase enzyme inhibition property ranges from 41.4% - 12.42% in the different concentration of aqueous extract and similarly pancreatic lipase inhibition potential at 50% with value of 100 μg/ml revealed the significant activity. Moreover, the formulation administered in the diabetic patient reduces the glucose level of fasting and postprandial level, notably; thus, it indicates that it is a promising drug candidate that proves antidiabetic activity.

**INTRODUCTION**

Diabetes is a group of metabolic disorder characterized via a high blood sugar level which results from defects in insulin secretion. Type II Diabetes mellitus is the commonest metabolic disorder in India. Heritable element, obesity, deskbound existence fashion and aging had been proven to raise the risk for diabetes. Changes in life style plays a major role in Diabetes. The proper medical care and an ordinary tracking of diabetes are crucial now not best to maintain the disorder under control; however, additionally to prevent Diabetes associated Troubles (Ananthan, 2003).

Diabetes mellitus affected nearly 7% of the world...
population and predicted that it would be the 7th leading cause of death by the year of 2030. Glucose-6-phosphatase is one of the key enzymes of gluconeogenesis which is involved in dephosphorylation of Glucose-6-phosphate to glucose, as the final step in gluconeogenesis and glycogenolysis. Fructose1,6-bisphosphatase is another enzyme that catalyzes the dephosphorylation of fructose1,6-bisphosphate to fructose-6-phosphate. Both of these enzymes will be elevated in diabetes, which is mainly due to insulin resistance. During the diabetic condition, the gluconeogenic enzymes are activated or increased, so it leads to the production of more glucose (Viswanathan et al., 2013).

The agents which are most commonly used for the treatment of diabetes mellitus include synthetic drugs and these are identified to be coupled with severe adverse effects such as hypoglycemia, weight gain, drug resistance (Selvam et al., 2020). Hence, in recent times medicinal plants have paid more attention for the treatment of diabetes mellitus because of low cost with less damaging consequences. The phytoconstituents present within the medicinal plants or natural method is known to make contributions towards the hypoglycemic assets of that unique plant thereby supporting in the management of diabetes mellitus (Sahariah et al., 2016). In Siddha System, diabetes is correlated as “Madhumegam” – Madhu meaning honey and megam meaning urine – which directly translates to increased sugar in the urine. It is also known as Pramegham, Neerizhivu noi, Thithippu neer. Mathumegam is one among the 20 types of Mega mentioned by the great Sage Yugi. Madhumegam comes under subclass pitha. Madhumegam deteriorates all the seven body constitutions, thus lead to emaciation (Sivaraj et al., 2011).

*Aegle marmelos* is commonly known as bael. It contains furocoumarins, including xanthotoxol and the methyl ester of alloimperatorin as well as flavonoids, rutin and marmesin a number of essential oils; and, among its alkaloids, a-fargarine (=allocryptopine), O-isopentenyldihydrofordinol, O-methylhafordinol. Aegeline (N-[2-hydroxy-2(4-methoxyphenyl)ethyl]-3-phenyl-2-propenamide) is a constituent that can be extracted from bael leaves. Leaf juice 10 to 15 ml can be given daily for treating Diabetes. Leaves may be soaked overnight time in the water then ground properly next morning and given with the remaining water to Diabetes patients. Powdered leaves of Aegle and Turmeric powder may be given inside the dose of half teaspoon two times or thrice an afternoon to deal with Diabetic ulcers. Siddha Medicine, Vilvam tablet is ideal to treat Hyperglycemia.

*Mimosa pudica* Linn is a common herb which grows in the course of in India. The stems are branched, with bristly hairs. The leaves are small leaflets on the stalk, and on touch, they fold collectively. *Mimosa pudica* Linn is traditionally utilized in Indian system of medication for the treatment of various sicknesses. This short-lived evergreen subshrub is typically handled as an annual. It is grown for its interest value- the fern-like leaves near up and hunch when touched, typically re-beginning inside minutes. It has prickly stems and small, fluffy, ball formed purple vegetation in the summertime. It grows to a height of five ft and spreads around 3 ft- a perennial plants, it grows to a top of 0.5m with a selection of 0.3m. In a few areas, this plant is becoming a noxious weed (Sundaresan and Radhiga, 2015). Mimosa owns the following movements Astringent, Styptic, Alterative and Aphrodisiac. The juice of the whole plant at the dose of 25-30ml in the early morning keeps the blood glucose stages within regular limits. In Siddha system, the leaves and the roots are dried and powdered and given in the dose of 2-5 gm for diabetes. *M. Pudica* is wealthy in medicinally critical secondary metabolites, consisting of carbohydrates, proteins, amino acids, tannins, phenolics, steroids, flavonoids, saponins, mucilage, and alkaloids. In the present study, the *M. Pudica* and *A.marmelos* were evaluated for the antioxidant activity and antidiabetic activity in *in-vitro* and healthy volunteer trial.

**MATERIALS AND METHODS**

**Collection and Formulation of the Antidiabetes drug *Mimosa pudica* and *Aegle marmelos***

The Fresh Leaves and roots of *Mimosa pudica* and leaves of *Aegle marmelos* were collected from Sri Sairam Siddha Medical College Herbal Garden, Tambaram. *Mimosa pudica* and *Aegle marmelos* were shade dried as a powder. Then it is formulated in the combination (1:0.5) and were dissolved in 200 ml of drinking water and heated up to half (100ml) then it was filtered as a concoction.

**In vitro antioxidant assay (DPPH)**

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay: A volume of 1.0 mL of 0.3 mM DPPH in methanol was added to 1.0 mL of formulated drug in different concentrations 50, 100, 150, 250 and 500 μg /mL in test tubes. Ascorbic acid was used as a reference. The contents were mixed and incubated in the dark for 30 min and after that absorbance was read at 517 nm against a reagent blank (Selvam et al., 2020; Vasanth et al., 2018).

**Inhibition of the alpha-glucosidase enzyme**
The inhibition of alpha-glucosidase enzyme activity was determined (Krishnaveni et al., 1984). Incubating a solution to starch substrate (2% w/v maltose) 1 ml with 0.2 M Tris buffer pH 8.0 and different concentrations (20–100 μl) of the formulated drug were added incubation for 5 min at 37°C. The reaction was initiated by adding 1 ml of the alpha-glucosidase enzyme (1 U/ml) to it followed by incubation for 40 min at 35°C. Then, the reaction was terminated by the addition of 2 ml of 6 N HCl. Then, the colour development was measured at 540 nm. Acarbose were used as a positive control for amylase inhibitor.

**In vitro Pancreatic Lipase Inhibitory Activity**

The inhibitory activity against pancreatic lipase was measured using p-nitrophenyl butyrate (p-NPB) as a substrate with a modified method from (Zhang et al., 2008). 10 mL of the formulated drug (prepared at concentrations of 20, 40, 60, 80 and 100 μg/mL), positive control (Orlistat, 100 mM) and were pipetted into respective wells of a 96 well plate. Freshly prepared porcine pancreatic lipase was added at fourfold the amount of the test samples, positive and negative controls (40 mL). The plates were initially
incubated at 37°C for 15 minutes. Thereafter 170 mL
of the substrate solution was added to the wells. The
plate was then incubated at 37°C and read at 405 nm.

A Pilot Clinical study

A 50-year-old man who was diagnosed with type 2
diabetes two weeks before admission to the hospi-
tal, Sri Sairam Siddha Medical College and Research
Center. He had a strong family history of type 2
diabetes. He smoked heavily (> 20 cigarettes/day)
and had no habit of alcohol consumption. His treat-
ment started following the first line of treatment.
His fasting blood glucose level was 181 and posted
prandial 246 mg/dl. His blood pressure was 140/80
mmHg, pulse was 85 bpm, and the temperature was
98.7°F, hemoglobin of 15.1 g/dl. His Liver Func-
tions and Renal functions were normal. He was
prescribed with the formulated Mimosa pudica and
Aegle marmelos for 30 days.

RESULTS AND DISCUSSION

After 30 days Fasting Blood Glucose reduced to
130 mg/dl and Post parandial back to 170 mg/dl. He
did not have any adverse effects during the course.

Radical scavenging activity study was performed
to determine the antioxidant activity of formulated
drug through DPPH radical scavenging activity. For-
mulated drug extract significantly (p<0.05) scaven-
ged 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free
radical, Ascorbic acid (standard) at 500 µg/mL−1 dis-
tilled water exerted a higher significant (p<0.05)
radical scavenging activity (Figure 1).

In-vitro Alpha Glucosidase Inhibitory Activity

The formulated drug revealed a considerable
inhibitory action of the alpha-glucosidase enzyme.
The percentage inhibition at a 20-100µl dose of
the formulated drug showed a dose dependent
increase in percentage inhibition. The percentage
inhibition varied from 41.4% - 12.42% for the
highest concentration on the lowest concentration
(Figure 2).

There was a dosage-dependent increase in per-
centage inhibitory activity against the pancreatic
enzyme. It showed 50% inhibition at a concentra-
tion of 100 µg mL−1 (Figure 3). In the in vitro
experiment, the ability to extract had condensed tannins
as an inhibitor of pancreatic lipase.

The lectin extract of Aegle marmelos has antibacte-
rial and anti-diabetic activity (18). The ethanolic
extract of Aegle marmelos cause a reduction in blood
sugar from the seventh day after continuous admin-
istration of the extract and on 28th day, the level of
the sugars was found to be reduced by 54% (Avula
et al., 2016).

The beta cells may be regenerated in Type 2 dia-
betic patients on GS4 of Gymnema and lectin of
aegle supplementation. GS4 supplementation for 18
- 20 months raised insulin levels in the serum of
patients (Baskaran et al., 1990). Preliminary human
research reports that Gymnema and its extract GS4
may be beneficial in patients with Type 1 or Type 2
Diabetes when it is added to diabetes drugs being
taken by mouth or to insulin (Bhavani, 2014). The
main cause of Type 2 diabetes is insulin resistance
and/or deficiency of this receptor for a hormone
which causes hyperglycemia. The key strategy in

Figure 3: Inhibition of pancreatic lipase by Formulated drug. Mean ± SEM, n =3, p<0.05 compared
to orlistat
treating the diabetic patient should be the maintenance of Normoglycemia (Chatham-Stephens et al., 2017).

From the results of alpha-glucosidase enzyme inhibition assay, it was observed that the formulation drugs reveals highest percentage inhibition ranges from 41.4% - 12.42% at the concentration of 10 μg/ml to 100μg/ml. The antidiabetic effect of *Mimosa pudica* is mainly due to the presence of phenolic and flavonoid compounds present in the plant *Mimosa pudica* (Lakshmibai et al., 2015; Rajendiran et al., 2017). *Mimosa pudica* alters the insulin and glucose levels and thus maintain the Glycated haemoglobin levels under control. *Mimosa pudica* treatment improves insulin sensitivity and attenuates fat accumulation in the liver (Muhammad et al., 2016).

Pancreatic lipase inhibition is one of such attempts and many researchers focused on the potential efficacy of natural products as antiobesity agents (Yun, 2010). According to Siddha literature evidences as for available now, it is mentioned that Diabetes occurs as a result of impairment in day to day activities or Lifestyle modifications (Khan et al., 2012; Mathew et al., 2008). These changes affect the body metabolism. Finally results in glucose intolerance.

Medicinal plant compounds are still the most accessible resource of pancreatic lipase inhibitors. Therefore, we investigated biologically active compounds from *Mimosa pudica* using different extraction ratios of petroleum ether to water. Extracts under different concentrations of *Mimosa pudica* were tested for α-glucosidase inhibitory activity. The methanolic extract showed higher pancreatic lipase activity than aqueous extract, whereas petroleum ether and chloroform extract did show moderate inhibit pancreatic lipase at all. Notably, neat alcoholic (methanolic) extracts exhibited stronger inhibitory effects than their corresponding aqueous mixtures. Methanolic extract of *Mimosa pudica* showed significant antidiabetic and antihiperlipidemic activities on streptozotocin-induced diabetes mellitus in rats (Parasaman et al., 2019).

**CONCLUSIONS**

The formulated drug *Mimosa pudica* and *Aegle marmelos* administered 100 mg concentration can significantly reduce the blood glucose level. Further, the in-vitro assays of antidiabetic and antioxidant of the formulated drug (herbal combination) demonstrate greater efficacy against anti-diabetes Mellitus and demonstrated property. Moreover, they are a low cost-effective when compared to the current medications used as hypoglycemic drugs.

**Conflict of Interest**

None.

**Funding Support**

None.

**REFERENCES**

Ananthan, R. 2003. Antidiabetic effect of Gymnema montanum leaves: effect on lipid peroxidation induced oxidative stress in experimental diabetes. *Pharmacological Research*, 48(6):551–556.

Avula, B., Chittiboyina, A., Wang, Y.-H., Sagi, S., Raman, V., Wang, M., Khan, I. 2016. Simultaneous Determination of Aegeline and Six Coumarins from Different Parts of the Plant Aegle marmelos Using UHPLC-PDA-MS and Chiral Separation of Aegeline Enantiomers Using HPLC-ToF-MS. *Planta Medica*, 82(06):580–588.

Baskaran, K., Ahamath, B. K., Shanmugasundaram, K. R., Shanmugasundaram, E. R. B. 1990. Antidiabetic effect of a leaf extract from Gymnema sylvestre in non-insulin-dependent diabetes mellitus patients. *Journal of Ethnopharmacology*, 30(3):295–305.

Bhavani, R. 2014. Antidiabetic activity medicinal plant aegle marmelos (linn.) on alloxan induced diabetic rats. *International research journal of pharmaceutical and biosciences (irjpbs)*, 1(1):36–44.

Chatham-Stephens, K., Taylor, E., Chang, A., Petersen, A., Daniel, J., Martin, C., Lewis, L. 2017. Hepatotoxicity associated with weight loss or sports dietary supplements, including OxyELITE ProTM United States, 2013. *Drug Testing and Analysis*, 9(1):68–74.

Khan, H. B. H., Vinayagam, K. S., Sekar, A., Palanivelu, S., Panchanadham, S. 2012. Antidiabetic and Antioxidant Effect of Semecarpus anacardium Linn. Nut Milk Extract in a High-Fat Diet STZ-Induced Type 2 Diabetic Rat Model. *Journal of Dietary Supplements*, 9(1):19–33.

Krishnaveni, S., Theymoli, B., Sadasivam, S. 1984. Phenol Sulphuric acid method. *Food chem*, 15:229–229.

Lakshmibai, R., Amirtham, D., Radhika, S. 2015. Preliminary phytochemical analysis and antioxidant activities of Prosopis juliflora and Mimosa pudica leave. *Int J Sci Eng Technol Res*, 4(30):5766–5770.

Mathew, A. J., Atulya, M., Joseph, A. 2008. The antioxidant and anti-diabetic activity of Mimosa pudica Linn. in streptozotocin-induced diabetic rats. *Biomed*, 3(2):155–164.
Muhammad, G., Hussain, M. A., Jantan, I., Bukhari, S. N. A. 2016. Mimosa pudica L., a High-Value Medicinal Plant as a Source of Bioactives for Pharmaceuticals. *Comprehensive Reviews in Food Science and Food Safety*, 15(2):303–315.

Parasuraman, S., Ching, T. H., Leong, C. H., Banik, U. 2019. Antidiabetic and antihyperlipidemic effects of a methanolic extract of Mimosa pudica (Fabaceae) in diabetic rats. *Egyptian Journal of Basic and Applied Sciences*, 6(1):137–148.

Rajendiran, S. D., Kandaswamy, S., Sivanesan, Radhakrishnan, K. G. S. 2017. Potential antidiabetic effect of Mimosa pudica leaves extract in high-fat diet and low dose streptozotocin-induced type 2 diabetic rats. *International Journal of Biology Research*, 2(4):55–62.

Sahariah, B. J., Sharma, R. K., Lahkar, M. 2016. A review on Mimosa pudica linn. With specific reference to antimicrobial activity. *CIBTech Journal of Microbiology*, 5(3):1–5.

Selvam, G. P., Vasanth, S., Bupesh, G., Prabhu, K., Kirshnamurthy, R. 2020. Phytochemical Screening. Invitro antidiabetic activity of Muntingia calabura leaves extract on alpha-amylase and alpha-glucosidase enzymes. *International Journal of Research in Pharmaceutical Sciences*, 11(1):1210–1213.

Sivaraj, R., Balakrishnan, A., Thenmozhi, M., Venckatesh, R. 2011. Preliminary phytochemical analysis of Aegle marmelos, Ruta graveolens, Opuntia delhini, Euphorbia royleana and Euphorbia antiquorum. *International Journal of Pharmaceutical sciences and research*, 2(1):132–136.

Sundaresan, A., Radhiga, T. 2015. Effect of Mimosa pudica Cured Extracts against High Fructose Diet-Induced Type 2 Diabetes in Rats. *International Letters of Natural Sciences*, 39:1–9.

Vasanth, S., Bupesh, G., Vijayakumar, T. S., Balachandrar, V., Gunasekaran, D. R. 2018. Evaluation Of In Vitro Antidiabetic And Antioxidant Potential Of Barleria Cristata Leaves Extracts. *Asian Journal of Pharmaceutical and Clinical Research*, 11(4):287–287.

Viswanathan, R., Sekar, V., Velpandian, V., Sivasaranavan, K. S., S. A. 2013. Anti-diabetic activity of thottal vadi choornam (Mimosa pudica) in alloxan-induced diabetic rats. *International Journal of Natural Product Science*, 3(5):13–20.

Yun, J. W. 2010. Possible anti-obesity therapeutics from nature - A review. *Phytochemistry*, pages 1625–1641.

Zhang, J., Kang, M. J., Kim, M. J., Kim, M. E., Song, J. H., Lee, Y. M., Kim, J. I. 2008. Pancreatic lipase inhibitory activity of taraxacum officinale in vitro and in vivo. *Nutrition Research and Practice*, 2(4):200–203.