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Anti-diabetic Potential of *Macrotyloma uniflorum* Leaves in an *in Vitro* and *in Vivo* Model

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KEYWORDS Anti-diabetic Activity. Carbohydrate Metabolizing Enzymes. *In Silico* Analysis. *Macrotyloma uniflorum*. Phytochemical Analysis

ABSTRACT To identify the anti-diabetic potential of *Macrotyloma uniflorum* (*M. uniflorum*) leaves. The *M. uniflorum* leaves were subjected to extract preparation by using the solvents, such as hexane, ethanol, and water. Then, phytochemical and *in vitro* and in vivo studies were carried out. The rats were divided into five groups. The streptozotocin was used to induce diabetes and the glibenclamide is used as a standard drug. The plant extract is treated to the toxin induced rats. The changes were noted. The secondary metabolites present in the leaves of all three extracts. The ethanolic extract is more potent than the aqueous extract when compared to the two extracts. *In vivo* studies the levels returned to normal after the treatment. The pancreatic cells are regenerated in the *M. uniflorum* leaves treated groups. The results proved that the leaves extract of *M. uniflorum* has an anti-diabetic efficacy in an animal model.

INTRODUCTION

Diabetes Mellitus (DM) is one of the important metabolic illnesses connected to hyperglycemia because of changes in the metabolism of lipids, proteins, and carbohydrates. According to Pavithra and Chaitanya (2015), diabetes is the primary risk factor for the emergence of numerous organ ailments, including kidney, eye, and heart conditions that result in myocardial infarction. Diabetes is an important public health problem of the 21st century. Worldwide there are 387 million people were predicted and the rate is increased to 592 million in the year 2035. In India, there 61.3 million people were affected by diabetes in the year of 2011 and it increased to 101.2 million in the year of 2030 (Gupta et al. 2015). A large number of antidiabetic drugs are available in the pharmaceutical market but it causes major medical problem as a side effect. Recently, some medicinal plants have been identified as having an anti-diabetic nature worldwide (Mitra et al. 1996), like *Psidiumguajava* (Manikandan et al. 2018), Andrographispaniculata, Rhinacanthusnasutus (Akilandeswari et al. 2023). Recent research has discovered that the lack of adverse effects makes medications derived from natural plants appealing. Macrotyloma uniflorum (Lam.) is an herb commonly called horse gram. It is highly distributed in the rural areas of south India. The *M. uniflorum* leaves contain alkaloids, steroids, tannins, polyphenols, and β- sitosterol. The major phytoconstituents include anthocyanins, flavonoids, phenolic acids with benzoic acid derivatives, phenolic acids with cinnamic acid derivatives, and tannins and phytic acid (Ahmed et al. 2016).

The current study was aims to find the secondary metabolites present in the various extracts of *T. catappa* leaves. The in vitro anti-diabetic analysis is carried out. Then the *in vivo* analysis were carried out to determine *M. uniflorum* leaf's anti-diabetic effectiveness in animal models.

MATERIAL AND METHODS

Extraction of Plant Material: The Department of Botany at St. Joseph College in Trichy validated the leaves of *M. uniflorum* after they were collected locally. *M. uniflorum* leaves were mechanically pulverized and kept in an airtight container after being shade dried. Soxhlet equipment was used to perform the extraction. The leaf extract was prepared using a variety of solvents, such as hexane, ethanol, and aqueous.

Phytochemical Screening: According to established protocols, freshly obtained hexane, ethanolic, and aqueous extracts of *M. uniflorum* leaves are subjected to a preliminary phytochemical examination to identify the phytoconstituents (Evans and Evans 2003).

In vitro Analysis: In vitro testing was done on the M. uniflorum ethanolic and aqueous extract. Acarbose is used as a reference drug to assess the inhibitory effects of the ethanolic and aqueous extract of M. uniflorum against the carbohydrate metabolizing enzymes such as alpha-amylase (Malik and Singh 1980) and alpha-glucosidase (Krishnaveni et al. 1984).

Preparation of Animal's Experimental Design

The adult male Albino Wistar rats (8 weeks old; 150-165 g) were obtained and kept in a 12 h light/dark cycle environment at a temperature of 25±10C with a standard pellet meal that included protein, fat, and fiber in a balanced ratio with carbohydrates, vitamins, and minerals, as well as water available at all times.

Rats received a single dose of *M. uniflorum* ethanolic leaf extract in sterile water (300 mg/kg b.w) post-orally by intubation in the early morning on each day of the trial. The rat population was divided into five groups of six at random.

- G1: Control Rats. (Basal Diet with saline water)
- G2: Negative control (Streptozotocin induced single diabetic dose (60 mg/kg b.w.i.p.)).
- G3: Normal rats + 300 mg/kg of ethanol extract of *M. uniflorum*.
- G4: Streptozotocin-induced rats + 300 mg/kg of ethanol extract of *M. uniflorum*.
- G5: Streptozotocin-induced rats + 3 mg/kg of glibenclamide

Biochemical Analysis

The serum samples were collected and analyze the glucose, insulin, glucokinase, G6Pase, FBPase, and kidney markers were evaluated. Pancreatic tissue was collected for histopathological studies.

Statistical Analysis

All data collected for the biochemical parameters were assessed for statistical significance using DMRT and one-way analysis of variance (SPSS®for Windows, V.17.0, Chicago, USA). Values from the table were presented as the mean and standard deviation. For all tests, statistical significance was defined as a probability value of 0.05 or less.

RESULTS AND DISCUSSION

Phytochemical Analysis of the Leaf Sample of *M. uniflorum*

The major source of bioactive compounds is the medicinal plants. The preservation of human health is greatly aided by these therapeutic herbs. The bioactive components derived from medicinal plants are valuable therapeutic agents (Kaushik et al. 2002). The biologically important elements of plants are secondary metabolites such as alkaloids, saponins, flavonoids, phenols, tannins, and phenolic compounds (Doss 2009). These phytoconstituents are highly used in the treatment of various health issues (Pandey et al. 2013). The bioactivity of the plant extract is directly proportional to the presence of phytoconstituents.

In the present study, the fresh leaves of *M. uniflorum* are collected, and dried and then the leaves are subjected to extraction by using hexane, ethanol, and aqueous. These *M. uniflorum* leaf extracts are tested to see if secondary metabolites such phenols, tannins, quinone, anthraquinones, flavonoids, total protein, and fixed oil are present. The phytoconstituents which are present in the various extracts of the leaves of *M. uniflorum* are listed in Table 1.

In the current study, the leaves of *M. uniflorum* extract which contain all the phytoconstituents such as alkaloids, carbohydrates, tannins,

Table 1: Phytochemical analysis of various extracts of leaf *M. uniflorum*

S. No.	*Phytochemical constituents*	*Name of the test*	*Hexane*	*Ethanol*	*Aqueous*
1.	Alkaloids	Mayer's test	+	+	+
2.	Carbohydrate	Molish test	+	+	+
3.	Tannin	Lead acetate test	+	+	+
4.	Terpenoids	Noller test	–	+	+
5.	Steroidal glycosides	Salkowaski test	–	+	–
6.	Quinones	H_2SO_4 test	–	+	–
7.	Anthraquinone	Borntrager's test	–	+	–
8.	Saponin glycosides	Froth test	–	+	–
9.	Flavonoids	Schioda's test	+	+	+
10.	Total protein	Millon's test	+	+	–
11.	Fixed oil	Spot test	+	+	–
12.	Phenols	$FeCl_3$ test	–	+	+
13.	Coumarin	NaCl test	–	+	+

+ = PRESENT – = ABSENT

terpenoids, steroidal glycosides, quinone, anthraquinone, saponin glycosides, flavonoids, total protein, fixed oil, phenols and coumarin. The aqueous extract contains alkaloids, tannins, terpenoids, flavonoids, carbohydrates, phenols, and coumarin. The hexane extract of leaves of *M. uniflorum* contains alkaloids, tannins, flavonoids, carbohydrates, protein, and fixed oil.

The medicinal activities of the plant do not depend on a single compound, it is the combined activity of various secondary metabolite compounds such as terpenoids, flavonoids, phenol, alkaloid and tannins. Suriyamoorthy et al. (2014) reported that the leaves of *M. uniflorum* contain the phytocompounds such as flavonoids, amino acids, carbohydrates, saponins, and terpenoids. The current study may potentially demonstrate this. Among the various extracts, the ethanolic leaf extract of *M. uniflorum* contains a large number of secondary metabolites. As a result, future research will only be conducted using ethanolic extracts of *M. uniflorum* leaves.

In Vitro, the Anti-diabetic Activity of *M. uniflorum* Leaves

By using the blocking activity of the enzymes alpha-amylase and alpha-glucosidase, the current study evaluates and compares the anti-diabetic properties of aqueous and ethanolic extracts of *M. uniflorum* leaves with the standard medicine acarbose in an *in vitro* model. The outcomes are displayed in Tables 2 and 3. All three extracts have an anti-diabetic activity by in vitro models.

Aqueous extract of *M. uniflorum* leaf produced 19.4 percent inhibition of alpha-amylase activity at 0.2 mg/mL and 75.2 percent at 1 mg/mL concentration, and its IC50 is found to be 0.58 mg/mL. Ethanolic extract shows 23.1 percent and 90.1 percent of inhibition at 0.2 mg/mL and 1 mg/mL respectively, and its IC50 is found to be 0.52 mg/mL. The standard drug confirmed 25.2 percent and 93.7 percent of inhibition at 0.2 mg/mL and 1.0 mg/mL respectively, and its IC50 is found to be 0.42 mg/mL (Table 2).

Table 2: Anti-diabetic activity of various extract of *M. uniflorum* leaves by alpha-amylase method

S. No.	*Concentration of the sample (mg/mL)*	*% of inhibition of aqueous extract*	*% of inhibition of ethanolic extract*	*% of inhibition of acarbose*
1.	0.2	19.4	23.1	25.2
2.	0.4	38.6	46.4	49.2
3.	0.6	52.9	57.8	62.3
4.	0.8	67.7	72.3	78.1
5.	1.0	75.2	90.1	93.7
	IC_{50}	0.58	0.52	0.42

Aqueous extract of *M. uniflorum* leaf showed 23.4 percent and 81.6 percent of inhibition on alpha-glucosidase at the concentration of 0.2 mg/mL and 1 mg/mL respectively, and its IC50 is found to be 0.49 mg/mL. The ethanolic extract exhibited 30.2 percent at 0.2 mg/mL and 88.3 percent of inhibition at 1 mg/mL concentrations, and its IC50 is found to be 0.38 mg/mL. The acarbose as a standard confirmed 34.2 percent of inhibition at 0.2 mg/mL and 92.5 percent at 1 mg/

Table 3: Anti-diabetic activity of various extract of *M. uniflorum*leaves by alpha-glucosidase method

S. No.	*Concentration of the sample (mg/mL*	*% of inhibition of aqueous extract*	*% of inhibition of ethanolic extract*	*% of inhibition of acarbose*
1.	0.2	23.4	30.2	34.2
2.	0.4	42.8	52.3	59.7
3.	0.6	59.2	66.5	70.3
4.	0.8	71.8	76.1	81.2
5.	1.0	81.6	88.3	92.5
	IC_{50}	0.49	0.38	0.32

mL concentrations, and its IC50 is found to be 0.32 mg/mL (Table 3).

To demonstrate the anti-diabetic properties of plant extracts, the inhibition of enzymes like alpha-amylase and alpha-glucosidase is crucial. Some synthetic chemical compounds successfully block the activity of the enzymes alpha-amylase and alpha-glucosidase, but they also have undesirable side effects such as bloating and diarrhea (Chakrabarti and Rajagopalan 2002). Polysaccharides are often converted into disaccharides and monosaccharides by enzymes (Lebowitz 1998; Inzucchi 2002). According to Cheng and Fantus (2005), the inhibition of these enzymes may halt the metabolism of carbohydrates and postpone the absorption of glucose. The previous studies of Manikandan et al. (2016) prove that the inhibition of this enzyme's action may reduce the glucose absorption in Psidium guajava leaves by inhibition of this alpha-amylaseandalpha-glucosidase enzyme action. In the current investigation, the ethanolic extract had a greater inhibitory effect on alpha-amylaseandalpha-glucosidase than the aqueous extract. Therefore, further research is limited to ethanolic extracts of *M. uniflorum* leaves.

Anti-diabetic Activity of *M. uniflorum* Leaves

After 45 days of daily administration of glibenclamide (3 mg/kg) and an ethanolic leaf extract of *M. uniflorum* (300 mg/kg). Table 4 displays the glucose, insulin, glucokinase, G6Pase, and FBPase concentration in STZ-induced diabetic rats. When compared to the control group, the STZ-induced diabetic rats have higher levels of glucose, G6Pase, and FBPase, while their levels of insulin and glucokinase are lower. However, after being treated with *M. uniflorum* leaves, the levels were significantly ($p \leq 0.05$) brought back to normal. The levels were restored to normal in the group that received the conventional medication, glibenclamide. There were no discernible changes in the group that received treatment with just the plant extract. A similar effect is already known by Manikandan et al. (2018) in *Psidium guajava* leaves and Lal et al. (2012) identified by Kyllinga triceps in STZ-induced diabetic rats.

The glucose concentration is indirectly proportional to the concentration of insulin. The insulin is secreted in our body in the β-cells. These cells are affected by some chemicals or other agents which destroy the β-cells. This destruction may reduce insulin secretion in our bodies. This effect directly affects the utilization of glucose. So, the reduction in the utilization of glucose increases the concentration of glucose in the blood. In the present study, the STZ is a chemical agent which destructs the β-cells, it may reduce insulin production and it reduces the utilization of glucose by the cells. It

Table 4: The concentration of glucose, insulin, G6Pase, FBPaseandglucokinasein diabetic and non-diabetic rats

Parameters	*Group I*	*Group II*	*Group III*	*Group IV*	*Group V*
Glucose	90.1± 4.32^{a}	276.3± 2.56^{b}	90.08 ± 1.92^{a}	102.6 ± 2.18^{c}	92.5 ± 2.61^{a}
Insulin	3.63 ± 0.31^{a}	0.92 ± 0.22^{b}	3.64 ± 0.16^{a}	3.58 ± 1.12^{c}	3.60 ± 0.62^{a,c}
G6Pase	7.81± 0.62^{a}	19.32± 0.31^{b}	7.83± 0.51^{a}	8.96± 0.13^{c}	8.02± 0.67^{a,c}
FBPase	36.83± 0.91^{a}	90.42± 0.63^{b}	36.31± 0.75^{a}	40.23± 0.95^{c}	39.62± 0.61^{c}
Glucokinase	227.52± 1.72^{a}	110.73± 1.96^{b}	228.65± 0.93^{a}	220.31± 2.62^{c}	221.65± 2.05^{c}

Values are expressed as means ± SD for six rats in each group
Values not sharing a common marking (a,b,c……) differ significantly at $p \leq 0.05$ (DMRT)
Glucose – mg/dl; Insulin – IU/L; G6Pase- µg of phosphorus liberated/min/g tissue; FBPase - µg of phosphorus liberated/min/g tissue; Glucokinase – mg/g.

favors the hyperglycemic condition in the blood. After the treatment of *M. uniflorum* leaves ethanolic extracts reduce the glucose concentration in blood. This result could be caused by the regeneration of β-cells and which favor insulin secretion and it reduces the blood glucose level in *M. uniflorum* leaves treated toxic induced rats.

The presence of different phytochemicals in *M. uniflorum* leaves may contribute to pancreatic β-cells regeneration. This regeneration induces insulin secretion. The increasing concentration of insulin may induce glycogen synthesis and the induction of cells to utilization of glucose may reduce the glucose concentration. It may also activate the insulin receptor, thus favoring the entry and utilization of glucose by the peripheral tissues. According to Sivaraj et al. (2009), STZ-induced diabetic rats treated with Cassia auriculata and Aegle marmelos also experienced a similar effect. According to Manikandan et al. (2018), the ethanolic extract of *Psidium guajava* decreases the glucose level in STZ-induced diabetic rats. The latest study makes a similar observation.

The elevated insulin concentration may alter the enzymes involved in carbohydrate metabolism. The body's ability to regulate blood glucose is greatly influenced by the enzymes that break down carbohydrates (O'Doherty et al. 1999). G6Pase and FBPase are two crucial enzymes that are involved in the processes of gluconeogenesis and glycogenolysis, respectively (Maiti et al. 2004). The liver cells of the STZ-induced rats in the current investigation were likewise injured. The concentration of several enzymes, including G6Pase and FBPase, may rise as a result of this damage. This mechanism demonstrates that the leaf extract of *M. uniflorum* also regenerates the damaged cells in the liver by lowering the amount of these enzymes after therapy. The processes of gluconeogenesis and glycogenolysis may be slowed down by the rising concentration of these enzymes. This could lower the blood glucose level. When compared to the groups that received plant extract alone, no significant differences are observed.

The major enzyme responsible for converting glucose into G6Pase is glucokinase. The relationship between the glucokinase concentration and glucose concentration is one of direct and indirect proportionality. In the current work, diabetic rats induced with STZ have lower glucokinase concentrations. The level of glucokinase increases after *M. uniflorum* leaves have been treated. The glycolysis process may be favored by the rising glucokinase concentration, which lowers the body's glucose concentration. The glucokinase enzyme level in the group treated with plant extract alone did not change significantly. contrasted with the control group.

Histopathological Studies

Figure 1 shows the histopathological study of the pancreas of diabetic rats. Group I-V shows the histopathological changes in the pancreatic tissues. Group I which is similar to group III shows there are no significant effects noted in the plant extract alone treated groups. Group II noted the changes in the cells due to the streptozotocin. After the treatment of M. uniflorun leaves the pancreatic cells are regenerated this effect was similar to the group V standard drug glibenclamide-treated rats. The ability of *M. uniflorum* to regenerate pancreatic cells may shed light on the beneficial effects of these substances on insulin production.

Ochei and Kolhatkar (2000) discovered a 50 percent decrease in the number of β-cells during diabetes. However, treatment of plant extracts from *M. uniflorum* led to both β-cell repair and islet size restoration. In the current study, rats given 300 mg/kg of the *M. uniflorum* leaves extract showed good activity after treatment. The results of the current investigation show that the ethanolic extract of *M. uniflorum* has hypoglycemic qualities because β-cells can regenerate after being damaged by STZ. The results of the current study demonstrate that *M. uniflorum* leaf extracts have an adjuvant function in the management of DM. The regeneration of pancreatic cells by M. uniflorum increases insulin production. The previous study of Vijayakumar et al. (2020) proves that the leaves of *P. guajava* protect the liver cells from the damage of streptozotocin in animal model. The similar effect is noted in the present study also.

CONCLUSION

The present study proves that various secondary metabolites are present in the *M. uniflorum* leaves. The *in vitro* results proved the anti-

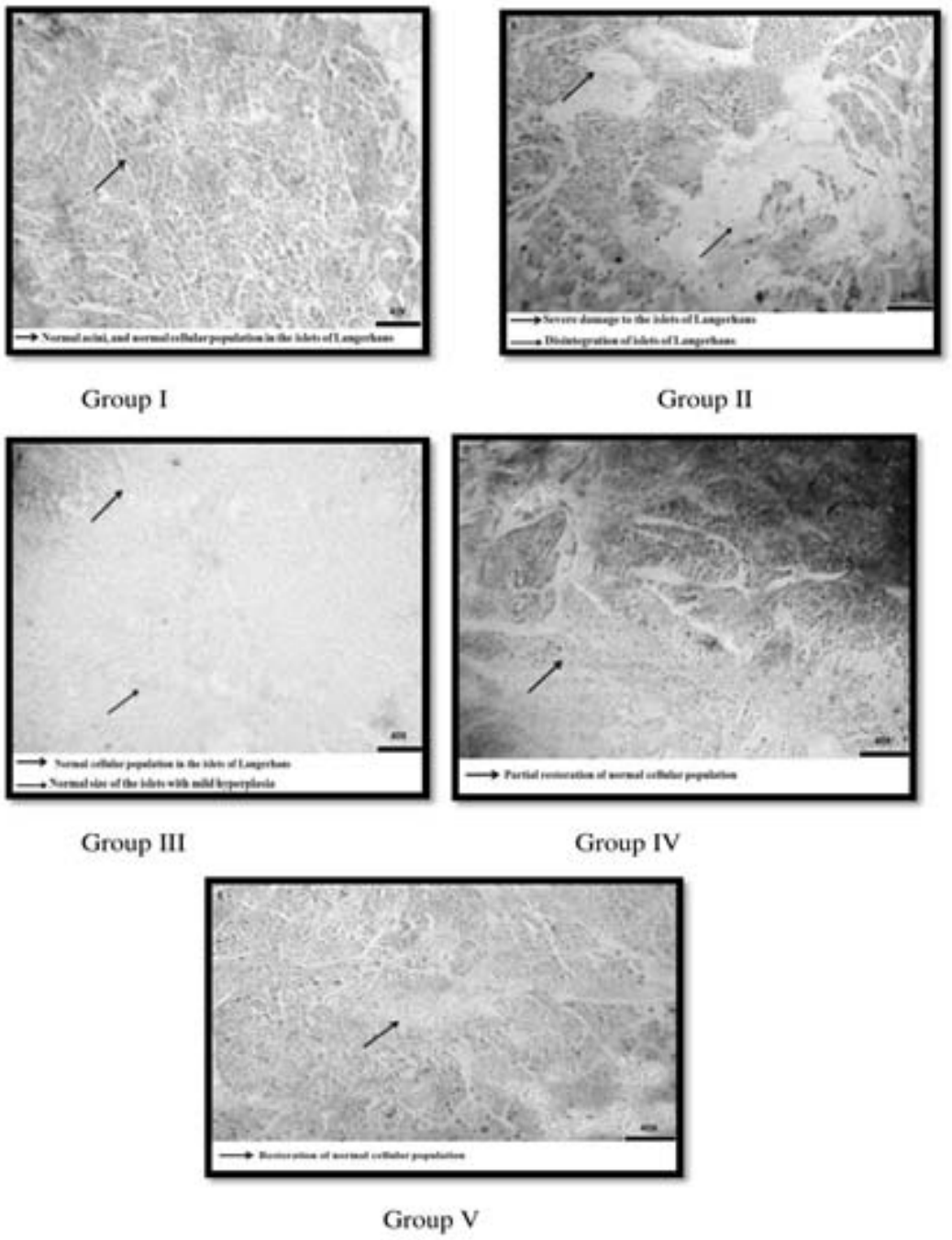

Fig. 1. Histopathological changes of pancreatic tissues in *M. uniflorum* leaves treated experiment rats

diabetic nature of the *M. uniflorum* leaves. The in vivo results were proved the anti-diabetic nature in an animal model.The isolation, purification, and mechanism of action of the chemical that gives it its anti-diabetic properties call for more research.

RECOMMENDATIONS

The results proved that the leaves extract of *M. uniflorum* has an anti-diabetic efficacy in an animal model. In the near future, it may be recommended as an effective drug against diabetes and its related complications.

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