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EVALUTION OF ANTI-DIABETIC ACTIVITY OF METHANOL EXTRACT OF *DIGERA MURICATA* (L) MART IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Digera muricata (L.) Mart (Amaranthaceae) commonly known as Cancali soppu widely used in traditional system of medicine for the treatment of diabetes mellitus In the present study, Methanol extract of *Digera muricata*(MEDM) leaves were subjected to phytochemical investigation and evaluated for antidiabetic activity in alloxan induced diabetic rats. MEDM (100, 200 mg/kg) and Glibenclamide (3mg/kg) were administered orally in alloxan (140 mg/kg, i.p.) induced diabetic rats. In acute oral toxicity (OECD Guide line 423) study, administration of MEDM no mortality upto 2000 mg/kg was observed. OGTT, Fasting blood glucose level, body weight, lipid profiles, HbA_{1c}, plasma insulin, and ALP were evaluated in normal and diabetic rats. preliminary phytochemical investigation revealed the presence of alkaloids, flavonoids, glycosides, tannins as the major constituents in the methanol extract .These results suggest that MEDM (200mg/kg) showed antihyperglycemic activity in alloxan induced diabetic rats.

INTRODUCTION: Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, abnormal lipid and protein metabolism along with specific long-term complications affecting the retina, kidney and nervoussystem [1]. Hyperglycemia is an important factor in thedevelopment and progression of the complications of diabetes mellitus [2] Regions with greatest potential are Asia and Africa, where DM rates could rise to two-to-three- folds compared with the present rates. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world for a range of diabetic presentation. *Digera muricata*(L) Mart is a Amaranthaceae Family .It is commonly distributed throughout the India. In Ayurveda the herb is considered as a cooling, astringent to the bowls and also used as laxative. The flowers and seeds are used to treat Urinary discharges [3].

The *Digera muricata* treatment augments the antioxidants defence mechanism against carbon tetrachloride induced toxicity and free radical mediated diseases. Annual herbs 30-60cm height branches glabrous spreading leaves 2-7.5 by 1.3-4.5cm variable, alternate, entire petiolate with angular branches. The reported studies of *Digera muricata* are Antimicrobial [4] protective effect [5] carbon tetra chloride nephrotoxicity [6, 7]. The present study was conducted to investigate the Antidiabetic activity of methanolic extract of *Abrus precatorius* in Alloxan induced diabetic rats.

MATERIALS AND METHODS:

Plant collection and Authentication: The Leaves of *Digera muricata* collected from the outer area of Vellore district, Tamil Nadu. The collected plants were authenticated by Mr. Jayaraman, Director, National Institute of N. Herbal Science Tamil Nadu. (No.-PARC/2010/233).

Preparation of extract: The leaves *Digera muricata* were dried under shade and then made into a coarse powder andt he powder of *Abrus precatorius* (520g) was extracted with methanol by Soxhlet extraction for 48hrs. The extracts were concentrated to dryness. It gives a thick greenish semisolid residue. These extract was suspended in distilled water and used for further studies. The extracts were stored in an air tight container for further use.

Animals: Male albino-Wistar rats weighing 150-250g were used in the present study. All rats were kept at room temperature of 25±2^{o}C in the animal house. All the procedures were followed the internationally accepted ethical guidelines for the care of laboratory animals. Prior to the experiments, rats were fed with standard food for one week in order to adapt to the laboratory condition, JKKMMRF College of Pharmacy. All the animals were followed the internationally accepted ethical guidelines for the care of laboratory animals (IAEC/2010/005).

Acute Toxicity Study: The procedure was followed as per (OECD 423 Acute classic method). The methanolic extract was tested for its acute toxicity in mice. To determine acute toxicity of methanolic extract of *Digera muricata* in different doses (100, 200mg/kg) were administered to different groups of mice and observed for signs of behavioral and mortality for 72hrs [8].

Oral Glucose Tolerance Test (OGTT): The overnight fasted (18 hr) normal rats were divided into four groups and each group consists of six animals. They were provided with drinking water only. Normal saline solution was administered to group I animals. *Digera muricata m*ethanol extract (100 mg/kg and 200 mg/kg) was administered, by oral route, to group II and III. Group IV animals were received *Glibenclamide* (3 mg/kg, b.w.) as a standard. Glucose (2g/kg) load was fed 30 minutes after the administration of dose. Blood was withdrawn from tail vein under mild ether anesthesia at initial, and 30,60, 90 minutes after glucose (glucose load, 2g/kg) administration and glucose levels were estimated using glucose strips and a glucometer (Standard diagnostics Ltd). Blood glucose levels were noted and reported

Induction of Experimental Diabetes: Diabetes is to be induced in overnight fasted adult Wistar albino rats weighing 150-250 gm by single i.p. injection of 140 mg/kg Alloxan monohydrate (9). Alloxan monohydrate was dissolved in normal saline (pH 4.5). Animals were fed with 5% glucose solution in order to prevent hypoglycemic shock for 18 hrs Hyperglycemia is to be confirmed by elevated blood glucose levels in plasma, determined at 72 hrs and then on day 0 after injection. The threshold value of fasting plasma glucose to diagnose diabetes was taken as >200mg/dL. Only the rats found with permanent diabetes were used for the antidiabetic study.

Experimental Design: Experimental rats were divided into five groups of six animals each and treated for 21 days as follows.

Group I: Normal control rats fed with vehicles only

Group II: Diabetic controls rats (Alloxan monohydrate 140 mg/kg body weight of rats, once i.p injection)

Group III: Normal rats treated with methanolic extract of *Digera muricata*, 100mg/kg, per oral, 0.5% in carboxy methyl cellulose (CMC).

Group IV: Diabetic rats treated with methanolic extract of *Digera muricata* 200mg/kg, per oral, 0.5% in carboxy methyl cellulose (CMC)

Group V: Diabetic rats treated with standard drug, *Glibenclamide* 3mg/kg body weight in 0.5% carboxy methyl cellulose (CMC).

Sample Collection: Blood sample was collected at weekly intervals from tail vein puncture till the end of study. In the Continuous 21 days of drug treatment, a blood glucose level of all animals was determined at the 0, 7, 14, 21 day. On day 21, blood was collected by cardiac puncture under mild ether anesthesia from over night fasted rats. Blood was collected in tubes

containing potassium oxalate and sodium fluoride as anticoagulant for estimation of fasting plasma glucose. Plasma and serum were separated by centrifuged at 4,000 rpm for 10 minutes; the clear supernatant was used for the analysis of various biochemical parameters [10, 11, 12].

Evaluation of Biochemical parameters: After 21 days of treatment, over night fasted rats were sacrificed and blood was collected from retro-orbital plexus using micro capillary technique and serum was separated. The biochemical estimation of serum Triglycerides, total Cholesterol, HDL Cholesterol, LDL Cholesterol, VLDL Cholesterol, HbA_{1C} ALP were estimated by using a commercial kit from Med source Ozone Biomedical Pvt. Ltd [13].

Statistical analysis: Values are given as mean ±SEM and were compared using one way ANOVA with Tukey-Kramer multiple comparison test. The values of $p<0.05$ were considered statistically significant.

RESULT AND DISCUSSION: The present study was under taken to investigate the Antidiabetic activity of methanolic extract of *Digera muricata* (Martt) in Alloxan induced diabetic rats In The phytochemical analysis of *Digera muricata* leaves extracts were shown in **table 1.** OGTT, the doses of Methanol extract of *Digera muricata* (100mg/kg, 200mg/kg) increased the tolerance for glucose suggesting increased peripheral utilization of glucose MEDM at dose, 200 mg/kg possessed significant ($p<0.01$) reduction on blood glucose level when compared to MEDM at dose of 100 mg/kg. Hence, the reduction in blood glucose level was dose dependent shown in **table 2** and **figure 3**.

Vehicles control animals were found to be stable in their body weight but significant reduction in diabetic control group during 21 days. Alloxan caused body weight reduction, which is slightly reversed by Methanol extract of *Digera muricata* treated (100mg/kg – 200mg/kg) groups after 21 days, but no significant reduction was noted in lower doses (100 mg/kg). While, significant ($p<0.01$) increase in body weight was observed in normal rats treated with methanol extract of *Digera muricata.* Hence, this extract caused increase in body weight of normal group. Glibenclamide to diabetic rats slightly decreased the body weight level are showed in **table 4**.

Treatment with MEDM at doses of 100mg/kg and 200mg/kg significantly decreased the blood glucose level in 21st day shown in **table 5** and **figure 4** and plasma insulin, glycosylated hemoglobin results are shown in **table 7**.

The serum lipid values of TC, TG, HDL, LDL and VLDL of those were treated with MEDM extract returned to values near to control group. The level of cholesterol and triglyceride increased in diabetic animals when compared to control animals. After MEDM treatment, the higher level of both cholesterol and triglyceride were restored towards the normal. The level of HDL in serum of diabetic animals was decreased. These lowered levels of HDL-cholesterol were restored significantly near to normal in MEDM treated diabetic groups. This showed that treatment with MEDM (200 mg/kg) significantly improved the lipid profile in Alloxan induced diabetic rats shown in **table 8**.

Serum alkaline phosphatase (ALP) level was increased in diabetic control group but this level was decreased significantly ($p<0.01$) in methanol extract of *Digera muricata*- treated groups (100, 200 mg/kg) and in normal rats treated with *Digera muricata* extract (Group III) due to 21 days of treatment. Normal ALP level was observed in normal control group (Group I). Hence, the methanol extract of *Digera muricata* possessed significant ($p<0.01$) effect on Serum alkaline phosphatase level shown in **table 9**.

TABLE 1: QUALITATIVE PHYTOCHEMICAL ANALYSIS OF *DIGERA MURICATA* LEAVES

Test	Pet. Ether extract	Methanol Extract	Aqueous Extract
Carbohydrates	-	+	+
Alkaloids	-	+	+
Flavonoids	-	+	+
Saponins	-	-	-
Tannins & phenolic compound	-	+	+
Proteins & amino acids	+	+	+
Steroids &sterols	+	-	
Glycosides	-	+	+
Fixed oil	+	-	-

(+) = indicates presence; (-) = indicates absence

TABLE 2: EFFECT OF MEDM AND GLIBENCLAMIDE IN OGTT OF CONTROL AND EXPERIMENTAL GROUPS OF RATS

Group	Treatment	Blood glucose level			
		Fasting	After 30 mins	After 60 mins	After 90 mins
I.	Glucose, 2gm/kg	66.85 ± 2.67	146.32 ± 2.76	135.83 ± 2.62	123.46 ± 4.55
II.	MEDM 100mg/kg	76.46 ± 3.85	122.83 ± 4.23*	117.52±5.64*	104.17 ± 3.65*
III.	MEDM 200mg/kg	71.33 ± 2.44	109.86 ± 3.84*	98.83±3.768	87.54 ± 2.45*
IV.	Glibenclamide, 3mg/kg	68.16 ± 3.36	95.2.5 ± 3.42	83.16 ±3.18*	78.84 ± 3.16*

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at *p<0.05. Diabetic rats were compared with control rats. MEDM treated diabetic rats were compared with diabetic Glibenclamide treated diabetic rats were compared with diabetic control rats

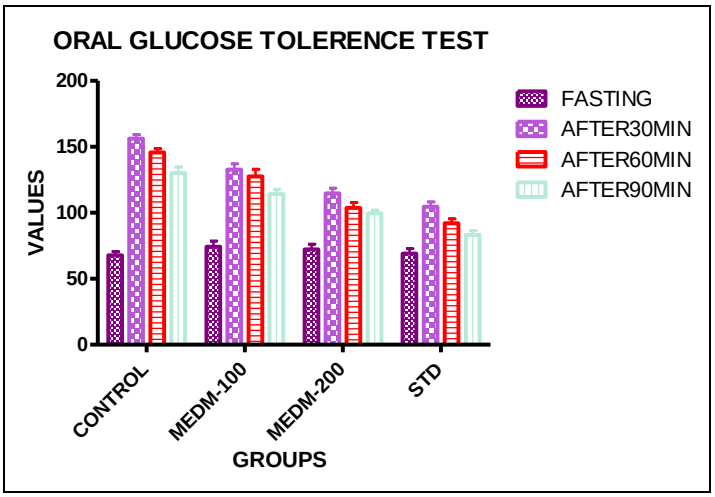

FIG. : EFFECT OF MEDM AND GLIBENCLAMIDE IN OGTT OF CONTROL AND EXPERIMENTAL GROUPS OF RATS

TABLE 4: BODY WEIGHT CHANGES IN METHANOL EXTRACT OF *DIGERA MURICATA* AND GLIBENCLAMIDE ON CONTROL AND EXPERIMENTAL GROUPS OF RATS

Group	Treatment/Dose	Body weight (kg) Initial	Body weight (kg) Final
I.	Normal Control rats + Vehicles only	186.16±11.46	212.33±13.32
II.	Diabetic Control rats	208.23±12*	174.26±9.52*
III.	Normal rats + EEAB (100 mg/kg)	179.62±22.58*	177.16±25.76*
IV.	Diabetic group + MEDM (200 mg/kg)	212.80±9.67*	214.62±9.28*
V.	Diabetic rats+ Glibenclamide (3mg/kg)	218.64±12.65*	217.31±15.88*

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at *p<0.01 Diabetic rats were compared with control rats. MEDM treated diabetic rats were compared with diabetic control rats Glibenclamide treated diabetic rats were compared with diabetic control rats

TABLE 5: CHANGES IN BLOOD GLUCOSE LEVEL

Group	Treatment	0 day	7 day	14 days	21 days
I	Control	100.76 ±5.13	96.98± 6.48	91.14 ±5.62	95.18 ± 3.65
II	Diabetic control	281.65±14.42*	293.12±21.22*	325.56±16.28*	344.83±17.68*
III	Medm100	271.02± 3.74*	269.32 ±8.21*	272.82±11.80*	270.48 15.02*
IV	Medm200	278.49± 4.66*	266.78±12.49*	255.40 12.62*	246.16±13.68*
V	Std	298.56 ±6.52*	298.24 ±3.44*	25.33 ±3.46 *	210.31±2.36*

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at *p<0.05. Diabetic rats were compared with control rats. MEDM treated diabetic rats were compared with diabetic control rats. Glibenclamide treated diabetic rats were compared with diabetic control rats

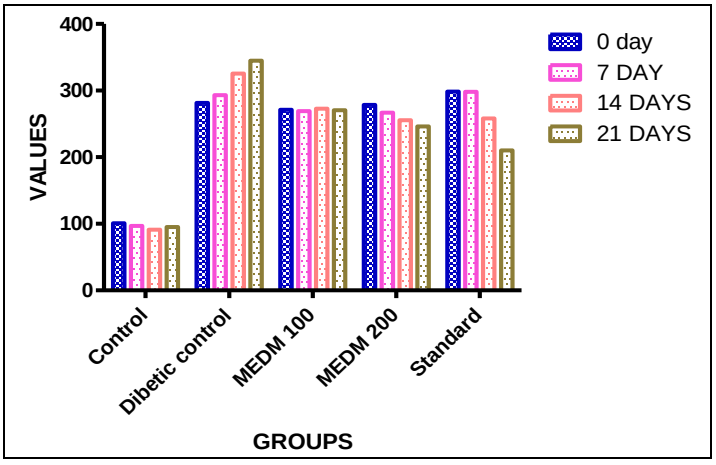

FIG. : CHANGES IN BLOOD GLUCOSE LEVEL

TABLE 7: EFFECT OF METHANOL EXTRACT OF *DIGERA MURICATA* AND *GLIBENCLAMIDE* ON PLASMA INSULIN, $HbA1_C$ OF CONTROL AND EXPERIMENTAL GROUPS OF RATS

Groups/Treatment/Dose	Insulin µU/ml	$HbA1_c$ %
I. Normal Control rats	31.8±1.58	3.70±.26
II. Diabetic Control rats	17.83±1.58*	6.42±.32*
III. Normal rats + MEDM 100mg/kg	19.16±0.76*	5.03±0.45*
IV. Diabetic rats + MEDM 200mg/kg	24± 1.04*	5.12± 0.32*
V. Diabetic rats + Glibenclamide	33.16 ±2.56*	3.69± 0.28*

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at *p<0.05. Diabetic rats were compared with control rats. MEDM treated diabetic rats were compared with diabetic control rats. Glibenclamide treated diabetic rats were compared with diabetic control rats

TABLE 8: EFFECT OF MEDM AND GLIBENCLAMIDE IN TOTAL CHOLESTEROL, TRIGLYCERIDES, HDL CHOLESTEROL, LDL CHOLESTEROL, VLDL CHOLESTEROL OF CONTROL AND EXPERIMENTAL GROUPS OF RATS

Group	Treatment	Total cholesterol (mg/dl)	HDL cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
I	Control	98.68±4.34	33.35 ± 1.35	108.56 ±1.43	46.8 ± 1.56*	16.4 ± 0.56*
II	Diabetic control (alloxan)	176.60 ±0.67*	22.34 ± 0.67*	171.5± 2.45*	118.4 ± 1.04*	33.4 ± 0.85*
III	MEDM (100mg/kg)	127.6 ± 1.05*	28.45 ± 1.05*	121.3 ± 1.93*	73.6 ± 1.8*	23.4 ± 0.09*
IV	MEDM (200mg/kg)	111.7± 2.57*	34.10± 0.43*	101.7 ± 3.76*	56.8 ± 2.2*	19.4 ± 1.30*
V	Glibenclamide	103.56± 3.638*	39.21 ± 1.06*	91.12 ± 0.86*	44.6 ± 1.7*	17.4 ± 0.32*

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at *p<0.05. Diabetic rats were compared with control rats. MEDM treated diabetic rats were compared with diabetic Glibenclamide treated diabetic rats were compared with diabetic control rat

TABLE 9: EFFECT ON MEDM CHANGES IN ALP LEVEL

Group	Treatment/dose	ALP U/L
I	Normal control	112.64 ± 2.51
II	Diabetic control	178.49± 4.38*
III	MEDM 100	153.14 ± 5.34*
IV	MEDM 200	157.15 ±5.18*
V	Glibenclamide	123.14 ±2.18*

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at *p<0.05. Diabetic rats were compared with control rats. MEDM treated diabetic rats were compared with diabetic Glibenclamide treated diabetic rats were compared with diabetic control rates

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CONCLUSION: In present study indicates that *Digera muricata* leaves of methanolic extract exhibited significant Antidiabetic activity in alloxan induced rats and also parameters like blood glucose level, body weight ,lipid profile along with plasma insulin, HbA1c, ALP.

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