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Anti-Diabetic Activity of *Melothria Heterophylla* (Lour.) Cogn. in Streptozotocin Induced Diabetic Rats

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ABSTRACT

Melothria heterophylla (Lour.) Cogn. (Cucurbitaceae) widely distributed in India and used ethnically by tribal people in India for controlling blood sugar. This promotes us to undertake a study to examine the possible antidiabetic activity of the plant extracts in normal and streptozotocin induced diabetic rats. A single dose study was studied in the normal rats for 12hrs. Oral glucose tolerance test was performed in normal rats after receiving glucose orally (2gm/kg). Diabetes was induced with streptozotocin (50mg/kg, i.p.) and a dose of 300mg/kg of Petroleum ether (PEMH), Methanol (MEMH) and Aqueous (AEMH) extracts were then administered orally to experimental diabetic rats for 21 days. Glibenclamide was used as standard reference. Fasting blood glucose levels, changes in body weight and liver weight, serum albumin, serum urea, total protein, total lipid profile, haemoglobin, SOD, GSH and TBARS were evaluated. Single dose study of extracts on normal rats showed a significant decrease in the fasting blood glucose levels when compared with the normal control rats. Oral glucose tolerance test clearly indicate that MEMH and PEMH extracts shown a significant reduce in the blood glucose levels, AEMH extract showed little effect. In diabetic rats, treatment with the PEMH, MEMH and AEMH showing significant reduction in the fasting blood glucose levels, serum cholesterol, serum triglycerides, LDL-C and VLDL-C levels. A significant escalation is seen in the levels of HDL-C, haemoglobin, body weight and liver weight. Whereas the antioxidant levels of SOD, GSH and TBARS improved than the untreated diabetic rats. The study reveals that the plant extracts of *Melothria heterophylla* showed significant antidiabetic activity in normal fasted rats, OGTT in normal rats and in STZ induced diabetic rats.
Keywords: Antidiabetic activity, Antioxidant, Glibenclamide, *Melothria heterophylla*, Streptozotocin.

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INTRODUCTION

Diabetes mellitus, often simply referred to as Diabetes. Diabetes is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger) [1]. Diabetes mellitus is often called 'The silent killer', because it causes serious complications without serious symptoms and can affect many of major organs in the body.

Diabetes mellitus is one of the common metabolic disorders with micro, macro vascular complications and affecting carbohydrate, fat and protein metabolism that result in significant morbidity and mortality [2]. It is considered as one of the five leading causes of death in the world. It is characterized hyperglycemia (an increased blood glucose levels) and also a defect in insulin secretion and insulin action or both.

According to world ethanobotanical information reports, almost 800 plants may possess antidiabetic potential. In the past decade, research has been focused on scientific evaluation of traditional drugs of plant origin and screening of more effective and safe hypoglycemic agents has continued to be an important area. However, lots of herbs are now being used in the management of Diabetes mellitus [3]. The mechanisms of action of most of the plants are not clear, although a few have been documented.

MATERIALS AND METHODS

Collection of Plant Material

The plant *Melothria heterophylla* most widely found in the India. The plant was collected from the forest near to Chittoor District (Andhra Pradesh). The plant was authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh, India.

Preparation of Extract

The whole plant was shade dried at room temperature and was chopped into small pieces. Dried plant were powdered and packed in air tight container. The coarse material was subjected to successive soxhlet extraction by using different solvents. Solvents are used based on their increasing order of polarity i.e., Petroleum ether (0.1, PEMH), Methanol (5.1, MEMH) and Water (10.2, AEMH). The extraction is carried at 60^0C for Petroleum ether and 65^0C for Methanol. The aqueous extraction was carried out by cold maceration process. The extracts were concentrated under reduced pressure and stored in desiccators [4,5].

Preliminary Phytochemical Screening of Extracts

Phytochemical analysis was carried out by using the standard procedures. alkaloids, carbohydrates, flavonoids, glycosides, phytosterols/terpens, proteins, tannins, saponins and lipids were qualitatively analysed [6].

Experimental Animals

Albino wistar rats weighing 160-220g was procured from Biogen, Bangalore. They were maintained in the animal house of Gautham College of Pharmacy, for experimental purpose. Animals were maintained under controlled condition of temperature at $27^{o} \pm 2^{o}$C and 12 hr light-dark cycles for one week. They were housed in polypropylene cages and containing paddy husk as bedding. They had a free access to standard pellets and water *ad libitum*. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Gautham College of Pharmacy, Bangalore (REF-GCP/IAEC/019/12/2010) according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

Determination of Acute Toxicity (LD_{50})

The procedure was divided into two phases. Phase I (observation made on day one) and Phase II (observed the animals for next 14 days of drug administration). Two sets of healthy female rats (each set of 3 rats) were used for this experiment. First set of animals were divided into three groups, each of one in a group. Animals were fasted overnight with water *ad libitum.* Animals received a single dose of 2000 mg/kg was selected for the test, as the test item was a source from herb. After administration of extract, food was withheld for 3-4 hrs. If the animal dies, conduct the main test to determine the LD_{50}. If the animal survives, dose four additional animals sequentially so that a total of five animals are tested. However, if three animals die, the limit test is terminated and the main test is performed. The LD_{50} is greater than 2000 mg/kg, if three or more animals survive. If an animal unexpectedly dies late in the study, and there are other survivors, it is appropriate to stop dosing and observe all animals to see if other animals will also die during a similar observation period. Late deaths should be counted the same as other deaths. The same procedure was repeated with another set of animals to nullify the errors [7].

Experimental Design

Effect of Extracts in Normal Rats [8]

Albino wistar rats weighing 160-220 mg/kg were divided into five groups of six in each group. Animals were fasted overnight for 16 hrs prior to the experiment. The blood glucose levels were measured just prior to and 1, 2, 4, 8 and 12 hrs after drug administration. The blood glucose levels were measured from the tail vein by using Sugarchek glucometer manufactured by Wockhardt.

Group-I: Saline will be supplied and served as control.
Group-II: Animals received a dose of 5 mg/kg of Glibenclamide p.o. and served as standard.
Group-III-V: Animals received a dose of 300 mg/kg of PEMH, MEMH and AEMH p.o respectively.

Oral Glucose Tolerance in Normal Rats [9]

The oral glucose tolerance test was performed in rats weighing 160-220g. The animals were fasted for 16 hrs before the experiment but allowed free access to water. These Rats were divided into five groups, six in each group. Rats of all groups were loaded with glucose 2 gm/kg p.o 30 min after drug administration. Blood samples were collected from the tail vein prior to drug administration and at 30, 90, and 150 min of glucose administration.

Group-I: Animals received Saline and after 30 min a glucose load of 2 gm/kg is administered p.o. which was served as control.

Group-II: Animals received a dose of 5 mg/kg of Glibenclamide p.o. and after 30 min a glucose load of 2 gm/kg is administered p.o which was served as standard.

Group-III-V: Animals received a dose of 300 mg/kg of PEMH, MEMH and AEMH p.o respectively and after 30 min a glucose load of 2 gm/kg is administered p.o.

Effect of Extracts in Streptozotocin Induced Diabetic Rats [10,11]

Experimentally Induced Diabetes Mellitus [12]

Female wistar rats weighing 160-220g were used for this study. The animals were overnight fasted for 16 hrs before the induction of Diabetes. Diabetes was induced by a single dose of 50 mg/kg body weight of Streptozotocin by intraperitoneal route. After a period of 3 days blood glucose levels were checked by snipping the tail of STZ treated fasted rats. Rats showing the blood glucose levels more than 300 mg/dl is taken into the study [13].

Experimental Procedure

Diabetes was induced in fasted female Albino wistar rats (160-220g) by intraperitoneal injection of 50 mg/kg body weight of STZ except Group I. After 72 hrs, rats with fasting blood glucose levels higher than 300 mg/dl were selected and used.

Group-I and II: Animals received Saline and served as normal control and diabetic control respectively.

Group-III: Animals received a dose of 5 mg/kg of Glibenclamide p.o. and served as standard.

Group-IV-VI: Animals received a dose of 300 mg/kg of PEMH, MEMH and AEMH p.o respectively.

The study was carried out for 21 days. Fasting blood glucose levels were measured before the administration of extracts. It was recorded as 0 day. The doses of the extracts (PEMH, MEMH and AEMH) along with the standard (Glibenclamide) were given daily to the animals for 21 days. The blood glucose levels were checked on 0, 7, 14, and 21 day of the

treatment period. Blood was collected from snipping of the rat tail. Blood glucose levels were measured by using the Sugarchek glucometer.

Collection of Blood and Serum Samples

At the end of the experiment, blood was collected by cardiac puncture from each rat under mild ether anaesthesia. The blood samples were used for the estimation of haemoglobin levels and remaining was allowed to clot for 30 min at room temperature and they were centrifuged at 3000 rpm for 10 minutes. The serum was used for the study of biochemical parameters.

Collection of Tissue

The animals were scarified and the Pancreas and Liver were collected. Pancreas was used for the histopathological study and liver was used for the estimation of SOD, GSH and TBARS.

Physical Parameters

Determination of Body Weight

Body weight of the all animals in each group was noted on the 0, 7, 14 and 21 day of the experiment period. The weight difference was calculated.

Determination of Wet Liver Weight

Animals were sacrificed and livers were isolated and washed with saline and weights determined by using an electronic balance. The liver weights were expressed with respect to its body weight i.e. gm/100gm.

Estimation of Biochemical Parameters

The following parameters are estimated by using standard procedures of span diagnostics estimating kits. Total Protein, Serum Albumin, Serum Urea, Hemoglobin (Hb) and Lipid Profile (HDL, LDL, VLDL, TG and Total Cholesterol).

Estimation of Antioxidant Activity

Livers of the animals were homogenized with ice-chilled 10% Phosphate buffer and centrifuge at 2000 rpm to 10 minutes. The supernatant liquid is used for the estimation of following parameters. Superoxide Dismutase (SOD), Thiobarbituric Acid Reactive Substances (TBARS) and Glutathione.

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Statistical Analysis

The values are expressed as Mean ± SEM. The data was analysed by using one way ANOVA followed by Dunnett's test using Graph pad prism software. Statistical significance was set at $P \leq 0.05$.

RESULTS

Extraction

Extraction of *Melothria heterophylla* (Lour.) Cogn. whole plant was carried out by using the soxhlet apparatus with different solvents (Petroleum ether, Methanol and Water) the percentage yield of each extract is given below in Table No-1.

Table No-1: Extractive Yield and Percentage Yield of *Melothria heterophylla.*

Sl. No.	Extracts	Yield in gms	Percentage Yield
1.	Petroleum Ether	10.75	2.59%
2.	Methanol	34.51	9.08%
3.	Aqueous	72.17	19.5%

Preliminary Qualitative Phytochemical Studies

Table No-2: Preliminary Phytochemical Screening of Extracts.

Sl. No.	Test	Petroleum Ether Extract	Methanolic Extract	Aqueous Extract
1	Alkaloids	+	+	+
2	Carbohydrates	+	+	+
3	Flavonoids	-	+	-
4	Glycosides	+	+	+
5	Phytosterols/Terpens	+	+	-
6	Proteins	+	+	+
7	Tannins	-	+	+
8	Saponins	-	-	+
9	Lipids	-	-	-

- Absent. **+** Indicates presence.

Acute Toxicity Studies (LD_{50})

In both phase I and Phase II procedures, none of the animals show any toxicity upon the single administration of PEMH, MEMH and AEMH (2000 mg/kg). Hence the procedure is repeated by increasing the dose of extracts (3000 mg/kg). None of the animals had shown

any toxicity. Thus, 1/10th of maximum dose (300 mg/kg) tested was selected for the present study.

Effect of Extracts in Normal Rats

Hypoglycaemic activity of PEMH, MEMH and AEMH were studied on normal rats and the results were tabulated in Table No-3. MEMH shows a significant reduction in the blood glucose levels at 1, 2, 4, 8 and 12 hours, onset of action is shown at 1 hour after the treatment. Less significant reduction in blood glucose levels was shown at 8th hour (P<0.01). A maximum blood glucose reduction was shown at 12th hour (P<0.001) compared to the normal untreated group. PEMH shows a significant action in reducing the blood glucose levels at the time interval of 2 hours (P<0.01) after the treatment. It also show a marked decrease in 4 hours (P<0.01) and 8 hours (P<0.05). But it has no significant activity at the time interval of 1 and 12 hours when compared to control. AEMH shows its action at time interval of 2 hour (P<0.05) and a much reduced activity at the time interval of 4 hours (P <0.05). It further did not show its effect after 8 hours of treatment. Glibenclamide showed it effect from 1 hour after treatment. The onset of Glibenclamide starts from 1 hour after the treatment. It reduces maximum blood glucose levels at 12th hour (P<0.001). Glibenclamide significantly reduced the blood glucose levels after treatment in normal rats. All the blood glucose levels of treated group were compared with the normal control group animals.

Table No-3: Effect of *Melothria heterophylla* Plant Extracts on Blood Glucose Levels in Normal Rats.

Groups	Treatment	Blood Glucose Levels (mg/dl)					
		0th hr	1st hr	2nd hr	4th hr	8th hr	12th hr
Group-I	Saline	94.00 ± 3.70	90.67 ± 3.16	87.67 ± 2.57	81.83 ± 2.61	75.00 ± 2.26	71.67 ± 2.33
Group-II	Glibenclamide (5mg/kg)	91.50 ± 3.95	61.33 ± 2.20***	55.33 ± 1.82***	43.67 ± 2.44***	40.50 ± 1.31***	36.33 ± 1.38***
Group-III	PEMH (300mg/kg)	94.33 ± 5.05	83.17 ± 3.07^{ns}	75.17 ± 1.57**	67.33 ± 2.36**	63.67 ± 1.70*	63.50 ± 3.13^{ns}
Group-IV	MEMH (300mg/kg)	94.33 ± 5.44	73.33 ± 3.43**	65.17 ± 3.20***	59.67 ± 3.12***	58.00 ± 2.88**	49.17 ± 1.83***
Group-V	AEMH (300mg/kg)	96.17 ± 2.68	85.50 ± 2.48^{ns}	78.50 ± 2.56*	70.50 ± 3.10*	69.83 ± 4.84^{ns}	75.00 ± 4.47^{ns}

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant.

Oral Glucose Tolerance Test in Normal Rats

The effect of PEMH, MEMH and AEMH on oral glucose tolerance test was tabulated in the Table No-4. Most significant decrease in blood glucose levels was observed when the MEMH was administered 30 min before glucose loading. Very significant reduction was observed at 150 min (P<0.001) and significant reduction was observed at 30 min and 90 min (P<0.01). Significant reduction was more at 90 min when compared with the 30 min.

Whereas PEMH also show a significant decrease in blood glucose levels, when administration 30 min before glucose loading. It showed a significant activity at the time intervals of 90 min and 150 min (P< 0.01). Significant reduction was more at 150 min when compared with the 90 min. But AEMH showed a much reduced activity (P<0.05) compare with the MEMH and PEMH extracts. It showed its effect at 30 min after glucose administration. Glibenclamide showed its potent antidiabetic activity in normal rats it bring backs the elevated blood glucose levels to normal levels compared to normal control group at 90 min (P<0.001).

Overall the different extracts of *Melothria heterophylla* had showed a significant decrease in the blood glucose levels when compared with the normal control group rats at time intervals 30, 90 and 150 min.

Table No-4: Effect of *Melothria heterophylla* Plant Extracts on Blood Glucose Levels on Oral Glucose Tolerance Test in Normal Rats.

Groups	Treatment	Blood Glucose Levels (mg/dl) and Time in min			
		0^{th} min	30^{th} min	90^{th} min	150^{th} min
Group-I	Saline	87.33 ± 2.81	132.70 ± 1.11	111.50 ± 1.40	96.67 ± 3.13
Group-II	Glibenclamide (5mg/kg)	81.83 ± 1.79	102.00 ± 2.88***	84.83 ± 2.38***	60.00 ± 1.71***
Group-III	PEMH (300mg/kg)	89.33 ± 2.77	120.80 ± 2.45^{ns}	94.67 ± 4.11**	77.17 ± 5.08**
Group-IV	MEMH (300mg/kg)	93.00 ± 3.34	111.00 ± 2.22**	95.67 ± 3.48**	67.17 ± 2.08***
Group-V	AEMH (300mg/kg)	88.33 ± 2.78	115.80 ± 6.74*	99.83 ± 2.05*	80.83 ± 3.77*

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant.

Effect of Extracts in Streptozotocin Induced Diabetic Rats

A chronic study of 21 days was done in STZ induced diabetic rats with *Melothria heterophylla* plant extracts and the results of blood glucose levels are tabulated in the Table No- 5. Blood glucose levels on day zero showed no significant intra group variation. Administration of streptozotocin (50mg/kg, i.p.) in 0.01M citrate buffer (pH= 4.5) showed a significant increase in fasting blood glucose levels (355.7 ± 13.37mg/dl). After 21 days, diabetic control rats exhibited significantly higher blood glucose levels (392.8 ± 17.05 mg/dl) as compared to the normal control rats (83.17 ± 6.05 mg/dl). Diabetic rats treated with a MEMH for 21 days lowers the blood glucose levels from 362.3 ± 7.41 to 154.3 ± 5.53 (P<0.001). It also shows a significant activity in decreasing the blood glucose levels on 7^{th} and 14^{th} day (284.7 ± 10.57 and 194.7 ± 5.69 respectively). Similarly, diabetic rats treated with the PEMH also shown a significant activity when compared with the diabetic control rats. Blood glucose levels on 21 day was 173.7 ± 7.62 which is less when compared with the initial blood glucose level 367.8 ± 16.82 on 0^{th} day of treatment. A daily treatment of AEMH for a period of 21 days lowers the blood glucose levels in diabetic treated rats. Blood glucose levels on 7^{th} and 14^{th} day (306.8 ± 9.92 and 211.8 ± 9.52 respectively) of treatment also show a significant reduce in blood glucose levels when compared with the diabetic control group of animals. Glibenclamide showed its potent antidiabetic activity and reduced the blood glucose levels of diabetic rats to the level significantly (360.7 ± 12.95 to 124.5 ± 4.63) at day 21. It elevated blood glucose levels on 7^{th} and 14^{th} days were 267.7 ± 14.73 and 179.8 ± 8.24 respectively. Which is having a high significant activity P<0.001.

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Table No-5: Effect of *Melothria heterophylla* Plant Extracts on Blood Glucose Levels in STZ Induced Diabetic Rats.

Groups	Treatment	Blood Glucose Levels (mg/dl)			
		0th day	7th day	14th day	21st day
Group-I	Saline	81.5 ± 4.33	90.0 ± 5.52***	85.0 ± 2.50***	83.17 ± 6.05***
Group-II	Saline + STZ (50mg/kg)	355.7 ± 13.37	373.7 ± 14.39	378.8 ± 15.59	392.8 ± 17.05
Group-III	Glibenclamide (5mg/kg) + STZ (50mg/kg)	360.7 ± 12.95	267.7 ± 14.73***	179.8 ± 8.24***	124.5 ± 4.63***
Group-IV	PEMH (300mg/kg) + STZ (50mg/kg)	367.8 ± 16.82	295.3 ± 10.74***	214.2 ± 8.27***	173.7 ± 7.62***
Group-V	MEMH (300mg/kg) + STZ (50mg/kg)	362.3 ± 7.41	284.7 ± 10.57***	194.7 ± 5.69***	154.3 ± 5.53***
Group-VI	AEMH (300mg/kg) + STZ (50mg/kg)	359.0 ± 16.89	306.8 ± 9.92**	211.8 ± 9.52***	189.3 ± 6.68***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001 and ** P<0.01. All the values are compared with the diabetic control group.

Effect of Extracts on Physical Parameters of STZ Induced Diabetic Rats

Body Weight

There is a significant change seen in the body weight of animals after the treatment inducing diabetes with STZ. The decreased body weight of the animals were significantly regains when compare with the diabetic control animals after treatment for 21 days with the extract. And also the body weight of normal control group was significantly increased compared to initial body weight. The changes in body weight of the animals during 0th, 7th, 14th and 21st days was tabulated in the Table No-6.

Wet Liver Weight

Rats treated with STZ shown a decrease in the liver weight of untreated diabetic rats, whereas in treated rats there is a significant restoration of wet liver weight which is near to the normal levels. The values of the wet liver weight were tabulated in the Table No-6.

Effect of Extracts on Biochemical Parameters of STZ Induced Diabetic Rats

Serum albumin levels were decreased in the diabetic animals, as compared with the normal control animals. Whereas albumin levels in the diabetic control group is 2.53 ± 0.12 g/dl. But albumin levels after treatment with the *Melothria heterophylla* plant extracts shows an increased the serum albumin levels. The values of the albumin levels are mentioned in the Table No-7.

Diabetic rats showed an increased in the levels of serum urea. Treatment of these rats with the extracts and glibenclamide showed a decrease in the urea levels when compared with the normal animals. The urea levels in the diabetic rats are 61.16 ± 4.70 mg/dl, where it is decrease to 40.00 ± 1.64 mg/dl in treated group and 31.89 ± 1.20 mg/dl in

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glibenclamide treated rats. Serum urea levels of treated and normal rats are expressed in the Table No-7.

Table No-6: Effect of *Melothria heterophylla* Plant Extracts on Body Weight and Liver Weight in STZ Induced Diabetic Rats.

Groups	Treatment	Body Weight (gms)				Liver Weight	
		0^{th} day	7^{th} day	14^{th} day	21^{st} day	Wet liver weight	Weight/100 gm body Wt
Group-I	Saline	183.3 ± 3.80	186.0 ± 4.49***	189.5 ± 4.89***	192.2 ± 4.58***	9.68 ± 0.22***	5.04 ± 0.16***
Group-II	Saline + STZ (50mg/kg)	172.3 ± 3.85	159.2 ± 1.93	154.7 ± 3.25	152.0 ± 2.49	5.81 ± 0.25	3.53 ± 0.07
Group-III	Glibenclamide (5mg/kg) + STZ (50mg/kg)	185.0 ± 4.78	182.3 ± 2.59***	187.2 ± 2.67***	189.5 ± 2.02***	8.28 ± 0.36***	4.47 ± 0.16**
Group-IV	PEMH (300mg/kg) + STZ (50mg/kg)	185.3 ± 3.82	181.2 ± 3.66**	184.5 ± 3.92***	188.5 ± 3.99***	7.58 ± 0.47**	4.02 ± 0.25^{ns}
Group-V	MEMH (300mg/kg) + STZ (50mg/kg)	180.8 ± 5.65	177.3 ± 5.57**	180.5 ± 4.92***	183.2 ± 4.95***	7.74 ± 0.25**	4.24 ± 0.22*
Group-VI	AEMH (300mg/kg) + STZ (50mg/kg)	189.2 ± 5.06	177.2 ± 3.71*	180.2 ± 3.67***	184.5 ± 3.99***	7.19 ± 0.31*	3.90 ± 0.20^{ns}

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All values are compared with diabetic control.

Table No-7: Effect of *Melothria heterophylla* Plant Extracts on Biochemical Parameters in STZ Induced Diabetic Rats.

Groups	Treatment	Serum Albumin Levels (g/dl)	Serum Urea Levels (mg/dl)	Serum Total Protein Levels (mg/dl)	Haemoglobin (mg/dl)
Group-I	Saline	3.99 ± 0.05***	29.23 ± 0.99***	6.03 ± 0.03***	12.38 ± 0.27***
Group-II	Saline + STZ (50mg/kg)	2.53 ± 0.12	61.16 ± 4.70	4.66 ± 0.13	9.83 ± 0.34
Group-III	Glibenclamide (5mg/kg) + STZ (50mg/kg)	3.57 ± 0.11***	31.89 ± 1.20***	5.92 ± 0.09***	12.15 ± 0.24***
Group-IV	PEMH (300mg/kg) + STZ (50mg/kg)	3.32 ± 0.09***	49.27 ± 1.39**	5.65 ± 0.07***	11.68 ± 0.32***
Group-V	MEMH (300mg/kg) + STZ (50mg/kg)	3.53 ± 0.08***	40.00 ± 1.64***	5.73 ± 0.07***	11.80 ± 0.26***
Group- VI	AEMH (300 mg/kg) + STZ (50mg/kg)	2.96 ± 0.16*	49.08 ± 2.04**	5.22 ± 0.14**	11.37 ± 0.22**

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All values are compared with diabetic control.

Serum protein levels are decreased (4.66 ± 0.13 mg/dl) in the untreated diabetic rats compares to the normal control rats (6.03 ± 0.03 mg/dl). After treatment with the Glibenclamide, MEMH and PEMH showed a significant increase in the serum protein levels compared with the diabetic control animals. AEMH showed a less significant activity in increasing the protein levels. The values of serum total protein levels are shown in the Table No-7.

A daily dose of the extracts for a period of 21 days showed an increased in the haemoglobin level of diabetic rats. But the AEMH showed a less significant activity when compared with the diabetic control rats. Glibenclamide restores the haemoglobin levels to normal levels after treatment. The values are tabulated in the Table No-7.

Serum Lipid Profile

The lipid profile was evaluated by estimating triglycerides (TG), total cholesterol (TC), HDL-Cholesterol (HDL-C), LDL-Cholesterol (LDL-C) and VLDL-Cholesterol (VLDL-C) in normal and diabetic animals. The STZ diabetic animals showed a significant increased in the TG, TC, LDL-C and VLDL-C levels and suppression of HDL-C levels compared to control group (Table No-8). But after treatment with the plant extracts and glibenclamide diabetic animals showed decrease in the TG, TC, LDL-C and VLDL-C levels and increase in the HDL-C levels compared to untreated diabetic rats.

Table No-8: Effect of *Melothria heterophylla* Plant Extracts on Serum Lipid Profile of STZ Induced Diabetic Rats.

Groups	Treatment	Serum Lipid Profile mg/dl				
		TC	TG	HDL-C	LDL-C	VLDL-C
Group-I	Saline	67.25 ± 3.06***	77.77 ± 4.54***	22.49 ± 0.89***	29.21 ± 2.09***	15.55 ± 0.90***
Group-II	Saline + STZ (50mg/kg)	122.2 ± 4.19	154.0 ± 7.13	16.36 ± 0.43	75.07 ± 4.46	30.79 ± 1.42
Group-III	Glibenclamide (5mg/kg) + STZ (50mg/kg)	82.45 ± 2.17***	92.06 ± 6.80***	21.06 ± 0.30***	42.99 ± 2.82***	18.41 ± 1.36***
Group-IV	PEMH (300mg/kg) + STZ (50mg/kg)	97.66 ± 2.29***	112.7 ± 4.54***	19.52 ± 0.36***	55.60 ± 3.15***	22.54 ± 0.90***
Group-V	MEMH (300mg/kg) + STZ (50mg/kg)	92.39 ± 2.66***	107.9 ± 8.39***	19.93 ± 0.34***	50.88 ± 3.26***	21.58 ± 1.68***
Group- VI	AEMH (300 mg/kg) + STZ (50mg/kg)	98.24 ± 2.02***	115.9 ± 5.72***	18.30 ± 0.40*	59.11 ± 2.90**	23.17 ± 1.14***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01 and * P<0.05. All values are compared with diabetic control.

Effect of Extracts on Antioxidant Levels

Diabetic rats exhibited significant lower SOD (9.92 ± 0.23) as compared to those of control rats (15.37 ± 0.31) treatment with the plant extracts significantly elevated the reduced SOD levels. MEMH, PEMH, AEMH and Glibenclamide showed a marked increase in the SOD levels (P<0.001) compared to the diabetic control. These values are tabulated in the Table No-9.

Rats treated with STZ had a TBARS level of 3.23 ± 0.15 nmoles of MDA/ 100 mg of tissue when measured on day 21. This was significantly higher when compared to levels in normal control rats of 0.91 ± 0.02 nmoles of MDA/ 100 mg of tissue.

Diabetic rats treated with MEMH had a lipid peroxidation levels of 1.63 ± 0.24 nmoles of MDA/ 100 mg of tissue and also rats treated with the PEMH and AEMH treated

rats having a TBARS levels of 1.97 ± 0.21 and 2.17 ± 0.16 nmoles of MDA/ 100 mg of tissue respectively. Whereas in glibenclamide treated rats the levels are restored to normal levels of 1.17 ± 0.07 nmoles of MDA/ 100 mg of tissue. These values are expressed in Table No-9. Rats treated with STZ had a tissue GSH level of 29.66 ± 2.45 mM/ 100 mg of tissue when measured on day 21. Treatment with PEMH, MEMH and AEMH show an increase GSH levels in STZ treated rats. These values are having a significant higher (P<0.001) when compared to GSH levels in diabetic control rats. The values are tabulated in the Table No-9.

Table No-9: Effect of *Melothria heterophylla* Plant Extracts on Antioxidant Levels in STZ Induced Diabetic Rats.

Groups	Treatment	SOD U/mg Protein	TBARS (nmoles of MDA/ 100 mg of tissue)	GSH (mM/ 100 mg of tissue)
Group-I	Saline	15.37 ± 0.31***	0.91 ± 0.02 ***	43.34 ± 0.74***
Group-II	Saline + STZ (50mg/kg)	9.92 ± 0.23	3.23 ± 0.15	29.66 ± 2.45
Group-III	Glibenclamide (5mg/kg) + STZ (50mg/kg)	13.89 ± 0.33***	1.17 ± 0.07 ***	42.84 ± 0.51***
Group-IV	PEMH (300mg/kg) + STZ (50mg/kg)	13.19 ± 0.13***	1.97 ± 0.21 ***	41.71 ± 0.39***
Group-V	MEMH (300mg/kg) + STZ (50mg/kg)	13.48 ± 0.15***	1.63 ± 0.24 ***	42.03 ± 0.51***
Group- VI	AEMH (300 mg/kg) + STZ (50mg/kg)	11.67 ± 0.26***	2.17 ± 0.16***	38.54 ± 1.92***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All values are compared with diabetic control.

Histopathological Study of Pancreas

Group –I (Normal Control + Saline)

Section studied shows pancreatic lobules separated by connective tissue septa. The pancreatic lobules consist largely of the exocrine acini and their intralobular ducts. Most of the lobules show small, round, light-staining islets of langerhans. The centre of islet cells consist of aggregates of small Beta-cells (70%, Short-arrow) having basophilic granules, while the periphery comprises of large Alpha-cells (25%, Long-arrow) having eosinophilic granules. Intervening these cells are seen thin walled capillaries.

Group –II (Diabetic Control + Streptozotocin 50mg/kg)

Section studied shows pancreatic lobules separated by connective tissue septa. The number of islets appears reduced in number. The center of islet cells consist of quantitative decrease in Beta-cells (30%, Long-arrow) having basophilic granules, while the periphery comprises of large Alpha-cells (65%, Short-arrow) having eosinophilic granules. Also seen are some degenerated beta cells and lymphocytic infiltration amidst these islet cells.

Group –III (Streptozotocin [50mg/kg] + Glibenclamide [5mg/kg])

Section studied shows pancreatic lobules separated by connective tissue septa. Most of the lobules show large areas of light-staining islets of langerhans. The center of islet cells

consist of Beta-cells (60%, long-arrow) having basophilic granules, while the periphery comprises of Alpha-cells (35%, short-arrow) having eosinophilic granules. Also seen are few congested vascular spaces amidst these islet cells.

Group – IV (Streptozotocin [50mg/kg] + PEMH [300mg/kg])

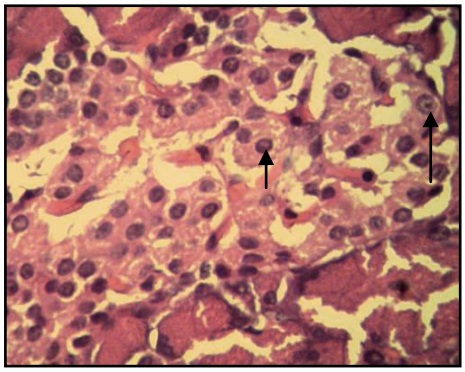
Section studied shows pancreatic lobules separated by thin fibrovascular septa. The center of islet cells consist of Beta-cells (70%, Short-arrow) having basophilic granules, while the periphery comprises of Alpha-cells having eosinophilic granules (25%, Long-arrow).

Group – V (Streptozotocin [50mg/kg] + MEMH [300mg/kg])

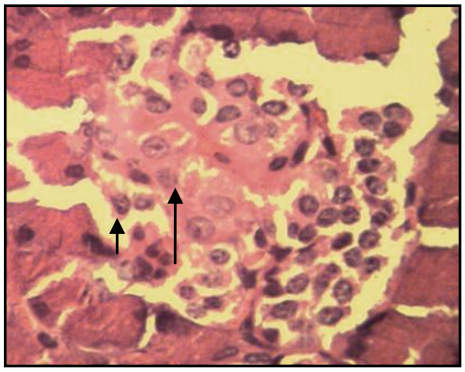
Section studied shows pancreatic lobules separated by thin connective tissue septa. The center of islet cells consists of Beta-cells (70%, Long-arrow) having basophilic granules, while the periphery comprises of Alpha-cells (25%, Short-arrow)) having eosinophilic granules.

Group – VI (Streptozotocin [50mg/kg] + AEMH [300mg/kg])

Section studied shows pancreatic lobules separated by connective tissue septa. The number of islets appears reduced in number. The center of islet cells consist of Beta-cells (45%, Short-arrow) having basophilic granules, while the periphery comprises of large Alpha-cells (50%, Long-Arrow) having eosinophilic granules. Also seen are few degenerated beta cells amidst these islet cells.



Group I



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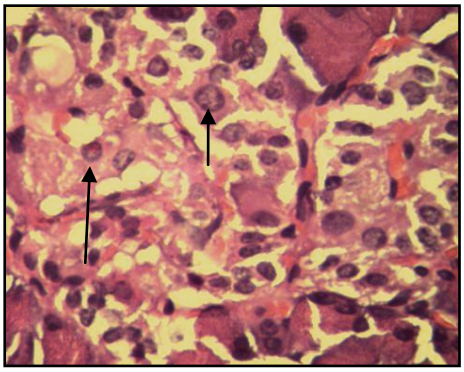

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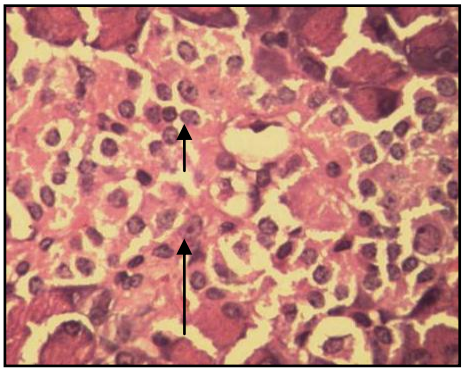

Group IV

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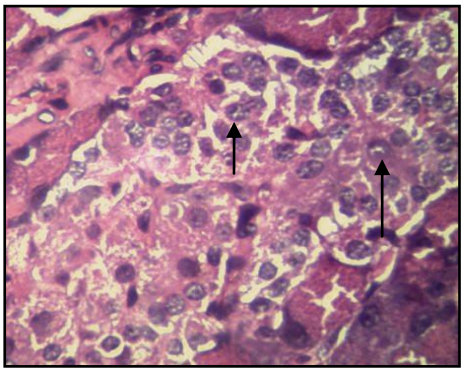
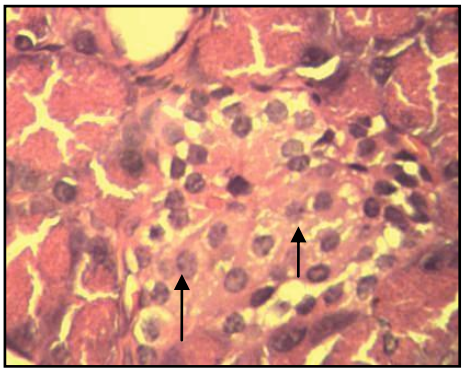


Group V



Group VI

Figure No-1: Histopathology of Pancreas.

DISCUSSION

Single dose study for 12 hrs was carried out in normoglycemic rats. MEMH showed maximum decrease in blood glucose levels at 12th hr compared to normal group. MEMH also showed a significant decrease from the 1st hr of the drug administration. PEMH showed a significant decrease in blood glucose level at 8th hr compared to normal levels. It also showed its activity from the 2nd hr after the drug administration when compared with the normal control and its effect did not last for 12 hrs. Glibenclamide (5mg/kg) showed a maximum decrease of blood glucose levels in normoglycemic rats at 12th hr of study. But AEMH did not show its effect for 12 hrs when compared with the normal rats.

Oral glucose tolerance test was studied on the normal rats. The lowering of glucose can be better seen in assay of glucose tolerance [14]. The fasting blood glucose levels decreases in glibenclamide, MEMH and PEMH treated rats. AEMH shows a reduced activity. Such a phenomenon was already seen in the indigenous plants and reported [15]. The lowering of glucose levels is may be due to the inhibition of intestinal absorption or it may act by potentiating the secretion of insulin and by increase in the utilization of glucose levels in muscles [16].

A prolonged treatment (21 days) of diabetic rats with the PEMH, MEMH and AEMH showed a significant reduction in the blood glucose levels than the untreated rats. This hypoglycaemic activity may be due to the stimulation of surviving β-cells to release more insulin. *Melothria heterophylla* may act by inhibiting hepatic gluconeogenesis or inhibiting α-glucosidase enzyme in the intestine, which is the enzyme helpful for breakdown of disaccharides to form glucose [17].

Induction of diabetes with STZ is associated with a characteristic reduce in body weight than the normal rats, this may be due to the wasting and loss of tissue protein. Whereas, diabetic rats treated with PEMH, MEMH and AEMH showed an improved result when compared with normal diabetic control. Which may be due to the protective effect in controlling muscle wasting i.e., reversal of gluconeogenesis and may also be due to the improvement of glycemic control [18].

A decrease in the liver weight observed in diabetic animals, this is may be due to insufficient release of insulin may causes decrease in the storage of glucose as glycogen in liver. After 21 days of treatment with *Melothria heterophylla* in diabetic animals, an increased in the liver weight is observed than the untreated rats. A less significant increase in liver weight is seen in AEMH treated diabetic rats. This result is may be the extract is having insulin like action which is reported as by Viswanathan [19].

A marked reduction in the levels of total protein and albumin levels was observed in the diabetic rats. The decrease in the albumin levels is due to the increased protein catabolism. Present study shows that the treatment of diabetic rats with the MEMH and glibenclamide showed a significant increase in the levels of albumin and protein levels compared to the normal untreated diabetic rats. An increased plasma urea levels was found in the diabetic rats when compared with the respective control group rats. While after the treatment with *Melothria heterophylla* levels were significantly decreased. Similar observations are also reported in the D-400 herbal formulation [20].

In the present study, the groups of normal rats have shown gain in the body weight while fasting serum glucose was maintained in the normal range throughout the study period. The serum cholesterol and serum triglyceride levels of the normal rats were found to be increasing within the normal range during the three weeks of study period and the haemoglobin levels was also found to be maintained within the normal range throughout the study period.

The haemoglobin levels of the diabetic group of rats were found to be reduced significantly as against the normal haemoglobin levels of the normal group of rats. This is due to an increased formation of its glycosylated form. During hyperglycaemic condition protein synthesis is attenuated or reduced in all the tissues and thus the synthesis of haemoglobin also reduced [21].

Diabetic rats treated with the MEMH, PEMH and glibenclamide has shown a significant decrease in the levels of TG, TC, LDL-C and VLDL-C, whereas it increases the levels of HDL-C when compared to the normal diabetic control rats. In AEMH treated rats HDL-C levels is less significant.

Diabetic rats treated with glibenclamide (5mg/kg) showed significant protection from the body weight loss and progressive reduction of 65.5% in fasting serum glucose levels after a daily dose for 21 days. The glibenclamide treatment also showed the reduced elevated serum cholesterol, albumin, total protein, urea levels and produced significant reduction in elevated serum triglyceride and allowed significant recovery of reduced haemoglobin content during the period of study when compared with the diabetic group of rats. In agreement with the present results, several studies have shown protection in body weight loss [22], anti-diabetic activity, reduction in serum cholesterol and serum triglyceride [23], and recovery in haemoglobin content upon glibenclamide treatment.

Superoxide dismutase is an enzymatic antioxidant which reduces superoxide radical to hydrogen peroxide and oxygen. A decrease in the antioxidant activity in liver results in

the accumulation of free radicals (hydroxyl radical) in diabetic rats. Administration of the PEMH, MEMH, AEMH and glibenclamide increased the activity of SOD levels to a significant level of P<0.001 the results were shown in the Table No-9. While the SOD levels of untreated diabetic control rats having lowered levels. The *Melothria heterophylla* plant may act by either directly scavenging the reactive oxygen metabolites or by increasing the anti-oxidant molecules.

In diabetes, lipid peroxidation is one of the characteristic features of chronic diabetes. The increased free radicals react with the polyunsaturated fatty acids in cell membrane leading to lipid peroxidation. This in turn results in the development of free radicals. Low levels of lipoxygenase peroxides stimulate the release of insulin. But, if the concentration of this peroxidase increases it results in uncontrolled release of lipid peroxidation. The most commonly used indicator of lipid peroxidation is TBARS. There was a significant elevation of TBARS in liver tissue in diabetic control animals when compared to the normal rats. Administration of PEMH, MEMH, AEMH and glibenclamide significantly reduce the TBARS levels. The effect shown is may be due to prevention of potential glycation of anti-oxidant enzymes.

Glutathione which is a tripeptide normally present at high concentrations intracellularly. Glutathione is helpful for reducing the toxic effects of lipid peroxidation. Decreased level of GSH in liver during diabetes represents its increased utilization due to oxidative stress. Significant increased levels of GSH were shown in the diabetic rats treated with the PEMH, MEMH, AEMH and glibenclamide.

The histological evidence shown the authenticated injury caused by STZ and the protection offered by PEMH, MEMH, AEMH and glibenclamide in pancreatic cells are shown. Microscopically examination revealed loss of architecture and cell necrosis with inflammatory collections in the central zone in STZ induced rats. Histopathological study shows that *Melothria heterophylla* has the capacity to increase islet cell mass. However, the expansion was better with MEMH and PEMH.

The hypoglycaemic activity of the *Melothria heterophylla* may be due to the presence of sterols, glycosides, alkaloids, carbohydrates, proteins, and flavonoids. One or more of the other chemical constituents of the plant especially played a crucial role in the hypoglycaemic action of the plant extract [24-26.

The plant *Melothria heterophylla* belongs to the family Cucurbitaceae. It is also reported that the plants belong to the Cucurbitaceae family are having the antidiabetic effect in diabetic rats.

CONCLUSION

The present investigation indicated that administration of PEMH and MEMH extracts at a dose of 300mg/kg produced significant antihyperglycemic activity in STZ induced diabetic rats. AEMH shows less effect then the PEMH and MEMH extracts in reducing the blood glucose levels. The acute toxicity study indicated that the extracts are devoid of major toxic effects. The effect of extracts in normal rats and glucose loaded rats also indicated that the extracts have better glycemic control compared with the normal control animals.

Besides this, the drug administered to treat STZ induced diabetic rats showed a significant reduces in blood glucose levels and the other serum biomarker levels and also increases the haemoglobin levels. The extracts also exhibited antioxidant activity in PEMH, MEMH and AEMH treated diabetic rats. The reports of histopathology study concluded there is an increased mass of β-cells in the pancreatic islets. The results showed in MEMH extract having a more similar to glibenclamide treated group which was used as reference standard. These observations concluded that the extracts of the plant *Melothria heterophylla* possess antidiabetic and antioxidant effects. These activities could be related to insulin secreting property or having increased glucose utilization in the body or decreased absorption of glucose in intestine.

Further studies will be necessary to establish the probable mechanism of action of plant extracts of *Melothria heterophylla*. The present investigation has also opened avenues for further research especially with reference to the development of potent phytomedicine for treatment of Diabetes mellitus from the title plant.

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