To Study The Effect Of *Lycopersicon Esculentum* Leaves Extract On Alloxan Induced Diabetes On Experimental Animals

[1]Shreyash Bale, [2]J.V. Vyas, [3]Dr. V.V. Paithankar, [4]DR. A.W. Wankhade

Department of pharmacology
Vidyabharati college of pharmacy, Amravati.

Abstract-
Lycopersicon esculentum (family: Solanaceae) commonly known as 'Tomato' widely used in traditional system of medicine for the treatment of diabetes mellitus. In the present study, methanol extract of Lycopersicon esculentum (MELE) leaves were subjected to phytochemical investigation and evaluated for antidiabetic activity on blood glucose level in alloxan induced diabetic rats. MELE (200 and 400 mg/kg) and metformin (250 mg/kg) were administered orally in alloxan (100 mg/kg, i.p.) induced diabetic rats. MELE (400 mg/kg) was found to have significant ($p<0.001$) blood glucose lowering effect. Preliminary Phytochemical investigation revealed the presence of alkaloids, phenolics, flavonoids, saponins and tannins as the major constituents in the ethanol extract. These results suggest that MELE (400 mg/kg) showed antihyperglycemic activity in alloxan induced diabetic rats.

Key words: Lycopersicon esculentum, Leaves, Alloxan, Metformin, Antidiabetic activity.

1. INTRODUCTION:

Diabetes mellitus is a syndrome of impaired carbohydrate, fat, and protein metabolism caused by either lack of insulin secretion or decreased sensitivity of the tissues to insulin. There are general types of diabetes mellitus:

1. Type I diabetes, also called insulin-dependent diabetes mellitus (IDDM), is caused by lack of insulin secretion.
2. Type II diabetes, also called non-insulin-dependent diabetes mellitus (NIDDM), and is caused by decreased sensitivity of target tissues to the metabolic effect of insulin. This reduced sensitivity to insulin is often called insulin resistance. In both types of diabetes mellitus, metabolism of all the main foodstuffs is altered. The basic effect of insulin lack or insulin resistance on glucose metabolism is to prevent the efficient uptake and utilization of glucose by most cells of the body, except those of the brain. As a result, blood glucose concentration increases, cell utilization of glucose falls increasingly lower, and utilization of fats and proteins increases.[1]
3. Gestational diabetes refers to the onset of glucose intolerance in women during pregnancy. It exclude women who were diabetic before pregnancy. If the women remains diabetic after pregnancy , she must be classified as type I or type II diabetic In short , all forms of DM are due to either a decrease in circulating free insulin(insulin deficiency , or a decrease in the response of peripheral tissue to insulin(insulin resistance).[2][3] Unfortunately, apart from having number of side effects, none of the oral synthetic hypoglycaemic agents has been successful in maintaining euglycemia and controlling long-term microvascular and macrovascular complications.[4] Medicinal plants continue to provide valuable therapeutic agents, in both modern medicine and in traditional system.[5] Tomato is labelled as a vegetable for nutritional purposes. The plant is useful for curing diabetes; it is also useful in the treatment of antifungal activity, rheumatism and severe headaches.[6] However, the antihyperglycemic activity of *Lycopersicon esculentum* leaves has not been reported. Hence, the present study was under-taken to explore Antidiabetic activity of *Lycopersicon esculentum* leaves extracts on alloxan induced diabetic rats.

2. MATERIAL AND METHODS:

1. Collection and Authentication of plant:

The plant of *Lycopersicon esculentum* has been collected from local area of Akot Dist. Akola, Maharashtra, India. The Plant were identified and authenticated by Ms. Kajal Aapale, Department of Botany, Vidyabharti Mahavidyalaya, Amravati.

2. Experimental Animals:

The experiment is performed on albino Wistar rats (weighing 150-250gm), which are obtained from the animal house of Department of Pharmacology. Vidyabharti college of pharmacy, Amravati.(1504/PO/RE/S/11/CPCSEA). All the animals are acclimatized to the animal house prior to use. They are kept in cages in animal house with n 12 h light: 12 h dark cycle. Animals are fed on pellets and top water ad libitum. The care and handling of rat were in accordance with the internationally accepted standard guidelines for use of animals (CPCSEA).Permission und approval animal studies were obtained from the Institutional Animal Ethics Committee (IAEC) of Vidyabharti college of Pharmacy, Amravati. SGI) Amravati University.

3. Drugs And Chemicals:

A) Inducing Agent:

Alloxan Monohydrate (100mg/kg) was used to induced diabetes on rats.

Product number: 00870 (10mg)

CAS Number: 2244-11-3

Was purchased from Loba Chemie, PVT,LTD, Mumbai, India

B) Standard Drug:

Metformin purchased by AbMole Bioscience.

C) Test Drug:

Lycopersicon esculentum plant were obtain from local areas.

D) Other Chemicals & Reagents:

Saline, Methanol, Citrate buffer. Fehling's Reagent, Benedict's Reagent, etc. The chemicals used and other solutions were of analytical grade. All drugs and reagents were prepared immediate for use.

C) Equipment:

Soxhlet Apparatus, Vacuum desiccator, Syringe, glucometer, test tube, China dish, petri dish, etc.

4. Preparation of Extract:

The Dried leaves of *Lycopersicon esculentum* (250 gm) were extracted with methanol : water (1:1) as solvent by Soxhlet extraction and solvents were evaporated and concentrated on water bath at controlled temperature. The yield of extract was found to be 13.83%.

The percentage yield of the extract was calculated and the extract was then subjected to different phytochemical tests.

5. Preparation of Diabetic Rat:

Male Wistar rats weighing 150-250 g are deprived of food 18 h prior to the experiment but are allowed free access to water are used. Alloxan monohydrate 100mg/kg body weight was dissolved in normal saline (0.9 % w/v) and injected intraperitoneally to induce hyperglycaemia in experimental rats.[7] The experimental animals were fasted for 18 hours before alloxan injection and the blood glucose level (BGL) was monitored after alloxanization in blood samples collected by tail tipping method using a Glucometer. Treatment was continued for 21 consecutive days, with twice a day dose (morning and evening).Before the treatment (0 day) and at the end of I and 21 day, blood samples were collected from the tip of the tail of each rat under mild ether anaesthesia .The blood glucose level was monitored after 72 h of alloxanization.[8].The blood sample was collected by tail vein and glucose level was estimated using glucometer.[9]. The animals which did not developed hyperglycaemia i.e. glucose level > 200mg/dl, were rejected and replaced with new animals.[8]

6. Experimental Design:

The rats were divided into five groups.

Group I (controlled group) administered with 0.5citrate buffer

Group II (untreated diabetic rats) Alloxan 100 mg/kg

Group III (diabetic rats receiving Metformin orally at 250mg/kg body weight in saline)

Group IV (receiving 200 mg/kg of body weight of test extract)

Group V (receiving 400 mg/kg of body weight of test extract respectively).

Metformin was used as the standard antidiabetic throughout the experimentation. They were carefully monitored every day. Animals described as fasted were deprived of food for at least 12 hours but allowed to

free access for drinking water. Fasting blood glucose measurement was done on day 1, 7th, 14th and 21st of the study. Blood glucose levels were measured by glucometer.

7. Statistical analysis:

The data obtained from the screenings were subjected to statistical analysis following one-way ANOVA followed by Dunnett's Multiple Comparison Test to assess the statistical significance of the results using GraphPad Prism-5 software. *p<0.001 were considered as statistically significant.

3. RESULT:

3.1 Pharmacognostical Examination.

% yield = (weight of the extract / weight of powder taken) × 100

Table 3.1 Percentage yield of Leaves of *Lycopersicon esculentum*

Drug	Leaves of *Lycopersicon esculentum*
Percentage of yield	13.83 % w/w

Table 3.2 Physical Examination of extract

Extract	**Colour**	**Odour**	**Solubility**
MELE	Brown	Characteristics	In water

3.2 Phytochemical Testing:

Table 3.3 Phytochemical Test:

Sr. No	**Chemical Constituents**	**Results**
1	Alkaloids	+
2	Flavonoids	+
3	Carbohydrate	-
4	Tannins	+
5	Glycosides	+
6	Phenols	+
7	Saponins	+
8	Steroids	-

Where, indicates (+) Present, (-) Absent.

3.3 Average Blood Glucose Profile:

In Alloxan treated diabetic rats, values of glucose level were elevated to high level during the study. Chronic treatment with the methanolic extract of *Lycopersicon esculentum* leaves at 200 and 400 mg/kg of body weight significantly ($P < 0.001$) causes decrease in blood glucose on 1, 7, 14th and 21st day as shown in table & figure.

Table 3.4 Average Blood Glucose Profile

Groups	Treatment	Average Blood Glucose Profile			
		Day 1	Day 7	Day 14	Day 21

I	Control	87.17±1.10*	83.17±1.99*	84.17±1.49*	88.50±1.66*
II	Alloxan (100mg/kg)	291.7±5.66	296.2±2.79	302.3±4.04	306.8±5.02
III	Alloxan +Metformin(250)	251.8±7.71*	180.7±1.28*	141.7±2.17*	104.5±0.99*
IV	MELE Extract (200 mg/kg)	288.5±4.38	211.0±6.18*	152.5±3.18*	123.8±1.37*
V	MELE Extract (400 mg/ kg)	286.5±5.17	196.7±1.16*	144.0±1.58*	115.5±1.31*

Values Expressed as Mean ± SEM: (n =6)
*p<0.001 compared with Alloxan(induced) group.

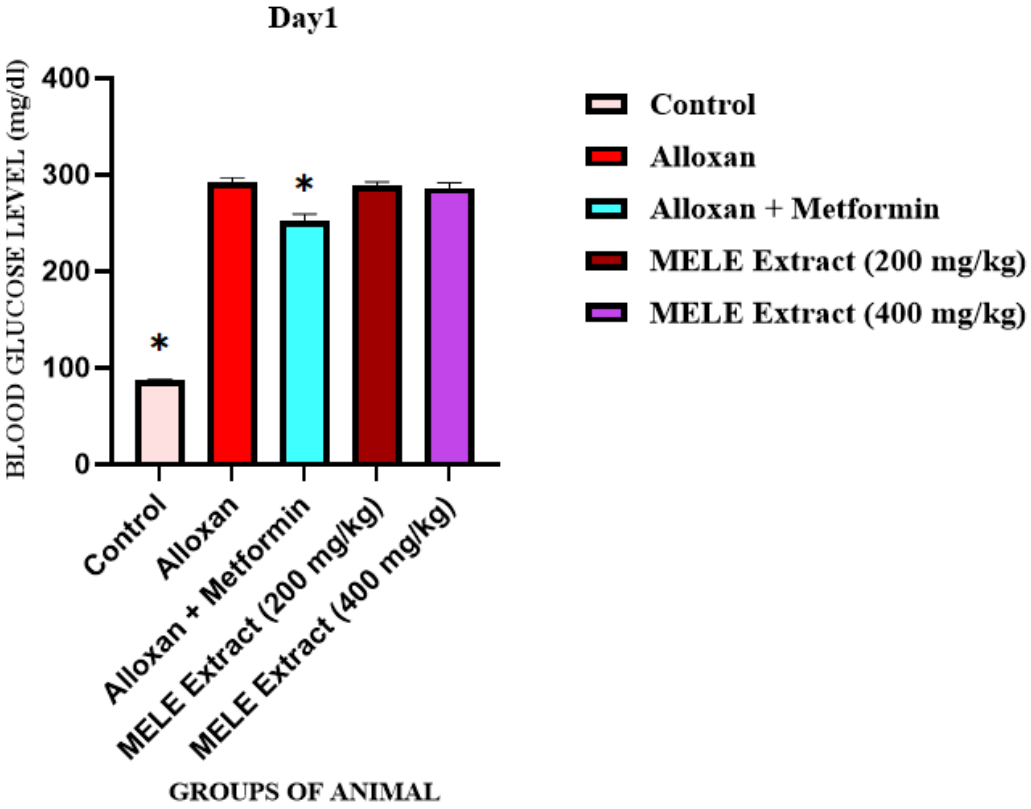

Figure3.1: Effect of *Lycopersicon esculentum* Leaves extract on Blood Glucose Level on 1st day.
All Values are expressed as mean ± SEM (n=6). Statistical comparisons between each treatment groups with Alloxan(induced) rats were carried out by one way ANOVA followed by Dunnet multiple comparison test. *P<0.001 when compared to Alloxan(induced) group.

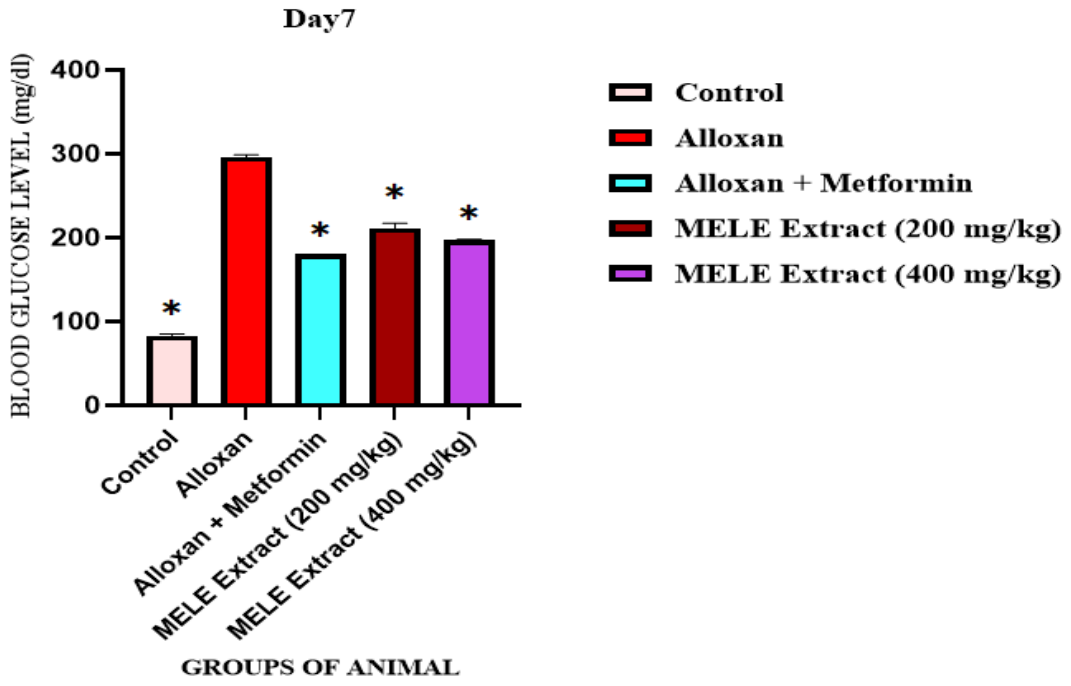

Figure3.2: Effect of *Lycopersicon esculentum* leaves extract on Blood Glucose Level on 7th day.
All Values are expressed as mean ± SEM (n=6). Statistical comparisons between each treatment groups with Alloxan(induced) rats were carried out by one way ANOVA followed by Dunnet multiple comparison test. *P<0.001 when compared to Alloxan(induced) group.

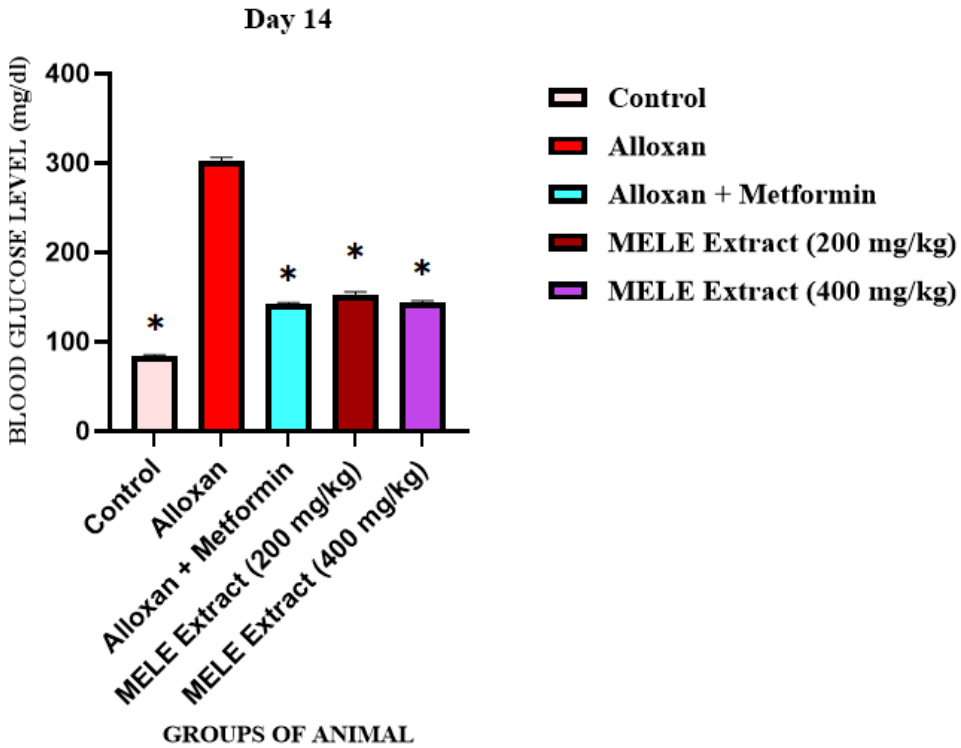

Figure3.3: Effect of *Lycopersicon esculentum* Leaves extract on Blood Glucose Level on 14th day.

All Values are expressed as mean ± SEM (n=6). Statistical comparisons between each treatment groups with Alloxan(induced) rats were carried out by one way ANOVA followed by Dunnet multiple comparison test. *P<0.001 when compared to Alloxan(induced) group.

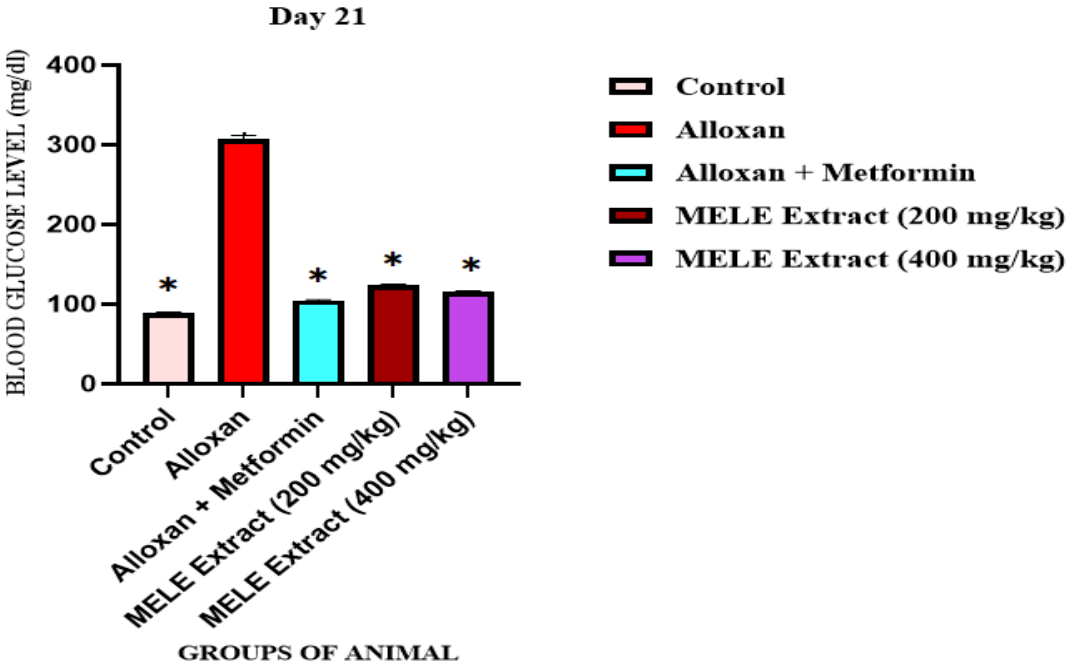

Figure3.4: Effect of *Lycopersicon esculentum* Leaves extract on Blood Glucose Level on 21st day.
All Values are expressed as mean ± SEM (n=6). Statistical comparisons between each treatment groups with Alloxan (induced) rats were carried out by one way ANOVA followed by Dunnet multiple comparison test. *P<0.001 when compared to Alloxan(induced) group.

3.4 Oral Glucose Tolerance Test of *Lycopersicon esculentum* Leaves Extracts on Alloxan Induced Diabetic Rats.

Table 3.5 Oral Glucose Tolarance Test:

Groups	Treatment	Oral Glucose Tolarance Test		
		Baseline	30 Min	90 Min
I	Control	88.50±1.66	97.80±1.31*	92.50±0.76*
II	Alloxan(100mg/kg)	306.8±5.02	329.2±4.50	355.0±2.67
III	Alloxan +Metformin(250)	104.5±0.99	168.3±3.19*	105.3±1.30*
IV	MELE Extract (200 mg/kg)	123.8±1.37	179.8±1.77*	126.0±1.15*
V	MELE Extract (400 mg/kg)	115.5±1.31	174.2±1.72*	117.8±1.81*

Values Expressed as Mean ± SEM: (n =6)
*p<0.001 compared with Alloxan(induced) group.

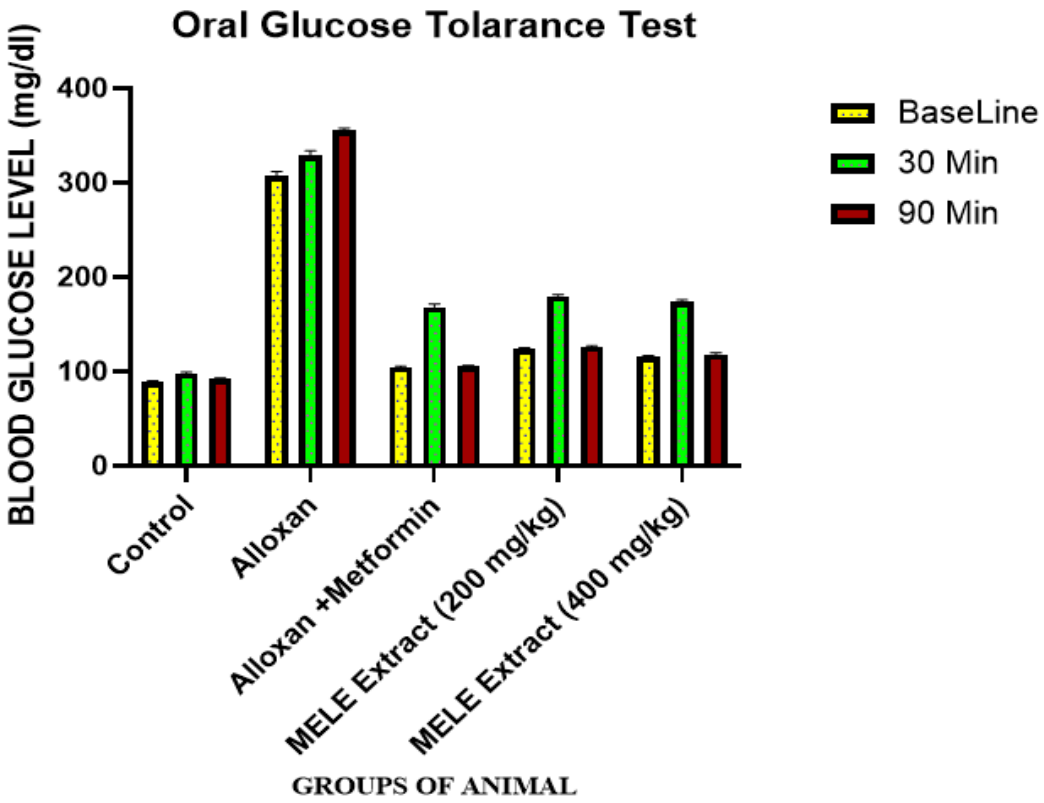

Figure 3.5: Oral Glucose Tolarance Test

All Values are expressed as mean ± SEM (n=6). Statistical comparisons between each treatment groups with Alloxan(induced) rats were carried out by one way ANOVA followed by Dunnet multiple comparison test. *P<0.001 when compared to Alloxan(induced) group.

3.5: HBA1C (Glycated haemoglobin Test):

Table3.6: HBA1C Test

Groups	Treatment	Before	After
I	Control	4.515±0.018	4.733±0.014*
II	Alloxan	4.698±0.057	12.33±0.017
III	Alloxan +Metformin	5.013±0.067	5.333±0.125*
IV	MELE Extract (200 mg/kg)	4.953±0.017	6.108±0.108*
V	MELE Extract (400 mg/ kg)	5.003±0.023	5.652±0.016*

Values Expressed as Mean ± SEM: (n =6)
* p<0.001 compared with Alloxan(induced) group.

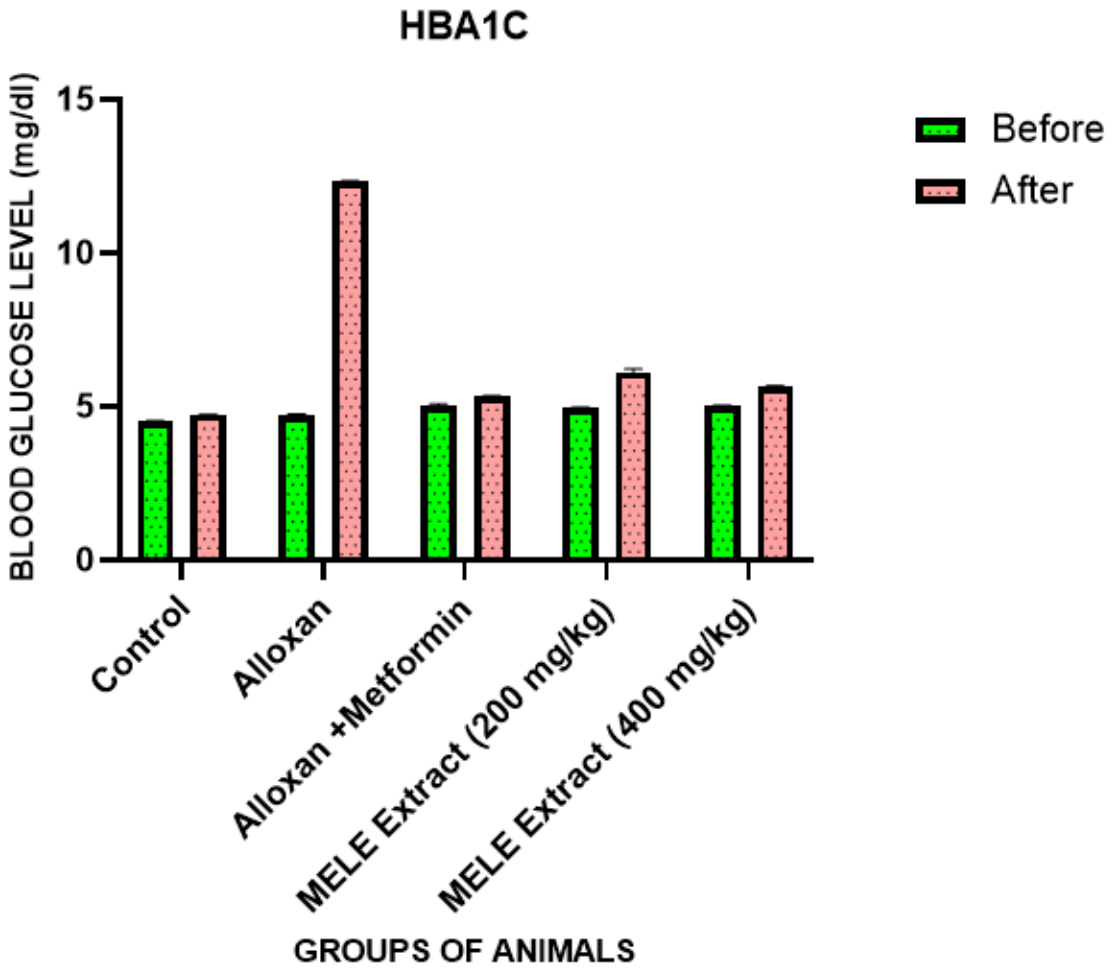

Figure 3.6: Hba1c Test on Before and After Treatment

All values are expressed as mean ± SEM (n=6). Statistical comparisons between each control before treatment groups & after alloxan (induced) rats were carried out by one way ANOVA followed by Dunnet multiple comparison test. * P< 0.001 when compared to Alloxan (induced) group.

4. DISCUSSION:

Diabetes mellitus is a persistent metabolic condition affecting the processing of carbohydrates, fats, and proteins in the body. It manifests as high levels of glucose in the blood, known as hyperglycaemia.

The primary cause of high blood sugar involves an excess of glucose production (through increased hepatic glycogenolysis and gluconeogenesis) and a decrease in glucose utilization by the body's tissues.[10] The development of type II diabetes primarily arises from the decline in pancreatic β-cell function,[11][12][13][14] leading to a reduced ability of patients to produce insulin when blood glucose levels rise. Metformin enhances insulin action directly and works effectively only when insulin is present. It remains to be determined whether MELE extract exhibit antidiabetic properties could be attributed to increased insulin secretion, similar to the mechanism observed with metformin. The findings of this study suggest that the MELE extract, at doses of 200 and 400 mg/kg effectively lowered glucose levels in both glucose-loaded and alloxan-induced diabetic animals. Alloxan is known to generate free radicals, leading to tissue damage, particularly in the pancreas.[15]

In this investigation, the methanolic extracts of MELE were assessed for their effectiveness in managing Alloxan-induced type 2 diabetes. Alloxan was selected at a dosage of (100 mg/kg) to specifically target certain pancreatic beta cells, imitating the condition of type 2 diabetes characterized by insufficient insulin secretion..

Following diabetes induction, rats were treated continuously with two doses of methanolic MELE extracts (200 and 400 mg/kg) for 21 days. Glucose levels were measured using a glucometer on days 1, 7, 14, and 21 of treatment. Throughout the 21-day treatment period, there was a gradual decrease in glucose levels. Post-diabetes induction, the rats glucose levels exceeded 300 mg/dl, which decreased to approximately 288.5±4.38 and 286.5±5.17 mg/dl after administering 200mg/kg and 400mg/kg extracts respectively on day 1. By the 7th day, glucose levels had decreased further to 211.0±6.18 and 196.7±1.16 mg/dl for the respective doses. By the 14th day, glucose levels had dropped to 152.5±3.18 mg/dl and 144.0±1.58 mg/dl, reaching near-normal levels by the 21st day, with values of 123.8±1.37 mg/dl and 115.5±1.31 mg/dl for the 200 and 400 mg/kg doses respectively.

The oral glucose tolerance test (OGTT) was conducted to assess the MELE Extract ability to regulate blood glucose levels in Alloxan-induced diabetic rats. The findings indicated that the Alloxan-induced diabetic rats, serving as the positive control group, demonstrated significantly elevated blood glucose levels at 30 minutes and 90 minutes compared to the negative control group. Conversely, rats administered with MELE Extract at doses of 200 mg/kg and 400 mg/kg exhibited decreased blood glucose levels in contrast to the positive control group. This suggests that MELE Extract administration may have the potential to improve glucose tolerance in diabetic conditions. Blood glucose levels were measured immediately by using Gluco-meter.

The research also examined how the MELE Extract affected glycosylated haemoglobin (HbA1c) levels in various treatment groups. HbA1c serves as an indicator of prolonged glycaemic regulation, with increased levels linked to diabetic complication development. Findings revealed that the positive control group (Alloxan-induced diabetic rats) had notably higher HbA1c levels in contrast to the negative control group (non-diabetic rats). Conversely, rats administered with MELE Extract at doses of both 200 mg/kg and 400 mg/kg displayed reduced HbA1c levels compared to the positive control group, suggesting that the MELE Extract can enhance long-term glycaemic regulation in diabetic conditions.

Phytochemical screening of *Lycopersicon esculentum* leaves extract shows that the crude methanolic extract contain the presence of alkaloids, flavonoids, tannins, glycosides, phenol, saponins these secondary metabolites which were responsible for the antidiabetic effect in other plants were also detected in this plant extract.[16][17] The Methanolic extract (400mg/kg) showed beneficial effects on blood glucose, thus it could serve as good adjuvant to other oral hypoglycaemic agents and seems to be promising for the development of phytomedicines for diabetes mellitus.

5. CONCLUSION:

In conclusion, it appear that extracts derived from *Lycopersicon esculentum* leaves possess the ability to effectively neutralize Reactive Oxygen Species, reduce fasting blood glucose levels, and potentially stimulate the regeneration of B-cells in cases of Alloxan-induced diabetes. The anti-diabetic activity is probably due to the presence of bioactive compounds like alkaloids, flavonoids, tannins, glycosides, phenol, saponins. The data obtained from this study indicates that the methanolic extracts of the leaves of *Lycopersicon esculentum* and are capable of exhibiting significant antihyperglycemic activities in diabetic rats. The extracts also showed improvement in parameters like regeneration of βcells of pancreas and so might be valuable in diabetes treatment. Further studies are required to confirm the exact mechanism underlying for the diabetic property of the extract.

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