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A Study on Anti-Diabetic Activity of Aegele marmelos, Musa paradisiaca and Ocimum sanctum

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ABSTRACT

Diabetes mellitus (DM) currently is a major health problem for the people of the world and is a chronic metabolic disorder resulting from a variable interaction of hereditary and environmental factors and is characterized by abnormal insulin secretion or insulin receptor or post receptor events causing glycosuria, hyperglycemia and a disturbance in carbohydrate, fat and protein metabolism and water and electrolyte balance in addition to damaging effect on liver, kidney and β cells of pancreas. Herbal medicines provide rational means for the treatment of many diseases that are obstinate and incurable in other systems of medicine but it is necessary to establish the scientific basis for the therapeutic actions of herbal plant medicines. The aim of the research work was to screen the anti-diabetic potential of methanolic extract of *Aegele marmelos*, *Musa paradisiaca* and *Ocimum sanctum* with the objective to evaluate histopathology study, effect on serum profiles and change in body weight analysis. Anti-diabetic activity was screened performing various parameters like oral glucose tolerance test, acute (single dose) and sub acute (multi dose) study, lipid profile, change in body weight and histopathology of pancreas. The result revealed that methanolic extracts of selected plants (parts) have significant anti-hyperglycemic effect due to improved glucose tolerance and by decreasing blood glucose levels in experimental animals. Total cholesterol (TC), triglycerides (TG) low density lipoproteins (LDL) and very low density lipoproteins (VLDL) levels were remarkably reduced whereas high density lipoproteins (HDL) level was increased in the animals treated with methanolic extracts. Histopathology of pancreas revealed substantial regeneration of β cells and cellular expansion of islets of langerhans in the animals treated with methanolic extracts.

Keywords: Acute Toxicity Study, Anti-diabetic Activity, Alloxan Induced, Diabetes Mellitus, Histopathological Studies, Methanolic Extract, Oral Glucose Tolerance Test, Type 2 Diabetes.

INTRODUCTION

Diabetes mellitus, one of the most common endocrine metabolic disorders has caused significant morbidity and mortality due to microvascular and macrovascular complications. Human body possesses enzymatic and non-enzymatic antioxidative mechanisms which minimize the generation of reactive oxygen species, responsible for many degenerative diseases including diabetes.

In India, Indigenous remedies have been used in the treatment of diabetes mellitus since the time of Charaka and Susruta. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The ethnobotanical information reports that about 800 plants may possess anti-diabetic potential. Several such herbs have shown anti-diabetic activity when assessed using

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presently experimental techniques.

The number of patients with DM is markedly increasing worldwide. DM is associated with impaired glucose metabolism that leads to an increase in free radical production and increase in triglyceride and lipoprotein levels. Oxygen free radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of anti-oxidant enzymes and play a role in the long-term complications of diabetes. Therefore, among the various therapeutic strategies, combination of anti-hyperglycemic, anti-hyperlipidemic and anti-oxidant activity can be beneficial in the prevention of DM and its complications.

It was observed that a large number of animal models and new drug entities were introduced treat diabetes. Also, in-vitro models were used for understanding the underlying mechanism of action of drugs. To induce diabetes in experimental animals most preferred drugs are streptozotocine (STZ, 69%) and alloxan (31%). Parenteral, intravenous, subcutaneous or intraperitonial administration of drug shows their diabetogenic effect. Depending upon animal species, route of administration dose to induce diabetes is slected for streptozotocine (STZ) and alloxan. It has been evaluated for its anti-diabetic acivity in streptozotocine - nicotinamide induced type 2 diabetes. As per traditional system of medicine the plant was widely used for management and treatment of diabetes mellitus.

As per traditional claims the plant was used for treating human ailments due to its varied therapeutic applications as hepato-protective, anti diarrheal, anti-bacterial and anti inflammatory effects. The experimental outcome of this study indicates anti diabetic effect against diabetic rats. The extract inhibits the production of reactive oxygen species which was due to the chemical constituents present in it.

MATERIALS AND METHODS

Animal Selection

Animals for the proposed activities are selected as per the experimental protocol approved by Institutional Animal Ethical Committee in accordance with OECD – 423 guidelines.

Determination of LD50

The method of OECD Guidelines was adapted for the determination of LD50. Female albino mice of 20-25 g weight were used.

Acute Toxicity Studies of Extracts of *Aegele marmelos, Musa paradisiaca, Ocimum sanctum* Using Swiss albino mice of 20-25 g Weight

Acute toxicity studies were performed following OECD guideline no. 423 (Acute Toxic Class Method), in which Swiss albino mice of either sex (20-25 g weight) were treated with methanolic extracts of *Aegele marmelos*, *Musa paradisiaca*, and *Ocimum sanctum*. It was observed that the test extracts did not show any remarkable undesired signs such as, change in behavior; change in body weight, mortality and morbidity even at 2000 mg/kg dose after completion of 14 days observation. Therefore, 1/10th (200 mg/kg b. w.) and 1/5th (400 mg/kg b. w.) of these doses were selected for further study.

Anti-Diabetic Activity of Plants

Pharmacological screening for Anti-diabetic activity was done by using male Wistar albino rats. The glucose tolerance test (GTT) study and evaluation of their effects (Single dose and Multidose treatment study) on blood glucose level, serum lipid profile and histopathology of liver in Alloxan induced diabetic rats was done.

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Drugs and Chemicals

Alloxan was purchased from Sigma Aldrich, USA. Daonil tablets were purchased from local market. Different diagnostic kits (HDL, Cholesterol, Triglycerides) were procured from ERBA diagnostics (Manheim Ltd. India).

Instruments Used

- Micro pipettes,
- Semi auto analyzer
- Epen-droff centrifuge
- UV-visible spectrophotometer (Shimadzu)

Table 1: Animal Experiment Model-Six animals in each group (n = 6)

Group (n = 6)	Group Type	Treatment Dose
Group-I	Control	Oral-1mL of 1% gum acacia suspension
Group-II	Diabetic Control	Alloxan 120 mg / kg
Group-III	Standard Drug	Glibenclamide-2.5 mg / kg
Group-IV	Methanolic extract of *Aegele marmelos* (Fruit)	200 mg / kg
Group V	Methanolic extract of *Musa paradisiaca (*Ripe fruit)	200 mg / kg
Group VI	Methanolic extract of *Musa paradisiaca* (Unripe fruit)	200 mg / kg
Group VII	Methanolic extract of *Ocimum sanctum* (aerial parts)	200 mg / kg

Oral Glucose Tolerance Test (OGTT)

Fasting blood glucose level of experimental animals was determined at zero time after overnight fasting with free access to water. Rats were divided into six groups each containing six rats. The first group of animals received 1 ml of 1% gum acacia suspension orally (Control animals). Remaining groups received Glibenclamide (2.5 mg/kg- standard) and methanolic extracts of selected plants (200 mg/kg) by oral route using an orogastric tube respectively. Glucose (2 gm/kg) was orally administered 30 min after the administration of extracts or Glibenclamide or gum acacia suspension. Blood samples were collected from the tail vein under ether anesthesia just prior to and 30, 60, 120 and 240 min after glucose loading. Glucose levels were estimated using glucose- oxidase-peroxidase reactive strips and a glucometer (Sugar-check, Wockhardt Ltd, Mumbai, India).

Effect of Methanolic Extracts on Blood Glucose Levels in Alloxan Induced Diabetic rats [Single dose (Acute) treatment]

A single intraperitoneal injection of 120 mg/kg of alloxan monohydrate was employed to induce diabetes in overnight fasted male Wistar albino rats weighing 170-200gm. After 72 hr, animals with blood glucose levels higher than 250 mg/dl were considered diabetic and

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were included in the study. Animals were divided into seven groups including six rats each shown in (Chart 1)**.** Blood samples were collected from the tail vein prior to and at 30 min, 60 min, 2, 4, and 6 h intervals after the administration of the extracts and blood glucose levels were estimated using glucometer.

- **Group I:** Normal control rats administered with 1 ml of 1% gum acacia suspension
- **Group II:** Diabetic control rats administered with 1 ml of 1% gum acacia suspension
- **Group III:** Diabetic rats administered with Glibenclamide (2.5 mg/kg)
- **Group IV:** Diabetic rats administered with methanolic extract of *Aegele marmelos* (200 mg/kg)
- **Group V:** Diabetic rats administered with methanolic extract of ripe fruit of *Musa paradisiaca* (200 mg/kg)
- **Group VI:** Diabetic rats administered with methanolic extract of unripe fruit of *Musa paradisiaca* (200 mg/kg)
- **Group VII:** Diabetic rats administered with methanolic extract of *Ocimum sanctum*

Chart 1: Division of animals into seven groups along with their respective doses in order to evaluate the effect of methanolic extracts on blood glucose levels in alloxan induced diabetic rats

Effect of Methanolic Extract on Blood Glucose Levels and Serum Lipid Profiles in Alloxan Induced Diabetic Rats [Multi Dose (Sub Acute) Treatment]

Diabetes was induced in overnight fasted adult male Wistar albino rats weighing 170-200gm by a single intraperitoneal injection of 120 mg/kg of alloxan monohydrate. After 72 hr, animals with blood glucose levels higher than 250 mg/dl were considered diabetic and were included in the study. Animals were divided into seven groups including six rats each as shown in Chart 2. Blood samples were collected from the tail vein prior to and at 30 min, 60 min, 2, 4, and 6 h intervals after the administration of the extract and blood glucose levels were estimated using glucometer. These rats were given the same doses of the extract once daily for 15 days in this study. Blood samples were collected from the tail vein of non fasted rats on days 0, 5, 10 and 15 of extract administration and blood glucose levels were estimated using glucometer. Serum lipid profiles on day 15 were measured by an autoanalyzer.

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- **Group I:** Normal control rats administered 1 ml of 1% gum acacia suspension
- **Group II:** Diabetic control rats administered 1 ml of 1% gum acacia suspension
- **Group III:** Diabetic rats administered Glibenclamide (2.5 mg/kg)
- **Group IV:** Diabetic rats administered with methanolic extract of *Aegele marmelos* (200 mg/kg)
- **Group V:** Diabetic rats administered with methanolic extract of ripe fruit of Musa paradisiaca (200 mg/kg)
- **Group VI:** Diabetic rats administered with methanolic extract of unripe fruit of *Musa paradisiaca* extract(200 mg/kg)
- **Group VII:** Diabetic rats administered orally with methanolic extract of *Ocimum sanctum*

Chart 2: Division of animals into seven groups along with their respective doses in order to evaluate methanolic extracts on blood glucose level and serum lipid profiles in alloxan induced diabetic rats Multidose (sub-acute treatment)

Histopathological Studies

Pancreatic tissues from rats of all groups of Multi dose (Sub acute) treatment were subjected to histopathological studies. The whole pancreas from each animal was removed after sacrificing the animal under anesthesia and was collected in 10% formalin solution and immediately processed by the paraffin technique. Sections of 5 µm thickness were cut and stained by hematoxylin and eosin (H and E) for histological examination.

Statistical Analysis

Values are presented as mean ± S.E.M. Statistical difference between treatments and the controls were tested by one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test using the "Stat" statistics computer program. A difference in the mean values of $P<0.05$ was considered to be statistically significant.

RESULT

Acute Oral Toxicity Study (OECD 423) The acute toxicity studies of *Aegele marmelos*, *Musa paradisiaca*, and Ocimum sanctum were determined as per the OECD guideline no. 423 (Acute Toxic Class Method) and 1/10th (200 mg/kg b. w.) and $1/5^{th}$ (400 mg/kg b. w.) of these doses were selected for further study.

Acute Oral Toxicity Study (OECD 423)

The acute toxicity studies of Aegele marmelos, Musa paradisiaca and Ocimum sanctum were determined as per the OECD guideline no. 423 (Acute Toxic Class Method) and 1/10th (200 mg/kg b.w.) and $1/5^{th}$ (400 mg/kg b.w.) of these doses were selected for further study The extracts are found to be non toxic and safe at 2000 mg/kg body weight and 1/10th of LD 50 was selected as therapeutic dose for the study.

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Table 2: Acute Oral Toxicity Study

Extracts	LD 50 (mg/kg Body Weight)	1/10th of LD 50
Aegele marmelos	**2000 mg/ kg**	**200 mg/ kg**
***Musa paradisiaca* (Ripe)**		
***Musa paradisiaca* (Unripe)**		
Ocimum sanctum		

LD50 was determined by performing acute toxicity study. Plant extracts were found to be safe (no adverse reactions and no mortality) even at a dose of 2000 mg/kg body weight. Therefore, 1/10th of 2000 mg i.e. 200 mg/kg body weight was selected as therapeutic dose for the study.

Oral Glucose Tolerance Test (OGTT)

The ethanolic extract of *Aegele marmelos*, *Musa paradisiaca* and *Ocimum sanctum* showed promising results in glucose tolerance test ($p < 0.05$) up to 4 h, as shown in Table 3, Fig. 1 and 2. The observed reduction in blood glucose level at the end of 2nd and 4th h was 26% & 11% for *Aegele marmelos*, 28% & 17% for *Musa paradisiaca* (Ripe), 23% & 3% for *Musa paradisiaca* (Unripe) and 13% & 3% for *Ocimum sanctum* respectively. The results are at par with the standard drug Glibenclamide.

Table 3: OGTT (Oral Glucose Tolerance Test)

Animal Group (n= 6)	Treatment	Blood Glucose Concentration (mg/dl) (Mean ± SEM)				
		0 min	30 min	60 min	120 min	240 min
I	Normal control (1% gum acacia)	92.8 ± 2.9	161.6 ± 4.1	162.7 ±2.6	161.2 ± 3.5	132.3 ± 3.2
II	Glibenclami de (2.5 mg/kg)	96.0 ±2.4	143.0 ±2.4 *	145.8 ±1.2 *	137.0 ±2.2 *	98.9 ±1.2 *
III	Met.AL (200 mg/kg)	98.2 ±4.2	130.0 ±2.7 *	133.2 ±3.5 *	124.7 ±2.6 *	109.0 ±1.4 *
IV	Met. BF (200 mg/kg)	97.6 ±3.9	131.0 ±3.0 *	132.7 ±3.2 *	125.7 ±2.9 *	114.0 ±1.4 *
V	Met.DM (200 mg/kg)	92.0 ±5.2	136.0 ±3.2 *	134.5 ±2.3 *	113.5 ±4.2 *	94.83 ±2.5 *
VI	Met.GG (200 mg/kg)	88.2 ±3.8	120.8 ±2.9 *	111.6 ±2.0 *	99.4 ±1.7 *	94.5 ±2.9 *

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***P< 0.01:** Significant, compared to normal control n = no of animals in each group
Met. AM : Methanolic extract of *Aegele marmelos* (Fruit)
Met. OS : Methanolic extract of *Ocimum sanctum* (Aerial parts)
Met. MP (R) : Methanolic extract of *Musa paradisiaca* (Ripe fruit)
Met. MP (U) : Methanolic extract of *Musa paradisiaca* (Unripe fruit)

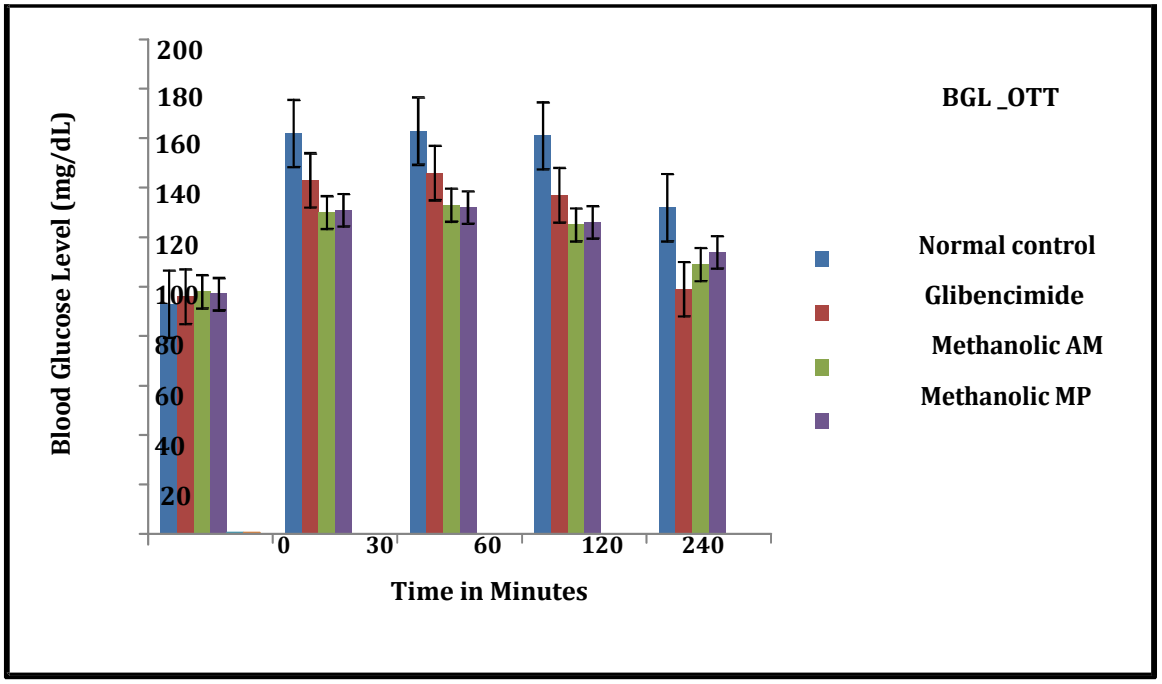

Figure 1: Effect of Methanolic Extract of *Aegele marmelos* and Methanolic Extract of Ripe Fruit of *Musa paradisiaca* on the Blood Glucose Levels in Glucose Loaded Rats (OGT)

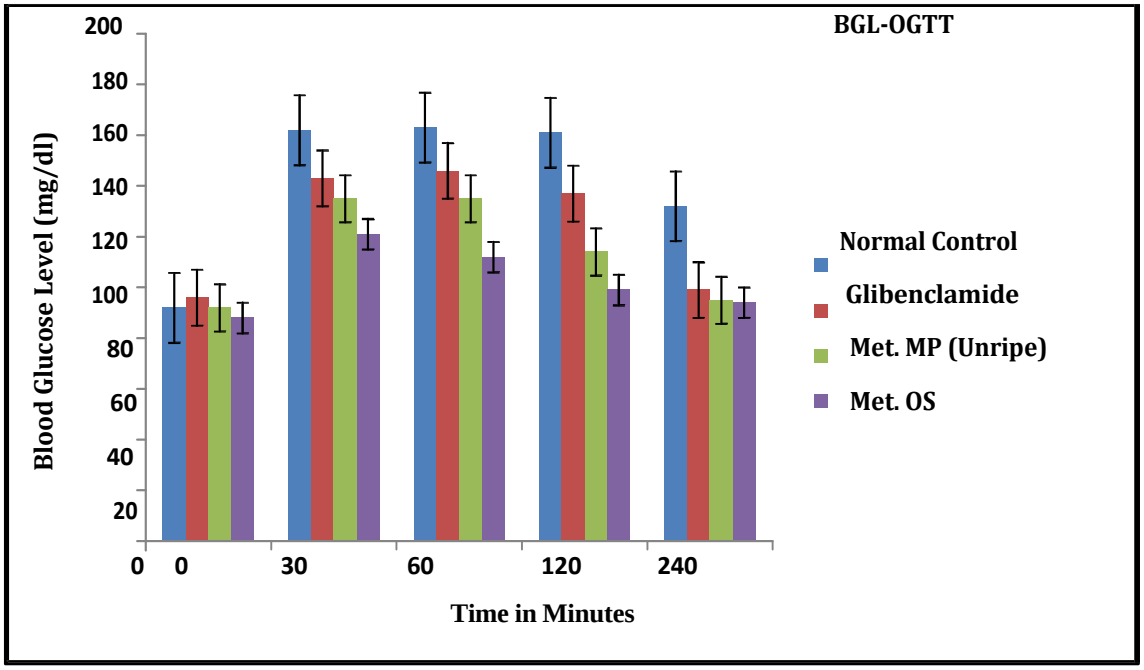

Figure 2: Effect of Methanolic extract of Unripe *M. paradisiaca* and Methanolic extract of *O. sanctum* on the Blood Glucose Levels in Glucose Loaded Rats (OGTT)

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BGL-Acute Study

The plant extracts were found to decrease the blood glucose level significantly in Alloxan induced diabetic rats in acute study (single dose study). All the extracts showed significant anti-hyperglycemic effect from 1 h up to 6 h after oral administration in comparison with normal and diabetic rats - Table 4, Fig. 3. The blood glucose lowering capacity was found to be 16%, 19%, 10% and 11% for each drug, which was at par with standard (20%).

Table 4: Blood Glucose Levels in Alloxan-Diabetic Rats
(Single Dose Treatment /Acute Study)

Animal Group	Treatment	Blood Glucose Concentration (mg/dl) (Mean ± S.E.M)					
(n= 6)		0 min	30 min	60 min	120 min	240 min	360 min
I.	Normal control (1% gum acacia)	87.2 ± 2.4	88.4 ± 2.1##	90.50 ± 2.3 ##	90.86 ± 1.8 ##	94.4 ± 2.2 ##	95.76 ## ± 3.1
II.	Diabetic control	288.8 ± 5.0	296.4 ± 5.1*	297.1 ± 5.2*	291.3 ± 4.1*	293.5 ± 3.5*	294.0 ± 4.1*
III.	Glibenclamide (2.5 mg/kg)	298.3 ± 6.2	285.1 ± 7.0*	271.3 ± 9.3*	259.0 ± 11.9* #	253.8 ± ## 12.1*	236.9 ± # 11.8*#
IV.	MEt. AM (200 mg/kg)	278.1 ± 5.0	273.4 ± 5.5*#	265.2 ± # 6.0* #	254.8 ±6.2*	248.1± ## 6.6*	234.7 ± ## 6.3*
V.	MEt. MP (Ripe) (200 mg/kg)	290.5 ± 6.7	278.2 ± 6.1* #	269.6 ± # 4.2* #	258.2 ±5.4*	250.8 ±5.1* ##	234.2 ± # 6.2*#
VI.	MEt.MP (Unripe) (200 mg/kg)	280.7 ±6.4	274.0 ±6.1* #	266.3 ±8.1* #	261.8 ±9.2*	256.2 ±8.8* #	252.2 ± 9.8* #
VII.	MEt.OS (200 mg/kg)	279.8 ± 5.0	272.2 ± 5.5* #	267.8 ± # 6.0* #	263.0 ±6.2*	256.7 ± 6.6* ##	249.8 ± 6.3* ##

*P< 0.01 Significant, compared to normal control

#P< 0.05

#P< 0.01 Significant, compared to diabetic control n = no of animals in each group

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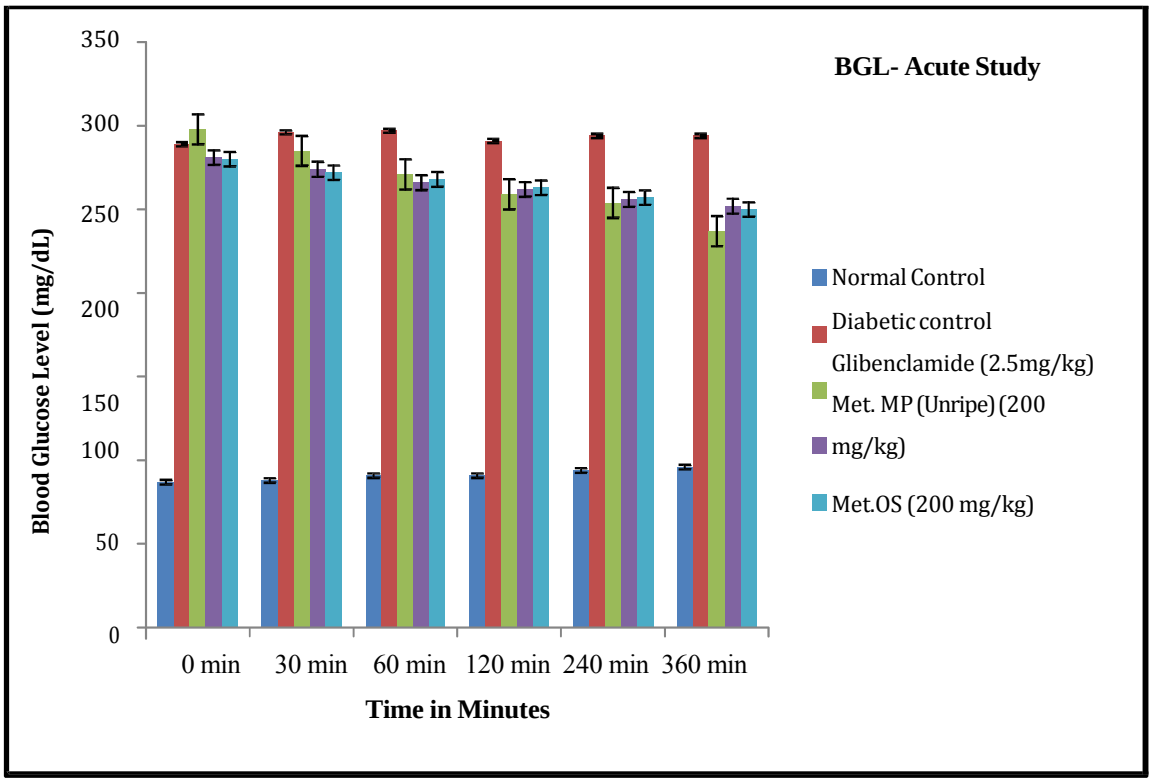

Figure 3: Effect of Methanolic Extract of unripe fruit of *Musa paradisiaca* and *Ocimum sanctum* on Blood Glucose levels (BGL) in Alloxan-Diabetic Rats (Single Dose Treatment/Acute Study)

BGL-Sub Acute Study

Methanolic extract of *Aegele marmelos*, *Musa paradisiaca* and *Ocimum sanctum* causes significant decrease in blood glucose levels on 5th, 10th and 15th day intervals, respectively, in comparison to diabetic rats. Also the comparison between initial as well as 15th day blood glucose levels was made in the same group of animals; it was found that, there is a significant reduction of 56% with methanolic extracts of *Aegele marmelos*, 43% with ripe fruit of *Musa paradisiaca*, 42% with unripe fruit of *Musa paradisiaca*, and 46% with *Ocimum sanctum* was observed where as standard drug Glibenclamide shows 43% reduction in blood glucose level as shown in Table 5, Fig. 4 and 5.

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Table 5: Blood Glucose Levels in Alloxan-Diabetic Rats
(Multi-Dose Treatment/Sub Acute Study

Animal Group (n= 6)	Treatment	Fasting Blood Glucose Concentration (mg/dl) (mean ± S.E.M)			
		0th Day	5th Day	10th Day	15th Day
I	Normal control (1% gum acacia)	87.35 ±2.2	87.75 ±2.8##	87.35± 2.3##	88.35± 1.8##
II	Diabetic control	288.2 ±5.2	277.6 ±7.1**	268.3± 4.2**	256.8± 4.1**
III	Glibenclamide (2.5 mg/kg)	300.3 ±6.9	222.3 ±12.3**##	190.3± ## 12.8**	172.3± 14.3**##
IV	MEt.AM (200 mg/kg)	278.2 ±8.7	194.2 ±	166.4± 9.6**##	122.8± ## 8.4*
V	MEt. MP (Ripe) (200 mg/kg)	292.3 ±6.0	218.0 ±	183.5± 9.4**##	166.5± ## 7.3*
VI	MEt. MP (Unripe) (200 mg/kg)	276.3 ±6.7	220.3± 12.5**#	166.3± 6.5**##	158.3± 6.4**##
VII	MEt. OS (200 mg/kg)	280.3 ±6.7	216.3± 12.5**#	168.3± 6.5**##	152.3± 6.4**##

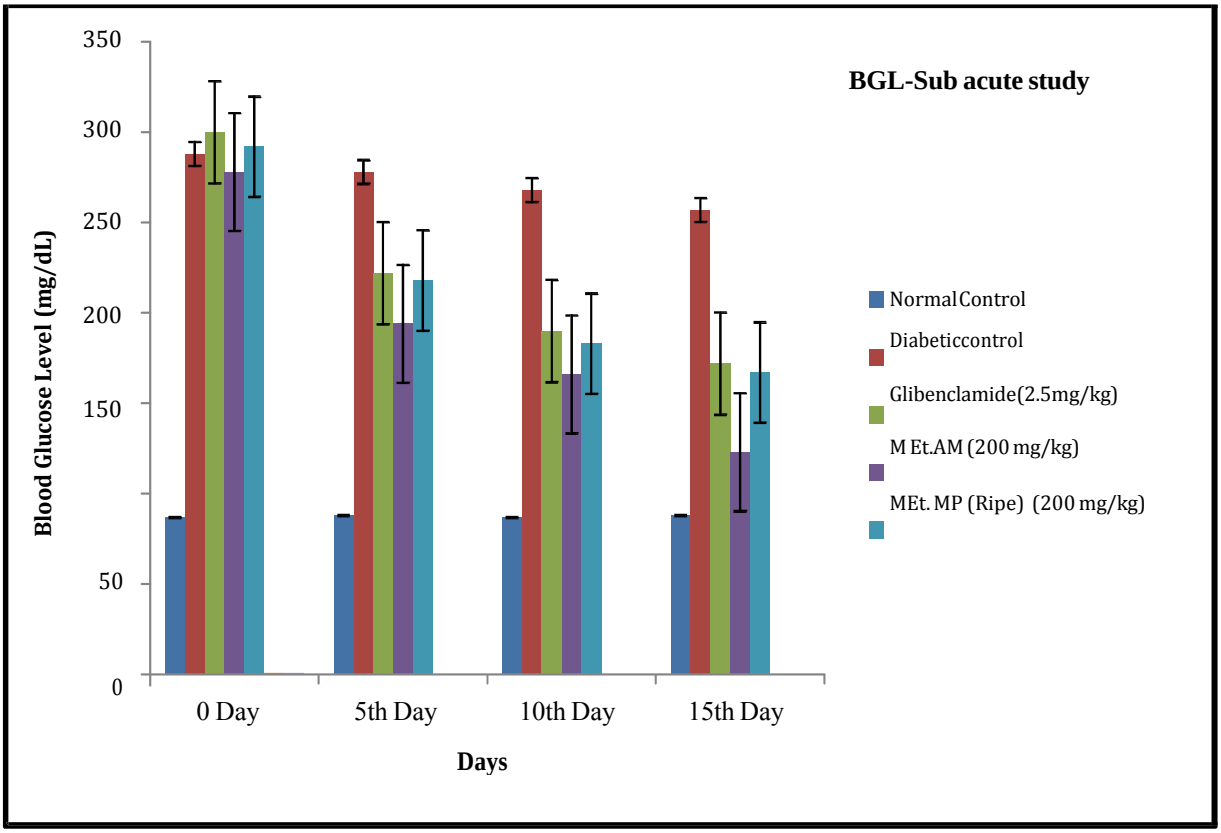

Figure 4: Histogram Showing effect of Methanolic Extract of *A. marmelos* and ripe fruit extract of *M. paradisiacal* on the Blood Glucose levels in Alloxan-Diabetic Rats (Multi-Dose Treatment / Sub Acute Study)

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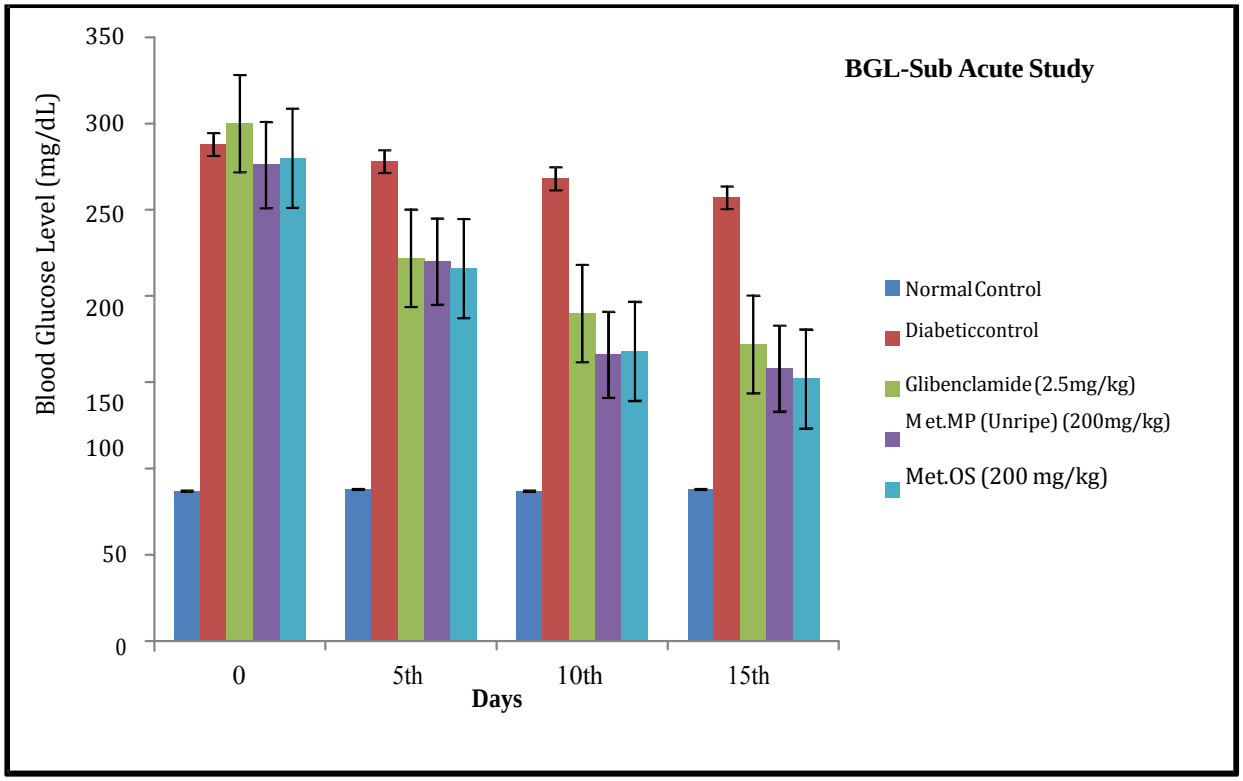

Figure 5: Histogram showing effect of Methanolic Extract of Unripe *M. paradisiaca* and Methanolic Extract of *O. sanctum* on the blood glucose levels in alloxan-diabetic rats (Multi-dose treatment / Sub acute study)

Results revealed that methanolic extracts of *Aegele marmelos*, *Musa paradisiaca* and *Ocimum sanctum* have significant anti- hyperglycaemic effect due to improved glucose tolerance; decreasing blood glucose levels in experimental animals in acute (single dose) and sub acute (multi dose) study. Glibenclamide was used as a reference standard which has a remarkable effect in decreasing glucose level in blood. It can be stated that the extracts are effective in regenerating the β cells in diabetes induced experimental animals. Alloxan destroys the β cells which produces insulin, in pancreas thereby leading to diabetes mellitus. In vitro study reports suggested that cell necrosis is due to selective toxicity of alloxan on pancreatic β cells. The toxic effect of alloxan is mediated through free radicals, with substantial elevated levels of cytosolic calcium which ultimately results in β cells damage/destruction.

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Table 6: Change in Body Weight Study

Animal Group (n= 6)	Treatment	Body weight of the animal (gm)				% Change in body Weight
		0th Day	5th Day	10th Day	15th Day	
I	Normal control (1% gum acacia)	202.5	207.3	212.3	217.3	7.4 %
II	Diabetic control	203.7	191.2	183	179.7	11.8 %
III	Glibenclamide (2.5 mg/kg)	203	206.8	209.5	213.7	4.9 %
IV	MEt.AM (200 mg/kg)	206.5	200.3	194.5	189.5	− 8.2 %
V	MEt. MP (Ripe) (200 mg/kg)	216	210.7	205.3	202.2	− 6.5 %
VI	MEt.MP (Unripe) (200 mg/kg)	217	209.2	202.3	195.8	− 9.7 %
VII	MEt.OS (200 mg/kg)	220.7	212.5	206.5	200.7	− 9.1 %

*Compared to day 0 (Zero) n= no of animals in each group

Plant extracts treated animals exhibited change in body weight. Diabetic group animals were compared with normal group animals whereas diabetic animals were compared with the animals treated with plant extracts. A significant prevention in reduction in body weight i.e. -8.2%, -6.5%, - 9.7% and - 9.1% was recorded with methanolic extracts of *Aegele marmelos*, *Musa paradisiaca*, and *Ocimum sanctum* respectively. [Table 6]

Table 7: Serum Profile in Diabetic Rats after 15 days of Treatment

Exp. Group	Treatment	TGL mg/dl	HDL mg/dl	VLDL mg/dl	LDL mg/dl	Total Cholesterol mg/dl
I	Normal control (1% gum acacia)	79 ± 3.01	44.7 ± 3.7	26 ± 1.9	21.2 ± 2.2	53.3 ± 2.5
II	Diabetic Control	120 ± 3.9	38.3 ± 0.9	49.7 ± 3.5	80.5 ± 4.2	88.2 ± 2.9

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III	Glibenclamide (2.5 mg/kg)	112 ± 4.6*	59.2 ± 1.9**#	53.3	45.8 ± 4.0**	75.5 ± 2.2
IV	MEt. AM (200 mg/kg)	90.7 ± 2.7**	42.5 ± 1.7*	22.5	32.7 ± 1.7**	60.7 ± 1.5**##
V	MEt. MP (ripe) (200 mg/kg)	98.3 ± 2.3**	41 ± 1.9	21 ± 0.9**	18.1 ± 2	59.5 ± 1.7**#
VI	MEt.MP (unripe) (200 mg/kg)	105 ±1.8**	39.2 ± 0.7#	24.2	27.2 ± 1.3**	69 ± 1.4**##
VII	MEt.OS (200 mg/kg)	92.7 ± 2.5**	45.7 ± 2.6*#	25.5 ± 1.5**	35.7 ± 1.7**#	63.2 ± 2.3**#

*P< 0.05 & **P< 0.01 Significant, compared to normal,

n = no of animals in each group

#P< 0.05 &# #P< 0.01 Significant, compared to diabetic control

Effect of ethanol extracts on serum lipid profiles in alloxan induced diabetic rats [Multi dose (sub-acute) treatment]

Hyperlipidemia is one of the associated complications in diabetes mellitus. After 15 days of treatment TC, TG, LDL, VLDL and HDL levels were estimated. The results obtained were compared with normal control group animals and diseased group animals. There was a remarkable decrease in TC, TG, LDL, VLDL levels whereas HDL levels were elevated (Table 7).

Blood glucose level, total cholesterol (TC), triglycerides (TG) low density lipoproteins (LDL) and very low density lipoproteins (VLDL) levels were remarkably reduced whereas high density lipoproteins (HDL) level was increased in animals treated with methanolic extracts of the above selected plants. This supports the claims made about these plants for their importance in management and treatment of diabetes. The results are also in confirmation with the earlier similar studies carried out.

Histopathological Studies

Histopathology of pancreas revealed substantial regeneration of β cells and cellular expansion of islets of langerhans in the animals treated with ethanol extracts of *Aegele marmelos*, *Musa paradisiaca* (ripe), *Musa paradisiaca* (Unripe) and *Ocimum sanctum*. This was evident by comparison with necroses occurred due to alloxan. From the figure it can be interpreted that normal group animals showed normal cellular population of islets of langerhans as well as normal acini. Diseased group animals showed damaged, irregular langerhans. Standard drug glibencamide exerts moderate effect by expanding cellular population and size of islet cells.

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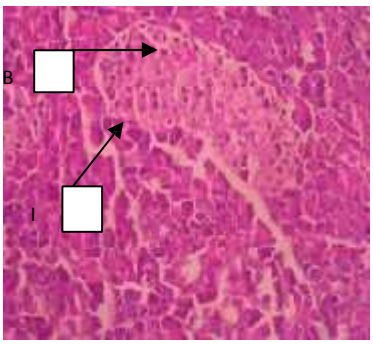
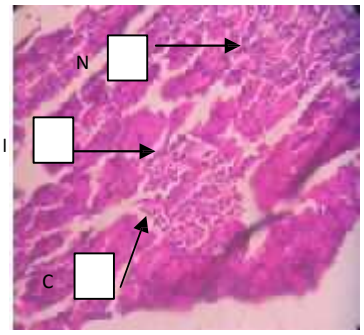
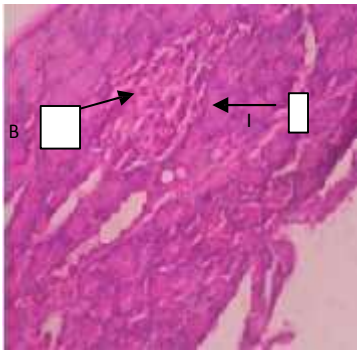
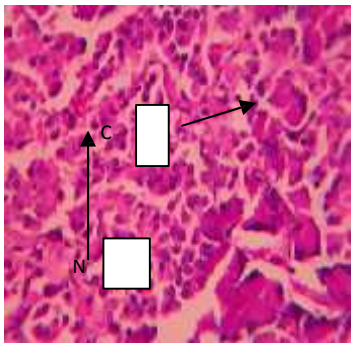
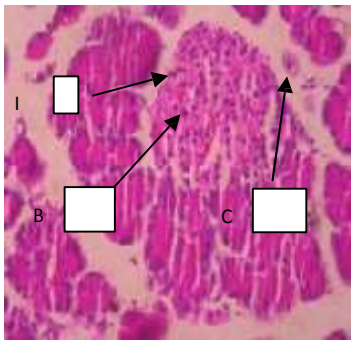
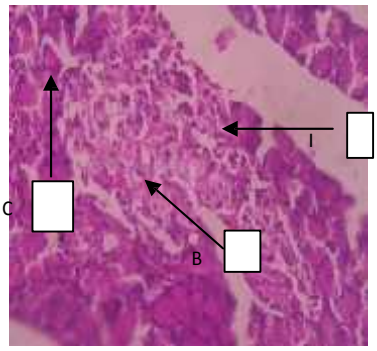
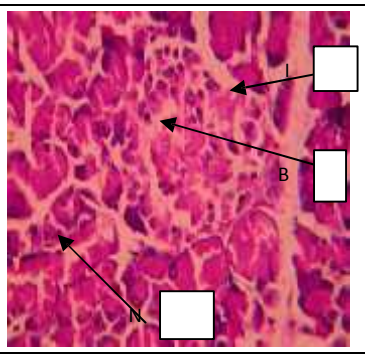
	
A- Normal control	**B- Diabetic group**
	
C- Glibenclamide	**D - MEt. AM**
	
E - MEt. MP (R)	**F- MEt. MP (U)**
	
G – MEt. OS	

I - Islets of Langarhans, B - Beta cells, C-Congestion, N-Neutrophils

Figure 6: Histopathology of Rat Pancreas after 15 days of Treatment with Ethanol Extract

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A. Normal control rats showed normal pancreatic tissue, while
B. Alloxan-induced diabetic rats had severe decrease in number of islets of Langerhans cells and β cells
C. Pancreatic tissue of diabetic rats treated with Glibenclamide (2.5 mg/kg)
D. Methanolic extract of *Aegele marmelos* (200 mg/kg) showed partial restoration of normal cellular population and size of islet cells
E. Methanolic extract of *Musa paradisiaca* ripe fruit (200 mg/kg) showed partial restoration of normal cellular population and size of islet cells
F. Methanolic extract of *Musa paradisiaca* unripefruit (200 mg/kg) showed partial restoration of normal cellular population and size of islet cells
G. Methanolic extract of *Ocimum sanctum* (200 mg/kg) showed partial restoration of normal cellular population and size of islet cells.

In histopathology study, pancreatic damage can be observed in diseased group i.e. animals treated with alloxan. Animals treated with standard drug glibenclamide, showed significant β cells regeneration. At par β cell regeneration was also observed in the animals treated with methanolic extract of *A. marmelos, M. paradisiaca* and *Ocimum sanctum.* From the photomicrographs it can be inferred that, healing of damaged pancreas after treatment with plant extract may be a probable underlying mechanism for their anti-diabetic effect. The activity may be attributed to the secondary metabolites such as flavonoids, triterpenoids present. Reports suggests that numerous plants of medicinal value showing hypoglycemic and anti-diabetic effects contains flavonoids

CONCLUSION

From the data acquired for various parameters evaluated, in is likely that free radical scavenging (antioxidant) effect of methanolic extracts of *A. marmelos*, *M. paradisiaca* and *Ocimum sanctum* is also responsible for anti-diabetic effect. Other significant probable mechanism may be regeneration and substantial growth in number and size of langerhans and β cells after treatment with plant extracts. However, diseased group animals when treated with plant extracts showed remarkable restoration of size and normal cellular population of islet cells. Another considerable outcome of change in body weight study reported that, after treatment with methanolic extract of these plants it was observed that there is a dose dependent decrease in body weight. Hence, it can be postulated that the plant products assists in proper functioning of metabolic pathways thereby regulating the sugar levels in normal range.

The results support the assumed antioxidant and anti diabetic activity of these mentioned plants in traditional system of medicine. However, the selected plants should further be studied on the basis of bioactivity guided fractionation and isolation of lead compound with anti oxidant and anti-diabetic activity and probable underlying mechanism. Most of available anti-diabetic drugs are synthetic drugs with some drawbacks or adverse effects for sure. Present study provides us with a reasonable potential alternative of natural drugs in treating diabetes mellitus and associated complications.

FUTURE PROSPECTS

The rapid occurrence of diabetes mellitus is serious threat to human physical condition in all over the world. There has been a revival of interest in polyherbal drugs in developed countries due to a huge amount on the preference of products from natural sources. Many

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polyherbal formulations are used by people and various native drugs are regularly being introduced into current therapeutics. Therefore, focus should be given for new active drugs extraction from plants which possess anti-diabetic activity with more effectiveness than oral hypoglycemic agents used in proven therapy. Awareness should be been drawn towards innovation of Diabetes mellitus treatment using polyherbal drugs having powerful pharmacological actions, free from side effects and increased therapeutic efficacy.

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