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DOSE DEPENDENT ANTIDIABETIC ACTIVITY OF HYDRO-METHANOLIC EXTRACT (3:2) OF FLOWER OF *M. BALBISIANA* IN STREPTOZOTOCIN INDUCED DIABETIC MALE RAT

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Keywords:

Musa balbisiana, Diabetes, Streptozotocin, Antioxidant enzymes, Thin Layer Chromatography

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ABSTRACT: Background: *Musa balbisiana* Colla (Musaceae family) is used as folkloric medicine throughout India. **Objective:** To search out the potent dose of a hydro-methanolic extract of *Musa balbisiana* flower for antidiabetic and antioxidative activities in streptozotocin-induced diabetic rat. **Material and Methods:** The extract of the said plant part was administered orally at the dose of 5, 10 or 20 mg /100 g body weight for 28 days. Diabetes was induced by a single intramuscular injection of streptozotocin at the dose of 4 mg / 0.1 ml of citrate buffer / 100 g body weight to Wistar stain albino male rat. Biochemical, histological and thin layer chromatographic profiles were studied. **Results:** Treatment with 10 mg or 20 mg doses exhibited a significant recovery from diabetes by decreasing fasting blood glucose and glycated hemoglobin levels along with increasing the serum insulin level. Significant recovery was noted in antioxidative and carbohydrate metabolic enzymes' activities in hepatic and skeletomuscular tissues in diabetic rat. The extract had no metabolic toxicity. From the qualitative phytochemical analysis of said extract, the presence of flavonoids, alkaloids, phenols, and triterpenes was noted. **Discussion and Conclusion:** Results indicated that 10 mg dose was considered as a minimum but effective dose. The higher dose was not more effective probably due to the saturation of the receptors of the concerned phytomolecules present in the extract, and therefore the remedial effect was not improved by higher doses. So, it may be concluded that 10 mg is the potent dose for the correction of diabetes as well as diabetes-induced oxidative stress.

INTRODUCTION: Throughout the world, many herbal plants are being used as herbal medicine, and the rate of their curative efficacy is mushrooming day by day. In the modern era, herbal medicine has become alternative medicine which contains so many phyto-compounds and cures many pathophysiological conditions. Research has shown their role in the management of cardiovascular disease, inflammation, renal disorder, male infertility and also diabetes.

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Diabetes mellitus is considered as one of the metabolic syndromes. In this condition, the body does not produce enough insulin or not responding to the insulin produced [1]. It is associated with disturbances in carbohydrate, protein and fat metabolism. Due to the high production of ROS (Reactive oxygen species) and low level of antioxidant, oxidative stress is noted in diabetic condition [2]. The percentage of diabetes is increasing day by day in the world as well as in India.

The prevalence rate of diabetes is increasing rapidly in our country. Approximately 25% population is suffering from this disease in world, and it recognized as the world's fastest growing chronic disease. According to the latest 2016 data from the 'World Health Organization,' the diabetic

population was nearly about 422 million worldwide [3,] and it is projected that the population of diabetic would be almost 762 million in 2030 and India would be considered as the 'capital of diabetes' [4].

Now a day's many synthetic drugs and insulin therapy are available in the market, but there are several side effects, *e.g*. liver problem, nausea, brain atrophy, vomiting, *etc*. whereas herbal medicine is considered as less toxic than synthetic medicine [5]. According to the ethnobotanical information, approximately 800 plants have antidiabetic activity [6]. *Musa balbisina* commonly found in our country belongs to the Musaceae family and cultivated in Eastern South Asia, Northern Southeast Asia, and Southern China. It is also known as a folk medicine for disease management like gout, gastritis, infertility, *etc*. [7]

Pilot work has been conducted in our laboratory to find out the antidiabetic activity of flower of *M. balbisiana*, and from the result, hydro-methanolic (3:2) extract of flower of *M. balbisiana* was selected as a potent extract for the management of the hyperglycaemic activity.

Very recently an experiment has been conducted regarding the anti-hyperglycemic activity of flower of *Musa balbisiana* with a few preliminary biochemical parameters, but other important glycaemic sensors, as well as antioxidant parameters along with histological changes after treatment with different doses, have not performed yet [8].

So, the current study was framed to explore the most potent dose of the hydro-methanolic extract of the flower of *M. balbisiana* on streptozotocin-induced diabetic rat for the management of diabetes.

MATERIAL AND METHODS:

Plant Material: During the summer season, flowers of *M. balbisiana* were collected from Midnapur local market, and it was authenticated and identified by a taxonomist in the Department of Botany and Forestry, Vidyasagar University, Midnapur. The voucher specimen has been submitted in the Herbarium of the Botany Department, Vidyasagar University (Ref. no. Bio-Med/V.U/M.B/16/09).

Preparation of Hydromethanolic (3:2) Extract of Flower of *M. balbisiana*: Fresh flowers were copped in small pieces and then dried in an incubator for 48 h at 37 °C, crushed by an electric grinder. Out of total powder, 30 g powder of flower of *M. balbisiana* has suspended in 500 ml hydro-methanol (3:2) solvent mixture and kept it overnight in the refrigerator. The hydro-methanolic mixture was filtered using a cotton and Whatman filter paper (no. 1). The filtrate was evaporated under low pressure using rotary evaporator (HAHN-SHIN HS-2000NS, Korea) then the residue was preserved in the refrigerator at 4°C for the experiment.

Chemicals: Streptozotocin (STZ) was purchased from Sigma Chemical (St Louis, MO, USA). All other analytical grade chemicals and reagents were obtained from Merck (KGaA, Germany) or standard manufacturers.

Selection of Animals and Animal Care: Thirty normoglycaemic male albino rats of Wistar strain (80-90 days old) having body weight 130 ± 10 g were used in this experiment. The rats were purchased from an authorized vendor and kept in our well-ventilated animal house (Vidyasagar University) at 22 - 25 ºC temperature and also maintained 12 h: 12 h light-dark cycles. Standard chow and water *ad libitum* were provided to animals for a specific duration.

Animal Ethics: The instruction and principles of laboratory animal care were given by the Committee for Control and Supervision of Experiments on Animals (CPCSEA) (Reg. no. 2013/GO/Re/S/18/CPCSEA, dated 1/5/2018, GOI) and approved by Institutional Ethics Committee (IEC) [IEC/9/4/18, dated 2/02/2018] were followed throughout the experiment.

Induction of Diabetes Mellitus: Diabetes was induced with streptozotocin as per standard method [9]. In 24 h fasting condition rats were kept and then single intramuscular injection of STZ at the dose of 4 mg / 0.1 ml citrate buffer (pH 4.5) / 100 g body weight/rat was injected to 24 rats that produce diabetes. Diabetes was confirmed after 7 days of STZ injection by determining the fasting blood glucose level.

Experimental Design: Before starting the treatment, thirty rats were divided into five group, and there are six rats in each group. Four groups were made diabetic by a single intramuscular STZ injection. The treatment of extract was continued for the next 28 days. An oral dose of distilled water or extract was given in fasting condition (at 11:00 a.m.). There was no feed supply for 2 h before and after extract treatment. This has been adopted to minimize the drug-nutrient interaction if any.

Group I: Vehicle Treated Control Group: Normoglycemic rats were taken in this group, *i.e*. having to fast blood glucose level 70-80 mg/dl and subjected to oral feeding of distilled water at the dose of 0.2 ml / 100 g body weight/day as vehicle treatment.

Group II: Vehicle Treated Diabetic Group: STZ induced diabetic rats of this group were also treated with 0.2 ml distilled water / 100 g body weight/day as vehicle treatment.

Group III: Diabetic + 5 mg Dose Treated Group: STZ induced diabetic rats of this group were treated with hydro-methanolic (3:2) extract of flower of *M. balbisiana* at 5 mg dose / 100 g body weight in 0.5 ml of distilled water/day for 28 days.

Group IV: Diabetic + 10 mg Dose Treated Group: In this group, diabetic rats were treated with hydro-methanolic (3:2) extract of flower of M. *balbisiana* at 10 mg / 100 g body weight in 0.5 ml of distilled water/day for 28 days.

Group V: Diabetic + 20 mg Dose Treated Group: Streptozotocin-induced diabetic rats of this group were treated with hydro-methanolic (3:2) extract of flower of *M. balbisiana* at 20 mg / 100 g body weight in 0.5 ml of distilled water/day for 28 days.

All the rats were sacrificed at fasting state by decapitation using euthanasia on the 29th day of treatment after recording their body weight. From the hepatic vein, blood was collected for fasting blood glucose estimation. After that, serum was separated by centrifugation at 4000 rpm for 10 min to measure the serum insulin. Different tissues, *i.e*. liver, pancreas, skeletal muscle were dissected out and stored at -20 ºC for the biochemical analysis and other.

Hyperglycaemic Profile Measurement:

Fasting Blood Glucose Level (FBG) Measurement: At the time of grouping of the animals, FBG (Fasting blood glucose) was measured. Blood was collected from the tail vein or by orbital puncture, and the FBG was measured by single touch glucometer [10]. FBG was recorded in all animals of all groups on every 7th day of treatment.

Assay of Glycated Haemoglobin (HbA1c): Glycated hemoglobin was measured according to standard protocol [11].

Assay of Serum Insulin: Serum insulin level was measured by ELISA (Enzyme-Linked Immuno-sorbent Assay) kit for rat (RayBio rat insulin ELISA kit, Norcross, GA 30092 U.S.A). The intra-assay variation was 4.9%. No inter-assay variation was considered as all the samples were measured at the same time [12]. The level of insulin in serum was expressed in µ IU/mL.

Evaluation of Carbohydrate Metabolic Markers: Hexokinase activities in hepatic tissue and skeletal muscle (biceps femoris) were measured spectrophotometrically according to standard protocol [13]. Tissue concentration was 50 mg/mL, and tissues were homogenized in ice-cold 0.1M phosphate buffer saline (pH 7.4). The assay mixture contained 7.5 mM $MgCl_2$, 3.7 mM glucose, 11 mM thioglycerol and 45 mM HEPES buffer. In a spectrophotometer cuvette, 0.9 ml of this assay mixture, 0.01 ml NADP and 0.03 ml of ATP were added and mixed well. After that, 0.1 ml of the tissue supernatant was added into the cuvette and absorbance was noted at 340 nm. One unit of hexokinase was expressed as µg/mg of tissue. The activity of glucose-6-phosphatase in hepatic tissue and skeletal muscle (biceps femoris) was measured by the method of Swanson [14].

Evaluation of Anti-oxidative Enzymes: The activities of catalase in hepatic tissues and skeletal muscles (biceps femoris) were measured biochemically [15]. Tissues were homogenized separately in 0.05 M Tris-HCl buffer solution (pH-7.0) at a concentration of tissue 50 mg of the concerned tissue/ml. Homogenized tissues were centrifuged at 10,000 rpm for 10 min at 4 ºC. In spectrophotometer cuvette, 0.5 ml of 0.00035 M H_2O_2 and 2.5 ml of distilled water were mixed

gently. After that, 40 µl supernatant samples were added into the cuvette and absorbance was noted at 240 nm. The kinetics was measured by recording successive six readings at the 30-sec interval.

The activities of peroxidase in hepatic tissue and skeletal muscles (biceps femoris) were measured according to the standard method [16]. Tissue was homogenized in ice-cold 0.1M phosphate buffer saline (pH 7.4), and tissue concentration was 50 mg/mL. The supernatant was collected from the homogenate, and 20 mM guaiacol was mixed with 0.1 ml supernatant. The time was recorded for an increase in the absorbance by 0.1 at 436 nm.

Superoxide dismutase activities in hepatic tissues and skeletal muscles (biceps femoris) were quantified by measuring the percentage inhibition in the pyrogallol auto-oxidation by superoxide dismutase according to standard protocol [17]. In a spectrophotometric cuvette, 2.04 ml of 50 mM Tris buffer (pH-8.2), 20 µl of sample and 20 µl of pyrogallol were taken.

Estimation of End Products of Lipid Per-oxidation: An estimation of lipid peroxidation was performed from the concentration of the thiobarbituric acid-reactive substances (TBARS) and conjugated dienes (CD) according to standard method [18]. Samples of hepatic tissue and skeletal muscle (biceps femoris) were homogenized separately in cold 0.1M phosphate buffer (7.4) at 50 mg/mL tissue concentration. All homogenate samples were centrifuged for 5 min at 10,000 × g at 4 ºC. The supernatant was collected from tissue homogenate for the spectrophotometrical analysis.

Estimation of Toxicity Markers: Activities of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were measured. Kits were supplied by Crest Bio-systems, Gitanjali, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex (Goa, India) [19].

Qualitative Phytochemical Screening of Hydro-Methanolic (3:2) Extract: Hydro-methanolic (3:2) extract of flower of *M. balbisiana* was subjected to qualitative analysis for different phytomolecules, *e.g*. flavonoids, alkaloids, phenols, glycosides, steroids, triterpenes as per the standard methods [20, 21].

Thin Layer Chromatographic Study (TLC): The hydro-methanolic (3:2) extract was dissolved with methanol for thin layer chromatography. The hydro-methanolic extract was loaded on the TLC plate using by capillary tube the plate was carefully dried. The plate was kept in a TLC chamber using methanol, chloroform, and acetic acid solvent system. The plate was dried and observed under ultraviolet light (254 nm and 366 nm), and the plate was kept for iodine maturation. The retention factor (R_f) for each active compound was calculated [22].

Histological Study of the Pancreas: Pancreas was dissected, fixed in 'Bouin's fixative.' Pancreatic tissue was cut into small pieces, and then pancreatic tissue was dehydrated in an ascending ethanol gradient and cleared with xylene. Tissue was subjected to paraffin embedding carefully after a proper dehydration process, followed by section cutting (5 micrometers thick) in Leica microtome.

The tissue section was allowed to hematoxylin and eosin staining after deparaffinization. Qualitative analysis of pancreatic islets histology was carried out on stained tissue from the viewpoint of cell density and islets size.

Statistical Analysis: Collected data of all parameters of this experiment were expressed as Mean ± SEM, (n=6). Statistical analysis was performed by 'Analysis of Variance (ANOVA)' followed by multiple comparisons two tail t-test [23]. Differences were considered significant at the level of $P<0.05$.

RESULTS:

Body Weight: A significant diminution in body weight was noted in vehicle-treated diabetic rat.

TABLE 1: BODY WEIGHT OF VEHICLE TREATED CONTROL, DIABETIC AND OTHER EXPERIMENTAL GROUPS

Body weight in (g)		
Groups	**Initial**	**Final**
Vehicle treated control	125.0 ± 4.5^a	128.0 ± 5.0^a
Vehicle treated diabetic	120.5 ± 4.0^a	108.0 ± 4.5^b
Diabetic + 5 mg treated	122.0 ± 3.5^a	112.0 ± 4.5^b
Diabetic + 10 mg treated	123.0 ± 4.5^a	132.0 ± 4.5^c
Diabetic + 20 mg treated	122.5 ± 4.5^a	135.0 ± 4.5^c

Data were expressed as Mean ± SEM (n=6) ANOVA followed by multiple comparisons of two-tail t-test. Values with different superscripts (a, b, c) in each column differ from each other significantly, $P<0.05$.

Oral administration of hydro-methanolic (3:2) extract of flower of *M. balbisiana* at 5, 10 or 20 mg dose / 100 g body weight/day for 28 days to diabetic rat showed significant recovery in body weight towards the vehicle-treated control rat **Table 1**.

Fasting Blood Glucose Level: Fasting blood glucose level was significantly elevated in vehicle-treated diabetic animals concerning the vehicle-treated control animal. Treatment with hydro-methanolic (3:2) extract at 5, 10 or 20 mg dose / 100 g body weight/day in STZ induced diabetic group resulted in a significant recovery in fasting blood glucose levels toward the control level but 10 mg dose showed most promising recovery in this concern **Table 2**.

TABLE 2: DOSE DEPENDENT EFFECT OF HYDRO-METHANOLIC (3:2) EXTRACT OF FLOWER OF *MUSA BALBISIANA* ON FBG LEVEL

Fasting Blood Glucose (mg/dl)						
Groups	**On the day of STZ injection**	**1st day (Treatment started)**	**7th day**	**14th day**	**21st day**	**29th day**
Vehicle treated control	69.0 ± 2.5[a]	69.0 ± 2.8[a]	68.0 ± 2.5[a]	72.0 ± 2.8[a]	69.0 ± 2.4[a]	70.0 ± 2.5[a]
Vehicle treated diabetic	68.5 ± 2.8[a]	358.0 ± 3.8[b]	352.6 ± 4.5[b]	348.0 ± 3.8[b]	354.0 ± 3.5[b]	349.5 ± 3.8[b]
Diabetic + 5 mg treated	67.0 ± 3.5[a]	345.0 ± 4.5[b]	265.5 ± 5.2[c]	260.0 ± 4.9[c]	245.3 ± 4.2[c]	224.6 ± 4.6[c]
Diabetic + 10 mg treated	69.8 ± 2.3[a]	352.0 ± 4.5[b]	186.4 ± 4.4[d]	178.0 ± 3.9[d]	125.0± 4.4[d]	98.1 ± 3.9[d]
Diabetic + 20 mg treated	66.0 ± 2.9[a]	354 ± 4.5[b]	178.0 ± 4.5[d]	169.3 ± 4.2[d]	119.4 ± 4.3[d]	95.5 ± 3.7[d]

Data were expressed as Mean ± SEM (n=6) ANOVA followed by multiple comparisons of two-tail t-test. Values with different superscripts (a, b, c, d) in each column differ from each other significantly, P<0.05.

Glycated Haemoglobin Level (HbA1c): In the vehicle-treated diabetic group, HbA1c level was elevated significantly in respect to the vehicle-treated control group. The supplementation of said extract at 5, 10 or 20 mg dose / 100 g body weight/day in the diabetic group resulted in a significant recovery in HbA1c level towards the vehicle-treated control group. Insignificant variation in the HbA1c level was noted between 10 mg and 20 mg extract treated groups **Fig. 1**.

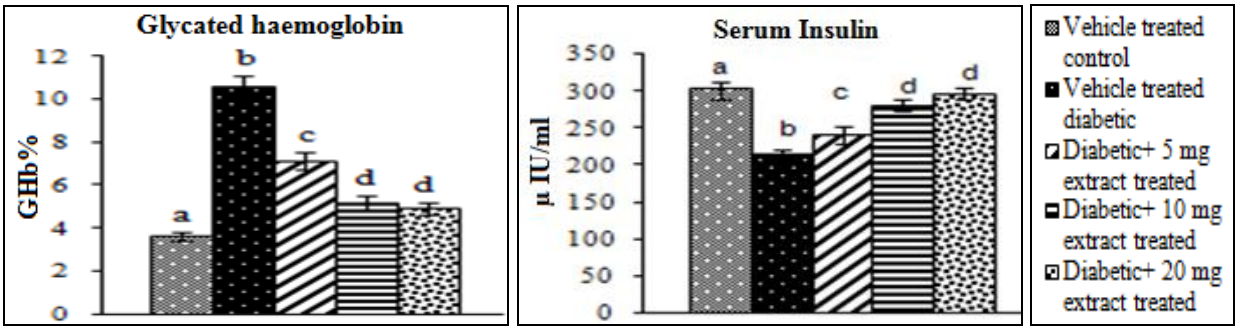


FIG. 1: RECOVERY IN GLYCATED HAEMOGLOBIN AND SERUM INSULIN LEVELS AFTER TREATMENT WITH HYDRO-METHANOLIC (3:2) EXTRACT OF FLOWER OF *M. BALBISIANA*. Data were expressed as mean ± SEM, n=6. Bars with different superscripts (a, b, c, d) differ from each other significantly; P<0.05 (ANOVA followed by multiple comparisons two tail t-test).

Serum Insulin Level: A significant decrease in the serum insulin level was observed in the vehicle-treated diabetic group concerning the vehicle-treated control group. Insulin levels of all extract treated groups were significantly recovered towards the vehicle-treated control group. But 20 mg dose did not show further recovery in comparison to 10 mg dose **Fig. 1**.

Hexokinase and Glucose -6 -phosphatase Activities in Liver and Skeletal Muscle: Hexokinase activity in hepatic tissue and skeletal muscle were significantly diminished in STZ induced vehicle-treated diabetic animal in comparison to the vehicle-treated control animal. Treatment with the extract in the doses mentioned above for 28 days focused significant recovery in the activity of hexokinase in hepatic tissue and skeletal muscle compared with a vehicle-treated diabetic rat. Similarly, oral administration of the extract at 5, 10 or 20 mg doses showed a significant decrease in the activity of glucose-6-phosphatase which was significantly elevated in the diabetic group.

Among all the treated doses, 10 mg dose exhibited maximum recovery as the higher dose (100 mg) showed a non-significant difference in the level of this parameter with 10 mg dose **Fig. 2**.

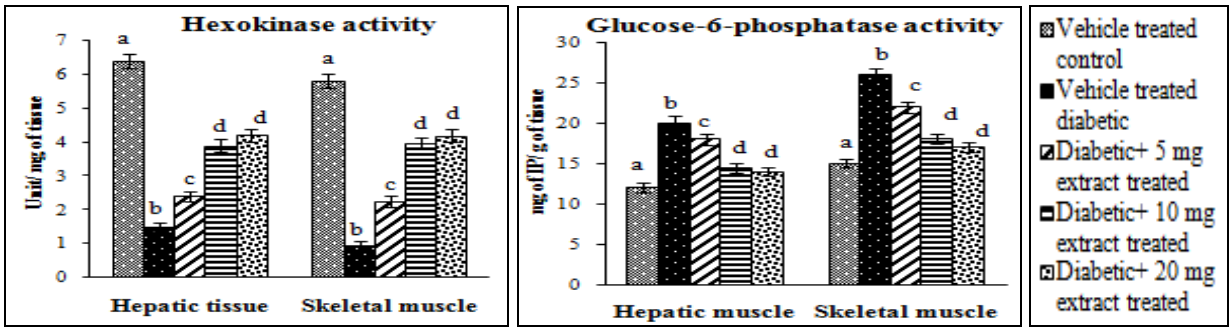


FIG. 2: EFFECT OF HYDRO-METHANOLIC (3:2) EXTRACT OF FLOWER OF *M. BALBISIANA* IN DIFFERENT DOSES ON THE ACTIVITY OF HEXOKINASE AND GLUCOSE-6-PHOSPHATASE IN HEPATIC TISSUE AND SKELETAL MUSCLE OF VEHICLE TREATED CONTROL, DIABETIC AND DIFFERENT DOSES TREATED GROUPS. Data were expressed as mean ± SEM, n=6. Bars with different superscripts (a, b, c, d) differ from each other significantly; P<0.05 (ANOVA followed by multiple comparisons two tail t-test).

Activities of Antioxidant Enzymes in Hepatic Tissue and Skeletal Muscle: Vehicle treated diabetic group showed a significant diminution in catalase, peroxidase, and superoxide dismutase activities in liver and skeletal muscle compared to the vehicle-treated control group. Treatment with hydro-methanolic (3:2) extract of flower of *M. balbisiana* at all the above-mentioned doses for 28 days to the diabetic animals resulted in a significant recovery in the activities of the above-mentioned enzymes towards vehicle-treated control group. The most effective recovery was noted at 10 mg dose in respect to other doses applied here **Fig. 3**.

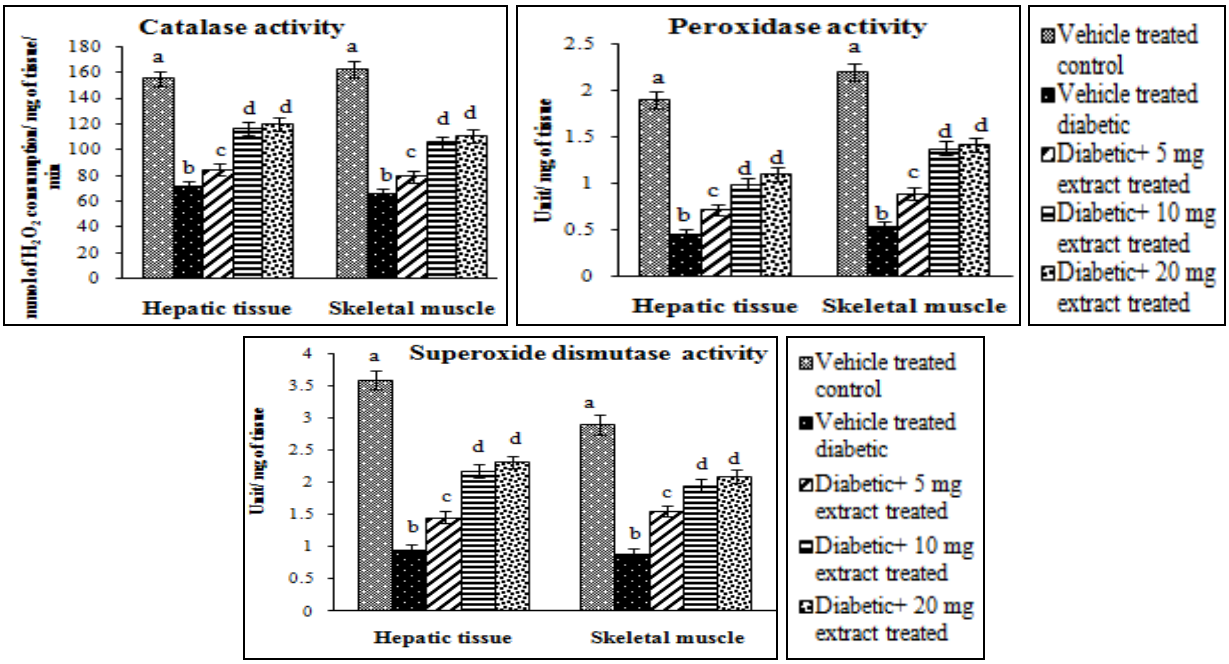


FIG. 3: ACTIVITIES OF CATALASE, PEROXIDASE AND SUPEROXIDE DISMUTASE IN HEPATIC TISSUE AND SKELETAL MUSCLE IN CONTROL, DIABETIC AND DIFFERENT DOSES TREATED GROUPS. Data were expressed as mean ± SEM, n=6. Bars with different superscripts (a, b, c, d) differ from each other significantly; P<0.05 (ANOVA followed by multiple comparisons two tail t-test).

Level of TBARS and CD: Levels of TBARS and CD in hepatic tissue and skeletal muscle were increased significantly in STZ induced vehicle-treated diabetic group in respect to vehicle-treated control. In contrast, levels of TBARS and CD in said tissues were significantly decreased after oral administration of the extract at 5, 10 or 20 mg dose / 100 g of bodyweight/day for 28 days. The minimum but the effective dose in this concern was 20 mg dose **Fig. 4**.

Toxicity Studies: Activities of SGOT, SGPT was increased in the vehicle-treated diabetic group when the comparison was made with the vehicle-treated control group. Hydro-methanolic (3:2) extract of flower of *M. balbisiana* treatment was able to recover the concerned enzyme activities in STZ induced diabetic rats at a significant level which was near to the vehicle-treated control group. The activities of SGOT and SGPT have decreased significantly at 10 mg or 20 mg doses treatment in comparison to 5 mg dose treatment **Fig. 5**.

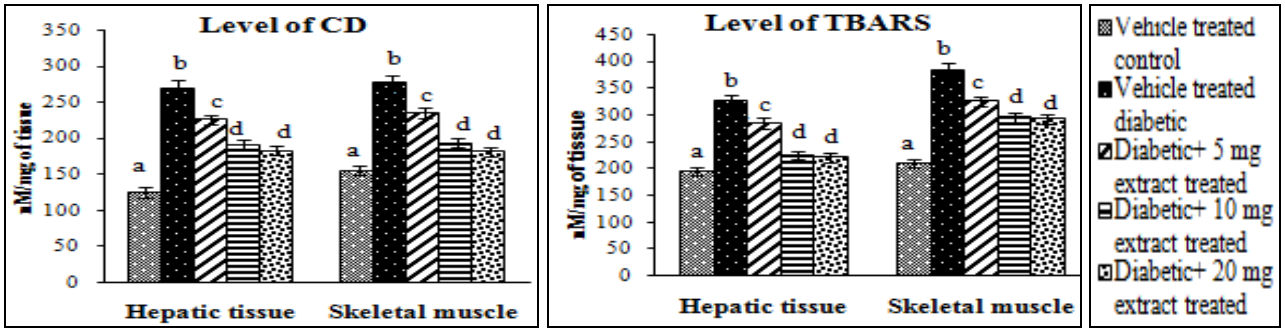


FIG. 4: EFFECT OF HYDRO-METHANOLIC (3:2) EXTRACT OF FLOWER OF *M. BALBISIANA* IN DIFFERENT DOSES ON CD AND TBARS IN HEPTIC TISSUE AND SKELETAL MUSCLE. Data were expressed as mean ± SEM, n=6. Bars with different superscripts (a, b, c, d) differ from each other significantly; P<0.05 (ANOVA followed by multiple comparisons two tail t-test).

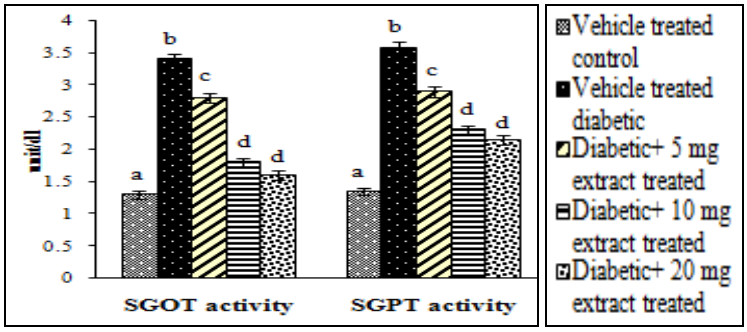


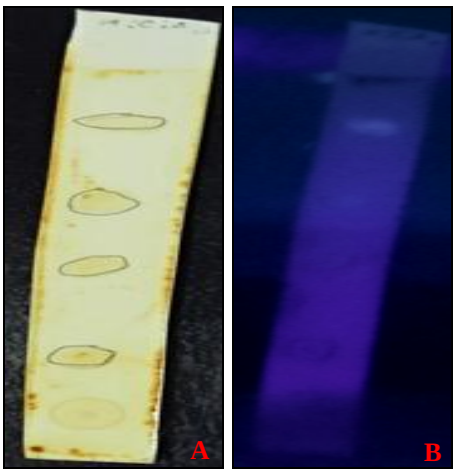
FIG. 5: ACTIVITIES OF SGOT AND SGPT IN CONTROL, DIABETIC AND EXTRACT TREATED GROUPS (mean ± SEM, n = 6). ANOVA followed by multiple comparisons of two tail t-tests. Values with different superscripts (a, b, c, d) on each bar differ from each other significantly (P<0.05).

Qualitative Analysis of Phytochemicals: The study of the phytochemical analysis of hydro-methanolic (3:2) extract indicated that extract contains flavonoids, alkaloids, phenols and triterpenes **Table 3**.

TABLE 3: PHYTOCHEMICAL SCREENING TEST OF HYRO-METHANOLIC (3:2) EXTRACT OF FLOWER OF *MUSA BALBISIANA*

Phytochemicals	Hydro-methanolic extract
Alkaloids	+
Flavonoids	+
Steroids	-
Glycosides	-
Phenols	+
Triterpenes	+

Thin Layer Chromatographic Study: Thin Layer Chromatographic study of hydro-methanolic (3:2) extract of *M. balbisiana* provided four spots.



Iodine maturation **UV exposure in long wave length of 366 nm**

FIG. 6: THIN LAYER CHROMATOGRAPHY OF HYDRO-METHANOLIC (3:2) EXTRACT OF FLOWER OF *MUSA BALBISIANA*. A SPOTS NOTED AFTER IODINE MATURATION. B, SPOT VISIBLE IN UV LONG WAVELENGTH (366 nm)

The initial spot was visible in long wavelength of UV light, and after iodine maturation, all the spots were visible **Fig. 6**. Values of retention factor of the compounds were 0.75, 0.66, 0.43, and 0.2.

Histological Study of the Pancreas: In STZ induced diabetic rat, cell population density and size of pancreatic islet were decreased in respect to the vehicle-treated control rat. After the treatment of hydro-methanolic (3:2) extract for 28 days to the diabetic rat, both were significantly restored towards the control but the most effective dose in this concern was 10 mg / 100 g body weight **Fig. 7**.

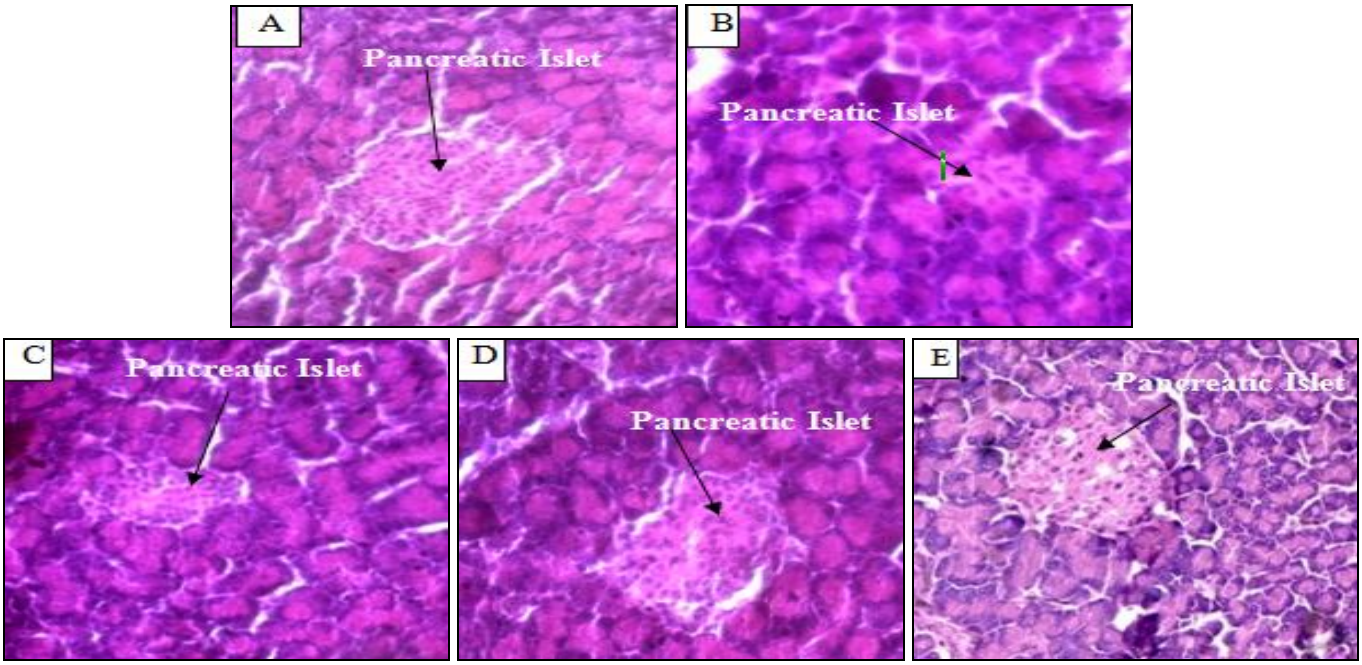


FIG. 7: HISTOLOGY OF PANCREAS WITH 'ISLETS OF LANGERHANS. A) Normal histoarchitecture of pancreatic Islet, in vehicle-treated control group. B) Diminution in the cell density in representative pancreatic islet tissue sample in vehicle-treated diabetic group. C) Recovery in islet's size by qualitative analysis in 5 mg extract treated diabetic group. D) Prominent recovery of the islet cell population in 10 mg extract treated diabetic group. E) Cell population and size of the islet recovered in 20 mg extract treated diabetic group. Hematoxylin-eosin stain, original magnification × 400.

DISCUSSION: Diabetes has now become a very common disease in urban sectors of developed as well as developing countries. It sometimes leads towards premature death due to its several complications. Treatment with herbal medicine has been proved to be a cost-effective, non-toxic, alternative way to cure the hyperglycaemic condition. So, the present study was designed to find out the most potent dose of hydro-methanolic (3:2) extract of flower of *M. balbisiana* for the management of STZ induced diabetic condition in the male rat. STZ is a pancreatic-selective *β*-cell toxin that induces *β*-cells destruction through DNA alkylation that leads to low insulin secretion. Besides its ability to accelerate the insulin-dependent diabetes mellitus or type 1 diabetes by *β* cell destruction, STZ is also responsible for developing peripheral insulin resistance that results in insulin secretion impairment from these cells [24]. The hydro-methanolic (3:2) extract of flower of *M. balbisiana* exerted the blood glucose lowering activity possibly due to the rectification in the resistance of the glucose transporter [25]. The level of glycated hemoglobin was increased in diabetic rat due to excess glucose in the blood which reacts with hemoglobin during erythropoiesis and forms a high level of glycated hemoglobin. After the treatment with extract for 28 days to STZ induced diabetic rat, glycated hemoglobin level was corrected significantly in all the treated doses towards the control due to a diminution in blood glucose level.

Hexokinase plays a vital role in the maintenance of glucose homeostasis. In liver and skeletal muscle of diabetic rat, the activity of this key enzyme of glucose catabolism was decreased due to the low level of insulin [26]. After administration of the

extract to the diabetic rats, a significant improvement in the enzymatic activity was noted probably due to the elevation in serum insulin [27] which was confirmed by ELISA study of this hormone as well as a qualitative study of islets of Langerhans. Glucose-6-phosphatase catalyzes the last step in both gluconeogenic and glycogenolytic pathways. Glucose-6-phosphatase cleaves the phosphate from glucose-6-phosphate to produce free glucose in the cell. Therefore, in the diabetic state, the activity of glucose-6-phosphatase is elevated that supports our results [28]. Our experiment has proved this fact where the said enzyme activity has been decreased in the diabetic state.

Levels of reactive oxygen species (ROS) generation in the hyperglycemic condition is high which results suppression in the activity of antioxidant enzyme [29]. In our present experiment, oxidative stress has been focused by assessing the antioxidant enzymes such as catalase, peroxidase, and superoxide dismutase activities. From our study, it may be stated that the extract can correct diabetes-induced oxidative stress due to an improved activity of antioxidant enzymes. Catalase and peroxidase along with superoxide dismutase activity were increased towards the control group by lowering the generation of free radical and end product of the oxidative stress which was possibly another important mechanism of the anti-oxidative effect of the said extract.

Further confirmation of the antioxidants activity of the extract was noted after the phytochemical screening where the qualitative analysis focuse on the presence of alkaloids, flavonoids, and phenols which are the well known natural antioxidant [30]. Presence of four compounds in the extract was further supported by four spots in TLC plate in the chromatographic study. The higher dose of the said extract is not more effective than the threshold dose, *i.e*. 10 mg may be due to the saturation of the receptor of the concerned phytomolecules present in the extract, and therefore the remedial effect of the extract was not improved further by higher dose, *i.e*. 20 mg. Though the 5 mg dose showed significant recovery compared to the diabetic group the desirable level of recovery did not achieve by this dose, so 10 mg dose was considered as potent dose. In diabetic condition, elevated activities of SGOT and SGPT were noted and that was related with increased ketogenesis and gluconeogenesis [31]. After treatment with the extract, activities of the said enzymes proved the non-toxic property of the extract which is similar to other work [32]. From this study, it may be predicted that hydro-methanolic (3:2) extract of flower of *M. balbisiana* at 10 mg has the maximum potentiality to recover the abnormalities in the diabetic state. From this experiment, it may be hypothesized that the extract of flower of *M. balbisiana* contains the phytomolecules which may have some direct role to regulate the synthesis and secretion of glycemic enzymes or the phytomolecules may generate the beta cell from stem cell for insulin secretion and thus control the glucose level in blood.

CONCLUSION: From the results, it may be concluded that the phytomolecules present in the *Musa balbisiana* flower can able to rectify the diabetes by controlling the blood glucose and managing the activities of glycaemic enzymes.

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REFERENCES:

1. Patil SS, Mehta M, Thakare V, Shende S, Shirure PA and Swami OC: Role of insulin in the management of type 2 diabetes mellitus. Int J Res Med Sci 2017; 5: 2282-92.
2. Newsholme P, Cruzat VF, Keane KN, Carlessi R and Bittencourt PI: Molecular mechanisms of ROS production and oxidative stress in diabetes. Biochem J 2016; 473: 4527-50.
3. World Health Organization. Global Report on Diabetes. Geneva, 2016: 1-88.
4. Kaveeshwar SA and Cornwall J: The current state of diabetes mellitus in India. Australas Med J 2014; 7: 145-48.
5. Mohammed SA, Yaqub AG, Sanda KA, Nicholas AO, Arastus W, Muhammad M and Abdullahi S: Review on diabetes, synthetic drugs and glycemic effects of medicinal plants. J Med Plant Res 2013; 7: 2628-37.
6. Kayarohanam S and Kavimani S: Current trends of plants having antidiabetic activity: A Review. Kayarohanam and Kavimani. J Bioanal Biomed 2015; 7: 55-65.
7. Borborah K, SK Borthakur and Tanti B: *Musa balbisiana* Colla-Taxonomy, Traditional knowledge and economic potentialities of the plant in Assam, India. Ind J Tradit Know 2016; 15: 116-20.
8. Borah M and Das S: Antidiabetic, antihyperlipidemic, and antioxidant activities of *Musa balbisiana* Colla. in type 1 diabetic rats. Ind J Pharmacol 2017; 49: 71-116.

9. Bera TK, Jana K, Maity S, Ghosh A, Kar SK and Ghosh D: Antihyperglycemic and antihyperlipidemic effects of diashis, a polyherbal formulation, in streptozotocin-induced diabetic male albino rat: an approach through toxicity study. World J Pharm Res 2015; 4: 1043-59.
10. Jana K, Ghosh A, Chatterjee K and Ghosh D: Antidiabetic activity of seed of *Eugenia jambolana* in streptozotocin-induced diabetic male albino rat: an apoptotic and genomic approach. Int J Pharm Pharm Sci 2014; 6: 407-12.
11. Chandalia HB, Sadikot S, Bhargava DK and Krisnaswamy PR: Estimation of glycosylated hemoglobin by a simple chemical method and its use in monitoring control of diabetes mellitus. J Assoc Physician Ind 1980; 28: 285-86.
12. Brugi W, Briner M, Fraken N and Kessler ACH: One step sandwich enzyme immunoassay for insulin using monoclonal antibodies. Clin Biochem 1988; 21: 311-14.
13. Jana K, Ghosh A, Bera TK and Ghosh D: Evaluation of the antidiabetic and antioxidative effect of the seed of *Eujenia jambolana* in the streptozotocin-induced diabetic male rat. World J Pharm Res 2014; 6: 2040-2056.
14. Swanson MA: Glucose-6-phosphatase from liver. Methods in Enzymol. Academic Press, New York, Vol. II, 1955: 541-43.
15. Beers RF and Sizer IW: Spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem 1952; 195: 133-40.
16. Maity R, Mishra DS and Ghosh D: Hypoglycemic and antioxidant potency of seed extract of *Tamarindus indica* Linn on streptozotocin-induced diabetes mellitus in the male rat: a dose-dependent study. Int J Pharma Bio Sci 2015; 6: 365-80.
17. Marklund S and Marklund G: Involvement of superoxide anion in the autoxidation of pyrogallol and a convenient assay of superoxide dismutase. Eur J Biochem 1974; 47: 469-74.
18. Bukan N, Sancant B, Yuvaz O, Koca C, Tuthun F, Ozcelikay AT and Altan N: Lipid peroxidation and scavenging enzyme levels in the liver of streptozotocin-induced diabetic rats. Ind J Biochem Biophys 2003; 40: 447-50.
19. Henry RJ, Chiamori M, Golub OJ and Berkman S: Revised spectrophotometric methods for the determination of glutamate-oxaloacetic transaminase, glutamate-pyruvate transaminase and lactic acid dehydrogenase. Am J Clin Pathol 1960; 34: 381-98.
20. Ramamurthy V and Sathiyadevi M: Preliminary phytochemical screening of methanol extract of *Indigotera trita* Linn. J Plant Biochem Physiol 2017; 5: 1-3.
21. Hossain MA, Khulood SA, AL-Mijizy ZH, Mohammed A and Qasim AR: Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*. Asian Pac J Trop Biomed 2013; 3: 705-10.
22. Mallick C, Bera TK, Ali KM, Chatterjee K and Ghosh D: Diabetes-induced testicular disorders vis-a-vis germ cell apoptosis in Albino rat: remedial effect of hexane fraction of root of *Musa paradisiaca* and leaf of *Coccinia indica*. J Health Sci 2010; 56: 641-54.
23. Tripathy A, Ghosh A, Dey A, Pakhira BP and Ghosh D: Attenuation of the cyproterone acetate-induced testicular hypofunction by a novel nutraceutical lycopene: A genomic approach. Andrologia 2016; 49: 1-11.
24. Tjalve H, Wilander E and Johansson EB: Distribution of labeled streptozotocin in mice: uptake and retention in pancreatic islets. J Endocrinol 1976; 69: 455-56.
25. Navale AM and Paranjape AN: Glucose transporters: physiological and pathological roles. Biophys Rev 2016; 8: 5-9.
26. Maity R, Mishra DS and Ghosh D: Hypoglycemic and hypolipidemic Effect of seed hydromethanolic extract of *Tamarindus indica* L. on streptozotocin-induced diabetes mellitus in rat. Am J Phytomed Clin Ther 2014; 2: 1416-29.
27. Miranda-Osorio P, Castell-Rodriguez A, Vargas-Mancilla J, Tovilla-Zarate C, Ble-Castillo J, Aguilar-Dominguez D, Juarez-Rojop I and Diaz-Zagoya J: Protective action of *Carica papaya* on β-Cells in streptozotocin-induced diabetic rats. Int J Environ Res Public Health 2016; 13: 1-9.
28. Xiong XP, Song Q, Han CC, Gan W, He F, Wei SH, Liu HH and Xu HY: Insulin promotes the expression of the gluconeogenic rate-limiting enzymes phosphoenolpyruvate carboxykinase (Pepck) and Glucose 6-Phosphatase (G6pase) through PI3k/Akt/mTOR signaling pathway in goose hepatocytes. Braz J Poultry Sci 2016; 18: 395-00.
29. Bera TK, Chatterjee K and Ghosh D: *In-vitro* antioxidant properties of the hydro-methanol extract of the seeds of *Swietenia mahagoni* (L.) Jacq. BGM 2015; 7: 18-24.
30. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG and Lightfoot DA: Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts. Plants 2017; 6: 1-23.
31. Marie PK and Jain SK: Hyperketonemia and ketosis increase the risk of complications in type 1 diabetes. Free Rad Biol Med 2016; 95: 268-77.
32. Ali MA, Wahed MI, Khatune NA, Rahman BM, Barman RK and Islam MR: Antidiabetic and antioxidant activities of ethanolic extract of *Semecarpus anacardium* (Linn.) bark. BMC Complement Altern Med 2015; 15: 1-10.

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