International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 640-669 www.ijprajournal.com ISSN: 2456-4494

Alterations in Hippocampal Oxidative Stress and Associated Spatial Memory Loss by Fruits of Momordica Dioica Extract In Streptozotocin-Induced Diabetes Type I in Rats Model

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Submitted: 15-11-2022 Accepted: 25-11-2022

Title: This present study aims to evaluate the effectiveness of Momordicadioicaextract on alterations in hippocampaloxidative stress and association spatial memory loss in STZ induced diabetes Type I in rat model.

Background and Objective: Diabetes mellitus occurs when the glucose level in the blood is high which in turn leads to several complications such as damage to the heart, blood vessels, eyes, kidneys and nerves. However, Diabetes mellitus patients when left untreated for long periods of time has been observed to show memory loss. Momordicadioicais a perennial, dioecious; cucurbitaceous climbing creeper (commonly known as kakrol, spiny gourd or teasle gourd) plant is used both in the prevention and cure of various diseases and in the food of humans. The fruits of Momordicadioicaused to management of diabetes and other health problems like; antimicrobial activity, antibacterial activity and fruits could be a good supplement for some nutrients, minerals, and fatties such as fiber, protein, carbohydrate, calcium, magnesium, oleic acid, and linoleic acid. The fruits could be promoted as a mineral and vitamin.

Methodology: evaluation of Momordicadioicaextract activity on alterations in hippocampal oxidative stress and association spatial memory loss in STZ induced diabetes Type I in rat model was performed firstly by inducing diabetes using a single dose of STZ intraperitoneal. The diabetes rats are further treated with Momordicadioicaat a dose of 250mg/kg b.w and 500mg/kg b.w orally for 21day after. Assessment of spatial memory and alteration in hippocampal oxidative stress was carried out by Morris's water maze test and estimation of MDA, SOD and CAT from isolated hippocampus.

Results: The oral administration of Momordicadioicaat low and high doses of 250 and 500 mg/kg respectively decreased blood glucose level, water consumption and urine output, body weight, increase serum insulin levels, pancreatic insulin; decrease HOMA-IR levels, shorter escape latency in Morris water maze test and elevation of MDA, SOD, and CAT levels in hippocampal tissue.

Interpretation and conclusion: The findings from this investigation suggest that Momordicadioicapossess spatial memory-enhancing activity and antioxidant activity.

Keywords: Momordicadioica, Diabetes Mellitus, Oxidative Stress, Spatial Memory Los

I. INTRODUCTION

Diabetes mellitus, often called diabetes, is a group of metabolic disorders associated with an increase in the glucose level of the blood. Insulin is the major hormone which is affected by this disease. Insulin is involved in the regulation of glucose in the blood; it controls the uptake of glucose in the blood of various cells of the body such as muscle cells, adipocytes liver, etc.[1] Beta cell of islet of Langerhansin pancreas secretes insulin depending on the glucose level of the blood especially after meal. Diabetes mellitus occurs when the cells of the body are unresponsive to insulin (Type I diabetes) or when the pancreas is unable to produce sufficient insulin Type 2 diabetes). Type 1 diabetes can be managed by treatment with insulin hence also called as insulin dependent diabetes- IDDM whereas Type 2 is in non-insulin dependent diabetes-NIDDM as is usually as a result of obesity and lack of physical exercise. Increased risk for diabetes is primarily associated with age, ethnicity, family history of diabetes, smoking, obesity, and physical inactivity.[2] Today, the challenges in treating diabetic subjects have increased due to the inefficiency of the anti-diabetic drugs in maintaining glycaemia levels over a long period of time thereby resulting in long term diabetic complications like nephropathy,

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Volume 7, Issue 6 Nov-Dec 2022, pp: 640-669 www.ijprajournal.com ISSN: 2456-4494

retinopathy, neuropathy, diabetic ketoacidosis, hypertension and cardiovascular complications.[4,5]
Diabetes when left untreated for a long period has been observed to have impact in one's cognitive abilities due to the destruction of nerve cells and blood vessels via increase in the level of blood glucose than normal hence one might experience memory loss. Hyperglycemia is a widely known cause of enhanced plasma free radical concentrations. Free radical production caused by hyperglycemia may occur via at least four different routes: i) increased glycolysis (ii) intercellular activation of sorbitol (polyol) pathway (iii) auto-oxidation of glucose and (iv) non-enzymatic protein glycation.[6,7.] One of the mechanismsof diabetes is oxidative stress which can result from imbalance between generation of free radicals and scavenging system of free radicals eventually this may lead to neurodegeneration, hippocampus is the major portion of the brain responsible for learning, memory and cognition.[8,9] Deficits in spatial learning and memory are found after lesions of the hippocampus and its extrinsic fiber connections.[10]
Numerous studies on untreated DM human patients have shown deposition of intraneuronal amyloid plaques in the brain which thereby leads to development of neuropathological symptoms of Alzheimer' disease (AD).[11] DM has been reported to be associated with its negative impacts on the function of central nervous system.
Evidence also suggests that successful management of DM does not result in complete reversal of the cognitive impairments.[12] The precise mechanism underlying the DM-induced cognitive dysfunction is not fully understood.

Momordicadioica

Momordicadioicais popularly known as spiny gourd or spine gourd or Kantola belonging to the family Cucurbitaceae. The Momordicagenus contains about 80 species. According to the latest revision of Indian Momordica, there are six well identified species of which four are dioecious and two are monoecious. Although this genus originated fromIndo-Malayan region, it is now found to grow in India, Bangladesh, SriLankan, Myanmar, China, Japan, SoutheastAsia, Polynesia, Tropical Africa, and South America. Its cultivation up to an altitude of 1500 meters in Assam and Garo hills of Meghalaya is reported. The roots fruits as well as tuberous roots of this plant are well known for their anti-diabetic activity. The fruits have hepatoprotective, diuretic, alexiteric stomachic laxative properties, and the leaves have aphrodisiac properties[31]. The aqueous extract of the roots has spermicidal activity and anthelmintic activity[32]. The root juice acts as a stimulant, astringent, and antiseptic.[33,34] The fruits could be promoted as a mineral and vitamin.[35]The whole plant is known for its use in the treatment of eye disease and poisoning and fever.[36]

Momordicadioicaalso shows Reno protective activity as well as anti-hyperglycemic action.[37,38] Moreover, its fruit is recommended as a nutritionally rich source of protein and good source of lipid, crude fiber, carbohydrate, iron, calcium, phosphorus.[39] Additionally, it is the highest amount of carotene (162 mg/100 g of edible portion) container amongst the cucurbitaceous vegetables.[40]

II. MATERIALS AND METHOD

Collection of plant material:

The fruit of MomordicaDioicawas procured from an authorized dealer in Bangalore in the month of August. The fresh fruits of MomordicaDioicawere identified by a botanist Dr. Geetanjli, (HOD of Botany department, Sree Sidda Ganga College Tumkur University, India) and reference No. 141/17-18 was provided for future reference.

Extraction of the plant material and sample preparation: The fruits of Momordicadioicawere thoroughly washed with water to free it from any debris, chopped into small pieces and dried under shade at room temperature. The dried fruits were powdered and passed through the sieve (coarse 10/40). The powder was used for the preparation of the methanolic extract. Each 50g powder was subjected to extraction with 500ml methanol in a reflux condenser for 3 cycles of 7hrs. each till the volume reduced to half. The extract was filtered throughWhatman filter paper no.1 and evaporated to dryness to get
constant weight. The extract will be concentrated on a rotary vacuum evaporator to get green semi solid residue.

Experimental animals:

Experimental animals: Wister male rats of age 8-10 weeks weighing 150-200g are used in the study. The animals are housed in polypropylene cages in groups of six to eight mice per cage and kept under controlled environmental conditions. The care of animals will be according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals. The study is approved by the Institutional Animal Ethics Committee (Sl. No. KCP/IAEC/09/21-22/06/18-12-21).

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At least 5 days prior to the start of the experiment, the Male rats of age 8-10 weeks weighing 150-200g were maintained at 25 ± 2°C RT with a 12:12 hr. light and dark cycle. The DM1 rat model was developed by intraperitoneal injection of STZ. On the day of DM1 induction, all rats were fasted for 6 to 8 hr. prior to STZ treatment.[47]

Water was provided as normal.

A batch of rats (disease group) was intraperitoneal injected with a single dose of freshly prepared Streptozotocin (STZ) (65 mg/kg BW in 0.1 M sodium citrate buffer, pH 4.5.The standard group, low dose group and high dose groups were also intraperitoneal injected with a single dose of freshly prepared Streptozotocin (STZ) (65 mg/kg BW in 0.1 M sodium citrate buffer, pH 4.5.The control rat group received equal volume of the vehicle (Citrate buffer) only. The rats were returned to their cages and normal food and 10% sucrose water was provided.[42,46,48]

The following day, the 10% sucrose water was switched to regular water. 10 days after administration of STZ, rats were made to fast for 6 to 8 hr. The blood glucose level was tested from a tail vein blood sample using a glucometer to check hyperglycemia.

Body weight, blood glucose content, water consumption (for evaluating the level of polydipsia) and volume of urine (to assess the condition of polyurea) were regularly determined before and after the

experiment. Rats with blood glucose level >250mg/dL) were considered as diabetic. The control rats treated with citrate buffer were further given oral treatment of normal vehicles for the rest of the time and used as control for the effects of MD. The low dose group received M. dioicaExtract of 500mg/kg B.W while the high dose group received M. dioicaExtract of 500mg/kg B.W, orally for 21 days while the standard group received insulin subcutaneously for 21 days.

All groups were maintained in individual metabolic cages and were regularly examined for their body weight, serum insulin content, HOMA-IR (insulin resistance), blood glucose level, water consumption and urine output.

At the end of the treatment, blood samples were collected, and animals were sacrificed using high doses of Phenobarbital (100 mg/kg).

Morris water maze test

The Morris water maze is widely used to study spatial memory and learning. The rats were placed in a pool of water that was colored opaque with powdered nonfat milk and were they must swim to a hidden escape platform.[49]

The maze was divided into four quarters – North, South, East and West. A rescue platform was in one of these quarters, positioned on the bottom of the tank with a metal stand, below the surface of water (out of the rats' sight).[50,51] The animals received five days training with the hidden platform, each day included four training sessions with a 60 s intersession interval. Each trial began by placing a rat at one of the three starting points, with its face toward the wall of the pool. The start location varied on each training trial and changed each day.[52] The trial was terminated when the animal entered the platform. If the rat did not find the platform within 60 seconds, it was carefully placed on the platform for 15 seconds. During acquisition of the spatial navigation task, all groups were given one session of four trials each day (day 1–5; trial 1–20).[53] Spatial memory was evaluated in the probe trial, on the sixth day (trial 21). The platform was removed, and animals were allowed to swim for 60 seconds. The time taken to reach the platform (latency) was measured.[41]

Isolation of hippocampus

The rats were treated with anesthesia chloroform and sacrificed by cervical dislocation .The rat was laid with it abdomen side facing down .The skull bone was carefully removed by bilateral incision and the intact brain was carefully dissected out from the skull and placed into ice-cold phosphate-buffered saline (PBS), the adherent blood was removed and transferred onto a wet filter paper placed on ice and the hindbrain and cerebellum were removed using surgical blade. The cortical layer of each hemisphere was laterally peeled out under a microscope. Thereafter, the hippocampus was exposed and removed from the brain by applying pressure to the medial white matter tracts with a needle and moving another needle slightly anteriorly and laterally. The hippocampal tissues were pooled, dipped into saline solution.[13]

4.5.3. Estimation of Malondialdehyde (MDA)

MDA content in the hippocampal tissue homogenate was determined by modified TBARS (Thiobarbituric acid-reacting substance) assay method.[54] The hippocampal tissue was homogenized in trichloroacetic acid (TCA) and the homogenate was used to estimate malondialdehyde (MDA). Briefly, lipid peroxidation was induced by adding ferric chloride (10 µl, 400 mM) and l-

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ascorbic acid (10 µl, 400 mM) to a mixture containing hippocampal homogenate (0.3 ml) in phosphate buffer solution (5 ml, pH 7.4, 0.2 M). After incubation for 1 hr. at 37°C, the reaction was stopped by adding HCl (2 ml, 0.25 N) containing TCA (1 ml, 15%

w/v) and TBA (0.5 ml, 0.375% w/v) boiled for 15 min, cooled, and centrifuged, and the absorbance of the supernatant was measured at 532 nm. The results were expressed as nmol MDA/g protein.

Estimate of Catalase (CAT)

Catalase activity (CAT) was determined by monitoring the H_2O_2 decomposition which was measured spectrophotometrically by the decrease in absorbance. Enzyme activity was calculated using a molar extinction coefficient of. /mM−1/cm−1 and expressed as µM/H_2O_2/minute/mg protein.[55]

Superoxide Dismutase Activity (SOD)

Superoxide dismutase activity (SOD) by to 2.78 ml sodium carbonate buffer (0.05 mM, pH 10.2), 100 µL of 1 mM EDTA and 20 µL tissue supernatant were added and incubated at 30°C for 45 min. The reaction was initiated by adding 100 µL of adrenaline. The change in the absorbance was recorded at 480 nm for 3 min. Sucrose was used as a blank. The activity of SOD was expressed as U/mg of protein.44

Statistical Analysis

The results are expressed as Mean ± S.E.M from N=6 rats in each group. The significance of difference among the groups was assessed using one-way analysis of variance (ANOVA) followed by Tukey's test compared between Normal control (Untreated) Vs all groups $p<0.05$ were considered significant.

III. RESULTS

Evaluation of Antidiabetic activity

The rats were divided into 5 groups for assessment of anti-diabetic activity of which the control group received an equivalent volume of vehicle only, disease control group received Streptozotocin (65mg/kg b.w. single i.p), Standard group received Streptozotocin (65mg/kg b.w. single i.p) and Insulin (4IU, S.C), low dose treatment group received Streptozotocin (65mg/kg b.w. single i.p) and M. dioicaExtract (250mg/kg, oral for 21 days) and high dose treatment group received Streptozotocin (65mg/kg b.w. single i.p) and M. dioicaExtract (500mg/kg, oral for 21 days).

The following parameters were observed:

*Urine output and food consumption was normal in normal control and other treatment group compared to the disease control which received only STZ injection.

Body weight before (0 days) and after (21 days) treatment

The data on effect of STZ reveals that STZ treatment leads to a significant increase in the body weight of STZ-treated rat compared to normal control mice. Treatment diabetic rats with STD drug (insulin, 4IU) results to decrease in body weight. Oral treatment of M.D at dose 250mg/kg BW leads to decrease in body weight and treatment of DM1 rats with its higher dose of 500 mg/kg BW leads to a significant decrease in the BW of DM1rats.

Table no 1: Body Weight

Group	Body Weight (In gm) Before (Day 0) Mean S.E.M	Body Weight (In gm) After (Day 21) Mean S.E.M
Group 1: Normal Control - Vehicle only	146.23±4.0619	147.15±4.1124

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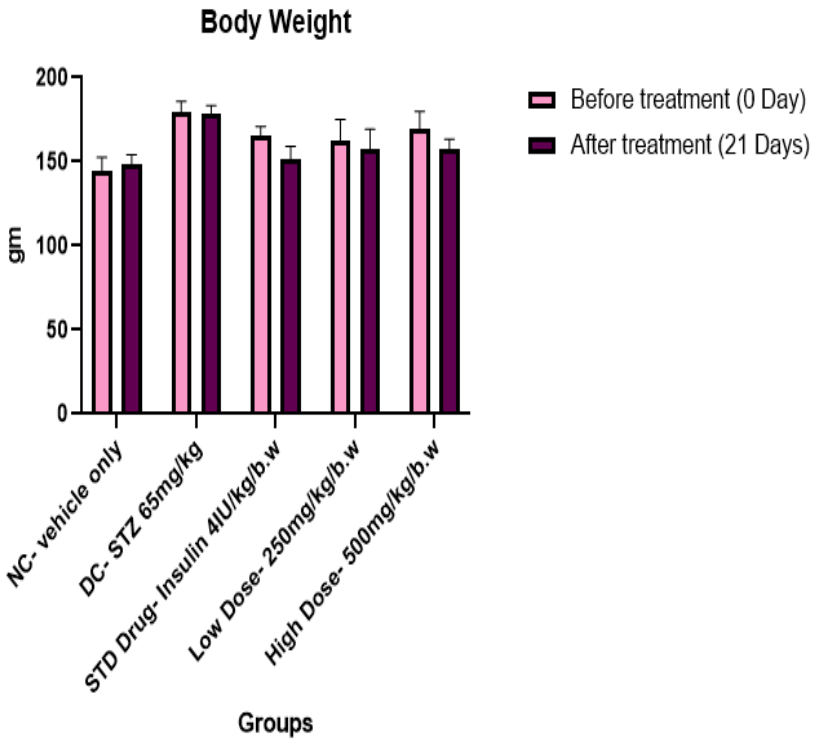
Group 2: DC – STZ 65mg/kg/b.w I.P (Single dose)	171.74 ±4.4848	171.39±4.8287
Group 3: STD Drug - Insulin (4IU, S.C) + Streptozotocin (65mg/kg b.w. Single i.p)	162.97±3.4210	152.41±2.1858
Group 4: Low Dose - M. dioica Extract (250mg/kg, oral for 21 days) + Streptozotocin (65mg/kg b.w. single i.p)	162.14±4.5578	155.36±2.1980
Group 5: High Dose - M. dioica Extract (500mg/kg, oral for 21 days) + Streptozotocin (65mg/kg b.w. single i.p)	166.503±4.9210	156.10±2.549

Values are expressed as Mean ± S.E.M (n=6).

Fig no 1: Body Weight



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Blood Glucose Level

Data obtained from our experiments on the effects of M.D on diabetes mellitus clearly indicates that streptozotocin treatment significantly increases the blood glucose levels compared to that in the control rats i.e., it successfully produces a diabetic rat model, which can be used for further experiments. Date shows treatment of diabetic rats with STD drug (insulin, 4IU) results to decrease in blood glucose level. Our data further suggests that lower dose of M.D 250mg/kg BW reduced the blood glucose content whereas its higher doses 500 mg/kg BWled to more significant decline in the blood glucose content.

Table no 2: Blood Glucose Level

GROUPS	**Blood Glucose level(mg/dl) Mean S.E.M**
Group 1: Normal Control - Vehicle only	84.44±2.6509
Group 2: DC – STZ 65mg/kg/b.w I.P (Single dose)	318.08±3.8372
Group 3: STD Drug - Insulin (4IU, S.C) + Streptozotocin (65mg/kg b.w. Single i.p)	117.32±1.6055
Group 4: Low Dose - M. dioica Extract (250mg/kg, oral for 21 days) + Streptozotocin (65mg/kg b.w. single i.p)	178.98±3.3436
Group 5: High Dose - M. dioica Extract (500mg/kg, oral for 21 days) + Streptozotocin (65mg/kg b.w. single i.p)	145.18±1.1372

Values are expressed as Mean ± S.E.M (n=6).

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Fig no 2: Blood Glucose level

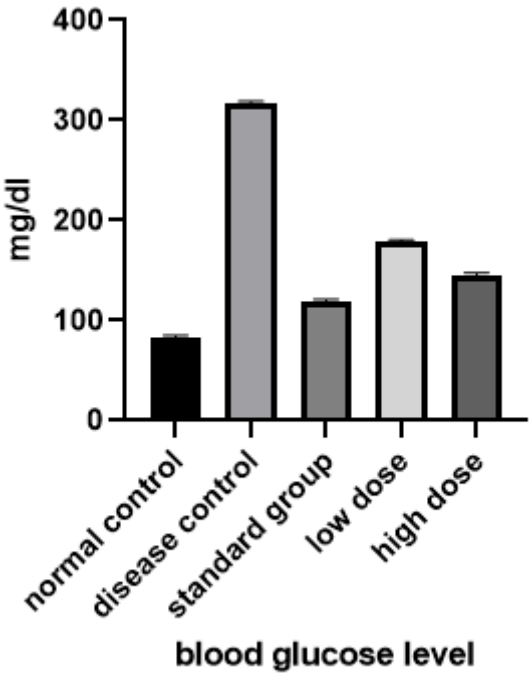

Table no 3: Statistics of blood glucose level -> comparison between the groups: Tukey's multiple comparisons test

Tukey's multiple comparisons test	Mean Diff	Summary	Adjusted P Value
Normal control vs. disease control	10.16	****	<0.0001
Normal control vs. Standard group	0.7850	ns	0.9159
Normal control vs. Low dose	7.212	****	<0.0001

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Normal control vs. High dose	4.535	***	0.0005
Disease control vs. Standard group	-9.375	****	<0.0001
Disease control vs. Low dose	-2.948	*	0.0312
Disease control vs. High dose	-5.625	****	<0.0001
Standard group vs. Low dose	6.427	****	<0.0001
Standard group vs. High dose	3.750	**	0.0041
Low dose vs. High dose	-2.677	ns	0.0590

Water Consumption

Water Consumption is one of the Cateria's used to evaluate diabetes-induced condition called polydipsia i.e., increase in water consumption (increased thirst) was measured as daily water intake by rats of all experimental groups. The results indicate that STZ treatment induces significant increase in the level of water consumption in diabetic rats. STD drug (insulin, 4IU) reduced water consumption. Low dose M.D (250mg/kg B.W) reduces the level of polydipsia. The higher doses of M.D (500 mg/kg BW) showed decline in daily water consumption among diabetic rats.

Water Consumption
Before (Day 0) and After (Day 21) of treatment Table no 4: Water Consumption

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Group	Water Consumption (ml/day) Before Treatment (Day 0) Mean S.E.M	Water Consumption (ml/day) After Treatment (Day 21) Mean S.E.M
Group 1: Normal Control - Vehicle only	10.832±1.1379	10.832±2.673
Group 2: DC – STZ 65mg/kg/b.w I.P (Single dose)	27.550±1.7779	33.820±2.1972
Group 3: STD Drug - Insulin (4IU, S.C) + Streptozotocin (65mg/kg b.w. Single i.p)	22.207±1.6414	17.980±1.6815
Group 4: Low Dose - M. dioica Extract (250mg/kg, oral for 21 days) + Streptozotocin (65mg/kg b.w. single i.p)	27.964±2.0602	25.426±1.7320
Group 5: High Dose - M. dioica Extract (500mg/kg, oral for 21 days) + Streptozotocin (65mg/kg b.w. single i.p)	26.223±2.8097	23.331±1.4832

Values are expressed as Mean ± S.E.M (n=6).

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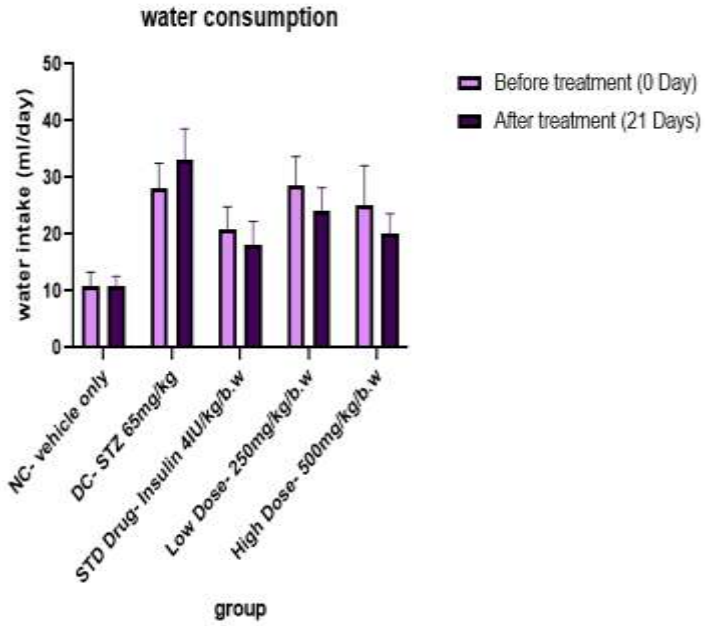
Fig. no 3: Water Consumption



Serum Insulin After the treatment

Table no 5: Serum Insulin level

GROUPS	**Serum Insulin (µU/mL) Mean S.E.M**
Group 1: Normal Control - Vehicle only	21.95±1.1388
Group 2: DC – STZ 65mg/kg/b.w I.P (Single dose)	8.594±0.9524
Group 3: STD Drug - Insulin (4IU, S.C) + Streptozotocin (65mg/kg b.w. Single i.p)	18.82±0.6565

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Group 4: Low Dose - M. dioica Extract (250mg/kg, oral for 21 days) + Streptozotocin (65mg/kg b.w. single i.p)	13.17±0.7638
Group 5: High Dose - M. dioica Extract (500mg/kg, oral for 21 days) + Streptozotocin (65mg/kg b.w. single i.p)	16.98±0.7292

Values are expressed as Mean ± S.E.M (n=6). \

Table no 6: Statistics of Serum Insulin -> comparison between the groups: Tukey's multiple comparisons test

Tukey'smultiple comparisons test	Mean Diff	Summary	Adjusted P Value
Normal control vs. disease control	11.23	****	<0.0001
Normal control vs. Standard group	1.632	ns	0.6763
Normal control vs. Low dose	6.322	***	0.0002
Normal control vs. High dose	3.668	*	0.0447
Disease control vs. Standard group	-9.600	****	<0.0001
Disease control vs. Low dose	-4.910	**	0.0041

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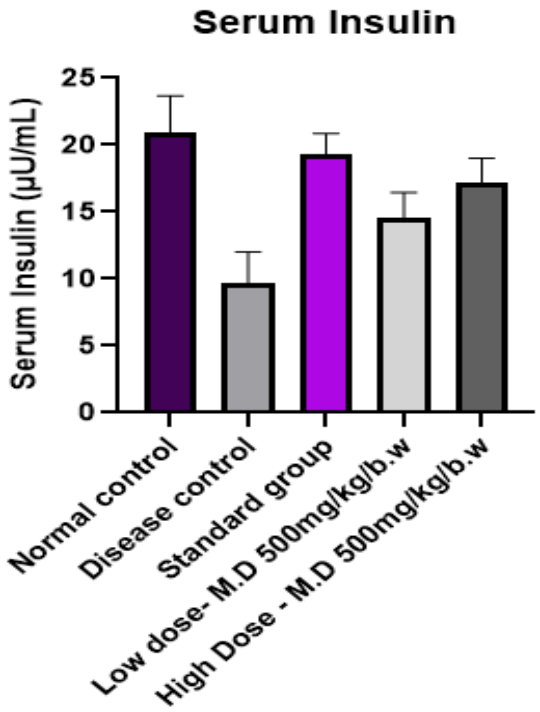
Disease control vs. High dose	-7.563	****	<0.0001
Standard group vs. Low dose	4.690	**	0.0064
Standard group vs. High dose	2.037	ns	0.4759
Low dose vs. High dose	-2.653	ns	0.226

Fig no 5.4: Serum Insulin Level



Serum insulin and measurement of insulin resistance (HOMA-IR).

The Data obtained reveals that STZ treatment lowered the serum insulin level and Pancreatic Insulin Level compared to that in the normal control rats. Treatment diabetic rats with STD drug (insulin, 4IU) results increase in serum insulin level as well as Pancreatic Insulin Level. Furthermore, Data on the effects of M.D at dose 250mg/kg BW and 500mg/kg BW indicate increase in both serum insulin level and Pancreatic Insulin Level. Our HOMA-IR data reveal that STZ treatment significantly increases the insulin resistance compared to the control rats. The data further indicate that the STD drug (insulin, 4IU), M.D at dose 250mg/kg BW and 500mg/kg BW when given to STZ-DM1 rats, reduced insulin resistance values compared to STZ treated rats.

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Pancreatic Insulin After the treatment

Table no 7: Pancreatic Insulin level

Group	**Pancreatic insulin (µU/mL) Mean S.E.M**
Group 1: Normal Control - Vehicle only	287.21±1.7520
Group 2: DC – STZ 65mg/kg/b.w I.P (Single dose)	29.86±0.6240
Group 3: STD Drug - Insulin (4IU, S.C) + Streptozotocin (65mg/kg b.w. Single i.p)	201.12±0.8206
Group 4: Low Dose - M. dioica Extract (250mg/kg, oral for 21 days) + Streptozotocin (65mg/kg b.w. single i.p)	186.13±0.8999
Group 5: High Dose - M. dioica Extract (500mg/kg, oral for 21 days) + Streptozotocin (65mg/kg b.w. single i.p)	245.43±0.6468

Values are expressed as Mean ± S.E.M (n=6).

Table no 8: statistics of Pancreatic Insulin -> comparison between the groups:
Tukey's multiple comparisons test

Tukey's multiple comparisons test	Mean Diff	summary	Adjusted P Value
Normal control vs. disease control	260.1	****	<0.0001

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Normal control vs. Standard group	87.52	****	<0.0001
Normal control vs. Low dose	99.35	****	<0.0001
Normal control vs. High dose	56.45	****	<0.0001
Disease control vs. Standard group	-172.6	****	<0.0001
Disease control vs. Low dose	-160.8	****	<0.0001
Disease control vs. High dose	-203.7	****	<0.0001
Standard group vs. Low dose	11.83	****	<0.0001
Standard group vs. High dose	-31.08	****	<0.0001
Low dose vs. High dose	-42.91	****	<0.0001

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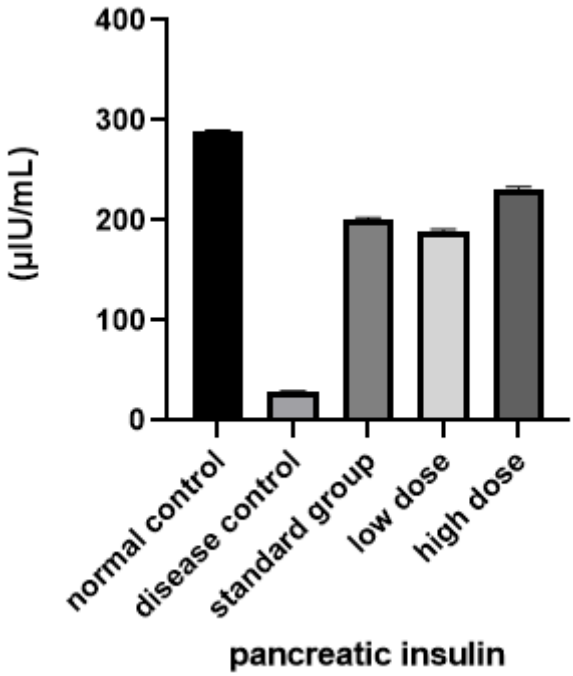
Fig no 5: Pancreatic Insulin Levels



HOMA-IR levels

Table no 9: HOMA-IR Levels

Group	**HOMA-IR Mean S.E.M**
Group 1: Normal Control - Vehicle only	78.614±0.7248
Group 2: DC – STZ 65mg/kg/b.w I.P (Single dose)	119.71± 0.6940
Group 3: STD Drug - Insulin (4IU, S.C) + Streptozotocin (65mg/kg b.w. Single i.p)	97.31±0.6018
Group 4: Low Dose - M. dioica Extract (250mg/kg, oral for 21 days) + Streptozotocin (65mg/kg b.w. single i.p)	105.46±0.4780

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Group 5: High Dose - M. dioica Extract (500mg/kg, oral for 21 days) + Streptozotocin (65mg/kg b.w. single i.p)	99.58±0.7694

Values are expressed as Mean ± S.E.M (n=6).

Table no 9: statistics of HOMA-IR-> comparison between the groups: Tukey's multiple comparisons test

Tukey'smultiple comparisons test	Mean Diff	Summary	Adjusted P Value
Normal control vs. disease control	-41.72	****	<0.0001
Normal control vs. Standard group	-19.90	****	<0.0001
Normal control vs. Low dose	-26.19	****	<0.0001
Normal control vs. High dose	-21.08	****	<0.0001
Disease control vs. Standard group	21.82	****	<0.0001
Disease control vs. Low dose	15.53	****	<0.0001
Disease control vs. High dose	20.64	****	<0.0001

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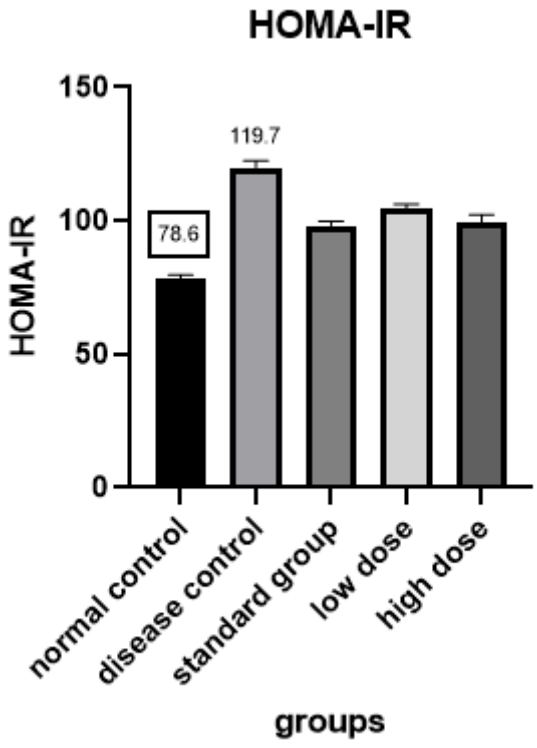
Standard group vs. Low dose	-6.285	***	0.0003
Standard group vs. High dose	-1.182	ns	0.8702
Low dose vs. High dose	5.103	**	0.0029

Fig no 6: HOMA-IR levels



Effect of Administration of MomordicaDioicaExtract on spatial Memory loss in STZ- Induced Type 1 Diabetic Rats

Our data from the Morris-water-maze test for assessing the level of spatial memory in rats reveals that the STZ-treated diabetic rats uses long escape latency in finding the hidden platform in the water tank compared to normal control rats. Hence, our data confirms that the conditions of diabetes mellitus led to spatial memory loss in diabetic rats. Our data suggest that treatment with STD drug (insulin, 4IU) was able to significantly decrease the escape latency time. Oral administration of M.D at dose 250mg/kg BW and 500mg/kg BW were also able to reduce the escape latency time.

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Morris water maze test

Table no 10: Escape Latency on Day 1 to Day 6

Group	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6 (Probe trail)	P
Group 1: Normal Control - Vehicle only	31.48±4.4	28.23±2.3	25.32±4.7	22.1±73.4	12.94±2.3	11.12±4.1	0.001
Group 2: DC – STZ 65mg/kg/b.w I.P (Single dose)	39.32±3.4	37.43±2.1	37.11±3.2	36.5±72.4	37.73±1.9	36.97±6.1	0.001
Group 3: STD Drug - Insulin (4IU, S.C) + Streptozotocin (65mg/kg b.w. Single i.p)	30.46±3.1	28.76±1.9	25.15±1.6	23.12±1.7	13.85±4.2	11.93±3.4	0.010
Group 4: Low Dose - M. dioica Extract (250mg/kg, oral for 21 days) + Streptozotocin (65mg/kg b.w. single i.p)	36.27±2.7	28.97±3.4	34.21±2.4	27.1.3±1.8	23.84±2.1	16.13±4.2	0.010

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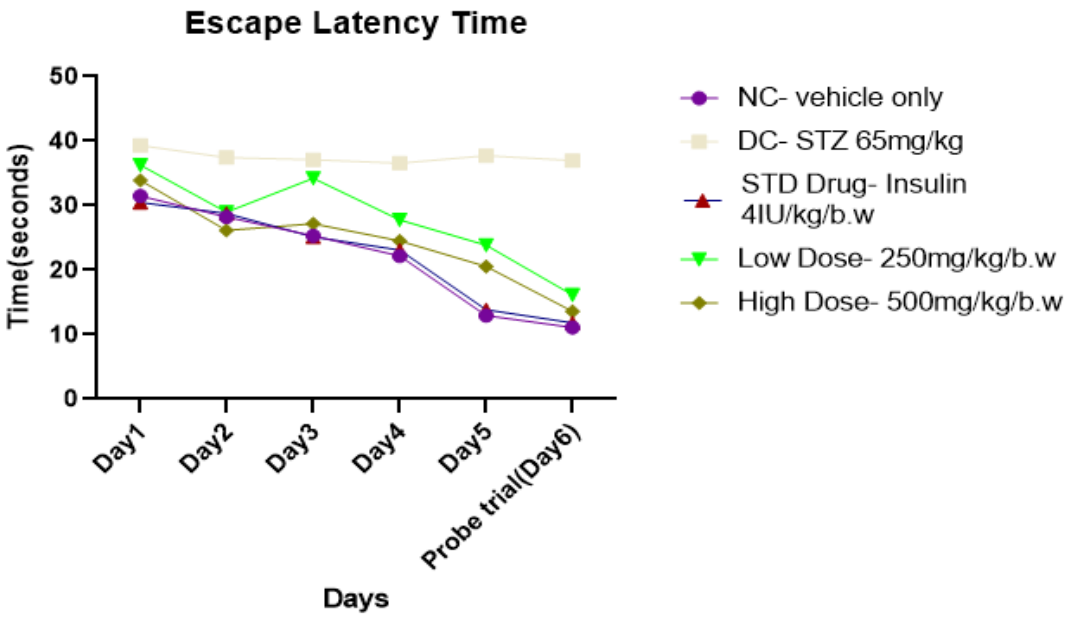
Group 5: High Dose - M. dioica Extract (500mg/kg, oral for 21 days) + Streptozotocin (65mg/kg b.w. single i.p)	33.91±4.2	26.13±2.5	27.19±3.5	24.52±3.5	20.57±2.4	13.59±1.3	0.001

Values are expressed as Mean ± S.E.M (n=6).

Fig no 7: Morris water maze test - Escape Latency on Day 1 to Day6



Effect of Administration of MomordicaDioicaExtract in Hippocampal oxidative stress in STZ- Induced Type 1 Diabetic Rats groups.

Our data indicates that STZ treatment leads to decrease in the level of SOD and CAT when compared with the same in normal control rats. Administration of STD drug (insulin, 4IU) leads significantly increases the level of SOD and CAT levels. M.D at dose 250mg/kg BW and 500mg/kg BW significantly increases the level of SOD and CAT, however, STZ treatment leads to increase in the level of MDA when compared with the same in normal control rats. its treatment with M.D at dose 250mg/kg BW and 500mg/kg BW as well as treatment with insulin had no further significant effect in its level.

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Table no 11: Antioxidant Enzyme

Group	SOD (Units SOD/mg protein)	CAT(µU/mL)	MDA (nmol/g)
Group 1: Normal Control - Vehicle only	20.908±0.6992	12.228±0.5849	1.9161±0.2086
Group 2: DC – STZ 65mg/kg/b.w I.P (Single dose)	8.4841±0.6633	6.7884±0.3908	2.6375±0.3011
Group 3: STD Drug - Insulin (4IU, S.C) + Streptozotocin (65mg/kg b.w. Single i.p)	39.026±0.7322	18.973±0.6610	2.6250±0.2498
Group 4: Low Dose - M. dioica Extract (250mg/kg, oral for 21 days) + Streptozotocin (65mg/kg b.w. single i.p)	28.612±0.6863	17.554±0.5505	2.9828±0.1562
Group 5: High Dose - M. dioica Extract (500mg/kg, oral for 21 days) + Streptozotocin (65mg/kg b.w. single i.p)	38.932±1.1425	19.576±0.6677	2.351±0.3062

Values are expressed as Mean ± S.E.M (n=6).

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Fig no 8: Superoxide dismutase (SOD) Levels

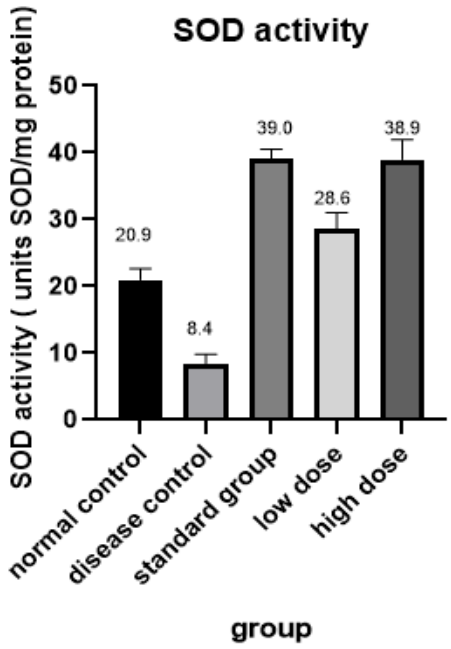

Table no 12: Statistics of SOD -> comparison between the groups: Tukey's multiple comparisons test

Tukey's multiple comparisons test	Mean Diff	summary	Adjusted P Value
Normal control vs. disease control	12.54	****	<0.0001
Normal control vs. Standard group	-18.07	****	<0.0001
Normal control vs. Low dose	-7.652	****	<0.0001
Normal control vs. High dose	-17.98	****	<0.0001

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Disease control vs. Standard group	-30.61	****	<0.0001
Disease control vs. Low dose	-20.19	****	<0.0001
Disease control vs. High dose	-30.52	****	<0.0001
Standard group vs. Low dose	10.42	****	<0.0001
Standard group vs. High dose	0.09167	ns	>0.9999
Low dose vs. High dose	-10.33	****	<0.0001

Catalase level

Table no 13: statistics of Catalase -> comparison between the groups: Tukey's multiple comparisons test

Tukey's multiple comparisons test	Mean Diff	summary	Adjusted P Value
Normal control vs. disease control	-0.6667	ns	0.8258
Normal control vs. Standard group	-0.6433	ns	0.8434

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Normal control vs. Low dose	-0.9217	ns	0.5949
Normal control vs. High dose	-0.3400	ns	0.9822
Disease control vs. Standard group	0.02333	ns	>0.9999
Disease control vs. Low dose	-0.2550	ns	0.9940
Disease control vs. High dose	0.3267	ns	0.9847
Standard group vs. Low dose	-0.2783	ns	0.9916
Standard group vs. High dose	0.3033	ns	0.9884
Low dose vs. High dose	0.5817	ns	0.8853

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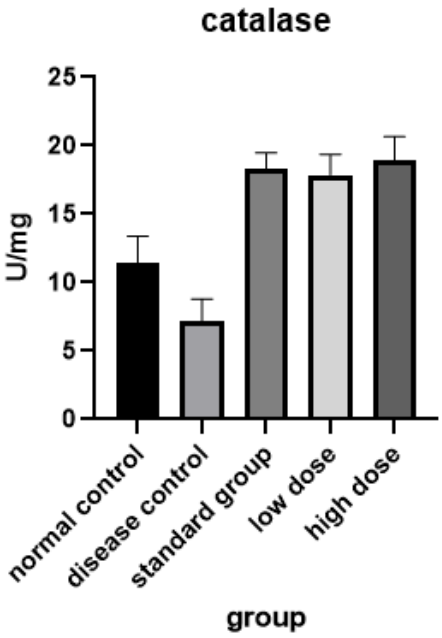
Fig no 9: Catalase levels



Malondialdehyde (MDA) Level

Table no 14: statistics of MDA -> comparison between the groups: Tukey's multiple comparisons test

Tukey'smultiple comparisons test	Mean Diff	summary	Adjusted P Value
Normal control vs. disease control	-0.6667	ns	0.8258
Normal control vs. Standard group	-0.6433	ns	0.8434
Normal control vs. Low dose	-0.9217	ns	0.5949
Normal control vs. High dose	-0.3400	ns	0.9822

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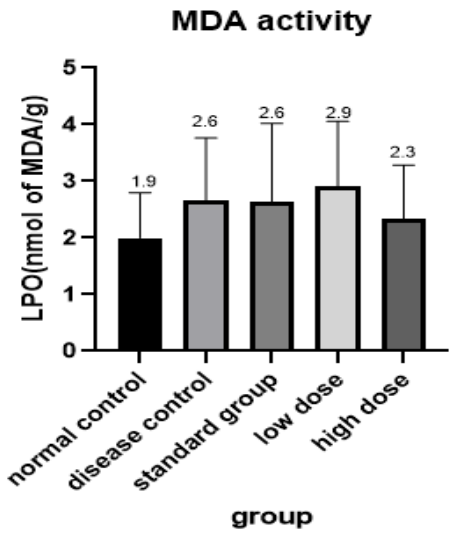
Disease control vs. Standard group	0.02333	ns	>0.9999
Disease control vs. Low dose	-0.2550	ns	0.9940
Disease control vs. High dose	0.3267	ns	0.9847
Standard group vs. Low dose	-0.2783	ns	0.9916
Standard group vs. High dose	0.3033	ns	0.9884
Low dose vs. High dose	0.5817	ns	0.8853

Fig no 10: MDA Levels



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IV. DISCUSSION

Diabetes mellitus is one of the most common non-communicable diseases that targets multi organ systems. In recent years, scientists over the globe have directed their efforts for the exploration of some novel food sources as hypoglycemic agents. In the early stages, drugs and diet can mediate the adverse consequences. If complications like cardiovascular disorders or renal malfunction, the strategies need to focus through some alternative arrangements. However, prevention of root causes and managing diabetes at early stages is the better remedy as diabetes when left untreated over a prolonged time may lead to reduced cognitive function.56

We have studied the effects of diabetes mellitus Type 1(DM1) on the spatial memory in rats and its association with the oxidative stress in the hippocampus as this is the major site in the brain for consolidation and storage of the long-term memory. To study above, we developed a Type 1 DM (DM1) mouse model by intraperitoneal injection of a selected dose of Streptozotocin and validated the model by examining the diabetic parameters like blood glucose content (hyperglycinemia), level of polydipsia (excess need of water intake) and polyuria (excessive urine discharge). Since DM1 is also associated with change in body weight and alterations in serum insulin level or its sensitivity; we also measured the body weight, serum insulin level and insulin resistance in the different groups. The DM1 rats were further used for analyzing alterations in the spatial memory and its association with oxidative stress at the level in the hippocampus.

The data gathered reveal that Streptozotocin-treated rats developed the characteristic features of diabetes mellitus such as increased blood glucose level (hyperglycemia), increased water consumption (polydipsia) and excessive urine discharge (polyuria). It was alsoobserved that STZ rats exhibited significant decline in body weight compared to the normal control rats, and the treatment of STZ rats with M. Dioica at low doses of 250 mg/kg BW had no remark- able effects on the body weight, however, the high dose of 500mg/kg BW caused slight increase in the body weight. To know whether these changes in DM1 rats are associated with serum insulin content or insulin resistance, we measured the serum insulin level, pancreatic insulin levels and insulin resistance value (HOMA-IR).

The results of this study showed increased serum insulin level in the high dose (500mg/kg./b.w) and low dose (250mg/kg./b.w) of M. Dioica compared to Disease control groups. There is also an increase in standard groups as compared to disease control groups. The increase in insulin levels suggested that M. Dioica would enhance the secretion of insulin from B-cells of islets of Langerhans.57

The data in this study shows that STZ-induced diabetes mellitus type 1 rats lead to increased HOMA-IR levels compared to normal control rats and the treatment of STZ rats with M. Dioica at low doses of 250 mg/kg BW and high dose of 500mg/kg BW caused decrease in the HOMA-IR levels.

One of the most important purposes of the current study was to assess whether DM2 has effects on learning and memory. Hence, we investigated whether the STZ-treated rats suffer from spatial memory loss/impairment by Morris-water-maze test. The rats were trained for five days, and behavior testing was carried out on day six (probe trial).58,59 The data obtained from this analysis suggests that STZ- induced diabetes mellitus Type 1 (DM1) leads to spatial memory loss as it showed longer escape latency where STZ-treatment group resulted in shorter escape latency. This decline in spatial memory has been correlated with the initial decline in the level of insulin in the blood due to toxic effects of Streptozotocin on the β cell of the pancreatic islets ofLangerhans and reduced insulin signaling in the hippocampus or the other memory related brain regions as they have been reported to have insulin receptor or increase in the insulin resistance value.60 Recovery of the memory loss by high dose (500m.g/kg./b.w) and low dose (250m.g/kg/b.w) of M. Dioica can be correlated with decline in insulin resistance which could lead to decline in the serum glucose towards normal control value and decline in oxidative stress as evident from our data.

The increased production of free radicals and oxidative damage is a feature of chronic diseases such as diabetes (Bayens and Thorpe, 1999). Hyperglycemia, due to diabetes, leads to the production of free radicals that are associated with the development of diabetic complications. This in turn reduces memory function over time. Free radicals are extremely toxic compounds that target biomolecules such as lipids with unsaturated double bonds and react with these lipids leading to lipid peroxidation. However, the increment of lipid peroxidation has been found to be involved in observed tissue damages in diabetes (Sathish Sekar and Subramanian, 2005). It was apparent from this study that the MDA, SOD and CAT levels in

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hippocampal tissue and plasma were significantly higher in the STZ- induced DM1 rat group than that of the normal group. According to the results of this study, levels of MDA, SOD and CAT in hippocampal tissue were increased by high dose (500m.g/k.g/b.w) and low dose (250m.g/kg./b.w) of M. Dioica.

The findings suggest the role of M. Dioica in recovery of the DM1- induced memory loss in rats by way of reversing the increased blood glucose level to normal by decreasing the insulin resistance and thereby decreasing the oxidative stress. Thus M. Dioica has a neuroprotective function in respect to cognitive loss due to diabetes mellitus as has been observed in earlier studies also. In addition to itsneuroprotective function, our study also suggests that M. Dioica has anti-diabetic potential. Therefore, the M. Dioica has therapeutic implications. So far as its role in treating diabetes mellitus is concerned, it may safely be used as a complementary drug with known anti-diabetic drugs; however, it needs a thorough study.

V. CONCLUSION

One of the mechanisms of diabetes is oxidative stress which can result from imbalance between generations of free radicals and scavenging system of free radicals eventually this may lead to neurodegeneration, hippocampus is the major portion of the brain responsible for learning, memory and cognition. Administration of Momordicadioica extract led to decrease blood glucose level, water consumption, urine output, body weight, increase in serum insulin levels, pancreatic insulin, and decrease HOMA-IR levels, shorter escape latency in MWM test and increased antioxidant activity

In conclusion, the results of the present investigation on alteration In hippocampal oxidative stress and associated spatial memory loss by fruits of Momordicadioica extract on STZ-induced diabetes type 1 in rat model has shown spatial memory-enhancing and antioxidant activities in low dose (250mg/kg/b.w) and high dose (500mg/kg/b.w).

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