RESEARCH PAPER

Amelioration of Diabetes-Induced Cognitive Dysfunction by Allium stracheyi in Rats

Bisht L[1, 2], Kumar N[1]* and Ram V[2]*

[1]School of Pharmaceutical Sciences, Shri Guru Ram Rai University, Patel Nagar, Dehradun-248001 Uttarakhand, India
[2]School of Pharmaceutical Sciences and Technology, Sardar Bhagwan Singh University, Dehradun-248001, Uttarakhand, India
[1,2]bishtlata155@gmail.com, [1]*neeraj_sidana2007@rediffmail.com and [2*]vrgodara@gmail.com
[1,2]ORCID ID: 0000-0002-9298-681X, [1*]ORCID ID: 0000-0002-5584-8522 and [2*] ORCID ID: 0000-0002-7564-2601

Received: 28th Feb, 2026; Revised: 6th March 2026; Accepted: 7th April, 2026; Available Online: 20th April, 2026

ABSTRACT

Background: Diabetic cognitive impairment is a metabolic neurodegenerative disorder associated with central nervous system. Unfortunately, there is no effective means by which to delay the cognitive symptoms of DM. Considering these limitations, there has been growing interest in investigating herbal alternatives for managing diabetes associated with cognitive deficit.

Objective: To investigate the neuroprotective role of methanolic leave extract of *Allium stracheyi* (MLEAS) Baker in diabetic rats.

Materials and Methods: Diabetes in rats were induced by 10% fructose solution for 14 days and single administration of streptozotocin (40mg/kg) in G2-G6 followed by 28 days treatment with glibenclamide (10mg/kg) and MLEAS at dose 100, 200 and 400mg/kg respectively. Vehicle control G1, received no treatment. Body weight, serum glycemic index, insulin marker, lipid indices, oxidative stress were evaluated using standard procedure. Neurobehavioural responses were measured using Elevated-Plus (EP) and Morris-Water (MW) Maze followed by estimation of acetylcholinesterase level. Pancreatic and brain tissue were retrieved for histopathological study.

Results: MLEAS significantly reinstated the body weight, fasting blood sugar, insulin resistance, lipid indices in diabetic rats. MLEAS, increased the glutathione (GSH) and superoxide dismutase (SOD) whereas decreases malondialdehyde (MDA) and nitric oxide (NO) levels. Furthermore, the AchE level was also resorted suggesting attenuation of cognitive impairment.

Conclusion: Overall findings showed that MLEAS exhibit neuroprotective effect against diabetes associated cognitive deficit. Thereby, opening new avenues for future research, which aim in developing potential compounds for effective disease management. Therefore, the plant emerges as a promising candidate that warrants further investigation to improve overall quality of life.
Keywords: *Diabetes, Cognition, Neuroprotective, Disease, Antioxidant.*
How to cite this article: Bisht L, Kumar N, Ram V, Amelioration of Diabetes-Induced Cognitive Dysfunction by Allium stracheyi in Rats. Int J Drug Deliv Technol. 2026;16(5): 61-78. DOI: 10.25258/ijddt.16.5.7
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

Diabetes mellitus (DM) is a chronic and complex metabolic disorder characterized by impaired metabolism of carbohydrates, lipids, and proteins, resulting in persistent hyperglycaemia. This condition arises primarily due to insufficient insulin secretion and insulin resistance in peripheral tissues such as skeletal muscles, adipose tissue, and the liver. Among the different forms of diabetes, type 2 diabetes mellitus (T2DM) is the most prevalent and is characterized by hyperglycaemia, impaired insulin secretion, and reduced insulin sensitivity. According to the International Diabetes Federation, more than 400 million people worldwide are currently affected by diabetes, and this number is expected to reach approximately 640 million by 2040. The progression of diabetes is often associated with several complications, including hypertension, hyperlipidaemia, renal dysfunction, vascular disorders, and neurological complications[1-5].

**Author for Correspondence: neeraj_sidana2007@rediffmail.com*

Cognitive function refers to the mental processes involved in learning, memory, reasoning, attention, and information processing. Maintenance of cognitive function is essential for independent living and decision-making in adults. However, cognitive decline, which often begins in early adulthood and accelerates with ageing, can ultimately lead to dementia. Increasing evidence suggests that T2DM significantly affects brain function and is associated with a higher risk of cognitive impairment and dementia. Individuals with T2DM have nearly a twofold higher risk of developing dementia compared to non-diabetic individuals. The association between diabetes and cognitive decline is complex and involves several underlying mechanisms, including oxidative stress, chronic inflammation, impaired insulin signalling, vascular damage, amyloid-β accumulation, and tau hyperphosphorylation in the brain[6].

Insulin plays a crucial role not only in glucose metabolism but also in several neurological processes such as synaptic plasticity, memory formation, and cognitive function. Impaired insulin signalling in diabetic conditions can disrupt neuronal metabolism, increase oxidative stress, and contribute to neurodegenerative changes. Experimental studies have shown that induction of diabetes using Streptozotocin in laboratory animals produces oxidative stress, inflammation, and neuronal damage, which resemble pathological features observed in neurodegenerative disorders such as Alzheimer's disease. Consequently, diabetic animal models are widely used to investigate mechanisms of diabetes-associated cognitive impairment.[7-10]

Although several pharmacological agents are currently available for the management of diabetes, including oral hypoglycaemic drugs and insulin therapy, these treatments are often associated with adverse effects such as hypoglycaemia, gastrointestinal disturbances, weight gain, and hepatic complications[11-13]. Therefore, there is growing interest in exploring natural products and medicinal plants as alternative therapeutic options due to their safety, accessibility, and diverse bioactive constituents.

Allium stracheyi Baker, a Himalayan medicinal herb belonging to the family Alliaceae, is traditionally used as a spice and folk remedy for various ailments. The plant is known to contain several bioactive phytochemicals, including organosulfur compounds, polyphenols, alkaloids, terpenoids, tannins, and saponins, which contribute to its antioxidant, anti-inflammatory, antimicrobial, and antidiabetic properties[14]. Previous studies have also indicated the potential of Allium species in protecting against metabolic and neurodegenerative disorders[15]. However, the role of *Allium stracheyi* Baker in diabetes-induced cognitive impairment has not been extensively investigated. Therefore, the present study was designed to evaluate the effects of methanolic extract of *Allium stracheyi* Baker on blood glucose levels, lipid profile, and learning and memory functions in fructose-streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Chemicals and Reagents

All the chemicals and reagents used in the research work were of analytical grade and purchased from authorised company.

Plant Material

The leaf of *A. stracheyi* Baker were collected from the Mana village, Joshimath, district Chamoli, Uttarakhand (UK) in 30 August; 2022 and was identified by the Botanical Survey of India (BSI), Kaulagarh road, Dehradun (UK). The plant sample was later placed in Herbanium unit with Accession no.: 1161.

Preparation Extract

The fresh leaves of *Allium straychei* Baker were dried in shed at room temperature for one week and pulverized into a required coarse powder form by mechanical grinder. The coarse powder weighing 250g were subjected for deffating using 1000ml of petroleum ether and extracted with methanol of analytical grade at temperature 40-60°C for 48hours in soxhlet apparatus. The extract of leaves was filtered and further evaporated to remove solvent till dryness at 60°C in rotary evaporator under reduced pressure. The percentage (%) yield of dark green-brown extract was calculated by formula mentioned by Harborne;1976 and further modified by Truong et al. The extract was then stored for further uses in desiccator.[15]

$$Percentage\ (\%)yield = \frac{weight\ of\ extract}{weight\ of\ leaves\ used} X\ 100$$

Phytochemical Screening of Extract

The phytochemical screening of methanolic extract of *Allium stracheyi* Baker was carried out using standard qualitative screening methods. Polyphenols, flavonoids, tannins, alkaloids, saponin and glycoside were screened.[16]

Ethical Consideration

The experimental protocol was approved by the Institution Animal Ethical Committee (IAEC) of Shri Guru Ram Rai University, Dehradun with ethical number 264/CPCSEA/IAEC/2022/12. The whole experiment was carried out according to the internationally accepted protocols provided by the Committee for Control and Supervision of Experiments on Animals (CCSEA).

Selection of Animals and Housing Condition

In the present study, healthy wistar albino rats (male) weighing between 200-250 gm were used and housed in a group of six animals per cage. The animals were maintained under standard conditions with temperature (18-29°c), relative humidity (30-70% RH), a photoperiod of 12-12 hour dark and light cycle and at an environment away from noise. Before initiation of experiment all the animals were acclimatize for a period of one week. During

acclimatization animals were provided with normal pallet diet and water *ad libitum*. The solid-bottomed cages with soft husk bedding were used to avoid discomfort to animals. Cage cleaning and bedding changing were done frequently to maintain sanitary conditions.[17]

Determination of LD_{50} Value (Acute oral toxicity study)
The acute oral toxicity of methanolic leaf extract of *A. stracheyi* Baker (MLEAS) was carried out in wistar albino rats as per the protocol given by OECD-Test guidelines-420. The animals were fasted overnight and the weight of each animal was recorded prior to the experiment. The different doses of MLEAS (5, 50, 300,1000 and 2000mg/kg BW p.o) were administered to the group of three animals. The animals were closely monitored and observed for a period of 30 minutes after administration of extract, periodically for next 24 hours for mortality and thereafter daily for a period of 14 days for the sign of abnormal behaviour, general toxic signs. Body weight of individual animal was recorded on weekly interval. LD_{50} was determined and 1/10th of LD_{50} was taken as therapeutic dose for the activity.[18]

Standard Drug Preparation
Glibenclamide 10mg/kg suspended in distilled water using 1% carboxy methyl cellulose (CMC) was used in the study as a standard treatment drug for evaluating hypoglycaemic activity.[19]

Induction of Diabetes and Selection of Diabetic Animals
Before induction rats were acclimatized for a period of seven days. On seventh day rats were randomly divided into vehicle control group (n=6) and diabetic control group (n=30). Vehicle control group (G1) received normal drinking water. Conversely, diabetic control groups (G2-G6), initially received 10 % w/v fructose supplied in the drinking water for a period of 2 weeks for initial induction of diabetes followed by administration of single dose of STZ (40 mg/kg i.p,) prepared in citrate buffer (pH 4.5) for induction of type 2 daibetes. STZ was dissolved in distilled water immediately before use. During the first 24hours after STZ injection, the experimental animals were supplied with 10% w/v of glucose solution to prevent acute hypoglycaemic state whereas the vehicle control group animals received distilled water (1ml/ 100gm). After 72 hours of STZ injection, blood sample of each animal were collected and the fasting blood glucose level was measured by GOD-POD method with the help of Erba enzymatic kit. Animals with blood glucose level 200-250mg/dl were considered as diabetic rat and selected for the study of antidiabetic and cognition enhancing activity of plant extract.[20]

Animals grouping
According to fasting blood sugar level, 30 diabetic rats were randomly divided into 5 groups (n=6/group) excluding vehicle control group. Diabetic rats in group G3-G6 were treated with glibenclamide (10mg/kg B.W) and MLEAS (100 mg/kg, 200 mg/kg and 400 mg/kg B.W respectively) once a day by oral intubation (p.o) between 10:00 am-11:00 am for 28 days. Overall, the animal groups were assigned as:

G1 (Vehicle control): Normal rats fed with standard pellet diet and distilled water.

G2 (Toxicant): Diabetic control rats

G3 (Standard): Diabetic rats treated with standard drug glibenclamide (10mg/kg OF BW; p.o)

G4 (Test): Diabetic rats treated with MLEAS (100 mg/kg of BW; p.o)

G5 (Test): Diabetic rats treated with MLEAS (200 mg/kg of BW; p.o)

G6 (Test): Diabetic rats treated with MLEAS (400 mg/kg of BW; p.o)

The fresh solution of standard drug and methanolic extract were administered everyday orally for a period of 28 days. After 28 days of respective treatments, the animals were subjected for evaluation of physical, biochemical and behavioural parameters for determining the effect of different doses of treatment on cognitive impairment in diabetic animals.

ESTIMATION OF PHYSICAL PARAMETER

Monitoring of body weight
The body weight (B.W) of experimental animals was measured on day 0, 7th, 14th, 21st and 28th using an adjusted electronic weighing balance and percentage change in weight were calculated for each group of animals by using formula[21]:

$$\text{Percentage (weight Change)} = \frac{\text{B.W (g) on 28th day} - \text{B.W (g)on 0th day}}{\text{B.W (g) on 0th day}} \text{X } 100$$

Collection of blood sample and serum separation
At the end of respective treatment, the animals were fasted overnight and approximate 1.5-2ml of blood sample was collected from each rat through retro-orbital technique under mild diethyl ether anaesthesia followed by collection of plasma after centrifugation for 10 min at 20,000 RPM using cold centrifugation machine (Model: Remi Centrifuge CE-412 LAG) for biochemical analysis using different diagnostic kit in semiautomated Autoanalyzer (Model: Erba Mannheim Chem 5X).[21]

ESTIMATION OF BIOCHEMICAL INDICES

Estimation of glycemic profile
The serum fasting blood glucose was measured periodically after overnight fasting by GOD-POD colorimetric method using erba diagnostic kit.[22]

Estimation of Insulin level
The serum insulin level of animals was measured by insulin ELISA diagnostic kit. Serum insulin level was quantified by procedure provided in kit by the manufacturer. It is based on the direct sandwich technique

in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation insulin in the sample reacts with anti-insulin antibodies bound to the microtitration well and with peroxidase-conjugated anti-insulin antibodies. A washing step removes unbound enzyme labelled antibody. The bound conjugate is detected by reaction with 3,3',5,5'-tetramethylbenzidine (TMB, a frequently used chromogenic in ELISAs). The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically at a wavelength of 450 nm using a microplate reader.[23]

Insulin Resistance Assessment

Insulin resistance and beta-cell functioning of diabetic and treated animals were assessed by Homeostasis model assessment index (HOMA-IR and HOMA-β) by using formula given by formula:[24-25]

$$\mathrm{HOMA-IR} = \frac{\text{Fasting Serum Glucose (mmol/l) X Fasting Serum Insulin (IU)}}{22.5}$$

$$\mathrm{HOMA}-\beta = \frac{\left(20\text{ X Fasting insulin }\left(\mu\frac{\mathrm{IU}}{\mathrm{ml}}\right)\right)}{\left(\text{Fasting glucose }\left(\frac{\mathrm{mmol}}{\mathrm{l}}\right)\right)} - 3.5$$

Conversion factor: Serum glucose (1mg/dl= 1/18 mmol/l)

Lipid profile Assesment

Total cholesterol, triglycerides and high-density lipoprotein (HDL) levels by using biochemical diagnostic kits on 28th day.[27]

The levels of serum very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) were calculated by using *Friedewald's* equation.[28-29]

Very low-density lipoprotein: $\mathrm{VLDL} = \mathrm{TG\ (mg/dl)}/5$

Low-density lipoprotein: LDL= CH – (HDL+VLDL)

Atherogenic index (AI) and Coronary artery risk (CR) index was also calculated to estimate the risk of atherosclerosis and cardiovascular disorders by using the formula:[30]

Atherogenic index (AI) = log [TG/HDL]

Coronary artery risk index (CARI): TC (mg/dl)/ HDL-cholesterol (mg/dl)

The effect of treatment on brain biomarkers

Behavioural models for analysis of memory and cognitive impairment in diabetic rats

Elevated plus-maze model (EPM)

The assessment of memory acquisition and retention was carried out using the elevated plus maze task, which tests spatial long-term memory. The key measure, transfer latency (TL), refers to the duration the animal takes to transition from an open arm to an enclosed arm, serving as a marker for evaluating learning and memory abilities. The EPM consisted of four arms, forming plus shape. All the arm are attached by a central field (10cm), in which two opposing arm were open (50x10cm) arms, having a 0.5cm wall to reduce the falling number and the other two closed (50x10x40cm) arm, closed by black wall that had a height of 40cm. the EPM was placed at height of 50cm from the floor. The learning memory of animals from each group was evaluated in two sessions by EPM. During trial session, animals were kept at distal end of open arm, facing away from central field and the TL_0 was recorded. Animals if failed to enter into enclosed arms within 90 secs, were pushed gently towards the any enclosed arms. The animals were permitted to explore the plus maze for another, 10 secs and then returned back to their respective cages. To analyse cognition, memory retention test session was performed after 24hr of trial session. On the first day (during trial session), the measured transfer latency of plus-maze served as an index of learning and acquisition, whereas reduction in time latency (TL_1), on 2nd day served as an index of retrieval and memory improvement. [41-43]. The TL was represented as the inflexion ratio (IR), calculated using the following formula: [31]

$$IR = \frac{TL1 - TL0}{TL0}$$

Moriss water Maze

Moriss water Maze

Morris water maze test was employed to assess learning and memory of the animal. Morris water maze is a swimming based model where the animal learns to escape on to a hidden platform. It consists of small circular pool of 150 cm in diameter, 45cm. in height, filled to depth of 30 cm with water maintain at 25±1°C. The tank was divided into four equal quadrants. To help the experimental animals with their spatial orientation, visual cues in the shape of red and blue tapes were positioned around the water tank; the cues locations remained constant during the experiment. During the acquisition phase, the submerged platform with 10 × 10 cm was maintained 1 cm above the water's surface. During acquisition, animals from each group were placed in tank separately with their back to the wall of tank and allowed to locate their platform within 120 sec. If rats failed to find

the platform within 120 seconds, they were guided gently onto platform and allowed to remaining there for 20 seconds. Each animal was subjected for four trials every day for four days with a 10-minute interval between trials. During each trail phase, the animal was carefully moved into each of the swimming tank's quadrants. During the retention phase, the water surface was kept 1 cm below the tank's water level and rendered opaque with milk powder to conceal the platform. On days 28, the animal's memory retention was assessed after they were placed in the quadrant facing towards the tanks wall. By timing how long it took the animal to find the secret platform in the water maze, escape latency was determined. Escape latency (EL) was measured by observing the time taken to relocate the water maze hidden platform.[32-33]

Collection of brain and pancreatic tissue

After completion of experiment and biochemical parameters on blood sample, animals from each group were euthanized by cervical dislocation. The pancreas and brain of animals were isolated and washed carefully with saline solution. The retrieved samples of organ were splitted symmetrically into two halves and one section was kept in 10% w/v saline for biochemical estimation. Whereas another section of tissue was stored separately in 4% w/v formaldehyde solution for histopathological evaluation.[31]

Preparation of homogenate

For biochemical estimation pancreatic and brain tissue sections were cut down into small pieces and further homogenized using tissue homogenizer in 0.1 M phosphate buffer solution, maintained at pH 7. The homogenates were subjected for centrifugation at 4000-5000rpm for 20 min at 4 C using cooling centrifuge (Remi). The supernatant was collected and stored for measuring oxidative stress level.[31,34]

Oxidative stress Indices

Antioxidant biomarkers were estimated in brain and pancreatic tissue homogenates. Reduced glutathione (GSH) levels were determined using Ellman's method by measuring absorbance at 412 nm[35]. Superoxide dismutase (SOD) activity was assessed according to the Kakkar method[36] based on inhibition of nitroblue tetrazolium reduction and measured at 560 nm. Lipid peroxidation was evaluated by estimating malondialdehyde (MDA) using the thiobarbituric acid reactive substances (TBARS) method at 532 nm.[37,38] Nitric oxide (NO) levels were determined using the Griess reagent colorimetric assay at 540 nm[39]. Acetylcholinesterase (AchE) activity in brain tissue was measured using Ellman's colorimetric method and expressed as µmol/min/mg protein.[40]

Histopathology

Pancreas and brain of vehicle, control and treated animals were isolated and fixed 4% w/v formaldehyde solution for histopathological analysis. Organ tissue were dehydrated, cleaned and embedded in paraffin. Organs were dissected into thin sections of 3-4 and stained using haematoxylin and eosin dye for histopathological evaluation.[40],[41]

Statistical Analysis

Evaluation of all the parameters were conducted in triplicate (n=3). Values of results were shown in Mean ± SEM. Analysis of biochemical and behavioural parameters were done by one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. Body weight and fasting blood glucose level were analysed by two-way ANOVA by using GraphPad Prism version. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Percentage (%) yield and Phytochemical Screening

The percentage (%) yield of methanolic leaf extract was found to be 12.90% and the results of qualitative phytochemical screening shows presence of tannins, alkaloids, phenols, flavonoids, steroids and saponin (**Table 1**).

Table 1: Percentage (%) yield and phytochemical constituents of methanolic leaf extract of *A. stracheyi* Baker

Plant Part and Extract	**Percentage (%) Yield**					
Methanolic Leaf Extract (MeOH)	**Colour**		**Consistency**		**% (w/w) Yield**	
	Dark Green		Semi-solid		12.60%	
Phytochemical test						
Tests	**Tannins**	**Alkaloids**	**Phenols**	**Flavonoids**	**Steroids**	**Saponins**
Inference	++	+	+++	+++	++	++

Notation: '+++': Highly present, '++': Moderately present and '+': Mild present

Acute toxicity study

The LD_{50} of MLEAS was evaluated by OECD guideline 420. **Table 2** illustrate that, no death of animals was observed at the highest dose 2000mg/kg B.W. Additionally, no or minimal adverse manifestations or behavioural deviations were observed, suggesting that the LD_{50} of MLEAS is greater than 2000mg/kg B.W. The 1/20th, 1/10th and 1/5th of 2000mg/kg B.W of MLEAS was used in the study as low, intermediate and highest dose i.e. 100, 200 and 400 mg/kg B.W.

Table 2: LD50 determination of MLEAS

Parameters	No. of animals treated	Doses of MLEAS (mg/kg B.W; P.O)						Control
		5	50	300	500	1000	2000	
Food Consumption	3	N	N	N	N	N	N	N
Water consumption		N	N	N	N	N	N	N
Body weight		N	N	N	N	N	N	N
Eye Colour		N	N	N	N	N	N	N
Skin/Pelage condition		N	N	N	N	N	N	N
Locomotion		N	N	N	N	N	N	N
Tremor/Convulsion		N	N	N	N	N	N	N
Sedation		N	N	N	N	N	AB (Mild)	N
Diarrhoea		N	N	N	N	N	AB (Mild)	N
Death		0/3	0/3	0/3	0/3	0/3	0/3	0/3

Key: 'N': Normal; 'AB': Adverse Behaviour

Effect of MLEAS on fasting blood sugar level

Fasting blood glucose (FBG) levels were measured on days 0, 7, 14, 21, and 28 in all groups. Following diabetes induction, groups G2–G6 showed a significant increase in FBG on day 0 compared with the vehicle control (G1) ($p < 0.001$). Treatment with glibenclamide (10 mg/kg) and methanolic leaf extract of *Allium stracheyi* (MLEAS; 100, 200, and 400 mg/kg) for 28 days significantly reduced elevated glucose levels in a dose-dependent manner. The highest dose of MLEAS (400 mg/kg) produced the most pronounced reduction in FBG ($p < 0.001$), followed by 200 mg/kg and 100 mg/kg ($p < 0.01$) on day 28 compared with day 0 (**Figure 1).** Although all treated groups showed significant improvement compared with the diabetic control (G2), FBG levels did not fully return to vehicle control values, indicating partial but therapeutically meaningful glycemic control.

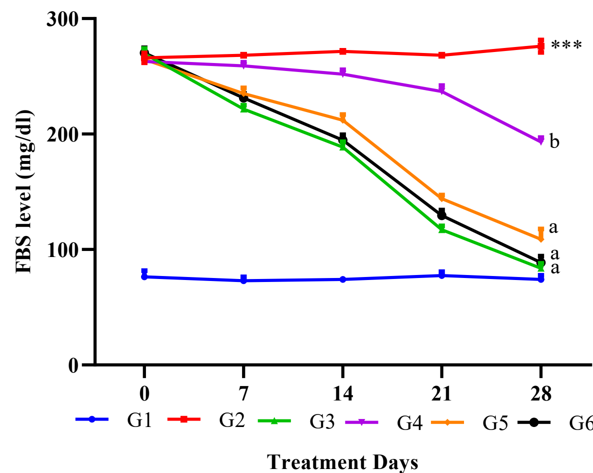

Figure 1: Effect of MLEAS on Serum Fasting Blood Sugar Level

P values: ***P<0.001 vs NC. P values: ^{b}P<0.01 and ^{a}P<0.001 when results of 28th day are compared with day 0.

Effect of MLEAS on body weight

The effect of treatment with different doses on body weight and percentage change in body weight are shown in **Figure 2(a) and (b)**. on 0th day (fructose 10%+STZ) before initiation of treatment the body weight of diabetic animals (G2-G6) was significantly decrease due to STZ induced impaired glucose utilization against vehicle control group. After four weeks (28 days) of treatment, diabetic animals (G3-G6) treated with glibenclamide and MLEAS (100, 200 and 400mg/kg) showed significant ($p<0.001$) increase in body weight by 9.23%, 10.24%, 12.44% and 16.94% respectively when compared with G2 i.e.-14.04%. Furthermore, there was a significant weight loss in G2 animals compared to G1 group animals (i.e.1.55%) reflecting reduced glucose utilization and metabolism due to insulin resistance. More interestingly, MLEAS at 400mg/kg increases the body weight more profoundly (16.94%).

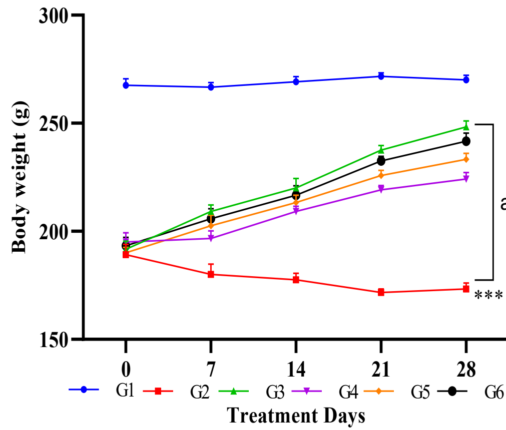

Figure 2: (a) Effect of MLEAS on Body Weight

***P<0.001 vs NC. P values: ^{c}P<0.05,^{b}P<0.01 and ^{a}P<0.001 when results of 28th day are compared with day 0.

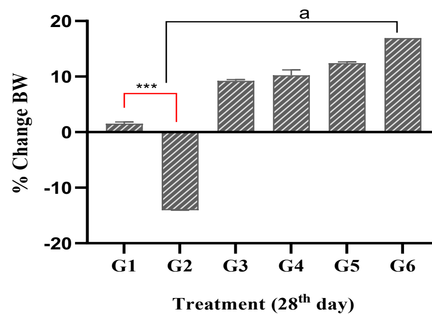

Figure 2: (b) Percentage (%) Change in Body Weight

***P<0.001 vs NC. P values: ^{c}P<0.05,^{b}P<0.01 and ^{a}P<0.001 when results of 28th day are compared with day 0.

Effect of MLEAS on insulin and insulin sensitivity

As illustrated in **Figure 3,** the insulin level of diabetic animals (G2-G6) was significantly (^{a}p<0.001) reduced on day 0 after administration of FrSTZ when compared to vehicle control G1 group. After treatment with glibenclamide (10mg/kg) and MLEAS (100, and 400mg/kg) for 28 days, the level of serum insulin was improved significantly on comparison with the G2, while the MLEAS 100 showed slight improvement in G4 animals but the effect was not statistically significantly.

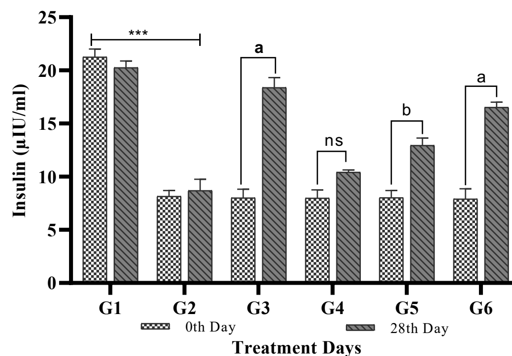

Figure 3. Effect of MLEAS on insulin (µIU/ml)

***P<0.001 vs NC. P values: ^{c}P<0.05,^{b}P<0.01 and ^{a}P<0.001 when results of 28th day are compared with day 0. ns: non-significant**.**

Effect of MLEA on HOMA-IR and beta-cell function (HOMA- β)

HOMA-IR and HOMA-β was calculated to measure the effect of MLEAS on insulin resistance and beta-cell function using fasting insulin (µIU/ml) and glucose (mg/dl) level. According to data illustrated in **Figure 4 and 5,** insulin resistance (HOMA-IR) was significantly (p<0.01) increased whereas HOMA-β was decreased in

all the diabetic animals on day 0 when compared with G1. Treatment of diabetic animals (G3,G5 and G6) with glibenclamide (10mg/kg) and MLEAS (100,200 and 400mg/kg) for 28 days, significantly reduces insulin resistance and increases HOMA-β level when compared with day 0.

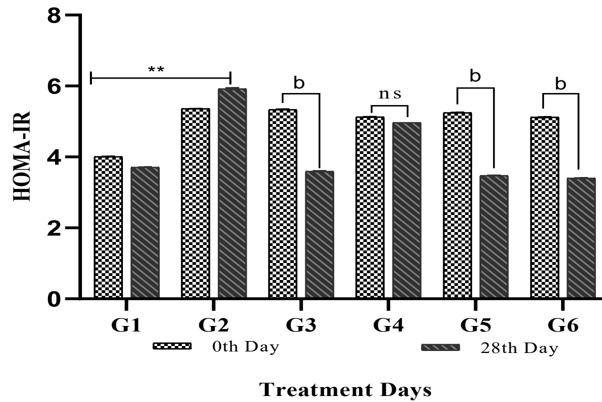

Figure 4. Effect of MLEAS on HOMA-IR

***P<0.001 vs NC. P values: [c]P<0.05,[b]P<0.01 and [a]P<0.001 when results of 28th day are compared with day 0. ns: non-significant

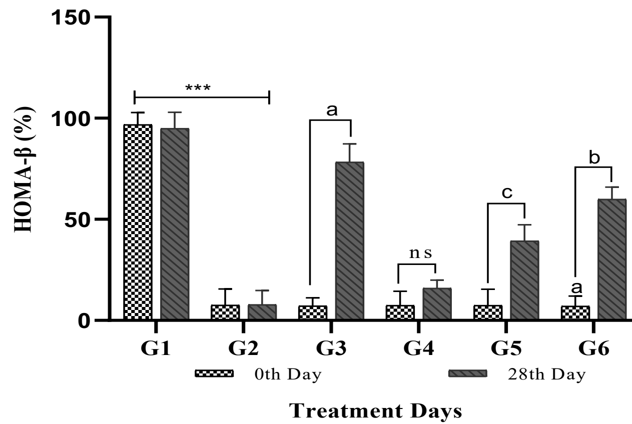

Figure 5: Effect of MLEAS on HOMA-β

***P<0.001 vs NC. P values: [c]P<0.05,[b]P<0.01 and [a]P<0.001 when results of 28th day are compared with day 0.ns: non-significant

Effect of MLEAS on lipid profile

Table 3 illustrate the serum lipid profile of experimental animals. The serum CH, TG, LDL and VLDL level of diabetic animals (G2-G6) after FrSTZ administration was significantly ($p<0.001$) increased whereas the serum HDL was significantly ($p<0.001$) reduced on day 0 when compared to the G1. Furthermore, treatment of diabetic animals with impaired lipid profile for 28th days with glibenclamide (10mg/kg) and MLEAS (200 and 400mg/kg respectively) significantly ($p<0.05$,$p<0.01$ and $p<0.001$) reduces the increased CH, TG, LDL whereas increases HDL level compared to day 0. Moreover, MLEAS 100 and 200mg/kg showed no significant difference in serum VLDL on day 28th, relative to day 0 whereas glibenclamideand MLEAS 400mg/kg caused significant ($p<0.01$) improvement in serum VLDL.

Table 3: Effect of Treatment of Serum Lipid Indices

Parameters	Days	G1	G2	G3	G4	G5	G6
CH (mg/dl)	0th (Baseline)	85.63±2.83	137.56±3.263	135.62±1.927	129.89±2.582	124.78±2.924	131.83±2.615
	28th (after treatment)	84.51±2.346	139.37±2.021***	96.86±2.167^{a}	120.59±2.167^{c}	112.11±2.304^{b}	89.67±2.732^{a}
TG (mg/dl)	0th (Baseline)	69.88±2.054	103.74±2.179	109.89±3.069	113.74±3.602	101.78±1.502	111.15±2.845
	28th (after treatment)	65.16±2.996	108.97±4.020***	72.67±2.808^{a}	100.8±2.409^{c}	91.63±2.628^{b}	85.26±3.120^{a}
LDL (mg/dl)	0th (Baseline)	29.834±1.20	93.86±0.458	85.31±0.369	77.75±0.33	85.77±0.91	89.75±0.03
	28th (after treatment)	13.03±0.593	21.74±0.804***	18.32±0.525^{a}	20.16±0.481^{a}	17.05±0.624^{a}	15.33±0.561^{a}
HDL (mg/dl)	0th (Baseline)	42.82±1.589	22.95±2.37	20.18±1.683	19.39±2.192	18.75±1.714	19.85±2.016
	28th (after treatment)	39.72±1.262	19.66±0.866***	36.32±1.083^{a}	24.03±0.848^{b}	27.88±0.683^{b}	32.485±1.08^{a}
VLDL (mg/dl)	0th (Baseline)	13.96±0.041	20.749±0.435	21.98±0.613	22.75±0.720	20.26±0.300	22.23±0.569
	28th (after treatment)	13.03±0.599	21.79±0.804^{a}	14.53±0.561^{b}	20.16±0.481^{ns}	18.33±0.525^{ns}	17.052±0.624^{b}

***P<0.001 vs NC. P values: ^{c}P<0.05,^{b}P<0.01 and ^{a}P<0.001 when results of 28th day are compared with day 0.ns: non-significant

Estimation of MLEAS on AI and CAR-I

Table 4 shows results of AI and CAR-I, which are strong and reliable markers of atherosclerosis and coronary heart disease risk were calculated from TG/HDL and CH/HDL ratio respectively for all the groups.)n day 0 the TG/HDL ratio and CAR-I of diabetic animals (G2-G6) was significantly (p<0.001) increasedwhen compared with G1 animals. Glibenclamide (G3) on 28th day decreases AI and CAR-I in contrast to day 0, showing 55.78% and 61.30% improvement after treatment. Similarly, on 28th day G6 animals with MLEAS (400mg/kg) the AI and CAR-I were decreases significantly (p<0.01) when compared to day 0, indicating 43.98 and 58.43% improvement. Furthermore, dose 200mg/kg (G5) caused significant (p<0.05) decrease in TG/HDL and CH/HDL ratio, showing 29.65% and 39.54 % improvement whereas MLEAS100mg/kg (G3) caused slight reduction (i.e. 18.88 and 18.77% respectively) but the changes were not significantly statistically (p>0.05).

Table 4: Effect of Treatment of Atherogenic Indices and Ratio:

Parameter →	AI			CAR-I		
Groups/ Days	**0th Day (Baseline)**	**21st Day (after treatment)**	**% Protection**	**0th Day (Baseline)**	**21st Day (after treatment)**	**% Protection**
G1	0.213±0.111	0.215±0.375	-	1.99±1.780	2.13±1.242	-
G2	0.655±0.283	0.744±0.667***	-13.58	5.99±1.376	7.089±2.33***	-18.34
G3	0.735±0.261	0.325±0.414^{b}	55.78	6.72±1.170	2.6±2.0^{b}	61.30
G4	0.768±0.215	0.623±0.453^{ns}	18.88	6.18±1.179	5.02±2.556^{ns}	18.77
G5	0.735±0.273	0.517±0.585^{c}	29.65	6.65±1.705	4.02±3.373^{c}	39.54
G6	0.748±0.149	0.419±0.4618^{c}	43.98	6.64±1.297	2.760±2.529^{b}	58.43

***P<0.001 vs NC. P values: ^{c}P<0.05,^{b}P<0.01 and ^{a}P<0.001when results of 28th day are compared with day 0.ns: non-significant

Effect of MLEAS on escape latency and transfer latency

Tabel 5 illustrate the effect of MLEAS on escape latency (EL) on 0 and 28th day. On day 0 after 14 days of induction with FrSTZ, diabetic animals (G2-G6), showed significant increase in EL and TL relative to G1 animals. On 28th day, the EL, TL, and IR time was significantly decreases in diabetic animals treated with glibenclamide (10mg/kg) and MLEAS in dose dependent manner respectively. Suggesting its potential role in memory consolidation.

Tabel 5: Effect of MLEAS on escape and transfer latency

Groups/Days	Escape Latency (Sec.)		Transfer Latency (sec.)		
	0th Day	**21 Day**	**0th Day**	**21th Day**	**Inflexion Ratio (IR)**
G1	19.201±0.5163	23.123±2.221	19.66±1.452	20.33±1.909	0.034±0.314
G2	70.34±1.843	67.08±1.751***	56.16±1.137	66.5±1.875***	0.184±0.649***

G3	61.66±0.843	29.66±1.887[a]	58.33±2.185	31.16±2.358[a]	0.466±0.079[a]
G4	63.34±1.087	57.09±1.358[c]	56.01±1.693	49.83±1.301[c]	0.110±0.231[c]
G5	65.83±2.548	46.166±2.427[b]	58.33±2.498	44.166±2.358[b]	0.248±0.056[b]
G6	59.166±1.514	34.05±0.683[a]	60.66±2.275	42.33±2.244[b]	0.302±0.013[b]

[***]P<0.001 vs NC. P values: [c]P<0.05,[b]P<0.01 and [a]P<0.001 when results of 28th day are compared with day 0.ns: non-significant

Effect of MLEAS on oxidative stress and antioxidants indices

The effect of MLEAS (100,200 and 400mg/kg) on oxidative stress and antioxidants was determined by evaluating lipid peroxidation (MDA) product, superoxide dismutase (SOD) and glutathione (GSH) level in the homogenate of brain and pancreatic tissue of animals. As illustrated in **figure 8,** animals in vehicle control group showed baseline MDA, SOD and GSH level, indicating normal LPO and antioxidants status. Moreover, the FrSTZ provoked diabetic demented animals (G2-G6) showed severe enhancement of MDA level and reduced SOD and GSH level on day 0, relative to vehicle control animals, reflecting enhanced oxidative stress in brain as well in pancreatic tissue. Conversely, administration of MLEAS for 28 days at different dose level to the diabetic demented rats caused appreciable significant (p<0.001) reduction in MDA level and improved the antioxidants levels in the tissue of rats when compared with diabetic control rats (G2), Glibenclamide at 10mg/kg (G3), also supressed the level of MDA and restored the antioxidants (SOD and GSH) levels (**Figure 6 (a, b and c)).** Among all the doses of MLEAS, the highest dose, 400mg/kg demonstrated the most potential role in restoring the MDA, SOD and GSH level, suggesting strengthened antioxidant defence mechanism.

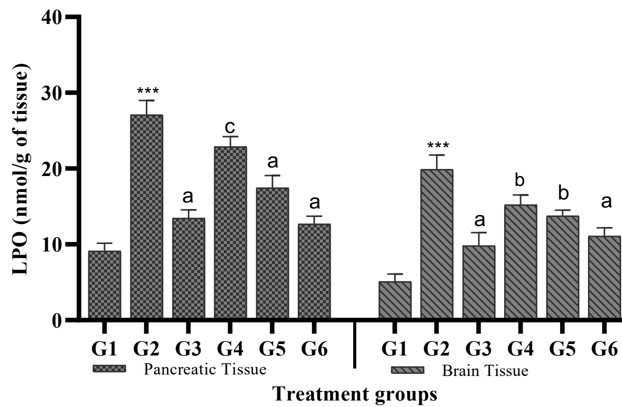

Figure 6a: Effect of MLEAS on Lipid Peroxidation

P values: [***]P<0.001 vs NC. P values: [c]P<0.05,[b]P<0.01 and [a]P<0.001 when results of 28th day are compared DC.

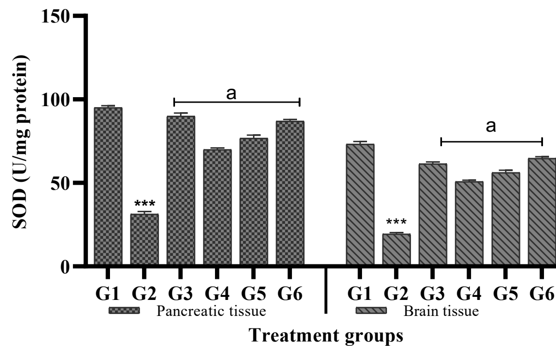

Figure 6b: Effect of MLEAS on Superoxide Dismutase

P values: [***]P<0.001 vs NC. P values: [c]P<0.05,[b]P<0.01 and [a]P<0.001 when results of 28th day are compared DC.

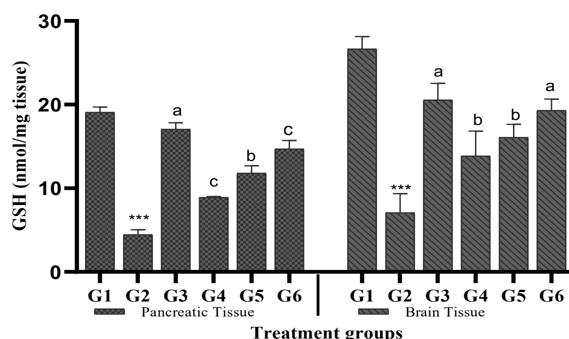

Figure 6c: Effect of MLEAS on Reduced Glutathione

P values: ***P<0.001 vs NC. P values: ^{c}P<0.05,^{b}P<0.01 and ^{a}P<0.001 when results of 28th day are compared DC.

Effect of MLEAS on nitrosative stress

The scavenging potential of MLEAS on nitrite production in pancreatic and brain tissue homogenate is illustrated in **figure 7**. The result showed that level of nitric oxide (NO) in the pancreas and brain of diabetic control animals (G2) were significantly (p<0.001) greater when compared with animals of G1 animals, indicating increased nitrosative stress level and neuronal injury in diabetic animals. Furthermore, administration of MLEAS for 28 days, decreased the NO level significantly (p<0.01 and p<0.001) in G4-G6 when compared with G2 animals. Findings of data suggested that, MLEAS at dose 200 and 400mg/kg caused most significant (P<0.001) decrease in NO level followed by MLEAS 100mg/kg (p<0.01). Simultaneously, glibenclamide at 10mg/kg, also reduces the nitrosative stress (NO) significantly. The result suggested that MLEAS and the standard Glibenclamide had remarkable protective effect against oxidant.

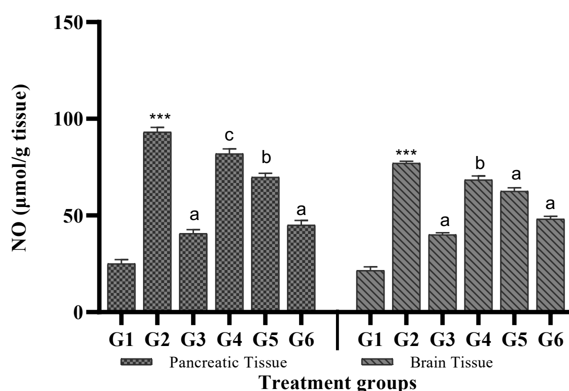

Figure 7: Effect of MLEAS on Nitric Oxide

P values: ***P<0.001 vs NC. P values: ^{c}P<0.05,^{b}P<0.01 and ^{a}P<0.001 when results of 28th day are compared DC.

Effect of MLEAS on AchE

Figure 8 illustrate the effect of MLEAS on AchE level on brain homogenate, determined by Ellman method. The brain of diabetic control animals showed significant (p<0.001) increase in the AchE level, relative to G1 animals. A significant dose-dependent reduction in the level of brain AchE was observed after treatment with MLEAS for 28 days, suggesting MLEAS have effective role in restoring cognitive function.

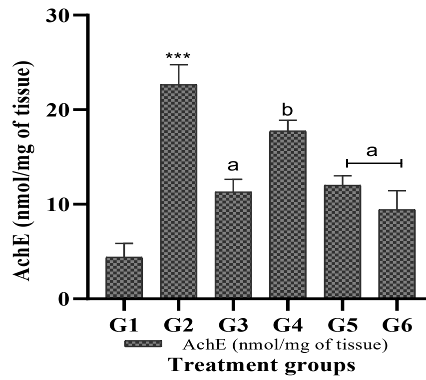

Figure 8: Effect of MLEAS on Enzyme Acetylcholinestrase

P values: ***P<0.001 vs NC. P values: ^{c}P<0.05,^{b}P<0.01 and ^{a}P<0.001 when results of 28th day are compared DC.

Effect of MLEAS on histopathological changes

Figure9a shows histopathology of pancreatic cell: Pancreatic section of G1 (vehicle control) showed normal histology of the pancreatic cells with no morphological changes in acinar cell and interlobular duct. G2 (Diabetic control) animals showed severe inflammation and damaged acinar cells with altered morphology of pancreatic langerhans and irregular arrangement of interlobular duct. G3 (Glibenclamide) animals: the islets of langerhans and acinar cells display normal histoarchitecture without any identifiable pathological changes, suggesting glibenclamidesignificantly restored normal number of cells as well as size of pancreatic cell. Additionally, the morphology of acinar cells exhibits no detectable changes. Pancreatic section of G4 (MLEAS 100) animals showed minimal restoration of pancreatic cell and morphological architecture. Interlobular duct showing restored organization. Histopathology of G5 (MLEAS 200) animals showed increased mass and number of pancreatic cells, relative to diabetic control animals. G6 (MLEAS 400) animals histopathology shows improvement in the pancreatic cells with normal histoarchitecture of acinar cells, suggesting its ameliorative effect.

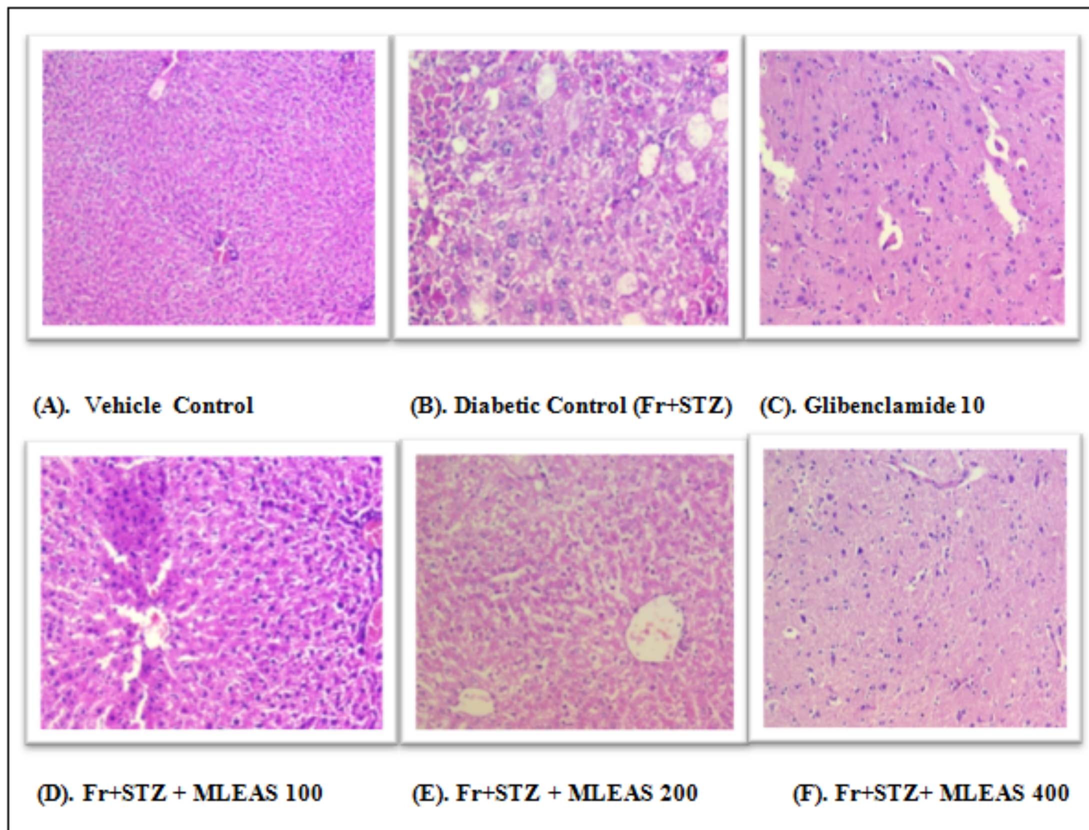

Figure 9a: Effect of MLEAS on histopathology of Pancreatic tissue

Figure 9b demonstrate the effect of MLEASon histopathological changes on diabetic rat brain tissue in the control and treatment group. G1 (vehicle control) rat: showed normal microarchitecture of neuronal tissue. In G2 (diabetic control), the pyramidal cells shrunken. The dilated congested blood vessels showed degeneration and pyknosis in neurons with focal amorphous eosinophilic materials in the cerebral cortex. Brain section shows necrosis of the neuron and formation of focal areas of malacia at the cerebral cortex, neuritic plaque and neurofibrillary tangles in different area of the brain with fibrotic changes and occasional eosinophilic amyloid plaques. Severe formation of neurofibrillary tangles was also observed in hippocampus together with haemorrhages and gliosis in brain, suggesting excessive neuronal loss and scarring of brain tissue. G3 (Glibenclamide) animals, showed normal pyramidal cells, granular cells with rounded open face nuclei and pink stained nucleus. The number and size of amyloid plaques was reduced with focal gliosis. No atypia or malignant component noted, suggesting mild degenerative changes. G4 (MLEAS 100) animals, showed moderate degeneration and necrosis of neurons. Fibrotic changes with presence of neuritic plaques and neurofibiliary tangles in few areas of brain. No atypia or malignant component noted. G5 (MLEAS 200) animals showed mild to moderate degeneration of neurons. Minimal perivascular inflammatory cell infiltration and few neurofibrillary tangles were observed than group IV. G6 (MLEAS 400): normal cell architecture with normal appearing ganglion and neuronal cells with occasional areas showing a very few neurofibrillary tangles, suggesting very mild degenerative changes. Histopathological findings suggested dissipation of neuronal cell damage by MLEAS treatment in fructose and STZ induced diabetic rats.

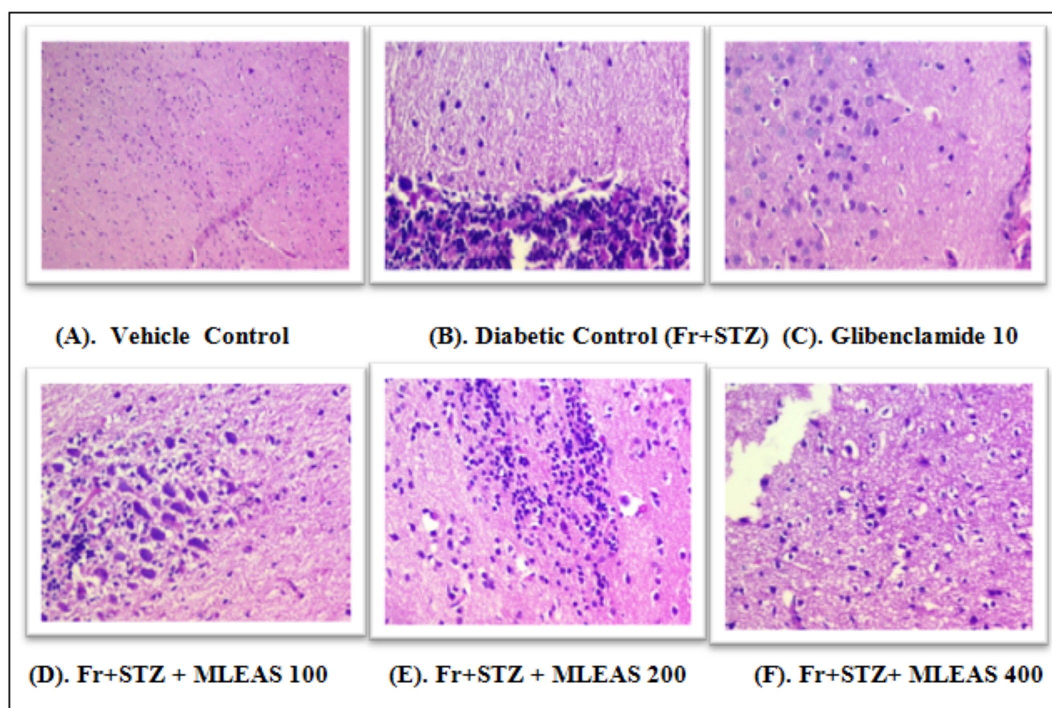

Figure 9b: Effect of MLEAS on histopathology of brain tissue

DISCUSSION

Diabetic cognitive impairment is a metabolic neurodegenerative disorder associated with central nervous system. Currently, no conventional drug is available that can effectively treat or prevent the manifestation of diabetic cognitive impairment. Moreover, development of novel drugs requires a long developmental process along with a huge economic investment. Therefore, understanding the mechanism of current treatment and their specific targets could be a successful strategy for treating diabetic cognitive impairment. Our study was undertaken to demonstrate the effect of methanolic extract of *Allium stracheyi* Baker (MLEAS) on diabetes and associated complications using fructose-STZ induced diabetic model.

Streptozotocin (STZ), a diabetogenic antibiotic, is widely used to induce experimental diabetes by damaging pancreatic β-cells through the GLUT2 transporter, leading to DNA damage and insulin-secreting cell necrosis[9,42]. In this study, type 2 diabetes was induced using low-dose STZ combined with 10% fructose. Epidemiological evidence suggests that STZ can also contribute to cognitive impairment by disrupting insulin receptor signalling in the brain[43]. Diabetes and dementia share common features such as impaired glucose metabolism, reduced insulin sensitivity, and increased oxidative stress[45-46]. Consistently, diabetic rats in this study showed significant deficits in learning and memory in behavioural assessments.

Fructose–streptozotocin (FrSTZ) administration significantly increased blood glucose levels and reduced serum insulin compared with the control group, confirming induction of diabetes. Treatment with glibenclamide and MLEAS for 28 days significantly lowered blood glucose levels, indicating antidiabetic potential. Diabetic rats also showed marked body weight loss due to metabolic disturbances, including enhanced proteolysis and reduced protein synthesis caused by insulin deficiency. MLEAS treatment significantly improved body weight, possibly by restoring normal metabolic pathways such as gluconeogenesis and glycogenolysis.[47]

Furthermore, diabetic animals exhibited decreased serum insulin levels, reflecting impaired pancreatic β-cell function. Evaluation using HOMA-IR and HOMA-β indices indicated increased insulin resistance and reduced β-cell activity in diabetic rats compared to vehicle control.[48] However, treatment with MLEAS improved insulin sensitivity and β-cell function. These findings suggest that hyperglycemia in diabetic rats is associated with glucose intolerance and peripheral insulin resistance, which were effectively ameliorated by MLEAS treatment.

Insulin resistance is strongly associated with metabolic syndrome, including cardiovascular disease, obesity, and hyperlipidaemia. Dyslipidaemia plays an important role in the development of diabetic microvascular and cardiovascular complications. Under normal conditions, insulin promotes triglyceride breakdown by stimulating lipoprotein lipase activity. In diabetes, insulin deficiency reduces lipid clearance, leading to accumulation of triglycerides and chylomicrons in the blood. It also increases mobilization of free fatty acids from adipose tissue due to enhanced hormone-sensitive lipase activity. These fatty acids are converted in the liver into cholesterol, phospholipids, and lipoproteins. [49-51] In this study, diabetic rats showed elevated levels of CH, TG, LDL, and VLDL along with reduced HDL levels. These changes indicate metabolic imbalance and reduced insulin sensitivity. However, treatment with MLEAS significantly decreased elevated lipid levels. Additionally, MLEAS increased HDL levels, suggesting improvement in lipid metabolism in diabetic rats. Atherogenic index (AI) of plasma and coronary artery risk indices (Castelli's risk indices) were calculated to assess cardiovascular disease (CVD) risk.[52] Diabetic animals showed significantly higher AI and CARI values compared with control animals, indicating increased risk of cardiovascular complications. However, treatment with MLEAS significantly reduced these indices in diabetic rats. This protective effect may be attributed to improved lipid metabolism and modulation of metabolic pathways by phytochemicals present in MLEAS.

Insulin, once considered mainly a peripheral hormone, also plays a crucial role in brain function. It crosses the blood–brain barrier and acts on insulin receptors present in regions such as the hippocampus, cortex, and hypothalamus. Neurons contain high densities of these receptors, particularly at post-synaptic sites. Brain insulin signalling regulates body weight, learning, memory, and food intake. Disruption of this signalling contributes to neuronal dysfunction and is associated with cognitive decline and neurodegenerative disorders like Alzheimer's disease including abnormal tau phosphorylation, APP processing, excessive deposition of neurotoxic insoluble Aβ. [53-54] Elevated Plus Maze and Morris Water Maze are reliable models for assessing cognitive impairment in rats as demonstrated by improvement in motor activity. In this study, MLEAS treatment significantly reduced transfer latency and escape latency in diabetic animals, indicating improvement in learning and spatial memory. MLEAS also decreased elevated acetylcholinesterase activity observed in diabetic rats, suggesting enhanced cholinergic function. These findings indicate the nootropic potential of MLEAS against diabetes-induced cognitive deficits.

Diabetes-associated insulin deficiency leads to mitochondrial damage and increased oxidative stress, resulting in lipid peroxidation and reduced antioxidant levels, which contribute to neuronal dysfunction and memory impairment. [55-57] MLEAS treatment significantly restored antioxidant levels (GSH) and reduced oxidative stress markers such as MDA and nitric oxide. Furthermore, histopathological analysis of pancreatic and brain tissues supported the biochemical and behavioural findings. Overall, these results confirm the therapeutic and neuroprotective potential of MLEAS in diabetes-induced cognitive impairment. Therefore, it can be interpreted that chronic administration of MLEAS could mitigate cognitive decline, suggesting its protective role on neurobehavioral deficits as well as on brain tissue damage caused by fructose and STZ induced diabetes.

Overall, administering MLEAS effectively improved hyperglycaemia, limited weight loss, and exhibited notable hypolipidemic effects in T2DM rats. The observed decrease in oxidative stress, indicated by elevated SOD and GSH activities, may stem from the enhanced glycaemic control provided by the extract. Additionally, restoration of biochemical and behavioural parameters observed in diabetic demented rats were further supported by histopathological study of pancreatic and brain tissue. Consequently, MLEAS appears to restore the antioxidant enzyme activity, leading to an enhancement in cognition, learning, and memory in diabetic rats. MLEAS contain numerous bioactive phytocompounds with broad spectrum traditional and pharmacological action including polyphenols, flavonoids, alkaloids, tannin, glycoside, phytosterol. Additionally, initial quantitative analysis of the methanolic extract of *A. stracheyi* revealed that it contains notably high levels of phenolic compounds and flavonoids.[21] Interestingly, flavonoids, and polyphenols exert both antidiabetic and cognitive-enhancing effects[58], and our findings are consistent with these observations.

CONCLUSION

Diabetes is increasingly recognized as a major contributor to cognitive dysfunction, increasing the risk of mild cognitive impairment and accelerating progression to

dementia. Cognitive decline is therefore considered a significant long-term complication of diabetes. Multiple factors such as hyperglycemia, insulin deficiency, insulin resistance, dyslipidemia, and oxidative stress collectively contribute to neuronal damage and cognitive deterioration. The present study evaluated the therapeutic potential of MLEAS against diabetes-induced cognitive impairment associated with chronic hyperglycemia and altered insulin signalling.

Our findings demonstrated that MLEAS improved insulin resistance, possibly through regeneration of pancreatic β-cells and enhanced insulin expression. Additionally, MLEAS strengthened antioxidant defenses, reduced free radical generation, and protected learning and memory in fructose-STZ induced diabetic rats. These beneficial effects may be attributed to the high phenolic and flavonoid content of the extract. Histopathological analysis further supported these results by showing reduced pancreatic and neuronal damage. Overall, plant-based therapies like MLEAS may provide a cost-effective strategy for managing diabetes-associated cognitive complications, although further studies are required to identify the active phytochemicals responsible for these effects.

ACKNOWLEDGMENT
The authors sincerely acknowledge the support of Shri Guru Ram Rai University, Patel Nagar, Dehradun and School of Pharmaceutical Sciences and Technology, Sardar Bhagwan Singh University, Dehradun. Authors also extend sincere appreciation to all the technical staff for their valuable assistance and support.

FUNDING
No Financial support

AUTHORS CONTRIBUTION
Neeraj Kumar and Veerma Ram designed the study. Lata Bisht conducted the study. Lata Bisht analysed and interpreted the result under the guidance of Neeraj Kumar and Veerma Ram. Lata Bisht wrote the paper. All authors have read and approved the paper.

CONFLICT OF INTEREST
All authors affirm that they have no conflicts of interest related to the publication of this manuscript.

ETHICAL APPROVAL
This research got ethical approval by the Institutional Animal Ethical Committee of the School of Pharmaceutical Sciences, Shri Guru Ram Rai University, Patel Nagar-248001, Dehradun, Uttarakhand, India

REFRENCES

1. Alam S, Dhar A, Hasan M, Richi FT, Emon NU, Aziz MA, Mamun AA, Chowdhury MN, Hossain MJ, Kim JK, Kim B. Antidiabetic potential of commonly available fruit plants in Bangladesh: updates on prospective phytochemicals and their reported MoAs. Molecules. 2022 Dec 8;27(24):8709.
2. Ajiboye BO, Ekundayo BE, Salami AW, Osukoya AO, Komolafe K, Gaur S, Oyinloye BE, Jeje TO, Ojo OA. Neuroprotective effect of Lanneaegregia Alkaloid- rich leaf extracts in streptozotocin-induced diabetic rats. Toxicol Rep. 2024 Dec 1;13:101742. DOI: 10.1016/j.toxrep.2024.101742
3. Min AY, Yoo JM, Sok DE, Kim MR. Mulberry fruit prevents diabetes and diabetic dementia by regulation of blood glucose through upregulation of antioxidative activities and CREB/BDNF pathway in alloxan-induced diabetic mice. Oxid Med Cell Longev. 2020;2020(1):1298691. DOI: 10.1155/2020/1298691
4. Muriach M, Flores-Bellver M, Romero FJ, Barcia JM. Diabetes and the brain: oxidative stress, inflammation, and autophagy. Oxid Med Cell Longev. 2014;2014(1):102158. DOI: 10.1155/2014/102158
5. McCrimmon R. J, Ryan C. M., and Frier B. M., Diabetes and cognitive dysfunction. Lancet. (2012) 379, no. 9833, 2291–2299. DOI: 10.1016/S0140-6736(12)60360-2
6. Wang ZY, Deng YL, Zhou TY, Liu Y, Cao Y. Effects of natural extracts in cognitive function of healthy adults: a systematic review and network meta-analysis. Front Pharmacol. 2025 Mar 27;16:1573034. DOI: 10.3389/fphar.2025.1573034
7. Gruber JR, Ruf A, Süß ED, Tariverdian S, Ahrens KF, Schiweck C, Ebner-Priemer U, Edwin Thanarajah S, Reif A, Matura S. Impact of blood glucose on cognitive function in insulin resistance: novel insights from ambulatory assessment. Nutrition & Diabetes. 2024 Sep 11;14(1):74. DOI: 10.1038/s41387-024-00331-0
8. Aderinto N, Olatunji G, Abdulbasit M, Ashinze P, Faturoti O, Ajagbe A, Ukoaka B, Aboderin G. The impact of diabetes in cognitive impairment: A review of current evidence and prospects for future investigations. Medicine (Baltimore). 2023 Oct 27;102(43):e35557. DOI: DOI: 10.1097/MD.0000000000035557.
9. Ma WX, Tang J, Lei ZW, Li CY, Zhao LQ, Lin C, Sun T, Li ZY, Jiang YH, Jia JT, Liang CZ. Potential biochemical mechanisms of brain injury in diabetes mellitus. Aging and dis. 2020 Jul 23;11(4):978. DOI: 10.14336/AD.2019.0910.
10. Chen L, Feng P, Peng A, Qiu X, Lai W, Zhang L, Li W. Protective effects of isoquercitrin on streptozotocin-induced neurotoxicity. J Cell Mol Med. 2020 Sep;24(18):10458-67. DOI: DOI: 10.1111/jcmm.15658
11. Naz S, Imran I, Farooq MA, Shah SA, Ajmal I, Zahra Z, Aslam A, Sarwar MI, Shah J, Aleem A. Hyperglycemia-associated Alzheimer's-like

symptoms and other behavioraleffects attenuated by Plumeria obtusa L. Extract in alloxan-induced diabetic rats. Front Pharmacol. 2022 Dec 16;13:1077570. DOI: 10.3389/fphar.2022.1077570

12. Infante-Garcia C, Jose Ramos-Rodriguez J, Marin-Zambrana Y, Teresa Fernandez- Ponce M, Casas L, Mantell C, Garcia-Alloza M. Mango leaf extract improves central pathology and cognitive impairment in a type 2 diabetes mouse model. Brain Pathol. 2017 Jul;27(4):499-507. DOI: 10.1111/bpa.12433
13. Ansari P, Samia JF, Khan JT, Rafi MR, Rahman MS, Rahman AB, Abdel-Wahab YH, Seidel V. Protective effects of medicinal plant-based foods against diabetes: a review on pharmacology, phytochemistry, and molecular mechanisms. Nutrients. 2023 Jul 24;15(14):3266. DOI: 10.3390/nu15143266
14. Bisht L, Kumar N, Ram, V. Phytochemical Investigation and In Vitro Antidiabetic Potential of Leaf Extracts of Himalayan Herb: Allium stracheyi Baker. J Nat Remedies. 2025;25 (3):141–150. DOI: 10.18311/jnr/2025/45179
15. Truong DH, Nguyen DH, Ta NT, Bui AV, Do TH, Nguyen HC. Evaluation of the useof different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of Severiniabuxifolia. J Food Qual.2019;2019(1):8178294. DOI: 10.1155/2019/8178294
16. Kumar M, Prabhakar P, Hameed S, Kumar M, Shekhar R, Kumar B, Dikshit H. Evaluation of the Antidiabetic Potential of Methanolic Extract of Clerodendrum infortunatum Compared to Metformin in Streptozotocin-induced Diabetic Rats. International Journal of Applied and Basic Medical Research. 2025 Apr 1;15(2):78-84. DOI: 10.4103/ijabmr.ijabmr_580_24
17. Yadav A. CPCSEA Guidelines for Laboratory Animal Facility. Int J Pharm Sci.2020;2(1):09-13. 10.33545/26647613.2020.v2.i1a.10
18. OECD. Test No. 420: Acute Oral Toxicity – Fixed Dose Procedure. Paris:Organisation for Economic Co-operation and Development .2002. DOI:10.1787/9789264070943-en.
19. Mehta BK, Banerjee S. Characterization of Cognitive Impairment in Type 2 Diabetic Rats. Indian J Pharm Sci.2017 Sep 1;79(5). DOI: 10.4172/pharmaceutical-sciences.1000292.
20. Oraby MA, El-Yamany MF, Safar MM, Assaf N, Ghoneim HA. Dapagliflozin attenuates early markers of diabetic nephropathy in fructose-streptozotocin-induced diabetes in rats. Biomed Pharmacother. 2019 Jan 1;109:910-20. DOI: 10.1016/j.biopha.2018.10.100.
21. Kaliaperumal K, Bhat BA, Subramanian K, Ramakrishnan T, Chakravarthy E, Al-Keridis LA, Ahmad I, Alabdallah NM, Saeed M, Karunakaran R. In-vivo anti-hyperglycemic effect of herbal extracts Tribulus terrestris (L) and Curcuma amada(R) on streptozotocin-induced diabetic rats and its associated histopathological studies. Heliyon. 2024 Jan 15;10(1). DOI: 10.1016/j.heliyon.2024.e24009
22. Motto AE, Lawson-Evi P, Eklu-Gadegbeku K. Antidiabetic and antioxidant potential of total extract and supernatant fraction of the roots of Anogeissus leiocarpus in HFD-fed and Streptozocin-induced diabetic rats. Biomedicine & Pharmacotherapy. 2022 Oct 1;154:113578. DOI: 10.1016/j.biopha.2022.113578.
23. Bristow AF, Das RG. WHO international reference reagents for human proinsulin and human insulin C-peptide. J Biol Stand. 1988 Jan 1;16(3):179-86. DOI: 10.1016/0092-1157(88)90005-4
24. Ram H, Kumar P, Purohit A, Kashyap P, Kumar S, Kumar S, Singh G, Alqarawi AA, Hashem A, Abd-Allah EF, Al-Arjani AB. Improvements in HOMA indices and pancreatic endocrinal tissues in type 2-diabetic rats by DPP-4 inhibition and antioxidant potential of an ethanol fruit extract of Withaniacoagulans. NutrMetabol. 2021 Apr 21;18(1):43. DOI: 10.1186/s12986-021-00547-2
25. Saha MR, Dey P, Sarkar I, De Sarker D, Haldar B, Chaudhuri TK, Sen A. Acacia nilotica leaf improves insulin resistance and hyperglycemia associated acute hepatic injury and nephrotoxicity by improving systemic antioxidant status in diabetic mice. Jethnopharmacol. 2018 Jan 10;210:275-86. DOI: 10.1016/j.jep.2017.08.036.
26. Iheagwam FN, Iheagwam OT, Onuoha MK, Ogunlana OO, Chinedu SN. Terminalia catappa aqueous leaf extract reverses insulin resistance, improves glucose transport and activates PI3K/AKT signalling in high fat/streptozotocin-induced diabetic rats. Sci Rep. 2022 Jun 23;12(1):10711. DOI: 10.1038/s41598-022-15114-9
27. Ashwini S, Bobby Z, Joseph M, Jacob SE, Padmapriya R. Insulin plant (Costuspictus) extract improves insulin sensitivity and ameliorates atherogenic dyslipidaemia in fructose induced insulin resistant rats: Molecular mechanism. J Funct Foods. 2015 Aug 1;17:749-60. DOI: 10.1016/j.jff.2015.06.024
28. Phatak RS, Khanwelkar CC, Matule SM, Datkhile KD, Hendre AS. Antihyperlipidemic activity of Murrayakoenigii leaves methanolic and aqueous extracts on serum lipid profile of high fat-fructose fed rats. Pharmacogn J. 2019;11(4). DOI: 10.5530/pj.2019.11.134.

29. Pellow S, Chopin P, File SE, Briley M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods. 1985 Aug 1;14(3):149-67. DOI: 10.1016/0165-0270(85)90031-7

30. Chikezie CM, Ojiako OA, Emejulu AA, Chikezie PC. Atherogenicity of diabetic rats administered single and combinatorial herbal extracts. Bull Fac Pharm Cairo Univ. 2018 Dec 1;56(2):169-74. DOI: /10.1016/j.bfopcu.2018.10.001

31. Auti ST, Kulkarni YA. Neuroprotective effect of cardamom oil against aluminium induced neurotoxicity in rats. Front Neurol. 2019 Apr 30;10:399. DOI: 10.3389/fneur.2019.00399

32. Shunan D, Yu M, Guan H, Zhou Y. Neuroprotective effect of Betalain against AlCl3- induced Alzheimer's disease in Sprague Dawley Rats via putative modulation of oxidative stress and nuclear factor kappa B (NF-κB) signaling pathway. Biomed Pharmacother. 2021 May 1;137:111369. DOI: 10.1016/j.biopha.2021.111369.

33. Ansari S, Uniyal S, Khali A, Gaur R, Kalita K. Ethanolic extract of Actinidia chinensis var. delicious fruit ameliorates scopolamine-induced cognitive impairment in rats. Pharmacol Res-Mod Chin Med. 2024 Sep 1;12:100476.

34. Neje P, Kulkarni S, Dabhekar S, Taksande B, Umekar M, Mangrulkar S. Neuroprotective and neuromodulatory role of agmatine in mitigating simulated microgravity-induced cognitive and behavioral deficits in rats. Life Sci Space Res. 2025 Sep 14. DOI: 10.1016/j.prmcm.2024.100476.

35. EllmanGL. Tissue sulfhydryl groups. Arch. Biochem Biophys. 1959 May 1;82(1): 70–77. DOI: 10.1016/0003-9861(59)90090-6.

36. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. J Biochem Biophys. 21, 130–132

37. JainkangL, Rei E, Hideaki K, Akitane M. 1990. Antioxidant action of guilingi in the brain of rats with FeCl 3 induced epilepsy. Free RadicBiol Med. 9: 451–454. DOI: 10.1016/0891-5849(90)90023-c.

38. Kumar MV, Gupta YK. Effect of different extracts of Centellaasiatica on cognition and markers of oxidative stress in rats. J ethnopharmacol. 2002 Feb 1;79(2):253-60. DOI: 10.1016/s0378-8741(01)00394-4.

39. Jasoria Y, Agrawal M, Kumar S, Chaudhary H, Sahu KK, Singhal M, Arora S, Chandolia P, Saha S, Singh K, Mahour S. In-vivo evaluation of neuroprotective effect of Chinese plant calendula officinalis Linn. Flower Extract against Aluminium chloride-induced Alzheimer's in Wistar rats. Pharmacol Res - Mod Chin Med. 2024 Sep 1;12:100458. DOI: 10.1016/j.prmcm.2024.100458

40. Ellman GL, Courtney KD, Andres Jr V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem pharmacol. 1961 Jul 1;7(2):88-95. DOI: 10.1016/0006-2952(61)90145-9.

41. Alsharif KF, Hamad AA, Alblihd MA, Ali FA, Mohammed SA, Theyab A, Al-Amer OM, Almuqati MS, Almalki AA, Albarakati AJ, Alzahrani KJ. Melatonin downregulates the increased hepatic alpha-fetoprotein expression and restores pancreatic beta cells in a streptozotocin-induced diabetic rat model: a clinical, biochemical, immunohistochemical, and descriptive histopathological study. Front Vet Sci.. 2023 Aug 16;10:1214533. DOI: 10.3389/fvets.2023.1214533

42. Elsner M, Guldbakke B, Tiedge M, Munday R, Lenzen S. Relative importance of transport and alkylation for pancreatic beta-cell toxicity of streptozotocin. Diabetologia. 2000 Nov;43(12):1528-33. DOI: 10.1007/s001250051564

43. Hoyer S, Lee SK, Löffler T, Schliebs R. Inhibition of the neuronal insulin receptor an in vivo model for sporadic Alzheimer disease?. Ann N Y Acad Sci. 2000 Dec;920(1):256-8. DOI: . 10.1111/j.1749-6632.2000.tb06932.x

44. Iwara IA, Mboso EO, Ibor OR, Elot K, Igajah C, Bassey AA, Eteng OE, Mgbeje BI, Igile GO, Eteng MU, Arukwe A. Modulatory effects of extract of Heinsiacrinita against fructose/streptozotocin-induced oxidative stress in diabetic rat models. Heliyon. 2023 Nov 1;9(11). DOI: 10.1111/j.1749-6632.2000.tb06932.x.

45. Xu WL, von Strauss E, Qiu CX, Winblad B, Fratiglioni L. Uncontrolled diabetes increases the risk of Alzheimer's disease: a population-based cohort study. Diabetologia. 2009 Jun;52(6):1031-9. DOI: 10.1007/s00125-009-1323-x

46. Clodfelder-Miller BJ, Zmijewska AA, Johnson GV, Jope RS. Tau is hyperphosphorylated at multiple sites in mouse brain in vivo after streptozotocin- induced insulin deficiency. Diabetes. 2006 Dec 1;55(12):3320-5. DOI: 10.2337/db06-0485

47. Liu ZH, Chen HG, Wu PF, Yao Q, Cheng HK, Yu W, Liu C. FlosPuerariae Extract Ameliorates Cognitive Impairment in Streptozotocin-Induced Diabetic Mice. EvidBased ComplementAlternat Med. 2015;2015(1):873243. DOI: 10.1155/2015/873243

48. Pan X, Olatunji OJ, Basit A, Sripetthong S, Nalinbenjapun S, Ovatlarnporn C. Insights into the phytochemical profiling, antidiabetic and antioxidant potentials of Lepionurussylvestris Blume extract in fructose/streptozotocin-induced diabetic rats. Front

Pharmacol. 2024 Jul 12;15:1424346. DOI: 10.3389/fphar.2024.1424346

49. Motto AE, Lawson-Evi P, Eklu-Gadegbeku K. Antidiabetic and antioxidant potential of total extract and supernatant fraction of the roots of Anogeissus leiocarpus in HFD- fed and Streptozocin-induced diabetic rats. Biomed Pharmacother. 2022 Oct 1;154:113578. DOI: 10.1016/j.biopha.2022.113578

50. Jifar WW, Debele GR, Kanfe SG, Mule CT. Evaluation of in vivo antidiabetic, Antidyslipidemic and in vitro anti-oxidant activity of extract and solvent fractions of Discopodium penninervum Hoschst Leaf in mice: Normoglycemic and Streptozocin- Induced Model. J Exp Pharmacol. 2022 Jan 1:317-30. DOI: 10.2147/JEP.S378166

51. Heeren J, Scheja L. Metabolic-associated fatty liver disease and lipoprotein metabolism. Mol metab. 2021 Aug 1;50:101238. DOI: 10.1016/j.molmet.2021.101238

52. Gomathi, D, Ravikumar G, Kalaiselvi M, Devaki K, Uma, C. Effect of Evolvulus alsinoides on lipid metabolism of streptozotocin induced diabetic rats. Asian Pac. J. Trop. Dis. 2013, 3, 184–188. DOI: 10.1016/S2222-1808(13)60037-7

53. Li Y, Feng Y, Li S, Ma Y, Lin J, Wan J, Zhao M. The atherogenic index of plasma (AIP) is a predictor for the severity of coronary artery disease. Front Cardiovasc Med. 2023 Jun 27;10:1140215. DOI: DOI: 10.3389/fcvm.2023.1140215

54. Kim B, Feldman EL. Insulin resistance as a key link for the increased risk of cognitive impairment in the metabolic syndrome. Exp Mol Med. 2015 Mar;47(3):e149. DOI: 10.1038/emm.2015.3

55. Sędzikowska A, Szablewski L. Insulin and insulin resistance in Alzheimer’s disease. Int J Mol Sci. 2021 Sep 15;22(18):9987. DOI: 10.3390/ijms22189987

56. Xu X, Guo L, Tian G. Diabetes cognitive impairments and the effect of traditional Chinese herbs. Evid Based Complement Altern Med. 2013;2013(1):649396. DOI: 10.1155/2013/649396

57. Comin D, Gazarini L, Zanoni JN, Milani H, de Oliveira RM. Vitamin E improves learning performance and changes the expression of nitric oxide-producing neurons in the brains of diabetic rats. Behav Brain Res. 2010 Jun 26;210(1):38-45. DOI: 10.1016/j.bbr.2010.02.001

58. Usha T, Middha SK, Brahma BK, Narzary D, Goyal AK. In silico and in vivo based scientific evaluation of traditional anti-diabetic herb Hodgsoniaheteroclita. Bangladesh J Pharmacol. 2017 May 15;12(2):165-6. DOI: 10.3329/bjp.v12i2.31122