NEUROPHARMACOLOGICAL EVALUATION OF *BRYOPHYLLUM PINNATUM* IN DIABETES INDUCED RODENT MODELS

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ABSTRACT

Diabetics frequently have brain dysfunction. However, an increasing amount of evidence suggests that medicinal herbs have positive effects on brain function and insulin sensitivity. Thus, the current study set out to examine how giving *Bryophyllum pinnatum* methanolic root extract affected the rat's blood glucose levels and learning and memory impairment in type 2 diabetics. This was accomplished by randomly assigning six healthy and twenty-four rats with type 2 diabetes to five groups: the diabetic group (D), the diabetic group getting 200 mg/kg of MEBP, the diabetic group receiving 400 mg/kg of MEBP, the diabetic group receiving Vidagliptin (3 mg/kg), and the healthy control group (Con). Nicotinamide (120 mg/kg) and streptozotocin (65 mg/kg) were administered intraperitoneally at 15-minute intervals to induce diabetes. The experimental groups received vidagliptin and *B. pinnatum* for 21 days. The lipid profile and blood glucose levels were assessed. The Cook's Pole Climbing test, the Novel Object Recognition Test, and the Shuttle Box Test were used to conduct the nootropic activity. Rats with diabetes showed a markedly elevated lipid profile and blood glucose levels in comparison to rats in good condition. In the PAL acquisition trial (STLa) and retention test (STLr), diabetic rats exhibited substantially shorter step through latency compared to both the control and treated groups. When comparing the diabetic group to the control and treated groups, the number of trials to acquisition is noticeably higher. Compared to the control and treated groups, the diabetic group's percentage of time spent with the novel object was much lower. Compared to the diabetes group, the control and treated groups took much less time to ascend the pole. The memory loss in streptozotocin-nicotinamide-induced type 2 diabetic rats was alleviated after 21 days of MEBP (400 mg/kg), presumably as a result of hypolipidemic and hypoglycemic effects. These findings imply that administering *B. pinnatum* root extract might be a useful strategy for treating behavioral abnormalities brought on by diabetes.

Keywords: Brain dysfunction, Insulin sensitivity, Memory impairment, Nootropic activity, Hypolipidemic, and Hypoglycemic.

INTRODUCTION

Alzheimer's disease (AD), the most prevalent form of dementia, is associated with Type II Diabetes. There is evidence from several epidemiological research that insulin resistance raises the risk of AD and dementia. Animal and *in vitro* research suggests that insulin resistance may play a role in the pathophysiology of AD via a variety of mechanisms. In AD, endocrine problems, particularly diabetes, are so prevalent that they are considered a form of diabetes. Diabetes has been shown to have a negative impact on synaptic connectivity, brain morphology (brain shrinkage), and memory processing (recognition and retrieval), all of which contribute to the pathophysiology of AD. Recent research suggests that AD is a type of diabetes that only affects the brain. T2D increases the risk of AD by many times and is the most prevalent and important co-morbidity of AD. Interestingly, AD has several characteristics with type-2 diabetes, including hyperglycemia, hyperinsulinemia, insulin resistance, metabolic dysfunctions, and chronic inflammation. Additional important elements that connect AD and T2D include oxidative stress, mitochondrial abnormalities, malfunctioning protein O-GlcNAcylation, skewed energy metabolism, and cholesterol alterations.

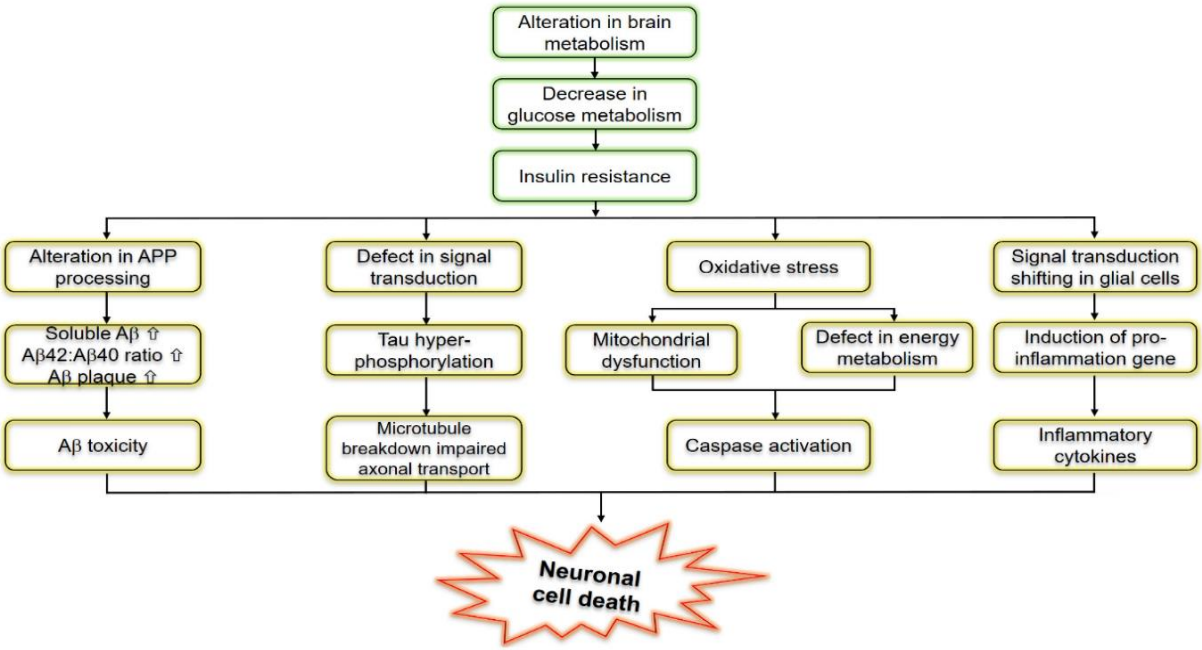

Figure 1: Schematic representation of molecular pathways linking insulin resistance and Alzheimer disease

Additionally, insulin is a key player in cognitive processes, and it is highly concentrated in areas like the hippocampus that are involved in memory consolidation and development. According to several recent research, memory and other executive functions are negatively impacted by decreased hippocampal insulin signaling. This is because insulin signaling declines and insulin resistance develops at the same time. This discussion supports the idea that hyperinsulinemia/insulin resistance and the resulting diseases, such as AD and T2D, are closely related. Insulin signaling in the central nervous system is reduced by peripheral insulin resistance, which then causes changes in brain metabolism. Central insulin resistance is linked to increased Aβ toxicity, Tau hyperphosphorylation, oxidative stress, and neuroinflammation, all of which contribute to neurodegeneration.

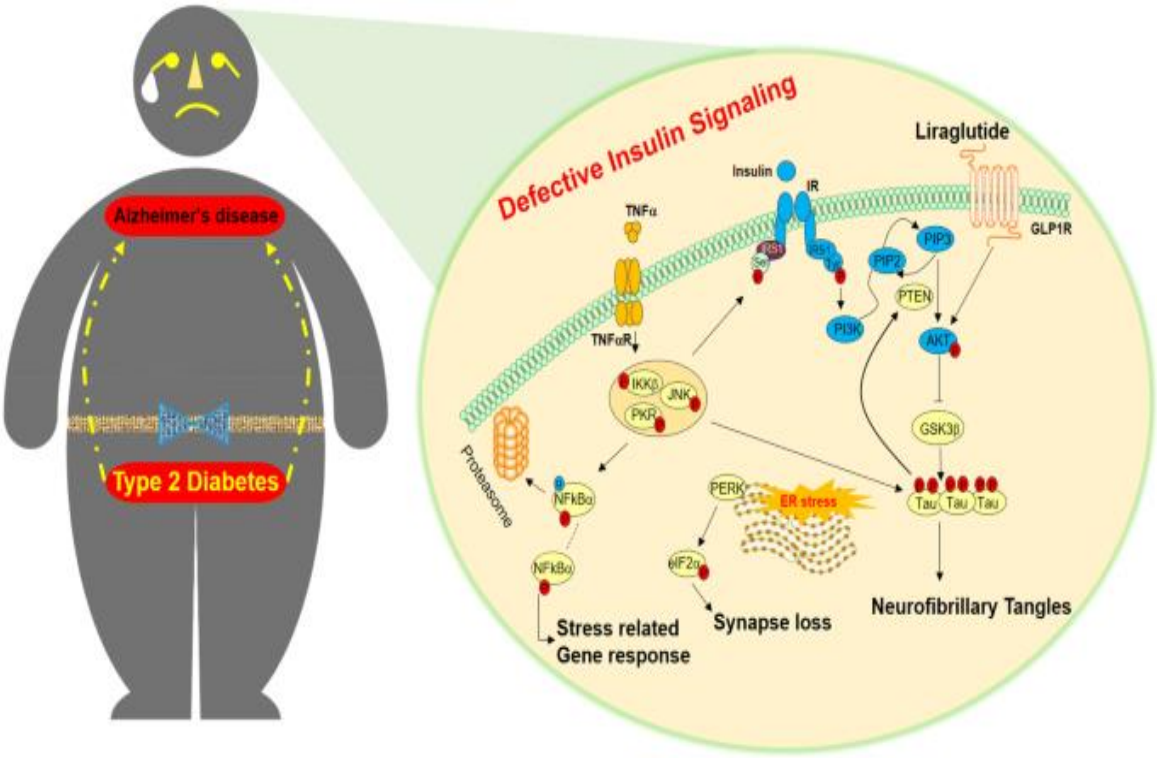

Figure 2: Potential molecular mechanisms underlying defective insulin signalling in Alzheimer's disease

MATERIALS & METHODS

Collection of Plant Materials and Extraction

Roots of *Bryophyllum pinnatum* were collected from a local area during December 2023. Crude plant material was identified and authenticated by Botanist from Government Degree College Kukatpally, Hyderabad. Roots were dried in the shade; coarsely powdered and powdered material was used for the extraction process.

Chemicals and reagents

Streptozotocin and nicotinamide used were a product of Tokyo chemicals Pvt. Ltd and Research Lab Fine Chem Industries, Mumbai, respectively. Vidagliptin drug used was a product of Torrent Pharmaceuticals Ltd. Biochemical kits and all other chemicals were of analytical grade.

Preparation of extract

The powdered crude material of Bryophyllum pinnatum roots was extracted with methanol by maceration and crude extract obtained was evaporated to a solid mass and stored in desiccators to remove remaining moisture, if present.

Selection of Animals

The selection consisted of thirty male Albino Wistar Rats, weighing between 200 and 250 g and aged between 6 and 8 weeks. Before the studies began, they were acclimated to the surroundings for a week. Ad libitum access to purified water and regular food was provided to all rats in their cages, which were kept under standard circumstances (12:12 hour's light/dark cycle, 22±2℃ temperature, and 50±5% humidity).

Acute toxicity studies

An acute toxicity study up and down procedure (OECD guideline-425) was carried out for methanolic root extract of *Bryophyllum pinnatum* on female Albino Swiss mice.

Research Design

Table 1: Design of Experimentation

S. NO	GROUP	TREATMENT
1	Control	Normal Saline
2	Disease Control	STZ-NA injection + Saline
3	MEBP (T1)	STZ-NA injection + MEBP (200 mg/Kg *p.o* for 21 days)
4	MEBP (T2)	STZ-NA injection + MEBP (400 mg/Kg *p.o* for 21 days)
5	Standard	STZ-NA injection + Vidagliptin (3 mg/Kg *p.o* for 21 days)

Introducing diabetes

All experimental rats were given no food for 12 hours following the adaption phase, and then they received injections to induce diabetes. Intraperitoneally, the rats received a single injection of streptozotocin (STZ; 65 mg/kg) diluted in 0.1 mol citrate buffer (pH 4.5). The rats were then given an injection of nicotinamide (120 mg/kg, soluble in normal saline) following the fasting interval before 15 minutes to receiving an injection of STZ. Injecting the same amount of buffer solution into healthy rats was done. Blood was extracted from the tail region after 72 hours, and a glucometer was used to determine the fasting blood sugar (FBS) levels. Diabetic rats were those whose fasting blood sugar levels were more than 200 mg/dL.

***In vivo* Screening Models**

The *in vivo* evaluation of nootropic activity of the methanolic extract of *Bryophyllum pinnatum* roots was carried out in the following models.

Passive Avoidance Learning Test: Shuttle box

To assess the animal's avoidance learning and memory skills, a shuttle box device was used in a passive avoidance learning (PAL) test. The apparatus was divided into portions that were illuminated and dark, with a guillotine door between the two and a grid floor made of stainless steel rods that was connected to a shock generator. Initially, two trials were conducted to acclimate the rats to the apparatus. After 30 seconds, the rat was put in a bright area and faced away from the entrance. The guillotine door was then manually opened. After demonstrating an innate preference for the dark area, the door was shut and the rat was put back in its original cage 30 seconds later. After 30 minutes, same test was conducted again, and the same interval before the initial acquisition trial was observed. The entry latency (also known as the step-through latency, or STLa) to the dark region was measured after the animal brought all four paws into the area. Following the lowering of the guillotine door, a 2-second, 0.8 mA electrical shock was administered. The rat was put back in its own cage after 30 seconds. The rat was given foot shocks each time it returned to the dark portion of the test, which was repeated two minutes later. Training was terminated and the number of trials was noted once the rat had been in the light area for 120 seconds in a row.

Twenty-four hours following the PAL acquisition experiment, the retention test was carried out. The step-through latency (STLr) was measured when the rat was placed in the light section, as in PAL training, and the guillotine door was opened to it five seconds later. Note that trials, STL-a, and STL-r are short-term indicators.

Novel Object Recognition Test

Rats may be tested for various stages of learning and memory using the object recognition test (ORT), sometimes called the novel object recognition test (NOR), which is comparatively quick and effective. Three days were allotted for the test: the days of habituation, training, and testing. Take the rat out of its cage and put it in the centre of the empty, open arena on the day of habituation. Give everyone five minutes to explore the arena at their leisure. After five minutes, take the rat out and put it in a holding cage. After 24 hours of habituation, on the day of training, take the rat out of its cage and put it in the middle of the arena, equally spaced from the two identical items. One new item and one utilized during the training session should be placed in opposing quadrants of the arena on the day of the test. For every mouse, utilize the same spots that were used for training. Place the rat in the middle of the arena, halfway between the new and the familiar objects, after removing it from its home cage. Let them explore freely for ten minutes. Remove the rat at the end of the experiment and put it in the holding cage.

In the testing portion of the experiment, the amount of time spent smelling or touching each object but not leaning against, sitting on, or standing on it was noted. The percentage of time spent with the items was computed.

Cook's Pole Climbing Test

To prevent foot shock, the rats had to learn how to hop on a pole. A foot shock of 1.0 mA served as the unconditioned stimulus, while a tone of 50 Hz served as the conditioned stimulus. The animal was first given a minute to adjust in the chamber before beginning the training process. After then, conditioned and unconditioned stimuli were presented one after the other for a duration of 15 seconds each. The experiment stopped either 30 seconds later or when the animal jumped on the pole in response, whichever came first. The experiment was conducted on an animal every day for ten days. In response to a buzzer, a trained animal would either react on its own or without waiting for a shock. Both before and after medication treatment, the ability to recall unpleasant stimuli that were established throughout the learning process was assessed. It was measured by how long it took the animals to leap on the pole and avoid shock.

Estimation of biochemical parameters

Blood samples were taken at the conclusion of the therapy, allowed to clot for 30 minutes at room temperature, and the animals were held for an overnight fast. Prior to analysis, the serum was separated and kept at -20°C after the blood samples were centrifuged for 20 minutes at

5000 rpm. With the use of a semi-auto-analyzer, the biochemical parameters for blood glucose, HDL, LDL, total cholesterol, and triglycerides were ascertained.

Statistical Analysis

The results were expressed as mean ± SEM. The effects were exposed to the statistical investigation by using one way ANOVA followed by Dunnett's test $P<0.05$, $P< 0.01$ and $P<0.001$ were considered to be statistically significant.

RESULTS & DISCUSSION

Methanolic extract of *Bryophyllum pinnatum* roots was explored for its antidiabetic and nootropic activities using suitable animal models. All the results obtained in the study were included below.

Preliminary Phytochemical analysis

The preliminary phytochemical investigation of methanolic extract of *Bryophyllum pinnatum* roots showed the presence of Alkaloids, Flavonoids, Phenolic compounds, Sterols, Carbohydrates, Protein, Amino acids, Tannins and Fixed oils.

Acute toxicity studies

Methanolic extract of *Bryophyllum pinnatum* roots was tested on albino Swiss mice up to a dose of 2000mg/kg bd. wt. The animal did not exhibit any signs of toxicity or mortality up to 2000mg/kg bd. wt. various morphological and behavioural characters were observed during the study. The other parameters, like food and water consumption, were also observed. All the animals were found to be safe even after 14days of observation. Hence the extract was found to be safe up to 2000 mg/kg bd. wt.

Passive Avoidance Learning Test: Shuttle box

Table 2: Comparison of the groups regarding (STL-a)

GROUP	TREATMENT	STL-a (Seconds)
I	Normal control	16±0.36
II	Disease control	$4\pm0.36^{***}$
III	MEBP (200 mg/kg)	$11\pm0.36^{**Bb}$
IV	MEBP (400 mg/kg)	$14\pm0.36^{*Bc}$

V	Vidagliptin (3 mg/kg)	15.5±0.22^{B}

The values are estimated as mean ± SEM (n=6). The results were analyzed using one way ANOVA and then Dunett's multiple comparison against control (***=p<0.001, **=p<0.01, *= p<0.05), against Disease control (B=p<0.01), against standard (b=p<0.01, c=p<0.05).

Table 3: The number of acquisition trials between the experimental groups

GROUP	TREATMENT	No. of Acquisition trials
I	Normal control	9±0.36
II	Disease control	19±0.36**
III	MEBP (200 mg/kg)	16.33±0.21^{**Cb}
IV	MEBP (400 mg/kg)	12.66±0.33^{*Bc}
V	Vidagliptin (3 mg/kg)	10.5±0.22^{B}

The values are estimated as mean ± SEM (n=6). The results were analyzed using one way ANOVA and then Dunett's multiple comparison against control (**=p<0.01, *= p<0.05), against Disease control (B=p<0.01, C=p<0.05), against standard (b=p<0.01, c=p<0.05).

Table 4: Comparison of the groups regarding (STL-r)

GROUP	TREATMENT	STL-r (Seconds)
I	Normal control	247.3±1.89
II	Disease control	75.6±1.40***
III	MEBP (200 mg/kg)	164.6±1.28^{**Bb}
IV	MEBP (400 mg/kg)	196±1.18^{*Bc}
V	Vidagliptin (3 mg/kg)	219.16±1.42^{B}

The values are estimated as mean ± SEM (n=6). The results were analyzed using one way ANOVA and then Dunett's multiple comparison against control (***=p<0.001, **=p<0.01, *= p<0.05), against Disease control (B=p<0.01), against standard (b=p<0.01, c=p<0.05).

Novel Object Recognition Test

Table 5: Comparison of the groups regarding the Percent of time spent with the objects

GROUP	TREATMENT	TRAINING PHASE (Percentage)	
		Familiar object	Novel object
I	Normal control	26±0.36	64±0.36
II	Disease Control	54±0.73***	26±0.73***
III	MEBP (200 mg/kg)	36.06±0.32**Bc	45.93±0.32**Cb
IV	MEBP (400 mg/kg)	32.16±0.27*B	52.83±0.27*Bc
V	Vidagliptin (3 mg/kg)	28.9±0.42B	61.1±0.42B

The values are estimated as mean ± SEM (n=6). The results were analyzed using one way ANOVA and then Dunett's multiple comparison against control (***=p<0.001, **=p<0.01, *= p<0.05), against Disease control (B=p<0.01, C=p<0.05), against standard (b=p<0.01, c=p<0.05).

Cook's Pole Climbing Test

Table 6: Comparison of the groups regarding Time taken to climb the pole

GROUP	TREATMENT	TIME TAKEN TO CLIMB THE POLE (Seconds)	
		Before treatment (Day 0)	After treatment (Day 21)
I	Normal control	13.26±0.31	12±0.36
II	Disease Control	26.63±0.35**	32.6±0.33***
III	MEBP (200mg/kg)	25.66±0.27**	20.56±0.42**Bb
IV	MEBP (400mg/kg)	27±0.36**	16.13±0.29*Bc
V	Vidagliptin (3mg/kg)	27.66±0.25**	13.7±0.34B

The values are estimated as mean ± SEM (n=6). The results were analyzed using one way ANOVA and then Dunett's multiple comparison against control (***=p<0.001, **=p<0.01, *= p<0.05), against Disease control (B=p<0.01), against standard (b=p<0.01, c=p<0.05).

Biochemical parameters

Blood glucose level

Table 7: Comparision of Blood glucose levels at 0th and 21st day

GROUP	TREATMENT	0TH DAY	21ST DAY
I	Normal control	93.1±0.81	94.75±1.01
II	Disease Control	227.817±0.77	245.83±1.29***
III	MEBP (200 mg/kg)	226.433±0.74	160.43±1.41**Cb
IV	MEBP (400 mg/kg)	227.8±1.08	126±0.88*B
V	Vidagliptin (3 mg/kg)	230±0.55	104.83±1.14B

The values are estimated as mean ± SEM (n=6). The results were analyzed using one way ANOVA and then Dunett's multiple comparison against control (***=p<0.001, **=p<0.01, *= p<0.05), against Disease control (B=p<0.01, C=p<0.05), against standard (b=p<0.01).

Lipid Profile

Table 4.13: Comparison of lipid levels at 0th and 21st day

GROUP	TREATMENT	TC		TG		HDL		LDL	
		Day-0	Day-21	Day-0	Day-21	Day-0	Day-21	Day-0	Day-21
I	Normal control	76.2± 1.21	77.5±0. 76	86.36 ±0.20	87.5±1. 33	52.43 ±0.43	52.5±0. 70	25.66 ±0.42	24.25± 1.13
II	Disease Control	134.6 ±1.35	142.83 ±0.60**	137.5 ±0.76	145.03 ±1.29**	16.8± 0.33	11.6±0. 66***	91.9± 0.58	95.8±1. 53***
III	MEBP (200 mg/kg)	136.2 ±0.76	124.3± 1.40**Cb	136.8 ±1.20	117.3± 1.22**Bb	15.8± 0.90	27.8±0. 72**Bb	92.7± 0.80	74.4±1. 45**Cb

IV	MEBP (400 mg/kg)	137.9 ±0.54	104.3± 1.05^{*Bc}	141±1 .18	109.1± 1.41^{*Bc}	16.4± 0.42	36.8±0. 60^{*Bc}	91.95 ±0.67	56±1.3 8^{*Bc}
V	Vidagliptin (3 mg/kg)	136.5 ±1.25	85.5±1. 43^{B}	139.9 ±0.73	94.8±1. 16^{B}	16±0. 51	46.2±0. 47^{B}	93.81 ±0.94	34±1.3 1^{B}

The values are estimated as mean ± SEM (n=6). The results were analyzed using one way ANOVA and then Dunett's multiple comparison against control (***=p<0.001, **=p<0.01, *= p<0.05), against Disease control (B=p<0.01, C=p<0.05), against standard (b=p<0.01, c=p<0.05).

Diabetes-related cognitive deterioration

According to the data, rats with type 2 diabetes produced by STZ–NA had higher blood glucose levels, total cholesterol, TG, and LDL than rats in good health. However, compared to healthy rats, diabetic rats had lower levels of HDL and insulin sensitivity. The lipid metabolism of the hippocampus is linked to elevated TG and total cholesterol levels in diabetes. It has been noted that diabetes impairs cognitive function. It is yet unclear how elevated lipid profiles relate to dysfunctional brain function. The hippocampus, the main brain region involved in memory storage and recall, can have its integrity partially compromised by an increase in lipid profiles, according to current research. According to the study's findings, diabetes induces a decline in cognitive memory, active avoidance, and general passive avoidance. These results are consistent with earlier research that documented behavioural abnormalities in animals caused by diabetes.

In diabetic individuals, glucose dysregulation and reduced insulin function are key factors in the onset and progression of dementia. Brain grey matter volume sadness and cognitive impairment are linked to elevated blood glucose levels. It has been suggested that a number of pathways are crucial in the diabetic cognitive abnormalities observed in rats with STZ-NA-induced diabetes. The accumulation of extracellular Aβ plaques and the development of neurofibrillary tangles are caused by insulin resistance. Neurodegeneration in AD and diabetic individuals was enhanced by both routes. Conversely, long-term hyperglycemia promotes oxidative stress and inflammation in the hippocampal region. In the dentate gyrus of the hippocampus and the sub ventricular zone, persistent inflammation and oxidative stress can cause damage to cell membranes, DNA, and protein structure, which can lead to decreased neurogenesis, disruption of neuronal functioning, loss of synapses, a decrease in neurotransmitter release, and increased neuronal death. Memory loss results from a reduction

in the quantity and functionality of neurons in the hippocampus. Patients with diabetes acquire dementia through a variety of mechanisms. Diabetic encephalopathy in diabetic people has a complicated and poorly understood molecular process.

Impact of MEBP on cognitive deficits caused by diabetes

The Shuttle box test, Cook's pole climbing test, and the Novel object recognition test were used to assess MEBP's *in vivo* nootropic activity. The diabetic group's STL-a and STL-r levels significantly decreased in the Shuttle box test as compared to the group receiving 400 mg/kg of MEBP. Where the diseased group had a considerably higher number of trials to acquisition than the treated group. During NORT, the diabetic group spent a lower percentage of time with the novel item than the familiar object, in contrast to the control and MEBP 400 mg/kg groups. Compared to the other group of animals, the diabetes group took longer to mount the pole in the CPC test.

According to the study's findings, compared to the sedentary diabetic rats, MEBP ingestion enhanced active avoidance memory, non-spatial cognitive memory, and passive avoidance memory. Additionally, MEBP raised HDL levels and glucose homeostasis while lowering total cholesterol, TG, and LDL levels. MEBP 400 mg/kg was shown to be more efficacious than MEBP 200 mg/kg in this research. Though the molecular mechanism behind this protective effect is unclear and requires more research, this is the first study to assess the preventive impact of *Bryophyllum pinnatum* methanolic root extract on behavioral impairment under hyperglycemic conditions. Glycemic control is the primary goal of diabetic treatment, and certain herbs, including B. pinnatum, are highly beneficial in this regard. However, further research is required to determine the precise components of the plant that provide the protection.

CONCLUSION

Based on the data, I deduce that long-term administration of 400 mg/kg of MEBP ameliorated the lipid profile and glucose levels, hence improving insulin sensitivity and memory deficit in STZ-NA-induced type 2 diabetic rats. According to these findings, *B. pinnatum* may be a useful therapy for behavioral deficits brought on by diabetes. Furthermore, this plant's noteworthy ability to improve cognition and reduce blood sugar levels may be linked to its high flavonoid, phenolic, and sterol content as well as its potent antioxidant properties. Clarifying the precise mechanism will require more preclinical and clinical research.

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