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# Evaluation of Nootropic Activity using Methanolic Extract of *Eichhornia Crassipes* against Streptozotocin Induced Diabetes in Rodent Models

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***ABSTRACT:*** *Type 3 diabetes (T3D) is a term used when Alzheimer's disease (AD) is triggered by insulin resistance in the brain. Type 2 diabetes (T2D) is a risk factor for causing dementia, vascular dementia, AD. Due to the pathophysiological similarity of T2D and AD, which includes insulin resistance and deficiency, protein aggregation, oxidative stress and advanced glycation end products (AGE's); AD is often referred to as "type 3 diabetes". The present study was aimed at evaluating the nootropic activity using methanolic extract of Eichhornia crassipes (MEEC) against streptozotocin (STZ) induced diabetes in rodent models by employing in vitro and in vivo Paradigm. MEEC was screened for in vitro antioxidant activity using hydrogen peroxide radical scavenging assay and reducing power assay and in vitro nootropic activity viz. inhibition of acetylcholinesterase inhibitory activity by microwell plate method and which was further proved by in vivo studies carried out by interoceptive model namely STZ induced diabetes model and exteroceptive models like actophotometer, morri's water maze test, elevated plus maze test, cook's pole climbing method. Oral administration of MEEC dose dependently (200 mg/kg and 400 mg/kg bd.wt) and significantly ($p < 0.01$) improved the basal activity score in actophotometer, reduced escape latency time in morris water maze test, transfer latency time in elevated plus maze test and the time taken to climb the pole in cook's pole climbing apparatus. The potential effect of MEEC in enhancing the brain memory in terms of increasing the concentration of neurotransmitter acetylcholine by inhibiting the enzyme acetylcholinesterase (AChE) which helps in enhancing the cholinergic transmission and improving the nootropic effect. The antioxidant potential along with AChE inhibitory activity due to presence of alkaloids and flavonoids might be contributed to the nootropic activity of extract.*

**KEYWORDS:** *Nootropic activity; Type 3 diabetes; Oxidative stress; Acetylcholinesterase; Eichhornia crassipes.*

## INTRODUCTION

Diabetes mellitus (DM) is not a single disease entity, but rather a group of metabolic disorders sharing the common underlying feature of hyperglycaemia. Hyperglycaemia in diabetes results from defects in insulin secretion, insulin action, or most commonly, both. The chronic hyperglycaemia and attendant metabolic dysregulation may be associated with secondary damage in multiple organ system, especially the kidneys, eyes, nerves, and blood vessels (Iyer SR et al., 2001). Type 1 diabetes (T1D) is characterized by an absolute deficiency of insulin caused by pancreatic β-cells destruction. It accounts for approximately 10% of all cases. Type 2 diabetes (T2D) is caused by a combination of peripheral resistance to insulin action and an inadequate secretory response by the pancreatic β-cells ("relative insulin deficiency"). Approximately 80% to 90% of patients have T2D. T3D is a term used when AD is triggered by insulin resistance in the brain. This condition is most often used to describe people who have T2D and are also diagnosed with Alzheimer's or dementia. AD is often referred to as "type 3 diabetes". Hyperglycaemia produces reactive oxygen species (ROS) as a result of glucose auto-oxidation, metabolism and the development of AGE's. In fact, diabetes is typically associated with increased generation of free radicles and impaired antioxidant defence qualifications, representing a central contribution for ROS in the onset, progression, and pathological consequences of DM, namely, vascular complications, nephropathy and cognitive impairment or dementia (Jellinger KN et al., 2008). T3D, is a term recently used by scientists and

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tried to define it as a metabolic syndrome that may lead to abnormalities linked to progressive brain insulin resistance with consequent impairment of central insulin signaling processes, accumulation of neurotoxins, neuronal stress, and resulting in a course of neurodegeneration (ND). According to Global Health and Aging (2010), 8% of the world's population is over 65 years of age in 2010, and the number of people over 65 years of age is expected to rise to 16% by the year 2050 (Ramachandran et al., 2001; Wang et al., 2016). Both T2D and AD are associated with increasing age. Additionally, there are at least 347 million diabetic patients and 44 million people suffering from AD. These numbers are expected to become double by the year 2030. Although T2D and AD are two independent diseases, evidences from epidemiological, pathophysiological and animal studies have indicated a close pathophysiological relationship between these diseases. The pathophysiology of T2DM and AD includes: Insulin resistance and deficiency, protein aggregation, oxidative Stress and mitochondrial dysfunction, advanced glycation end products (AGE's) (Figure 1).

Treatment with a second-generation drug, glibenclamide an oral antidiabetic drug. Sulfonylureas are a class of organic compounds used in medicine and agriculture. They are widely used in the management of T2D. They act by increasing insulin release from the beta cells in the pancreas. Sulfonylureas bind to close ATP-sensitive $K^+$ (KATP) channels on the cell membrane of pancreatic beta cells, which depolarizes the cell by preventing potassium from exiting. This depolarization opens voltage-gated $Ca^{2+}$ channels. The rise in intracellular calcium leads to increased fusion of insulin granule with the cell membrane, and therefore increased secretion of mature insulin. Gliclazide is more specific for pancreatic in comparison to cardiac or neuronal KATP channels at concentrations used in clinical practice. Glyburide (glibenclamide) and glipizide have been investigated for their effects on memory and cognition in patients with diabetes. Sulfonylureas are used primarily for the treatment of T2D (Craft, 2007).

Eichhornia crassipes commonly known as water hyacinth (Family: Pontederiaceae), a free-floating aquatic macrophyte consists of broad, thick, glossy and ovate leaves float above the water surface. They have long, spongy and bulbous stalks and violet blue with dark purple and yellow color flowers are present. It is widely distributed all over the world (Gunnarsson & Petersen., 2007). Phytochemical analysis of the MEEC revealed the major secondary metabolites to be alkaloids, phenolic compounds and terpenoids. *E. crassipes* contains flavonoids (luteolin, apigenin, tricin, chrysoeriol, kaempferol, gossypetin and orientin) (Della Greca et al., 2009) It is used as a carotene rich table vegetable. The flowers are used for medicating the skin of horses. Leaves used for hepatoprotective activity in traditional therapy. However, there were no reports concerning the influence of

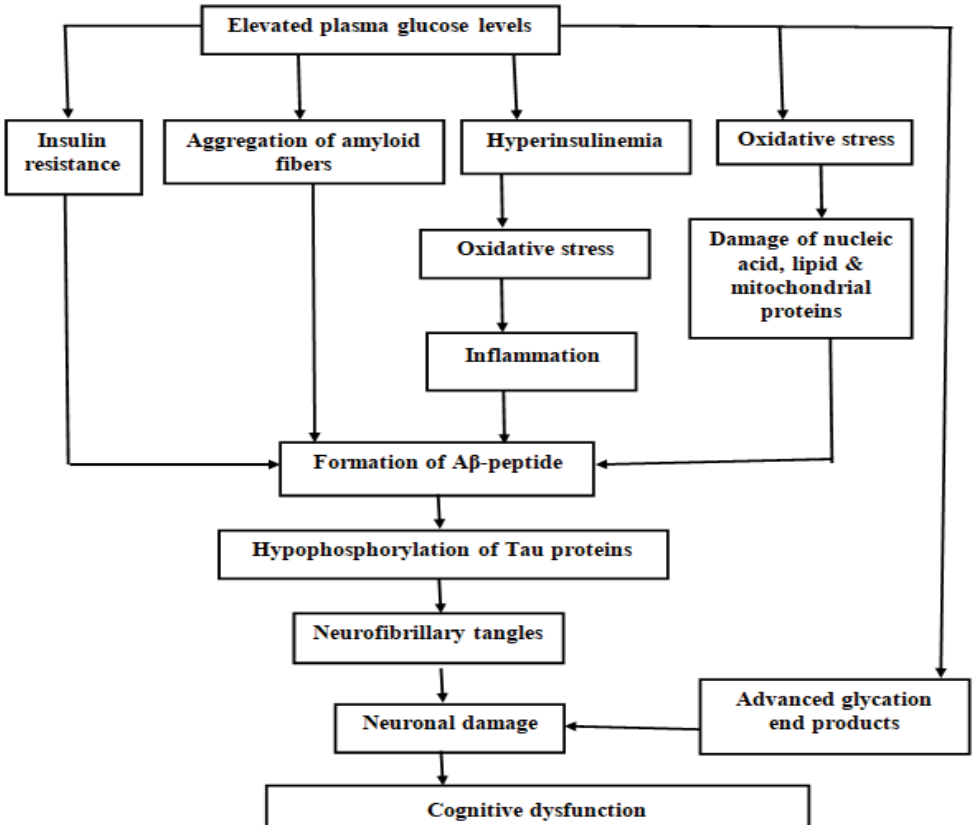


**Figure 1.** Pathophysiology of Type 3 diabetes.

*E. crassipes* against diabetes induced cognitive dysfunction. Therefore, the present study was designed to investigate the protective role of *E. crassipes* on cognitive dysfunction in streptozotocin induced diabetic rats (Gunnarsson and Petersen 2007).

## MATERIALS AND METHODS

### SUBJECTS

Adult male wistar rats reared in the animal house of the Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, India. (Reg.No.1175/PO/Re/S/08/CPCSEA) from a stock originally purchased from albino research, Hyderabad, India. Young healthy male rats (200-250 g) were group housed (three per cage) and maintained at 23±2º C under 12:12 h light /dark cycle with free access to rodent's chow and tap water. The animal studies were approved by the institutional animal ethics committee (IAEC), constituted for the purpose of control and supervision of experimental animals by ministry of environment and forests, Government of India. New Delhi, India. Animals were naïve to drug treatment and experimentation at the beginning of all studies. All tests were conducted between 09:00 and 13:00h.

### DRUGS AND CHEMICALS

Streptozotocin (Albino Research laboratories), Glibenclamide (Apollo pharmacy), Methanol (Crescent trading company), Potassium ferric cyanide, Hydrogen peroxide, Acetyl thiocholine iodide, Naphthyl acetate, Fast blue B salt, Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB), (SD Fine Chem. Limited), Trichloro acetic acid (SD Fine Chem. Limited), Ferric chloride, Potassium dihydrogen phosphate, Sodium hydroxide, Disodium hydrogen phosphate, Sodium dihydrogen phosphate (Himedia laboratories), Glucose estimation kit (K.K. Diagnostics).

### PLANT MATERIALS AND PREPARATION OF EXTRACT

The whole plant of Eichhornia crassipes was collected from a river named sundalavani kunta, Balanagar, Hyderabad, India during the month of December 2018. This plant material was identified and authenticated by Botanist. The freshly collected plant was cleared from dirt and then the plant were cut into pieces and dried under shade for about 7 days and coarsely powered in a mixer grinder. The powdered material was taken up for Soxhlet extraction process using methanol as a solvent. The solvent was completely removed under reduced pressure using rotary evaporator. The present yield of methanolic extract was found to be 11.2% w/w respectively.

### EXPERIMENTAL INDUCTION OF DIABETES

Diabetes was induced in rats by using earlier reported method (Umathe et al., 2009). In brief, STZ was dissolved in 0.1M sodium citrate buffer, (pH 4.4) and administered at the dose of 55 mg/kg i.p. route. STZ treated rats received 5% glucose solution instead of water for 24 h after injection of STZ in order to reduce death due to hypoglycaemic shock. Blood sample is collected through retro-orbital puncture and Fasting blood glucose level will be estimated by glucose oxidase - peroxidase method using semi autoanalyzer. (Reitman and Frankel, 1957). Only animals with fasting blood glucose levels over 250 mg/dl were considered diabetic and used for the further (Figure 2-7).

### TREATMENT SCHEDULE

After diabetes induction, a group of rats (n = 6) were orally administered MEEC at different doses (200 and 400 mg/kg, b.w, p.o.) and standard drug glibenclamide at a dose of (10 mg/kg, b.w, i.p.) was administered for 21 days and the rats were subjected to further behavioural studies like locomotor activity, morris water maze test, elevated plus maze (EPM) test, cook's pole climbing apparatus.

### ASSESSMENT OF COGNITIVE FUNCTION

#### Locomotor Activity:

Albino Wistar rats weighing 200 - 250 g will be used for this study. Two doses of MEEC and glibenclamide (10 mg/kg, b.w, i.p.) will be administered. Depending on CNS depressant action of the drug, the animals show reduced locomotor activity. The digital counts, as the number of line crossings by animal due to beam interruptions, will be recorded. The counts correspond to locomotor activity. The cut off time period will be 10 minutes. After 30 min and one hour, animals will be individually placed in the actophotometer and the digital counts, as the number of line crossing by animal due to beam interruptions, will be recorded for 10 minutes (Nikajoo, et al., 2009).

#### Morris water maze test:

Wistar albino rats weighing 200 - 250 g will be used for this study. The test apparatus was circular water tank (180 cm in diameter and 60 cm high, filled up to a height of 30 cm with white colored water maintained at 25°C) made up of dark grey plastic and the surface is painted white. The tank is hypothetically divided into four equal quadrants and a platform (12.5 cm in diameter and 29 cm high) is located in the center of one of the four quadrants. Two doses of MEEC and glibenclamide (10 mg/kg, b.w, i.p.) will be administered and each animal is subjected to four consecutive trials, during which they are allowed to escape on to the hidden platform and allowed to remain there for 20 sec. Escape latency time to locate the hidden platform in water maze is noted as an index of learning. In case the animal is unable to locate the hidden platform within 90 sec, it is gently guided by hand to the platform and allowed to remain there for 20

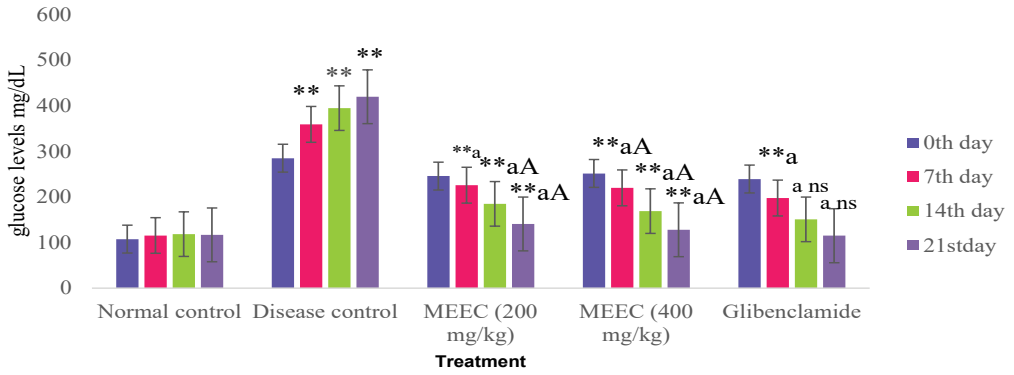


**Figure 2.** Effect of MEEC on blood glucose levels by STZ induced diabetic rats.

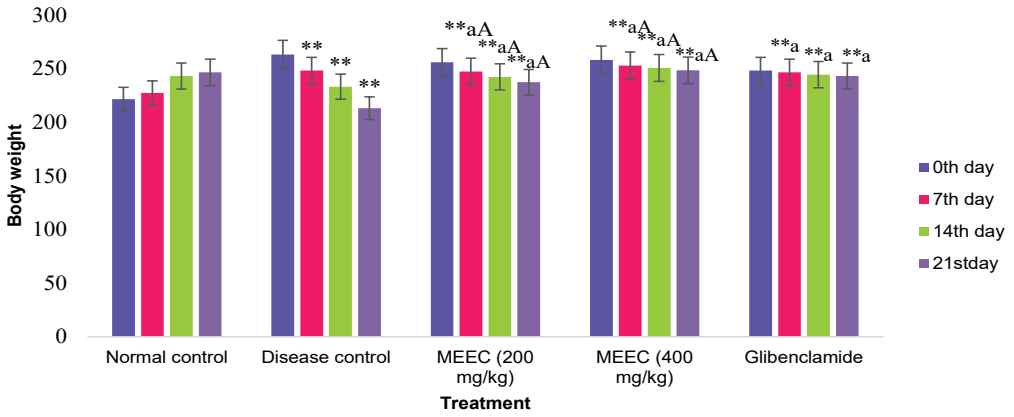


**Figure 3.** Effect of MEEC on Body weights by STZ induced diabetic rats.

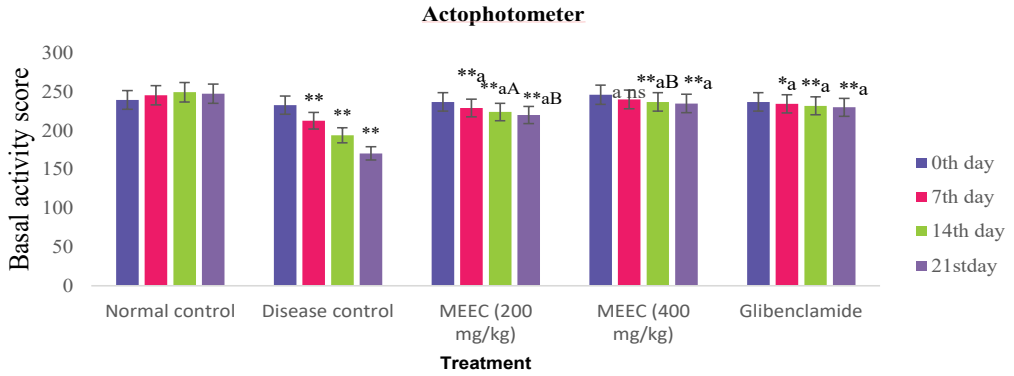


**Figure 4.** Effect of MEEC on Basal activity score in actophotometer by STZ induced diabetic rats.

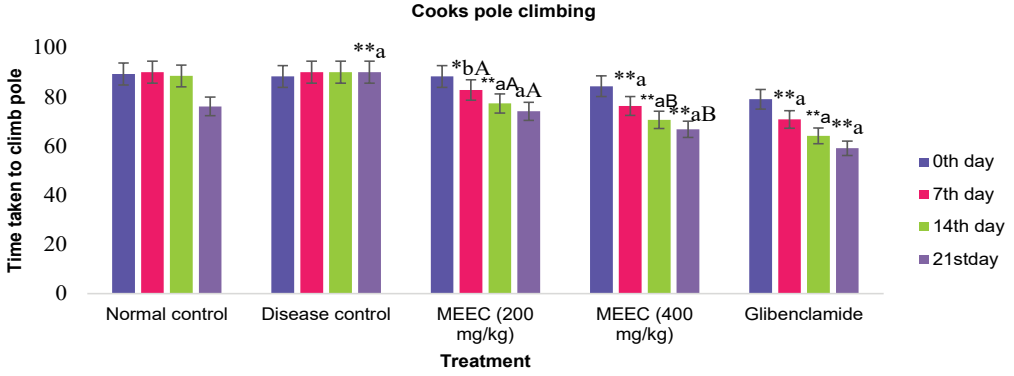


**Figure 5.** Effect of MEEC on Cooks pole climbing by STZ induced diabetic rats.

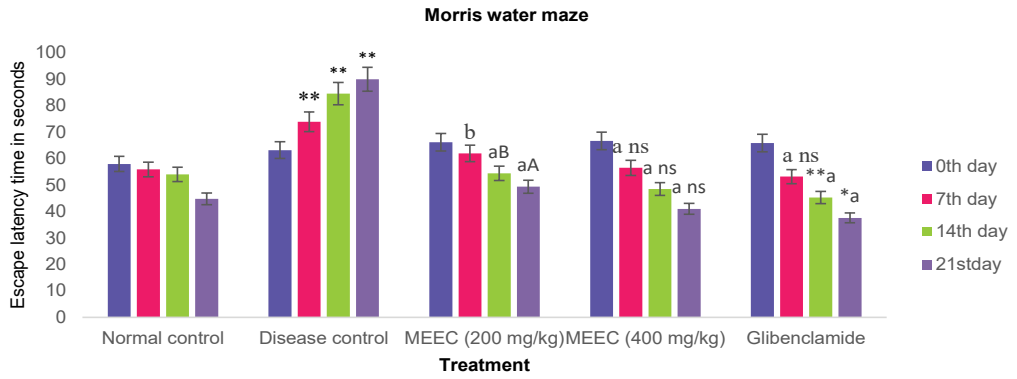


**Figure 6.** Effect of MEEC on Morris water maze test by STZ induced diabetic rats.

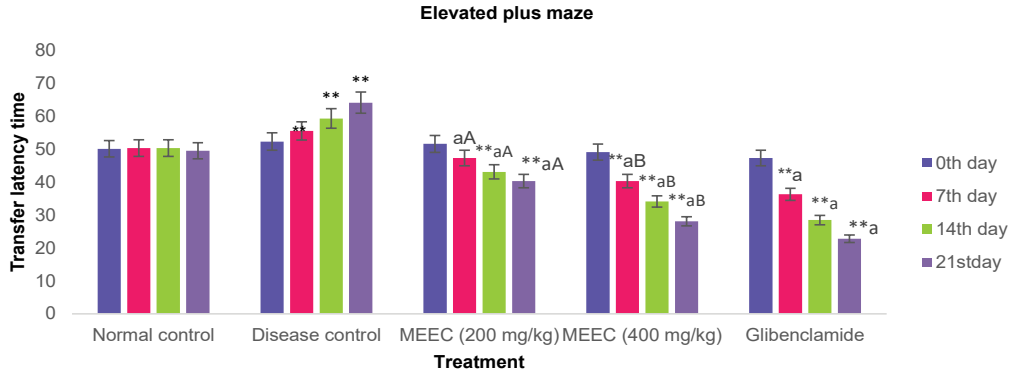


**Figure 7.** Effect of MEEC on Elevated plus maze test by STZ induced diabetic rats.

sec. On fifth day, platform is removed and time spent by the animal in each quadrant is noted. The time spent by the animal in target quadrant searching for the hidden platform is noted as an index of retrieval. (Kamal et al., 1998) To test possible deficits in sensorimotor processes, rats were tested in the water maze with a visible platform on a new location on the final day of training (Kamal et al., 2000).

The test with the visual platform does not require special orientation (McNamara & Skelton, 1993) and was used to show possible deficits in sensorimotor processes. For the test, target platform was placed inside the pool 1 cm above the water line. Rats were allowed to swim for 60 s. Time to reach the platform was recorded as escape latency. After completion of the last trial, rats were gently dried with a towel, kept warm for an hour and returned to their home cages. In memory consolidation test, a probe trial was performed wherein the extent of memory consolidation was assessed (Tiwari et al., 2009; Tuzcu & Baydas, 2006). The time spent in the target quadrant indicates the degree of memory consolidation that has taken place after learning. The probe trial was conducted on day 36, wherein the individual rat was placed into the pool as in the training trial, except that the hidden platform was removed from the pool. The time spent in target quadrant was measured for 90 s. In probe trial, each rat was placed at a start position directly opposite to where the platform was located (Kamal et al., 1998).

## Elevated plus maze (EPM) test:

Cognitive behaviour was evaluated by using the EPM learning task, which measures spatial long-term memory (Reddy & Kulkarni, 1998). Transfer latency (the time in which the animal moves from the open arm to the enclosed arm) was utilized as an index of learning and memory processes. The principle in this experiment is based upon the aversive behaviour of rodents to open and high spaces. The animals dislike open and high spaces and move from them to an enclosed arm, the protected areas of the maze. Albino Wistar rats weighing 200 - 250 g will be used for this study. Two doses of MEEC and glibenclamide (10 mg/kg, b.w, i.p.) will be administered for 21 days and the animals will be free to food and water. On $0^{th}$, $7^{th}$, $14^{th}$ & $21^{th}$ day after 60 mins after the drug administration. The animals were randomly assigned to the different experimental and control groups. In the acquisition session each rat was gently placed at the distal end of an open arm of the apparatus facing away from the central platform and the time it took to move from the open arm to either of the enclosed arms (transfer latency) was recorded. Training (repeated exposure of animal to open arms) shortens this parameter, possibly as a consequence of learning acquisition and retention. If the rat did not enter the enclosed arm within 90 s, it was excluded from further experimentation. The criterion of an animal's entry into the enclosed arm was crossing with all four legs of an imaginary line separating the enclosed arm

from the central space. After entering the enclosed arm, the rat was allowed to move freely in the maze regardless of open and enclosed arms for 10 s. Then, the rat was returned to its home cage. The retention session followed 24 h after the acquisition session. The rats were put into the open arm and the transfer latency was recorded again. The maze was cleaned after each rat (Hlinak et al., 2002).

### Cook's pole climbing apparatus:

Pole climbing apparatus is based on conditioned avoidance response, in which animal learns to avoid foot shock by climbing on to a wooden pole, hanging in the middle of the chamber. A chamber (25×25×40 cm) with grid floor (shock zone) to provide electric shock (1.5 mA), a wooden pole (2.5 cm in diameter) hanging in centre (shock free zone), a 2.8 kHz speaker and lights are situated on top of the chamber. Albino Wistar rats weighing 200 - 250 g is given with two doses of MEEC and glibenclamide (10 mg/kg, b.w, i.p.) will be administered for 21 days and subjected to a neutral conditioned stimulus (light or sound) and after 20 sec to an aversive unconditioned stimulus (foot shock). After 25 trials animal learns to avoid the foot shock by climbing on the pole immediately after hearing the sound is called conditioned avoidance response (Table 1).

## IN VITRO EVALUATION OF NOOTROPIC ACTIVITY

### Evaluation of AChE inhibitory activity using a NEW Micro-Well Plate assay (NA-FB):

A new assay to evaluate the activity of ache, using B-naphthyl acetate as the substrate, and fast blue B salt as the colour reagent instead of DTNB. The enzyme hydrolyses the substrate B-naphthyl acetate to naphthol and acetate. Naphthol is allowed to react with fast blue B. This reaction resulted in the development of a stable purple colour. The colour intensity of the product was estimated visually, which allowed qualitative detection of the enzyme inhibition, or was measured by UV spectrophotometer, which allowed quantitative detection of the enzyme inhibition. The male Wistar rats are sacrificed by cervical decapitation under light anaesthesia & whole brain was carefully removed from the skull. The fresh whole brain is weighed, kept on ice bath, rinsed with ice cold isotonic saline & homogenized in a homogenizer. The homogenate is centrifuged at 3000 rpm for 10 mins & the resultant cloudy supernatant is used for estimation. The experiment was organized using 96-microwell plates. In each well, 10 µL plant extract, 50 µL (0.25 mg/ml) of β-naphthyl acetate dissolved in methanol, and 200 µL of AChE solution (3.33 U/ml) were added. The mixture was incubated at 4°C for 40 min. Later, 10 µL (2.5 mg/ml) fast blue b dissolved in water were added to the mixture and the inhibition was observed visually. Qualitative Determination of acetylcholinesterase inhibitory activity (AChEI). The newly modified method using, β-naphthyl acetate as a substrate and fast blue B salt as colour reagent resulted in the production of a purple colour from the reaction between naphthyl and the reagent. A scale of 0-3 was used to evaluate qualitatively the inhibition of AChE by plant extracts: Scores: 0 represents no inhibition activity with the solution colour dark purple, 1 represents mild inhibition activity with the solution colour purple, 2 represents moderate inhibition activity with the solution colour light purple, 3 represents strong inhibition activity with no change in solution colour (Lan et al., 2012).

## IN VITRO ANTIOXIDANT ASSAYS

### Reducing power assay:

Phosphate buffer pH 6.6: Potassium dihydrogen phosphate (62.5 ml 0.2M) was added to 250 ml volumetric flask and also 20.5ml of 0.2 M NaOH and made up to volume 250ml with distilled water. Potassium dihydrogen phosphate (0.2 M) solution: Potassium dihydrogen phosphate (2.72 g) was dissolved in distilled water and volume made up to 100 ml. Sodium hydroxide solution (0.2 M) solution: 0.8 g of sodium hydroxide was dissolved in distilled water and volume was made up to 100 ml. Potassium ferric cyanide (1% w/v) solution: Potassium ferricyanide (l g) was dissolved in water and volume was made up to 100 ml in volumetric flash. Ferric chloride solution (0.1% w/v): Ferric chloride (25 mg) was dissolved in distilled water and volume was made up to 25 ml in volumetric flask (Kanatt et al., 2007).

Method: To 1 ml of test and standard compounds, 2.5 ml of potassium ferricyanide (1% WM, 2.5 ml of phosphate buffer pH 6.6 were added and incubated at 50°C for 30. To 2.5 ml of above supernatant liquid 2.5 ml of distilled water and 0.5 ml of Kcl; solution (0.1% w/v) were added. The absorbance of ferric complex was measured using phosphate buffer pH 6.6 as control at 700 nm using UV-Visible spectrophotometer and estimated the increase in absorbance. The percent increase in reducing power was calculated using the following equations,

**Table 1.**

Scores of AChEI activity of MEEC

| S.no | Type of inhibition | Scores | % Scores |
|---|---|---|---|
| 1 | No inhibition | 0 | 0 |
| 2 | Mild inhibition | 1 X 31 | 19 |
| 3 | Moderate inhibition | 2 X 48 | 57 |
| 4 | Strong inhibition | 3 X 12 | 23 |

Percentage increase in reducing power (%) = Abs test - Abs blank X 100

Abs blank

Where 'Abs test' is absorbance of test solution, 'Abs blank' is absorbance of blank.

### Hydrogen peroxide radical scavenging assay:

Phosphate buffer solution ph 7.4: Add 250.0 ml of 0.2 M potassium dihydrogen phosphate to 393.4 ml of 0.1 M sodium hydroxide and make up the volume to 1000 ml with the distilled water. Potassium dihydrogen phosphate (0.2 M) solution: Potassium dihydrogen phosphate (2.72 g) was dissolved in distilled water and volume made up to 100 ml. Sodium hydroxide solution (0.1 M) solution: 0.4 g of sodium hydroxide was dissolved in distilled water and volume made up to 100 ml (Jaing et al., 1992) (Figure 8).

Method: A solution of hydrogen peroxide (2 mmol/l) was prepared in phosphate buffer (Pb 7.4). Test compounds (10-50 ug/M) were added to hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide and compared with ascorbic acid, the reference compound.

Abs control - Abs sample

% $H_2O_2$ activity = × 100

Abs control

Where, Abs (control): Absorbance of the control, Abs (sample): Absorbance of the extract/standard.

## EVALUATION OF BLOOD GLUCOSE LEVELS AND BODY WEIGHT

Blood glucose levels were measured with a semi autoanalyzer (Tulip, India). In brief, blood sample was withdrawn from the retro orbital puncture and Fasting blood glucose level will be estimated by glucose oxidase - peroxidase method using semi auto analyzer. During the experiment, blood glucose levels and body weights were verified in the interim (0, 7th, 14th, 21st days) of the treatment.

## STATISTICAL ANALYSIS

Results were expressed as mean ± SEM. The data were analysed by one- way analysis of variance (ANOVA) followed by Dunnett's test respectively. Statistical significance was considered at *p<0,005, **p<0.01.

## RESULTS

### Preliminary Phytochemical analysis

The Preliminary Phytochemical investigation of methanolic extract of whole plant of Eichhornia crassipes showed the presence of phenolic compounds, Alkaloids, Flavonoids, phenols, Sterols, Terpenoids, Tannins.

### Acute toxicity studies of MEEC

MEEC was treated on albino swiss mice up to a dose of 2000 mg/kg b.w. The animal did not exhibit any signs of toxicity or mortality up to 2000 mg/kg b.w. various morphological and behavioral 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 14 days of observation. Hence the extract was found to be safe up to 2000 mg/kg b.w.

**Effect of MEEC on blood glucose levels (mg/dl):**

Values are expressed as mean ± SEM, (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test. Results were expressed as (**=p<0.01, ns= non-significant) when compared to normal control, (a = p<0.01, b = p<0.05) when compared to disease control, (A= p< 0.01, B= p<0.05) when compared to standard. There was a significant increase (P<0.01) in BGL's in disease control group when compared to Normal control on 0th (285.1 ± 8.43) 7th (359.5 ± 8.61) 14th (395 ± 1.93) and 21st (420 ± 0.96) days indicating the induction of diabetes. Treatment with MEEC at 200 and 400 mg/kg doses significantly reduced the BGL's on 7th, 14th and 21st days. The effect of 400 mg/kg (128 ± 2.36) of MEEC was found to be better than that of 200 mg/kg (141 ± 0.96). The antioxidant potency of 400 mg/kg dose of MEEC was found to be comparable to that of standard glibenclamide (115.1 ± 0.6).

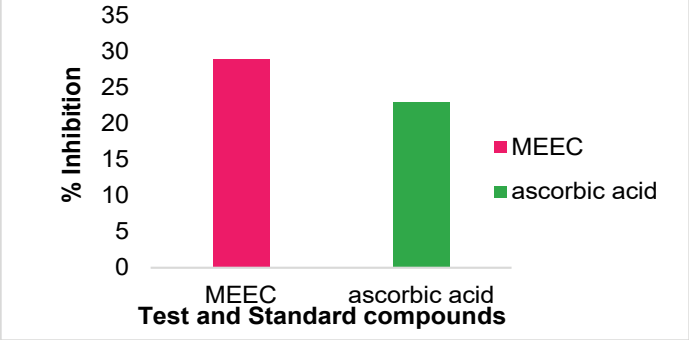


**Figure 8.** Reducing power radical assay of MEEC.

### Effect of MEEC on Body weight:

Values are expressed as mean ± SEM, (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test. Results were expressed as (** = $p < 0.01$) when compared to normal control, (a = $p < 0.01$) when compared to disease control, (A = $p < 0.01$) when compared to standard. Administration of streptozotocin (55mg/kg) resulted in significant attenuation of body weights in disease control group indicting the induction of diabetes (213.3 ± 0.08). Treatment of diabetic animals with MEEC at 200 and 400 mg/kg doses significantly antagonised the streptozotocin induced reduction of body weights. The effect of 400 mg/kg of MEEC (248.6 ± 0.02) was found to be better than that of 200 mg/kg (237.5 ± 0.06). The efficacy of 400 mg/kg dose of MEEC was found to be similar to that of standard glibenclamide (243.3 ± 0.06).

### Cognitive parameters:

The effect of streptozotocin induced diabetes on cognitive parameters like memory was assessed by actophotometer, cook's pole climbing apparatus, morris water maze test, elevated plus maze test.

### Basal activity score actophotometer:

Values are expressed as mean ± SEM, (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test. Results were expressed as (**=$p<0.01$, ns= non-significant) when compared to normal control, (a = $p<0.01$) when compared to disease control, (A= $p< 0.01$, B= $p<0.05$) when compared to standard. Administration of streptozotocin showed significant reduction of basal activity score (170.6 ± 0.76) in disease control group. Treatment with 200 and 400 mg/kg doses of MEEC significantly enhance the basal activity score compared to disease control. The effect of MEEC at 400 mg/kg dose (235 ± 3.45) was found to be better than that of 200 mg/kg dose (220 ± 0.63) the potency of 400 mg/kg of MEEC was found to be comparable to that of standard glibenclamide (230 ± 0.96).

### Cooks pole climbing:

Values are expressed as mean ± SEM, (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test. Results were expressed as (**=$p<0.01$) when compared to normal control, (a = $p<0.01$, b = $p<0.05$) when compared to disease control, (A= $p< 0.01$, B= $p<0.05$) when compared to standard. There was significant increase in the time taken to climb the pole of disease control animals (90 ± 0.25). Compared to normal control animals (76.1 ± 1.7). Administration of 200 and 400 mg/kg doses of MEEC significantly decrease pole climbing time compared to disease control. The activity of 400 mg/kg of MEEC (66.8 ± 2.04) was found to be better than that of 200 mg/kg (74.1 ± 2.03). The potency of 400 mg/kg of MEEC was found to be comparable to that of standard glibenclamide (59.1 ± 1.6).

### Morris water maze test:

Values are expressed as mean ± SEM, (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test. Results were expressed as (**=$p<0.01$, *=$p<0.05$, ns= non-significant) when compared to normal control, (a = $p<0.01$, b = $p<0.05$) when compared to disease control, (A= $p< 0.01$, B= $p<0.05$) when compared to standard. Administration of streptozotocin significantly increased the escape latency time of disease control animals (90 ± 3.04) compared to normal control animals (44.8 ± 3.04). Treatment with MEEC at 200 and 400 mg/kg doses significantly reduced escape latency time compared to disease control. The activity of 400 mg/kg dose of MEEC (41 ± 0.96) was found to be better than that of 200 mg/kg (49.4 ± 1.5). The efficacy of 400 mg/kg dose of MEEC was found to be compared to that of standard glibenclamide (37.6 ± 0.6).

### Elevated plus maze:

Values were expressed as mean ± SEM, (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test. Results were expressed as (**=$p<0.01$, *=$p<0.05$), when compared to normal control, (a = $p<0.01$, b = $p<0.05$) when compared to disease control, (A= $p< 0.01$, B= $p<0.05$) when compared to standard. There was a significant elevation in the transfer latency time of disease control animals (64.1 ± 0.6) compared to that of normal animals (49.5 ± 1.18) treatment with MEEC at 200 and 400 mg/kg doses. Significantly reduced transfer latency time compared to disease control. The effect of 400 mg/kg dose of MEEC (28.1 ± 1.08) was found to be better than that of 200 mg/kg dose (40.3 ± 0.8). The efficacy of 400 mg/kg of MEEC was found to be comparable to that of standard glibenclamide (22.8 ± 1.1).

## IN VITRO ANTIOXIDANT ASSAYS

### Reducing power assay:

The MEEC has shown antioxidant activity in reducing power assay. The reducing ability of a compound generally depends on the presence of a compound generally depends on the presence of reductants which have been exhibiting antioxidative potential by breaking the free radical chain and donating a hydrogen atom. The reducing capacity of a compound can be known by measuring $Fe^{3+} – Fe^{2+}$ complex. $Fe^{3+}$ reduction is due to electron donating activity of MEEC, which is an important mechanism of antioxidant action. Increase in absorbance indicates an increase in reductive ability of MEEC. The reducing power activity of MEEC might be due to presence of hydroxyl groups. It might be due to the presence of phenols and flavonoids in the extract with adequate number of hydroxyl group.

MEEC had shown dose dependent inhibition of free radicals and its IC50 value was found to be 29 µg/mL. The potential of the extract was comparable to that of standard ascorbic acid (IC50 = 23 µg/mL).

### Hydrogen peroxide scavenging assay:

The in vitro antioxidant activity was performed using hydrogen peroxide radical scavenging assay. The IC50 value of the MEEC was 28 µg/mL and ascorbic acid was 22 µg/mL respectively. From the results it is clear that the MEEC showed antioxidant activity. With increase in concentration the % inhibition of MEEC was found to increase and its IC50 value was found to be 28 mg/ml. The antioxidant potential of MEEC was found to be similar to that of standard ascorbic acid (IC50 = 22 mg/dl). The antioxidant activity of the extract was further confirmed by hydrogen peroxide radical scavenging assay. The standard ascorbic acid showed prominent dose dependent inhibition of hydrogen peroxide radicals and its IC50 value was found to be 22 µg/mL. Similarly, the test extract exhibited the dose dependent inhibition of free radicals and its IC50 value (28 µg/mL) was comparable to that of standard. The MEEC has shown antioxidant activity in hydrogen peroxide radical scavenging assay (Figure 9).

Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) group. It can cross cell membrane rapidly: once inside the cell, it can probably react with $Fe^{2+}$ and possibly $Cu^{2+}$ ions to form hydroxyl radicals and this may be the origin of many of its toxic effects. The scavenging capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activities of antioxidants have been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity and radical scavenging. The MEEC have shown dose dependent inhibition of hydrogen peroxide and good scavenging ability which was comparable to ascorbic acid. It might be due to the presence of phenols and flavonoids in the extract with adequate number of hydroxyl groups.

### Invitro evaluation of nootropic activity:

Evaluation of AChE Inhibitory activity using a new micro-well plate AChE Inhibition assay:

Inhibition of AChE activity was measured using a 96- micro-well plate based on Ellman's method. The AChEI activity of MEEC was determined based on the intensity of stable colour that resulted by the reaction of naphthol and fast blue B salt which allowed qualitative detection of the enzyme inhibition. A scale of 0-3 was used to evaluate qualitatively the inhibition of AChE by MEEC is as follow:

## HISTOPATHOLOGY

One rat each from five groups were sacrificed by decapitation the brain was isolated by clearing its extraneous connections with cranium. Later it was cut in to two vertical halves. Cerebral cortex was separated and stored in 10 percent formalin solution; these specimens stored were used for Histopathological studies.

## DISCUSSION

The incidence of cognitive disfunction or dementia appears to be double in elderly subjects with diabetes millets. Hyperglycaemia in diabetes millets results in overproduction of oxygen free radicals, which contributes to the progression of diabetes. The development of complication during diabetes is also associated with oxidative stress. The neurodegenerative disease as AD or dementia has also been related to oxidative stress during diabetes millets. The neurochemical changes causing cerebrovascular alterations are also considered as potential mechanism for diabetic cognitive decline (Wang XP et al., 2008). Evidence is accumulating the people with diabetes millets as at a risk of developing cognitive impairments. It has also been demonstrated that acute hyperglycaemia in people with T2D significantly impaired speed of information processing; working memory has been suggested to be associated with the changes in hippocampal synaptic plasticity (kanat et al., 2007).

Antidiabetic, psychotropic drugs, antioxidants like vitamin A, C & E and superoxide dismutase (SOD), catalase (CAT)

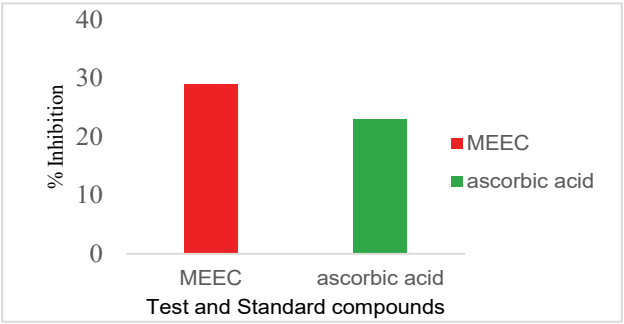


**Figure 9.** Hydrogen peroxide radical assay of MEEC.

enzymes have been found to prevent the progression of diabetes and occurrence of complications resulted from DM. However, these drugs are associated with several adverse effects which have provoked research in field of traditional system of medicine to deduce the drugs with less toxicity and better tolerability (Tsuyoshi Hamaguchi, 2009).

From the vast array of materialmedica of the indigenous system, many plants have been reported to have activity against DM and CNS disorders that act as very useful remedies for the alleviation of human suffering. E. crassipes is one of them and it is reported to contain phytoconstituents like alkaloids, flavonoids, and phenols etc which have been reported to decrease the levels of blood glucose and glycosylated haemoglobin significantly and increase the serum insulin to normal level.

The MEEC has been studied for its effect on diabetes and diabetes induced cognitive decline by streptozotocin model. Streptozotocin is capable of inducing diabetes upon intraperitoneal injection in experimental animals been taken up cellularly by the glucose transporter 2 (GLUT2) which is a low affinity protein situated in cell membrane of insulin producing β – cells of the pancreas as well as in cell of other organs like kidney and liver, leading to selective toxicity due to DNA alkylation resulting into cellular necrosis. The resulting hyperglycaemia due to reduced insulin secretion results in enhanced formulation of reactive oxygen species both centrally and peripherally. These free radicals contribute to the increased neuronal death by oxidizing protein, damaging DNA, and augmenting lipid peroxidation. It causes brain energy metabolism malfunction and cholinergic deficiency in the hippocampus, leading to cognitive discrepancies (Zhong-he Liu et al., 2015).

In this study, the effect of MEEC towards hyperglycaemia and its complications in the learning and memory decline work ascertained. MEEC significantly ameliorated the fasting blood glucose levels at both 200 and 400 mg/kg doses. This might be given by their antioxidant properties in reducing oxidative stress in the pancreas caused by streptozotocin induced effect, hence reduces the glucose metabolism in diabetic rats. The streptozotocin induction exhibits a significant lower bodyweight in diabetic control group as compared to the normal control. Treatment with MEEC caused a significant improvement in body weight as compared to the diabetes control rats. This proved the beneficial effect of the selected plant in DM. The effect of MEEC on diabetes induced cognitive decline was assessed by in vitro model such as Elliman's method and in vivo models which include actophotometer, elevated plus maze, cooks pole climbing and morris water maze test.

Cholinergic system as an integral role in the memory and cognition and its degeneration is one of the hallmark features in AD patients and AChE inhibitors are the most widely used 1st line therapy for AD management in the present study, MEEC showed moderate inhibition of AChE activity in 96 microwell plate method. It tells us that the extract can enhance AChE levels centrally and can augment cholinergic mediated memory and cognition.

Basal activity score of animals by using actophotometer was carried out in order to rule out the possible role of locomotor changes in these cognitive studies. There was no prominent change in the basal activity of animal treated with MEEC when compared with their initial values. It indicates the lack of the effect of MEEC on the locomotor movement mediated by the cortex. But there was significant difference in the basal activity score of various groups which might be due to inter individual differences.

The elevated plus maze task relays on rat's characteristic explorative behaviour using transfer latency as the outcome variable. The transfer latency indicates the extent of exploration of open arms by the rats without any impact of handling by the experimental and hence helps in the assessment of cognitive impairments. Streptozotocin significantly reduced the transfer latency indicating the induction of dementia. MEEC showed prominent improvement in the transfer latency indicating and enforcement of memory that has been attenuated by streptozotocin.

Passive avoidance in cooks pole climbing apparatus indicates the retention of memory i.e., acquired during acquisition trials. Elevation of time taken to climb the pole in streptozotocin treated animals showed the induction of memory loss. The significant reduction in the time taken to climb the pole after treatment with MEEC indicates its reinforcement effect on the memory which has been reduced by streptozotocin. Ability to avoid the 4th coming punishment in the form of shock is a good indicator of memory.

Morris water maze test performance in diabetic rats was severely impaired as compare with non-diabetic rats, conforming earlier findings the current study explored those findings demonstrating that diabetes reduced learning and memory performance. Furthermore, the present findings indicate that the impaired performance of diabetic rats is related to cognitive impairment rather than to sensory mortar deficits, since performance of diabetic rats were similar to non-diabetic rats in the task with the visible platform. The significant reduction of escape latency in memory consolidation test by MEEC indicates its cognitive enhancing effect (McNamara & Skelton., 1993).

There was prominent neuronal loss in hippocampus and cerebral cortex with subsequent deposition of β – amyloid plaques as revealed by histopathological studies treatment with MEEC showed treatment with MEEC showed significant reduction in neuronal loss and amyloid deposition indicating its neuroprotective effect. This studying substantiates the in vivo findings of the plant (Figure 10).

The role of oxidative stress in complications of diabetes has been studied extensively in experimental diabetes has

been studied extensively in experimental diabetes models and diabetic patients. Due to the hyperglycaemia associated with diabetes enhanced formation of reactive oxygen occurs, which contributes to the increased normal death by oxidising proteins, damaging DNA and augmenting lipid peroxidation. Oxidative damage to the rat synapse in the cerebral cortex and hippocampus has been previously reported to contribute to the deficit of cognitive functions. Therefore, antioxidants might be of general use in the prevention of the neurodegeneration deficits and cognitive impairments associated with diabetes. In the present study, MEEC significantly scavenged hydrogen peroxide radicles and their subsequent reducing power. The antioxidant property of *E. crassipes* may reduce oxidative damage to the synapse in the hippocampus and cortex and therefore improve learning and memory deficits (Figure 11).

In general, it was found that *E. crassipes* significantly ameliorated the cognitive impairment in diabetes rats. The exact mechanism of *E. crassipes* in preventing learning and memory deficits is still in diabetes. The presence of phytoconstituents like phenols, alkaloids, flavonoids, terpenoids, steroids, tannins might be responsible for the beneficial role of E. crassipes in diabetes and its related complications.

In conclusion, MEEC treatment ameliorated cognitive disfunction in diabetic rats, which may find clinical application in treating neuronal deficit in the diabetic

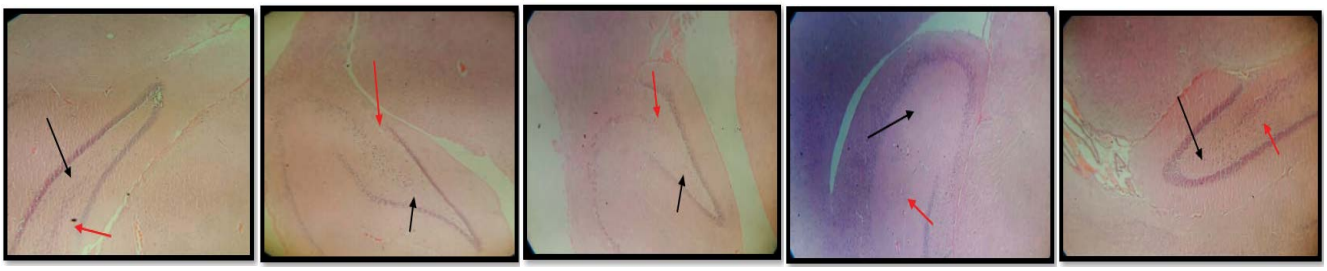


**Figure 10.** Histopathology of Hippocampus.

A. Normal control: Hippocampus was found to be intact with 8-10 layers. There was a presence of significant number of reactive astrocytes and absence of β - amyloid plaques.

B. Disease control: There was prominent neuronal loss in hippocampus with moderate deposition of β - amyloid plaques. Few reactive astrocytes were observed.

C. MEEC (200mg/kg): There was moderate neuronal loss with deposition of β - amyloid plaques. Few reactive astrocytes were observed.

D. MEEC (400mg/kg): There was absence of neuronal loss and deposition of β - amyloid plaques. More number of reactive astrocytes was observed.

E. Standard drug Glibenclamide: Neuronal loss and deposition of β - amyloid plaques was not observed. The hippocampus was found to be intact and a more reactive astrocytes was observed.

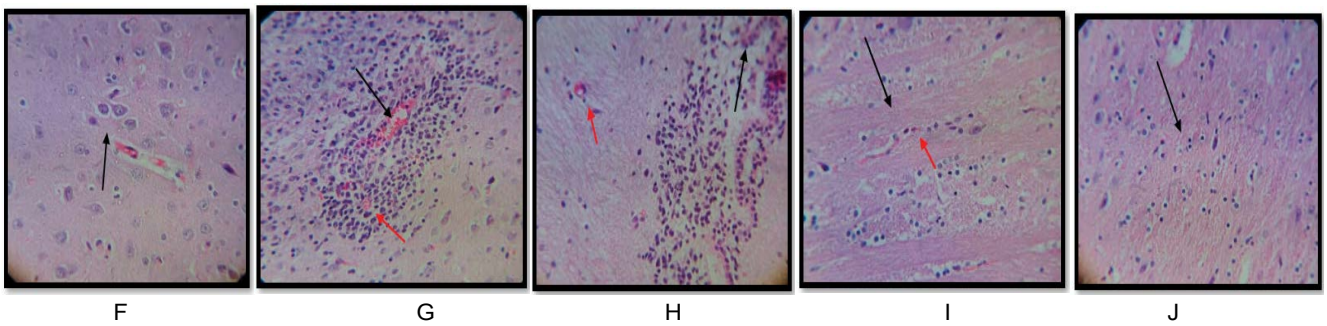


**Figure 10.** Histopathology of Cortex.

F. Normal control: The cellular texture of cortex was found to be intact with clear meninges surrounding it. There was no degeneration or loss of neurons.

G. Disease control: There was prominent damage to the cellular texture of cortex with presence of inflammatory cells of lymphocytes, histiocytes and many lymphoplasmacytes. There was clear degeneration or apoptosis of neurons.

H. MEEC (200mg/kg): There was mild damage of cellular texture of cortex with many lymphocytes and few histiocytes. There was less degeneration or apoptosis of neuronal cells.

I. MEEC (400mg/kg): There was no damage to the cellular texture of cortex but few lymphocytes were observed. Degeneration or apoptosis of neurons was not seen.

J. Standard drug Glibenclamide: The cellular texture of the cortex was found to be intact with the absence of inflammatory lymphocytes or histiocytes and astrocytes. Degeneration and apoptosis of neuronal cells were completely absence.

patients. The protective actions of MEEC may be attributed to its multiple pleiotropic effects like antioxidants, antihyperglycemic and anticholinesterase activity.


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## References


Craft S, Asthana S. Schellenberg G, Cherrier M, Baker LD, Newcomer J. & Grimwood, K. (1999). Insulin metabolism in Alzheimer's disease differs according to apolipoprotein E genotype and gender. *Neuroendocrinol*. *70*(2), 146-152.

DellaGreca M, Previtera L, Zarrelli A. (2009). Structures of new phenylphenalene-related compounds from Eichhornia crassipes (water hyacinth). Tetrahedron. 65:8206–8208

Gunnarsson CC, Petersen CM. (2007). Water hyacinths as a resource in agriculture and energy production: a literature review. *Waste Manag.* 27:117–129.

Iyer, S. R., Iyer, R. R., Upasani, S. V., & Baitule, M. N. (2001). Diabetes mellitus in Dombivli--an urban population study. *J Assoc Physicians India*. *49*, 713-716.

Jellinger KA. (2008). Neuropathological aspects of Alzheimer disease, Parkinson disease and frontotemporal dementia. *Neurodegener Dis*. 5(3-4):118–121.

Kamal A, Biessels GJ, Duis, SE, & Gispen, WH. (2000). Learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: Interaction of diabetes and ageing. *Diabetologia,* 43:500–506.

Kanatt S.R., Chander R. and Sharma A. (2007). Antioxidant potential of mint (Mentha spicata L.) in radiation-processed lamb meat. *Food Chem*. 100:451– 458.

McNamara RK, & Skelton RW. (1993). The neuropharmacological and neurochemical basis of place learning in the Morris water maze. Brain Research. *Brain Res Rev*. 59:193–204.

Ramachandran A, Snehalatha C, Kapur, A., Vijay V, Mohan V, Das, AK. & Nair, J. D. (2001). High prevalence of diabetes and impaired glucose tolerance in India: National Urban Diabetes Survey. *Diabetologia. 44*(9), 1094-1101.

Reddy DS, & Kulkarni, SK. (1998). Possible role of nitric oxide in the nootropic and antiamnesic effects of neurosteroids on aging- and dizocilpine-induced learning impairment. *Brain Res*. 799:215–229

Tiwari V, Kuhad A, & Chopra, K. (2009b). Suppression of neuro-inflammatory signaling cascade by tocotrienol can prevent chronic alcohol-induced cognitive dysfunction in rats. *Behavioural Brain Res*. 203:296–303.

Tsuyoshi Hamaguchi (2009). Phenolic compounds prevented the development of AD pathology by affecting different Aβ aggregation, *Int J Alzheimer's Dis*. (7):1647-1655.

Umathe, S. N, Bhutada, PS, Jain NS, Dixit PV, & Wanjari MM. (2008). Effects of central administration of gonadotropin-releasing hormone agonists and antagonist on elevated plus-maze and social interaction behavior in rats. *Behavioral Pharmacol*. 19:308–316.

Wang (2016). Neuroprotective effect in streptozotocin-induced AD rat model ameliorating spatial learning and memory impairment. *Neurosci.* 3:789-796.

Wang XP, Ding HL (2008). Alzheimer's disease: epidemiology, genetics, and beyond. *Neurosci Bull*. 24(2): 105–109.

Liu ZH., Chen HG, Wu PF, Yao Q, Cheng HK., Yu, W, & Liu C. (2015). Flos puerariae extract ameliorates cognitive impairment in streptozotocin-induced diabetic mice. *Evid Based Complementary Altern Med* 2015.