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Evaluation of Anti-alzheimer activity of Methanolic extract of leaves of Jatropha gossypifolia in ICV Amyloid-β Alzheimer's model.

S.Baby vanitha,[1] B.Jayakar[2]

[1]Assistant Professor, Department of Pharmacology, Vinayaka Mission's College of Pharmacy, Vinayaka missions research foundation (DU), Salem, Tamilnadu, India.

[2]Professor, Registrar, Vinayaka missions research foundation (DU), Salem, Tamilnadu, India.

Email: vanithabprakash@gmail.com,

ABSTRACT

The aim of this study was to evaluate the anti-alzheimer activity of methanolic extract of leaves of *Jatropha gossypifolia* in ICV Amyloid-β Alzheimer's disease model. The leaves of *Jatropha gossypifolia* (Family:Euphorbiaceae) extracted with methanol and studied by *in vivo* $A\beta_{1-42}$ induced model in rats. *In vivo* behavioural study results showed decreased RME, CWME and IWME in cognitive declined rats in RAM indicates the cognitive enhancement potential of the plant extracts. Also reduced the IL and STL period in $A\beta_{1-42}$ infused rat evidences the cognitive enhancement activity of above extracts in Alzheimer's like pathology.

Met-L-JG in $A\beta_{1-42}$ infused rats have shown significant decrease in LPO, NO, and Increased the level of SOD, GSH, and CAT represents the anti-oxidant activity of the extract in Alzheimer's by *in -vivo*. Met-L-JG has drastically attenuated the brain TNF-α and IL-1β levels in the $A\beta_{1-42}$ treated rats represent the anti-neuroinflammatory activity of the extract in Alzheimer's. Met-L-JG (200 mg/kg) reduced the hippocampal glutamate level in comparison to $A\beta_{1-42}$ infused rats indicates the anti-excitotoxicity capacity of above extract in neurodegenerative conditions like Alzheimer's disease. Histopathological studies showed that met-L-JG (100 mg/kg), met-L-JG (200 mg/kg) have reduced the neuronal damage with decreased necrotic, swelling and neuronal degeneration with restored intact cells which are comparable with SO group. In the view of above results we concluded that methanolic extracts of leaves o*f Jatropha gossypifolia* were showed to be potent anti-alzheimer's in experimental animals.

Keywords: Amyloid beta, Excitotoxicity, Glutamate, *Jatropha gossypifolia*

INTRODUCTION

Alzheimer's disease is a neurodegenerative disorder caused by protein misfolding and aggregation. The brain possesses various protective mechanisms that control the accumulation of protein aggregates. Chaperone proteins which bind to misfolded proteins and direct them to fold correctly and the ubiquitination reaction which prepares proteins for destruction within the cell. Accumulation of protein deposit occurs when these protective mechanisms are unable to cope. Neuronal death pathologically recognized by cell swelling, vacuolization, ca^{2+} overload, lysis and membrane damage. Cells can also die by necrosis and apoptosis which occur in many neurodegenerative diseases. Excitotoxicity, Oxidative stress kill the cells directly by necrosis or if less intense induce them to undergo apoptosis. Both processes are possible target for neuroprotective drug therapy.

In 1906 Dr.Alois Alzheimer first discovered Alzheimer disease. It presents as progressive memory decline initially followed by other cognitive dysfunctions such as visuospatial abnormalities, navigation difficulties, executive problem and language disturbance and pathological appearance of senile plaques and neurofibrillary tangles and decrease acetylcholine level. Various risk factors like oxidative stress, obesity, diabetes, hypertension, air pollution, smoking, and hypercholesterolemia have significant role in the formation AD.

Currently two pharmacotherapies are approved by Food and Drug Administration (FDA) i.e. Acetylcholinesterase inhibitors like Tacrine, Donepezil, Rivastigmine, Galantamine and N-methyl D-aspartate, glutamate antagonist (NMDA) – Memantine.

Phytochemicals are chemical molecules that modify the function of different receptor excitatory and inhibitory neurotransmitters in the brain and maintain or alter the chemical level of the brain. Based on this several plants were identified with medicinal value to treat cognitive disorders. Various herbal remedies are available in the market as mood enhancers, memory boosters or agents to slow down or prevent Alzheimer's disease. Dietary supplements marketing do not require the rigorous scientific research and clinical trials by the U.S. Food and Drug Administration (FDA) for the approval.

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Materials and Methods

Collection and Authentication of Plant Material

The plants *Jatropha gossypifolia* leaves had been collected from ABS botanical garden, Salem, Tamil Nadu, India. The plant was identified and authenticated by the botanist Mr. A. Balasubramanian (consultant – central siddha research) Executive Director ABS botanical, Salem, Tamil Nadu.

Extraction of Plant Material

The fresh leaves of *Jatropha gossypifolia, a*re collected and dried under shade, sliced into small pieces and ground into powder with mechanical grinder and the powder was sieved by Sieve no.30 and preserved in a container.

Extraction procedure

The dried powder of leaves of *Jatropha gossypifolia* was defattedwith petroleum ether in Soxhlet apparatus by hot percolation. The defatted powder material (marc) thus obtained was further extracted with Chloroform and methanol. The solvent was removed by distillation under reduced pressure and evaporation. The resulting semisolid mass was vaccum dried by using rotary flash evaporator.

Preliminary Phytochemical Studies [1-4]

The extract was subjected to preliminary phytochemical investigations to identify various phytoconstituents present in the leaves of *Jatropha gossypifolia*

In vivo study

Based on the acute toxicity studies reports and according to OECD 425 guidelines 100 mg/kg ($1/20^{th}$ dose of 2000 mg/kg) 200 mg/kg ($1/10^{th}$ of 2000 mg/kg) doses of methanolic extract of leaves of *Jatropha Gossypifolia* were selected for the pharmacological screening evaluation for Alzheimer's disease .

Animals

Sprague Dawley (SD) rats (Male, 200-280 g) were obtained from Central Animal House facility of Sri Venkataeswara Enterprises, Bangalore. Experiment done in between 09:00am to 5:00 pm. The food was reserved 12 to 18 hours before the surgical procedure. The experimental procedures were permitted by the Institutional Animal Ethics Committee (IAEC), Vinayaka Mission's College of Pharmacy, Salem 8.The experiment was done in the agreement with the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA), guidelines of Government of India on animal experimentation. **IAEC proposal number: P.col/18/2019 IAEC/VMCP.**

Preparation of $A\beta_{1-42}$ and plant extract

Preparation of $A\beta_{1-42}$ solution

Artificial cerebrospinal fluid (a CSF) is generally used as a vehicle for the administration of test supplements to the CNS of test animals. Fresh solution was prepared daily just previously to the surgery (Artificial CSF: 2.9 mM KCl: 147 mM NaCl; 1.6 mM $MgCl_2$; 2.2 mM dextrose; and1.7 mM $CaCl_2$). Prior to injection, the Aβ1–42 peptide was dissolved in an artificial cerebrospinal fluid (CSF) at a concentration of 5 mg/ml and incubated at 37°C for 72 h to induce aggregation. Each animal was given with 2.0µl (5µg/1µl) on each site of bregma through ICV injection using stereotaxic apparatus.

Preparation of plant extracts

The methanolic extract of leaves of *Jatropha Gossypifolia* have weighed equivalent to 100 mg/kg, 200 mg/kg and suspended in the 0.3% Carboxy Methyl Cellulose (CMC) to prepare the oral suspension of above extracts. The extract treatments were made through oral route in the respective group.

Table 1: Groups and treatments

Groups	Treatments	No. of animals
I	Control (ICV-2µl aCSF/site)	9
II	Amyloid-β ($A\beta_{1-42}$) (ICV- 10µg/2µl aCSF/site	9
III	$A\beta_{1-42}$ + methanolic extract of leaves of *Jatropha Gossypifolia* (100 mg/kg)	9

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IV	$A\beta_{1-42}$ + methanolic extract of leaves of *Jatropha Gossypifolia* (200 mg/kg)	9

Experimental Induction of Alzheimer's disease (ICV-$A\beta_{1-42}$) model

Rats were anesthetized using intra-peritoneal administration of ketamine (80mg/kg) and xylazine (10mg/kg). After anesthesia, ICV administration of $A\beta_{1-42}$ was done followed by using the stereotaxic apparatus. The scalp was incised and retracted, and the head position was adjusted to place bregma and lambda in the same horizontal plane. The rats skull were opened, and burr holes were drilled at the corresponding position to allow for the ICV injection of Aβ1–42 intra-hippocampal injection (anteroposterior: -3.8 mm from Bregma, medial/lateral: ±2.2 mm and dorsal/ventral: -2.7 mm). For Aβ1–42 infusion, two small holes were made, and Aβ1–42 (2.0 µl per side) was injected bilaterally into the lateral ventricles through a stainless steel cannula using a Hamilton microsyringe. Sham rats were bilaterally injected with same volume of saline (2.0 µl per side, ICV) into cerebroventricular. The injection lasted 5 min, and the needle with the syringe was left in place for 2 min after the injection to ensure the complete infusion of the drug. After surgery, two stainless steel obturators were inserted into the guides to prevent cannula occlusion. Penicillin was applied daily to prevent the infection, and the rats were allowed 7 days to recover from surgery. Then the rats were subjected to behavioural evaluation followed by neurobiochemicals estimation and histopathological study as mentioned above (Zhang et al., 2015; Ghumatkar et al., 2019).

Cognitive Behavioural studies

Radial Arm Maze Task

The RAM consists of 8 equidistantly spaced arms and length of each arm is 4 feet which is radiating from a small circular central platform. The height of this rotatable maze was elevated 90cm above the floor which is supported by a wooden stand. In case of trail period each arms were allotted for food reward and the pattern of baited and un-baited arms stayed same throughout test sessions. A food container is kept at the edge of each arm with food pellets as a reward for the rat and at the end of the arm a protection the goal box. Rails (2.5 cm high) were made for preventing the animal from falling.

The habituation training for the animal was done for eight days until the animal had gone into all eight arms or ten minute time. Inside the goal box the animal was allowed to remain in there for 1 minute. When the rat inserted only its head into the incorrect space of opening and if remain there more than one minute immediately it was replaced at the centre of the the maze. Animals which are proceeded through the non-spacial strategies were omitted from the present study. Each arm was washed with 70% of ethanol to wash out the previous animal traces (faeces and urine) when all of the paws are inside an arm was considered as arm entry. During this retention and acquisition phases the total behavioural performances were measured by latency to find the goal box and the recorded parameters were described below (Hritcu et al., 2014)

- Number of entry into an un-baited arm is denoted reference memory error
- Number of baited arm re-entries is denoted correct working memory error
- Number of un-baited arm re-entries are denoted as incorrect working memory error .

Step-through Passive Avoidance Test

The passive avoidance task measures the retention levels in rats. The task was performed in a box comprising of illuminated chamber connected to a dark chamber with a floor constructed with stainless steel rods. The light and dark chambers were separated by a guillotine door. The electronic stimulator was connected to the surface of floor rods in the dark chamber. The rats were familiarized to the apparatus for 5 min for 2 days prior the acquisition phase (Day 20). In training trials, the rats were individually placed in the lightened chamber, and when the rat arrive the dark chamber, the door was closed and the inescapable electric shock was delivered to the rat through the grid floor at 0.3 mA for 2 s once. Then the rat was removed from the apparatus 30s later. In this test, the initial latency of entering into the dark chamber was recorded on day 20. After twenty four hours of initial latency the same procedure without foot shock was repeated to assess the retention levels. The step-through latency on day 21 was measured for 300 sec by calculating the interval between the placements into the light chamber and entering into the dark chamber **(Yabuki et al., 2017)**.

IL-Time spent of animal in the dark chamber with inescapable electric shock in 30s.

STL-Time taken for the animal to enter the dark chamber from light chamber without electric shock in 300s

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Neurobiochemicals Evaluation
Anti-oxidant parameters
Brain Isolation

After completion of behavioural assessments, the rats were sacrificed by excessive anaesthesia. Then the brain samples were quickly isolated and hippocampus region was micro-dissected and washed with chilled saline and stored at -80°C till further evaluations. The whole brain samples were homogenized with 10% ice-cold KCl (a quantity of 100µl KCl for a quantity of 10mg tissue) for following anti-oxidant parameter analysis (Justin et al., 2014).

Lipid peroxide (LPO)

Lipid peroxidation was evaluated by measuring the TBAR content according to the thiobarbituric acid (TBA) test described by Ohkawa et al. (1979) with slight modifications. The incubation mixture consists of 0.5 ml of aliquot, 0.2 ml of 8 % sodium dodecyl sulphate, 1.5 ml of 0.9 % aqueous solution of thiobarbituric acid and double distilled water bath for 30 min. After cooling, the red chromogen was extracted into 5 ml of mixture of n-butanol and pyridine (15.1v/v) centrifuged at 4000 rpm for 10 min. The absorbance of the organic layer was taken at 532 nm (UV, Shimadzu, Japan). 1, 1, 3, 3-Tetra ethoxy propane was used as an external standard in the concentration range of 80–240 nmol (Ohkawa et al., 1979).

Nitric oxide (NO)

Nitric oxide (NO) was indirectly measured in the form of nitrates and nitrites taking 0.2 ml of 10 % homogenate followed by the addition of 1.8 ml of saline and 0.4 ml of 35 % sulphosalicylic acid for protein precipitation. The precipitate was removed by centrifugation at 4000 rpm for 10 min. To 1 ml aliquot of supernatant, 2 ml Griess reagent (1 g of sulphanilamide was dissolved in a small volume of water to which 2 ml of orthophosphoric acid and 100 mg of naphthyl ethyl diamine was added and the volume was made up to 100 ml) was added. The mixture was allowed to stand for 20 min under dark condition. The colour intensity was read at 540 nm (UV, Shimadzu, Japan). Standard calibration was plotted using sodium nitrite in the concentration range 200–1000 ng (Green et al., 1982).

Superoxide dismutase (SOD)

The sodium pyrophosphate buffer (0.025 M, pH 8.3) in a quantity of 0.3 ml was added to 0.05 ml of homogenate. To this mixture, 0.025 ml and 0.075 ml of PMS (186 µM) and NBT (300 µM in buffer, pH 8.3) were added. The initiation of the reaction was commenced by the instillation of 0.075 ml of NADH. The mixture was then incubated at temperature of 30 °C for a period of 90 sec. 0.25 ml of glacial acetic acid was added in-order to arrest the ongoing reaction. N-butanol (2 ml) was shaken vigorously along with the reaction mixture; later the mixture was centrifuged at 4000 rpm for 1 min. The colorimetric analysis was carried out at 560 nm using spectrophotometer, with n-butanol (1.5 ml) serving as blank (Kakkar et al., 1984).

Catalase (CAT)

A small quantity of brain homogenate (100 µl) or sucrose (0.32 M) was subjected to incubation with potassium phosphate buffer (2.25 ml) 65 mM at pH 7.8 for 30 min at 25 °C. The reaction was initiated by the addition of hydrogen peroxide (7.5 mM; 650 µl). The absorbance change was measured for a period of 2 to 3 min at 240 nm (UV, Shimadzu, Japan) (Beers and Sizer, 1952).

Reduced glutathione (GSH)

GSH content was estimated by following the method of Jollow et al. (1974). 0.25 ml of brain homogenate was added with equal volume of ice-cold 5 % TCA. The precipitate was removed by centrifugation at 4000 rpm for 10 min. To 1 ml aliquot of supernatant, 0.25 ml of 0.2 M phosphate buffer (pH 8.0) and 0.5 ml of DTNB (0.6 mM in 0.2 M phosphate buffer, pH 8.0) were added and mixed well. The absorbance was measured at 412 nm using spectrophotometer (UV, Shimadzu, Japan) (Jollow et al., 1974).

Inflammatory cytokines estimation

The hippocampal regions of the brain samples were renovate with buffer (0.1 % BSA, 81 mM Na_2HPO_4, 50 mM NaCl, 19 mM $NaH2PO_4$, 0.1 % Triton X-100, pH 7.4) and the level of IL-1β and TNF-α pro-inflammatory cytokines levels were quantified using ELISA kit (Quantikine and Invitrogen, USA) as per manufacturer's instruction (Justin et al., 2014).

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Neurotransmitter – Glutamate estimation

The hippocampal region was homogenized with 0.1 N HCl in 80 % ethanol (a fraction of 200 µl of ethanol for every 10 mg of brain tissue), the homogenate was transmitted to polypropylene tubes and were subjected to centrifugation at a rate of 4500 rpm for 20 min and 25 °C temperature was maintained. The resultant supernatant was obtained and stored in micro centrifuge tubes for the estimation of glutamate. The glutamate content was estimated by HPTLC (CAMAG — version 1.3.4, USA) chromatographic condition - silica gel GF254 as stationary phase, n-butanol: glacial acetic acid: water (65:15:25 v/v) as mobile phase; applicator-Linomat V, Scanner - CAMAG TLC scanner III, Developing chamber - twin trough glass chamber (20 × 10), Developing mode -Ascending mode (multiple development), Detection reagent - 0.2 % ninhydrin in acetone, Scanning wavelength - 486 nm, and experimental condition - 25 ± 2 °C room temperature, 55–65 % RH. Standard solutions of L-glutamic acid (20–200 ng/spot) was prepared for plotting the calibration curve (Justin et al., 2014).

Histopathology - Hematoxylin and Eosin staining

The brain samples were submerged in formalin for fixation and soaked in alcohol to remove the lipid debris. Then brain samples were fixed in paraffin wax and 5µm coronal sections were obtained in CA1 hippocampus region of the brain. Brain sections processing was done followed by staining with the hematoxylin and eosin (H&E). The sections were dehydrated using ethanol. After that, the sections were microscopically observed under 40X objective and the CA1 hippocampus region of the brain was photographed in order to understand the drug effects (Aras et al., 2015).

Statistical analysis

The values were expressed as the Mean ± SEM. All the data were statistically analyzed by using Graphpad Prism 6.0 software. Statistical significance was determined by One Way ANOVA followed by Tukey's multiple comparison tests to assess statistical differences between the groups. Values were considered statistically significant if $P<0.05$.

RESULTS

Table 2: Preliminary phytochemical studies of various extract of leaves of *Jatropha gossypifolia*

Phytochemical analysis of methanolic extract of leaves of *Jatropha gossypifolia* showed the presence of Carbohydrates, Glycosides, Alkaloids, Terpenes, Phytosterol, Saponins, Phenolic compounds and Tannins, Flavonoids.

Radial arm maze test

Table 3: Effect of methanolic extract of leaves of *Jatropha Gossypifolia* (met-L-JG) on Reference Memory Error (RME), Correct Working Memory Error (CWME), and Incorrect Working Memory Error (IWME) in Radial Arm Maze (RAM) test in ICV-Amyloid-β rat model.

S.No	Groups	RME	CWME	IWME
1	SO	1.39 ± 0.37	1.26 ± 0.28	2.00 ± 0.57
2	Aß(1-42)	$5.23 \pm 0.34^{***}$	$5.68 \pm 0.39^{***}$	$5.71 \pm 0.49^{***}$
3	met-L-JG(100 mg/kg)	$3.11 \pm 0.57^{\#}$	$3.67 \pm 0.32^{\#}$	3.88 ± 0.42
4	met-L-JG (200 mg/kg)	$2.23 \pm 0.64^{\#\#}$	$2.31 \pm 0.24^{\#\#\#}$	$3.10 \pm 0.44^{\#\#}$

Statistical significance was determined by one way ANOVA followed by Tukey's multiple comparison tests using Graph pad prism Version 6.0. Values are represented as Mean ± SEM. Superscript *** denotes $p<0.001$ vs SO group, ### denotes $p<0.001$; ## denotes $p<0.01$; # denotes $p<0.05$ vs $A\beta_{1-42}$treated group respectively. (SO: Sham Operated, Aβ: Amyloid beta)

Step through Passive Avoidance Test

Table 4: Effect of methanolic extract of leaves of *Jatropha Gossypifolia* (met-L-JG) on initial latency (IL) period, and Step through latency (STL) in Passive Avoidance Test (PAT) in ICV-Amyloid-β rat model.

S.NO	Groups	Initial Latency (Sec)	Step-through Latency (Sec)
1	SO	24.77 ± 2.64	253.66 ± 5.45
2	Aß(1-42)	$49.32 \pm 0.49^{***}$	$122.39 \pm 2.90^{***}$

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3	met-L-JG (100 mg/kg)	47.91 ± 1.81	125.27 ± 2.67
4	met-L-JG (200 mg/kg)	34.13 ± 1.87$^{###}$	162.48 ± 3.21$^{##}$

Statistical significance was determined by one way ANOVA followed by Tukey's multiple comparison tests using Graph pad prism Version 6.0. Values are represented as Mean ± SEM. Superscript *** denotes p<0.001 vs SO group, ### denotes p<0.001; ## denotes p<0.01; # denotes p<0.05 vs $A\beta_{1-42}$treated group respectively. (SO: Sham Operated, Aβ: Amyloid beta)

Neurobiochemicals Estimation

Table 5 : Effect of methanolic extract of leaves of *Jatropha Gossypifolia* (met-L-JG) on brain hippocampal Lipid Peroxide (LPO), Nitric oxide (NO), Superoxide dismutase (SOD), Catalase (CAT), Reduced Glutathione (GSH) level in ICV-Amyloid-β rat model.

S.NO	Groups	Malondialdehyde (uM of MDA/g tissue)	Nitrate/ Nitrite (uM/g tissue)	SOD (U/g tissue)	Catalase (U/ g tissue)	GSH (U/g tissue)
1	SO	112.27 ± 7.14	26.63 ± 2.62	16.45 ± 2.09	9.23 ± 0.47	29.55 ± 1.55
2	Aß(1-42)	440.79 ± 24.02***	92.86 ± 4.21***	3.63 ± 0.46***	1.83 ± 0.36***	5.41 ± 0.46***
3	met-L-JG (100 mg/kg)	371.49 ± 13.36	81.85 ± 1.78	4.89 ± 0.87	3.63 ± 0.23	7.60 ± 0.42
4	met-L-JG (200 mg/kg)	281.12 ± 9.82$^{##}$	49.51 ± 2.80$^{###}$	10.97 ± 0.96$^{##}$	7.45 ± 0.16$^{###}$	15.13 ± 1.27$^{###}$

Statistical significance was determined by one way ANOVA followed by Tukey's multiple comparison tests using Graph pad prism Version 6.0. Values are represented as Mean ± SEM. Superscript *** denotes p<0.001 vs SO group, ### denotes p<0.001; ## denotes p<0.01; # denotes p<0.05 vs $A\beta_{1-42}$treated group respectively. (SO: Sham Operated, Aβ: Amyloid beta)

Brain cytokines

Table 6: Effect of methanolic extract of leaves of *Jatropha Gossypifolia* (met-L-JG) on brain hippocampal interleukin-1β (IL-1β), Tumor necrosis factor alpha (TNF-α) level in ICV-Amyloid-β rat model.

S.NO	Groups	IL-1β (pg/ml)	TNF-α (pg/ml)
1	SO	137.20 ± 12.14	167.46 ± 4.19
2	Aß(1-42)	869.00 ± 11.04***	645.92 ± 22.13***
3	met-L-JG (100 mg/kg)	771.80 ± 8.945$^{#}$	556.13 ± 13.31$^{#}$
4	met-L-JG (200 mg/kg)	530.16 ± 14.29$^{###}$	463.26 ± 27.45$^{###}$

Statistical significance was determined by one way ANOVA followed by Tukey's multiple comparison tests using Graph pad prism Version 6.0. Values are represented as Mean ± SEM. Superscript *** denotes p<0.001 vs SO group, ### denotes p<0.001; ## denotes p<0.01; # denotes p<0.05 vs $A\beta_{1-42}$treated group respectively. (SO: Sham Operated, Aβ: Amyloid beta)

Neurotransmitters

Glutamate

Table 7: Effect of methanolic extract of leaves of *Jatropha Gossypifolia* (met-L-JG) on brain hippocampal glutamate level in ICV-Amyloid-β rat model.

S.No	Groups	Glutamate (µmoles/g tissue)
1	SO	3.55 ± 0.119
2	Aß(1-42)	11.72 ± 0.258***
3	met-L-JG (100 mg/kg)	10.22 ± 0.49
4	met-L-JG (200 mg/kg)	7.90 ± 0.44$^{###}$

Statistical significance was determined by one way ANOVA followed by Tukey's multiple comparison tests using Graph pad prism Version 6.0. Values are represented as Mean ± SEM. Superscript *** denotes p<0.001 vs SO group, ### denotes p<0.001; ## denotes p<0.01; # denotes p<0.05 vs $A\beta_{1-42}$treated group respectively. (SO: Sham Operated, Aβ: Amyloid beta)

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Histopathology studies

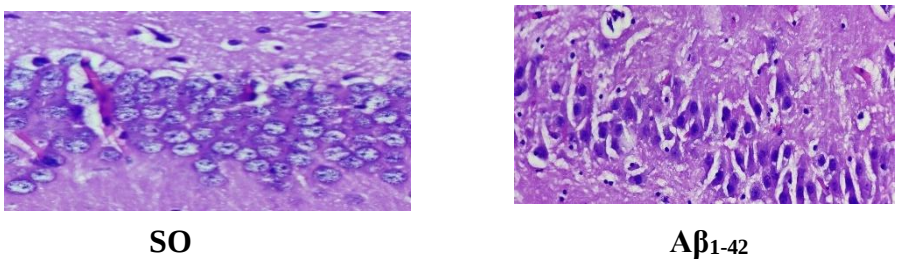

Figure 1: Sections of CA1 hippocampus region of brain stained with Haemotoxylin and Eosin staining

DISCUSSION

Radial arm maze

The radial arm maze has been widely used to assess memory in a number of settings, including the impact of psychoactive drugs and treatments.[5,6] The RAM has been used to investigate the impact of a variety of substances on rat cognitive performance.[7,8] In RAM experimental studies, we measured reference memory error (RME) and working memory error (WME). RM error is defined as the number of times an animal discovered a path that they entered in the same block, regardless of whether that path was rewarded. WM error was defined as the number of times an animal re-entered paths that were not rewarded.

In reference memory error (RME), **i**ntracerebroventricular (ICV) administration of $A\beta_{1-42}$ in rats has increased the RME score in comparison to SO rats. Post-treatment with met-L-JG (100 and 200 mg/kg), have notably decreased the RME score in cognitively declined rats by dose depended manner that indicates the cognitive enhancement potential of plant extracts treatment. Similarly, correct working memory error (CWME) and incorrect working memory error (IWME) test show the $A\beta_{1-42}$administered group has shown an increase in CWME score indicates the loss of cognitive function upon ICV administration of $A\beta_{1-42}$in rats when compared to SO group animals. However, treatment with met-L-JG (100 and 200 mg/kg), have remarkably decreased the CWME and IWME score in $A\beta_{1-42}$administered rats by dose dependent manner indicate that the plant extracts possess, enhancement of cognitive performance activity against $A\beta_{1-42}$ insulted toxicity in rats.

Passive Avoidance Test

The Passive Avoidance Task is a fear-aggravated test used in rat models of CNS diseases to assess learning and memory. Subjects learn to avoid situations in which they have previously been exposed to an aversive stimulus (such as a foot shock) in this test. The Passive Avoidance task can be used to assess the impact of a new medicine on learning and memory, as well as to research cognition systems.[9]

In this experiment, we measured the latency period to cross through the gate between the compartments. The initial latency (IL) period in passive avoidance test, the $A\beta_{1-42}$administered group has shown to increase in IL

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period when compared to SO group, which denotes the declining of memory function after administration of $A\beta_{1-42}$in rats. Post-treatment with met-L-JG (100 and 200 mg/kg), have notably reduced the IL period in $A\beta_{1-42}$infused rats, results indicate that the plant extract had ability to enhancement of cognitive activity.

The Step-through latency (STL) in passive avoidance test, the $A\beta_{1-42}$ administered group has reduced STL period when compared to SO group. At the same time, post-treatment with met-L-JG (100 and 200 mg/kg) in passive avoidance test, the $A\beta_{1-42}$ administered group has significantly ($p<0.001$; $p<0.01$) increases in STL period when compared to $A\beta_{1-42}$ administered alone group.

Antioxidant studies *in vivo* experiments:

The numerous experimental and clinical studies have demonstrated that oxidative damage plays a key role in the loss of neurons and the progression to dementia.The production of β-amyloid, a toxic peptide often found present in Alzheimer's patient's brain, is due to oxidative stress and plays an important role in neurodegenerative process.[10,11]There is strong evidence that oxidative brain damage is a precursor to Alzheimer's disease.

Many different types of antioxidant have been investigated in relation to Alzheimer's disease,[12] but most experiments have been conducted *in vitro*. However, we conducted experiments in *in vivo* models using Aβ1-42 in rats respectively to evaluate the antioxidants status of plant extracts in AD conditions.

Recent studies indicate that amyloid beta- peptide (Aβ) can be neurotoxin by mechanisms involving the generation of H_2O_2, ROS and lipid peroxidation.[13,14]In our experimental studies, administration of $A\beta_{1-42}$ in rats has remarkably increased ($p<0.001$) the LPO and NO levels in hippocampus region of the brain when compared to SO vehicle treated rats. The administration of met-L-JG (100 and 200 mg/kg), have significantly ($p<0.001$; $p<0.01$) altered the hippocampal LPO and NO levels in$A\beta_{1-42}$ infused rats

It has been suggested that the brain of the AD patients are affected by inordinate oxidative stress.[15] This may be due to decreased SOD, CAT and GSH activities, leading to elevation of H_2O_2 concentration in AD. The generated H_2O_2 is used for hydroxyl radical production via Fenton and Haber-Weiss reactions.[16,17] Identifying marker(s) that can be used as a measure of oxidative stress associated with pathologic alterations in the brain of people with Alzheimer's disease is therefore extremely important.

On contrarily, drastically attenuated ($p<0.001$) the hippocampal SOD, CAT and GSH level was observed in $A\beta_{1-42}$ intoxicated rats when compare to normal group animals. On the other hand, met-L-JG extracts restore the levels of SOD, CAT and GSH that indicates the anti-oxidant potential of extracts in $A\beta_{1-42}$ intoxicated rats.
Brains of people with Alzheimer's disease appear to have higher levels of natural antioxidants responsible for 'clearing up' excess free radicals, suggesting that the body is trying to combat this damage. Our finding is in accordance with previous studies and the epidemiological data, demonstrating that antioxidants may have a beneficial effect on many age-related diseases, such as AD.[18,19]

In vivo studies have been observed that beta amyloid aggregated peptide, a characteristic feature of AD, is toxic to neurons is likely through generation of free radicals and by induction of lipid oxidation.[20] Marcus *et al*[21] have reported that the temporal lobes of AD patients consistently showed significant differences in activity for the above studied SOD, CAT, GSH, LPO and NO, suggesting that abnormalities in the antioxidant system may lead to neuronal cell death in AD. For these facts, antioxidant properties of met-L-JG extracts are promising in the prevention and treatment of oxidative stress-related diseases, which is the case for ADs.

Inflammation Associated AD

Inflammation of the brain is a pathological hallmark of Alzheimer's disease. Inflammation is observed in pathologically susceptible areas of the AD brain, with elevated production of acute phase proteins and proinflammatory cytokines not seen in the normal brain.[22-25]The inflammatory response is caused by microglia, astrocytes, and neurons. Beta-amyloid (A)-induced neuroinflammation plays a key role in the aetiology of Alzheimer's disease (AD), and decreasing Aβ induced neuroinflammation could be a promising therapy option. Interleukin-1β is a proinflammatory cytokine that modulates neurotoxic neurotransmission IL-1 β is a proinflammatory cytokine that is upregulated early in AD development and is considered crucial for β-amyloid plaque deposition.[26] IL-1β may play a key role in local brain tissue reactions came from demonstrations of elevated IL-1β have been reported within brain lesions from patients with Alzheimer's disease (AD).[27] These findings have since been reproduced in corresponding animal models of disease for AD.[28]

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During an inflammatory response, TNF- plays a critical role in both initiating and controlling the cytokine cascade. It starts out as a 26 kDa membrane-bound precursor molecule, which is then cleaved by the TNF-converting enzyme to yield a 17 kDa active cytokine.[29]

The levels of TNF-*α* expression in healthy individual brain are generally low, making it difficult to determine its precise role under physiological conditions. In inflammatory or disease states, TNF-*α* along with several other proinflammatory mediators and neurotoxic substances are predominantly produced by activated microglia. Neuronal production of TNF-*α* has been demonstrated, [30] although brain-derived TNF-*α* is mostly synthesized by glial cells in response to pathological stimuli.

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In our studies, *in vivo* experiments show the $A\beta_{1-42}$ administration through ICV route has significantly elevated ($p<0.001$) the hippocampal IL-1β and TNF-α levels in comparison to SO group indicates the intensity of neuroinflammation occurred upon $A\beta_{1-42}$intra-hippocampal infusion. Administration of met-L-JG (100 and 200 mg/kg), have drastically attenuated the brain IL-1β and TNF-α levels in the $A\beta_{1-42}$ treated rats represents the anti-neuroinflammatory activity of met-L-JG extracts in neurodegenerative conditions. The concomitant release of anti-inflammatory mediators by met-L-JG (100 and 200 mg/kg), extracts treatment may partly antagonize this action ultimately leading to chronic disease.

GLUTAMATE IN AD

Glutamate is a potent excitatory neurotransmitter of nerve cells in the brain release. It's in charge of transferring messages between nerve cells, and it's crucial for learning and memory under normal circumstances. Glutamate is the brain's most abundant fast-acting neurotransmitter, and it plays a critical role in learning, memory, and cognition.[31] Glutamate is required for brain activities such as learning and memory at normal concentrations. However, at high concentrations the increased cellular activity caused by glutamate results in over-excitation of nerve cells, which eventually leads to cell death.[32] Administration of $A\beta_{1-42}$ in rats has remarkably increased ($p<0.001$) the glutamate level in hippocampus region of the brain when compared to SO vehicle treated animals indicates excitotoxicity in neurons after $A\beta_{1-42}$ infusion. Administration of met-L-JG (100 and 200 mg/kg), have significantly reduced ($p<0.001$; $p<0.01$) the hippocampal glutamate level in comparison to $A\beta_{1-42}$ infused rats indicates that the plant extracts possess anti-excitotoxicity capacity against $A\beta_{1-42}$ insulted neurodegenerative conditions.

HISTOPATHAOLOGICAL STUDY

Intra-hippocampal injection of $A\beta_{1-42}$through ICV route has caused significant neuronal morphology changes and the neurons were noticeably degenerated and became necrotic, swelling and their arrangement was messy. Post-treatment with met-L-JG (100 mg/kg) has shown some degree of restoration of neuronal morphology from $A\beta_{1-42}$induced neurodegeneration while met-L-JG (200 mg/kg) have reduced the neuronal damage with decreased necrotic, swelling and neuronal degeneration with restored intact cells which are comparable with SO group.

CONCLUSION

In an *in-vivo* behavioural study, results showed that decreased RME, CWME and IWME in cognitively declined rats in the Radial arm maze indicates the enhancement of cognitive potential of the plant extracts. Also, notably reduced the IL and increased the STL period in Aβ1-42 infused rats evidence that enhancement of the cognitive activity of the tested plant extracts. The met-L-JG extracts restore the levels of LPO, NO, SOD, CAT and GSH which are altered by the $A\beta_{1-42}$ intoxication. This indicates the antioxidant potential of extracts in $A\beta_{1-42}$ intoxicated rats. The antioxidant properties of met-L-JG extracts are promising in the prevention and treatment of oxidative stress related ADs. In *in vivo* experimental studies, the plant extracts have shown a significant reduction in neuroinflammatory agents such as TNF-α and IL-1β in Aβ1-42 intoxicated SH-SY5Y cell lines and experimental rats, indicating the anti-neuroinflammatory potential of the extracts in neurotoxicity. Hence, decreasing Aβ induced neuroinflammation by met-L-JG extracts could be a promising therapy option. The met-L-JG extracts reduced the hippocampal glutamate levels in $A\beta_{1-42}$ intoxicated rats, indicating that the plant extracts possess the anti-excitotoxicity capacity in neurodegenerative conditions like Alzheimer's disease.In view of the above results, methanolic extracts of leaves o*f Jatropha gossypifolia* plants concluded that they have potent anti-Alzheimer's activity in experimental animals. Hence, our research strongly indicates that met-L-JG extracts treatment improved the cognitive enhancement activity in $A\beta_{1-42}$ insulted ADs conditions. Also, support the possible development of these plants into suitable formulations for further in-depth preclinical studies and clinical studies as pharmacological targets in neurotoxic situations in the future.

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