

NEUROPROTECTIVE EFFECT OF *LEUCAS ASPERA* IN STREPTOZOTOCIN INDUCED ALZHEIMER RAT MODEL

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ABSTRACT: The present investigation deals with the assessment of neuroprotective effect *Leucas aspera* in streptozotocin (STZ) induced Alzheimer's disease (AD) in rats. AD was induced by administering STZ (3 mg/kg, ICV) on day one and 3rd day after surgery. Surgery was performed on anesthetized rats with the help of the stereotaxic apparatus. STZ induced AD rats were treated with ethanolic extract of *Leucas aspera* (100, 200, and 400 mg/kg, p.o.) for 28 days. Effect of *Leucas aspera* root in AD rats was assessed by estimating the alteration in the behavior (Y maze apparatus and single trail passive avoidance) and biochemical parameter in the brain tissue {Oxidative stress parameters (SOD, CAT, and LPO), amyloid β peptide (A β) and acetylcholinesterase (AChE). Treatment with *Leucas aspera* shows significant ($P < 0.01$) increased in the % of alteration in the behavior and step-through latency in Y maze task and single-trial passive avoidance test compared to AD rats. *Leucas aspera* significantly ($P < 0.01$) decreases the AChE in the brain tissue compared to AD rats, whereas treatment with *Leucas aspera* significantly reduces the oxidative stress level in AD rats. The present study concludes the neuroprotective effect of *Leucas aspera* extract in AD rats by reducing oxidative stress and AChE in the brain tissue.

Keywords:

Leucas aspera, Alzheimer's disease, Amyloid β peptide, Acetylcholinesterase, Oxidative stress

INTRODUCTION: Alzheimer's is associated with neuronal degeneration, characterized by memory loss and altered behavior. Many pathological conditions like increased oxidative stress, amyloid β (A β) plaque formation, neuroinflammation results in AD ¹. Free radicals and the oxidative stress they generate have been implicated as the prime factors responsible for the neuronal changes which mediate these behavioral effects.

Therefore, free radical scavengers and antioxidants have been proposed as agents that may delay or inhibit the progression of such neurodegenerative disorders ². *Leucas aspera* belonging to the family Lamiaceae is well-known for its wide medicinal applications. It is used traditionally as an anti-inflammatory, a stimulant in the treatment of jaundice, cough, asthma, conjunctivitis, diabetes, malaria, skin diseases, snakebite, toothache, and wound healing.

L. aspera is scientifically evaluated for anti-inflammatory activity, analgesic activity and cobra venom-induced mortality in mice, anti-parasitic activity, antibacterial activity against *M. pyrogenes*, *V. aureus*, and *Escherichia coli*. It is toxic to the filarial vector mosquito, antinociceptive, anti-oxidant, and cytotoxic activity ³⁻¹². The plant revealed the presence of triterpenoids, oleanolic acid, ursolic acid, and 3-sitosterol ^{13, 14}. Aerial parts are reported to contain nicotine, sterols, two new alkaloids (α -sitosterol and β -

sitosterol), reducing sugars (galactose), and glucoside ¹⁵. The present study was undertaken to evaluate the neuroprotective of *Leucas aspera* in STZ induces AD in Wistar rats.

MATERIALS AND METHODS:

Collection and Authentication of Plant Material: Roots of *Leucas aspera* were collected from local areas around Nilgiris, Tamil Nadu, India. The plant material was authenticated by Dr. S. Rajan, Field Botanist, Survey of Medicinal Plants, and collection Unit.

Preparation of Extracts: In this study, the dried root was coarsely powdered and kept it into a plastic container with ethanol for 72 h. The extract was concentrated using a rota vapor at reduced temperature and pressure in order to remove the solvent completely. It was dried and kept in desiccators till experimentation (Yield 8.5% w/w).

Animals: Healthy male Wistar rats (180-250 g) at about 8 weeks of age were used for the pharmacological screening in the present study. The animals were housed at 20 ± 2 °C temperature, 12 h light/dark cycle, and $60 \pm 5\%$ of relative humidity. Rats were fed with standard diet and water *ad libitum*. Protocols of the present investigation for all the animal studies were approved by the Institutional Animal Ethical Committee.

Acute Toxicity Study: The acute toxicity study was performed for the ethanolic extract of *Leucas aspera* according to the OECD 423 guidelines ¹⁶. The extract at different doses of 5, 50, 300, and 2000 mg/kg, p.o., was administered to the rats and observed closely for 14 days. All the rats were observed for behavior changes and mortality at the end of the experiment. The extract was found safe up to 2000 mg/kg.

Induction of AD: Male Wistar rats were anesthetized by IP injection of a combination of ketamine and xylazine (100 and 5 mg/kg, respectively) and then all the rats were operated by using stereotaxic apparatus (Stoelting, USA). Stereotaxic atlas was used for the surgery, rats scalp washed by using iodine solution, incised on midline and a hole was drilled through the skull 0.8 mm post bregma, 1.4 mm lateral from midsagittal line and 3.4 mm below the dura. Rats were divided into 5 different groups: control (Sham-operated), Streptozotocin treated rat (STZ, 3 mg/kg, icv), Streptozotocin plus *Leucas aspera* 100 mg/kg (STZ + LA 100 mg/kg, p.o.), Streptozotocin plus *Leucas aspera* 200 mg/kg (STZ + LA 200 mg/kg, p.o.), Streptozotocin plus *Leucas aspera* 400 mg/kg (STZ + LA 500 mg/kg, p. o.). The STZ groups received bilateral ICV injection of streptozotocin (3 mg/kg, body weight), which was dissolved in citrate buffer (pH 4.4). STZ concentration was prepared so as to deliver 5 μ l / injection site of the solution. Rats in the control group received ICV injection of the same volume of citrate buffer as in STZ treated, and the injection was repeated on day 3. AD rats were treated for 4 weeks with *Leucas aspera* extract and its anti-alzheimer's activity was assessed by using behavioral models (Y maze task and single trial passive avoidance) and biochemical parameters in the brain tissues of rats (AChE, oxidative stress parameters) ¹⁷.

Behavioral Models:

Y-maze Task: Short-term spatial memory performance was assessed by recording spontaneous alternation behavior during a single session in a Y-maze ¹⁸⁻¹⁹. This method is based on the tendency of rodents to enter an arm of a Y-maze that was not explored in the last two choices. Each rat, naive to the maze, was placed at the end of one arm and allowed to move freely through the maze during an 8 min session. The series of arm entries was recorded visually. Arm entry was considered to be completed when the base of the animal's tail had been completely placed in the arm. Alternation was defined as successive entries into the three arms on overlapping triplet sets. The effect was calculated as percent alternation according to the following formula

Percentage alteration = (Number of alterations / Total number of entries-2) \times 100%

Single Trial Passive Avoidance Test: This test was done 3 days after Y-maze task ²⁰. The apparatus consisted of an illuminated chamber connected to dark chamber by a guillotine door. Electric shocks were delivered to the grid floor by an isolated stimulator. On the first and second days of testing, each rat was placed on the apparatus and left for 5 min to habituate to the apparatus. On the third day, an acquisition trial was performed. Rats were individually placed in the illuminated chamber. After a habituation period (2 min), the guillotine door was opened, and after the rat entering the dark chamber, the door was closed, and an inescapable scrambled electric shock (40 V for 3 s once) was delivered. In this trial, the initial latency (IL) of entrance into the dark chamber was recorded, and rats with IL greater than 60 s were excluded from the study. Twenty-four hours later, each rat was placed in the illuminated chamber for retention trial. The interval between the placement in the illuminated chamber and the entry into the dark chamber was measured as step-through latency (STL, up to a maximum of 600 s as a cut-off).

Estimation of Biochemical Parameter:

Brain Tissue Homogenate Preparation: Rats were sacrificed and brain dissected out, washes it thoroughly with saline solution, and divided into two halves. One-half of the brain of each rat was homogenized instantaneously in a solution containing Tris-Hcl (50 mM, pH 7.4) and sucrose (300 mM). The tissue homogenate was centrifuged at 10000 RPM for 10 min at 4 °C, and the supernatant was separated for the below given biochemical estimation ²¹.

Estimation of β Amyloid: A β was measured in brain tissue extracts using an ELISA Kit. A β was captured by antibody-coated plates (BNT77) and detection of A β 1-40 and A β 1-42 peptides achieved with the horseradish peroxidase-conjugated antibodies BA27 or BC05, respectively. Developed plates were analyzed at an optical density of 450 nm ²².

Determination of AChE Activity in Brain: Cholinergic dysfunction was assessed by measuring AChE levels in brain tissue ²³. The assay mixture contained 0.05 ml of supernatant, 3 ml of 0.01 M sodium phosphate buffer (pH 8), 0.10 ml of acetylthiocholine iodide, and 0.10 ml 5, 5, dithiobis (2-nitro benzoic acid) (Ellman reagent). The change in absorbance was measured at 412 nm for 5 min.

Results were calculated using molar extinction coefficient of chromophore ($1.36 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$).

Estimation of Markers of Oxidative Stress: Superoxide dismutase (SOD) was estimated in the brain tissue of STZ treated AD rats by using riboflavin sensitized method. The alteration in absorbance was observed for 4 min at 460 nm ²⁴. The level of lipid peroxidation (LPO) was estimated the method given by Ohkawaka in the brain tissue of rats. The quantity of malondialdehyde (MDA) was estimated at 532 nm ²⁵. The activity of catalase (CAT) in the brain tissue was assessed on the ability of catalase to oxidize H₂O₂. The change in absorbance was recorded for 3 min at 1 min interval at 240 nm ²⁶.

Statistical Analysis: Data were expressed as mean \pm SEM (n=6). Data were statistically analyzed using one way ANOVA (Dunnett post hoc test). P<0.05 was considered statistically significant.

RESULTS:

Estimation of Change in Behavior Parameters: Alzheimer's disease was induced by STZ, and after completion of the treatment protocol, the effect of treatment was assessed by estimating alteration in the behavior by Y maze control and step-through latency by single-trial passive avoidance test. **Fig. 1** shows the % of spontaneous alteration in the behavior by using Y maze in control (Vehicle treated), negative control (STZ treated), STZ + LA (100 mg/kg, p. o.), STZ+LA (200 mg/kg, p. o.), STZ + AC (400 mg/kg, p. o.) group of rats. There was a significant decrease (P<0.05) in the % of alteration in the behavior of the negative control group compared to the control group of rats.

This decrease in the % alteration in behavior confirms the AD in a negative control group, i.e., STZ only treated rats. Whereas, treatment with LA at the dose of 100.200 and 400 mg/kg were significantly ($P<0.05$) increased the % of alteration in behavior compared to AD rats. **Fig. 2 and 3** show the effect of LA on STL in the passive avoidance test in STZ treated AD rat. For STL, there was decrease time significantly ($P<0.05$) in STZ only treated rats compared to the control group. This decreased time was recovered with the treatment significantly at the dose of LA 100, 200, and 400 mg/kg ($P<0.05$, respectively).

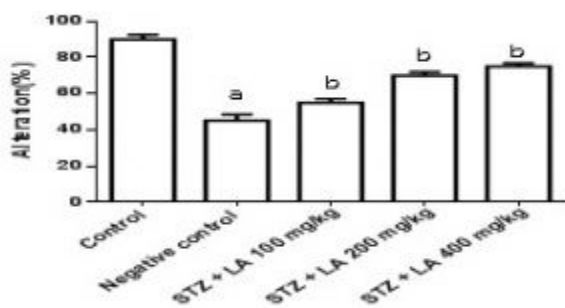
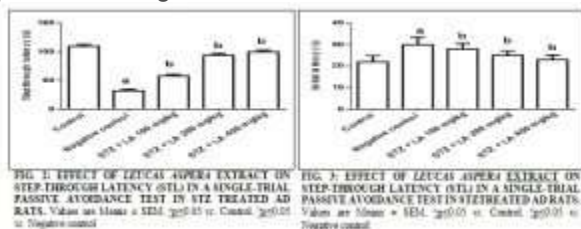
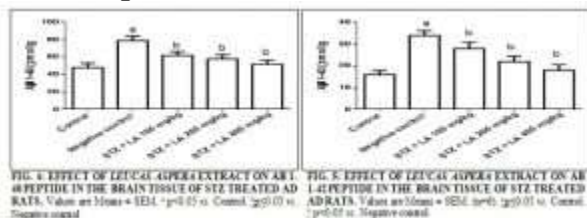


FIG. 1: EFFECT OF *LEUCAS ASPERA* EXTRACT ON ALTERATION IN BEHAVIOR IN THE Y-MAZE MODEL IN STZ TREATED AD RATS. Values are Means \pm SEM. a $p<0.05$ vs. Control. b $p<0.05$ vs. negative control



Estimation of Biochemical Parameters:

Estimation of β Amyloid in Brain Tissue: β amyloid 1-40 and 1-42 peptides were estimated in the brain tissues of rats. **Fig. 4 and 5** show the estimation of A β 1-40 and A β 1-42, these subunits of β amyloid peptide in brain tissues of control (Vehicle treated), negative control (STZ treated), STZ+LA (100 mg/kg, p.o.), STZ + LA (200 mg/kg, p.o.), STZ + LA(400 mg/kg, p.o.) treated rats. It was observed that level of A β 1-40 and A β 1-42 in the brain tissue of the negative control group (STZ only treated rats) increases significantly ($P<0.05$) compared to the control group of rats. However, this increased level of A β 1-40 and A β 1-42 was found to be decreased significantly ($P<0.05$) in LA treated group compared to AD rats (negative control group). This decrease in the level of A β 1-40 and A β 1-42 plays a role in the decrease in neuron degeneration.



Estimation of AchE in Brain Tissue: Effect of ethanolic extract of LA on AchE activity in STZ induced AD rats, as shown in **Fig. 5**. STZ administration produced significant elevation ($P< 0.05$) in brain AchE activity compared to the control group of rats. However, treatment with LA extract of 100, 200, and 400 mg/kg

resulted in significant inhibition ($P < 0.05$) of AchE activity in brain tissue as compared with the negative control group of rats.

Estimation of Oxidative Stress Parameters: Effect of LA extract on superoxide dismutase, lipid peroxidation, catalase in the brain tissue of STZ treated AD rats were shown in **Table 1**. STZ induced AD, results in significant ($P < 0.05$) decrease in the SOD and increase ($P < 0.05$) in the LPO and CAT level compared to the control group of rats. However, treatment with LA extract significantly improved the SOD level in the brain tissues compared to negative control group of rats. LPO and CAT levels were found to be significantly decreased ($P < 0.05$) in the brain tissues of LA extract-treated group of rats compared to negative control group of rats. Moreover, the study result also suggested that this improvement in the level of oxidative stress parameters is dose-dependent.

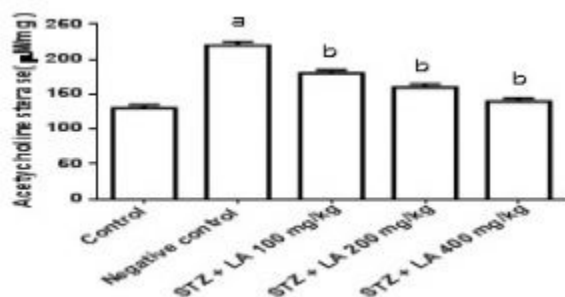


FIG. 6: EFFECT OF *LEUCAS ASPERA* EXTRACT ON ACHE IN THE BRAIN TISSUE OF STZ TREATED AD RATS. Values are Means \pm Sem $p < 0.05$ vs. control. ^b $p < 0.05$ vs. negative control

Values are Means \pm SEM. ($n=8$); @ $p < 0.01$ (vs. Control group), * $p < 0.05$, ** $p < 0.01$ (vs. Negative control group)

DISCUSSION: AD induced by STZ produces progressive deficits in cognitive function in rats, which is similar to sporadic kind of AD, as indicated by behavioral tests including passive avoidance paradigm and spatial cognitive deficit in Y-maze task ²⁷.

Literature suggested that improvement in the % alteration and step-through latency behavior in Y maze and a single-trial passive avoidance test, respectively, confirms the improvement in memory and cognitive performance in AD rats ²⁸. Results of present study demonstrated that treatment with LA (100 mg/kg, 200 mg/kg & 400 mg/kg) ameliorates the cognitive function in STZ induced AD rats.

Reported studies reveal that spatial cognitive deficits are characterized by changes at the level of various neurotransmitters and related markers ²⁹. Cholinergic system is the most severely affected body system in spatial cognitive deficits, and elevation of the Ach level might be helpful in attempts to improve the symptoms of cognitive deficits in Alzheimer's disease ³⁰.

The result of this study suggested that treatment with LA extract reduces the AchE level in the brain tissues of AD rats. Increased level of β A peptide in the brain tissues increases the levels of AchE, and on the basis of this, it results in neurodegeneration ³¹.

Treatment with LA extracts found to reduce the concentration of β A peptide significantly in the brain tissue of STZ induced AD. ICV administration of STZ elevates the oxidative stress and thereby alters the cholinergic markers and β A peptide level in the brain tissues of AD rats ³².

LA extracts possess strong antioxidant properties by improving SOD, LPO, and CAT level in the brain tissues of STZ induced AD rats.

CONCLUSION: The present study concludes that LA possesses a neuroprotective effect in STZ induced AD rats. The study also postulates the mechanism of its action as treatment with LA reduces oxidative stress, which decreases the concentration of β A peptide in the AD rat. This decreased concentration of β A peptide in the AD rat attenuates the AchE level, which increases the concentration of acetylcholine in the brain tissue, and thereby, it protects the neurodegeneration in STZ induced AD rats.

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CONFLICTS OF INTEREST: Nil

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