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ORIGINAL PAPER

Baicalein's neuroprotective effects in a lansoprazole-induced Alzheimer's model in Wistar rats

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ABSTRACT

Introduction and aim. Alzheimer's disease (AD) is a devastating neurodegenerative disorder and the most frequently diagnosed type of dementia. Baicalein, a flavonoid found in the roots of *Scutellaria baicalensis*, was useful in preventing neuronal injury in various neurodegenerative models. The present study was designed to determine the neuroprotective effect of baicalein in lansoprazole-induced AD in Wistar rats.

Material and methods. We used male Wistar rats randomly divided into six groups: control, vehicle, lansoprazole-induced, and baicalein+lansoprazole or baicalein treated. The lowest dose, lansoprazole (30 mg/kg), was administered orally for 28 weeks to induce AD-like pathology. Baicalein (10 mg/kg) was coadministered with lansoprazole in the treatment group. Cognitive functions were evaluated using a Morris water maze (MWM) and a novel object recognition test. Acetylcholinesterase (AChE) activity, determination of oxidative stress, including malondialdehyde, superoxide dismutas, and reduced glutathione levels, and inflammation-related cytokines such as tumor necrosis factor α were biochemically analyzed. We collected hippocampal sections to assess amyloid beta deposition and neuronal integrity histochemically.

Results. Lansoprazole-induced rats showed marked cognitive decline accompanied by enhanced AChE activity, increased oxidative stress, and levels of pro-inflammatory cytokines compared to controls ($p<0.01$). Co-administration of Baicalein significantly improved cognitive performance, AChE activity was reduced to control levels, oxidative stress markers decreased to near-normal values in the brain and blood, and inflammatory cytokines were significantly lower compared to rats treated with the lansoprazole-treated rats ($p<0.01$). Baicalein treatment attenuated plaque deposition and neuronal injury, as revealed by histopathological analysis in vivo.

Conclusion. Baicalein has a protective effect on lansoprazole-induced AD in Wistar rats. These appear to be due to the flavonoid's antioxidative, anti-inflammatory, and enzyme-inhibiting activities on AChE. These results propose baicalein as a potential therapeutic agent for further study with respect to prevention and treatment, especially in patients under long-term administration of proton pump inhibitors.

Keywords. Alzheimer disease, anti-inflammatory, baicalein, lansoprazole, oxidative stress

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Introduction

Alzheimer's disease (AD) is the most common type of dementia that affects the elderly population. It is marked by behavioral changes, cognitive decline, and a decline in social functioning.[1] The defining characteristic of AD is the gradual deterioration of cognitive functions, with recent fact memory, spatial orientation, attention, and executive functions among the first to be compromised.[2] Subsequently, there are issues with speech and behavior that impact daily life.[2] Several elements potentially play a role in the onset of AD, including genetic anomalies, mitochondrial dysfunction, modified processing of amyloid precursor protein, the emergence of neurofibrillary tangles, insufficiency in neurotrophic factors, as well as heightened oxidative stress.[3] The accumulation of extracellular amyloid in the brain stimulates microglia activation, leading to the release of various proinflammatory cytokines such as interleukin (IL)-1β, tumor necrosis factor α (TNF-α), and IL-6. Furthermore, amyloid accumulation induces mitochondrial dysfunction by hindering oxidative phosphorylation, increasing reactive oxygen species (ROS) production, disrupting mitochondrial energy, and interacting with various mitochondrial proteins.[4]

The potential link between proton pump inhibitors (PPIs) and AD has been a subject of growing interest in recent years. PPIs such as omeprazole, lansoprazole, and pantoprazole are commonly prescribed for acid-related diseases such as gastroesophageal reflux disease and peptic ulcers.[5] However, recent findings suggest that their long-term use may have unintended effects on cognitive function and neurodegeneration. Other research indicates that PPIs can encourage the buildup of beta-amyloid (Aβ), a characteristic of AD.[6] Some studies show that some PPIs can modify lysosomal function and decrease Aβ clearance, which could be a factor in plaque development in the brain.[7-8] PPIs such as omeprazole and lansoprazole have been found to modulate gamma-secretase activity, an enzyme involved in Aβ generation, and result in increased amyloidogenic processing.[9] PPIs can modulate acetylcholine concentration by inhibiting choline acetyltransferase, an enzyme responsible for acetylcholine synthesis. Acetylcholine deficiency is a characteristic of cognitive impairment in AD.[10]

According to their hypothesis, PPIs penetrate the blood-brain barrier and block vacuolar-type ATPase proton pumps. Due to this suppression of V-ATPases, microglial lysosomes have a higher pH and microglial phagocytosis breaks down less Aβ.[11] It is known that amyloid precursor protein (A-42 and A-42) and amyloid beta (A-40) are localized in mitochondrial membranes, interact with mitochondrial proteins, obstruct nuclear-encoded mitochondrial proteins from entering mitochondria, disrupt the electron transport chain, enhance ROS, damage mitochondria, and affect neuronal function.[12]

In addition, buildup of amyloid beta in the synaptic terminals in AD patients may contribute to synaptic degeneration and cognitive loss.[13] Therefore, the production of reactive oxygen species is caused by the release of electrons and the formation of free radicals. However, inhibition of the electron transport chain does not ensure a direct increase in ROS production of ROS within the mitochondria.[14] An excess of reactive oxygen species in the brain can lead to oxidative stress together with neuroinflammatory release, which can harm the central nervous system as well as result in neurodegenerative diseases such as AD.[15]

The current investigation was carried out to establish lansoprazole-induced neurological modification as an animal model of AD in rats.[16] Furthermore, the model was verified by modulation of various neurobehavioral and neurochemical parameters.[17] Baicalein is a flavonoid compound isolated from the roots of *Scutellaria baicalensis*, known as Baikal skullcap or Chinese skullcap, a Chinese herbal medicine.[18] Baicalein is a traditional Chinese medicine used for centuries to treat inflammatory disorders, infections, and even cancer. Baicalein has been supported by modern scientific research that reveals its numerous pharmacological activities suggesting that it may also be of significant interest in modern medicine.[19] Baicalein, on the other hand, is highly effective in reducing inflammation, which is a complex biological response necessary for recovery but when prolonged is associated with diseases such as arthritis, cardiovascular diseases, and neurodegenerative disorders to name a few.[20] Modulation of multiple signaling pathways and molecular targets is done by inhibiting cyclooxygenase-2 (COX-2) and lipoxygenase (LOX), the two enzymes that are mandatory in the process of inflammation. This reduces the production of pro-inflammatory mediators such as prostaglandins and leukotrienes and relieves inflammation.[21]

In addition to its anti-inflammatory activity, baicalein also demonstrates considerable antioxidant action. Oxidative stress caused by an imbalance of free radical and antioxidant levels is known to be associated with many chronic diseases, such as cancer, diabetes, or heart diseases.[22] Baicalein possesses a shielding activity against oxidative damage to the cells and helps to restore cell levels, thereby reducing the incidence of oxidative stress-related diseases.[21] It achieves this by scavenging free radicals and boosting the activity of natural antioxidant enzymes, two oxidative stress prevention techniques.[22] For the central nervous system, the effects of associations with baicalein are however worth noting. Baicalein confers neuroprotective actions along several pathways such as antioxidant protection, anti-inflammation, synaptic modulation, inhibition of apoptosis, induction of autophagy, and stabilization of the blood-brain barrier. Through these mechanisms, it

is considered a promising candidate for the therapeutic treatment of Alzheimer's disease, Parkinson's disease, stroke, epilepsy, and other neurodegenerative conditions. More scientific and clinical work is needed to confirm its efficacy against human neurological conditions.[23] It has also been shown in animal models of AD that baicalein is capable of preventing amnesia as well as improving their cognitive abilities.[24]

Aim

The aim was to investigate the neuroprotective effects of baicalein in a lansoprazole-induced Alzheimer and model in Wistar rats.

Material and methods

Animals

After approval of the study by the Institutional Animal Ethics Committee (IAEC) Reg. No.711/PO/ReRc/S/02/CPCSEA and date of registration: 29 October 2002, Meerut Institute of Engineering and Technology, Meerut, 36 female Wistar rats weighing 200-300 g were raised at the Animal House Facility (Meerut Institute of Engineering and Technology, Meerut U.P., India). The animals were confined in polypropylene cages measuring 49x34x26 cm, with unrestricted access to food and water. All animals were housed under the same standard conditions, including a 12-hour cycle of light and darkness, a quiet chamber, and twice-weekly bedding changes. The temperature was maintained at 23°C±2°C, and a relative humidity of 65±5%. All behavioral experiments were conducted between 9:00 am and 5:00 pm. The study protocol complied with the institutional policies and the Institutional Animal Ethics Committee for the care and use of experimental animals. Everyone worked together to minimize the amount of animals and any discomfort they might have had. According to the laws of the Government of India, the Institutional Animal Ethics Committee (IAEC) authorized the study protocol. It was registered with 711/PO/ReRc/S/02/CPCSEA 29/10/2002 as protocol number IAEC/MIET/CPCSEA/Meeting No: 04/2023/Protocol No.192. The rodents were acclimatized to the laboratory environment before the experimentation phase.

Experimental design and procedure

For the purpose of this study, 36 female Wistar rats, weighing between 200 and 300 g, were used. The rats used in the study were divided into sex groups each with 6 rats (n=6) that are detailed in the (Table 1). The study lasted 42 days, while the detailed experimental design is given in (Fig. 1). The rats were placed in individual cages that had 6 rats per cage before getting used to behavioral tests. Group I (vehicle group): rats were administered normal saline orally for 42 days. Group II (per se group): rats were given 10 mL/kg of test drug (baicalein) orally dissolved in distilled water for 14 days. Group III (toxin group): rats received 30 mL/kg (orally) lansoprazole dissolved in distilled water to induce AD up to 28 days. Group IV (toxin+low dose): rats received 30 mL/kg (orally) of lansoprazole dissolved in distilled water to induce AD for up to 28 days and then administered baicalein 5 mg/kg orally for 14 days. Group V (toxin+high dose): rats received lansoprazole 30 mL/kg (orally) dissolved in distilled water to induce AD for up to 28 days

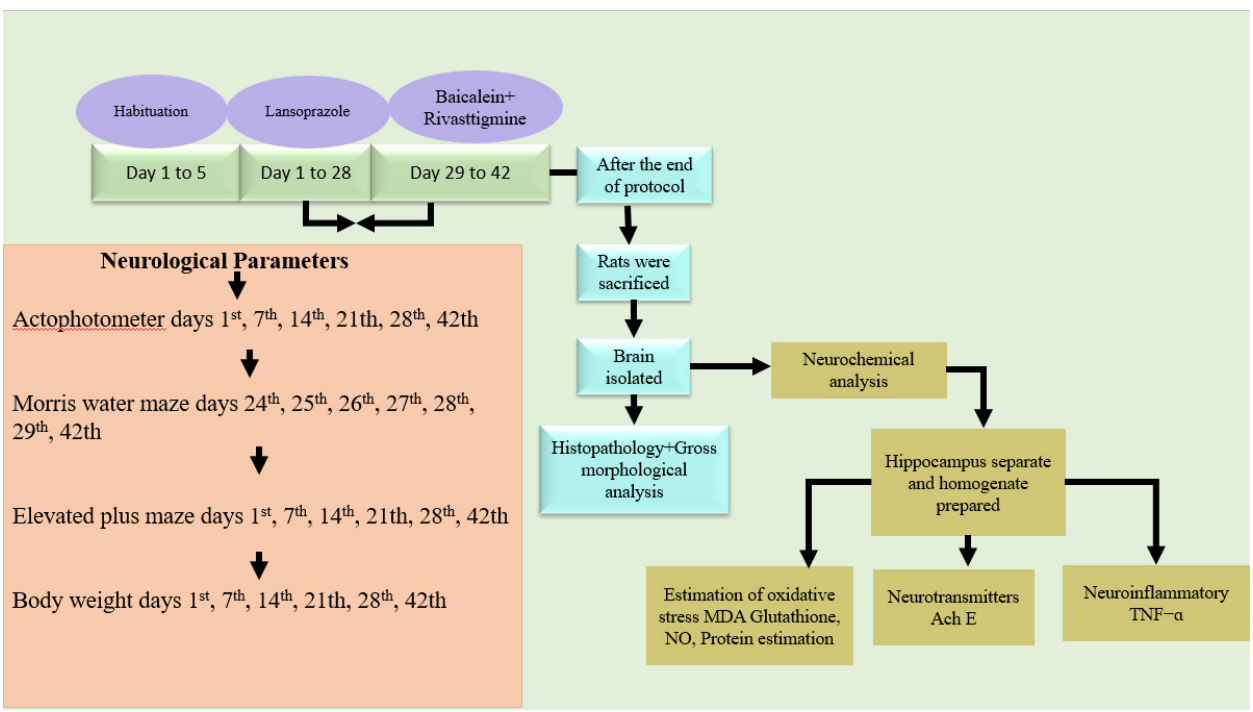

Fig. 1. Representation of the experimental procedure

and then baicalein 10 mg/kg orally for 14 days. Group VI (toxin+dose of std): rats received lansoprazole 30 mL/kg (orally) dissolved in distilled water to induce AD up to 28 days and then administered Rivastigmine 0.3 mg/kg intraperitoneally for 14 days**.**

Table 1. Representation of experimental grouping

S. No.	Groups	Animal species	Number of animals
1	Normal group (normal saline 10 mg/kg orally)	Albino Wistar rats	6
2	Perse group		6
3	Toxin group (lansoprazole 30 mg/kg)		6
4	Toxin group (lansoprazole 30 mg/kg)+test drug (baicalein 5 mg/kg)		6
5	Toxin group (lansoprazole 30 mg/kg)+test drug (baicalein 10 mg/kg)		6
6	Toxin group (lansoprazole 30 mg/kg)+standard drug (rivastigmine 0.3 mg/kg)		6

Drugs and chemicals

The following substances were used: Sigma Aldrich co., an NMDA receptor antagonist at 50 mg/mL *i.p.;* lansoprazole PPI (proton pump inhibitor) and administered orally at 30 mg/kg. dimethyl sulfoxide (DMSO), and EDTA from CDH; baicalein was obtained from Shipra Herbal (An ISO and GMP Certified Company), and administered orally at 5 mg/kg and 10mg/kg.[25] The selective baicalein antagonist Rivastigmine (API) was purchased from Sigma Aldrich Co. and administered intraperitoneally at 0.3 mg/kg. Sodium carbonate, sodium hydroxide, sodium bicarbonate, hydroxylamine, and CDH hydrochloric acid. All medications and chemicals were of analytical quality and were prepared immediately before injection.

Induction of AD

Lansoprazole, a widely used PPI drug for treating disorders related to acids, has also been involved in cognitive impairment and AD pathology. AD-like pathology due to the administration of lansoprazole 30 mg/kg (for 28 days p.o.) was established in rodent models by modifying Aβ metabolism, oxidative stress, neuroinflammation and neurotransmitter systems.[26]

Body weight

The experiment included the measurement of body weight on days 1, 7, 14, 21, 28, 35, and day 42 as a major predictive variable in the logistic regression study for AD due to lansoprazole. The test drug, baicalein, was administered to evaluate its therapeutic effect in the prevention of neurodegenerative effects. Changes in body weight were correlated with disease progression, and in fact, flight time is suggested to be induced by baicalein-mediated prevention of lansoprazole-induced Alzheimer's pathology- associated with overall loss of body weight.[27]

Neurobehavioral parameters

Morris Water Maze assessment

The Morris Water Maze (MWM) is a standard behavioral test to measure spatial memory and learning in rodents. It involves a circular swimming pool with opaque water and a submerged hidden escape platform just below the surface. Rodents learn to use extra-maze visual cues to swim and find the platform during training trials. Escape latency, path length, and time in the target quadrant are measured to assess learning and memory retention. The test trial, which is performed without the platform, assesses the capacity to recall. Reversal learning can be tested by moving the platform to examine cognitive flexibility. MWM is widely applied in neuroscience studies to investigate cognitive impairment, neurodegenerative disorders, and the impact of pharmacological treatments. Its sensitivity and reliability to hippocampal function make it an important tool for evaluating memory-related disorders and possible therapeutic interventions.[28]

Locomotor activity

Using a digital actophotometer, the impulsive locomotion was measured on days 1, 7, 14, 21, 28, and 42. Mental activity was also observed. Rats were used to measure mental move activity after training for 180 seconds in the actophotometer chamber. A digital actophotometer prepared with infrared light-sensitive photocells was used to measure locomotor activity, and the counts per minute were noted for the intervals of five minutes.[29]

Elevated plus maze

Rats were placed individually at the end of each open arm in an exploratory situation (new situation), facing away from the central platform; thus, we used this situational test to demonstrate a level of new situations with the possibility of fear faced by animal so-called elevated plus maze (EPM) on days 1, 7, 14, 21, 28 and 42. The latency to cross from the open arm into either enclosed arm was recorded for each rat. Once this delay was noted, the rat was allowed to explore the plus-maze for the following 20 s freely spared of restraints or researcher interference in selecting open vs. closed arms. Shorter transfer latency values were associated with better long-term memory.[30]

Biochemical parameters

Brain homogenate preparation

Rats in each group were individually anesthetized at a low dosage of ether, killed, and given PBS perfusion when the 10-week experiment was complete. The brains were immediately washed in cold water and removed from the skull. The hippocampal were separated in separate containers, and after cooling PBS at pH 7.4, phosphate buffer (0.1 M) was used to homogenize the tissues

at 800×g for five minutes at 4°C, or ten times their volume. The resulting supernatant was centrifuged for 10 minutes at 10,000×g, at 4°C. To perform additional biochemical calculations, the supernatant was separated and stored at -80°C.[31]

Estimation of AChE level

Ellman's techniques are used to measure the amount of AChE in the hippocampus of the brain. A mixture of three milliliters (mL) of PBS (25 mM, pH 7.4), 0.1 mL of DTNB (5,5-dithio-bis-(2-nitrobenzoic acid, 10 mM in 15 mM $NaHCO_3$) and 0.2 mL of 75 mM acetyl-thiocholine iodide are used for reaction. The mixture was incubated for ten minutes at a temperature of 25°C. Following evolution, 0.2 mL of the hippocampus homogenates' supernatants were combined. From Perkin Elmer, the twenty spectrophotometers measured the optical density at 412 nm in five minutes, recording variations in absorbance every thirty seconds. Nano moles of hydrolysis of acetylthiocholine iodide per milligram of proteins were detected.[32]

Estimation of lipid peroxidation (LPO)

The hippocampal region of the brain was using TBARS as a lipid peroxidation indicator. Using Will's method, the concentration of MDA was determined. A total of 0.5 mL of Tris-HCL and 0.5 mL of supernatant were mixed and incubated at 37°C for two hours. One mL of 10% trichloroacetic acid was added to the reaction mixture after incubation, and the mixture was centrifuged for ten minutes at 1000×g. Therefore, thiobarbituric acid was diluted to 0.67% using 1 mL of quiet supernatant. Boiling water was poured over the reaction tube for ten minutes. One ml of distilled water was added once it had cooled. At a wavelength of 532 nm, the absorbance was measured. Millimoles of TBARS were recorded for each milligram of protein.[33]

Assessment of catalase (CAT)

CAT amount determined using Calibre's (1985) method. A reaction mixture comprising 0.05 ml of supernatant, 1 mL of hydrogen peroxide (H_2O_2, 0.019 M), and 1.95 mL of supernatant was combined with phosphate buffer (0.5 M; pH 7.0) to modify the final product's volume to 3 mL. The PerkinElmer Lambda 20 spectrophotometer was used to record changes in absorbance at 240 nm every 30 seconds. Measurement of CAT activity was expressed in nmol of H_2O_2 / min / mg protein.[35]

Estimation glutathione (GSH)

To conduct the GSH test, 1 mL of the 4% w/v sulfosalicylic acid precipitate and 100 mL of the tissue homogenate supernatant were combined. Then store the reaction mixture in a refrigerator between 2 and 8°C. Samples were centrifuged at 1200×g for 15 minutes at 4°C after an hour. The particle was removed once the supernatants were removed. For the purpose of obtaining a faint yellow tint, 100 µL of this supernatant was mixed with 200 µL of 5,5-dithiobis-2-nitrobenzoic acid, 2.7 mL of 0.1 M phosphate buffer (pH 8), and 2.7 mL of this supernatant. It was discovered that the generated color was 412 nm using a UV-visible spectrophotometer (Perkin Elmer, USA). To calculate this, the molar extinct coefficient was used.[34]

Estimation of superoxide dismutase (SOD)

The Marklund-developed approach was used to assess the mobility of the SOD (Turf) protein for particular changes. The capacity of the Grass protein to prevent pyrogallol from autoxidizing serves as a gauge for the efficacy. Ten microliters of hippocampi homogenate were rapidly combined with nine hundred microliters of Tris-HCl cushion (1 mL of EDTA and one millimoles of Tris, at a pH of 8.2). Subsequently, 20 µL of room temperature pyrogallol (13 mm) was combined with the previously indicated response mixture. Spectrophotometry was used to measure changes in absorbance at 420 nm during a 30-second rest interval. The amount of material projected to restrict 50% of the total pyrogalol autooxidation per minute is known as one Grass action unit (U/min/mg protein).[35]

Estimation of the nitrite level

Hippocampal nitrite levels were not entirely restored by the Griess reagent. The Griess reagent, which contained 0.1%N-(-naphthyl ethylenediamine dihydrochloride, 1% sulphanilamide, and 5% phosphoric acid), was mixed 1:1 with the supernatant. The cakes were then cooked for 10 minutes at room temperature. A Perkin Elmer Lambda 20 spectrophotometer was one of the instruments used to measure the absorbance at 540 nm. The protein nitrite concentration was expressed in milligrams per milligram using the sodium nitrite average curve.[36]

Estimation of neuroinflammatory marker

Neuroprotective effects of baicalein in the model of the TNF-α alterations in lansoprazole-induced AD rat. The outcomes were evaluated by one-way ANOVA followed by the post hoc Tukey multiple comparison test and demonstrated that lansoprazole-induced AD decreased the level of TNF-α in the rat hippocampus and the whole brain. Data are obtainable as mean±SD (n=6).[37]

Gross morphological and histopathological examination

Following the completion of the experimental protocol, rats were sacrificed by deep anesthesia induction and intraperitoneal injection of sodium phenobarbitone at a

concentration of 270 mg/mL. After that, the rat brain is removed and stored at a pH of 7.4 in 4% paraformaldehyde for later. The rats' brains were sectioned into 5-mm thick sections after the 42-day experimental schedule, arranged coronally (from the cerebral cortex anterior to the posterior poles) on a white background and captured on camera using a high-end digital camera to allow clear visualization of the various regions of the brain and damaged areas.[38] After this, the hippocampus was separated and preserved in saline phosphate buffer. saline. Each slide in the histological investigation was kept in a variable ethanol concentration. The molds were created with the aid of a tissue processor. Hematoxylin and eosin staining was then used to help them stand out from each other and imaging was done with fluorescence microscope.[39]

Statistical analysis

The recorded values from several tests were statistically analyzed using Prism software (GraphPad Prism 8.0.1, San Diego, CA, USA). To assess the neurochemical results, Tukey's Post-Hoc Test for multiple comparisons and the One-Way ANOVA with repeated measures were used. Additionally, to evaluate differences in neurobehavioral evaluation across treatment groups, a two-way analysis of variance (ANOVA) was used, followed by a post-hoc Bonferroni's test. A graphic representation of the experimental data was created using mean and standard deviation (SD). The probability threshold of $p<0.01$ was established as the significance criterion for the findings.

Results

Body weight

On days 1, 7, 14, 21, 28, and 42 of the investigation, the weight changes of the six groups were compared. On the first day of the experiment, there were no significant differences in weight between the groups. The body weight of the animals that received continuous lansoprazole-induced lansoprazole decreased from day 1 to day 28. On days 21 and 28, rats in the lansoprazole-induced groups weighed significantly less than rats in the vehicle group treated, perse group-treated. from day 29-42, rats were treated with baicalein and rivastigmine, that is, lansoprazole+5 mg/kg baicalein and lansoprazole+10 mg/kg baicalein and lansoprazole+0.3 mg/kg rivastigmine, resulting in significant restoration in body weight compared to lansoprazole-induced rats. Upon completion of the protocol, it was determined that there was a dose-related increase in the restoration of body weight of rats in the 10 mg/kg treatment group compared to the lansoprazole+baicalein 5 mg/kg treatment group of lansoprazole+baicalein (Fig. 2, F value: 5.40).

Neuroprotective effects of baicalein on neurobehavior alterations in AD rats

Morris water maze

Lansoprazole-induced AD rats had significantly lower TSTQ levels compared to the vehicle group-treated, and those treated with the perse group. Furthermore, treatment with Baicalein by oral route at doses of 5 mg/kg significantly and 10 mg/kg improved TSTQ significantly in rodents with lansoprazole-induced AD. From day 29-42 rats were treated with baicalein and rivastigmine, that is, lansoprazole+5 mg/kg baicalein and lansoprazole+10 mg/kg baicalein and lansoprazole+0.3 mg/kg rivastigmine, which results in a significant increase in TSTQ compared to lansoprazole-induced rats. Upon completion of the protocol, it was determined that there was a dose-related increase in TSTQ of rats in the lansoprazole+baicalein 10 mg/kg treatment group of 10 mg/kg compared to the treatment group (Fig. 3, F value: 6.35).

Locomotor

Actophotometer measurements of impulsive locomotion were conducted on days 1, 7, 21, 28 and 42 of the experimental protocol. On the first and seventh day of the investigation, there was no noticeable distinction between the locomotions of the groups. On day 14, 21 and 28, AD-induced rats with AD exhibited significantly distinct locomotion compared to the vehicle group treated, per se group-treated from day 29-42 rats were treated with baicalein and rivastigmine, i.e., lansoprazole+5 mg/kg baicalein and lansoprazole+10 mg/kg baicalein and lansoprazole+0.3 mg/kg rivastigmine, which results of significant restoration in locomotor activity as compared to lansoprazole-induced rats. At the end of the protocol, it was determined that there was a dose-related increase in the locomotion of rats in the lansoprazole+bacalein 10 mg/kg treatment group compared to the lansoprazole+bacalein 5 mg/kg treatment group (Fig. 4, F value: 1.8).

EPM

On days 1, 7, 14, 21, 28, and 42, inactivity (immobility) was assessed in rats against baicalein. On days 14, 21 and 28 the lansoprazole group exhibited distressing behavior and had a significantly prolonged period of immobility increased as compared to the vehicle group treated and perse group-treated. from day 29–42 rats were treated with baicalein and rivastigmine, that is, lansoprazole+5 mg/kg baicalein and lansoprazole+10 mg/kg baicalein and lansoprazole+0.3 mg/kg rivastigmine, which results in significant restoration in immobility time compared to lansoprazole-induced rats. Upon completion of the protocol, it was determined that there was a dose-related increase in immobility of rats in the lansoprazole+bacalein 10 mg/kg treatment group as compared to the

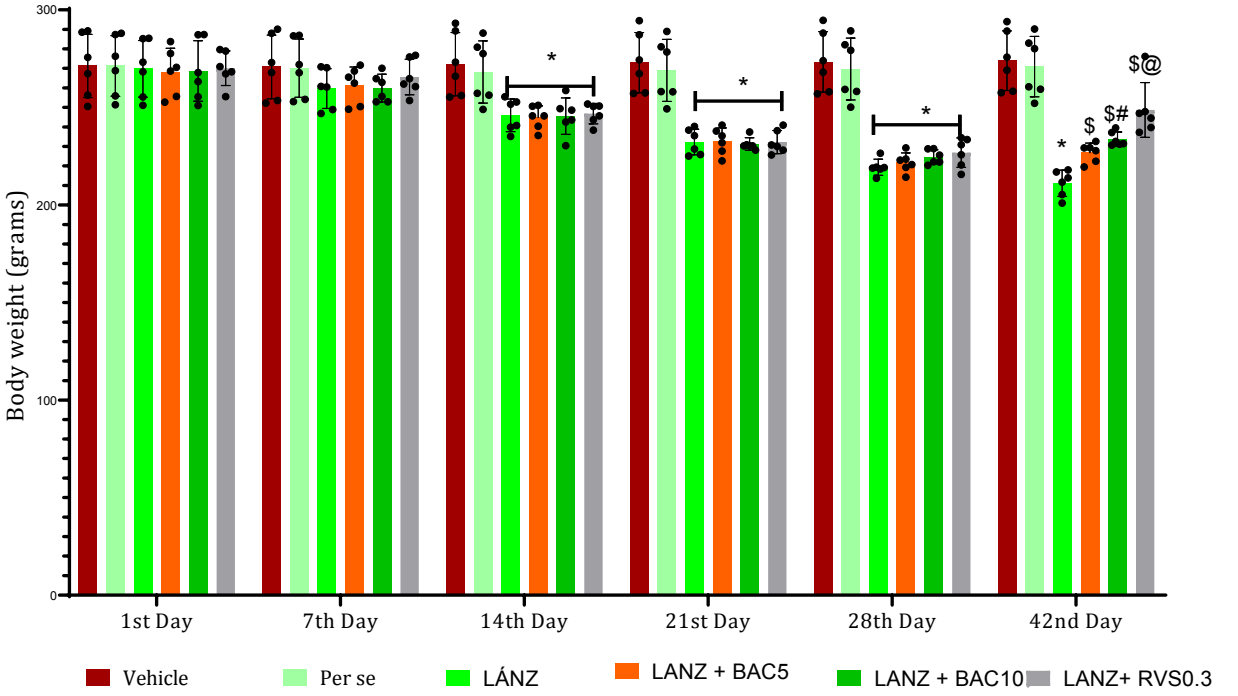

Fig. 2. Baicalein improves body weight alterations in AD rats, the statistical data analysis presented by two-way ANOVA using the Bonferroni post hoc test, values presented as mean±SD ($p<0.01$); [n=six Wistar rats in each experimental group], * versus vehicle and per se; $# versus lansoprazole (LANZ), lansoprazole+bacalein (BAC) 10; $ versus lansoprazole, lansoprazole+bacalein 5, lansoprazole+bacalein 10 and lansoprazole+RVS 0.3; $@ versus lansoprazole, lansoprazole+bacalein 5 and lansoprazole+bacalein 10

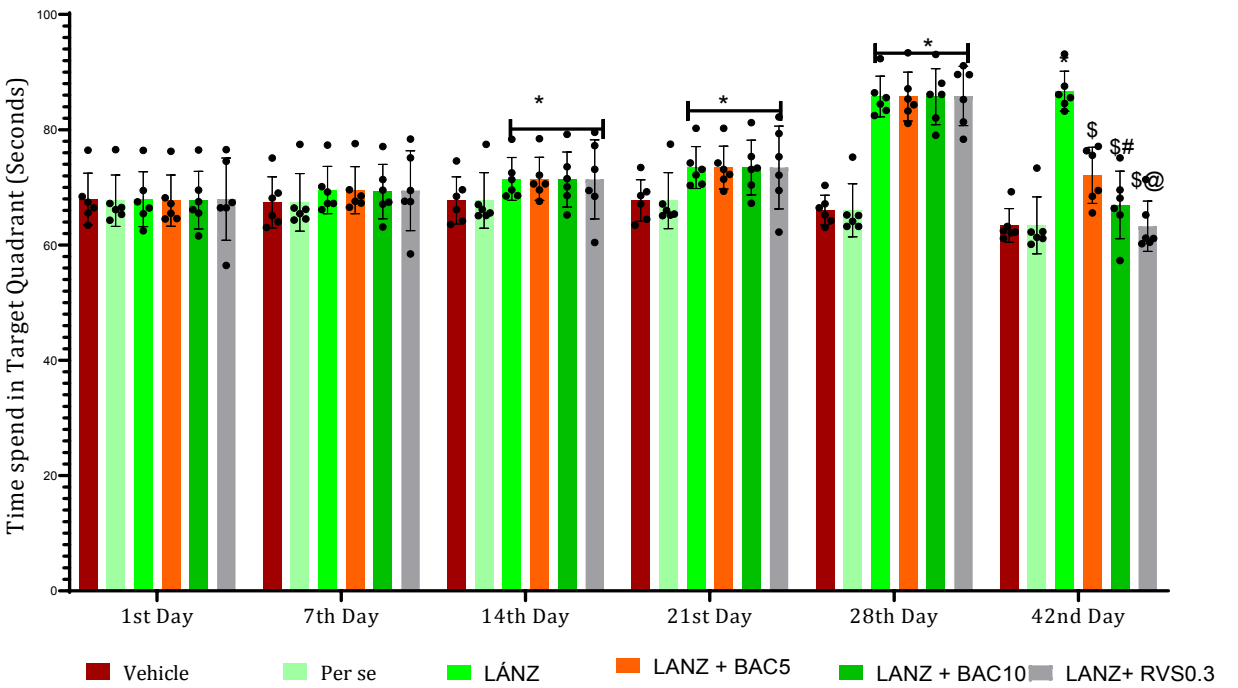

Fig. 3. Baicalein improves memory and cognition in AD rats induced by experimental lansoprazole, statistical data analysis presented by two-way ANOVA employing the Bonferroni post hoc test, values presented as mean±SD ($p<0.01$); [n=six Wistar rats in each experimental group], * versus vehicle and per se; # versus lansoprazole (LANZ), lansoprazole+baicalein (BAC) 10; $ versus lansoprazole, lansoprazole+baicalein 5, lansoprazole+baicalein 10 and lansoprazole+RVS 0.3; $@ versus lansoprazole, lansoprazole+baicalein 5 and lansoprazole+baicalein 10

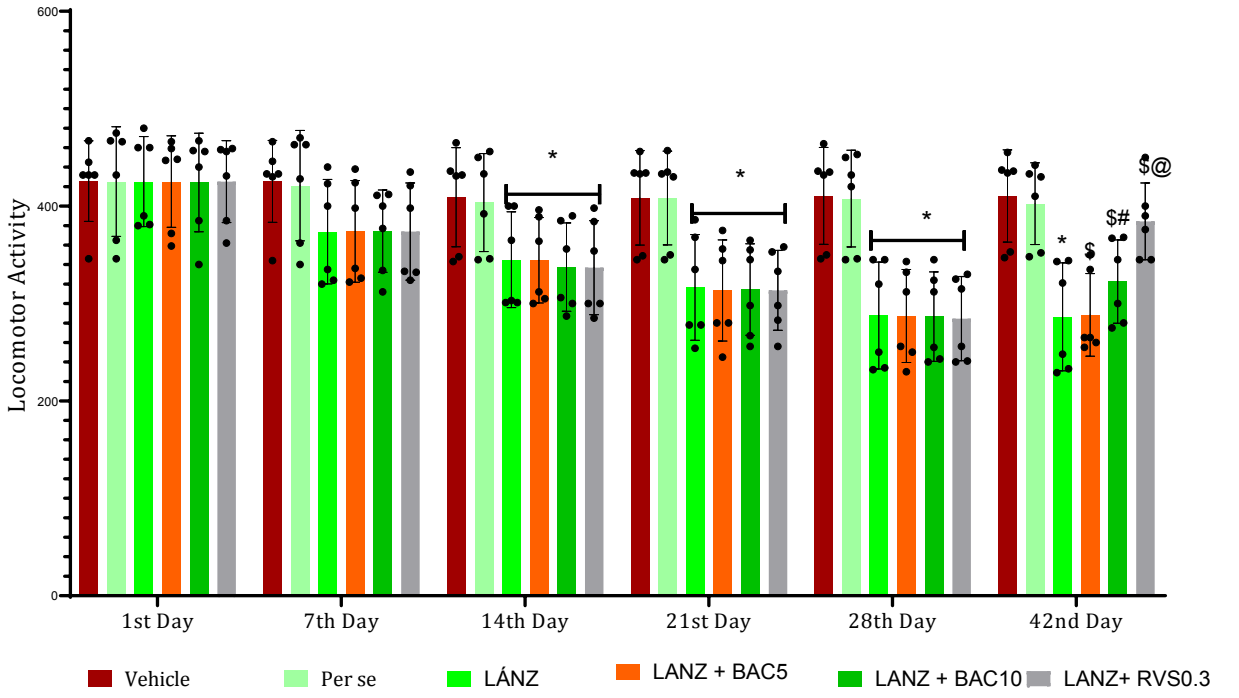

Fig. 4. Baicalein improves locomotion behavior in AD rats induced by experimental lansoprazole, statistical data analysis presented by two-way ANOVA employing the Bonferroni post hoc test, values presented as mean±SD ($p<0.01$); [n=six Wistar rats in each experimental group], * versus vehicle and per se; # versus lansoprazole (LANZ), lansoprazole+baicalein (BAC) 10; $ versus lansoprazole, lansoprazole+baicalein 5, lansoprazole+baicalein 10 and lansoprazole+RVS 0.3; $@ versus lansoprazole, lansoprazole+baicalein 5 and lansoprazole+baicalein 10

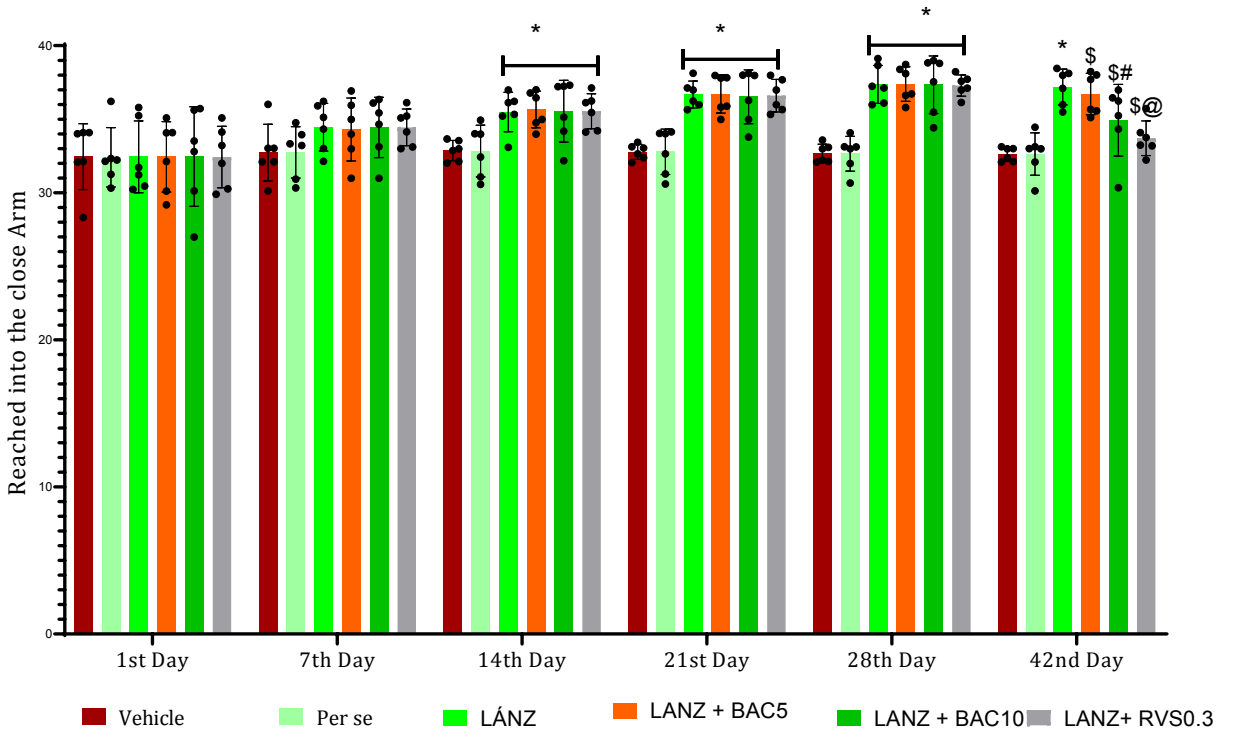

Fig. 5. Baicalein reduces immobility time in AD rats induced by experimental lansoprazole, statistical data analysis presented by two-way ANOVA employing post-hoc test Bonferroni, values presented as mean±SD ($p<0.01$); [n=six Wistar rats in each experimental group], * versus vehicle and per se; # versus lansoprazole (LANZ), lansoprazole+baicalein (BAC) 10; $ versus lansoprazole, lansoprazole+baicalein 5, lansoprazole+baicalein 10 and lansoprazole+RVS 0.3; $@ versus lansoprazole, lansoprazole+baicalein 5 and lansoprazole+baicalein 10

lansoprazole+baicalein 5 mg/kg treatment group (Fig. 5, F value: 2.1).

Neuroprotective effects of baicalein on neurotransmitter alterations in lansoprazole-induced AD rats

Figure 6 represents the result of baicalein in the AChE in lansoprazole-induced AD rats. The outcomes were evaluated by one-way ANOVA followed by the post hoc Tukey Kramer multiple comparisons test and showed that baicalein decreased the level of AChE in the rat hippocampus. Data are obtainable as mean ± SD (n=6). The signs indicate a difference compared to the Lansoprazole-induced AD group. Two different doses of baicalein (5 mg/kg and 10mg/kg) were administered to rats from day 29 to 42nd days (F value: 4.11).

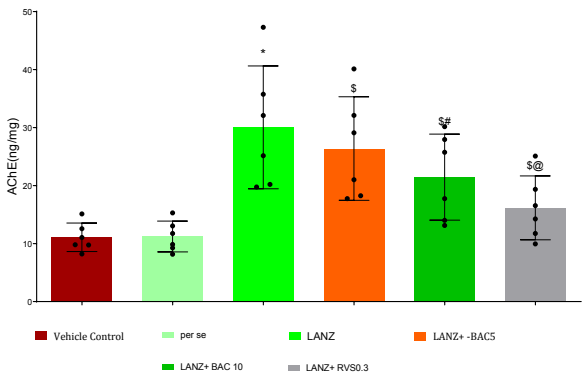

Fig. 6. Baicalein decreases the AchE level in AD rats induced by experimental lansoprazole, statistical data analysis presented by one-way ANOVA employing post-hoc test Tukey, values presented as mean±SD ($p<0.01$); [n=six Wistar rats in each experimental group], * versus vehicle and per se; # versus lansoprazole (LANZ), lansoprazole+baicalein (BAC) 10; $ versus lansoprazole, lansoprazole+baicalein 5, lansoprazole+baicalein 10 and lansoprazole+rvs 0.3; $@ versus lansoprazole, lansoprazole+Baicalein 5 and lansoprazole+baicalein 10

Neuroprotective effects of baicalein on neurochemical alterations in lansoprazole-induced AD rats

Neuroprotective effects of baicalein on LPO alterations in lansoprazole-induced AD rats

Figure 7 represents the outcome of baicalein on the level of LPO in the Lansoprazole-induced AD rats. The results were evaluated by one-way analysis of variance followed by the Tukey multiple comparison post hoc test and demonstrated that baicalein decreased the level of LPO in the rat hippocampus. Data are obtainable as mean±SD (n=6). Signs indicate a difference compared to the lansoprazole-induced AD group. Two different doses of baicalein (5mg/kg and 10mg/kg) were administered to rats from 29 to 42nd days (F value: 0.95).

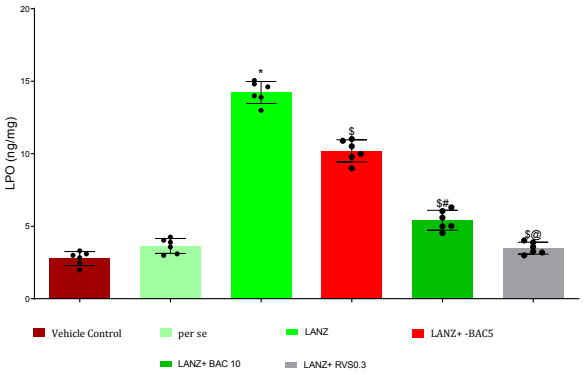

Fig. 7. Baicalein decreases the level of LPO in experimental lansoprazole-induced AD rats, the statistical data analysis presented by one-way ANOVA using the Tukey post hoc test, values presented as mean±SD ($p<0.01$); [n=six Wistar rats in each experimental group], * versus vehicle and per se; $# versus lansoprazole (LANZ), lansoprazole+Baicalein (BAC) 10; $ versus lansoprazole, lansoprazole+Baicalein 5, lansoprazole+baicalein 10 and lansoprazole+rvs 0.3; $@ versus lansoprazole, lansoprazole+Baicalein 5 and lansoprazole+baicalein 10

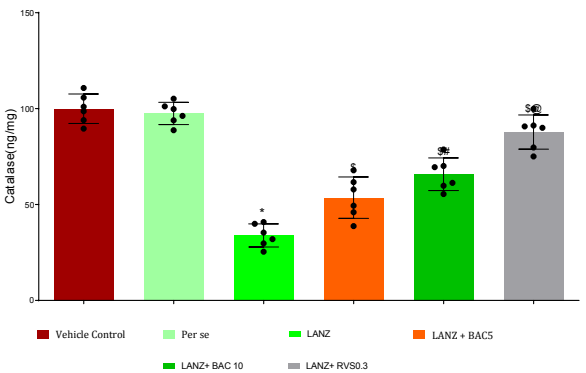

Fig. 8. Baicalein restores the catalase level in experimental lansoprazole-induced AD rats, the statistical data analysis presented by one-way ANOVA employing Tukey post hoc test, values presented as mean±SD ($p<0.01$); [n=six Wistar rats in each experimental group], * versus vehicle and per se; # versus lansoprazole (LANZ), lansoprazole+bacalein (BAC) 10; $ versus lansoprazole, lansoprazole+bacalein 5, lansoprazole+baicalein 10 and lansoprazole+rvs 0.3; $@ versus lansoprazole, lansoprazole+baicalein 5 and lansoprazole+baicalein 10

Neuroprotective effects of baicalein on CAT alterations in lansoprazole-induced AD rats

Figure 8 represents the outcome of baicalein on CAT level in lansoprazole-induced AD rats. The results were evaluated by one-way analysis of variance (ANOVA) followed by the post hoc Tukey multiple comparisons test and showed that baicalein decreased the level of CAT in the rat hippocampus. Data are obtainable as mean±SD (n= 6). Signs indicate a difference compared to the lan-

soprazole-induced AD group. Two different doses of baicalein (5 mg/kg and 10 mg/kg) were administered to rats from day 29 to 42nd days (F value: 63.43).

Neuroprotective effects of baicalein on GSH alterations in lansoprazole-induced AD rats

Figure 9 represents the result of baicalein on GSH level in lansoprazole-induced AD rats. The outcomes were evaluated by one-way ANOVA followed by the post hoc Tukey multiple comparison test and demonstrated that baicalein decreased the level of GSH in the rat hippocampus. Data are obtainable as mean±SD (n=6). Signs indicate a difference compared to the lansoprazole-induced AD group. Two different doses of baicalein (5 mg/kg and 10 mg/kg) were administered to rats from 29 to 42nd days (F value: 2.12).

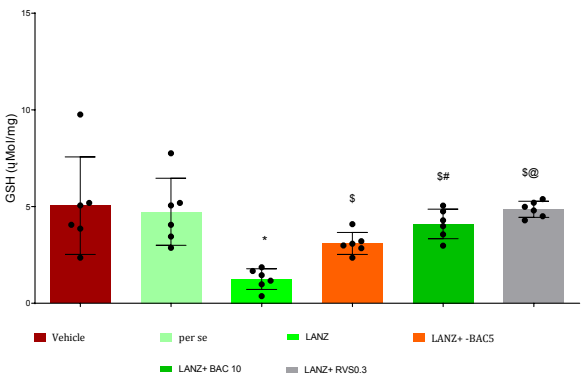

Fig. 9. Baicalein restores the level of GSH in experimental lansoprazole-induced AD rats experimental, the statistical data analysis presented by one-way analysis of variance (ANOVA) employing Tukey post hoc test, values presented as mean±SD (p<0.01); [n=six Wistar rats in each experimental group], * versus vehicle and per se; # versus lansoprazole (LANZ), lansoprazole+bailein (BAC) 10; $ versus lansoprazole, lansoprazole+baicalein 5, lansoprazole+baicalein 10 and lansoprazole+rvs 0.3; $@ versus lansoprazole, lansoprazole+baicalein 5 and lansoprazole+baicalein 10

Neuroprotective effects of baicalein on SOD alterations in lansoprazole-induced AD rats

Figure 10 represents the outcome of baicalein on SOD level in the lansoprazole-induced AD rats. The results were evaluated by one-way ANOVA followed by the post hoc Tukey multiple comparison test and demonstrated that baicalein decreased the level of SOD in the rat hippocampus. Data are obtainable as mean±SD (n=6). Signs indicate a difference compared to the lansoprazole-induced AD group. Two different doses of baicalein (5 mg/kg and 10 mg/kg) were administered to rats from 29 to 42nd days (F value: 0.38).

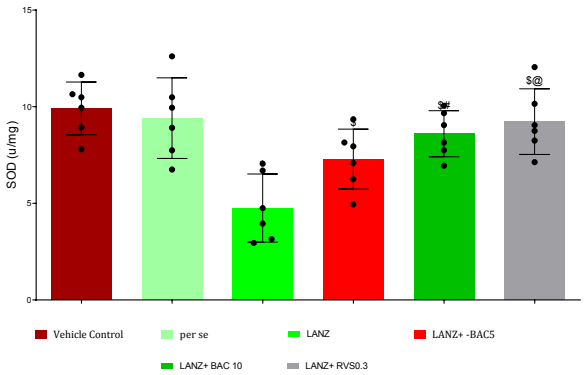

Fig. 10. Baicalein restores the level of SOD in experimental lansoprazole-induced AD rats, the statistical data analysis presented by one-way ANOVA employing the Tukey post hoc test, values presented as mean±SD (p<0.01); [n=six Wistar rats in each experimental group], * versus vehicle and perse; $# versus lansoprazole (LANZ); $ versus lansoprazole, lansoprazole+bacalein (BAC) 5 and lansoprazole+baicalein 10

Neuroprotective effects of baicalein on nitrite alterations in lansoprazole-induced AD rats

Figure 11 represents the outcome of baicalein on the level of nitrite in lansoprazole-induced AD rats induced by lansoprazole. The results were evaluated by one-way analysis of variance followed by the Tukey multiple comparisons test and demonstrated that baicalein decreased the level of nitrite in the rat hippocampus. Data are obtainable as mean±SD (n=6). Signs indicate a difference compared to the lansoprazole-induced AD group. Two different doses of baicalein (5 mg/kg and 10 mg/kg) were administered to rats from 29 to 42nd days (F value: 2.20).

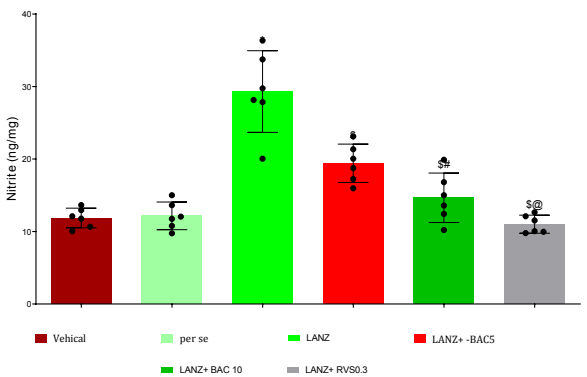

Fig. 11. Baicalein decreases the level of nitrite in experimental lansoprazole-induced AD rats, the statistical data analysis presented by one-way ANOVA using Tukey post hoc test, values presented as mean±SD (p<0.01); [n=six Wistar rats in each experimental group], * versus vehicle and per se; # versus lansoprazole (LANZ); $ versus lansoprazole, lansoprazole+baicalein (BAC) 5 and lansoprazole+baicalein 10

Neuroprotective effects of baicalein on neuroinflammatory markers in AD rats

Figure 12 represents the outcome of baicalein on the TNF- level in AD rats induced AD rats. The results were evaluated by one-way analysis of variance followed by the post hoc Tukey multiple comparisons test and demonstrated that baicalein decreased the level of TNF-α in the rat hippocampus. Data are obtainable as mean±SD (n=6). The signs indicate differences compared to the lansoprazole-induced AD group. Two different doses of baicalein (5 mg/kg and 10 mg/kg) were administered to the rats from the 29th to 42nd day (F value: 3.00).

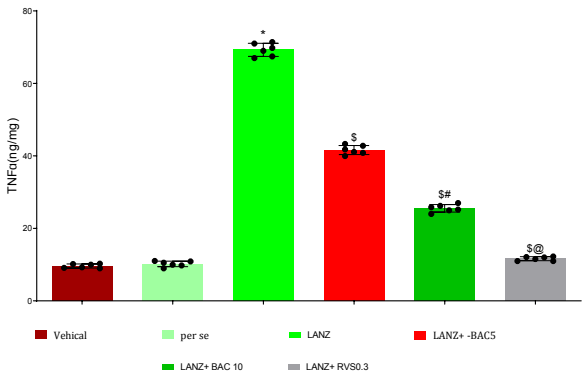

Fig. 12. Baicalein decreases TNF-α in AD rats induced by experimental lansoprazole, statistical data analysis presented by one-way ANOVA employing post-hoc test Tukey, values presented as mean±SD ($p<0.01$); [n=six Wistar rats in each experimental group], * versus vehicle and perse; $# versus lansoprazole (LANZ); $ versus lansoprazole, lansoprazole+baicalein (BAC) 5 and lansoprazole+baicalein 10

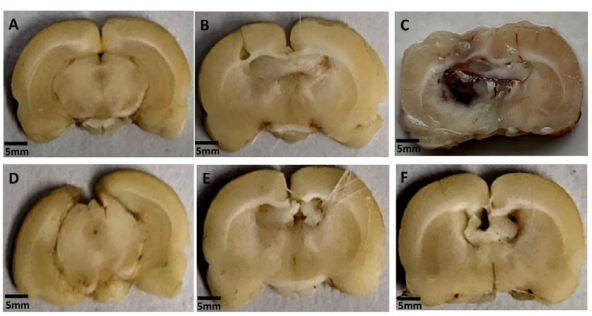

Fig. 13. Baicalein effects on gross pathological deficiencies in experimental lansoprazole-induced AD rats, the statistical data analysis presented by one-way ANOVA using Tukey post hoc test, values presented as mean±SD ($p<0.01$); [n=six Wistar rats in each experimental group], A: vehicle, B: per se, C: lansoprazole, D: lansoprazole+baicalein 5, E: lansoprazole+baicalein 10, F: lansoprazole+rsv 0.3 mg/kg

Neuroprotective effects of baicalein on gross pathological deficits in lansoprazole-induced AD rats

Figure 13 shows the gross morphological changes in the rat brain from day 1 to day 42 in different groups, which shows degeneration in the lansoprazole treated group as compared to vehicle control and perse control group. In the following Figures D and E show restored degeneration in the rat brain compared to the lansoprazole-induced AD rat brain, which shows potential effects of baicalein on degeneration caused by toxin-induced rats. Figure F shows standard drug-treated rats as compared to toxin control and baicalein-treated groups.

Neuroprotective effects of baicalein on histopathological deficits in lansoprazole-induced AD rats

Upon examination of each slide under a microscope, it was noted that the group treated with lansoprazole exhibited more pronounced signs of plaque formation and irregular tangle formation compared to the control group. In addition to reducing the number of unfolded tangles inside the brain, baicalein, as well as the entire brain, which was employed as a treatment group, showed some improvement in the hippocampal region (Fig. 14).

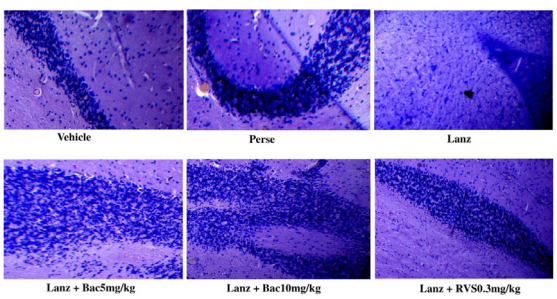

Fig. 14. Effects of baicalein on histopathological deficits in AD rats

Discussion

AD poses a major and increasing challenge within the global healthcare system, affecting cognitive function, memory, and behavior progressively.[40] The main pathological features of AD are extracellular amyloid-beta plaques and intracellular hyperphosphorylated tau-associated neurofibrillary tangles NFT.[41] Oxidative stress, neuroinflammation, and synaptic dysfunction are exceedingly involved in the pathogenic processes leading to the pattern of progressive neuronal loss seen in AD.[42] Conventional medical management is inadequate at preventing, or reversing the progression of this disease and as a result, new therapeutic pathways need to be explored.[43]

Recently, natural compounds like flavonoids have gained attention due to their potential ability to protect neurons. Baicalein, a flavonoid obtained from the roots of *S. baicalensis*, has emerged as one of the effective

neuroprotective agents in several models of neurodegenerative diseases, which is attributed to its antioxidant, anti-inflammatory, and other beneficial effects.[44] A model of this type is the use of lansoprazole, a proton pump inhibitor commonly used to treat gastrointestinal diseases, but in experimental animal studies linked with worsening of AD-like pathology.[45] If anything, Lansoprazole treatment has been shown to drive amyloid-beta accumulation and tau hyperphosphorylation mimicking two of the major features seen in AD pathology, providing a consistent model that is amenable to the evaluation of potential therapeutic interventions.[46]

The present study was undertaken to determine the neuroprotective property of baicalein in lansoprazole-induced AD model in Wistar rats. From day 29 to 42nd of the study, all the animals were administered two different doses of baicalein i.e. 5mg/kg and 10 mg / kg, and standard drug used for Alzheimer's disease, ie rivastigmine 0.3mg/kg for 14 days. The study used 28 days for the introduction of a lansoprazole, whereas the introduction of Baicalein lasted 14 days. Cognitive function was periodically assessed during the study by performing behavioral assessments such as the Morris water maze, the actophotometer, and EPM.[47] The biochemical evaluations compared brain tissue of treatment rats with controls and focused on molecular and cellular markers of oxidative stress, neuroinflammation, and neurodegeneration.[48] During the lansoprazole induction phase, rats showed clear deficits in spatial learning and memory retention indicated by longer escape latency from the Morris water maze and lower discrimination index in the novel object recognition task. These behavioral deficits were accompanied by biochemical changes consistent with neurodegeneration, particularly ROS and lipid peroxidation products as well as pro-inflammatory cytokines in the brain of rats treated with lansoprazole[49] All these facts confirm the negative influence of lansoprazole on neuron functioning and prove the model to successfully depict AD-like pathological characteristics.

In contrast, treatment with baicalein throughout the 14-day intervention period proved to be significantly more beneficial for neuroprotection during the 14-day intervention period. Rats supplemented with baicalein had better outcomes in both of the behavioral tests compared to rats who received lansoprazole alone. For example, baicalein supplementation decreased the escape latency in the Morris water maze, which implied improved spatial learning and memory retention.[50] Similarly, in the object recognition paradigm, baicalein-treated rats achieved better discrimination indices, thus indicating maintained recognition memory and cognitive faculties as well as processing. Aggressive behavioral assessments led to the corresponding decrease in markers of oxidative stress and neuroinflammatory mediators in the brains of baicalein-treated rats in comparison to controls.

The potent neuroprotective properties of baicalein were more specifically observed at the molecular level through mechanistic studies. Free radical scavenging and lipid-peroxidation inhibited baicalein extended to the neuronal membranes, demonstrating baicalein was an antioxidant. Baicalein was able to shield such neurons from oxidative damage and mitochondrial dysfunction attributed to cell-based neurodegenerative processes, particularly in AD by alleviating the oxidative stress. Furthermore, baicalein suppressed the release of TNF-α. As a result, the neuronal integrity and synaptic functions of treated rats, compared to control rats, were preserved in part by these anti-inflammatory effects.

Considering the fact that baicalein has so many positive effects for the preservation of neurons, it seems quite promising in the treatment of AD. In addition to antioxidant and anti-inflammatory actions, baicalein is believed to modulate further signal transduction pathways that promote the survival of neurons and the plasticity of synapses. In addition, baicalein may also alter the expression and processing of APP, thereby decreasing amyloid-beta peptide synthesis and deposition in the brains of AD patients. These molecular mechanisms support the multifaceted effects of baicalein in the treatment of AD by ameliorating the pathological conditions that compromise cognitive function.

Conclusion

The research investigated the neuroprotective effects of baicalein in wistar rats with AD induced by lansoprazole, a proton pump inhibitor associated with an increased risk of AD. Baicalein, a flavonoid of *S. baicalensis*, is recognized for its antioxidant and anti-inflammatory properties. In this study, the rats were divided into groups and treated with lansoprazole to induce AD, followed by the administration of baicalein. Cognitive functions, oxidative stress markers, and neuronal integrity were evaluated using behavioral tests, biochemical assays, and histopathological analyzes. The findings indicated that baicalein markedly improved cognitive performance, improving memory and learning capabilities on maze tests. Additionally, it reduced oxidative stress markers, such as MDA, and elevated levels of antioxidant enzyme levels like SOD and catalase. Histopathological analysis revealed a reduction in amyloid-beta plaques and neuronal loss in the hippocampus, indicating a neuroprotective effect. These results suggest that baicalein offers neuroprotection through its potent antioxidant properties, which reduce amyloid beta aggregation and help preserve neuronal integrity. Consequently, baicalein shows promise as a therapeutic agent for mitigating the symptoms and progression of Alzheimer's disease, particularly in cases triggered by external fac-

tors such as lansoprazole. More studies, including clinical trials, are necessary to validate these protective effects in humans and to elucidate the underlying molecular mechanisms.

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Declarations

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Author contributions

Conceptualization, A.G. and P.N.T.; Methodology, A.G.; Software, F.G.; Validation, P.N.T., A.G. and S.N.T.; Formal Analysis, F.G.; Investigation, P.S.; Resources, A.G.; Data Curation, P.S.; Writing – Original Draft Preparation, P.S.; Writing – Review & Editing, F.G.; Visualization, S.N.R.; Supervision, P.N.T.; Project Administration, P.S.

Conflicts of interest

All authors declared the absence of any personal conflicts related to this work.

Data availability

Data will be made available according to the Journal policy.

Ethics approval

The protocol number is IAEC/MIET/CPCSEA/Meeting No: 04/2023/Protocol No.192.

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