UNLOCKING THE POTENTIAL OF ANNONA SQUAMOSA LINN. FRUIT PULP: EXPLORING THE COGNITIVE EFFECT IN RATS WITH ALUMINIUM CHLORIDE-INDUCED ALZHEIMER'S DISEASE

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DOI:10.48047/ecb/2023.12.si4.749

ABSTRACT

Objective: The current study analysed the fruit pulp of ethanol extract of Annona squamosa that diminish AlCl3-induced behavioural, metabolic, and neurochemical changes that match symptoms of Alzheimer's disease (AD).

Materials and Methods: Various behavioural and biochemical parameters that were carried out to evaluate the activity of fruit pulp of ethanol extract of Annona squamosa (FEAS) on AlCl3 intoxicated rats. To determine the therapeutic significance of FEAS on AD, different behavioural tests such as Morri's water maze test, passive avoidance test and some biochemical tests along with neurochemical findings were done. **Results:** AlCl3 caused physical and behavioural deformity in animals, including abnormal posture, weak grip strength, and motor deficit.

Biochemical analysis in brain homogenates of FEAS treated rats showed modified oxidative stress and raised lipid biomarkers. Neurochemical alterations of the striatum of FEAS treated rats exhibit altered levels of catecholamines. FEAS treated for 90 days significantly enhanced motor function and behaviour tasks and further restored the invitro antioxidant changes in the brain. Furthermore, FEAS-II treatment significantly improved oxidative damage, which is denoted by the alterations in neurochemical changes of rat brain. **Conclusion:** In this experimentation, FEAS-I & II (200 mg/kg & 400 mg/kg) provided a remarkable neuroprotective impact, which was evidenced by behavioural and biochemical tests. It recovered the behavioural and biochemical alterations caused by AlCl3 and authenticated the strong neuroprotective mechanism of FEAS in AlCl3-intoxicated behaviour and motor abnormalities.

Keywords: AlCl3, Annona squamosa fruit, Alzheimer's disease, Dopamine, Neurological disorder.

INTRODUCTION

Memory and learning are the main cognitive functions that are affected by Alzheimer's disease (AD), a chronic, progressive neurodegenerative condition. Amyloid beta peptides (Aβ) store up in neurons and the development of intracellular neurofibrillary tangles in the brain are its defining features. (1) Memory loss, mood and behaviour changes, confusion, anger, and a withdrawal from social connections are few of the symptoms of AD that can vary greatly from patient to patient. (2) The disease can also lead to serious complications such as pneumonia, immobility, and malnutrition. (3) These complications are often the primary cause of death in people with AD, rather than the disease itself. (4)

The underlying causes of AD are complex and involves multiple mechanism. According to studies, development of the disease is influenced by neuroinflammation, oxidative stress, and the development of amyloid plaques. (5) Moreover, studies have shown that a major component in the pathogenesis of AD is the increased activity of the nuclear factor-κB (NF-κB) pathway, which is triggered by Aβ accumulation. Moreover, excessive Aβ synthesis causes more acetylcholine (ACh) breakdown, which is essential for normal memory and cognitive function. (6)

With an estimated 50 million affected people, AD is a significant global health concern. By 2050, it is anticipated that this population would increase to 152 million. AD is more common in developing nations, with the bulk of cases exists in South Asian nations, China, and India. (7)

Because of the late diagnosis and lack of access to appropriate care, AD places a heavy financial and social burden on families and care givers of those who are pathetic. Despite the fact that there are treatment alternatives available in developed nations, these medicines have a limited potential to reverse AD. Given the increasing incidence of AD and its associated disability and mortality, research into alternative treatments that are both effective and accessible is urgently needed. A diet high in some medicinal ingredients may assist to lower the prevalence of AD, according to recent studies. (8)

Many studies have demonstrated the strong connection between heavy metals like aluminium (Al) and neurological diseases like Alzheimer (AD). (9) Al is a major heavy metal that plays a role in the development and progression of these disorders as it affects various metabolic processes in the nervous system. The use of aluminium chloride (AlCl3) is widespread, as it is found in many commonly used products like medicines, toothpaste, food, and packaged drinking water. (10) AD is often connected to exposure to metal toxins like Al through occupational exposure, food contaminants, drinking water contaminants, and cooking with Al cookware. (11) Al can also damage the blood-brain barrier, leading to accumulation of the metal in the brain, making it a risk factor for neurological disorders. (12) Additionally, Al can inhibit antioxidant enzyme activity, alter brain neurochemistry, and cause DNA damage in the brain. (13) Also, studies have denoted that long lasting exposure to Al leads to neuronal symptoms and there is changes in brain structure similar to those seen in advanced neurodegeneration. Animal models of AD induced by AlCl3 are considered an accurate representation of the human disease. (14,15)

Despite significant advancements in AD research in recent years, current drugs such as donepezil, rivastigmine, galantamine and memantine can only temporarily improve cognitive symptoms and cannot change the course of the progressive neurodegenerative process. These synthetic drugs can cause various side effects, and their high cost makes them unfeasible for some patients, and they are not effective for all cases of AD. (16) Also, current treatment options for AD are limited and often ineffective, highlighting the need for new and innovative therapies. (17, 18)

Due to their low cost and limited side effects, herbal plants have been utilised as an alternative type of medicine for a very long time. Several plant-based substances have recently been demonstrated to prevent neuronal damage, making them an essential treatment alternative for

various brain illnesses. In order to ascertain the effect of Annona squamosa fruit pulp in a rat model of AD caused by aluminium chloride, ($AlCl_3$) this experiment was carried out.

MATERIALS AND METHODS

Plant protocol

Fruits from the Annona squamosa Linn. plants were collected in Tamil Nadu's Namakkal District and certified by the Coimbatore branch, Botanical Survey of India. The fresh fruits were washed, and the flesh was extracted from the seeds using a vacuum inside a glovebox. The resulting pulp was then freeze-dried at a low temperature(-50ºC), and the powder was mixed with 70% ethanol through a cold maceration process for 72 hours with continuous stirring, resulting in a brownish yellow extract.

Qualitative phytochemical analysis

The extract underwent a qualitative phytochemical examination using the conventional procedure described by Trease and Evans. (40)

Total phenolic content analysis

The phenolic content of plant extract was ascertained using the Folin-Ciocalteu technique. (19) For this study, 2.5 ml of a 10% dilution of Folin-reagent Ciocalteu's in water were combined with 0.5 ml of a ethanolic solution of the extract at 1 mg/ml concentration, and the mixture was left to stand for 3 minutes. After that, 2.5 ml of a 7.5% $NaNo_2$ solution was added, and the combination was allowed to sit at normal room temperature for two hours. Using a UV spectrophotometer to measure the samples' absorbance at 765 nm, the data were compared to a calibration curve made using different amounts of gallic acid (10, 20, 30, 50, and 100 mg/ml) as a standard. Per gramme of extract, the total phenol content was calculated as milligrammes of gallic acid equivalents.

Animals

Wistar rats of male gender, weighing between 230-250 grams, were kept in controlled laboratory cages, and given food and water *ad libitum*. They were continued a week to adjust to the experimental conditions before the start of the experiment. The institution's Animal Ethics Committee examined and authorised the experiment, with the permission number, KMCRET/Ph.D/07/2015-16 after precautions were taken to reduce the animals' suffering.

Experimental Design

A total of 30 rats were methodically split into five separate groups, each with six rats, before the experiment began. The dosage administered was calculated based on their body weight and administered on a daily basis for six consecutive weeks to the following groups: The first group, known as the control group, was given a solution of normal saline orally for the duration of the 90-day experiment. The second group was given with a solution of AlCl3 in their drinking water from day 01 to 30, with the dose being 17 mg/kg of body weight. The third group received an oral dosage of rivastigmine (0.3 mg/kg) for the next 60 days after exposed to AlCl3 from the 1st to 30th day. The fourth and fifth groups were given a dosage of FEAS I and FEAS II (200 mg/kg and 400 mg/kg of body weight) for 60 days respectively, after exposed to AlCl3 from day 1 to 30.

AlCl3 treatment group allows it to penetrate and restore in the brain and induce neuronal toxicity and cognitive impairment (i.e., representing an early stage AD), which was assessed after the pilot study before the main work, along with the available literature reporting on an interruption in the content of brain catecholamine, (20) a raise in CNS oxidative stress, (21) pathological hallmarks shows damage in the brain tissues (22) and an attenuation in total rats' weights, (23) after AlCl3 treatment with short-term (01-30 days). A visual representation of the experimental process undertaken in this study can be observed in the diagram illustrated in graphical abstract.

During the study, the weight, diet, water intake, temperature, and urine volume of the rats were monitored before treatment and on the final day. After the study, the rats underwent behavioural testing before being sacrificed. They were given pentobarbital sodium (40 mg/kg) as anaesthesia and their brains were collected for pathological and biochemical examination.

Morris water maze behavior

Rats were tested on their capacity for spatial learning and recall using the Morris water maze. (24,25) The experiment includes an acquisition trial and a probe trial in a circular pool with a depth of 30 cm and a diameter of 160 cm. In order to provide four different starting places and divide the pool into four quadrants, the 4 points around the border of the tank were given the designations North (N), South (S), East (E), and West (W) (NW, NE, SE, and SW). The invisible platform was positioned in the northeast quadrant 2 cm below the water line. The rats were trained to discover the platform over a period of five days by being given up to 90 seconds and various beginning points around the pool. The platform was taken away on the sixth day, and

each rat's time spent swimming in the destination quadrant was noted. Comparison of rats with Alzheimer's disease, healthy memory-functioning rats were predicted to spend more time looking for the platform in the target quadrant.

Passive avoidance test

A slider door with a 90 mm diameter connects a bright and a dark compartment (depth 270 mm, width 370 mm, and height 360 mm), in the Passive Avoidance (PA) test to gauge the affective memory of rats. (27,28) A shock generator that could produce shocks of 0.5 mA was linked to a metal grid that covered the instrument's floor and was spaced 0.9 cm apart. (29,30) A retention trial and an acquisition trial were both part of the test. Each rat was placed during the acquisition session in the light compartment and administered a 0.5 mA electric shock for three seconds upon entering the dark container. Then measured the escape latency as the length of time it took the rat to enter the dark compartment (EL). After 24 hours, a retention trial was carried out in which no shock was administered, and the retention latency (RL) up to 600 seconds was calculated based on how long it took the rat to return to the dark compartment. The apparatus was cleaned with 70% ethanol after each test, to get rid of any odour indicators. (26)

Biochemical Studies

After conducting behavioral assessments on the animals with 90 days treatment, they were put under deep anesthesia and their brains were carefully removed and stored in a freezer at minus 80°C. To prepare the brains for a biochemical study, they were fixed using a Microwave Fixation System. After homogenization of the tissue in a PBS solution with a pH of 7.4, the sample was centrifuged at high speeds for 20 min at a cool temperature of 4°C. (31) To withdraw the nuclear debris, the brain tissue was centrifuged at 800 x g for 5 min at 4°C. Malondialdehyde (MDA/LPO) and the activity of the enzyme acetylcholine esterase were both examined in the resultant liquid, referred to as the supernatant. The post-mitochondrial supernatant was obtained by centrifuging the residual supernatant a second time at 10,000 x g for 30 min at 4 °C. This supernatant was then examined for the presence of glutathione (GSH) and the action of the enzymes superoxide dismutase (SOD) and catalase (CAT). (24)

Lipid peroxidation (LPO) activity

A technique established by Jain *et al*., was used to gauge the degree of lipid peroxidation (LPO) in the tissue (32) In a nutshell, PBS, BHT, and 30% TC were combined with the tissue homogenate before being incubated for an hour at room temperature. A water bath at 80 °C was

used to heat the solution for 20 minutes after it had been centrifuged at 3000 g for 20 minutes. The supernatant that was left over was then combined with EDTA and 1% TBA. The amount of malondialdehyde (MDA) present was then quantified by the solution's absorbance at 532 nm and reported in nmol/mg of wet tissue.

Superoxide dismutase activity

Oberley's approach was used to quantify the superoxide dismutase (SOD) enzyme's activity. (33) In order to conduct the assay, 1 ml of reaction mixture including 960 µl of 100 mM sodium carbonate buffer (pH 7.8), 0.1 mM xanthine, 0.025 mM nitroblue-tetrazolium (NBT), and 0.1 mM EDTA, 20 µl of xanthine oxidase, and 20 µl of brain supernatant was mixed. The creation of blue formazan generated changes in absorbance at 560 nm, which were measured to track the reaction. The quantity of enzyme necessary to reduce the rate of NBT oxidation by 50% was used to measure SOD activity. Per milligramme of protein, SOD activity was expressed in units per minute.

Catalase activity

A technique Sinha described was used to evaluate the enzyme catalase's activity. (34) In a nutshell, 200 µl of hydrogen peroxide (200 mM) was mixed with 50 µl of tissue homogenate or plasma and 750 µl of phosphate buffer (0.01 M; pH 7.0). The reaction was then allowed to run for 60 seconds. By adding 2 ml of a dichromate and acetic acid solution (1:3 v/v of 5% potassium dichromate with concentrated acetic acid), the process was stopped. The absorbance was measured at 570 nm using a blank solution of 50 µl of 0.9% NaCl after the tubes had been heated at 100 °C for 10 minutes and cooled in an ice bath. The amount of hydrogen peroxide absorbed by catalase per minute per milligramme of protein was measured.

GSH activity

For this study, we used Ellman's technique to quantify glutathione concentration. (35) 900 µl of Ellman's reagent was produced in a tris-HCl buffer solution and 100 µl of tissue homogenate was added to the mixture (0.1 M, pH 6.5). After mixing, the solution was kept at room temperature to 30 minutes. At 412 nm, absorbance was measured using blank sample. Molecular extinction coefficient DTNB ($\varepsilon = 1.36 \times 105$ M− 1 cm− 1) was utilised to ascertain glutathione concentration. Millimoles per gramme of protein were the units of measurement used.

Total protein content

The amount of thiols in the samples was calculated by observing how much yellow colour developed after reacting sulfhydryl groups with a reagent called DTNB. Lowry *et al*., technique's was used to calculate the protein concentration in the samples. (36)

Assessment of nitric oxide activity

By employing Griess reagent, as described by Tracey *et al*., to determine the concentration of nitric oxide (NO) in the tissue. 96-well plates for cell culture were priorly coated with poly-L-lactic acid (PLLA) and tissue homogenates were diluted with PBS before being incubated at 25°C for 15 minutes. Afterward, the spectrophotometer was used to detect the absorbance at 540 nm and compare it to a blank sample. (37)

Neurochemical analysis

Assessment of acetylcholinesterase (AchE) activity

Ellman's approach was used to analyze the acetylcholinesterase (AChE) activity. (38) 250 µL of brain supernatant (25 mg/kg and 50 mg/kg) and 170 ml of Tris-HCl (50 mM) were placed in a falcon tube, followed by 10 µL of AChE (6.67 U $ml^{-}1$) and 20 µL of DTNB (10 mM) (5,5′-dithio-bis [2- nitrobenzoic acid]) in buffer. Ten minutes were spent incubating the solution at 37 degrees Celsius. The absorbance was then measured at 412 nm after adding 10 µL of 200 mM acetylthiocholine iodide. The percentage of enzyme inhibition was determined by:

% Inhibition = 100 – change of sample absorbance / change of blank absorbance * 100

Assessment of transmembrane protein activity

Na+/K+ ATPase (NKA) activity was determined following a protocol proposed by Li et al (39) 0.2 millilitres of buffer, 0.2 millilitres of calcium chloride, 0.2 millilitres of adenosine triphosphate, and 0.2 millilitres of distilled water were added to the supernatant from homogenates of brain tissue. After 10 minutes incubation at 37°C, 2.0 ml of 20% TCA was added to the mixture. After adding 1.0 millilitres of ammonium molybdate and 0.8 millilitres of 4-aminonaphthol sulphonic acid, a blue colour was produced. The resulting solution's absorbance was measured at 650 nm in a spectrophotometer.

Statistical Analysis

The study presented the findings as mean ± standard error mean (SEM). The researchers analyzed the behavior data using a statistical method called ANOVA, which compares multiple

groups, and a follow-up test called Dunnett's test to find the specific differences. A p-value of less than 0.05 was used for determining statistical significance of the analysis.

RESULTS

Effect of FEAS on AlCl3 induced determination of total phenolic content

The extract was subjected to preliminary phytochemical screening. The chemicals were evaluated using the Trease GE and Evans WC procedures (40), and it was discovered that several substances were present including alkaloids, carbohydrates, proteins, steroids, sterols, phenols, tannins, flavonoids, gums, mucilage, glycosides, saponins, and terpenes. Using gallic acid as a standard, the total phenol content of the Annona squamosa L. fruit extract was calculated. The phenolic content of the ethanol extract was very high (302.820.8319). The data were presented as GAE milligrammes per gramme of plant extract [Table 1].

Because of their hydroxyl groups, phenols play a crucial role in plants and may potentially have antioxidant effects. (41) It can be shown in Fig. 1, the high concentration of phenolic compounds in Annona squamosa L. fruit extract is a good predictor of radical scavenging capability. Using gallic acid standard curve (range, 0-120 mg/ml, Y = 0.0017x + 0.2122, R^2 = 0.8846), we were able to convert the phenolic content to GAE/g of plant material.

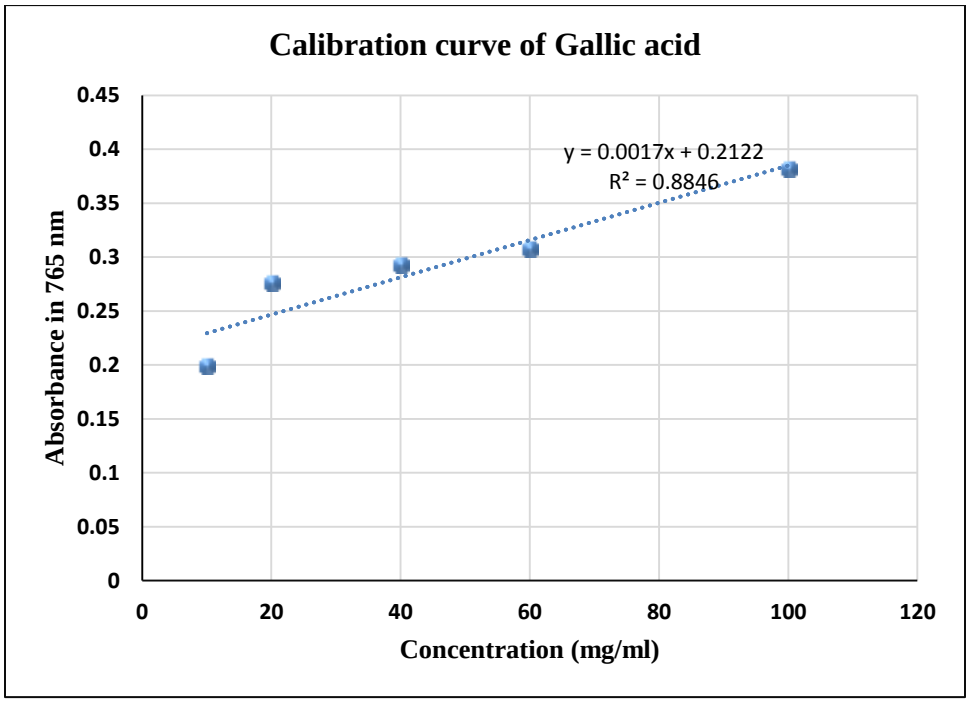


Fig. 1. Calibration curve of Gallic acid- standard

Sample	Total Phenolics (mg GAE/g)

FEAS	302.82 ± 0.8319

Table 1. Determination of Total phenolic content of Fruit extract of Annona squamosa Linn.

Effect of FEAS on AlCl3 induced decreased in body weight, food and water intake, urinary output and urinary ions.

When compared to the control rats, the rats induced with Alcl3 had a significant decrease in body weight, food intake, water consumption, and urinary output volume, as well as a decrease in urinary sodium and potassium concentration. Treatment with Rivastigmine resulted in an increase in these values for the Alcl3-induced rats, while the Alcl3 + FEAS-I and Alcl3 + FEAS-II treatments resulted in varying degrees of change in these values when compared to the control rats. Overall, the study suggests that Alcl3 had a negative impact on the rats' weight and consumption, while the treatments with Rivastigmine and FEAS-I and II had varying significant effects on these values shown in figures.

Fig.2 Percentage change in body weight ofAlcl3 induced rats

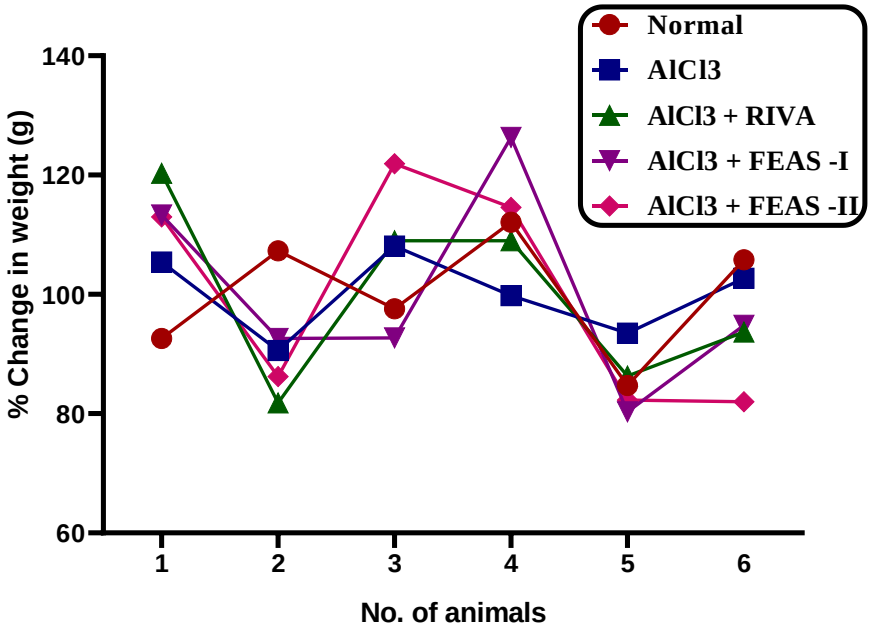


Fig.3 Percentage change in food intake ofAlcl3 induced rats

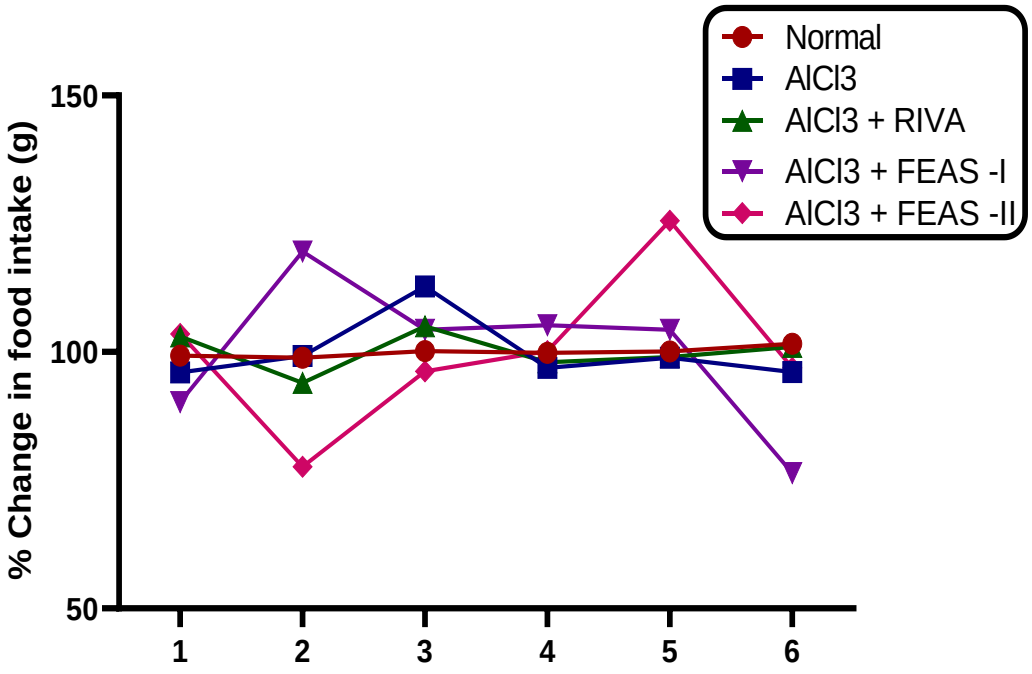


Fig.4 Change in body weight ofAlcl3 induced rats

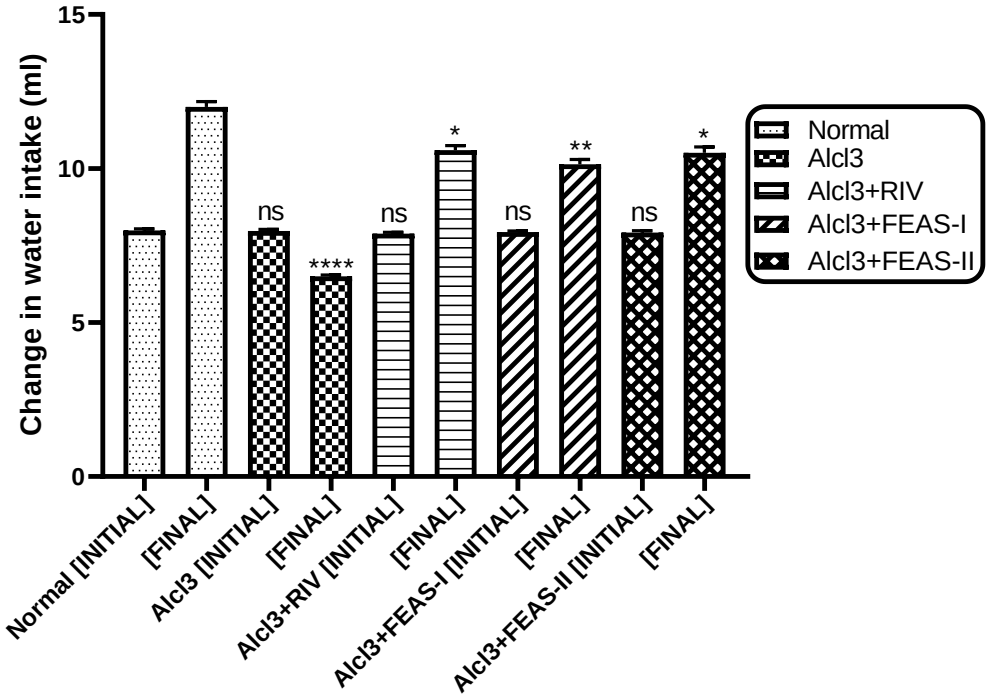


Fig.5 Change in urinary volume ofAlcl3 induced rats

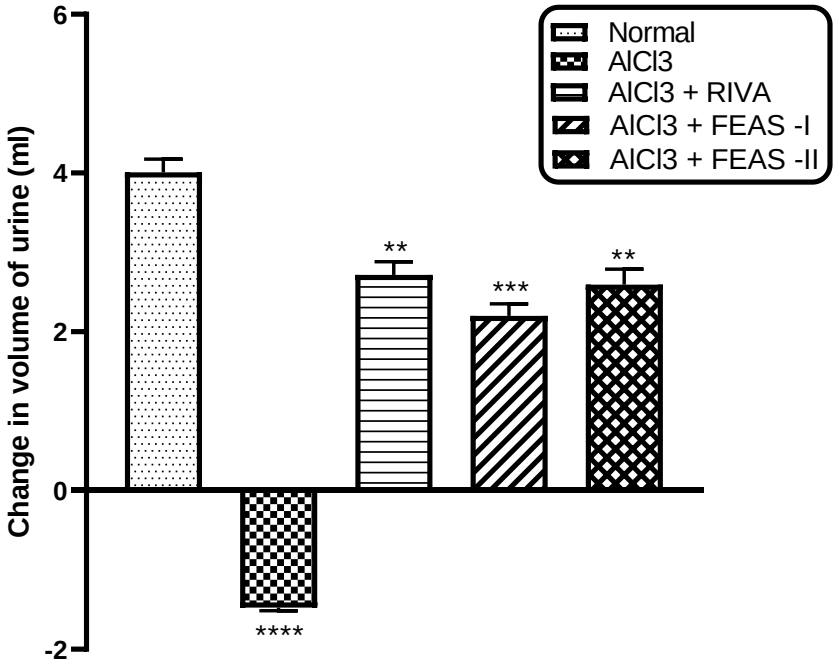


Fig.6 Change in urinary sodium level ofAlcl3 induced rats

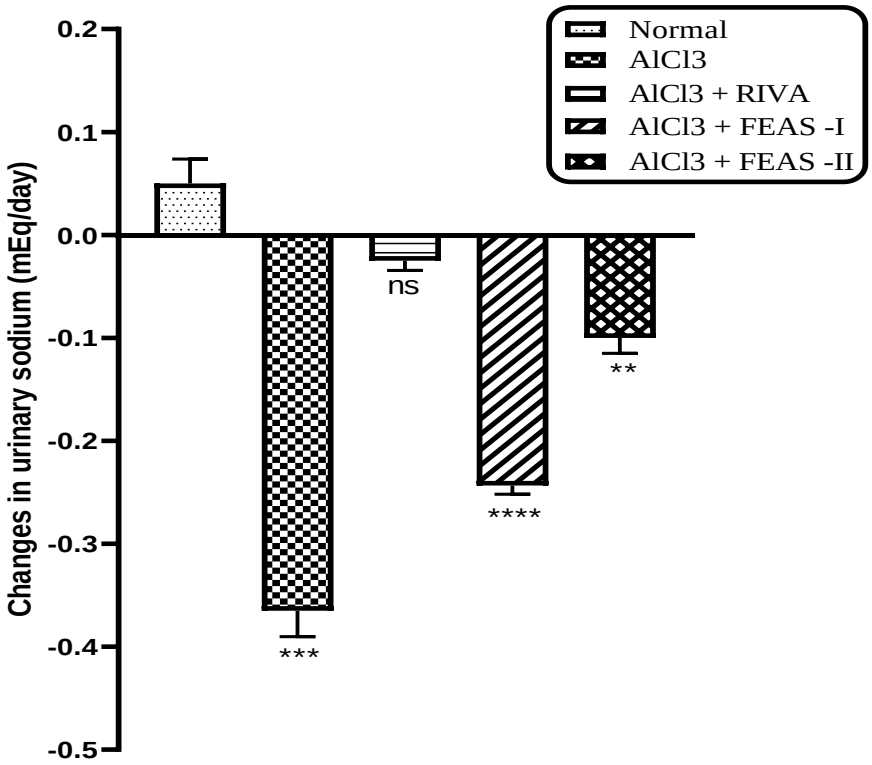
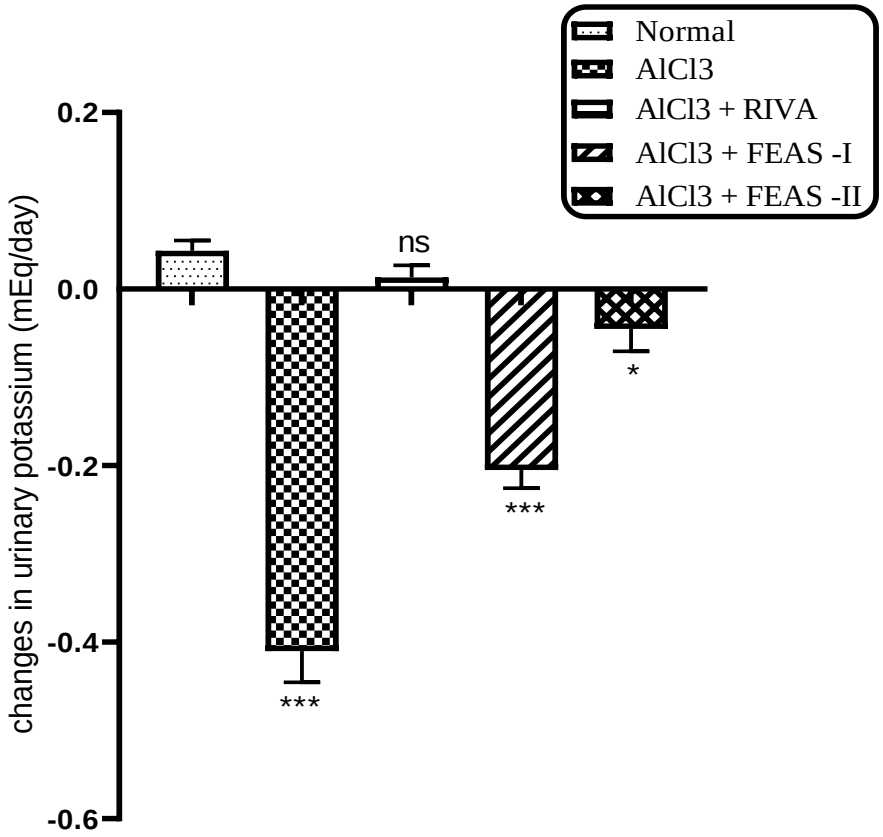


Fig. 7 Change in urinary sodium level of AlCl3 induced rats



Effect of FEAS treatment on AlCl3 induced changes in Morris water maze test of rats

The results presented that AlCl3 impaired the rats' ability to learn and memorise, but FEAS treatment was able to reverse the effect and improve the rats' spatial memory and the learning ability. This suggests that FEAS could be used to treat the cognitive impairments caused by aluminium exposure. The duration of mean latency to escape the platform, denoted in seconds on days 1, 10, 30, 60, and 90, is shown in Fig. 8, and time spent in each quadrant is shown in Fig. 9 which denotes that the duration of search strategy of the particular quadrant containing platform by AlCl3 was higher as compared to control and AlCl3 + FEAS- II takes lesser duration as compared to control to reach that platform.

Fig. 8: Effect of FEAS on duration of mean escape latency of AlCl3 induced deficit in Morris water maze test

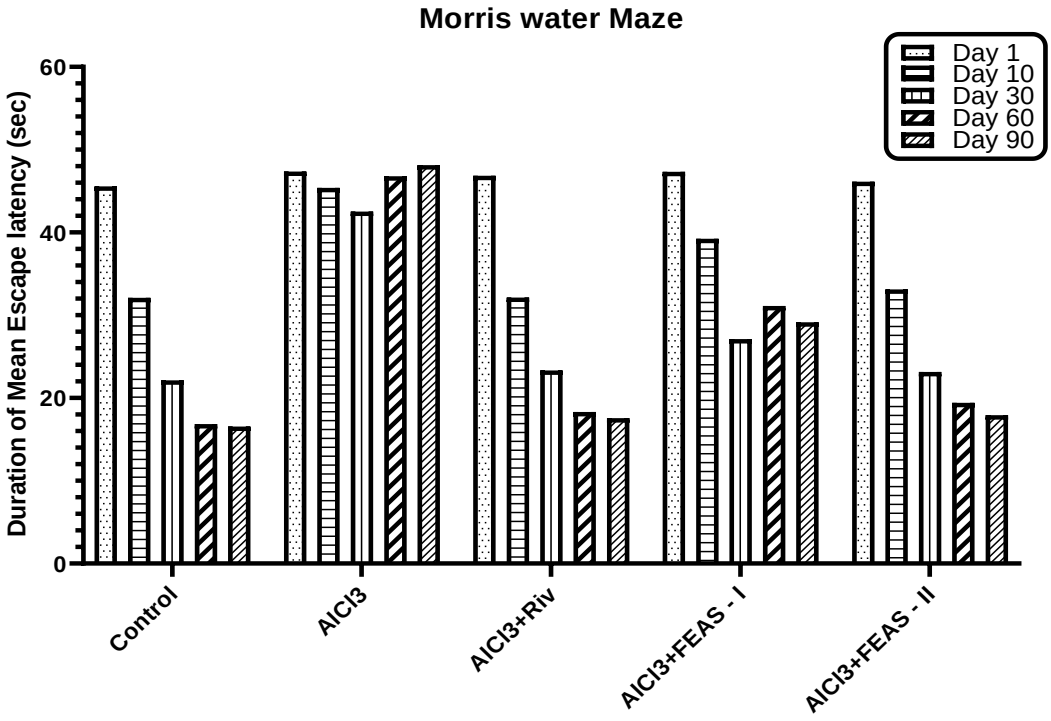


Fig. 9: Effect of FEAS treatment in the search strategies of Morris water maze test using AlCl3 induced Alzheimer model in rat

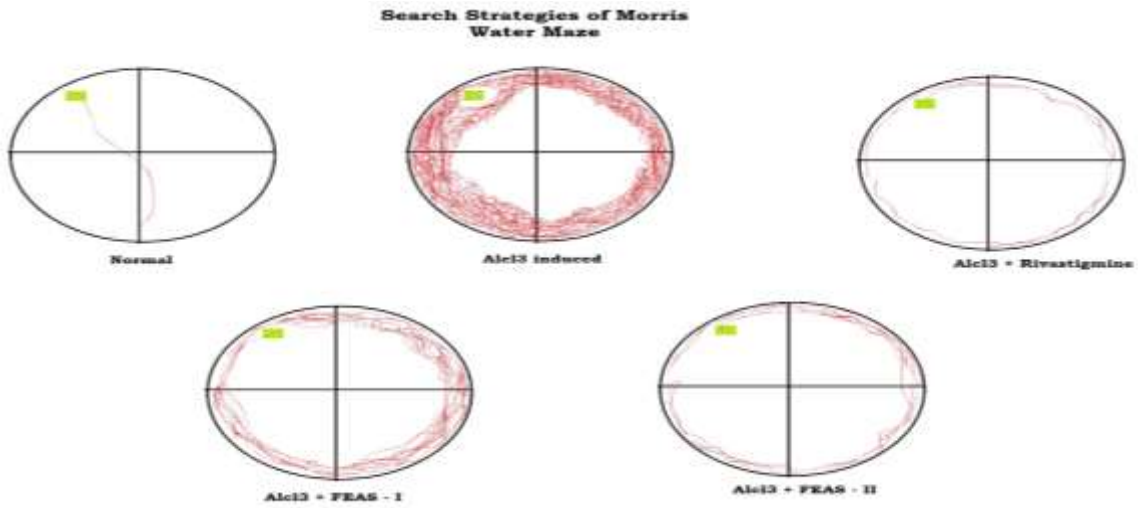


Fig.10 Effect of FEAS treatment in the step through latency in passive avoidance test using Alcl3 induced Alzheimer model in rat

Effect of FEAS on AlCl3 induced changes in passive avoidance test in rats

The study found that 24 hours after receiving an electric shock during the retention phase, rats treated with aluminum chloride had a shorter time spent in a dark compartment (103.7 ± 0.88) compared to the normal control group (235.8 ± 2.24). However, rats given both FEAS-I and FEAS-II, as well as rivastigmine (215.3 ± 0.88), spent longer time in the dark compartment because they remembered the shock due to electricity from the learning phase, as shown in Fig.10a,b. These differences were found to be statistically significant ($p < 0.0001$ for NC versus AlCl3, $p < 0.01$ for NC versus AlCl3+ Rivastigmine, $p < 0.001$ for NC versus AlCl3+FEAS-I, and $p < 0.001$ for NC versus AlCl3+FEAS-II). The researchers also counted the number of times the rats crossed between the light and dark compartments. As shown in Fig.11a,b, the aluminium chloride group had more crossings (4.00 ± 0.36) than the normal control group (2.16 ± 0.41), which was statistically significant ($p < 0.001$, NC vs. AlCl3). The number of crossings was not significantly different between the groups treated with rivastigmine and FEAS-I & II ($p > 0.05$ for NC vs. AlCl3+Rivastigmine, $p > 0.05$ for NC vs. AlCl3+FEAS-I & II).

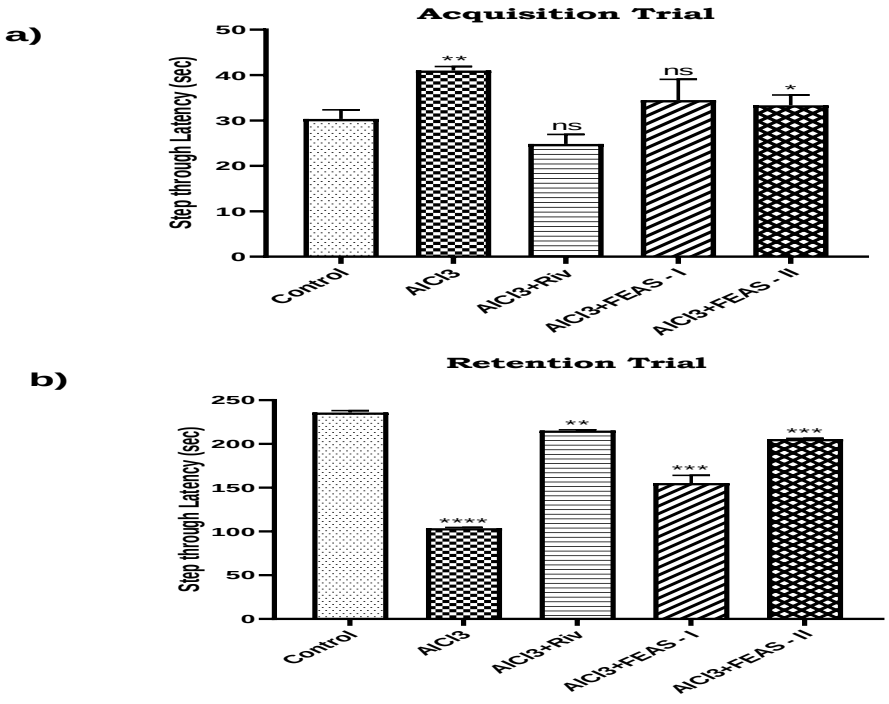
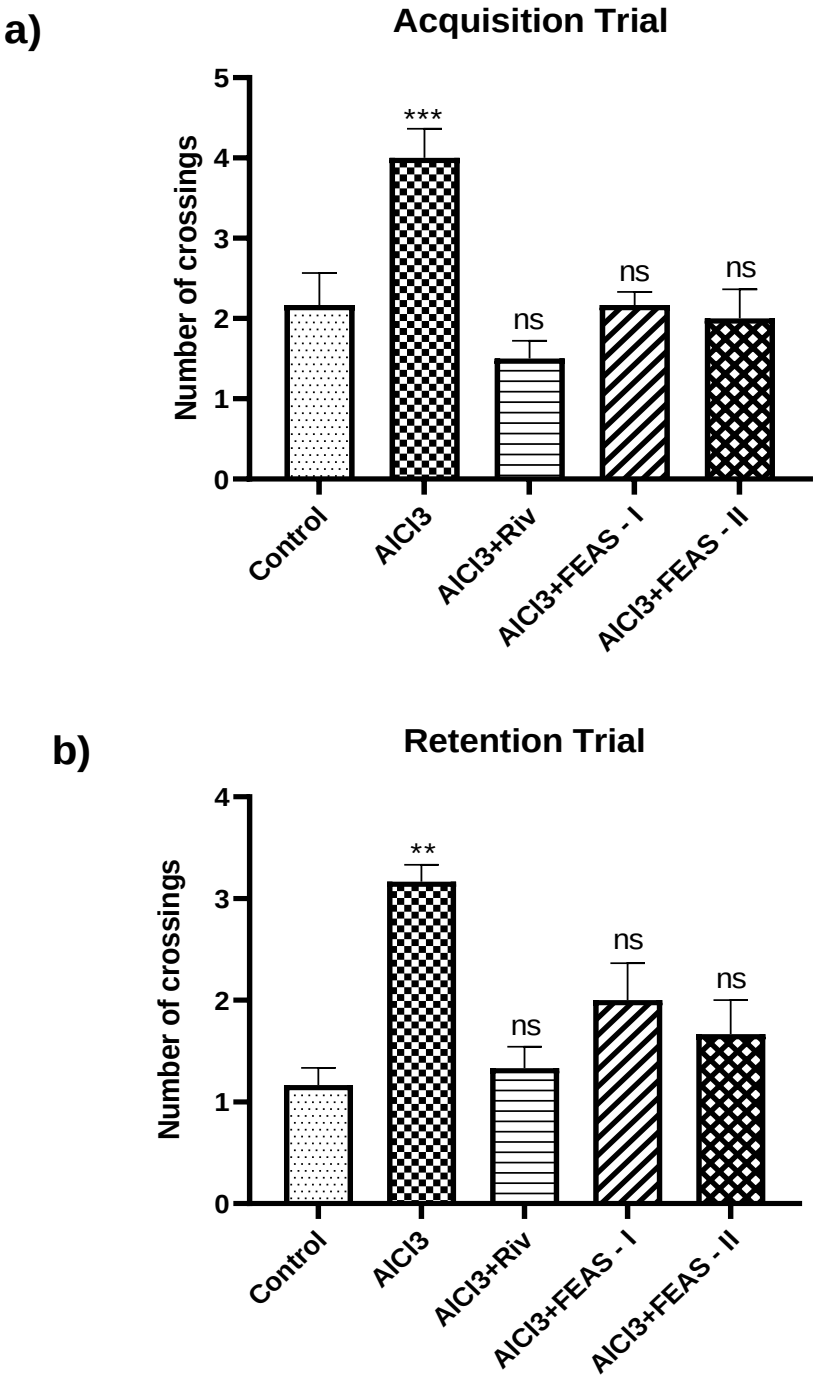


Fig.11 Effect of FEAS treatment in the Number of crossings in passive avoidance test using Alcl3 induced Alzheimer model in rat

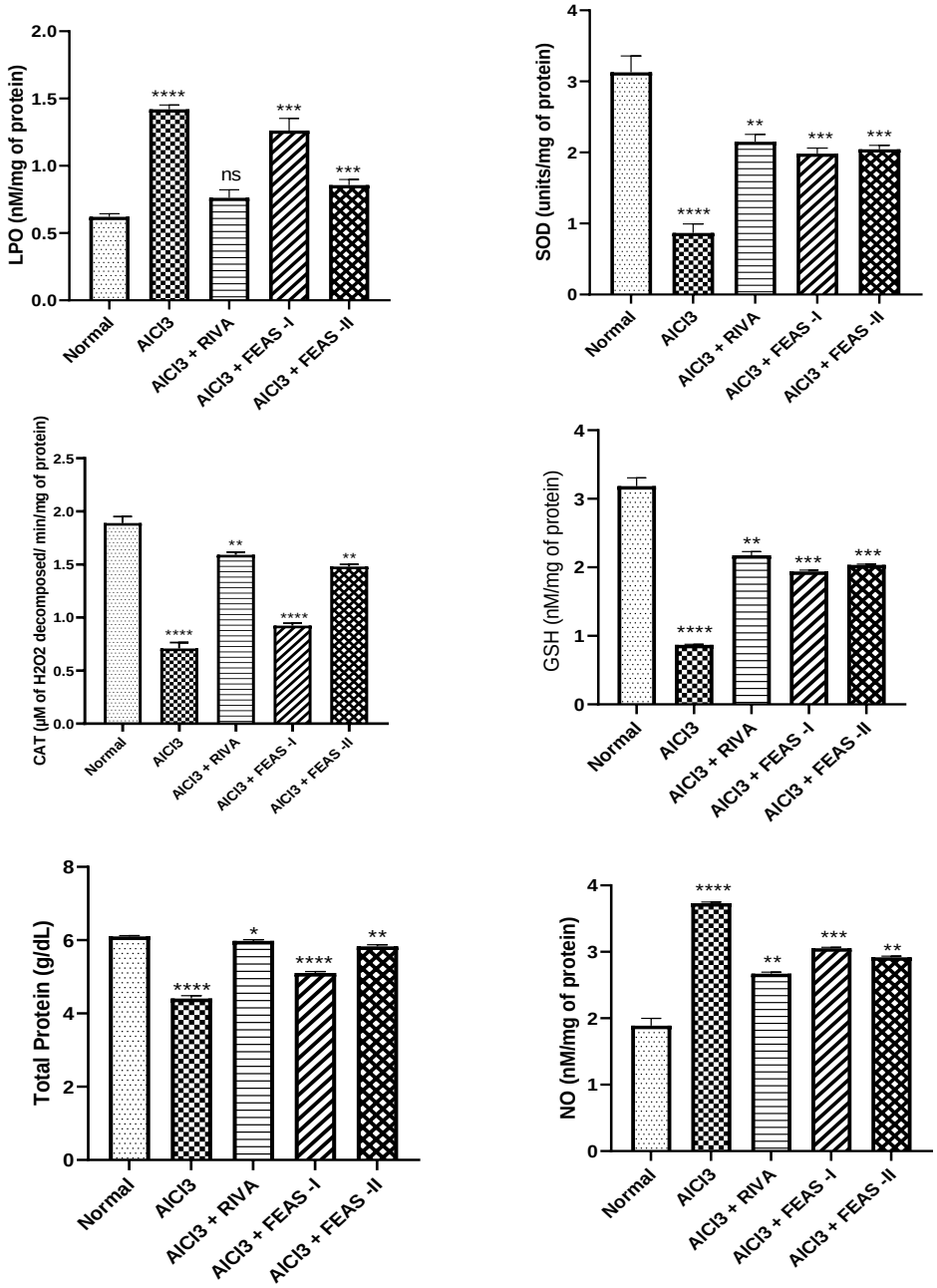


Effect of FEAS on AlCl3 induced changes in brain antioxidant levels in rats

Animals were found to suffer from oxidative damage after being given AlCl3. When compared to the control group, the AlCl3 group has higher levels of pro-oxidant markers (LPO and NO) and lower levels of antioxidant enzymes (CAT, SOD, TP, and GSH). However, treatment with

Rivastigmine, FEAS-I & II , (ns; ***$p < 0.001$; ***$p < 0.001$ and **$p < 0.01$; ***$p < 0.001$; **$p < 0.01$ respectively) resulted in remarkable decrease in the levels of LPO and NO and increased levels of antioxidant enzymes (CAT, SOD, TP and GSH) compared to the control group. These effects on cerebral antioxidants are depicted in Figure 12.

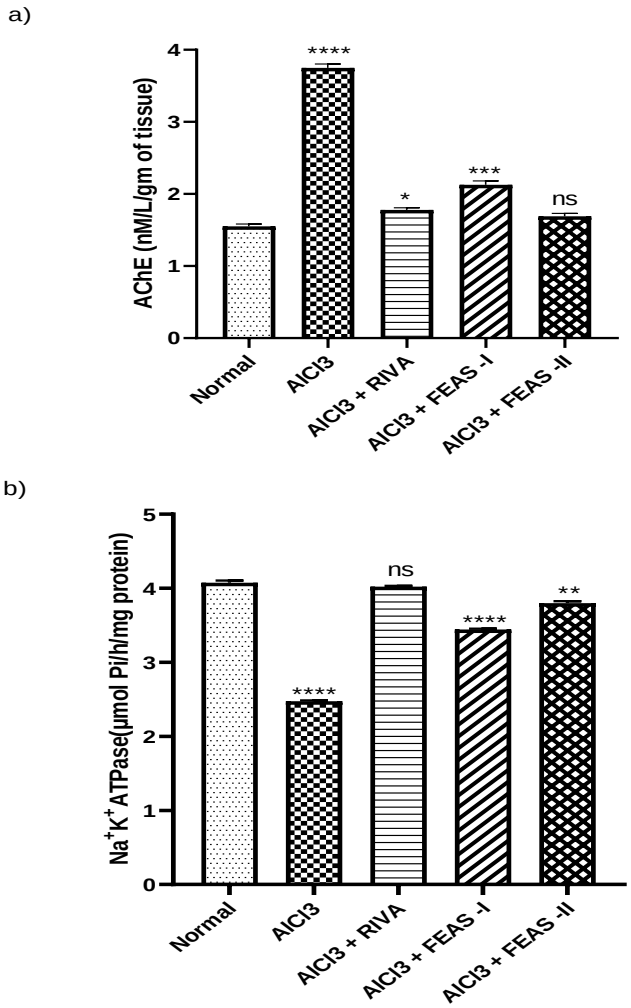
Fig.12 Effect of FEAS treatment in the cerebral antioxidant level of Alcl3 induced Alzheimer model in rat



Effect of FEAS treatment on cholinergic function in AlCl3 induced rats

Figure 13 depicts the effect of oral FEAS administration on cholinergic function by measuring the activity of acetylcholinesterase (AChE) and transmembrane protein (Na^+K^+ATPase) in brain tissue in the normal control and AlCl3 treatment groups. Figures 13a and 13b show that rats from the AlCl3-induced group had higher AChE levels and lower Na^+K^+ATPase levels, with a significant difference (p 0.05) between the two groups. Following that, FEAS treatment significantly suppressed (p 0.05) AChE and raised Na^+K^+ATPase levels in rats from Group IV (FEAS-I) and V (FEAS). This can be explained by FEAS treatment improving cholinergic function, which is responsible for knowledge, memory, and movement control in the brain.

Fig.13 Effect of FEAS treatment in the cholinergic functions of Alcl3 induced Alzheimer model in rat



DISCUSSION

A new study using UPLC-ESI-MS/MS reveals the composition of phenolic compounds in Annona squamosa fruit pulp. The fruit extract contained 16 free, 15 bound, and 13 esterified phenolic compounds, according to the study. (42) These phenolics' antioxidant properties stem from their ability to act as reducing agents, hydrogen donors, and neutralise singlet oxygen, thereby enhancing their role in nutrition. (43-47) Phenolic compounds are also important in protecting lipids from oxidation and inhibiting oxidising enzymes. (48, 49) Variations in flavonoid structures and substitutions affect the stability of phenoxyl radicals, and thus their antioxidant properties. (50) The phenolic content of fruit pulp was determined to be 302.82 mg GAE/g in the current study.

Extensive exposure to aluminium chloride (AlCl3) has been linked to cognitive impairments such as memory loss and learning difficulties, according to research. AlCl3, which is used in a variety of industries, can find its way into our food and drinking water, causing accumulation in the brain, particularly in the hippocampus - the memory and learning centre. Aluminium levels in Alzheimer's disease patients' brains have also been found to be elevated. Researchers conducted a study on rats to monitor the impact of AlCl3 on associative memory and extinction learning in order to gain a better understanding of this neurotoxicity and potentially discover a treatment for Alzheimer's disease. (51)

Aluminium causes its harmful effects by promoting an intracellular environment rife with oxidative stress, which leads to a variety of health complications. The study's findings show that chronic exposure to aluminium reduces antioxidant levels/activities such as SOD, CAT, TP, and GSH while increasing oxidative stress markers LPO and NO. This oxidative stress is directly responsible for the decline in cognitive function in rats due to ageing and oxidative damage to the brain. (52)

A mismatch between oxidative stress and antioxidant defences is thus linked to sudden cognitive decline and may be an early sign of Alzheimer's disease progression. (53) Aluminum administration caused oxidative stress, according to a study by HH Ahmed et al., as evidenced by an increase in MDA levels and a decrease in antioxidant enzyme secretion (SOD, CAT, GSH). However, Betalain treatment reduced MDA levels and restored antioxidant enzyme secretion (SOD, CAT, GSH). Many herbal compounds have been shown in aluminium chloride-induced Alzheimer's disease to counteract oxidative stress via antioxidant mechanisms. (54)

The cholinergic pathway is essential for memory and learning. Acetylcholine is a cholinergic neurotransmitter that regulates the activity of AChE, the enzyme that degrades acetylcholine. (55) Through transmembrane protein (Na^+K^+ATPase) activity, AChE also maintains the integrity of cholinergic neuron membranes. The researchers discovered that giving rats AlCl3 increased both AChE and transmembrane protein activity. However, FEAS treatment reduced their activities, indicating that Annona squamosa fruit pulp has neuroprotective effects by inhibiting AChE activity. This adds to the evidence that Annona squamosa fruit pulp has a neuroprotective effect against aluminium chloride-induced neurotoxicity in rats.

CONCLUSION

In conclusion, the findings suggest that Annona squamosa fruit pulp can protect memory, brain tissue, and cholinergic function from the damaging effects of aluminium chloride. This is most likely due to the fruit pulp's potent antioxidant properties, which effectively combat oxidative stress and maintain transmembrane protein levels. These findings suggest that Annona squamosa fruit pulp could be used to treat neurodegenerative diseases like Alzheimer's. However, more research is needed to establish the fruit pulp's anti-effects Alzheimer's in trials with other Alzheimer's models before proceeding with clinical trials.

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