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EVALUATION OF ANTI-ALZHEIMER ACTIVITY OF ETHANOLIC EXTRACT OF *ANNONA SQUAMOSA* LEAVES IN WISTAR ALBINO RATS

Nitesh Gupta and Imtiyaz Ansari *

Department of Pharmacology, Oriental College of Pharmacy, Sector 2, Behind Sanpada Railway Station, Sanpada West, Navi Mumbai - 400705, Maharashtra, India.

Keywords:

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Correspondence to Author:
Mr. Imtiyaz Ansari

Assistant Professor,
Department of Pharmacology,
Oriental College of Pharmacy,
Sanpada West, Navi Mumbai - 400705, Maharashtra, India.

E-mail: imtiyaz.ansari@ocp.edu.in

ABSTRACT: Alzheimer's (AD) has been the most prevalent kind of dementia, generally producing linguistic and visuospatial deficits, frequently combining comfort ability problems such as apathy, aggression and depression, *via* the gradual deterioration of the episodic memory and learning. A noteworthy pathophysiological cause is the presence in the neuronal cytoplasm of extracellular plaque of insoluble ß-amyloid Peptide (Aβ) and neurofibrillary (NFT) tangles containing hyperphosphorylated tau protein (P-tau). Genetics could be linked to approximately 70% of the probability of development of AD. In the present study, the efficacy of ethanolic extract of *Annona squamosa* leaves (ASE) was evaluated against scopolamine-induced Alzheimer in the Wistar albino rat. Donepezil at dose of 2.5 mg/kg, ASE was given in rat at a dose of 100 mg/kg body weight, 200 mg/kg body weight and 300 mg/kg body weight. Anti-Alzheimer activity was assessed by Morris water maze, Novel object recognition test and Y-maze. And by biochemical test like neurotransmitter Acetyl cholinesterase activity, catalase activity, malondialdehyde and Nitric oxide assays. Results showed that extracts treatment prevents Alzheimer and increases the level of acetylcholine, catalase and phytochemical studies identified the presence of Phenolic compounds, flavonoids and alkaloids in the ASE. Hence *Annona squamosa* Leaves extract seems to be a potent candidate for enhancing learning and memory and it would be beneficial for the treatment of amnesia and Alzheimer's disease.

INTRODUCTION: The German psychiatrist and neuropathologist Dr. Alois Alzheimer is credited with describing for the first time a dementing condition which later became known as Alzheimer's disease [1]. Alzheimer's disease (AD) is characterized by a progressive deterioration of the cognitive functions (dementia) associated with specific histological lesions of the cerebral cortex.

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The disease is age-related. An early form begins between 45 and 60 years and occurs in about 0.1% of the population at risk. A late form, which has increased in frequency in developed countries due to longer life expectancy, is seen in subjects aged over 60 years. Alzheimer's disease occurs in about 5-6% of the population over 65 years of age.

The prevalence increases by a factor of two for every 5 years, thereby reaching 20 percent of the population beyond the age of 80. The disease usually begins insidiously, with three types of symptoms of memory impairment, behavioral problems and mental failure steadily developing [2]. Alzheimer's disease (AD) is a prolonged,

pathophysiologically well-designed neuro-degenerative disease, which affects primarily lateral brain stem and associative neocortical regions. The pathologic signs of AD include neurofibrillary plaques and tangles, related respectively to amyloid-beta peptide (Aβ) buildup in brain tissues and the cytoskeletal modification of neuronal tau protein associated with hyper phosphoryls.

According to the AD amyloid hypothesis, Aβ' surplus synthesis is a result of disturbance of homeostatic mechanisms that control amyloid protein cleavage protein (APP). Genetically, ageally and ecologically, the metabolic change leads to the amyloidogenic treatment of APP, at the expense of the physiologic, secretory processing pathways. Aβ-peptides are produced by the subsequent APP Cleavage, which has recently been described as part of the presenilin Complex (BACE-1) and Gamma-Secretase. In AD pathogenesis, Aβ1−42 played a major role in various beta-amyloid Isoforms with minor variations according to C-terminal amino acid amounts. The neurotoxicity potential of Aβ peptide arises in the aggregation of insoluble oligomers and protofibrils through its biochemical characteristics. These are also the buildup of fibrillary Aβ into senile and neuritic plaques. These mechanisms and the lowering of the brain Aβ clearance contribute to the extracellular buildup of Aβ, with activation of neurotoxic cascades, leading to cytoskeletal modifications, neuronal impairment, and cellular damage. Intracerebral amyloidosis is age-dependent in Dementia, however recent data suggests that it might be observed in certain people [3–9].

Current Treatment of Alzheimer's disease in Medical Science: Currently available conventional pharmacotherapy for AD which is approved by the Food and Drug Administration (FDA) are mainly of two types:

Acetylcholinesterase inhibitors - tacrine, donepezil, rivastigmine, galantamine.

N-methyl-D-aspartate glutamate antagonist (NMDA antagonist)- memantine.

Annona squamosa: *Annona squamosa* (Annonaceae) is a tiny semi-deciduous tree 3-7 m high and abundantly accessible in India with a wide-opened crown or unevenly distributed branches. In diagnosing specific ailments and renowned for its pharmacological and nutraceutical properties, various components of a tree-like fruit, leaves, barks, and roots. In India, the commercial value is somewhat high and well-known as that country's indigenous fruit. However, during the last 500 years, this has been a secondary diversification center. This fruit has an intriguing history with regard to its name, namely Sitaphal. It is widespread in tropics and is extensively dispersed in the east parts of India. It is a terrestrial course, woody, feathered and perennial tree with characteristic harsh odour, ash-gray bark with apparent leaf scars and smooth to gently fissured in plates, inner bark pale yellow and somewhat bitter. The blue leaf is dazzling green above and green below; with smallpox 0,7 to 1,5 cm; ovate elliptical to lanceolate; lamellan measures about 10 to 5 cm. The plants are blooms from 2.0 to 2.5 centimeters; somewhat fragrant, lonely or in 2 to 4 flavors, with three green sepals and six petals in two containers. The flowers feature many spirally organized, conglomerated stems underneath and around a high globe-shaped dome of several joined carpets. The fruit body is aromatic, delicious, white and light golden. The fruit is split into 20 - 38 segments each with harsh, sparkling brownish-black seed, fleshy, even if some plants produce seedless fruit[10].

Materials and Methods:

Plant Material: The fresh leaves of *Annona squamosa* were collected in November 2020 from the medicinal garden of Oriental College of Pharmacy Mumbai, Sanpada. The sample specimen voucher was submitted to Dr. Rajendra D. Shinde, Director, Blatter Herbarium of St. Xavier's College, Mumbai, for plant authentication. The specimen matched with the Blatter Herbarium specimen R-2503 of R. R Fernandez. *Annona squamosa* leaves were washed and then kept for shade drying. After drying the leaves was powdered.

Preparation of Extract: *Annona squamosa* powdered leaves (180 g) were placed in the Soxhlet extractor's extraction compartment using cotton inserts to filter the solvent (ethanol 90 percent v/v) returning from crude powder to the round bottom flask. The Soxhlet extractor was mounted on the

ethanol-containing round bottom flask. The condenser was installed towards the other end of the Soxhlet extractor to avoid solvent loss due to vapor formation and vapors condensation. The solvent dissolves the powder's soluble constituents and changes color. The siphon tube drained the compartment, with the solvent flowing back into the bottom round flask.

This loop was replicated until the powder was consumed, which was detected when the liquid in the siphon tube was clear. Next, in the petri plates, all the contents of the round bottom flask were drained and the solvent was allowed to dry up.

After the extracts had been evaporated to get the dried crude form, they were then placed in an appropriate container and stored in a refrigerator at 4°C till the time for their use. The ethanolic extract percentage yield was 18.50 percent w/w. The entire study was then done on the basis of Ethanolic extract of *Annona squamosa*.

Medicinal Properties: Antioxidant, Antitumor, Antimalarial, Antiulcer, Anthelmintic, Anti-genotoxic, Antibacterial, wound healing, Hepatoprotective, Antimicrobial, Antidiabetic, Cytotoxic, Anti-arthritic, Anti-inflammatory, Analgesic, Anti-HIV, Stress, Depression, Prevent Ageing, Antihyperlipidemic, Larvicidal, Anticancer, and Antithyroid activity.

Toxicity: The alcoholic extract's LD_{50} was estimated to be 2000 mg/kg. This plant has also known to be edible, making it safe, and accordingly, $1/10^{th}$ and $1/20^{th}$ doses were selected for study, *i.e.*, low dose 100 mg/kg, intermediate-dose 200 mg/kg, and high dose 300 mg/kg for the test groups with dose conversion.

Drug Treatment: The ethanolic extract was suspended in Normal saline in doses of 100, 200, 300 mg/kg p.o. for the pharmacological tests. The doses were selected based on the previous studies on ethanolic extract.

Annona squamosa leaves extract (ASE) was administered to individual rats in groups 4,5,6,7. None of the rat was dead due to treatment till the end of the observation period. The *Annona squamosa* leaves extract (ASE) caused no abnormality till the duration of treatment.

Qualitative Phytochemical Screening: Preliminary chemical studies on Annona squamosa alcoholic extract were performed. The presence of the following phytoconstituents was assessed: Molisch test, Benedict test, and Selwinoff test were done for carbohydrates, Sodium hydroxide (NaOH) test, Alkaline reagent test and Shinoda test for flavonoids, Modified Borntrager's test for glycosides, Ferric chloride test, and dil. Iodine solution test for tannins, Foam test for saponins, Sodium hydroxide solution test for fixed oils, Dragendroff reagent test, Mayer's reagent test, and Hager's reagent test for alkaloids, dil. Potassium permanganate test for phenolic compounds, Liebermann Burchard test and Salkowski test for sterols, Liebermann Burchard test for terpenoids, and Biuret test for proteins.

Equipments and Chemicals: Electronic balance, Morris water maze, Y-maze, novel object apparatus, syringes, and needles, ethanol, NS, and scopolamine. Donepezil tablet, 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB), acetylcholine, thiobarbituric acid (TBA), Trichloroacetic acid (TCA), Hydrogen peroxide (H2O2), Griess reagent.

Animals: The CPCSEA registered Institutional Animal Ethics Committee (IAEC) of Oriental College of Pharmacy; Sanpada approved the animals needed for the research work with protocol no. OCP/IAEC/2020-2021/06 entitled "Evaluation of anti-Alzheimer activity of ethanolic extract of *Annona squamosa* leaves in Wistar albino rats". Animals procured were female Swiss Albino mice (15 to 20 g) for carrying out Acute oral toxicity study & both male and female Albino Wistar rats (100 to 120 g) were procured from National Institute of Biosciences, Pune. The animals were kept in well ventilated, air-conditioned animal house at a constant temperature of 24 ± 2 ° C, with 12:12 hours dark: light cycle and with a relative humidity of 55-60%. The animals were placed on bedding material, in spacious polypropylene cages with a paddy husk. The animals were held on normal diet with pellets and filtered water.

Acute Oral Toxicity Study: Acute oral toxicity analysis was carried out for the ethanolic extract of dried Leaves of *Annona squamosa* as recommended in OECD Guideline 423. The animals were noted keenly for the display of any

toxic signs or symptoms at different time intervals of 0, 30 min, 1, 2, 4, 6, 8, 12 h and then daily for 14 days. When this was conducted, any kind of toxic signs were not noted in clinical parameters during acute toxicity study even at the highest dose of 2000mg/kg. Hence, it can be concluded that the LD_{50} of the ethanolic extract of dried Leaves of *Annona squamosa* is greater than 2000 mg/kg.

Experimental Design: Grouping of Animals: Animals were divided into seven groups, each of six animals.

Group I: Control group oral administered by Distilled water (20 ml/kg).

Group II: Intraperitoneal injection by scopolamine hydrochloride (0.5 mg/kg) (Disease control).

Group III: Animal oral administered by donepezil tablet standard (2.5 mg/kg) and Alzheimer's induced with scopolamine (Standard).

Group IV: Animal's oral is administered by extract dissolved in NS (100 mg/kg) and Alzheimer's induced with scopolamine (Low dose).

Group V: Animal's oral is administered by extract dissolved in NS (200 mg/kg) and Alzheimer's induced with scopolamine (Intermediate dose).

Group VI: Animal's oral administered by extract which is dissolved in NS (300 mg/kg) and Alzheimer's induced with scopolamine (High dose).

Group VII: Animal's oral is administered by extract dissolved in NS (300 mg/kg) (Extract only).

Anti-Alzheimer Study:

Morris Water Maze: The apparatus consists of a circular pool (45 cm in height and 100 cm in diameter) with a featureless inner surface. The pool was filled with opaque water (maintained at 22 ± 2 °C) to a height of 30 cm and divided into four quadrants of equal area marked by different visual cues. There was a platform (29 cm × 6 cm) placed one centimeter below the level of water at the center of one of the four quadrants (the target quadrant). The position of the platform was unaltered throughout the duration of the experiment. The test was commenced on day 10^{th} of the treatment period, where the rats were allowed to swim for 120 s without the platform to acclimatize. During the next four consecutive days, each animal had received four learning trials of 120 s with an intertrial interval of 60s. For each learning trial, the rat was placed in the water facing the pool wall diagonally opposite to the quadrant in which the platform was kept. The time taken by the animal to locate the submerged platform was recorded as the escape latency time for each trial. The animal was unable to locate the platform within 120 s, it was directed to the platform and allowed to rest there for 60 s, and in this case, the escape latency time was recorded as 120 s. These sessions were recorded as the hidden platform trials or acquisition tests. On day 15 (24 h after the final learning trial), the platform was removed from the pool, and the rats were subjected to a probe trial session to assess memory retention. Each rat was placed into the water diagonally opposite the target quadrant and for 60s were allowed to swim and find the quadrant in which the platform was previously placed. The time spent by the animal in the target quadrant was recorded [11].

Novel Object Recognition Test: The procedure consists of three different phases: a habituation phase, an acquisition phase, and a retention phase. Initial day (habituation Phase) rats were individually subjected to an adaptation session of 10 min, during which they were introduced in the unfilled area to become familiar with the apparatus. On the 2^{nd} day (acquisition phase), animals were subjected to a 10-min session, during which floor-fixed two objects (A and B) were placed in a symmetric situation in the central line of the area. The two objects, prepared by the same material with a similar color and smell, were different in shape but identical in size. Rats were allowed to explore the objects in the open field. The exploration time on each object was shown (as seconds) to indicate the exploring activity of rats. On the 3rd day (retention phase), rats were allowed to explore the open field in the presence of two objects: the recognizable object A and a novel object C in different shapes but in similar color and size (A and C). A recognition index (for retention session), calculated for each rat, was expressed as the ratio recognition index (RI) = time exploring novel object t / (Time exploring novel object + time exploring familiar object) × 100 [12].

Y Maze Test: Y maze consist of 3 arms, each arm was 40 cm long, 12 cm height and 3 cm wide at the

base and 10 cm wide at the top and converged in an equilateral triangular central area. Each rat was placed at the end of one arm and allowed to travel freely through the maze during an 8 min session. The capability to alternate requires that the rat know which arm they have already visited. The sequences of arm entries, including possible returns into the same arm, were recorded visually. Instant working memory performance was assessed by recording spontaneous alternation behavior.

Entry was considered to be completed when the rat's hind paws had completely entered the arm. Alternation was defined as successive entries into the three different arms (A, B, and C) on overlapping triplet sets. % Alteration was calculated by the formula % Alteration = (Number of arm alteration/total arm entry-2) *100 [13].

Biochemical Test:

Preparation of Brain Sample: After assessing the learning and memory paradigms in scopolamine-induced amnesia, rats from each group were euthanized by using a carbon dioxide chamber; brains were removed quickly and placed in ice-cold saline.

The frontal cortex, hippocampus, and septum (any other region of interest) were quickly dissected out on a petri dish chilled on ice crushed (this part of the brain cannot be identified in a small rat brain; therefore, the whole brain was taken). The tissues were weighed and homogenized in 0.1M Phosphate buffer (pH 8). The samples of rat brain homogenates were collected in different test tubes and tested for acetylcholine esterase, catalase, nitric oxide, MDA, *etc*. The supernatant was used for enzymatic assays.

Estimation of Acetyl Cholinesterase Enzyme Levels in the Brain: 0.4ml aliquot of the homogenate was added to a cuvette containing 2.6 ml phosphate buffer (0.1M, pH 8) and 100µl of DTNB. The content of the cuvette was mixed thoroughly by bubbling air, and absorbance was measured at 412 nm in a spectrophotometer. 20µl of substrate, *i.e.*, acetyl thiocholine, was added, and a change in absorbance was recorded. Change in the absorbance per minute was thus determined [14].

Determination of Catalase (Cat) Activity: 0.1 ml of supernatant was added to the cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). The reaction was started by adding 1.0ml of newly prepared 30 mM H_2O_2. The rates of decomposition of H_2O_2 were measured spectrophotometrically from changes in absorbance at 240 nm. The activity of catalase was expressed as units/mg protein. The absorbance was measured at wave length 240 nm[15].

Determination of Nitric Oxide (No): The production of nitric oxide was estimated by the accumulation of nitrate in the supernatant, which was determined by a colorimetric assay with the Griess reagent (1:1 solution of 1% sulfanilamide in 5% H3PO4 and 1% naphthylamine diamine dihydrochloric acid in water).

An equal volume of the supernatant and the Griess reagent was mixed, and this mixture was incubated for 10 min in the dark at room temperature. The absorbance was taken at 540 nm using a spectrophotometer. The nitrite concentration in the supernatant was estimated from a sodium nitrite standard curve [16].

Determination of Malonyldialdehyde (Mda): Suspension mediums were taken from the tissue homogenate in a tube. 0.5 ml. of Trichloroacetic acid (TCA) was added to it, followed by 0.5 ml. of 8% Thiobarbituric acid (TBA) reagent. The tubes were covered with aluminum foil and kept in the water bath for 30 min. at 80ºC.

After 30 minutes, the tubes were taken out and placed in the cold water for 30 minutes. These tubes were centrifuged for 15 min at 3000 rpm.

The absorbance of the supernatant was taken at 540 nm, at room temperature against the appropriate blank solution. MDA value was expressed as moles MDA/mg of protein [16].

Statistical Analysis: The data were analyzed using BioStat Pro (version 5.9.8). For each category, the results are expressed as mean ± SEM. A one-way variance analysis (ANOVA) was used to analyze statistical differences, followed by the Tukey-Kramer test.

Tests at $P \leq 0.05$. were considered statistically significant. *** indicated $p \leq 0.001$, ** indicates $p \leq 0.01$, * Indicates $p \leq 0.1$

RESULTS:

Qualitative Phytochemical Screening:

TABLE 1: RESULT OF PHYTOCHEMICAL ANALYSIS OF ETHANOLIC EXTRACT OF THE DRIED LEAVES OF *ANNONA SQUAMOSA*

S. no.	Phytochemicals	Tests performed	Inference	Alcoholic extract of the dried leaves of *Annona squamosa*
1	Carbohydrates	Molisch test	Violet ring at the junction	+
		Benedict test	Red ppt	+
		Selwinoff test	Red colour	+
2	Flavonoids	Sodium hydroxide (NaOH) test	Yellow ppt	+
		Shinoda test	Pink to red solution	+
		Alkaline reagent test	Colourless	+
3	Glycosides	Modified Borntrager's test	Pink to red colour	+
4	Tannins	Ferric chloride test	Deep black colour	+
		dil. Iodine solution test	Transient red colour	+
5	Saponins	Foam test	Persistent foam	-
6	Fixed oils	Sodium hydroxide solution test	Clear blue solution	+
7	Alkaloids	Dragendroff reagent test	Reddish brown ppt	+
		Mayer's reagent test	Buff coloured ppt	+
		Hager's reagent test	Yellow ppt	+
8	Phenols	dil. Potassium permanganate test	Decolouration of $KMnO_4$ solution	+
9	Sterols	Liebermann Burchard test	Green upper layer	+
		Salkowski test	Red lower layer	+
10	Terpenoids	Liebermann Burchard test	Red upper layer	+
11	Proteins	Biuret test	Violet/ pink colour	+

Present (+) / Absent (-)

Acute Oral Toxicity Studies: Acute oral toxicity analysis was carried out for the ethanolic extract of dried Leaves of *Annona squamosa* as recommended in OECD Guideline 423. The animals were noted keenly for displaying any toxic signs or symptoms at different time intervals of 0, 30 min, 1, 2, 4, 6, 8, 12 h and then daily for a period of 14 days.

When this was conducted, any toxic signs were not noted in clinical parameters during acute toxicity study even at the highest dose of 2000mg/kg. Hence, it can be concluded that the LD_{50} of the ethanolic extract of dried Leaves of *Annona squamosa* is greater than 2000 mg/kg.

Anti-Alzheimer Study:

Morris Water Maze: During the acquisition sessions (days 11–14) scopolamine treatment resulted in significantly longer escape latencies when compared to the controls; this effect was mitigated by co-administration of the test formulation.

Significant decreases in escape latencies were observed on days 2–4 (treatment days 12–14) compared to day 1 (treatment day 11) of the acquisition sessions in the control group but not in the scopolamine-treated rats. These differences were restored in rats co-administered with scopolamine and the test formulation.

TABLE 2: EFFECT OF ETHANOLIC EXTRACT OF *ANNONA SQUAMOSA* LEAVES ON ESCAPE LATENCY TIME IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Day / Group	Escape Latency Time (Seconds)			
	Day 11	Day 12	Day 13	Day 14
Control	86.458±4.0412	77.208±0.6272	56.625±0.8915	27.3333±0.9212
Disease Control	99.5±2.698$^{\#}$	85.375±0.9827$^{\#}$	65.375±0.826$^{\#}$	37.9583±1.0595$^{\#}$
Standard Drug	81.083±3.2357***	55±0.7528***	27.9167±0.7207***	15.2083±1.2373***
Low Dose (100 Mg/Kg)	91.417±0.9302***	74.542±1.0255***	57.25±1.3229***	17.458±17.458***
Medium Dose (200 Mg/Kg)	92.708±0.9946***	69.75±1.3979***	59±1.2162***	15.958±0.7512***
High Dose (300 Mg/Kg)	92.625±0.7548***	69.75±0.6325***	37.875±1.1542***	18.958±0.5531***
Extract Only (300 Mg/Kg)	78.083±2.1052***	55.083±2.045***	35.625±2.1878***	21.292±0.435***

In the probe trial session on day 15, scopolamine-treated rats spent significantly less time swimming in the target quadrant when compared to the controls, whereas the time spent by the rats co-administered with the test formulation and scopolamine in the target quadrant was more than the disease controls.

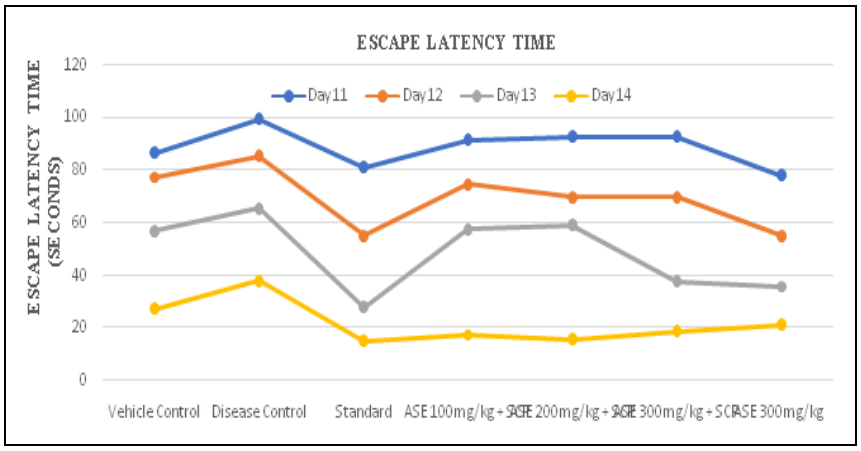

FIG. 1: EFFECT OF ETHANOLIC EXTRACT OF *ANNONA SQUAMOSA* LEAVES ON ESCAPE LATENCY TIME IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RAT

All the four-test groups of ethanolic extract of leaves *Annona squamosa* showed a dose-dependent increase in Escape latency time when compared against the negative control group (scopolamine).

TABLE 3: EFFECT OF ETHANOLIC EXTRACT OF *ANNONA SQUAMOSA* LEAVES ON TIME SPENT IN TARGET QUADRANT IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Groups	Time Spent In Target Quadrant (Seconds)
Control	12.833±0.600
Disease Control	7.166±0.749
Standard Drug	22±1.211
Low Dose (100 Mg/Kg)	14±0.577
Medium Dose (200 Mg/Kg)	15.167±0.792
High Dose (300 Mg/Kg)	18.5±0.341
Extract Only (300 Mg/Kg)	20.333±0.494

Values are the mean ± SEM of n=6 rats/treatment Significance ***p ≤0.001.

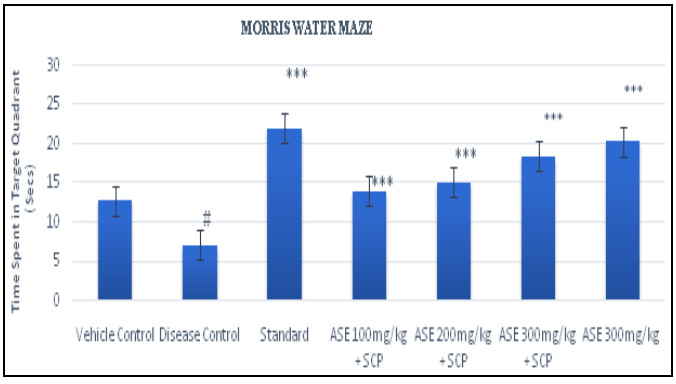

FIG. 2: EFFECT OF ETHANOLIC EXTRACT OF *ANNONA SQUAMOSA* LEAVES ON TIME SPENT IN TARGET QUADRANT IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RAT

Novel Object Recognition Test: All the four-test groups of ethanolic extract of leaves Annona squamosa showed a dose-dependent increase in recognition index when compared against the negative control group (scopolamine).

TABLE 4: EFFECT OF ETHANOLIC EXTRACT OF *ANNONA SQUAMOSA* LEAVES ON RECOGNITION INDEX IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Groups	Recognition Index
Control	55.68±1.062
Disease Control	42.16±1.306
Standard Drug	60.75±1.272
Low Dose (100 Mg/Kg)	56.027±1.064
Medium Dose (200 Mg/Kg)	57.18±1.281
High Dose (300 Mg/Kg)	59.13±2.259
Extract Only (300 Mg/Kg)	59.83±1.873

Values are the mean ± SEM of n=6 rats/treatment Significance ***p ≤0.001.

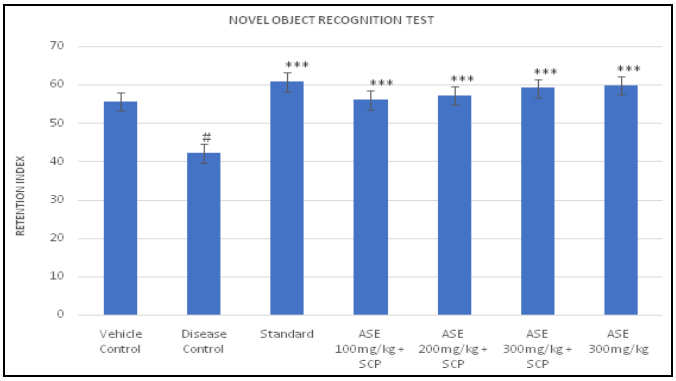

FIG. 3: EFFECT OF ETHANOLIC EXTRACT OF *ANNONA SQUAMOSA* LEAVES ON RECOGNITION INDEX IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RAT

Y-Maze: All the four-test groups of ethanolic extract of leaves *Annona squamosa* showed dose-dependent increase in percentage alteration when compare against the negative control group (scopolamine).

TABLE 5: EFFECT OF ETHANOLIC EXTRACT OF *ANNONA SQUAMOSA* LEAVES ON % ALTERATION IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Groups	Percentage Alteration
Control	54.29±0.803
Disease Control	32.28±0.609
Standard Drug	57.21±0.542
Low Dose (100 Mg/Kg)	45.51±0.162
Medium Dose (200 Mg/Kg)	47.96±0.473
High Dose (300 Mg/Kg)	53.22±0.741
Extract Only (300 Mg/Kg)	55.83±0.822

Values are the mean ± SEM of n=6 rats/treatment Significance ***p ≤0.001.

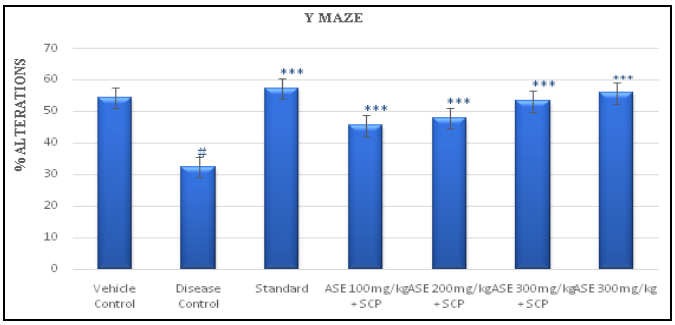

FIG. 4: EFFECT OF ETHANOLIC EXTRACT OF *ANNONA SQUAMOSA* LEAVES ON % ALTERATION IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RAT

Biochemical Test:

Estimation of Acetyl Cholinesterase Enzyme Levels in the Brain: All the four test doses of the ethanolic extract of leaves of *Annona squamosa* showed dose-dependent decrease in acetyl cholinesterase level when it was compared against negative control group (scopolamine).

TABLE 6: EFFECT OF ETHANOLIC EXTRACT OF *ANNONA SQUAMOSA* LEAVES ON ACETYLCHOLINE ESTERASE LEVEL IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Groups	Enzyme Level
Control	0.081±0.0003
Disease Control	0.092±0.0003
Standard Drug	0.071±0.0003
Low Dose (100 Mg/Kg)	0.079±0.0014
Medium Dose (200 Mg/Kg)	0.078±0.0004
High Dose (300 Mg/Kg)	0.075±0.0003
Extract Only (300 Mg/Kg)	0.074±0.0004

Values are the mean ± SEM of n=6 rats/treatment Significance ***p ≤0.001.

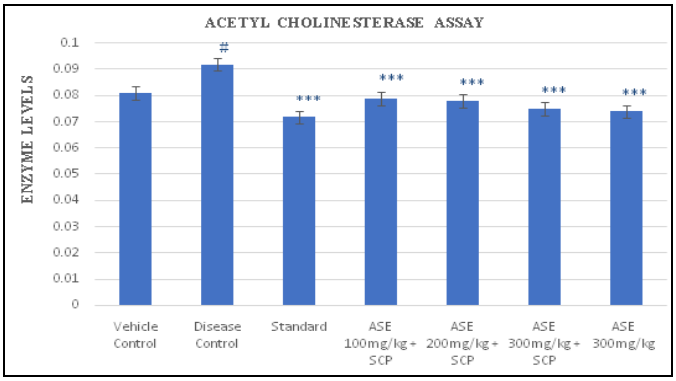

FIG. 5: EFFECT OF ETHANOLIC EXTRACT OF *ANNONA SQUAMOSA* LEAVES ON ACETYLCHOLINE ESTERASE IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RAT

Determination of Catalase (Cat) Activity: All the four test doses of the ethanolic extract of leaves of *Annona squamosa* showed a dose-dependent increase in catalase activity when it was compared against the negative control group (scopolamine).

TABLE 7: EFFECT OF ETHANOLIC EXTRACT OF *ANNONA SQUAMOSA* LEAVES ON CATALASE ACTIVITY IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Groups	Catalase Level
Control	34.67±0.120
Disease Control	27.38±0.116
Standard Drug	39.39±0.154
Low Dose (100 Mg/Kg)	33.78±0.178
Medium Dose (200 Mg/Kg)	35.69±0.135
High Dose (300 Mg/Kg)	37.55±0.073
Extract Only (300 Mg/Kg)	38.13±0.199

Values are the mean ± SEM of n=6 rats/treatment Significance ***p ≤0.001.

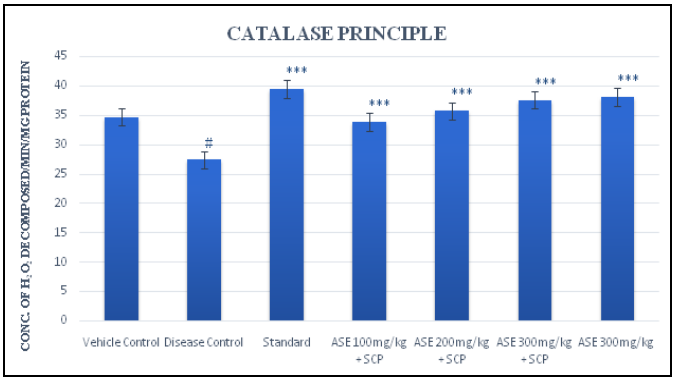

FIG. 6: EFFECT OF ETHANOLIC EXTRACT OF *ANNONA SQUAMOSA* LEAVES ON CATALASE LEVELS IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RAT

Determination of Nitric Oxide (No): All the four test doses of the ethanolic extract of leaves of *Annona squamosa* showed dose dependent increase

in nitric oxide activity when it was compared against negative control group (scopolamine).

TABLE 8: EFFECT OF ETHANOLIC EXTRACT OF *ANNONA SQUAMOSA* LEAVES ON NITRIC OXIDE ACTIVITY IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Groups	No Level
Control	7.06±0.021
Disease Control	20.0475±0.243
Standard Drug	9.14±0.104
Low Dose (100 Mg/Kg)	14.78±0.242
Medium Dose (200 Mg/Kg)	13.25±0.141
High Dose (300 Mg/Kg)	11.92±0.215
Extract Only (300 Mg/Kg)	10.39±0.088

Values are the mean ± SEM of n=6 rats/treatment Significance ***p ≤0.001.

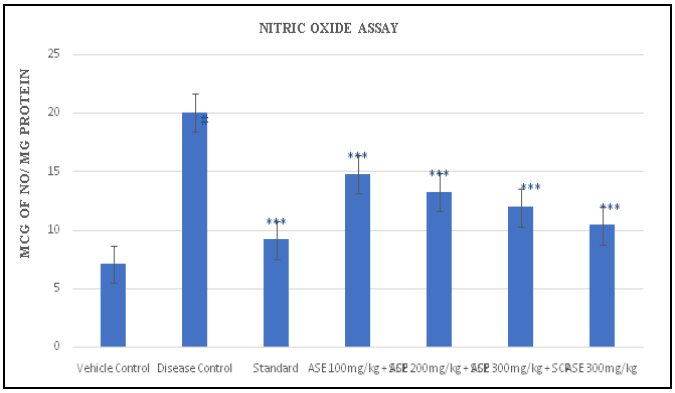

FIG. 7: EFFECT OF ETHANOLIC EXTRACT OF *ANNONA SQUAMOSA* LEAVES ON GLUTATHIONE PEROXIDES LEVEL IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Determination of Malonyldialdehyde (Mda): All the four test doses of the ethanolic extract of leaves of Annona squamosa showed a dose-dependent decrease in MDA level activity when it was compared against negative control group (scopolamine).

TABLE 9: EFFECT OF ETHANOLIC EXTRACT OF *ANNONA SQUAMOSA* LEAVES ON MDA LEVEL IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Groups	MDA Level
Control	0.025±0.001
Disease Control	0.030±0.0003
Standard Drug	0.0103±0.0004
Low Dose (100 Mg/Kg)	0.0205±0.0004
Medium Dose (200 Mg/Kg)	0.0195±0.0004
High Dose (300 Mg/Kg)	0.0168±0.0004
Extract Only (300 Mg/Kg)	0.0139±0.0004

Values are the mean ± SEM of n=6 rats/treatment Significance ***p ≤0.001.

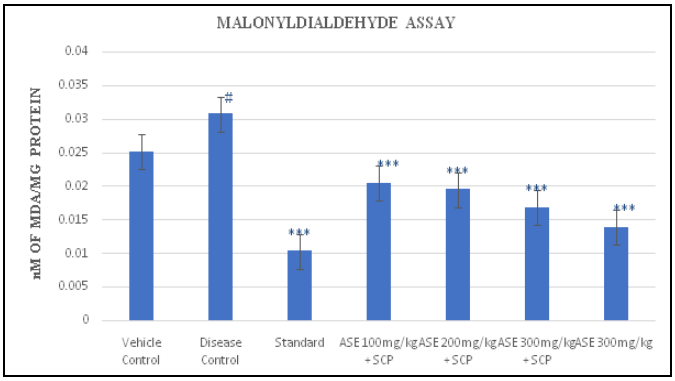

FIG. 8: EFFECT OF ETHANOLIC EXTRACT OF *ANNONA SQUAMOSA* LEAVES ON MDA LEVEL IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

DISCUSSION: Alzheimer's disease is a neurodegenerative disorder associated with a decline in cognitive abilities. Patients also frequently have non-cognitive symptoms, such as depression, apathy and psychosis that impair daily living, Alzheimer's disease can occur at any age, even as young as 40 years. It is mainly caused by beta-amyloid plaques, which are the dense deposit of protein and cellular material that accumulate outside and around nerve cells. Neurofibrillary tangles are the twisted fiber that builds up inside the nerve cell.

Annona squamosa tested for anti-Alzheimer model in Morris water maze test it showed decrease in Escape latency time and increase in time spent in target quadrant, novel object recognition test it showed increase in recognition index and Y maze showed increase in % alteration. All of it indicates improve in the spatial memory of rats.

Direct analysis of neurotransmitter content in the cerebral cortex of AD rats pretreated with ethanolic extract of *Annona squamosa* leaves shows a reduction in Ach level, an increase in catalase activity has been observed, and a decrease in the levels of the lipid peroxidation product Malonyldialdehyde (MDA) have been observed. Oxidative stress has been described in AD brains before the accumulation of Aβ and phosphorylated tau due to increasing levels of reactive oxygen and nitrogen species, respectively. The AD rats pretreated with ethanolic extract of *Annona squamosa* leaves showed a significant decrease in nitrite levels when compared against the negative control group.

Reactive oxygen species are responsible for the age-related degradation of cognitive performance, which ultimately may cause Alzheimer's disease in elderly persons *Annona squamosa* has been reported to possess antioxidant properties as well. The neuroprotective effect of ethanolic extract of *Annona squamosa* leaves may be due to its antioxidant property; hence, the susceptible brain cells are exposed to lesser oxidative stress, thereby reducing brain damage. The symptoms of dementia are associated with impaired neurotransmission in the affected brain regions.

CONCLUSION: The findings of the current study indicate that ethanolic extract of *Annona squamosa leaves* possess anti-Alzheimer activity by improving spatial memory and also showed a significant increase in the onset of action and decrease in duration of action and recovery of time as compared to the negative control, thus justifying its anti-Alzheimer activity which may be due to presence of flavonoid, alkaloid, phenol, triterpenoid and glycoside, as a phytoconstituent present. Additional studies are suggested to classify the active phytochemicals and illustrate the mode of action.

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CONFLICT OF INTEREST: The authors have no conflicts of interest regarding this investigation.

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