



Evaluation of Solanum- xanthocarpum as anti- alzheimers in obesity induced rodent model

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Abstract: Alzheimer's disease is a neurological illness that primarily affects the elderly where gradual neuronal loss is observed. The hippocampal region of the brain is home to senile plaques, which contain deposits of beta-amyloid protein, which is linked to the most prevalent type of dementia. The use of herbs has greatly improved global health. Despite the significant advancements in modern medicine over the past few years, plants continue to play a significant role in healthcare. The goal of the current investigation is to assess *Solanum xanthocarpum* (SX) in vivo using mice that have been given AD. The current study uses both in vitro and in vivo methods to examine the anti-Alzheimer's activity of the *solanum xanthocarpum* ethyl acetate extract (EASX). Runway avoidance and the elevated plus maze experiments were used in in vivo investigations after inducing obesity, whereas modified Ellman's method was used in in vitro investigations. I.e. The increased lipid levels were decreased, and the rodent models likewise had positive outcomes. A histological analysis verified the recovery. Efficacy towards AD has been demonstrated by the plant extract (200 and 400 mg/kg).

From the above results it is evident that ethyl acetate extract of *solanum xanthocarpum* possess obesity induced anti-alzheimer's activity.

Keywords: *Solanum xanthocarpum*, Runway avoidance, Elevated plus maze, Ellman's assay, acetylcholinesterase, donepezil, imipramine.



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INTRODUCTION

Alzheimer's disease (AD) is a neurological illness that is typified by cognitive inefficiency, behavior degradation, diminished memory, as well as cognitive difficulties ^[1]. Nucleic acid, protein, and mitochondria in the brain sustain damage from oxidation that leads to a buildup of malfunctioning associated with cognition and the nervous system ^[2]. Brains of individuals with Alzheimer's Disease exhibit 2 separate characteristics: i) Extracellular deposits of amyloid-beta ($A\beta$), a peptide produced by breaking down $A\beta$ precursors, are found in senile plaques. $A\beta$ depositions can also be aberrant in blood vessels. ii) Alzheimer's patients have neurofibril tangles, which are dense clumps of aberrant fibers in the cytoplasm of neurons made of a modified version of the microtubular-associated protein. ^[3-6] AD is responsible for most dementia cases reported worldwide and is marked by the loss of memory and aggregation of Amyloid beta peptide (ABP) and Neurofibrillary tangle (NFT) ^[7]. Broad range of treatment approaches aimed at correcting presynaptic cholinergic function loss have been developed ^[8].

Patients who are obese frequently have low-grade systemic inflammation in adipose tissue at basal levels and are more

vulnerable to immune-related illnesses. ^[9-10] Feeding-induced free fatty acids (FFA) set off a chain of inflammatory reactions that is triggered by stimulation of Toll-like receptor 4. This results in the release of cytokines that promote inflammation, including TNF- α and interleukins IL-1 β and IL-6. ^[11-13] Elevated FFA concentrations have the potential to further impede insulin's anti-lipolytic effect, accelerating the release of FFA into the bloodstream. ^[14] When FFA binds to BBB endothelial cells, it changes their permeation, which permits FFA to enter the brain. ^[15] Therefore, harmful processes such as ceramide synthesis, identification of patterns receptor activation, inflammation, and endogenous retinoic stress are triggered by an elevation in cerebral FFA. ^[16-17] It results from the activation of microglia by the brain upon detection of a disturbance in homeostasis. ^[18] Research findings suggest that lipidemic imbalance in metabolic illnesses may cause the blood-brain barrier to become permeable to FFAs, which in turn can kick a series of processes that ultimately result in inflammation of neurons and glial cell activation. Simultaneously, elevated AGE levels in hyperglycemia may exacerbate brain pathology that leads to AD and RAGE-mediated breakdown of BBB integrity. ^[19-22]

Research findings suggest that lipidemic imbalance in metabolic illnesses may cause the blood-brain barrier to become permeable to FFAs, which in turn can kick off a series of processes that ultimately result in inflammation of neurons and glial cell activation. Simultaneously, elevated AGE levels in high blood sugar levels may exacerbate brain pathology that leads to AD and RAGE-mediated breakdown of Blood Brain Barrier integrity. [23].

Solanum xanthocarpum commonly known as yellow fruit nightshade(kantakari) is a prickly diffuse, bright green perennial herb, woody at the base, 2–3 m height, found throughout India, mostly in dry places as a weed along roadsides and waste lands [24]. Fruits are consumable, because the locals use them as conventional medications to alleviate inflammation issues including infections of the throat. Fruit juice is useful in sore throats and rheumatism [25]. Orissa, India's nomadic and tribal communities cure diabetes with a drink made from the plant's fruits. The fruits are eaten as an anthelmintic and for indigestion [26]. Aim of the investigation is to evaluate anti- Alzheimer's activity of ethyl acetate extract of *solanum xanthocarpum* on obesity induced animals using runway avoidance and elevated plus maze models in rodents.

Materials and methods

Collection of *Solanaum xanthocarpum* fruit

SX fruits were collected from various fields, the material's was identified and authenticated by professor and head of the department of botany, government degree college, Hyderabad. Fruits were cleaned shade dried, coarsely powdered and stored up for extraction process.

Preparation of plant extract

Soxhlet extraction is a comprehensive extraction technique usually employed with compounds that are adequately stable under thermal conditions. The sample is extracted in the hot solvent by gradually boiling and condensing the extraction solvent via the matrix. The procedure is non-selective aside from the solvent system selection, therefore further hygiene and concentration are frequently required [27].

Phytochemical analysis

Phytochemical screening was done in order to screen the presence of secondary metabolites in ethyl acetate extract of *Solanum xanthocarpum* [28].

GC-MS studies

It's having internal diameter of 0.25mm, thickness of 0.25µm, He at a same flow 1mL/min is used as a carrier. Injector and temperature of ion source was 250°C and 280.0°C. Oven temperature was programmed from 110°C with an increase in 10°C/min, to 200°C, then 5°C/min to 280°C, ends with in 9 min isothermal at 280°C and total GC running time was 41 min.

Mass Spectrometer: Ionization energy of 70 eV for the positive electron ionization mode.

Solvent delay: 0-3.00 min

Scan interval: 0.50 sec

Source temperature: 280°C and 250°C

Acute toxicity studies

This investigation aimed to determine whether the extract was safe or unsafe to animals. The OECD specifications are followed in

carrying it out. To screen for severe oral toxicity, one executes an up-and-down process (OECD guideline - 425) [29].

Screening models for anti-alzheimer's

In vitro model

Evaluation of acetylcholinesterase enzyme (AChE) inhibitory activity using micro well plate assay.

This method is also called as modified Ellman's method. It is of 2 types "qualitative and quantitative"

Qualitative Method

"β-naphthyl acetate" is a substrate which is hydrolyzed to naphthol and acetate by the enzyme. The fast blue B reacts with naphthol.

Take new microwell plate to each plate add

- 10µL of plant extract.
- 50µL (0.250 microgram/milli liter) of beta naphthyl acetate dissolved inCH₃OH.
- 200µL acetylcholinesterase enzyme (03.33µl/milliliter) was added.

Above mixture were incubated at 4°C for forty minutes. Later on, 10µl (2.50 microgram/milliliter) fast blue B dissolved in H₂O, was combined with solution. Stable purple color change was exhibited finally. Visual estimation of the intensity of color allows for the qualitative detection of AChE blockage. to evaluate qualitative inhibition of AChE by plant extract scoring is given from 0-3.

SCALE:

- If a dark purple color is observed score of 0 is given it is determined that EAESX possess no activity.
- If the color of mixture is purple then it is determined that EAESX processes mild inhibition activity
- when solution color light purple score is "2" Moderate inhibition activity is determined.
- if no change in mixture is observed score of "3" is given which shows Strong inhibition activity. (Positive control).

Quantitative Analysis:

- 10µl plant extract.
- 50µl (0.250microgram/milliliter) of β-naphthyl acetate dissolved in CH₃OH.
- 200µl of Acetylcholinesterase (3.33µl/ml) was combined.

Above solution was incubated at 4°C until 40min. After, 10µl (02.5) fast blue b combined in H₂O was combined to the mixture and the absorbance were measured at 600nm. The absorbance that was measured before incubation was deducted from the absorbance measured after the introduction of pigment in order to correct for the inaccuracy in measuring the absorbance caused by the tint of the herbal extracts [30].

$$\% \text{ inhibition} = (A \text{ sample} / A \text{ control}) \times 100$$

In-vivo screening models for anti-alzheimer's

Runway avoidance

For this activity, female albino rats are utilized. An overhead light source illuminates the device uniformly. To offer sound conditioned stimuli, a speaker situated 50.0 centimeter above the starting point of box is used. Animal is given five minutes to investigate the entire device. Rat is then kept at the light-starting section once the guillotine door has been closed. Conditioned stimuli is kept on after 10 seconds, and the door is opened at the same time. 5 seconds later, shock is activated. CS keeps going till the animal gets to the safe spot. The procedure is repeated after one

minute, and training continues until the animals meet the requirement 9 avoidances out of 10 trials. The process is repeated

until the same learning objective is met on the next day. It is measured how long it takes to get to the safe region ^[31].

Table 1: Treatment schedule for Runway avoidance

S. No	Groups	Treatment	No. of animals
1.	Normal	H ₂ O/saline	6
2.	Disease control	H ₂ O/saline	6
3.	EAESX 200mg/kg	HFHCC diet + EAESX 200mg/kg	6
4.	EAESX 400mg/kg	HFHCC diet + EAESX 400mg/kg	6
5.	Donepezil	HFHCC diet + Donepezil (10 mg/kg <i>p.o</i>)	6

Elevated Plus Maze:

Swiss albino mice (20-25g) are used. Six animals each make up each of the five groups into which the animals are separated. 2 open arms (30 cm 15 cm) and 2 closed arms (35 cm 5 cm 15 cm) made of wood are connected by an uncovered middle base which is positioned 40 cm above floor height. The outermost portion of the wide arm was enclosed by an elevated platform that was three millimeters high and one millimeter thickness. The three week induction of obesity. Mice were given a vehicle or drug injection for a period of two weeks prior to the test, and on the actual day of the test, the animal was put in the equipment' center, exposing an open arm, and given five minutes for exploring the equipment. All four paws must be inside the arm to qualify as an arm entrance. % of both open and closed arm entries, as well as % amount of time spent in each arm, were recorded at the culmination of the procedure ^[32].

Table 2: Treatment schedule for Elevated plus maze

S. No	Groups	Treatment	No. of animals
1.	Normal	H ₂ O/saline	6
2.	Disease control	H ₂ O/saline	6
3.	EAESX(T ₁)	HFHCC diet + EAESX 200mg/kg	6
4.	EAESX(T ₂)	HFHCC diet + EAESX 400mg/kg	6
5.	Standard control	HFHCC diet + Donepezil (10 mg/kg <i>p.o</i>)	6

Results

Ethyl acetate extract of solanum xanthocarpum was explored for its anti-alzheimers activity. All the results in the study are included below

Soxhlation is the method used to extract the solanum xanthocarpum fruits and the formula that is stated below is utilised to find yield

$$\% \text{ yield} = \frac{\text{Weight of crude extract (g)} \times 100}{\text{Weight of plant material (g)}}$$

$$= 18 / 180 \times 100.0 = 10 \% \text{ w/w.}$$

Quantity of EAESX collected at end is 10 % w/w.

Preliminary phytochemical screening

Preliminary phytochemical analysis indicates presence of alkaloids, flavonoids, glycosides, terpinoids, sterols, steroids, phenols, absence of coumarins, carbohydrates, saponins, lignins, tannins.

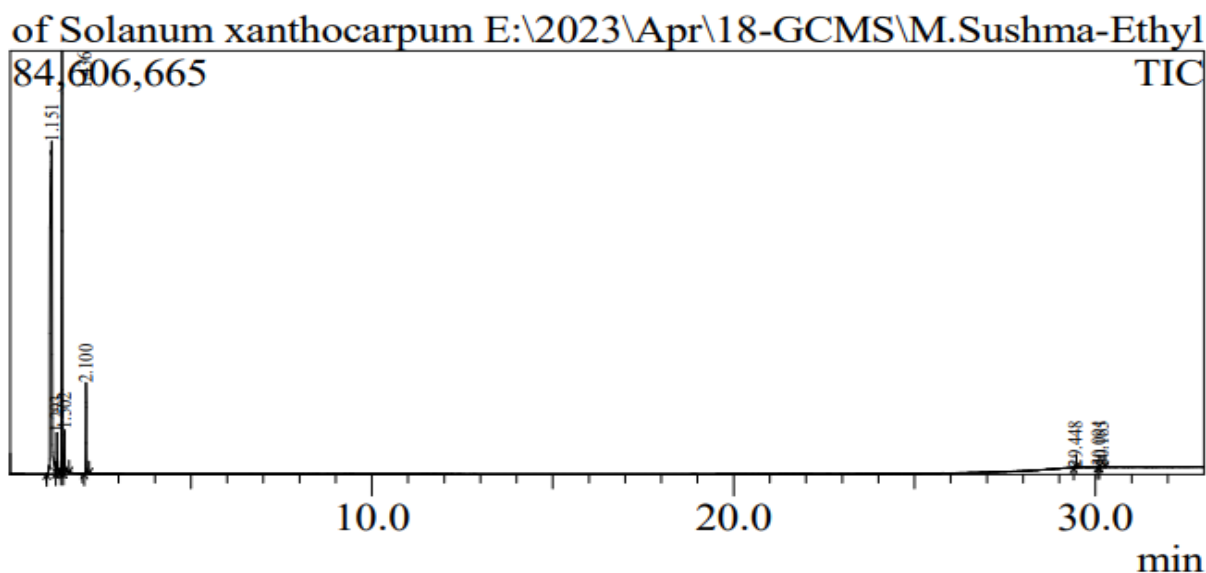
Table 6: List of phytoconstituent's identified from GC-MS

Sl.no	Compound	Molecular weight	Peak area
1	Methylsulfidtiole	82.1 g/mol	61.5
2	1,3- pentanidiole	104.1 g/mol	2.9
3	2- ethoxyethylamine	89.1 g/mol	27.9
4	1-propanol	60.09 g/mol	3.01
5	d-mannitol	182.1 g/mol	0.05
6	gamma-sitosterol	414.7 g/mol	0.05
7	Pentasiloxane	354.77 g/mol	0.06

GC-MS of ethyl acetate extract of solanum xanthocarpum

Sample Information

Analyzed by : B. Sammayya
 Analyzed : 4/18/2023 4:47:47 PM
 Sample Name : M.Sushma-Ethyl Acetate extract of Solanum xanthocarpum
 Injection Volume : 1.00
 Data File : E:\2023\Apr\18-GCMS\M.Sushma-Ethyl Acetate extract of Solanum xanthocarpum.QGD
 Method File : C:\GCMSsolution\Data\Project1\GC_MS Fatty acid.qgm
 Instrument Model : GCMSQP2010, SHIMADZU



Acute toxicity studies

2000mg/kg was found to be safe after performing acute toxicity studies. 1/10th and 1/20th i.e 200 and 400 mg/kg was used to perform the in-vivo models.

Evaluation of acetylcholinesterase (AChE) inhibitory activity using modified Ellman's method

Test extract at different concentration (10,20,30,40,50,60,70,80,90,100) were added to tissue suspension in micro well plate method. A score of 2 is given light purple color change was observed at 30µg/ml which indicate that extract has moderate acetylcholinesterase inhibition.

Inducing of obesity

Obesity was induced for 21 days by using HFHCC diet as mentioned above the animals had exhibited 40-50 raise in its body weight.

Increase in body weights of rats after 21 days of HFHCC

Table 8: Progressive Raise of body weight in rats

Groups	Body weight			
	0 th Day	7 th Day	14 th Day	21 th Day
Normal	201.5±2.06	201.5±1.9	201.8±1.9	202.6±1.6
Diseased control	203.66±2.48	231.5±2.5	257.8±2.1	287.3±1.5
EAESX 200 mg/kg	213.83±2.20	238.6±2.0	256±1.8	280±1.6
EAESX400mg/kg	207.16±3.0	233.33±2.9	250.3±2.3	278.8±2.0
Standard	210.5±2.15	229.5±2.09	247.5±1.9	270±1.6

Lipid profile after inducing obesity and during treatment

The lipid profile was studied using respective kits and equipment called semi-autoanalyzer and the results obtained are as shown below:

Table 9: Comparison of LDL levels at regular time intervals

	LDL		
Groups	0 th Day	7 th Day	14 th Day
Normal	50.45±1.79	49.06±1.54	48.7±1.426924
Diseased control	162.6±2.79	163.01±2.674788	161.55±1.51
EAESX (200mg/kg)	153.45±2.73	131.81±2.06	70.08±1.95
EAESX (400mg/kg)	157.6±2.46	114.94±1.90	59.57±2.36
Standard	162.73±2.29	120.6±1.68	78.27±1.007

LDL level at day 7 and 14 are compared with values at day 0, decrease in the LDL levels was observed.

Table 10: Comparison of TG levels at regular time intervals

	TRIGLYCERIDES		
Groups	0 th Day	7 th Day	14 th Day
Normal	97.58±2.44	97.5±2.65	97.63±1.82
Diseased control	222.51±3.52	225±3.530	228.66±4.08
EAESX (200mg/kg)	253±2.43	190.933±2.555	140.33±2.60
EAESX (400mg/kg)	249±1.82	183.51±2.354	133±2.34
Standard	252.3±2.498	172.66±2.259	129.38±1.06

Triglycerides level at day 7 and 14 are compared with values at day 0, decrease in the Triglycerides levels was observed.

Table 11: Comparison of HDL-D levels at regular time intervals

	HDL-D		
Groups	0 th Day	7 th Day	14 th Day
Normal	53.56±0.9	53±1.210739	53.4±1.166
Diseased control	28.45±1.0	27.95±1.14	27.95±1.14
EAESX (200mg/kg)	20.66±0.98	33.35±0.93	45.83±1.690
EAESX (400mg/kg)	27±1.19	38.05±1.179	51.31±0.80
Standard	21.73±0.8	40.95±0.90	51.28±1.22

HDL-D level at day 7 and 14 are compared with values at day 0, increase in the HDL levels was observed

Table12: Comparison of total cholesterol levels at regular time intervals

	TOTAL CHOLESTEROL		
Groups	0 th Day	7 th Day	14 th Day
Normal	32.85±0.75	31.8±0.4	32.16±1.6
Diseased control	238.16±2.83	240±2.7	240.31±2.2
EAESX (200mg/kg)	208.5±2.38	138.3±2.1	53.5±0.5
EAESX (400mg/kg)	216.5±2.31	130.6±1.28	50.8±0.4
Standard	193.66±1.59	120.3±7.161	43.6±1.1

Total cholesterol level at day 7 and 14 are compared with values at day 0, decrease in the TC levels was observed.

4.7 *in vivo* models for evaluation of anti-alzheimers activity

EAESX was investigated for its anti-alzheimers using models like:

1. Runway avoidance
2. Elevated plus maze

Runway avoidance**Table 13:** Effect of EAESX on runway avoidance test.

Sl. No	Groups	Conditioned avoidance
1	Normal	89±6.32
2	Diseased control	19 ± 5.9***
3	EAESX (200mg/kg)	72.77 ± 6.52* ^{Ans}
4	EAESX (400mg/kg)	76 ± 10.48*** ^{Ans}
5	Standard	79.6 ± 4.16*** ^A

The estimates are given as mean SEM (n=6). Dunnett's multiple comparison and one-way ANOVA were used to examine the results against Control * = 0.05, ** = 0.01, *** = 0.001, against diseased control A = 0.001, against standard ns = non-significant.

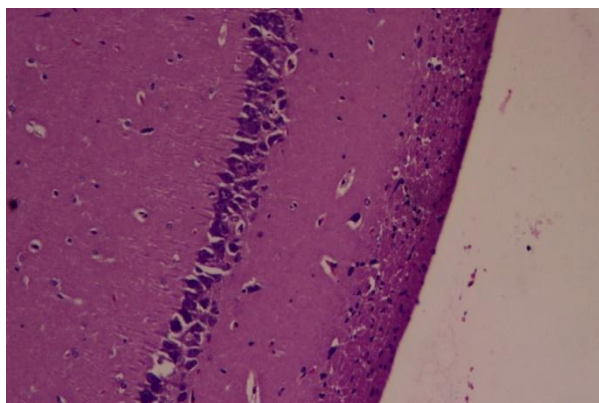
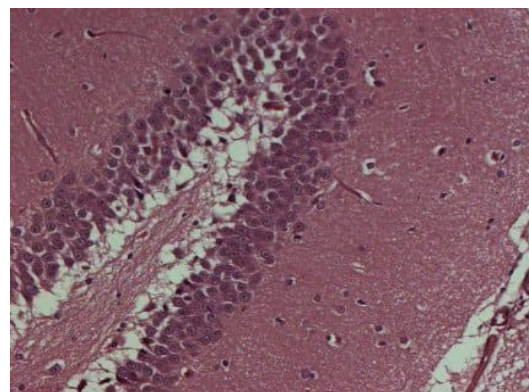
Elevated plus maze**Table 14:** Effect of EAESX on elevated plus maze

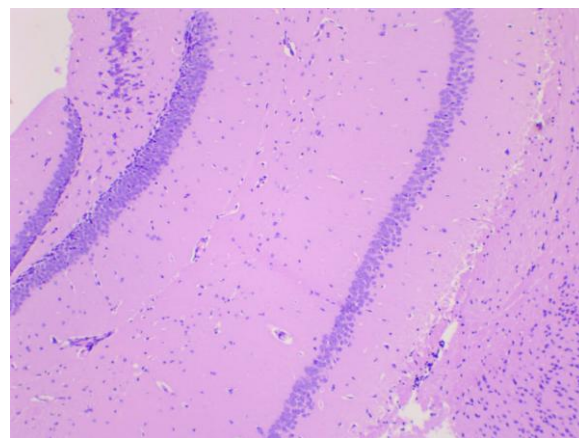
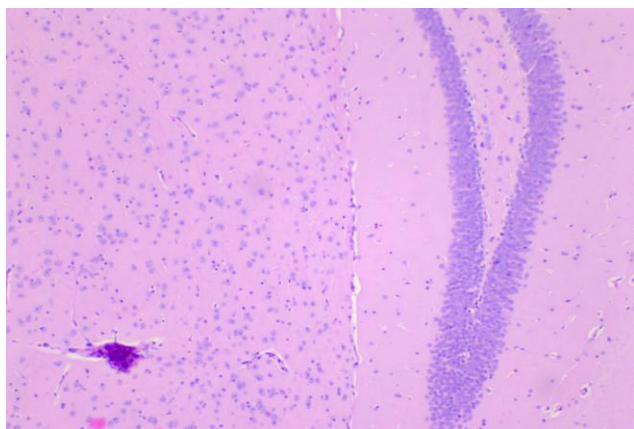
Groups	Time spent in arms (sec)		No. of entries	
	Open	Closed	Open	Closed
Normal	230.83±0.9	46.67±1.3	17.16±0.51	5.83±0.56
Diseased control	34±1.1***	229±1.2***	7±0.77***	16.73±0.78***
EAESX (200mg/kg)	237.66±1.7** ^{Ans}	32.76±0.9** ^{Ans}	17.66±0.66 ^{Ans}	4.67±0.1** ^{Ans}
EAESX (400mg/kg)	247.5±1.2** ^{Ans}	27.66±0.36* ^{Ans}	15.16±0.87*** ^A ns	5±0.3** ^{Ans}
Standard	255.66±1.9*** ^A	24.167±1.0* ^A	14±0.34*** ^A	3.86±0.26*** ^A

The estimates are given as mean SEM (n=6). Dunnett's multiple comparison and one-way ANOVA were used to examine the results against Control * = 0.05, ** = 0.01, *** = 0.001 against diseased control A = 0.001, against standard ns = nonsignificant.

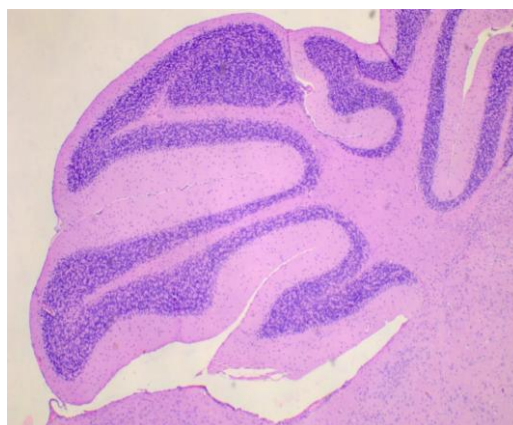
Histopathology studies

The animals used in elevated plus maze were sacrificed, brain samples of rats were isolated and are given for histopathology studies:

Normal control chow diet + H₂O**C. HFD + EAESX 200 mg/kg****B. Disease control HFD + H₂O****D. HFD + EAESX 400 mg/kg**



E. FFD + Donepezil



- A. In normal control animals' hippocampal region is normal which is central switching point for memory in brain.
- B. In disease control group the brain of animals showed hemorrhage, vacuolization and neurodegeneration is more when compared to other groups.
- C. Animals treated with 200 mg/kg has showed slight neurodegeneration in eosinophilic neurons.
- D. Animals treated with 400 mg/kg has showed normal hippocampal region no neurodegeneration was found but slight vacuolization was found.
- E. Normal hippocampal region is found no degeneration, hemorrhage or vacuolization is found.

DISCUSSION

The current study has investigated the effect of SX fruit extract on obesity induced neurodegeneration and the alteration of AChE activity in hippocampus. Obesity causes circulating free fatty acids to increase which causes major physiological or metabolic changes.

Obesity was induced to rats using high fat high collate cholesterol diet (HFHCCD). Raise in body weight and lipid levels confirmed induction of obesity. Lipid profile was also analysed by collecting blood samples at regular time intervals. Elevated fatty acids penetrate blood brain barrier and releases inflammatory cytokines that lead to neuronal inflammation it also plays a role in the amyloid beta neurotoxicity. Ones the obesity is induced using HFHCCD for 3weeks treatment schedule was started from 22nd day. Lipid profile was screened at regular time intervals it was found their levels to be high at 22nd day (i.e at the beginning of treatment schedule) but decline in lipid levels was observed after completion of treatment schedule.

In run way avoidance animals were not entering in the dark chamber and thus avoided the shock given in the dark chamber. SX extract was exhibiting dose dependent activity animals with

200mg/kg has showed 72.7 % avoidance, animals treated with 400mg/kg has shown 76 %avoidance whereas animals treated with standard drug donepezil has showed 79.6 % avoidance. In elevated plus maze test animals treated with200 mg/kg had spent 237.6 seconds in open arm 32.7 seconds in closed arm remaining time in neutral zone it has shown 17 entries into open arm and 4 entries into closed arm. Animals treated with 400 mg/kg had spent 247.5 sec in open arm 27.6 sec in closed arm it has shown 15 entries into open arm and 5 entries into closed arm. Animals treated with donepezil which is a marketed anti-depressant had spent 255.6 sec in open arm and 24.1 sec in closed arm it has shown 14 entries in to open arm and3 entries in to closed arm.

This study demonstrated that the memory enhancing effect of SX fruit extract might occur partly via the decreased oxidative stress and the enhanced cholinergic function. other concerning mechanisms might be suppression of monoamine oxidase (MAO) which gave rise to the enhanced dopaminergic function induced by SX fruit extract might also play the pivotal role in the cognitive enhancing effect of SX fruit extract. A growing body of research indicates that phytosterols can help avoid disorders of the brain.Owing to their distinct BBB penetration properties,

considerable attention has been given to the exploration of their neuroprotective effects [33-36].

CONCLUSION

Solanum xanthocarpum has significant neuropharmacological potential, as demonstrated by the results of this investigation. Rats with memory impairments showed promise for improvement after receiving ethyl acetate extract over an extended period of time. By using in vitro research, the outcomes was subsequently confirmed. Certain plant-based compounds identified by GC-MS analysis may have antioxidative qualities, which could explain these advantageous effects. It is possible that the SX sterol components are reducing the reported neurological deficits by acting as a neuroprotectant in a variety of ways. Isolating and characterizing these plant elements as well as assessing their molecular effects will help to further support the results of this research.

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