Research Article

J. innov.appl. pharm. Sci, 8(2) 2023, 1-9

Journal of Innovations in Applied Pharmaceutical Science (JIAPS)

Content available at: www.saap.org.in ISSN: 2455-5177

EVALUATION OF NEUROPROTECTIVE ACTIVITY OF CHRYSIN NANOPARTICLES USING SCOPALAMINE INDUCED NEUROINFLAMMATION IN THE RAT MODEL OF ALZHEIMER'S DISEASE

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Article History

Received: 15-02-2023
Revised: 27-02-2023
Accepted: 29-03-2023

Keywords:
scopolamine, neuroinflammation, chrysin solid lipid nano particles.

Abstract

Background: scopolamine induced neuroinflammation limits its long-term clinical use. Several agents/strategies were attempted to prevent scopolamine neuroinflammatory but were not found suitable for clinical use.

Objective: To investigate the neuroprotective activity of chrysin nanoparticle on scopolamine induced neuroinflammation.

Material and Method: Male Albino wistarrats (180- 230 grms) were evenly divided into 5 groups. Group I&II served as control (distilled water 10ml/kg) and diseases control (scopolamine) 100mg/kg per os treated for 4 weeks, group III served as standard group which received Donepezil (1.5mg/kg) on ,while group IV and V are simultaneously treated with (50mg/kg and 100 mg/kg) orally with chrysin nanoparticle up to 4 weeks. On 29 and 30 day behavioral parameters and brain were isolated for biochemical parameters at the same time rat brains were used for histological studies.

Results: Treatment of scopolamine caused neuroinflammation as the evidence by marked elevation in AchE, GSH, catalase, super oxide Dismutase, Elevated plus maze,Y-maze and Open field test. SLN chrysin decrease in AchE, catalase, super oxide dismutase, GSH time respectively. SLN chrysin increase activities of SOD, crossing, Rearing, Grooming. Histopathological changes also showed the protective nature SLN chrysin induced neuroinflammation

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https://doi.org/10.37022/jiaps.v8i2.452

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1. Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disorder with a devastating impact on public health. AD is the most common cause of dementia among people 65years or older. Scopolamine is a non-selective muscarinic receptor antagonist it impairs learning /memory by blocking cholinergic signalling and some indirect mechanisms [1]. Scopolamine-treated rats display impaired spatial learning/ memory abilities, increased Aβ deposition, activation of oxidativestress [2]. Flavonoids belong to a group of natural substance with phenolic groups and are found in different plant tissues or products such as fruits , vegetables, grains, bark, roots, stems, flowers, tea, and wine [3]. The therapeutic efficacy of chrysin is limited due to its compromised bioavailability. One of the good strategies to increase the bioavailability of chrysin could be its inclusion in nanoparticles made from solid lipids, called solid lipid nanoparticle. These are spherical particles, nanometre rang, which dispersed in water or in aqueous surfactant solution [4] and represent an alternative drug delivery system to colloidal carriers such as lipid emulsions, liposomes and polymeric nanoparticle and an excellent candidate for the encapsulation of drugs with poos water solubility [5]. Moreover SLN are taken up readily by the brain because of their lipidic nature and the bio acceptable and biodegradable nature of SLNs makes them less toxic as compare to polymeric nanoparticle [6].

Therefore, in our present study we proposed that chrysin, with its antioxidant and anti- inflammatory properties, may exhibits beneficial effects on improving learning and memory impairment in rats induced scopolamine. Furthermore, we investigated the therapeutic effect of CN-SLNs alt low dose of 50mg/kg and 100mg/kg body weight regard to higher dose of free CN (50mg/kg and 100mg/kg) in treatment of biochemical, behavioural and histopathological changes induced after orall administration of scopolamine.

2. Material and Methods

2.1 Chemical and reagents

Chrysin were purchased from otto Kemi, glyceryl mono-stearate and soya lecithin are purchased from otto Kemi, Tween 20 purchased from Burgyne laboratory , chloroform purchased from Alpha chemika, donepezil Alkem, Acetylthiocholine iodide Kanata fine chem, DTNB kemphosol, hydrogen Molychem, Tris- HCL buffer 0.lml and Tris-HCL buffer 0.05 ml are purchased from Vijaya Lakshmi chemical, pyrogallol , ethanol,0.2M phosphate buffer, 0.6mM of Elman's reagent, 10%TCA, standard glutathione and 10%sodium citrate were purchased from sigma Aldrich. All chemical used were of analytical grade.

2.2 method of chrysin nanoparticle preparation

Chrysin loaded SLN were prepared by using glyceryl monostearate (GMS) as lipid, soya-lecithin as a co emulsification and evaporation method was selected to prepare SLNs due to convenience of lab scale equipment and stability of the method. In this method, accurately weigh amounts of 60mg lipid; 500mg drug and 20mg co-emulsifier, which were dissolved in 1 ml of organic solvent, chloroform. In 50ml beaker, 10ml of 1.0% Tween 20 solution is taken. Then the organic phase was added to 10% Tween 20 solution and homogenised 12400rpm for 3 min, in order to get a coarse o/w emulsion. Further, this coarse emulsion was subjected to ultrasonication for 10 min using a probe sonicator at 45% amplitude. During sonication, due to solvent emulsification and evaporation, SLN were precipitated at settled down. Thus the chrysin loaded SLN were formed [7].

2.3 Fourier- transform infrared (FTIR)

To determine of drug- excipient interaction was evaluated by ATR-FTIR spectroscopy (Bruker Vertex 70 spectrophotometer). Sample loading liquid sample: Introduce the liquid sample (1 drop) on to the ATR crystal keep anvil the up position. Solid sample: place the sample so that it covers the ATR crystal. Gently press down on the anvil till it make contact the sample. If necessary, rotate the anvil arm to ensure full contact is made with the crystal. In most case solid sample that are ground to a finer powder, result in sharp FTIR spectra [8].

2.4 Animals.

Male Wister rats (200-220) will be procured &maintained under standard conditions (temperature 22± 2°c, relative humidity 50± 5%) and 12 h light/ dark cycle). The animals will be housed in sanitized polypropylene cage sterile paddy husk as bedding. They will have free access to standard pellet diet and water libitum. The Institutional Animal Ethics committee approved the experimental protocol (120/PO/Re/S/08/CPCSEA Dated 28/08/2017). All the animals received human care according to the criteria outline in the "Guide for the care and use of Laboratory Animals" prepared by the "National Academy Of Sciences". All the procedures will be performed in accordance with Institutional Animal ethics constituted as per the direction of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), All the studies conducted were approved by the institutional animal ethics committee with the approval number IAEC/ANCP/2018-19/10 under ministry of animal welfare division, Government of India, New Delhi, India.

2.5 Experimental design

Animals were classified into five group (6 rats each). Treatment was given for four successive days. One hour after last dose of test agents, all animals were oral with scopolamine (100mg/kg) except the first group (control group). Animals were treated according to following scheme: groups I and II normal saline group III and IV received donepezil hydrochloride (1.5mg/kg) or chrysin nano particles (50 and 100 mg/kg), respectively. Elevated plus maze, Y-maze spontaneous alteration test were conducted 30 min and open field test after scopolamine oral. Immediately after performing the behavioral test, rats were sacrificed by decapitation, brains were rapidly isolated. Each brain was dissected through the midline into two hemispheres. Each brain weight one of the two hemisphere was homogenized in ice-cold acidified butanol to obtain 10 % homogenate that was used for the determination of brain dopamine (DA), norepinephrine (NE) and serotonin (5-HT) contents. The other hemisphere was homogenized in ice-cold 50mM phosphate buffer (pH 7.4) to prepare 10% homogenate aminobutyric acid (GABA), lipid peroxides, reduced glutathione (GSH), nitric oxide (NO) and TNF contents as well as AchE activity. Finally, brain of 2-3 rats from each group were preserved in 10% formalin and kept for histopathologic examination.

Table 01: Treatment Protocol

Groups	Treatment	Duration	No of Animals
1	Control	Normal saline(0.5ml/kg) for 4weeks	6
2	Disease control	Scopolamine (100mg/kg)for 4weeks	6
3	Standard	Donepezil(1.5mg/kg)+scopolamine(100mg/kg)for 4 weeks	6
4	Chrysin nanoparticle low dose(50mg/kg).	Chrysin nanoparticle low dose(50mg/kg)+ scopolamine (100mg/kg) for 4 weeks	6

2.6.1 Elevated plus maze

The EMP device was comprised of four arms sharing the same dimensions, i.e., two open arms (50x10cm) that crossed over two closed arms with 40 cm high walls. These arms were connected using a central square (10x10cm), thus giving the apparatus plus sign look. Furthermore the EMP was 50 cm above floor level. This technique is almost similar one reported by Halder *et al. (*2011). The behavioural testing was conducted between 9:00 am and 6:00 pm under dim red-light illumination. During the training phase, each rat was placed at end of an open arm and by using a stopwatch, transfer latency time(s) which is the time each rat took to enter (with all four paws) into either closed arm, was noted. The maze was clean with 70% ethanol between runs to minimize scent trails. To evaluate memory retention, a test phase was conducted 24h (retention) after a training section. The cut-off time for each rat to explore the maze in both the phases was 90s. A drop in transfer during test sessions was taken as an index of memory improvement [9].

2.6.2 Y-maze spontaneous alteration test

The Y-maze spontaneous alteration is a behaviour altest which employed to investigate the spital working memory. The apparatus were made up of black painted wood. Each arm of Y-maze was about 40 cm in length, 13 cm height, 3 cm bottom width, 10 cm top width and further converges at an equal angle. Rat were placed at end of one arm and allowed to move freely through the maze during a 5 main session. Spontaneous alteration was examine by visually recording the pattern of entrance into each arm in the maze for each rat. Alternation was define as successive entries into the three arms on overlapping triplet set. Accordingly, the spontaneous alternation performance score, spontaneous alternation percentage (SAP%)and total arm entries (TAE) were calculated [10].

2.6.3 Open field test

Exploratory activity and anxiety- like behaviour were measured using an open-filed apparatus (50x50x50cm). Each rat was placed in the centre of the open field apparatus. The center zone was defined as a square, 10 cm away from the wall. Distance travelled and time spent in the center zone by each animal was observed for 10 min [**11]**.

2.7 Biochemical studies

After the experimental period of 21 days, all the animals were sacrificed and their brains were removed quickly and their hippocampus were collected and rinsed with ice cold 0.9%Nacl. The Hippocampus were then transferred to the ice cold 0.1 M phosphate buffer (pH 8) and homogenized.

2.7.1Acetyl cholinesterase activity

AchE is a marker of extensive loos of cholinergic neurons in the forebrain. The AchE activity was assessed by the Ellman method (Ellman et al., 1961). The above 2 to 5gm of rat brain homogenate preparation gives the linear rat of reaction up to 10-15min. To 4ml homogenate is measured at 412nm. When absorbance reaches stable 20µ of Acetylthiocholine iodide was added change in the absorbance was measured at 5 minutes intervals. Change absorbance per minute was measured. AchE activity was calculated with the following formula [12].

2.7.2 Catalase

Catalase activity (CAT) was assayed by the method of Aebi (1984). Tissue was homogenized with isotonic buffer and centrifuged at 3000rpm for 10min. The supernatant liquid was collected and 0.01ml of ethanol per ml for supernatant liquid was added. Then the sample were incubated for 30 min in a ice water bath. At end of the incubation period,10% Triton X was added to 0.1ml of supernatant and used for catalase estimation was read at 240 nm at 15 sec interval for a total of 30 sec [13].

2.7.3 superoxide Dismutase (SOD)

Superoxide dismutase activity was measured according to the method describe by Marklund and Marklund (1974), with some minor modifications. Tissue was homogenized in 0.25 M tris-sucrose buffer. Then the homogenized tissue was centrifuged at 10,000rpm for 15min at 4°c. The supernatant was collected, to which 50% ammonium sulphate was added,

vortexed, and the reaction mixture was kept for incubation at 4°c for 4h. After the incubation period it was again centrifuged at 12,000rpm for 30min at 4°c. The sample were then kept overnight for dialysis in 0.0025 Tris HCL buffer. The next day, appropriate volumes of the samples to maximum of 1.2ml were taken. To it 1.2ml sodium pyrophosphate, 0.1ml phenazine methosulphate, 0.3ml of NBT were added. The final volume was made up to 3ml distilled water. After adding NADH, it was immediately incubated for 90sec at 30°c and the reaction was stopped by adding 1ml acetic acid to the reaction mixture. 4ml of butanol was added and after 10min. centrifuged at 3000rpm for 10min. The organic layer was separated and absorbance was observed at 560nm in a spectrophotometer. Data expressed as IU/mg protein [14].

2.7.4 Reduced Glutathione (GSH)

The reaction mixture containing aliquot of 1.0ml of the suspwnsion was precipitated by using 10% 0f TCA. The precipitate formed was then removed by centrifugation at 1500Xg for 10 minutes. To the supernatant, phosphate buffer (2.0ml) and DTNB (0.5ml) were added, mixture well and the volume was there by made up to 3.0ml by utilizing double distilled water. Standerds were also similarly treated as discussed above. The colour developed was read at 412nm. The amount of GSH had been as µg of glutathione/mg of protein [15].

2.8 histopathological examinations of brain tissues

Histopathologic assessment was performed on the brains of 2-3 rats randomly selected from each group. Brains were immediately fixed in 10% phosphate buffered formaldehyde, embedded in paraffin, and 5µm longitudinal sections were performed. The section were stained with hematoxylin and eosin (H&E) and examined microscopically.

2.9 Statistical analysis

Data represent mean ± S.D Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Multiple comparison tests by prism graph pad version 9.

4. Results

4.1 AIFTR studies:

In this due to the compatibility studies the drug -excipients interaction study was carried out using the AFTIR studies. Show in fig 1

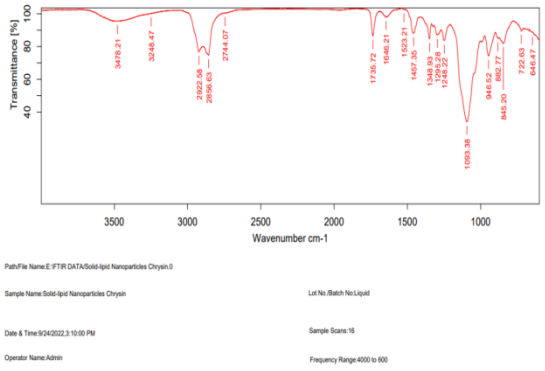


Fig 01: AT-FTIR studies chrysin drug along with excipient

Table 02: Summary Data of Functional group present in FTIR Spectra's

MATERIALS	Groups with assigned Wave number				
	OH	C-H	C=O	C=C	C-O-C
Chrysin	3402.95	3010.41	1652.26	841.31	1024.68

Glycerol Mono-Stearate	**3309.76**	**293.93**	**1737.45**	**1391.76**	**1046.35**
Soya Lecithin	**3351.04**	**2922.63**	**1742.82**	**1377.64**	**973.37**
Tween 20	**3480.30**	**2862.75**	**1734.20**	**-**	**1094.17**
SLN chrysin	**3478.21**	**2922.58**	**1735.72**	**845.20**	**1093.38**

Hence considering the above mentioned therapeutic values and constituents of chrysin nanoparticles.

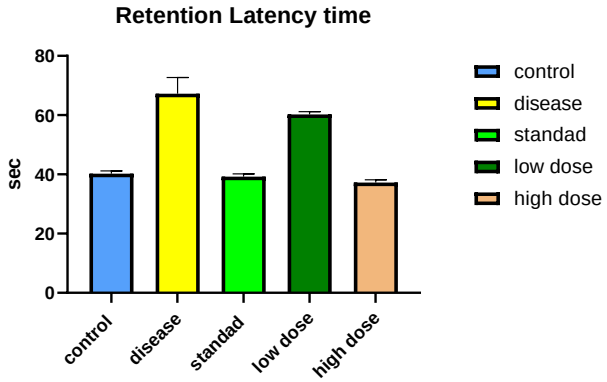
4.2 behavioural parameters

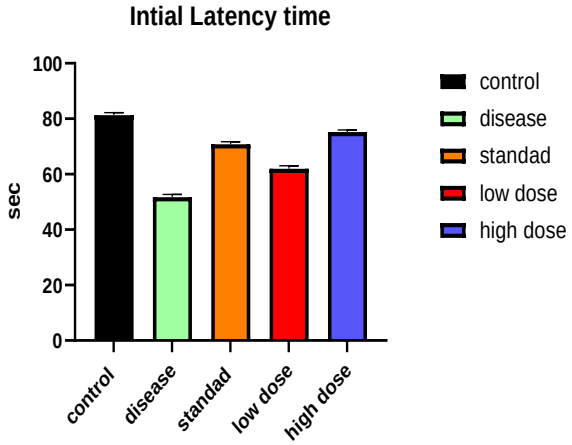
4.2.1 ELEVATED PLUS MAZE

Table 03: Effect of chrysin nanoparticle Behavioural Parameter elevated plus maze Test

S.NO	GROUPS	TREATMENT	INITIAL LATENCY TIME	Detention LATANCEY TIME
1	CONTROL	Distilled water	81.250±0.935***	40.250±0.935***
2	DISEASE	Scopolamine	51.667±1.080	67.250±5.447
3	STANDARD	Donepezil	70.750±0.935****	39.250±0.935***
4	LOW DOSE	Low dose SLN chrysin	61.917±1.158***	60.250±0.935***
5	HIGH DOSE	High dose SLN chrysin	75.000±0.791***	37.250±0.935**

Values represent mean±SD analysed by One way ANOVA followed by dunnet's multiple comparison test ****P<0.0001 compared to disease.





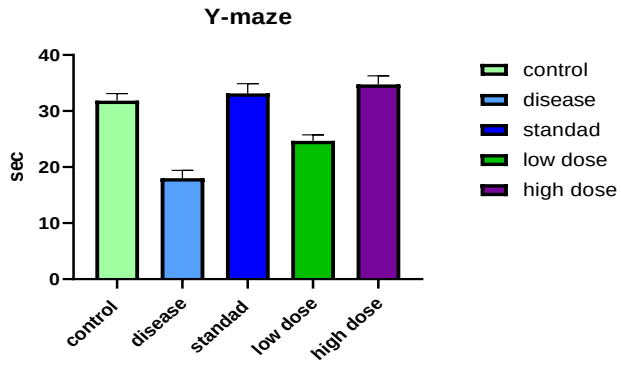
Graph 01 and 02 effect of chrysin nanoparticle on elevated plus maze

4.2.2 Y- MAZE

Table 04: Effect Of chrysin nanoparticle Behavioural Parameter Y- maze Test

S.no	GROUP	TREATMENT	% Altration
1	CONTROL	Distilled water	31.833±1.291****
2	DISEASE CONTROL	Scopolamine	18.000±1.414
3	STANDARD	Donepezil	32.000±0.894*****
4	LOW DOSE	Low dose SLN Chrysin	24.667±1.080*****
5	HIGH DOSE	High dose SLN Chrysin	37.833±1.472*****

Values represent mean±SD analysed by One way ANOVA followed by dunnet's multiple comparison test ****P<0.0001 compared to disease.



Graph 03: Effect of chrysin nanoparticle on Y – maze

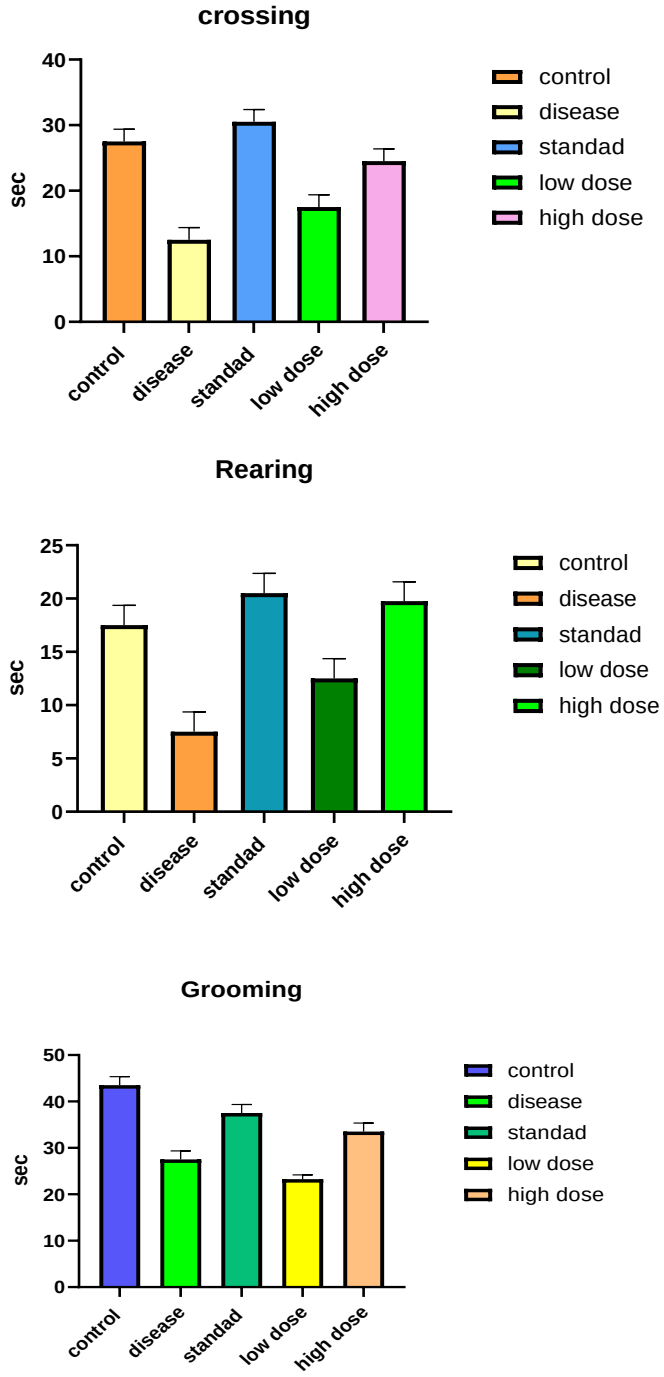
4.2.3 Open field test

Table 05: Effect of chrysin nanoparticle on Behavioural Parameter (open field test)

S.NO	GROUPS	TREATMENT	CROSSING	REARING	GROOMING
1	CONTROL	Distilled water	27.500±1.871****	17.500±1.871***	43.500±1.871****
2	DISEASE	scopolamine	12.500±1.871	7.500±1.871	27.500±1.871

3	STANDARD	Donepezil	30.500±1.871****	20.500±1.871***	37.500±1.871****
4	LOW DOSE	Low dose of SLN chrysin	17.500±1.871****	12.500±1.871****	23.500±0.935*
5	HIGH DOSE	High dose of SLN chrysin	24.500±1.871****	19.767±01.802***	33.500±1.871****

Values represent mean±SD analyzed by One way ANOVA followed by Dunnett's multiple comparison test *P<0.1, **P<0.01, ***P<0.001, ****P<0.0001 compared to disease



Graph 04: Effect of chrysin nanoparticle on open field test

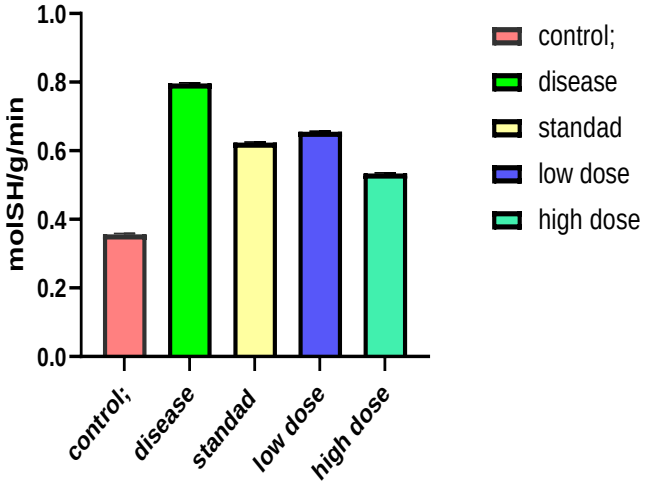
4.3 BIOCHEMICAL PARAMETER

4.3.1 Acetylcholine esterase activity:

Table 06: Effect of chrysin nanoparticle Acetyl Choline Esterase Activity

S.NO	GROUP	TREATMENT	MEAN+-SD
1	CONTROL	Distilled water	0.356±0.002**
2	DISEASE	Scopolamine	0.796±0.002
3	STANDARD	Donepezil	0.624±0.002***
4	LOW DOSE	Low dose SLN chrysin	0.534±0.002***
5	HIGH DOSE	High dose SLN chrysin	0.656±0.001***

Values represent mean±SD analysed by One way ANOVA followed by dunnet's multiple comparison test ****P Value 0.0001 compared to disease



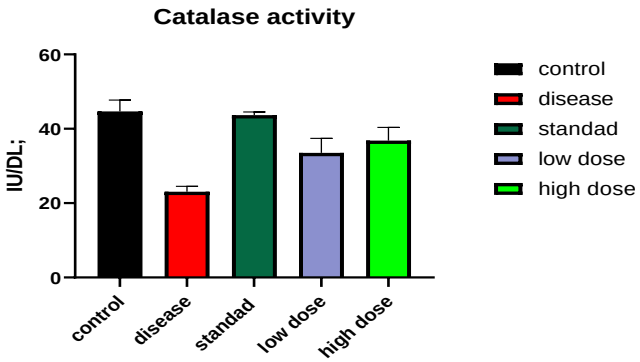
Graph 05: Effect of chrysin nanoparticle on Acetyl Choline Esterase Activity

4.3.2 **Catalase**

Table 07: Effect of chrysin nanoparticle on catalase

S.no	GROUP	TREATMENT	MEAN±SD
1	CONTROL	Distilled water	44.500±862****
2	DISEASE	Scopolamine	18.000±1.306
3	STANDARD	Donepezil	46.000±1.304***
4	LOW DOSE	Low dose SLN chrysin	33.500±1872****
5	HIGH DOSE	High dose SLN chrysin	41.083±1.043****

Values represent mean±SD analyzed by One way ANOVA followed by Dunnett's multiple comparison test **P< 0.01 ****P< 0.0001 compared to diseases.



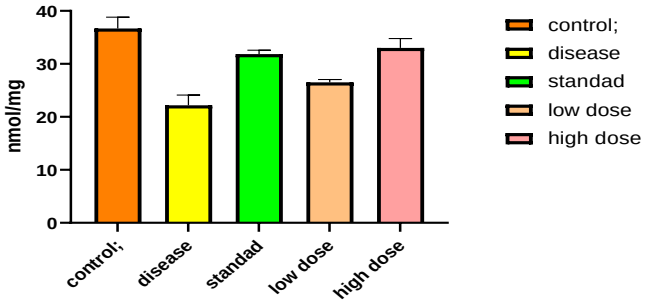
Graph 06: Effect of chrysin nanoparticles on catalase

4.3.3 Super oxide dismutase (SOD)

Table 08: Effect of chrysin nanoparticle onSOD

S.no	GROUP	TREATMENT	MEAN±SD
1	CONTROL	Distilled water	36.667±2160*
2	DISEASE CONTROL	Scopolamine	22.167±1.941
3	STANDARD	Donepezil	31.831±6.753****
4	LOW DOSE	Low dose SLN chrysin	26.500±0.548****
5	HIGH DOSE	High dose SLN chrysin	33.00±1.789**

Values represent mean±SD analyzed by One way ANOVA followed by Dunnett's multiple comparison test ****P<0.0001 compared to disease.



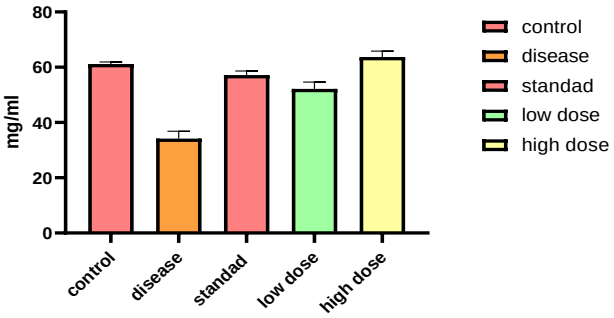
Graph 07: Effect of chrysin nanoparticle on SOD

4.3.4 Reduced Glutathione (GSH)

Table 09: Effect of chrysin nanoparticles on GSH

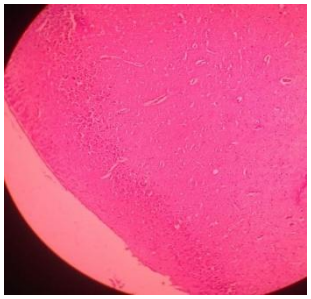
S.no	GROUP	TREATMENT	MEANSD
1	CONTROL	Distilled water	53.833±1941****
2	DISEASE CONTROL	Scopolamine	34.167±2.639
3	STANDARD	Donepezil	73.833±2.317****
4	LOW DOSE	Low dose SLN Chrysin	43.833±2.483****
5	HIGH DOSE	High dose SLN Chrysin	63.667±2.160****

Values represent mean±SD analysed by One way ANOVA followed by dunnet's multiple comparison test ****P<0.0001 compared to disease.

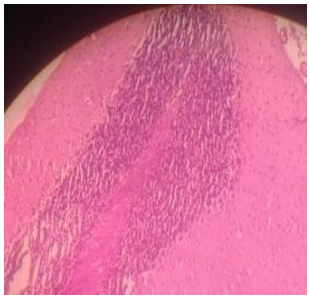


Graph 05: Effect of chrysin nano particles on GSH

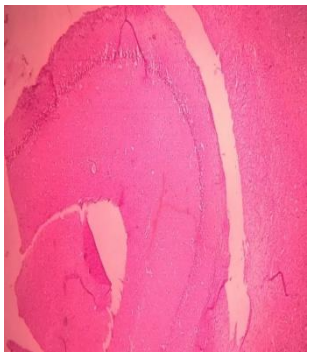
4.4 Histopathological studies



A Control

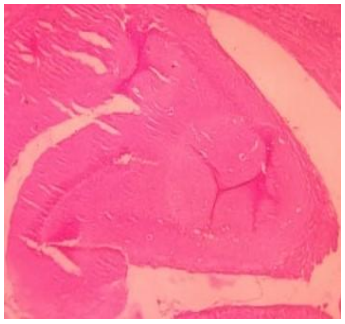


B Disease control



C Standard

D Low Dose



E High dose

a. Section of brain from control rats stained with hematoxylin and eosin revealed normal nerve cell.
b. The section of rat brains intoxicated with scopolamine for 4weeks showed the presence of fatty fibres, necrosis of the brain, spongy appearance, plaques and normal structure.
c. The brain section of standard drug treated animals shows somewhat normal nerve cell appearance.
d. Brain section of rats treated with low and high dose of chrysin solid lipid nanoparticles appeared more or less like normal section but with some dark neurons.

5. Discussion

Alzheimer's disease (AD) is a progressive neurological disorder that causes the brain to shrink (atrophy) and brain cells to die. Alzheimer's disease is the most common cause of dementia a continuous decline in thinking, behavioural, and social skill that affects a person's ability to function independently. The early signs of the disease include forgetting recent events or conversations. As the disease progresses, a person with Alzheimer's disease will develop severe memory impairment and lose the ability to carry out everyday tasks. Medications may temporarily improve or slow the progression of symptoms. These treatments can sometimes help people with Alzheimer's disease maintain function and independence for short period of time. Different programmes and services can help support people with Alzheimer's and their caregivers.

There is no treatment that cures Alzheimer's disease or alters the disease process in the brain. In advance stage of the disease, complications from severe loos of brain function, such as dehydration, malnutrition, or infection, result in death. No treatment has been proven to stop AD. The USFDA has approved four drugs to treat AD: donepezil. Memantine, rivastigmine and galantamine. However these drugs don't stop or revers AD and appear to help patients only for a few months to a few months to a few years.

The literature survey reveals that flavonoids, isoflavonoids and antioxidants are responsible for neuropharmacological effects. The present study is designed to assess the neuroprotective activity of chrysin as a plant-derived bioflavonoid in the treatment of neurological disorders especially Alzheimer's disease.

Chrysin (CN) is a phenolic compound showing various neuroprotective properties (He *et al.,*2012; Zhang *et al.,*2015). Due to its poor oral bioavailability, CN may not be successfully used as a dietary flavonoid for neuroprotection. On of the most promising application of current nanotechnology is targeted drug delivery to treat neurodegenerative disease. Nanoparticles play an important role in treatment AD. Due to the compatibility studies the drug- excipient interaction study was carried out using the AFTIR studies.

In a FT- IR drug – excipient interaction study, it was found that chrysin was compatible with all excipients used in the formulation; no extra peaks were observed. As result, the excipients use in the formulation was discovered to be compatible with the active ingredient and to have no physical interaction with it.

Hence, considering the above -mentioned therapeutic values and constituents of chrysin nanoparticles, a literature reveals that anti-Alzheimer's activity has not been reported. In this the present research work is carried out to evaluate the role of chrysin nanoparticles in scopolamine induced in rat.

The present research work includes the induction of behavioural changes by scopolamine administered orally and investigation of the possible effect of chrysin nanoparticle treatment as well as donepezil on the improvement of these behavioural changes. Three behavioural tests were used in this study: plus maze, Y-maze and open field tests. In addition, biochemical testing for Acetylcholinesterase, catalase, superoxide dismutase (SOD), and reduced glutathione and histopathological examination of the brain are performed to confirm the neuroprotective effects of chrysin nanoparticles.

The plus maze is a behavioural parading used to assess learning and memory. It represents the model of memory especially spital memory. This model is very helpful for analysing the reversal of the amnesic effect with an investigational drug. Moreover it provides a clean validation platform for comparing the escape latency of test and control animals. Decrease escape latency in the plus maze task demonstrates intact learning and memory function. The escape latency of disease control group rats increase when compare to the normal group, suggesting impairment in memory due to scopolamine. Scopolamine given orally to donepezil- treated and chrysin nanoparticle treated rat on the other hand significantly reduced time to reach the hidden platform in the plus maze.

The Y-maze is a behavioural paradigm used to asses learning and memory. This model is very helpful for analysing the reversal off the amnesic effect with an investigational drug. Moreover, it provides a clean validation platform for comparing the escape latency of test and control animals.

A neuroinflammatory increase the time spent in central area and the frequency of entries into the open field in the test. In the present study, high dose and low dose of chrysin solid lipid nanoparticle are significantly reversed the average time spent in crossing,rearing and grooming induced by scopolamine and show effect. Donepezil demonstrated effects as did donepezil treated with chrysin solid lipid nanoparticle.

Acetylcholine (Ach) is the most important neurotransmitter involved in the regulation of cognitive functions. Cholinergic transmission is terminated mainly by acetylcholine hydrolysis through enzyme acetylcholinesterase (AchE). The increase AchE activity in scopolamine-induced group was significantly reduced in animals treated with 50 and 100mg/kg of chrysin solid lipid nanoparticle and donepezil (1.5mg/kg) when compered with the scopolamine induced control group. However, a 50mg/kg dose was observed to be more effective than a 100mg/kg dose in redusing the increasing in AchE activity cause by scopolamine administration. The finding indicated that chrysin solid lipid nanoparticle posses AchE inhibitory in rats and may be considered a natural AchE inhibitor.

In the study of catalase estimation, control group values were increased in the scopolamine treated group compared with the control group, and treatment 1 (low dose) values are lower when compare with the standard group. In treatment 2(high dose) control group values were higher in the scopolamine-treated group compared with the control group. And treatment 1(low dose) values are lower when compared with the standard group. In treatment 2(high dose) values are slightly higher compared with low-dose group. It suggests that a lower dose is more effective than a higher dose.

The values in the study of the super oxide dismutase (SOD) disease control group are significantly lower than the values in the control group, and low dose of chrysin solid lipid nanoparticle show significantly higher values compared to the disease group. High dose of chrysin solid lipid nanoparticle show slightly compare with low doses.

GSH levels were found to be lower in the brains of alcohol - intoxicated rats in the GSH study. GSH is a nonenzymatic antioxidant that potential role in protecting the brain tissue against free radical production and neurodegeneration. In this

disease control group values are significantly higher than dose of control group. Furthermore the low dose chrysin solid lipid nanoparticle values are significantly higher than dose of the control group. Furthermore the high -dose chrysin sloid lipid nanoparticle have slightly higher values than the low-dose group.

Several histopathology finding of previous studies showed normal degeneration in hippocampal region of scopolamine induced in rat brain. In our study the histopathological changes caused by scopolamine showed never vacuolar degeneration and neuronal cell. Treatment with chrysin solid lipid nanoparticle show less sign of degeneration in dose dependent manner.

6. Conclusion

The finding of this investigation proved the therapeutic potentials of chrysin solid lipid nanoparticles against the scopolamine triggered neuroinflammation in the rat model of Alzheimer's diseases. The administration of chrysin solid lipid nanoparticle treated eased the scopolamine stimulated neuro-inflammation through its strong anti- inflammatory action. Hence it can be promising curative agent to treat the Alzheimer's disease in the future. However the further research work was still needed in the future to understand the exact mechanisms of chrysin sloid lipid nanoparticle against the Alzheimer's disease.

Funding

No Funding

Conflict of Interest

Authors are declared that No Conflict of Interest

Ethical Consideration

Not Applicable

Inform Consent

Not Applicable

Author Contribution

All authors are contributed equally.

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