



Ameliorating Effects of Chrysin on Scopolamine Induced Memory Impairment in Rats: A Pilot Study on Behavioural, Biochemical and Histological Approach

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Received: 14 Jan 2019 / Accepted: 12 Mar 2019 / Published online: 1 Apr 2019

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Abstract

Dementia is a neurodegenerative disorder characterized clinically by a progressive loss of memory and cognitive functions. The primary cause of dementia is brain's acetylcholine (ACh) deficiency and oxidative stress which analyzed principle pathogenic factors. The present study was to investigate the anti- amnesic effect of chrysin on scopolamine induced behavioural and neurochemical changes in rats. Chrysin (CN) (25mg and 50mg/kg) were orally administrated for 7 days. Dementia was induced on the 7th day by a single injection of Scopolamine (SCP) (1 mg/kg i.p). Study across three cognitive domains spatial, recognition, and associative memory and associated alterations in their oxidative status and neurochemical profile to select appropriate dementia model. Results showed significant decline in different aspects of memory function in all dementia models which was more significant in scopolamine-injected rats. A significant decline in the levels of acetylcholine was also observed. In addition, significant alterations were also seen in oxidative profile indicating that cognitive decline could be associated with increased oxidative stress. Thus, the study reveals the ameliorative effect of Chrysin which has its therapeutic strategies against cognitive dysfunctions.

Keywords

Dementia, Scopolamine, Chrysin, Coginition, neuroprotection, Acetylcholinesterase

1. INTRODUCTION

Dementia is a chronic neurodegenerative disease that results in loss of neurons or malfunction of neurons causes changes in one's memory, behaviour and ability to think especially in elder peoples [1]. In Worldwide, around 50 million people have dementia.

Every year, there are nearly 10 million new cases. Estimated proportion of the general population aged 60 and over with dementia at a given time is between 5 to 8 per 100 people. The total number of people with dementia is projected to reach 82 million in 2030 and 152 in 2050 [2]. Dementia is characterised

by deterioration in cognitive abilities, memory impairment [3]. Cognitive deficits is produced by the accumulation of amyloid plaques, [4] hyper phosphorylation of tau protein, oxidative stress [5] neuro inflammation leading to neuronal death and deficiency in cholinergic neurotransmission [6]. The loss of cholinergic neuronal signals and transmission leads to an impairment of higher cortical functions, related to the progressive deterioration of memory and cognitive process in both animals and human [7]. The neurological changes in demented patients is due to the loss of cholinergic markers choline acetyltransferase [8]. By inhibiting the acetylcholine esterase activity, the elevated levels of acetylcholine is maintained in the neuronal synapses [9]. Acetylcholine esterase inhibition down regulate the extra synaptic metabolism of ACh, and elicit the levels of ACh at the synaptic cleft and enhances postsynaptic stimulation [10]. Muscarinic receptors is the main target for restoration of cognitive functions in order to enhance synaptic plasticity and memory potential [11,12]. In demented condition, anti-mutagenic drugs interfere with the activity of acetylcholine esterase inhibitors [13]. Especially, Scopolamine, a cholinergic receptor antagonist used to study cognitive deficits in experimental animals. Scopolamine is a cholinergic muscarinic receptor antagonist, which blocks acetylcholine receptor and interrupts their neurotransmission leading to the development of cognitive amnesia in animals [14]. Scopolamine suppresses the two major fore brain cholinergic systems (nucleus basalis-cerebral cortical and hippocampal pathways) and block the central cholinergic activity causing transient memory impairment in animals [15]. Because of this effect, scopolamine induced neural impairment is a viable model for studying cognitive disabilities.

In Scopolamine-treatment express oxidative stress elicited by mitochondrion derived ROS led to the decreased level of ACh, resulting in the cholinergic dysregulation. The decreased level of antioxidant enzymes activity, such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) have been reported in the early stages of the dementia. In addition, scopolamine reduced the expression of BDNF, a neurotrophin, that plays a crucial role in synaptic plasticity and memory formation, and the expression of cAMP response element-binding protein (CREB), which regulates the level of BDNF [16,17,18].

Flavonoids are natural polyphenolic phytochemicals that are ubiquitous in plants and have anti-inflammatory, anti-oxidative, anti-viral, anti-tumor properties and neuroprotective activity [19]. Chrysin

is a natural flavonoid found in many plant extracts, honey, and propolis. It is found in passion flowers such as *Passiflora caerulea*, *Passiflora incarnata* and *Oroxylum indicum*. Chrysin possess neuroprotective effect on inflammatory neuropathies including diabetic neuropathy, hypoxic-ischemic brain injury and experimental autoimmune encephalomyelitis [20,21]. Chrysin is predominantly based on its function as an agonist of peroxisome proliferator-activated receptors gamma (PPARc) and an antagonist of NF-kB, which ultimately downregulated the production of iNOS and COX-2 [22].

Therefore, complementary and alternative therapies utilizing phytochemicals from medicinal plants and foods for the prevention or treatment of dementia are highly desirable and promising. In this study, we evaluated the effects of chrysin on learning and memory in hippocampus tissue. To assess its effect on learning and memory on Scopolamine induced cognitive dysfunctions.

2. MATERIALS AND METHODS

2.1 chemicals and reagents:

Chrysin were purchased from Sigma Aldrich. Scopolamine Hydro bromide were purchased from TCI chemicals. Glutathione reduced form (GSH), glutathione oxidized form (GSSG), 1-chloro-2, 4-dinitrobenzene (CDNB), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) were purchased from SRL. β -Nicotinamide adenine dinucleotide phosphate reduced (NADPH) was purchased from CDH. All other chemicals used were of analytical grade.

2.2 Animals and treatment schedule

Male Wister rats weighing 300-350 g were obtained from Central Animal House, Dr. ALMPGIBMS, University of Madras, Tamil Nadu, India. Rats were accommodated in polypropylene cages separately under hygienic conditions and fed standard pellet diet. Rats were placed on a 12 h light and dark cycles with ad libitum condition. All experiments and protocols described in the present study were approved by the Institutional Animal Ethics Committee (IAEC) (IAEC No: 02/18/2017) of Dr. ALMPGIBMS, University of Madras, Taramani, Chennai-113, India.

Animals Were Classified into Five Groups

Treatments were given p.o for seven successive days. One hour after the last dose of test agents, all animals were injected with scopolamine hydro bromide (1 mg/Kg) (i.p) except the first group (Control group). Animals were treated according to the following scheme:

Group I - Control rats.

Group II - Animals received scopolamine (SCO 1 mg/kg) intraperitoneally on 7th day.

Group III- Animals were treated with 25 mg/kg of Chrysin (CN) for 7 days intraperitoneally and on the 7th day Scopolamine (1mg/kg) were given intraperitoneally.

Group IV- Animals were treated with 50 mg/kg of Chrysin (CN) for 7 days intraperitoneally and on the 7th day Scopolamine (1mg/kg) were given intraperitoneally.

Group V- Animals were treated with (50mg/kg) of Chrysin (CN) for 7 days intraperitoneally

Immediately after performing the behavioural tests, rats were sacrificed by decapitation, brains were rapidly isolated.

2.3 Behavioural Observations

All the behavioural studies were carried out at room temperature without any outside interference. All the experiments were conducted between 10.00 am and 6.00 pm.

2.3.1 Novel Object Recognition Test

The novel object recognition test is widely used to evaluate the memory in rodents. The test was a simple behavioural assay for memory that rely primarily on a rodent's innate exploratory behaviour in the absence of externally applied rules or reinforcement. The NOR task has become a widely used model for the investigation into memory alterations. It is used to measure the working memory, attention, anxiety, and preference for novelty in rodents. It is also very useful to study short-term memory, intermediate-term memory, and long-term memory, through manipulation of the retention interval. The amount of time animals must retain memory of the sample objects presented during the familiarization phase before to the test phase, when one of the familiar objects is replaced by a novel one. During training session, two different objects A and B were placed in testing area. Each animal was allowed to explore the objects for 5 mins. The rat was considered to be exploring the object when touching or sniffing the object. The total time spent exploring each object was recorded and expressed as percentage of total exploration time. In retention session, one identical and one novel object placed were used. The rat was allowed to explore the objects for 5min, and the time spent exploring each object was recorded [23].

2.3.2 Morris Water Maze Test

The Morris water maze test was used to assess the learning and memory of the animals [25,25]. It is a swimming-based model where the animal learns to escape on to a hidden platform. It was performed to evaluate the changes caused by SCP on spatial

memory and learning. The circular water tank which was 55 cm in height and 160 cm in diameter was used. The rats were trained to find the platform. Latency time (time to find the platform) and swim distance were recorded in each trial. The average over 3 trials/day for seven consecutive days was used for statistical analysis. The probe trial was conducted on the sixth day while the platform was removed. The animal was placed in the water diagonally from the target quadrant; each rat was allowed to swim [26,27]. Time taken by the rat to reach the platform (initial acquisition latency) was recorded.

2.3.3 Open Field Test

The open field test is designed to measure behavioural responses such as anxiety, locomotor activity and exploratory behaviour. This test used to measure horizontal activity, time spent in various regions of the open field, and the total distance travelled. The open field test is a widely used model of anxiety-like behaviour developed to evaluate emotionality in animals. The test consists of measuring the activity of rats in an open novel space, from which escape is prevented by a surrounding wall. Each animal was kept in the center of the open field apparatus which was a circle made of wood with 90cm in diameter. To monitor the activity animals taken out from their home cages were placed in the central square of the open field (one at a time). Activity in open field was determined by monitoring latency period and counting number of squares crossed. Time taken for each animal spent in central area was noted between the control and experimental rats [28].

2.4 Preparation Of Tissue Homogenate For Biochemical Studies

On the day 7, after the neurobehavioral studies all the biochemical estimations were carried out. The animals were sacrificed by cervical decapitation and the brains were dissected out. Hippocampus region was separated and placed on ice. Using 0.1 M phosphate buffer (pH 7.4), 10% (w/v) tissue homogenate was prepared. The homogenate was centrifuged at 10,000 × g for 15 min. Aliquots of the supernatant was separated and used for biochemical estimation

2.5. Oxidative Stress Parameters

2.5.1 Estimation of Lipid Peroxidation Level (LPO)

Oxidative stress induced by reactive oxygen and nitrogen species has been implicated in the pathogenesis of various diseases. Lipid peroxidation is the oxidative degradation of lipids. In this process free radicals take electrons from the lipids, resulting in cell damage. Estimation of lipid peroxidation is essential to assess oxidative stress in

pathophysiological processes. In the present study, the thiobarbituric acid reactive substance was used to as an indicator to find out lipid peroxidation. 0.2 ml homogenate was pipetted and incubated at $37 \pm 1^\circ\text{C}$ in a metabolic water bath shaker at 120 strokes up and down for 60 min; 0.2 ml of homogenate was pipetted and placed at 0°C incubation. After 1 h of incubation 0.4 ml of 0.67% TBA and, 0.4 ml of 5% TCA was added in both samples (i.e., 0°C and 37°C) and centrifuged at $3500 \times g$ for 15 min by transferring the reaction mixture from the vial to the tube. The supernatant was transferred to another tube and placed in a boiling water bath for 10 min. Then the test tubes were cooled and the absorbance of the color was read at 535 nm. The rate of lipid peroxidation expressed as nmol of MDA released/min/mg protein. The method of [29] was modified for the estimation of malondialdehyde (MDA) an end product of lipid peroxidation.

2.5.2. Determination Of Protein Carbonyl Content

The protein carbonylation has been used as an indicator of protein oxidative damage. The assay of carbonyl groups in proteins provides a convenient technique for detecting and quantifying oxidative modification of proteins. 2,4- dinitrophenyl hydrazine (DNPH) reacts with protein carbonyls to produce hydrazones. In this, 100 μl of supernatant from brain homogenate was incubated with 0.5 ml of 2, 4-dinitrophenylhydrazine for 60 min. Subsequently, the protein was precipitated from the solution using 20% trichloroacetic acid. The pellet was washed after centrifugation ($3400 \times g$) with ethyl acetate: ethanol (1:1 v/v) mixture, to remove excess of 2, 4-dinitrophenylhydrazine. The final protein pellet was dissolved in 2.5 ml of 6 M guanidine. Protein carbonyl content determination was based on the reaction of carbonyl groups with 2, 4-dinitrophenylhydrazine to form 2, 4-dinitrophenylhydrazone. The carbonyl content was evaluated in a spectrophotometer at 370 nm, the values were calculated using molar extinction coefficient ($22,000 \text{ M}^{-1} \text{ cm}^{-1}$) and expressed as nmol per mg protein [30].

2.5.3. Estimation of Nitrite (NO)

Griess reaction were employed to detect nitrite and nitrate as products of nitric oxide synthase in biological systems. These include a constitutive, low-output, neuronal isoform that modulates synaptic plasticity and immune inflammatory isoform that functions as an effector component of the cell-mediated immune response. By using Greiss reagent (0.1% naphthyl ethylene diamine dihydrochloric acid and 1% sulfanilamide in 2.5% phosphoric acid) nitrite levels were determined. Equal volumes of Greiss

reagent and supernatant were mixed, the mixture was incubated for 10 min at room temperature in dark and the absorbance was determined at 540 nm [31]. The concentration of nitrite in supernatant was determined from a sodium nitrite standard and expressed as nmol per mg protein.

2.6 Antioxidant Enzyme and Non-Enzyme Parameters

2.6.1. Determination of Superoxide Dismutase Activity

Superoxide dismutase activity were measured using the following method. 0.25 ml of ethanol and 0.15 ml of chloroform was added and kept in a mechanical shaker for 15 min and centrifuged. To 0.5 ml of supernatant, 2.0 ml of pyrogallol was added to 1 ml of homogenate. In optical density 0, 1, 2, 3 min at 420 nm were read in spectrophotometer. Against a buffer blank, control tubes containing 0.5 ml of water were also treated in a similar manner. The enzyme activity was expressed as units/mg protein. One unit is equivalent to the amount of SOD required to inhibit 50% of pyrogallol autooxidation was measured according to the method described [32].

2.6.2 Determination Of Catalase Activity

Catalase is also an antioxidant enzyme which has capability to detoxify oxidative free radicals. To estimate catalase activity in the mixture consisted of 0.05 M phosphate buffer (pH 7.0), 0.019 M H_2O_2 , and 0.05 ml PMS in a total volume of 3.0 ml. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated in terms of $\mu\text{mol H}_2\text{O}_2$ consumed/min/mg protein [33].

2.6.3. Determination of Glutathione Reductase Activity (GR)

Glutathione reductase activity was measured by the method of [34]. In a total volume of 2.0 ml, the assay system consisted of 0.1 M PB (pH 7.6), 0.5 mM EDTA, 1 mM GSSH, 0.1 mM NADPH and PMS (0.1 ml). At room temperature the enzyme activity was quantitated by measuring the disappearance of NADPH at 340 nm and was calculated as $\mu\text{mol NADPH}$ oxidized/min/mg protein.

2.6.4. Determination of Glutathione Peroxidase Activity (GPx)

About 0.2 mM H_2O_2 , 1 mM GSH, 1.4 unit of GR, 1.43 mM NADPH, 1 mM sodium azide, PMS (0.1 ml) and PB (0.1 M, pH 7.0) in a total volume of 2.0 ml was considered to be reaction mixture. The NADPH disappearance at 340 nm was recorded at room temperature. The enzyme activity was calculated as nmol NADPH oxidized/min/mg protein GPx activity was measured at 37°C by the coupled assay method [35].

2.6.5. Estimation of Glutathione-S-Transferase Activity (GST)

Glutathione-s-transferase activity were measured by the assay mixture consisted of 2.7 ml of phosphate buffer, 0.1 ml of reduced glutathione, 0.1 ml 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate and 0.1 ml of supernatant. The changes in absorbance were recorded at 340 nm, and the enzyme activity was calculated as nmol CDNB conjugate formed/min/mg protein was assayed by the method [36].

2.6.6. Determination of Reduced Glutathione Content (GSH)

The level of reduced glutathione within the hippocampus was calculated based on the following method. To the 10% trichloroacetic acid equal quantity of homogenate was mixed and centrifuged to separate the proteins. To 0.1 ml of this supernatant, 0.4 ml of double distilled water and 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5, 5-dithiobis (2-nitrobenzoic acid) were added. The mixture was vortexed, and the absorbance was read at 412 nm within 15 min. The concentration of reduced glutathione was expressed as μmol of GSH/min/mg protein, according to the method [37].

2.6.7. Assay on Acetylcholinesterase (Ache) Activity

Acetylcholinesterase is an enzyme participating in cholinergic neurotransmission. It breaks down acetylcholine which terminates the neurotransmission process [38]. The method using an alternative substrate acetylthiocholine and 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB). The reaction results in production of 5-thio-2-nitrobenzoate that has yellow color due to the shift of electrons to the sulphur [39]. To the aliquot containing 2.85 ml phosphate buffer (0.1 M, pH 8.0), 50 μl of DTNB (10 mM), 50 μl sample and 20 μl acetylthiocholine iodide (78 mM) were added and the change in absorbance was monitored at 412 nm for 5 min in a spectrophotometer. The enzyme activity was expressed as nmol of substrate hydrolyzed/L/min/mg protein was determined according to the method [40].

6.8. Na^+/K^+ ATPase (Adenosine Triphosphatase)

The mixture containing 1.0ml of Tris-HCL buffer, 0.2ml each of magnesium, potassium chloride, sulphate, sodium chloride, ATP, EDTA and the tissue homogenate was made to incubated at 37°C for 15 minutes. By the addition of 1.0ml of 10% TCA the reaction was arrested with simultaneous mixing and centrifugation. The quantity of phosphorus was

estimated, and the enzyme activity was expressed as μmoles of phosphorus liberated/min/mg of protein [41].

2.6.9. Ca^{2+} ATPase (Adenosine Triphosphatase)

At 37 OC 0.1ml of Tris-HCL buffer, ATP, magnesium chloride and enzyme preparation was incubated for 15 minutes, after which the reaction was arrested by the addition of 1ml of 10%TCA. The amount of phosphorus liberated was estimated and the enzyme activity was expressed as μmoles of phosphorus liberated/min/mg of protein

2.7 Histological Evaluation on Hippocampus

On day 7, cervical decapitation was done by sacrificing the animal. The brain was carefully removed without any injury after opening the skull. Then the Hippocampal region was removed and made fixed in 10% buffered formalin. The samples were dehydrated with ethanol followed by xylazine. It was then embedded in paraffin at 56°C in hot water over 24 hours. The paraffin wax tissue blocks were prepared for sectioning at 4 μm thickness by slide microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin and eosin, and was examined using a light microscope

2.8. Statistical Analysis

The data were analysed by using analysis of variance (ANOVA) followed by Tukey's *Post-hoc* test. All the values are expressed as mean $\pm$ SEM. In all tests, the values with $P < 0.05$ was considered statistically significant.

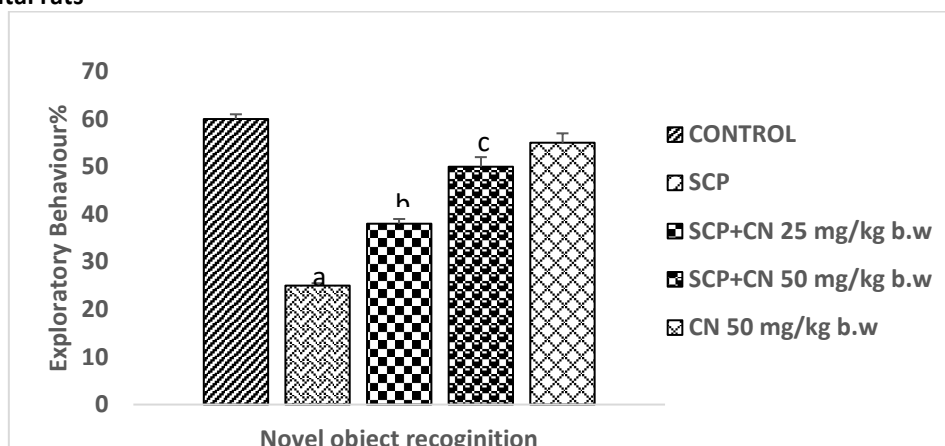
3. RESULTS

3.1. Behavioural Studies

3.1.1 Effect of chrysin on scopolamine induced changes in novel object recognition test in control and experiment animals

Memory test was assessed using a novel object recognition test. Compared to the training session, SCP induced group showed significantly less frequent exploratory ($P < 0.001$) behaviour to a novel object than a familiar object when compared to the control group. Whereas the exploratory behaviour to a novel object was found to be significant in treatment with chrysin (25 mg/kg) improved the memory performance on 7th day significantly ($P < 0.05$) against SCP administered group. In animals treatment with Chrysin (50 mg/kg) significantly increased ($P < 0.01$) the cognitive function against SCP induced group on 7th day (Figure 1)

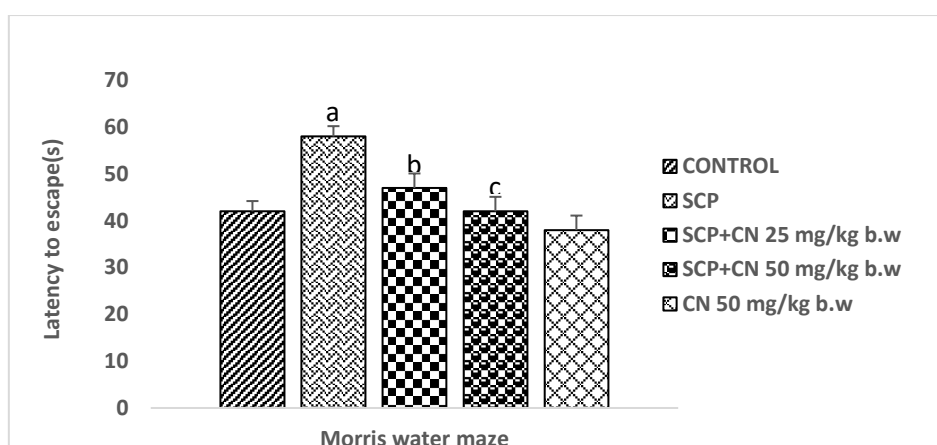
Figure 1: Effect of Chrysin on SCP Induced alteration in memory using object recognition test in control and experimental rats



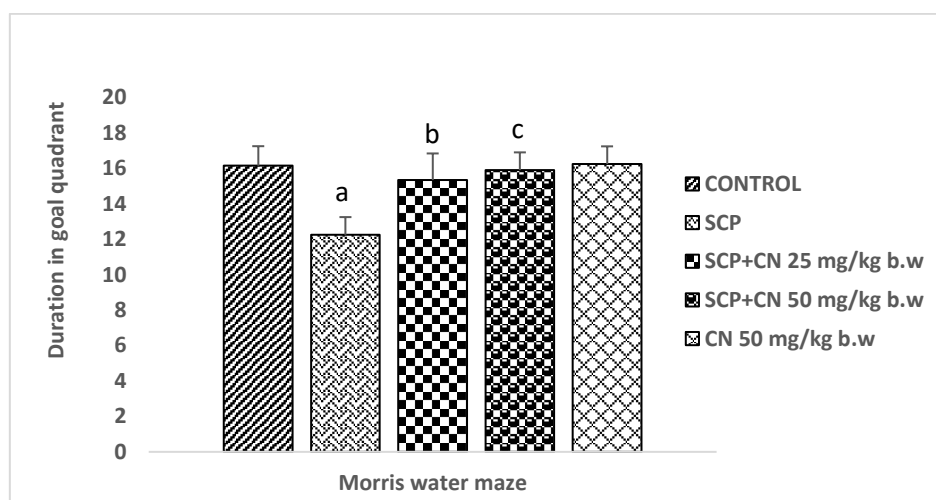
Data represents the memory retention animals mean values $\pm$ SD (n=6 animals each groups). ^ap<0.01, is significantly different from control. ^bp<0.05 and ^cp<0.01 is significantly different from SCP exposed group; one-way ANOVA with Tukey's post hoc test was used for statistical analysis.

Figure 2(A&B): Effect of Chrysin on SCP induced variations in the cognitive behavior by Spatial navigation task in control and experimental rats

A



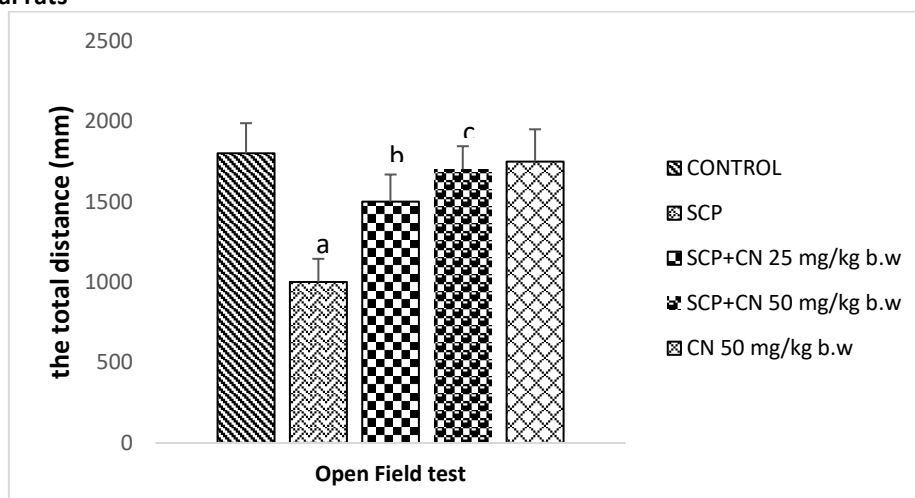
B



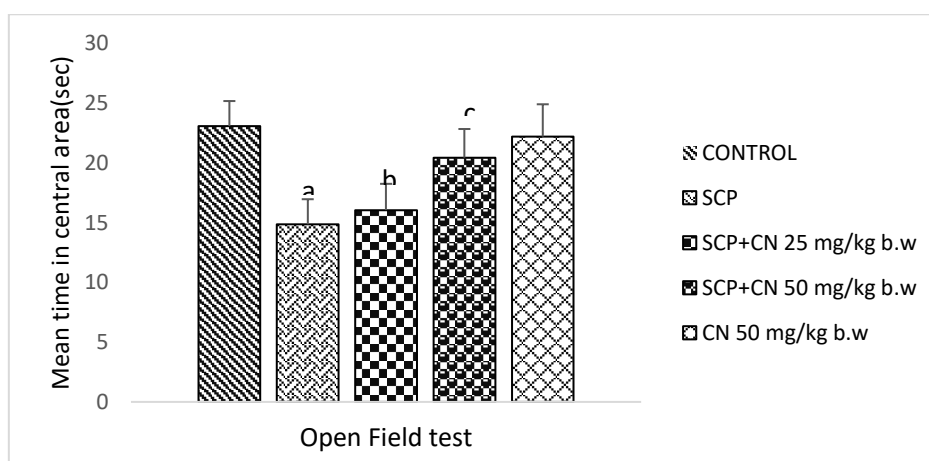
Data represents duration spent in target quadrant of the animals mean values $\pm$ SD (n=6 animals each groups). ^ap<0.01, is significantly different from control. ^bp<0.05 and ^cp<0.01 is significantly different from SCP exposed group; one-way ANOVA with Tukey's post hoc test was used for statistical analysis.

Figure 3(A and B): Effect of Chrysin on SCP induced locomotory and exploratory task in control and experimental rats

A

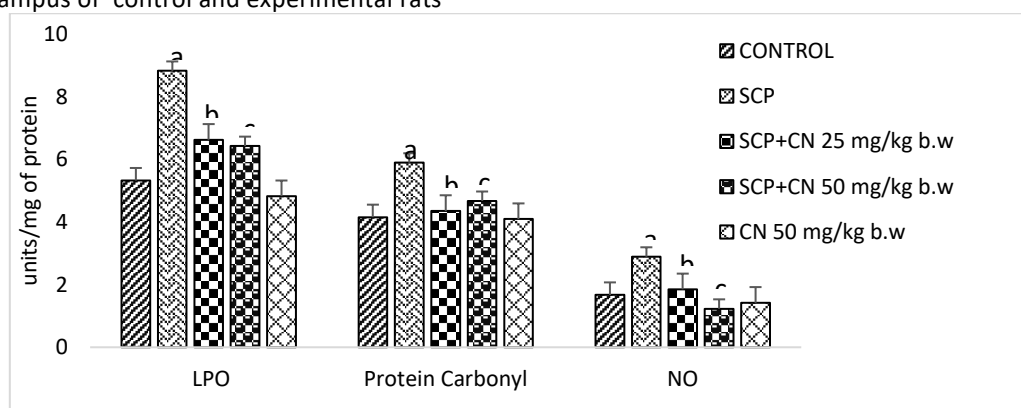


B



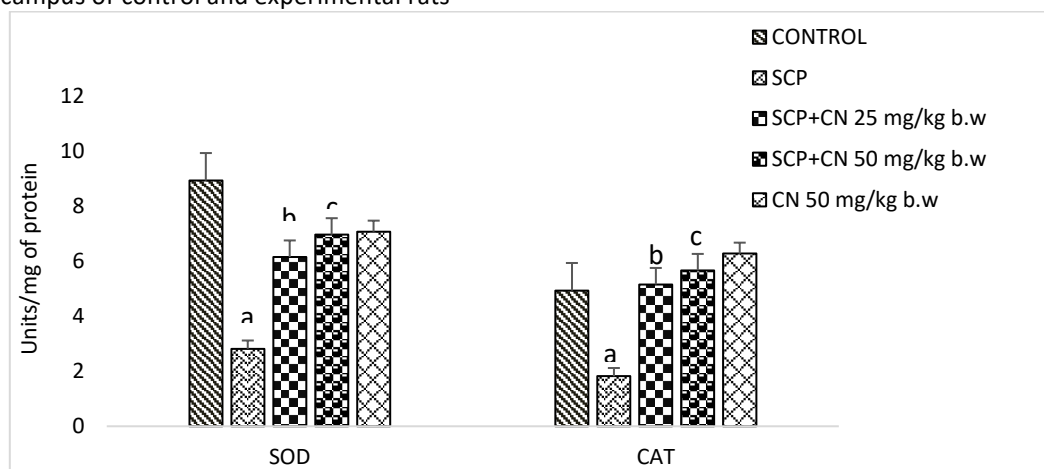
Data represents time spent in central area of the animals mean values $\pm$ SD (n=6 animals each groups). ^ap<0.01, is significantly different from control. ^bp<0.05 and ^cp<0.01 is significantly different from SCP exposed group; one-way ANOVA with Tukey's post hoc test was used for statistical analysis.

Figure 4: Effect of Chrysin on SCP induced alterations in the levels of LPO, Protein carbonyls and Nitrite in hippocampus of control and experimental rats



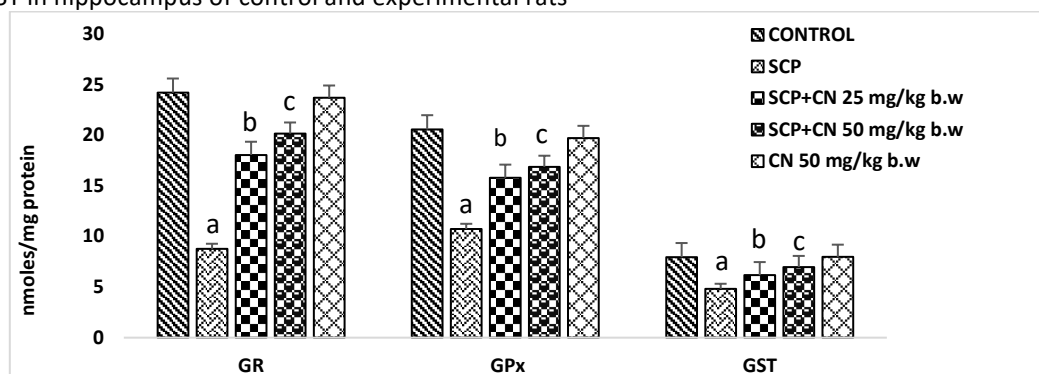
Data represents alteration LPO, carbonylated protein and NO level in hippocampus represents mean $\pm$ SD (n=6) ^ap<0.01, is significantly different from control. ^bp<0.05 and ^cp<0.01 is significantly different from SCP exposed group; one-way ANOVA with Tukey's post hoc test was used for statistical analysis

Figure 5: Effect of Chrysin on SCP induced alterations in the levels of enzymic antioxidant SOD and CAT in Hippocampus of control and experimental rats



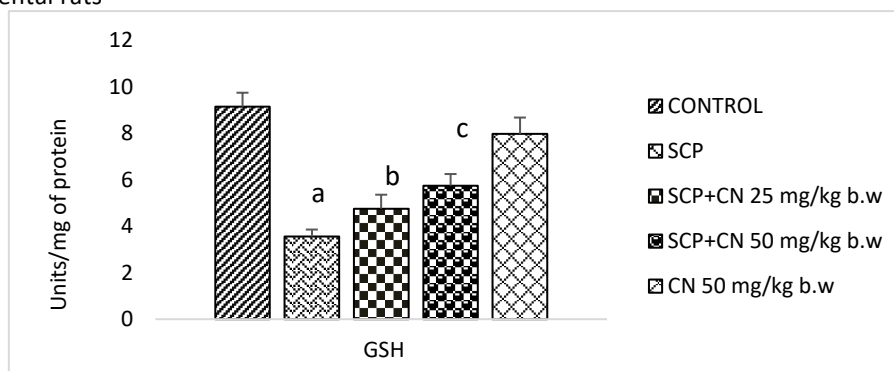
Data represents mean values $\pm$ SD (n=6 animals each groups). ^ap<0.01, is significantly different from control. ^bp<0.05 and ^cp<0.01 is significantly different from SCP exposed group; one-way ANOVA with Tukey's post hoc test was used for statistical analysis

Figure 6: Effect of Chrysin on SCP induced alterations in the levels of Glutathione family enzymes GR, GPx and GST in hippocampus of control and experimental rats



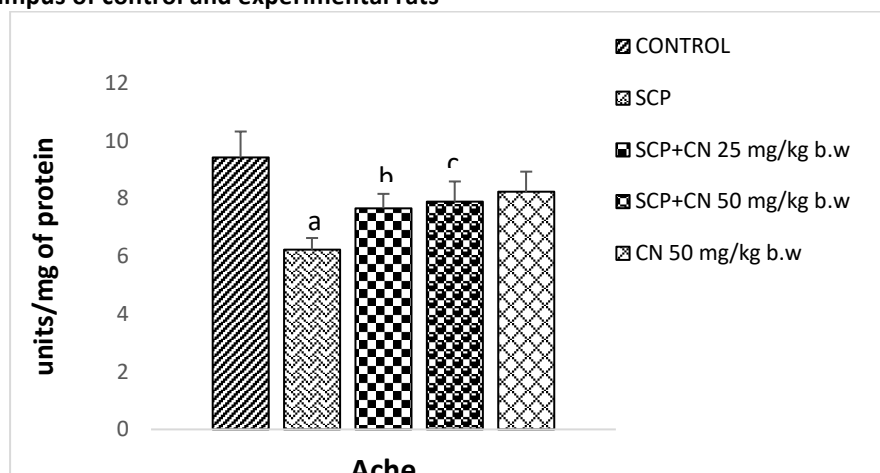
Data represents mean $\pm$ SD (n=6). GPx activity was expressed in nmol of GSH consumed/min/mg protein. GR activity was expressed in μ mol of NADPH oxidised/min/mg protein. Unit of GST activity was expressed as nmol of CDNB conjugated/min/mg protein. Data represents mean values $\pm$ SD (n=6 animals each groups). ^ap<0.01, is significantly different from control. ^bp<0.05 and ^cp<0.01 is significantly different from SCP exposed group; one-way ANOVA with Tukey's post hoc test was used for statistical analysis.

Figure 7: Effect of Chrysin on SCP induced alteration in the level of GSH enzymes in hippocampus of control and experimental rats



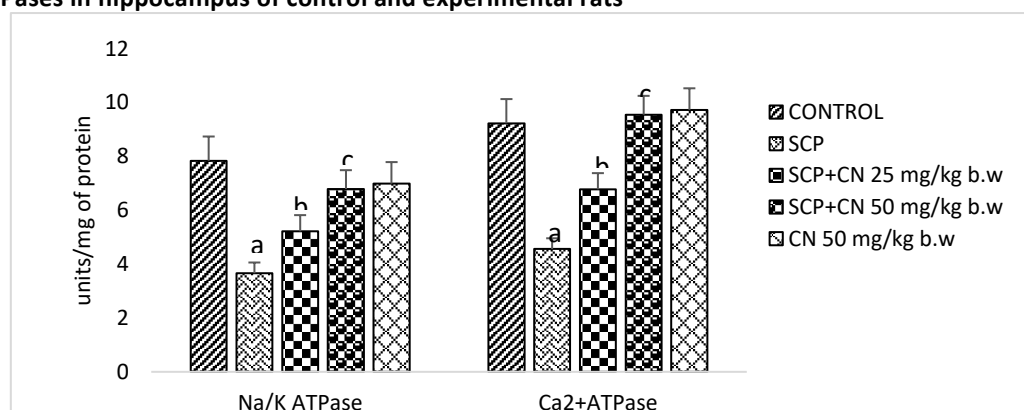
Data represents mean values $\pm$ SD (n=6 animals each groups). ^ap<0.01, is significantly different from control. ^bp<0.05 and ^cp<0.01 is significantly different from SCP exposed group; one-way ANOVA with Tukey's post hoc test was used for statistical analysis

Figure 8: Effect of Chrysin on SCP induced alteration in the activity of Achetylcholine esterase enzyme in hippocampus of control and experimental rats



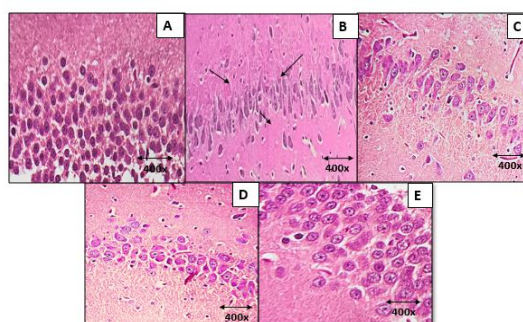
Data represents mean values $\pm$ SD (n=6 animals each groups). ^ap<0.01, is significantly different from control. ^bp<0.05 and ^cp<0.01 is significantly different from SCP exposed group; one-way ANOVA with Tukey's post hoc test was used for statistical analysis

Figure 9: Effect of Chrysin on SCP induced alterations in the levels of enzymes Na⁺/K⁺ATPases and Ca²⁺ATPases in hippocampus of control and experimental rats



Data represents mean values $\pm$ SD (n=6 animals each groups). ^ap<0.01, is significantly different from control. ^bp<0.05 and ^cp<0.01 is significantly different from SCP exposed group; one-way ANOVA with Tukey's post hoc test was used for statistical analysis

Figure 10: Effect of Chrysin on SCP induced histological changes in Hippocampus sections of control and experimental rats



Hematoxylin and eosin stained sections were visualized under microscope at a magnification of 400x. Figure A: Control rats showing normal neuronal structure of hippocampus region. Figure B: SCP induced rats showing greater extent of inflamed neurons (denoted by arrow) and neurodegenerative cells. Figure C: SCP+ CN (25 mg/kg bw) treated showing mild recovery of inflamed and degenerative neurons. Figure D: SCP + CN (50 mg/kg bw) treated showing reduced number of inflamed cells and recovering positive healthy neurons. Figure E: CN (50 mg/kg bw) alone administered rats showing no inflammatory alterations they resemble that of control histology.

3.1.2. Effect of chrysin on scopolamine induced changes in morris water maze test in control and experiment animals

The cognitive function was assessed by Morris water maze. Test SCP induced animals showed a significantly ($P<0.01$) delayed mean latency on 7th day as compared to the control group. Subsequently, treatment with chrysin (25 mg/kg) improved the memory performance on 7th day significantly ($P<0.05$) against SCP administered group. In animals treatment with Chrysin (50 mg/kg) significantly ($P<0.01$) improves the cognitive function against SCP induced group on 7th day. (Figure 2 A)

Chrysin treated (25mg/kg and 50 mg/kg) efficiently enhanced the time in target quadrant with a significance of ($P<0.05$ and $P<0.01$) respectively on 7th day compared to SCP treated which significantly ($P<0.01$) reduced the same. The memory defect and time spent in target quadrant were improvised well in Chrysin treated animals. (Figure 2 B)

3.1.3. Effect of Chrysin on SCP induced Open field test in control and experimental rats.

The animals induced with SCP showed a significant ($P<0.01$) increase in the duration of immobility on 7th day. Administration of 25mg/kg and 50mg/kg of SCP significantly ($P<0.05$ and $P<0.01$) respectively improved the mean time spent in central area of animals versus SCP induced animals on 7th day. (Figure 3 A)

The total distance covered by SCP induced animals in open field test was significantly ($P<0.01$) reduced compared to control animals. In Chrysin treated groups (25mg/kg and 50 mg/kg) shows the significant increase in total distance covered ($P<0.05$ and $P<0.01$) respectively on 42nd day compared to SCP induced group. (Figure 3B)

3.2. Biochemical Observations

3.2.1. Effect Of chrysin on SCP induced changes in the levels of LPO, Protein carbonyls and Nitrite in the hippocampus of control and experimental rats

The SCP induced rats showed a significantly ($P<0.01$) increased level of LPO and when compared to control animals. The level of LPO was found to be decreased significantly ($P<0.05$ and $P<0.01$) respectively on treatment with Chrysin (25mg/kg and 50mg/kg) . (Figure4)

In SCP treatment, there was a significantly ($P<0.01$) elevated levels of protein carbonyls and NO as compared to control group. However, treatment with Chrysin (25mg/kg and 50mg/kg) significantly ($P<0.05$ and $P<0.01$) respectively reduced those oxidative stress markers level when compared to SCP administered animals.

3.2.2. Effect of Chrysin on SCP induced changes in activities of enzymic antioxidant SOD and CAT in the Hippocampal region of control and experimental rats

Administration of SCP caused significant ($P<0.01$) reduction in the activities of CAT and SOD when compared to control animals. Upon simultaneous treatment with Chrysin (25mg/kg and 50mg/kg) significantly ($P<0.05$ and $P<0.01$) respectively improved their activities as compared to SCP administered animals .(Figure 5).

3.2.3. Effect of Chrysin on SCP induced changes in activities of GR , GPx and GST in the hippocampus region of control and experimental rats

Administration of SCP resulted in significant ($P<0.01$) loss of activities of Glutathione family enzymes . GR and GPx as compared to control animals. Subsequent treatment with Chrysin (25mg/kg and 50mg/kg) raised those antioxidant enzyme activities significance of ($P<0.05$ and $P<0.01$) respectively when compared to SCP induced animals respectively (Figure 6)

3.2.4. Effect of Chrysin on SCP induced changes in the activities of GSH in the hippocampus region of control and experimental rats

Administration of SCP resulted in significant ($P<0.01$) loss of Gsh level as compared to control animals. Subsequent treatment with Chrysin (25mg/kg and 50mg/kg) raised those level significances of ($P<0.05$ and $P<0.01$) respectively when compared to SCP induced animals respectively (Figure 7).

3.2.5 Effect of Chrysin on SCP induced change in the activities of Ache in hippocampus of control and experimental rats

SCP induced group resulted in significant ($P<0.01$) decrease in levels of GST, as compared to control animals. Subsequent treatment with Chrysin (25mg/kg and 50mg/kg) raised those antioxidant enzyme activities significantly ($P<0.05$ and $P<0.01$) when compared to SCP induced animals (Figure 8)

3.2.6. Effect of Chrysin on SCP induced change in the activities of Na⁺/K⁺ATPase and Ca²⁺ATPase activities in Hippocampus of control and experimental rats.

Animals with SCP administration group showed significantly ($P<0.01$) decreased Na⁺/K⁺ATPases activity as compared to control animals. Upon treatment with Chrysin (25mg/kg and 50mg/kg) raised those activities in the significance of ($P<0.05$ and $P<0.01$) respectively when compared to SCP induced animals (Figure 9). The SCP administration significantly ($P<0.01$) decreased Ca²⁺ATPases activity as compared to control animals. Upon treatment with Chrysin (25mg/kg and 50mg/kg) raised those activities significantly ($P<0.05$ and

$P < 0.01$) when compared to SCP induced animals respectively (Figure 9)

3.3. Histological examination of hippocampus sections

3.3.1. Effect of Chrysin on SCP induced pathological changes in the H&E stained hippocampus sections of control and experimental rats

Hippocampus of brain tissues of control, SCP-induced dementia, and treated groups were examined using H&E staining for examination of abnormal structures. A) The hippocampus of control rats showed normal neuronal tissue formed of glial cells and astrocytes without any signs of neurodegeneration. B) In SCP-induced memory impairment group showed more degenerative cells and congested blood vessels compared with the other Chrysin treated groups. This is indicated by neuron swelling, vacuolation, gliosis with condensed nuclei and acute inflammation. C) In Chrysin (25mg/kg) treated animals hippocampus region showed degenerative neurons and astrocytes and normal neuronal cells along with glial cells. D) In Chrysin (50mg/kg) treated animals hippocampus region showed lesser degeneration and healthy neuron, astrocytes and glial cells indicates the neuroprotective effect of Chrysin against Scopolamine induced cognitive impairments. (Figure 10).

4. DISCUSSION

Cognition deficits produced by cholinergic antagonism mimic the cognitive symptomology of Dementia. Dementia is characterized by an insidious degradation of memory, associated with neurobehavioral disturbances [43]. Therefore, alternative and complementary phytochemicals and extracts are being utilized in the management of memory impairment [44]. In the present study, we investigated the effects of chrysin on the scopolamine induced dementia. The effect of Chrysin were measured using the morris water maze test, open field test and the novel object recognition test. Scopolamine-induced dementia has been used extensively to assess potential therapeutic agents for treating cognitive impairment and memory loss.

Scopolamine is a muscarinic cholinergic receptor antagonist associated with cholinergic dysfunction, which causes performance deficits in learning and memory [45]. In this study, scopolamine was administered to rodents for 1 week to induce cholinergic neurodegeneration along with cognitive deficits. Following 6 days of scopolamine administration, the scopolamine treated group had less than 20% of the recognition index of other

groups. Treatment with Chrysin ameliorated memory impairment caused by scopolamine. comparison of scopolamine treated group in a dose-dependent manner.

The rats exposed with SCP have undergone neurobehavioral changes which was elucidated by Morris water maze, novel object recognition and open field test to examine the excellence of Chrysin because of its neuroprotective activity which on 7th day reading was recorded significantly raised the mean fall-off time in SCP delayed the transfer latency and reduced the time spent in target quadrant in morris water maze experiment, suggesting cognitive impairment. Our results in open field test of SCP resulted in rats showed greater period of immobility during induced condition compared to Chrysin treated rats which symptoms resembles behavioural abnormalities

Lipid peroxidation is an important indicator of neurodegeneration of brain. The neuronal membranes contain a very high percentage of long chain polyunsaturated fatty acids because they are used to construct complex structures needed for high rates of signal transfer. ROS are generated continuously in nervous tissues during normal metabolism and neuronal activity. The brain is subjected to free radical induced lipid peroxidation because it uses one-third of the inspired oxygen. Lipids and proteins, the major structural and functional components of the cell membrane, are the target of oxidative modification by free radicals in neurodegenerative disorders. [46] In this study the level of LPO was apparently increased in SCP treated rats which was perceivably decreased in Chrysin treated groups in dosage of 50mg/kg/b.w compared to 25mg/kg/b.w, the higher dosage shows a decline of LPO.

In our study the level of protein carbonyls are increased in SCP treated rats, commonly there is an elevated of carbonylated protein in Dementia symptom which is said to be a biomarker of oxidative injury within the CNS. The Chrysin treated (50mg/kg/b.w) rats shows the decline in the level of carbonylated protein compared to induced group. The level of nitric oxide was elevated in SCP treated rats mainly involved in Hippocampal injury, due to the appearance of the inducible form of nitric oxide synthase (iNOS.) The level of NO was subsequently recessed in Chrysin treated groups. In comparing, the dosage 25mg/kg/b.w and 50mg/kg/b.w, the higher dosage shows an apparent decline of NO.

Recently, many studies have reported that memory impairments is associated to oxidative damage in the scopolamine-induced dementia in rats [47].

Reactive oxygen species causes permanent irreversible oxidative damage to neurons which further aggregates to loss of memory and dementia [48]. The alterations in level of antioxidants such as superoxide dismutase (SOD), catalase, reduced Glutathione (GSH) Glutathione peroxidase (GPx), Glutathione-S-transferase (GST), non-enzymatic antioxidants Glutathione reductase (GR) and nitrate finally may lead to changes in neurochemicals Na^+ - K^+ ATPase, Ca^{2+} -ATPase which are increasingly considered to play a causative role in the pathogenesis and pathophysiology of developing Dementia

The key players in β -oxidation and lipid synthesis are peroxisomes, produces higher amounts of H_2O_2 which is normally unloaded by peroxisome-bound catalase (CAT) and SOD. In this present study SCP treated rats reported to have decreased amount of CAT which was remarkably increased in Chrysin treated groups, the level was slightly higher in rats treated with 50mg/kg/b.w groups. . In SCP treatment, the rate of $\text{O}_2 \bullet^-$ scavenging and conversion to H_2O_2 is low in Hippocampal tissues as the level of SOD was reduced whereas in Chrysin treated rats showed the significantly increased level of SOD.

Lipid peroxidation may enhance due to depletion of GSH content in the brain, which is often considered as the first line of defence of the cell by this endogenous antioxidant against oxidative stress [49]. Glutathione peroxidase require Glutathione (GSH) as an electron donor for the conversion of H_2O_2 to H_2O + O_2 which there is a reduction in GSH, GST and GPx levels during SCP treatment while in our results suggest that the level of antioxidant enzymes are markedly increased in Chrysin treated rats but distinguish the level was higher in 50mg/kg/b.w Chrysin treated rats than 25mg/kg/b.w.

The model of dementia shows, changes in Na^+ / K^+ ATPase and Ca^{2+} ATPase level which would be expected to import injurious levels of Ca^{2+} . The ATPase predicted to have a protective effect, either by prevention.

The central cholinergic system plays a vital role in memory processes. Dysfunction of ACh-containing neurons among age people with cognitive impairments [50,51] The present data were with [52], which indicated that SCO produced severe deficits in cholinergic neuron and augmented AChE activity and expression in the hippocampus enhancing the neurodegeneration in the brain. In this study the level was decreased in SCP treated groups whereas the level was abrogated in Chrysin treated group but significant increase was noticed in

50mg/kg/b.w treated groups. The level of Ache was commonly reduced in SCP model of Dementia also shows the decreased level of Ache while in Chrysin (25mg/kg/b.w and 50mg/kg/b.w) treated groups showed the variable difference compared to induced groups.

Histopathological evidence shows the neurodegeneration, gliosis, congestion of blood vessels and acute inflammation of neuronal cells in hippocampus region of scopolamine treated on comparing with the control animal normal hippocampus neuron cells. In CN (25mg/kg) with SCP treated showed mild recovering of neuronal cells along with inflammation while comparing with SCP+ CN (50 mg/kg) treated shows well recovered neuron, astrocytes and glial cells.

The presented data in this study also suggests that chrysin possesses potent antioxidant activity by scavenging ROS and exerting a neuroprotective effect against oxidative damage induced by scopolamine. Predominant role of AChE inhibition, antioxidant activity reveals an important contributory factor to the beneficial effects of chrysin against dementia. Higher dose of chrysin i.e. 50 mg/kg, i.p. was found more neuroprotective in all behavioural and biochemical evaluations. At lastly, the neuroprotective effects of chrysin might result from the regulation of AChE and the anti-oxidative defence system. These results suggest that chrysin can be used as a preventive herbal drug to impede cholinergic dysfunctions and oxidative stress.

5. CONCLUSION

It was concluded that administration of scopolamine could cause dementia via increase AChE levels and oxidative stress. Scopolamine mediated dementia is mainly associated with cognitive and memory impairments in behavioural models. On the basis of this study, the vital biomarkers of Dementia acetylcholine esterase enzymes and histopathological changes were evaluated according to current protocol schedule to confirm and justify the strong evidence of chrysin treated scopolamine mediated dementia. Chrysin diminished the acetylcholinesterase level and improves the antioxidant defence system. Further, Chrysin downturned the cognitive impairments induced by scopolamine. Therefore, Chrysin possess neuroprotective effect against the scopolamine induced dementia in rats.

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