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EVALUATION OF THERAPEUTIC POTENTIAL OF *CLEOME VISCOSA* ON CORTICOSTERONE INDUCED EXPERIMENTAL DEMENTIA IN MICE

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ABSTRACT
The present study was aimed to assess the effect of *Cleome viscosa* extract on learning and memory, brain cholinesterase levels and the oxidative stress markers in corticosterone treated mice. Mice weighing about 25-30 g were randomized into 12 groups. The extract was administered orally in three doses (100, 200 and 400mg/kg) for a period of 21 days, 30 minutes before corticosterone injection. Piracetam (200mg/kg i.p.) was used as a standard drug. Corticosterone in a dose of 5mg/kg was administered subcutaneously for 21 days. Behavior models, elevated plus maze and Morris water maze were used to assess the cognitive functions. Effect of extract on biochemical parameters (brain cholinesterase level, lipid per oxidation, superoxide dismutase and reduced glutathione) was also assessed in the brain tissue of mice. Corticosterone treated mice showed significant impairment in acquisition and retention as compare to vehicle control group. Pre treatments with *Cleome viscosa* extract for 21 days showed significant decrease in transfer latency in elevated plus maze and increase in time spent in target quadrant in Morris water maze compared to the negative control. Corticosterone cause significant increase in brain cholinesterase level, level of lipid per oxidation and reduce the level of antioxidant enzymes, superoxide dismutase and glutathione. Pre treatment with *Cleome visocsa* extract resulted significant decrease in brain cholinesterase level and lipid per oxidation and increase in the reduced glutathione and superoxide dismutase levels as compared to negative control. These results suggest that plant have a potential role in the management of cognitive dysfunctions.

KEYWORDS- Alzheimer's disease, dementia, elevated plus maze, morris water maze, lipid per oxidation, glutathione, superoxide dismutase.

INTRODUCTION
Dementia is a disorder in which there occurs a significant difficulty with functioning daily activities due to problems with thinking and memory. It is a clinical syndrome characterized by global cognitive impairment, which represents a decline in the previous level of functioning and also associated with impairment in functional abilities, behavioral and psychiatric disturbances.[1] Sign and symptoms of dementia are gradual increasing loss of memory, confusion, delusions, loss of interest in daily activities and loss in thinking ability. Risk factors of dementia are Sociodemographic factors (Age and education, gender and hormonal effects) and Stroke and vascular risk factors. Dementia is classified as Alzheimer disease, vascular dementia, Dementia with lewy bodies, Parkinson's disease dementia, Frontotemporal lobe dementia.[2]

Alzheimers disease
Alzheimer's disease is a form of cortical dementia which is characterized by massive loss of neurons and disrupted signaling between cells in the brain, it is the most common form of the dementia which occurs among older people above the age of 60 years.[3]

The early and most affected feature of Alzheimer's disease is memory impairment. It is a progressive and irreversible neurodegenerative disorder that is characterized by the appearance of amyloid fibrils and plaques.[4] These plaques are formed by the polymerization of the β-amyloid (Aβ) proteins that are

derived from the *β*-amyloid precursor protein. APP is hydrolyzed by *β*-secretase and by the *γ*-secretase complex.[5]

Various theories are there for understanding the pathophysiology of alzheimer's disease[6]

- Senile plaques
- Neurofibrillary tangles
- Acetylcholine deficit

Oxidative stress

- Inflammation
- Excitotoxicity
- Mitochondrial dysfunction

Senile plaques- Senile plaques are polymorphous β -amyloid protein deposits which are found in the brain of Alzheimer disease and normal aging. This β -amyloid protein is derived from a larger precursor molecule of which neurons are the principal producers in brain. Amyloid precursor protein (APP)-immunoreactive neurites are involved in senile plaques formation in Alzheimer dementia.[7] The genetic evidence suggests that aggregation of *A*β to form senile plaques (SP) is an essential component of AD pathophysiology.[8] These deposits of *A*β are insoluble and the formation process of *A*β is irreversible. In alzheimer's brain *A*β deposits form β pleated sheets which serves as a major constituent of senile plaques.[9]

Neurofibrillary tangles- The another major cause of Alzheimer's disease (AD) is a predominant occurrence of neurofibrillary tangles (NFTs).[10] The neurofibrillary tangles are thought to interfere with the cytoskeletal integrity and to induce neuronal as well as synaptic death. Limbic areas play essential role in the neural control of memory function and they are selectively vulnerable to the formation of NFT in the course of aging.[11]

Acetylcholine deficit- Acetylcholine is an important neurotransmitter which is found in brain regions involved in memory. In Alzheimer patient there occurs a decrease in the level of acetylcholine in brain due to cholinergic abnormalities like down regulation of choline acetyltransferase (an enzyme involved in synthesis of acetylcholine) in hippocampus and frontal cortex. The cholinergic neurons count in the nucleus basalis is generally lowered in Alzheimer's brain.[10, 11]

Oxidative stress- Oxidative stress occurs when there is an imbalance between the production and quenching of free radicals from oxygen species. These reactive oxygen species (ROS) play a role in many chronic diseases including mitochondrial diseases, neurodegenerative diseases, renal disease, arteriosclerosis, diabetes, cancer and systemic lupus erythematosus.[12] Three of the important aspects of mitochondrial oxidative phosphorylation for disease pathogenesis are- energy production, generation of free radicals, regulation of programmed cell death.[13] [14]

It is also reported that aggregated Aβ peptides can also induce production of proinflammatory cytokines (IL-1b and TNF-a), chemokines (MIP-1a, MIP-1b and MCP-1), and nitric oxide (NO) mainly by microglia.[14]
Therefore, this approach aimed at reducing oxidative stress associated with Alzheimer's disease may prove worthwhile in the management of this disease.

MATERIALS AND METHODS

Plant collection and extraction- The plant *Cleome viscosa* was collected from local areas of Dehradun Uttarakhand. The authentication of *Cleome viscosa* was confirmed from Forest Research Institute, Dehradun, after submission of a herbarium.

Sample of plant (upper part) was air dried at room temperature and powdered. The powdered material of plant *Cleome viscosa was* refluxed with 90% of ethanol in a Soxhlet extractor for 48 hrs in batches of 350g each. Every time, before extracting with the next solvent the marc was dried. The extract so obtained was concentrated in vacuum using rotary flash evaporator. Finally the solvent was removed completely over the water bath. The extract so obtained was weighed and the yield was calculated in terms of grams percent of the weight of the powdered plant.

Animal- Swiss albino mice of either sex weighing 25-30g, three to four month of age were procured from animal house facility of Shri Guru Ram Rai Institute of Technology and Science, Patel Nagar, Dehradun, Uttarakhand, India. Animals were housed in an air conditioned animal room at 23±2°C with 12/12 h light and dark photo period, with free access to food and water. The animals were acclimatized to the laboratory conditions for at least seven days prior to the behavioral experiments.

The care of laboratory animals and all the procedures involving animals were performed in strict accordance with the CPCSEA, Ministry of forest and environment Government of India. The IAEC (Regd. No.264/CPCSEA) approved the experimental protocol prior to the commencement of experiments (No.-Mph/IAEC/01/2014/ECC-11)

Experimental induction of dementia- The animals were dosed for 21 days with corticosterone (5mg/kg s.c.) 30 min after the drug administration.

Experimental protocol

Six groups to be employed in present study for elevated plus maze and each group contain six animals, as follows
Group 1- Normal control group- Vehicle treated
Group 2- Disease induced group- Corticosterone (5mg/kg, s.c.) was administered for 21 days

Group 3- Standard group treated with Piracetam (200mg/kg i.p.) for 21 days
Group 4- Test group treated with 100 mg/k of *Cleome viscosa* for 21 days
Group 5- Test group treated with 200 mg/kg of *Cleome viscosa* for 21 days
Group 6- Test group treated with 400 mg/kg of *Cleome viscosa* for 21 days

Six groups to be employed in present study for Morris water maze and each group contain six animals, as follows

- Group 1- Normal control group- Vehicle treated
- Group 2- Disease induced group- Corticosterone (5mg/kg, s.c.) was administered for 21 days
- Group 3- Standard group treated with Piracetam (200mg/kg i.p.) for 21 days
- Group 4- Test group treated with 100 mg/kg of *Cleome viscosa* for 21 days
- Group 5- Test group treated with 200 mg/kg of *Cleome viscosa* for 21 days
- Group 6- Test group treated with 400mg/kg of *Cleome viscosa* for 21 days

Behavioral models for evaluation of learning and memory

Eleavated plus maze- The elevated plus maze serves as an exteroceptive behavioral model for the evaluation of learning and memory. The apparatus for mice consisted of two open arms (16 cm × 5cm) and two closed arms (16cm × 5 cm ×15cm) which are extended from a central platform and the maze is elevated at a height of 25cm from the floor. On the first day of experiment the mouse was placed at the end of the open arm, facing away from the centre of maze. For each animal the transfer latency was recorded at the first day of experiment. Transfer latency is defined as the time taken by the mouse to move into any one of the two covered arms from the open arm with all its four legs. If the animal was not able to enter into one of the covered arms with in 90 sec then it was guided and pushed into one of the covered arms and the transfer latency was assigned as 90s. After this the mouse was allowed to explore for 10 s and then returned to its cage. The evaluation of memory retention was done on the next day that is the second day of experiment, 24 hrs after the trial of first day. The transfer latency on the first day served as an index of learning and acquisition and on the second day served as an index of retrieval and memory.[15]

Morris water maze- Morris water maze for mice consisted of a circular pool (60 cm in diameter, 25 cm in height) which filled to be a depth of 20 cm with water and the temperature maintained at 25°C. The water was made opaque with milk or some other non toxic white colored dye. The tank was divided into four equal quadrants with the help of two threads, fixed at right angle to each other on the rim of the pool. A submerged platform (painted white with top surface 6 cm × 6 cm) was placed 1cm below the surface of water inside the target quadrants (Q4 in present study) of this pool. The position of platform was kept unaltered throughout the training session. Each animal was subjected to four consecutive trials each day with a gap of 5 min for four consecutive days, during which they were allowed to escape on to the hidden platform and to remain there for 20 s. During the training session, the mouse was gently placed in the water between quadrants, facing the wall of pool with drop location changing for each trial, and allowed 120 s to locate submerged platform. If the mouse failed to find the platform within 120 s, it was guided gently on to the platform and allowed to remain there for 20 s. Escape latency (EL) is the time taken by the animal to move from the starting quadrant to find the hidden platform in the target quadrant. EL was recorded for each animal. Each animal was subjected to training trials for four consecutive days, the starting position was changed with each exposure as mentioned below and target quadrant (Q4 in the present study) remained constant throughout the training period.

Day 1	Q1	Q2	Q3	Q4
Day 2	Q2	Q3	Q4	Q1
Day 3	Q3	Q4	Q1	Q2
Day 4	Q4	Q1	Q2	Q3

On the fifth day of the experiment, the platform was removed and mouse was placed in any of the three quadrants and allowed to explore the target quadrant for 120s. Mean time spent in all the three quadrants that are, Q1, Q2, and Q3 were recorded. The mean time spent in the target quadrant in search of the missing platform was noted as index of retrieval or memory. The observer must stand at the same position on each day of experiment. Care was taken not to disturb the relative location of water maze with respect to other objects in the laboratory. [16]

Biochemical parameters for evaluation of learning and memory

Estimation of brain cholinesterase activity- Brain cholinesterase activity was estimated by method of Ellman GF *et al.* The esterase activity is measured by providing an artificial substrate, acetylthiocholine (ATC). Thiocholine released because of the cleavage of ATC by acetyl choline esterase is allowed to react with the –SH reagent 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB), which is reduced to thionitrobenzoic acid, a yellow colored anion with an absorption maxima at 412 nm. The extinction coefficient of thionitrobenzoic acid is 1.36×10^4 /molar/centimeter. The concentration of thionitrobenzoic acid is detected using a UV spectrophotometer and taken as a direct estimate of the AChE activity. [17]

Estimation of lipid peroxidation

The estimation of malondialdehyde (MDA), a product of lipid peroxidation was done by this method. One molecule of malondialdehyde reacts with two molecules of thiobarbituric acid (TBA) under mildly acidic

conditions to form a pink color chromogen, the intensity of whose was measured in spectrophotometer at 535nm.[18]

Estimation of reduced glutathione

Glutathione is a major non protein thiol and endogenous antioxidant that counters balance of free radical mediated damage. It is involved in the protection of normal cell structure and function by maintaining the redox homeostasis, quenching of free radicals groups. 5, 5 di thio 2- nitrobenzoic acid (DTNB), a disulphide compound gets easily attacked by tissue sulfhydryl group and forms a yellow colored anion the intensity of which is measured at spectrophotometer at 412 nm.[19]

Estimation of superoxide Dismutase

Superoxide dismutase is involved in the antioxidant defense against ROS by lowering the steady state oxygen level. SOD scavenges the superoxide ions produced as cellular by-products. This enzyme is a major defense for aerobic cells combating the toxic effect of superoxide radicals. It has the ability to inhibit the auto oxidation of epinephrine to adenochrome at pH 10.2. This inhibition can be measured with spectrophotometer at 480 nm. One unit of SOD is defined as the amount of enzyme requires producing 50 % inhibition of epinephrine auto-oxidation.[20]

Estimation of total protein- Total protein estimation was done by the method of Lowry OH et al, 1951. The absorbance was determined spectrophotometrically at 750 nm against prepared blank. The amount of total protein was expressed in mg.[21]

Statistical analysis: The statistical analysis was carried out using Graph pad 5 software. All values were presented as mean ± SEM. The statistical significance of difference between means was calculated by one way Analysis of Variance (ANOVA), followed by Tukey's multiple comparison test in all behavioral and biochemical evaluations except for escape latency in Morris water maze where two way ANOVA was used followed by Bonferroni's post test. Difference level at $P< 0.05$ was considered statistically significant.

RESULTS

Physical characteristics and percentage yield of extract- The yield and physical characteristics of *Cleome viscosa* extract are described in table 1.

Table 1: Physical characterstics and percentage yield of extract

Extract	Colour	Odour	% Extractive value
90 % hydroethanolic	Dark green	Characteristic	7.06%

Preliminary phytochemical screening of *Cleome viscosa* exract- The phytochemical screening of Cleome visocsa extract was performed as per standard tests.[22]

The results of test are given in table 2.

Table 2: Phytoconstituents in *Cleome viscosa* extract

Phytoconstituents	Test	Presence(+) or absence(-) in *Cleome viscosa* extract
Alkaloid	Dragendroff's test	-
Carbohydrate	Molisch test	+
Glycoside	Modified borntragers test	-
Saponins	Froth test Foam test	- -
Phytosterols	Salkowaski's test	-
Phenols	Ferric chloride test	+
Tannins	Gelatin test	+
Flavanoids	Alkaline reagent test	+
Proteins	Xanthoprotic test	+
Amino acids	Ninhydrin test	+

Acute oral toxicity test- The hydroethanolic extract of *Cleome viscosa* when evaluated for acute toxicity studies as per guidelines OECD 423. The observations show grooming during the 14 days after the administration of three doses of *Cleome viscosa* (50 mg/kg, 300 mg/kg and 2000 mg/kg). There were no sign of any hyper reactivity, sedation and convulsion after drug administration. On the basis of acute toxicity study, extract of *Cleome viscosa* was deemed to be extremely safe even at a high dose of 2000 mg/kg. The doses selected for present study

were 100mg/kg (CV100), 200mg/kg (CV200) and 400mg/kg (CV400).

Behavioral evaluation

Effect of *Cleome viscosa* on transfer latency of corticosterone treated mice in elevated plus maze

Administration of hydro-ethanolic extract of *Cleome visocsa* (100mg, 200mg and 400mg p.o.) significantly (P<0.001) reduce the rise in transfer latency (TL) induced by corticosterone as compared to disease induced group indicating protection from corticosterone induced dementia. The effect of CV 200 and CV 400 mg/kg were observed to be comparable to standard treatment of Piracetam while effect of 100mg/kg CV were significantly lesser than that of other treated groups.

Effect of *Cleome viscosa* on escape latency and time spent in target quadrant of corticosterone treated mice in morris water maze

Administration of hydro-ethanolic extract of *Cleome viscosa* (100 mg, 200 mg and 400 mg p.o.) significantly enhanced learning during acquisition trials and resulted in decrease in ELT by 4th day. Further it significantly (P<0.001) increased TSTQ as compared to disease control group indicating protection from corticosterone induced dementia. The effect of CV 400 mg/kg was observed to be comparable with the standard treatment of Piracetam while the effect of CV100 mg/kg and 200 mg/kg were significantly lesser than that of the treated group.

Biochemical Estimation

Effect of ethanolic extract of *Cleome viscosa* on brain AChE activity of corticosterone treated mice

Corticosterone produced significant (P<0.001) increase in brain AChE activity in comparison with vehicle control. However the treatment with ethanolic extract of *Cleome viscosa* (100mg, 20mg and 400mg p.o.) significantly inhibited the corticosterone induced rise in brain AChE activity as compared to disease control group. All doses of CV extract produced comparable effect to the standard treatment of Piracetam.

Effect of ethanolic extract of *Cleome viscosa* on lipid peroxidation activity of corticosterone treated mice

Corticosterone produced a significant (P<0.001) increase in brain oxidative stress which is determined by the levels of MDA during lipid per oxidation in comparison with vehicle control. The treatment with ethanolic extract (100 mg, 200 mg and 400 mg p.o.) significantly (P<0.001) inhibited the corticosterone induced rise in brain oxidative stress. .

Effect of ethanolic extract of *Cleome viscosa* on reduced glutathione level of corticosterone treated mice

Corticosterone produces a significant decrease in glutathione level in comparison with vehicle control group. However the CV extract treated group significantly protected brain from corticosterone induced decrease in brain GSH levels. In other side the standard treatment of Piracetam also significantly attenuated the effect of corticosterone on glutathione level. CV 200 mg/kg and CV 400 mg/kg shows more significant results compare to the low dose of CV 100 mg/kg.

Effect of ethanolic extract of *Cleome viscosa* on superoxide dismutase levels of corticosterone treated mice

Corticosterone treated mice showed a significant decrease in SOD levels in comparision with vehicle control group. However the treatment with ethanolic extract of *Cleome viscosa* (100 mg/kg, 200 mg/kg and 400 mg/kg p.o.) significantly protected the brain from corticosterone induced decrease in brain SOD levels.

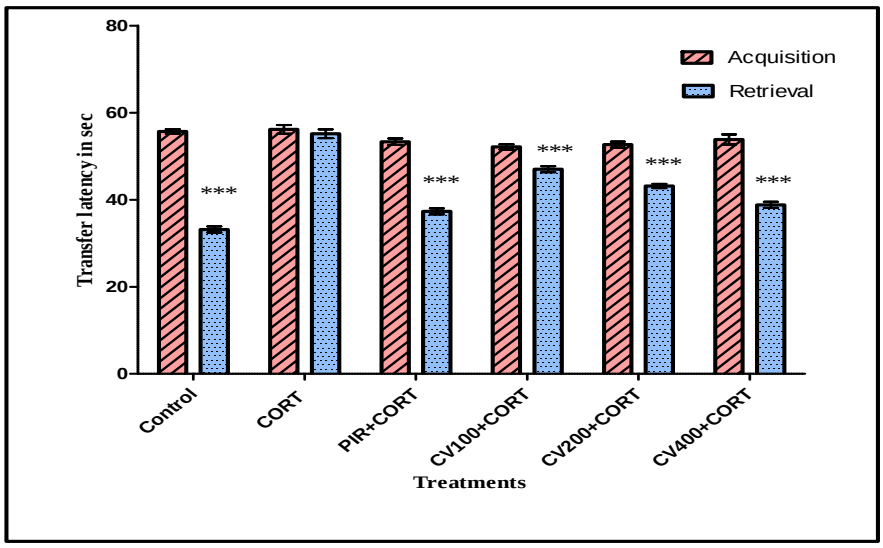

Fig 1: Transfer latency of mice on acquisition and retrieval by elevated plus maze

CORT represents administration of corticosterone for 21 days, PIR+CORT represents administration of 200 mg/kg piracetam 30 min before corticosterone administration, CV100+CORT represents administration of 100 mg/kg of *Cleome viscosa* 30 min before corticosterone administration, CV200+CORT represents administration of 200 mg/kg of *Cleome viscosa* 30 min before corticosterone administration and CV400+CORT represents administration of 400 mg/kg of *Cleome viscosa* 30 min before corticosterone administration
This indicates significance versus acquisition in control group, PIR+CORT group, CV100+CORT group, CV200+CORT group and CV400+CORT group. *** represents significant at p<0.001. All values are expressed as Mean±SEM (n=6)

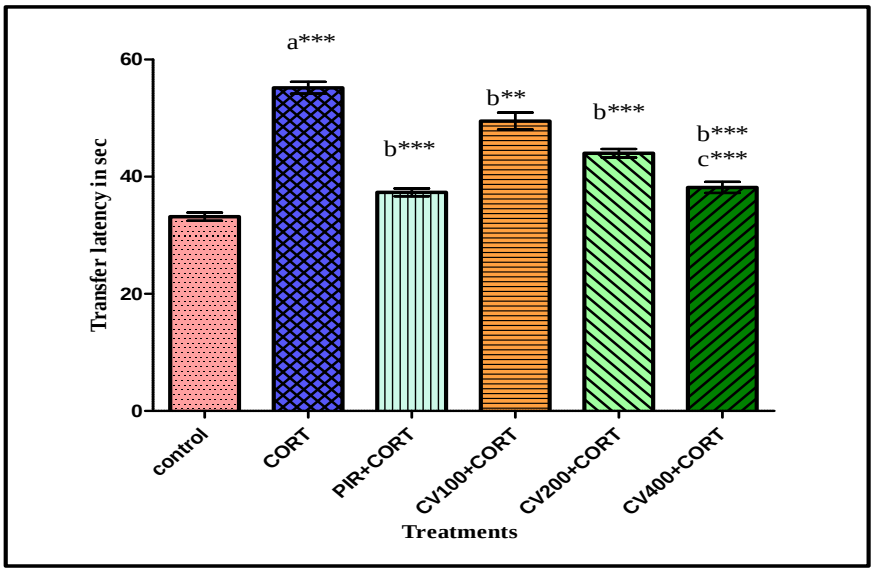

Fig2: Transfer latency of mice on retrieval by elevated plus maze.

CORT represents administration of corticosterone for 21 days, PIR+CORT represents administration of 200 mg/kg piracetam 30 min before corticosterone administration, CV100+CORT represents administration of 100 mg/kg of *Cleome viscosa* 30 min before corticosterone administration, CV200+CORT represents administration of 200 mg/kg of *Cleome viscosa* 30 min before corticosterone administration and CV400+CORT represents administration of 400 mg/kg of *Cleome viscosa* 30 min before corticosterone administration a indicates significance versus vehicle control, b indicates significance versus disease control, c indicates significant versus CV 100 treated group. ** represents highly significant at p<0.01, *** represents very significant at p<0.001. All values are expressed as Mean±SEM (n=6).

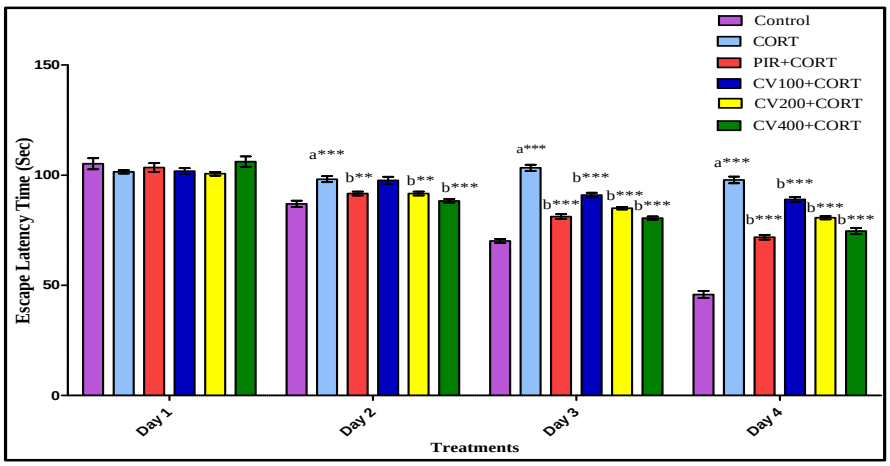

Fig3: **Escape latency of mice using morris water maze for 4 successive days (in sec)**

CORT represents administration of corticosterone for 21 days, PIR+CORT represents administration of 200 mg/kg piracetam 30 min before corticosterone administration, CV100+CORT represents administration of 100 mg/kg of *Cleome viscosa* 30 min before corticosterone administration, CV200+CORT represents administration of 200 mg/kg of *Cleome viscosa* 30 min before corticosterone administration and CV400+CORT

represents administration of 400 mg/kg of *Cleome viscosa* 30 min before corticosterone administration.

'a' indicates significance versus control group, b indicates significance versus diseased (CORT) group. *** represents significant at p<0.001. All values are expressed as Mean±SEM (n=6).

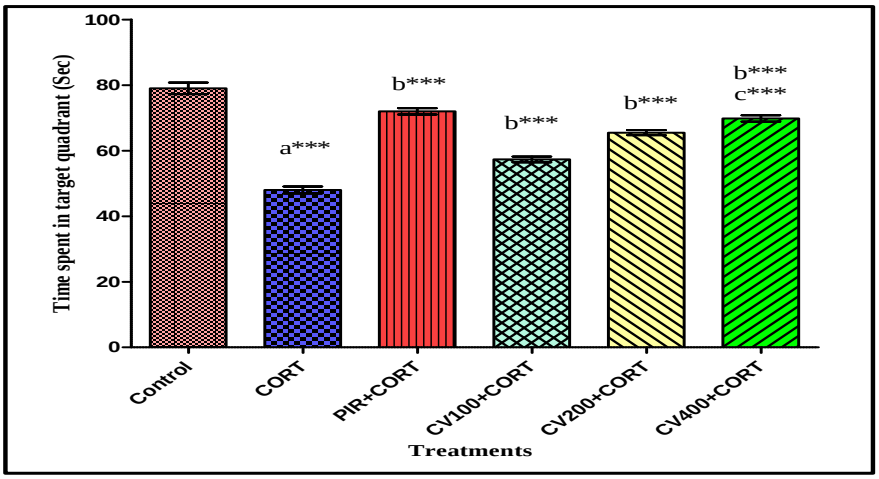

Fig 4: Effect of CV extract on time spent in target quadrant in corticosterone treated mice in Morris water maze

CORT represents administration of corticosterone for 21 days, PIR+CORT represents administration of 200 mg/kg piracetam 30 min before corticosterone administration, CV100+CORT represents administration of 100 mg/kg of *Cleome viscosa* 30 min before corticosterone administration, CV200+CORT represents administration of 200 mg/kg of *Cleome viscosa* 30 min before corticosterone administration and CV400+CORT represents administration of 400 mg/kg of *Cleome viscosa* 30 min before corticosterone administration.

'a' indicates significance versus vehicle control, 'b' indicates significance versus disease control, 'c' indicates significance versus CV 100 treated group. *** represents significant at p<0.001. All values are expressed as Mean±SEM (n=6)

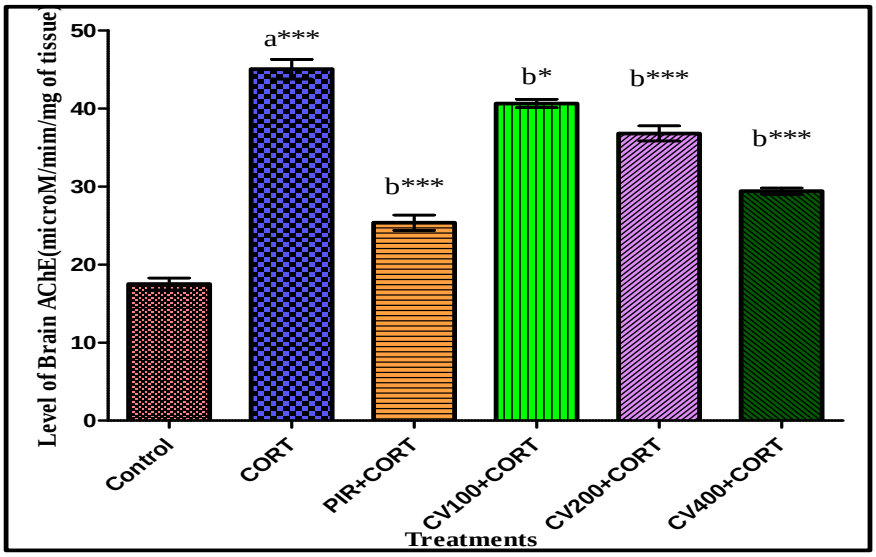

Fig 5:Effect of CV extract on brain Cholinesterase activity in corticostrone treated mice.

CORT represents administration of corticosterone for 21 days, PIR+CORT represents administration of 200 mg/kg piracetam 30 min before corticosterone administration, CV100+CORT represents administration of 100 mg/kg of *Cleome viscosa* 30 min before corticosterone administration, CV200+CORT represents administration of 200 mg/kg of *Cleome viscosa* 30 min before corticosterone administration and CV400+CORT represents administration of 400 mg/kg of *Cleome viscosa* 30 min before corticosterone administration

'a' indicates significance versus vehicle control, 'b' indicates significance versus disease control. *represents

significant at p<0.05. *** represents very significant at p<0.001. All values are expressed as Mean±SEM (n=6).

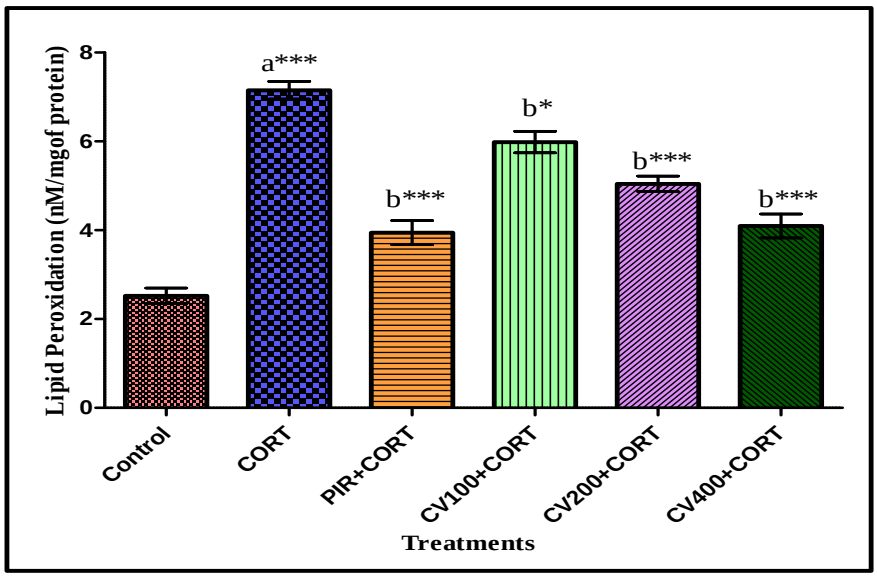

Fig 6: Effect of CV extract on level of lipid peroxidation in corticostrone treated mice.

CORT represents administration of corticosterone for 21 days, PIR+CORT represents administration of 200 mg/kg piracetam 30 min before corticosterone administration, CV100+CORT represents administration of 100 mg/kg of *Cleome viscosa* 30 min before corticosterone administration, CV200+CORT represents administration of 200 mg/kg of *Cleome viscosa* 30 min before corticosterone administration and CV400+CORT represents administration of 400 mg/kg of *Cleome viscosa* 30 min before corticosterone administration

'a' indicates significance versus vehicle control, 'b' indicates significance versus disease control. * represents significant at p<0.05, ** represents highly significant at p<0.01, *** represents very significant at p<0.001. All values are expressed as Mean±SEM (n=6).

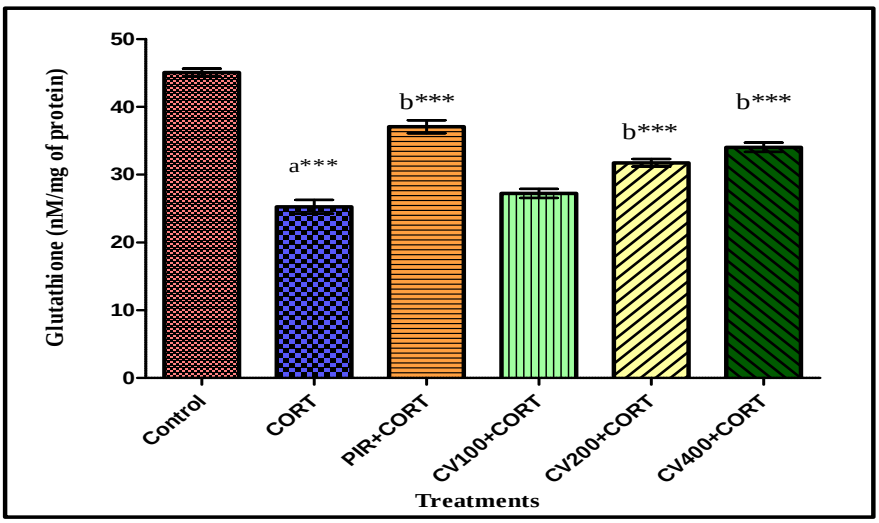

Fig 7: Effect of CV extract on level of glutathione in corticosterone treated mice in Morris water maze

CORT represents administration of corticosterone for 21 days, PIR+CORT represents administration of 200 mg/kg piracetam 30 min before corticosterone administration, CV100+CORT represents administration of 100 mg/kg of *Cleome viscosa* 30 min before corticosterone administration, CV200+CORT represents administration of 200 mg/kg of *Cleome viscosa* 30 min before corticosterone administration and CV400+CORT represents administration of 400 mg/kg of *Cleome viscosa* 30 min before corticosterone administration

a indicates significance versus vehicle control, b indicates significance versus disease control, *** represents very significant at p<0.001. All values are expressed as Mean±SEM (n=6)

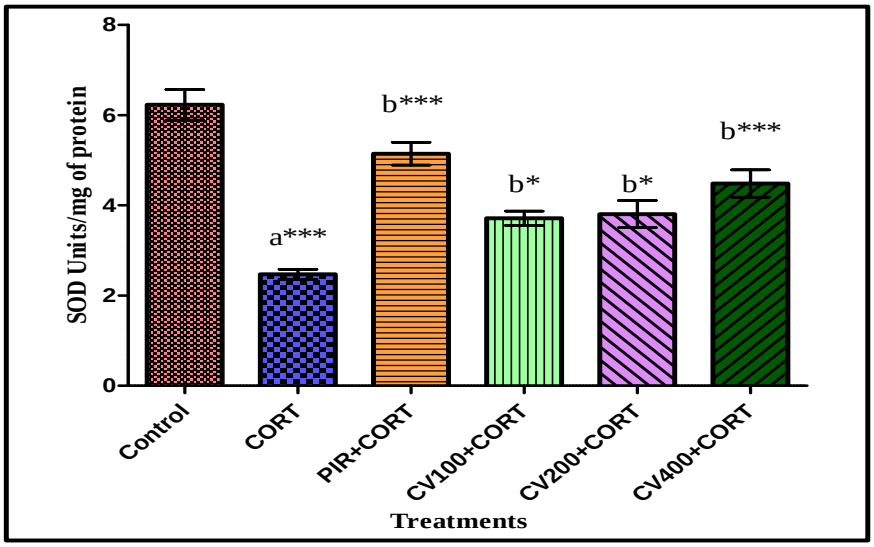

Fig 8: Effect of CV extract on level of superoxide dismutase in corticosterone treated mice.

CORT represents administration of corticosterone for 21 days, PIR+CORT represents administration of 200 mg/kg piracetam 30 min before corticosterone administration, CV100+CORT represents administration of 100 mg/kg of *Cleome viscosa* 30 min before corticosterone administration, CV200+CORT represents administration of 200 mg/kg of *Cleome viscosa* 30 min before corticosterone administration and CV400+CORT represents administration of 400 mg/kg of *Cleome viscosa* 30 min before corticosterone administration

'a' indicates significance versus vehicle control, 'b' indicates significance versus disease control, * represents significant at $p<0.05$, *** represents very significant at $p<0.001$. All values are expressed as Mean±SEM (n=6).

DISCUSSION

Oxidative stress plays a central role in the pathogenesis of AD leading to neuronal dysfunction and cell death. The increased level of oxidative stress in the AD brain is reflected by increased protein and DNA oxidation, enhanced lipid peroxidation, decreased level of cytochrome c oxidase and advanced glycosylation end products. [23]

In present study two behavioral models Elevated plus maze and Morris water maze were used to evaluate the learning and memory in mice. Elevated plus maze, which is very useful for the evaluation of antianxiety agents, has been also helpful for measuring the cognitive performances. Transfer latency of the first day is associated with acquisition of the information and memory while the transfer latency of the second day shows the retention of learning and memory. Our study shows a significant reduction in transfer latency.

Morris water maze test is employed as this is a most widely accepted model which has been used for the evaluation of learning and memory in rodents. Present study shows that the significant decrease in the escape latency time (ELT) which was observed during the exposure to Morris water maze, significant decline was observed in escape latency time at 4th day, and the time spent in target quadrant was found to be increased at 5th day of experiment.

Estimation of brain acetyl cholinesterase activity- AD is associated with intellectual malfunction and subsequent decline in cognitive, behavioral and motor function. Increased levels of AChE in AD patient has lead to the hypothesis that cognitive decline is related to cholinergic degeneration. Therefore promising approach for treating AD is to enhance Acetylcholine concentration in the brain.

Acetylcholinesterase is an important therapeutic target. Reversible inhibitors of this enzyme have been used as cognitive enhancers in the treatment of Alzheimer's disease and other dementia disorders. The activity of this enzyme is found to be lower in Alzheimer disease brain compare to normal brains.

In the present study there was a very significant rise in AChE in the corticosterone treated mice. This rise in enzyme level leads to rapid cleavage of Acetylcholine and there by reduces concentration and turnover of Ach. A significant inhibition of AChE activity has been found in the mice treated with CV extract 100mg/kg, 200mg/kg and 400mg/kg for 21 days. Thus, the plant extract is found to inhibit the rise in AChE activity.

Antioxidant parameters- The excessive generation of free radicals or reactive oxygen species can lead damage to cell and tissue, which results in aging and cell death. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) belongs to the class of main defense antioxidants that prevent the formation of new free radical species. SOD converts superoxide into

hydrogen peroxide whereas GPx changes hydrogen peroxide into harmless molecules. [24]

Present study revealed that the oxidative stress induced by administration of corticosterone for 21 days in mice depletes the superoxide dismutase and glutathione peroxidase and the treatment with the hydro ethanolic extract of CV could restore the activity of both these antioxidant enzymes and it may possibly reduce the generation of free radical and neuronal damage.

Lipid peroxidation- Increase in the level of lipid peroxides in the brain reflects the neuronal damage. The depletion of antioxidant defenses and/or raise in free radical production deteriorates the pro-oxidant antioxidant balance, leading to oxidative stress and cell death.[25] The oxidative stress induced by corticosterone has been associated with increased amount of lipid per oxidation.[26] Indeed the administration of the hydro ethanolic extract of *Cleome viscosa* in our study was potentially effective in reducing the oxidative stress, this suggest that *Cleome visocsa* has antioxidant activity to reduce the oxidative stress induced lipid per oxidation.

CONCLUSION

The purpose of the present study was to evaluate the therapeutic potential of *Cleome viscosa* on corticosterone induced dementia in mice and also found the possible mechanism of action which is involved in its effect on learning and memory. This was confirmed by the following parameters and considerations.

Firstly the phytochemical constituents in the hydro ethanolic extract of *Cleome viscosa* were found to be tannins, flavanoids, proteins, amino acids, phenols and carbohydrate. Corticosterone treated mice showed a significant increase in the acetylcholinesterase activity whereas, increased activity is reversed by treatment with extract of *Cleome viscosa.* This reduced activity of AChE would increase the availability of acetylcholine by decreasing the breakdown. This decrease in the acetylcholinesterase activity by *Cleome viscosa* extract may indirectly or directly influence the cholinergic based learning and memory.

In case of corticosterone treated groups the mice showed decrease in reactive oxygen species scavenging enzyme activities such as superoxide dismutase and glutathione. The activity of these enzymes restored significantly after the treatment with *Cleome viscosa* extract by reducing the generation of free radicals.

In the present study, treatment by *Cleome viscosa* reduced the lipid peroxidation which is indicated by decrease in the toxicant elevated levels of thiobarbituric acid reactive substances (TBARS) like Malondialdehyde. Hence it was concluded that this plant have a potential role in the management of cognitive dysfunctions.

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