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EVALUATION OF NEUROPROTECTIVE EFFECT OF *STEPHANIA GLABRA* (ROXB.) MIERS TUBERS EXTRACT AGAINST COLCHICINE INDUCED COGNITIVE IMPAIRMENT AND OXIDATIVE DAMAGE IN MICE

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ABSTRACT

Aim: The present study was carried out to investigate the neuroprotective effect of *Stephania glabra* (Roxb.) Miers tubers extract against Colchicine induced cognitive impairment and oxidative damage in mice.

Methods and materials: Colchicine (3µg/10 µl), a microtubule disrupting agent when administered intracerebrally in mice resulted in poor memory retention in both Morris water maze, Passive avoidance paradigms and caused marked oxidative stress as indicated by significant increase in malondialdehyde, nitrite levels, depletion of Superoxide dismutase, catalase, and reduced glutathione levels. It also caused a significant increase in the Acetylcholinesterase activity.

Results: Chronic administration of *Stephania glabra* extract (100 and 200 mg/kg; p.o.) for a period of 25 days, starting 4 days prior to Colchicine administration resulted, an improvement in memory retention, attenuation of oxidative damage indicated by significant decrease in malondialdehyde, nitrite levels, and significant increase in Superoxide dismutase, catalase, reduced glutathione and restoration of Acetylcholinesterase activity.

Conclusion: Present study substantiates a neuroprotective effect of *Stephania glabra* (Roxb.) Miers tubers extract against Colchicine induced cognitive impairment and oxidative damage.

INTRODUCTION

Alzheimer's disease (AD) is an age related neurodegenerative disorder comprising complex neurobiochemical and neuropathological events (Hardy et al., 1992). It is characterized by extracellular amyloid-

beta deposits intraneuronal neurofibrillary tangles and selective neuronal loss (Dai et al., 2002). A large number of studies have shown that the cholinergic system plays an important role in learning and memory (Musial et al., 2007, Sivaprakasam et al., 2006).

Central administration of Colchicine inhibits microtubule polymerization by binding to tubulin, causes cognitive dysfunction and oxidative stress in animals (Yu et al., 19997, Kumar and Gupta 2002, Kumar et al., 2007). Sporadic dementia of Alzheimer type (SDAT) is shown to be associated with microtubule dysfunction, which is associated with cognitive impairment (Fu et al., 1986; Grundke-Iqbal et al., 1986). It induces neurofibrillary degeneration by binding to tubulin, the principal structural protein of microtubule (Walsh et al., 1986), which is associated with loss of cholinergic neurones and decrease in acetylcholine transferase, thereby resulting in impairment of learning and memory (Kevin et al., 1989).

Stephania glabra (Roxb.) Miers (Menispermaceae), a large glabrous, climbing shrub indigenous lo the lower Himalayas of India. In traditional medicine, the tubers of the plant have long been used in dysentery, fever, asthma and tuberculosis (Chopra et al., 1958). A number of the most active isoquinoline compounds are quaternary alkaloids, such as berberine, palmatine, 11-hydroxypalmatine, and gindarudine (Semwal and Rawat., 2009b, Semwal et al., 2010b). The activity of protoberberine alkaloids on cholinesterases has been evaluated by numerous authors, mainly regarding the quaternary compounds berberine and palmatine (Ji and Shen 2011, 2012).

The plant has also been evaluated pharmacologically and shown to possess psychopharmacological activity (Khanna et al., 1972), analgesic and antipyretic activity (Semwal et al., 2011), anthelmentic activity (Das et al., 2009, Tandon et al., 2004), antihyperglycemic activity (Semwal et al., 2010a), In-vitro H_1-receptor antagonist activity (Khan et al., 2010), antimicrobial activity (Semwal et al., 2009, 2009a), in- vitro Acetylcholinesterase inhibitory activity (Ingkaninan. K et al., 2003). Due to presence of palmatine, berberine and in- vitro Acetylcholinesterase inhibitory activity the present study is planned to investigate the neuroprotective effect of ethanolic extract of *Stephania glabra* on Colchicine induced cognitive impairment and oxidative damage in mice.

Materials and Methods

Chemicals

5, 5'- dithiobis (2-nitrobenzoic acid) (DTNB), 2-thiobarbituric acid (TBA), 1,1,3,3 tetraethoxypropane (TEP), bovine serum albumin (BSA), Griess reagent were purchased from Sigma chemical (St. Louis, MO). Chloral hydrate, trichloracetic acid (TCA), Acetylthiocholine iodide were purchased from Himedia Co. All others unlabelled chemicals and reagents were analytical grade.

Plant material

Fresh tubers of *Stephania glabra* were collected from the Champawat region of Uttarakhand in the month of April 2012. The tubers were authenticated by Dr. A.K. Gupta, Reader, Department of Botany, Meerut College, Meerut. The voucher specimen (MCM/Bot-1/2012) of the plant material has been deposited in the Department.

Preparation of ethanolic extract and fractionation into tannins fraction

Stephania glabra tubers were dried under shade, coarsely powdered and passed through 40 mesh sieves. Successive solvent extraction scheme was followed for the preparation of extract. Coarsely powdered tubers were defatted with petroleum ether (40-60□C), the dried marc (80 g) was further extracted with ethanol using Soxhlet apparatus for 24 hr to produce the original extract. The solvent was evaporated by rotary evaporation at 35°C, and the remaining solvent was removed by Lyophilization, yielding a dark brown colour mass (12.35g) and stored at 4°C. The yield of the ethanolic extract was found to be 15.43%. Which was subsequently dissolved in water and extraction with chloroform was done in separating funnel and 10% NaCl solution was added drop wise to the aqueous layer in order

to precipitate out the tannins 12.56% (Bruneton. 1999).

Phytochemical Screening

Phytochemical screening of the prepared extract was conducted with various qualitative tests to identify the presence of chemical constituents by standard procedures (Harborne. 1984, Trease, 1972).

Determination of total phenols

Folin-Ciocalteu (FC) reagent was used for the assessment of total phenolic content. The determination was carried out spectrophotometrically according to (Slinkard & Singleton, 1977) with some modifications. 0.1 ml of extract (1mg/ml) was taken in a test tube and diluted with 1.9 ml distilled water and 1.0 ml of Folin– Ciocalteau's reagent was added in a tube, and then 1.0 ml of 100 g/l Na_2CO_3 was added. The mixture was incubated at room temperature for 2 hours and the absorbance of the solution was taken at 765 nm. Total phenol content was estimated from standard curve of gallic acid. The total phenolic compounds of the plant extracts were expressed as gallic acid equivalents (GAE) which indicated the phenolic content equal to the gallic acid (mg) in one gram of dry material.

Experimental model selected for this work: Healthy Swiss albino mice (25-30 g) were used for neuroprotective screening and safety study respectively.

Animal care and selection: adult male swiss albino mice were obtained from the Animal House of M.I.E.T, Meerut. They were housed in polypropylene cages in groups of six mice per cage and kept in a room maintained at 25±2 °C with a 12-h light/dark cycle. They were allowed to acclimatize for 1 week before the experiments and were given free access to standard laboratory feed and water ad libitum. Protocol is approved by Institutional animal ethics committee (711/02/a/CPCSEA/2013).

***3.2.* Acute toxicity study:** The acute toxicity study was performed according to OECD guideline 423, acute toxic class (three animals used). The adult Albino Swiss mice (25-30g) were randomly divided into five different groups containing three animals in each group. The animals were fasted overnight, *Stephania glabra* extract was administered orally at various dose levels (250, 500, 1,000, and 2,000 mg/kg, BW) suspended in 0.5% gum acacia. Fifth group was maintained as control and administered the vehicle only. The animals were observed continuously for 2 h, then occasionally for a further 24 h, and finally any mortality. The behaviour of the animals and any other toxic symptoms were also observed for 72 h, and they were kept under observation for up to 8 days.

Intracerebral (i.c.) administration of Colchicine

Colchicine was injected intracerebrally (i.c.) according to the method of (Haley and McCormick, 1957) in mice anesthetized with chloral hydrate (300 mg/kg, i.p.). A midline sagittal incision was made in the scalp. A 27 gauge hypodermic needle attached to a 100 µl Hamilton syringe was inserted (2.5 mm depth) perpendicularly through the skull into the brain. Colchicine (3 µg/10 µl), dissolved in freshly prepared artificial CSF (aCSF), was administered slowly in a volume of 10 µl by intracerebral (IC) route. The site of injection was 2 mm from either side of the midline on a line drawn through the anterior base of the ears. The syringe was left in the place for an additional 2 min for proper diffusion of Colchicine.

Experimental Design

Group 1: Control mice treated with vehicle of extract (0.5 % (w/v) gum acacia).

Group 2: aCSF (10 µl i.c.) + vehicle of extract (0.5% (w/v) gum acacia).

Group 3: Colchicine treated group (3 µg/10 µl i.c.) + vehicle of extract

Group 4: SGE extract (200 mg/kg, p.o.) + aCSF (10 µl i.c.) *Per se group*

Group 5: SGE extract (100 mg/kg, p.o.) + Colchicine (3 µg/10 µl i.c.)

Group 6: SGE extract (200 mg/kg, p.o.) + Colchicine (3 µg/10 µl i.c.)

To study neuroprotective effect of *Stephania glabra* extract (SGE) in Morris water maze, and step through passive avoidance, extract

was administered at doses of 100 and 200 mg/kg orally daily for a period of 25 days starting from 4 days prior Colchicine administration.

Tests employed for learning and memory functions

For the behavioural tests, each group comprised of 6 mice and during behavioural testing only one animal was tested at a given time.

Morris water maze Test

The acquisition and retention of a spatial navigation task was examined using a Morris water maze (Morris, 1984). Animals were trained to swim to reach a platform in a circular pool (120cm diameter, 50cm height,) located in a darkened test room. The pool was filled with water (26±2ºC) to a depth of 30 cm. Four equally spaced points around the edge of the pool were designed as N (North), E (East), S (South) and W (West). A black coloured round platform of 8 cm diameter was placed 1 cm below the surface of water in a constant position in the middle of the NE quadrant in the pool; the starting point was in the SW quadrant in all the trials. The water was colored with non-toxic black dye to hide the location of the submerged platform. Trials were given for 5 consecutive days in order to train mice in the Morris water maze. The mice were given a maximum time of 60 s (cut-off time) to find the hidden platform and were allowed to stay on it for 30 s. The experimenter put the mice on platform himself that failed to locate the platform. The animals were given a daily session of 3 trials per day. Latency time to reach the platform was recorded in each trial. A significant decrease in latency time from that of 1st session was considered as a successful learning (Tota S et al., 2009).

A probe trial was performed 1 h after the last water maze session (on day 21) to access the extent of memory consolidation. The time spent in the target quadrant indicates the degree of memory consolidation that had taken place after learning. The individual mouse was placed into the pool as in the training trial, except that the hidden platform was removed from the pool. The time spent in target quadrant was measured for 60 s. In probe trial, each mouse was placed at a start position directly opposite to platform quadrant. Further the number of times crossing over the platform site of each mouse was also measured and calculated (Rajasekar et al., 2013).

Passive avoidance test

The mice were subjected to the passive avoidance test by placing in a compartment with light at intensity of 8W in a shuttle box. The light compartment was isolated from the dark compartment by a guillotine door. After an acclimatization period of 30 s, the guillotine door was opened and closed automatically after entry of the mouse into the dark compartment. The subject received a low-intensity foot shock (0.5mA; 10 s) in the dark compartment. Infrared sensors monitored the transfer of the animal from one compartment to another, which was recorded as transfer latency time (TLT) in seconds. The 1st trial was for acquisition and retention was tested in a 2nd trial (1st retention) given 24 h after the 1st trial. Further, 2nd, 3rd and 4th retention trials were given on alternate days. The duration of a trial was 270 s. The shock was not delivered in the retention trials to avoid reacquisition. The criterion for learning was taken as an increase in the TLT on retention (2nd or subsequent) trials as compared to acquisition (1st) trial (Tota S et al., 2009).

Assessment of gross behavioral activity

Gross behavioural activity was observed on day 1, 7, 14 and 21 following i.c. Colchicine injection. Each animal was placed in a square (30 cm) closed arena equipped with infra-red light sensitive photocells using digital photoactometer. The animals were observed for a period of 5 min and the values were expressed as counts/5 min. The apparatus was placed in a darkened, light and sound attenuated and ventilated test room (Reddy and Kulkarni., 1998).

Estimation of biochemical parameters

Acetylcholinesterase and biochemical parameters of oxidative stress, MDA, GSH, SOD, Catalase and nitrite were measured in the brain on the 21st day after Colchicine injection.

Brain tissue preparation:

The mice were decapitated under ether anaesthesia. The skull was cut open and the brain was exposed from its dorsal side. The whole brain was quickly removed and cleaned with chilled normal saline on the ice. A 10% (w/v) homogenate of brain samples (0.03M sodium phosphate buffer, pH 7.4) was prepared by using a homogenizer. The homogenized tissue preparation was used to measure Acetylcholinesterase, NO, MDA, GSH, SOD and Catalase.

Measurement of MDA

MDA, which is a measure of lipid peroxidation, was measured spectrophotometrically by the method of (Colado et al., 1997) using 1, 1, 3, 3-tetraethoxypropane as standard. MDA is expressed as nanomoles per mg protein. To 500 µl of tissue homogenate in phosphate buffer (pH 7.4), 300µl of 30% trichloroacetic acid (TCA), 150µl of 5N HCl and 300µl of 2% (w/v) 2-thiobarbituric acid (TBA) were added and then the mixture was heated for 15 min at 90°C. The mixture was centrifuged at 12,000 g for 10 min. Pink colored supernatant was obtained, which was measured spectrophotometrically at 532 nm.

Measurement of GSH

GSH was determined by its reaction with 5, 5'-dithiobis (2-nitrobenzoic acid) (Ellman. 1959) to yield a yellow chromophore which wasmeasured spectrophotometrically. The brain homogenate was mixed with an equal amount of 10% trichloroacetic acid (TCA) and centrifuged (Remi cold centrifuge) at 2000 g for 10 min. at 4°C. The supernatant was used for GSH estimation. To 0.1 ml of processed tissue sample, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) and 0.4 ml of double-distilled water were added and the mixture was shaken vigorously on vortex. The absorbance was read at 412 nm within 15 min.

Nitrite estimation

Nitrite was estimated in the mice brain using the Greiss reagent and served as an indicator of nitric oxide production (Green LC et al., 1982). 100µl of Greiss reagent (1:1 solution of 1% sulphanilamide in 5% phosphoric acid and 0.1% napthaylamine diamine dihydrochloric acid in water) was added to 100µl of supernatant and absorbance was measured at 542nm. Nitrite concentration was calculated using a standard curve for sodium nitrite.

Superoxide dismutase activity

SOD activity was assayed by the method of (Kono, 1978). The assay system consisted of EDTA 0.1 mM, sodium carbonate 50 mM and 96 mM of nitro blue tetrazolium (NBT). In the cuvette, 2 ml of the above mixture, 0.05 ml of hydroxylamine and 0.05 ml of the supernatant was added and the auto-oxidation of hydroxylamine was measured for 2 min at 30 s intervals by measuring the absorbance at 560 nm using Schimadzu spectrophotometer.

Catalase activity

Catalase activity was assessed by the method of (Luck., 1971), wherein the breakdown of hydrogen peroxide is measured. Briefly, the assay mixture consisted of 3 ml of H_2O_2 phosphate buffer and 0.05 ml of the supernatant of the tissue homogenate. The change in absorbance was recorded for 2 min at 30 s.

Acetyl cholinesterase (AchE) activity

AchE is a marker of extensive loss of cholinergic neurons in the forebrain. The AchE activity was assessed by the Ellman method (Ellman et al., 1961). The assay mixture contained 0.05 ml of supernatant, 3 ml of sodium phosphate buffer (pH 8), 0.1 ml of acetylthiocholine iodide and 0.1 ml of DTNB (Ellman reagent). The change in absorbance was measured for 2 min at 30 s interval at 412 nm using Schimadzu spectrophotometer. Results were expressed as micromoles of acetylthiocholine iodide hydrolyzed per min per mg protein. The specific activity of Acetylcholinesterase is expressed in micromoles/min/mg of protein.

Protein estimation

Protein was measured in all brain samples according to the method of (Lowry et al. 1951)

using bovine serum albumin (BSA) (1 mg/ml) as standard.

Statistical analysis

The results are expressed as mean ± S.E.M. Statistical analysis of passive avoidance, Morris water maze and biochemical values were performed by one-way analysis of variance (ANOVA) followed by Tukey test.

Results and Discussion

Preliminary Phytochemical Screening

Preliminary qualitative phytochemical screening of *Stephania glabra* extract (SGE) showed the presence of alkaloids, phenols, cardiac glycosides, tannins, saponins and steroids and absence of flavonoids.

Determination of Total Phenols

The total phenolic compounds of the plant extracts were expressed as gallic acid equivalents (GAE) which indicated the phenolic content equal to the gallic acid (mg) in one gram of dry material. The total phenolic compounds in *Stephania glabra* extract were found to be (286.12 ± 3.14 mg/g of dry material)

Acute Toxicity Study

From the present study, the ethanolic extract of *Stephania glabra* tubers did not show any mortality and toxic manifestations upto the dose of 2000 mg/kg, orally. Further dosing was not performed to estimate the LD_{50} (lethal dose) value. According to the OECD guidelines 423, for the acute toxicity, an LD_{50} dose of 2000 mg/kg is categorized as unclassified and hence the drug was found to be safe. Based on acute toxicity studies, the doses 100 and 200 mg/kg of the plant extracts had been selected as therapeutic doses.

Tests employed for learning and memory functions

Morris water maze Test

The effect of extract on spatial learning was evaluated using the Morris water maze test. The control ($P < 0.001$) and aCSF ($P < 0.001$) groups had shown improved performance in Morris water maze task in five consecutive trials as revealed by significant decrease in latency time as compared to acquisition trial. However, no significant decrease in latency time ($P > 0.05$) was observed throughout all the sessions in Colchicine treated animals. Colchicine treated group showed significantly higher latency time as compared to the control and aCSF groups ($P < 0.001$). Oral administration of SGE showed dose-dependent effect on Colchicine induced memory deficit in mice. SGE (100 and 200 mg/kg) treatment significantly decreased mean latency time at the 3rd, 4th and 5th sessions (retention) as compared to 1st session (acquisition) ($P < 0.001$). Further, SGE (200 mg/kg) per se treated group had no significant ($P > 0.05$) effect during any session as compared to control and aCSF groups (Fig. 1).

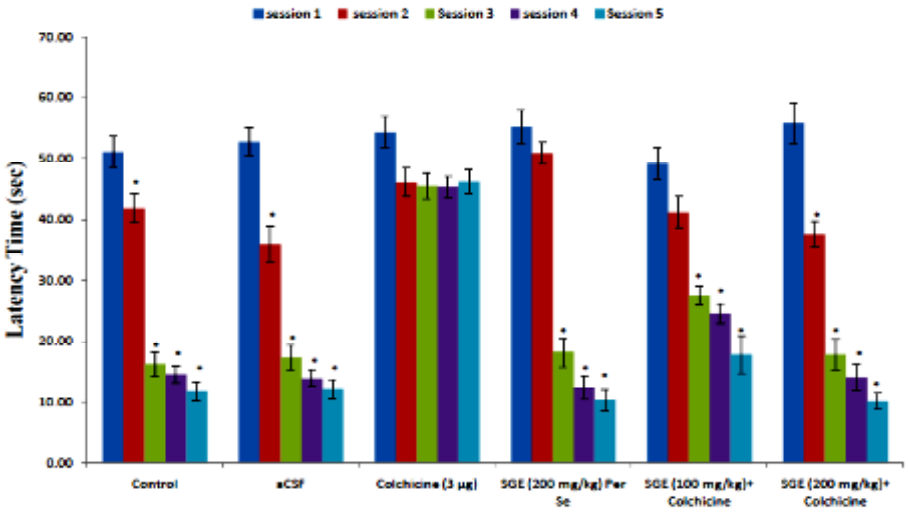

Fig. 1: Effect of SGE on Colchicine (i.c.) induced memory deficit in Morris water maze test in mice, significant decrease in latency time, (*P <0.001) vs. acquisition trial.

The time spent in target quadrant was significantly high in control, aCSF and Per se groups ($P < 0.01$) in comparison to the Colchicine treated group. It was further observed that the target quadrant preference was lost in Colchicine treated mice ($P > 0.05$). The treatment with SGE (100 mg/kg and 200 mg/kg) prevented the memory impairment as indicated by the significant ($P < 0.01$) increase in the time spent in target quadrant as compared to Colchicine treated group (Fig. 2a).

Probe trial study also suggested that Colchicine treated animals showed significantly less ($P < 0.01$) platform crossings when compared with control and aCSF groups, indicating their poor search efficiency for the hidden platform. SGE (100 mg/kg and 200 mg/kg) administration in Colchicine treated mice improved search accuracy as indicated by significantly higher ($P < 0.01$) platform crossings in comparison to Colchicine treated group (Fig. 2b).

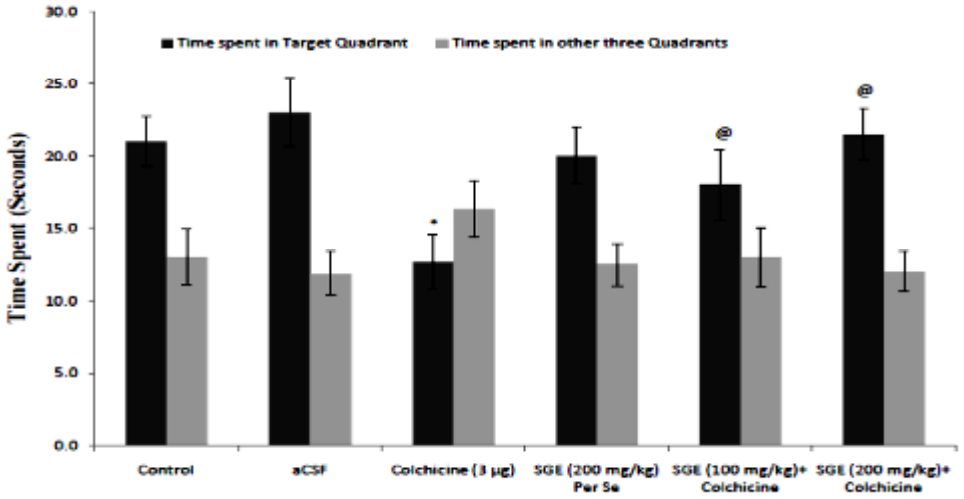

Fig. 2 (a): Effect of SGE on probe trial performance in Morris water maze. Probe trial performance as measured by comparing time spent in the target quadrant with an average time spent in all three non-target quadrants. significant difference in time spent in target quadrant($^{*}P < 0.01$) vs Control and aCSF group and significant difference in time spent in target quadrant ($^{@}P < 0.01$) vs Colchicine (i.c.) treated group.

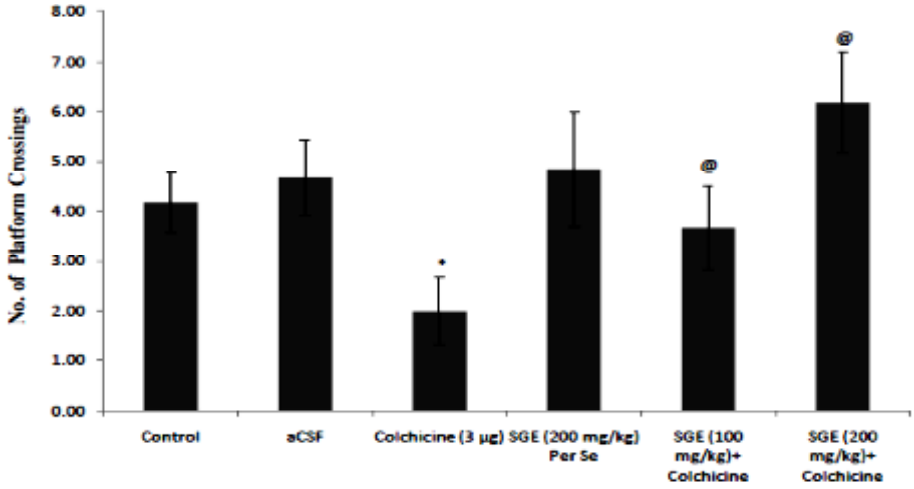

Fig. 2 (b): Effect of SGE on probe trial performance in Morris water maze. Number of platform crossings of training site. During the probe trial, significant decrease ($^{*}P < 0.01$) vs Control and aCSF group and significant increase ($^{@}P < 0.01$) vs Colchicine (i.c.) treated group.

Passive avoidance test

The transfer latency time (TLT) significantly increased in the 1st, 2nd, 3rd and 4th retention trials as compared to acquisition trial in control, aCSF and Per se groups ($P < 0.001$). In the IC Colchicine treated group there was no significant increase in the TLT in the 1st, 2nd, 3rd and 4th retention trials as compared to acquisition trial ($P > 0.05$). However, in the SGE (100 mg/kg and 200 mg/kg) treated groups the TLT of retention trials were significantly higher ($P < 0.001$) than that of the acquisition trial (Fig. 3).

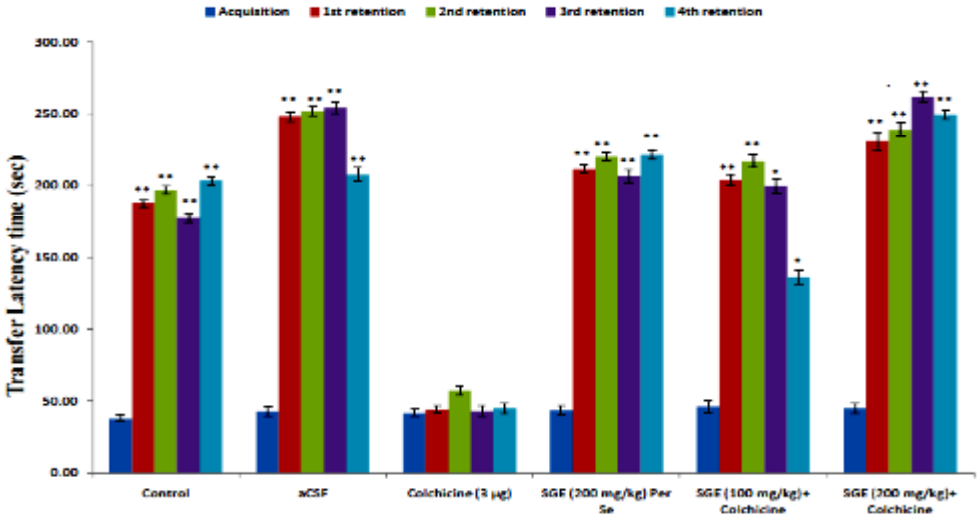

Fig. 3: Effect of SGE on Colchicine (i.c.) induced memory deficit in passive avoidance test in mice, significant increase in transfer latency time (*P <0.01 and **P <0.001) vs. acquisition trial.

Assessment of gross behavioural activity

The spontaneous locomotor activity did not differ significantly among different groups ($P > 0.05$).

Estimation of biochemical parameters

Measurement of MDA

The MDA level (nmol/mg protein) in the brain was measured on day 21 after the IC administration of Colchicine. The level of MDA rise significantly in IC Colchicine treated group ($P < 0.001$) as compared to control and aCSF treated group. . Further, there was no significant difference in the brain MDA level due to SGE (200 mg/kg) per se treatment group as compared to aCSF injected group. On the other hand, both SGE (100 mg/kg) ($P < 0.001$) and 200 mg/kg SGE ($P < 0.001$) significantly decreased MDA level as compared to Colchicine treated group (Fig. 4).

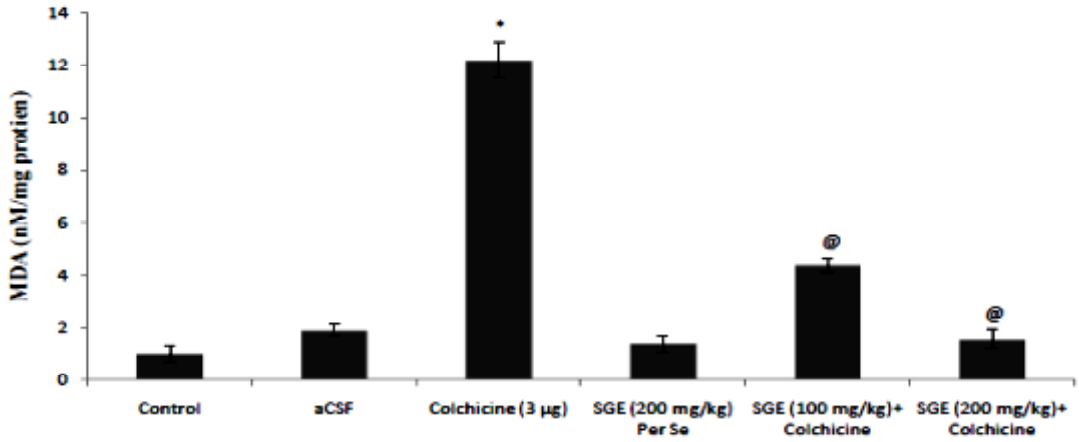

Fig. 4: Effect of SGE treatment on MDA level, significant increase ($^{*}P < 0.001$) vs. control and aCSF group and significant decrease ($^{@}P < 0.001$) vs. Colchicine (i.c.) treated group.

Measurement of GSH

The GSH level (µg/mg protein) in the brain was measured on day 21 after the IC administration of Colchicine. The level of GSH decrease significantly in IC Colchicine treated group ($P < 0.001$) as compared to control and aCSF treated group. . Further, there was no significant difference in the brain GSH level due to SGE (200 mg/kg) per se treatment group as compared to aCSF injected group. On the other hand, both SGE (100 mg/kg) ($P < 0.001$) and 200 mg/kg, SGE ($P < 0.001$) significantly increased GSH level as compared to Colchicine treated group (Fig. 5).

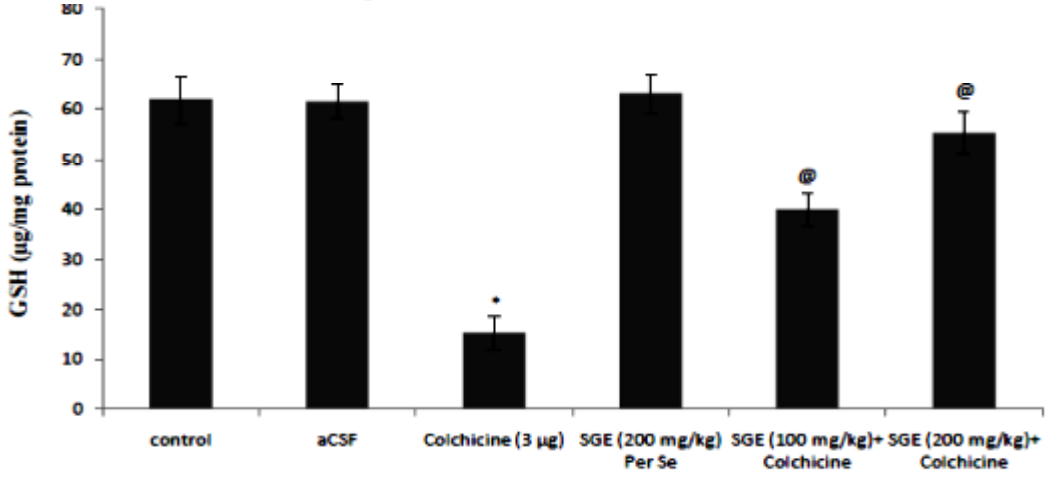

Fig. 5: Effect of SGE treatment on GSH level, significant decrease ($^{*}P < 0.001$) vs. control and aCSF group and significant increase ($^{@}P < 0.001$) vs. Colchicine (i.c.) treated group.

Nitrite estimation

The Nitrite level (µg/mg protein) in the brain was measured on day 21 after the IC administration of Colchicine. The level of nitrite rise significantly in IC Colchicine treated group ($P < 0.001$) as compared to control and aCSF treated group. . Further, there was no significant difference in the brain nitrite level due to SGE (200 mg/kg) per se treatment group as compared to aCSF injected group On the other hand, SGE (100 mg/kg) ($P < 0.001$) and 200 mg/kg, SGE ($P < 0.001$) significantly decreased nitrite level as compared to Colchicine treated group (Fig. 6).

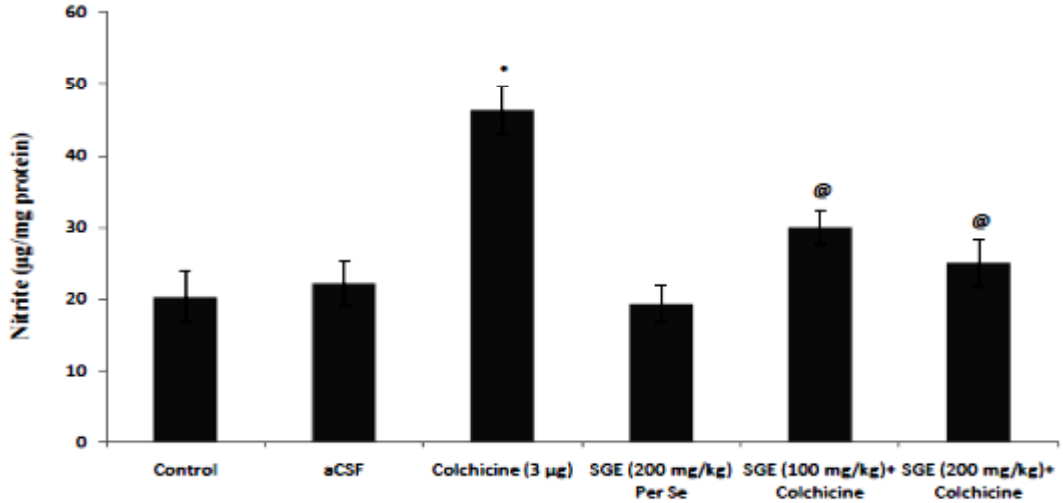

Fig. 6: Effect of SGE treatment on Nitrite level, significant increase ($^{*}P < 0.001$) vs. control and aCSF group and significant decrease ($^{@}P < 0.001$) vs. Colchicine (i.c.) treated group.

Superoxide dismutase activity

The SOD level (U/mg protein) in the brain was measured on day 21 after the IC administration of Colchicine. The level of SOD decrease significantly in IC Colchicine treated group ($P < 0.001$) as compared to control and aCSF

treated group. . Further, there was no significant difference in the brain SOD level due to SGE (200 mg/kg) per se treatment group as compared to aCSF injected group. On the other hand, SGE (100 mg/kg) ($P < 0.01$) and 200 mg/kg, SGE ($P < 0.01$) significantly increased SOD level as compared to Colchicine treated group (Fig. 7).

Catalase activity

The Catalase level (U/mg protein) in the brain was measured on day 21 after the IC administration of Colchicine. The level of Catalase decrease significantly in IC Colchicine treated group ($P < 0.001$) as compared to control and aCSF treated group. . Further, there was no significant difference in the brain Catalase level due to SGE (200 mg/kg) per se treatment group as compared to aCSF injected group. On the other hand, SGE (100 mg/kg) ($P < 0.01$) and 200 mg/kg, SGE ($P < 0.01$) significantly increased Catalase level as compared to Colchicine treated group (Fig. 8).

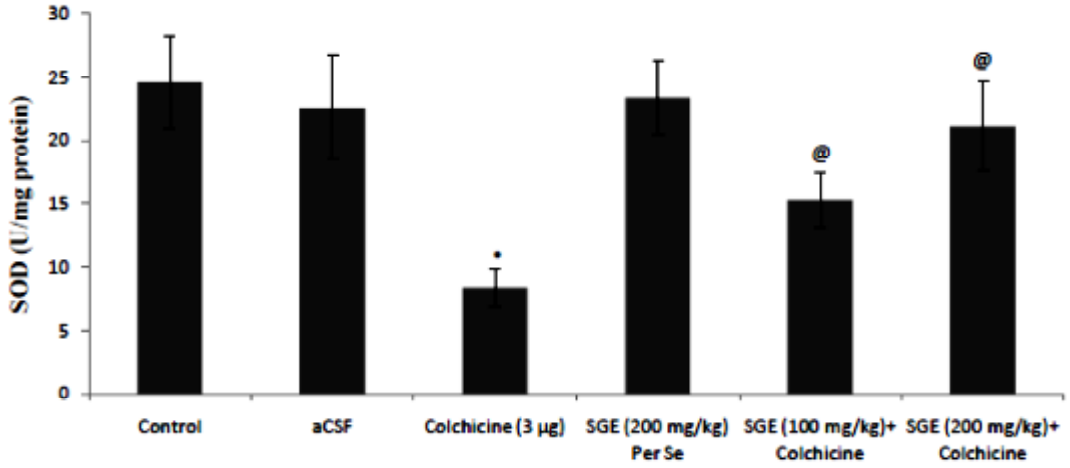

Fig. 7: Effect of SGE treatment on Superoxide dismutase level, significant decrease ($^{*}P < 0.001$) vs. control and aCSF group and significant increase ($^{@}P < 0.01$) vs. Colchicine (i.c.) treated group.

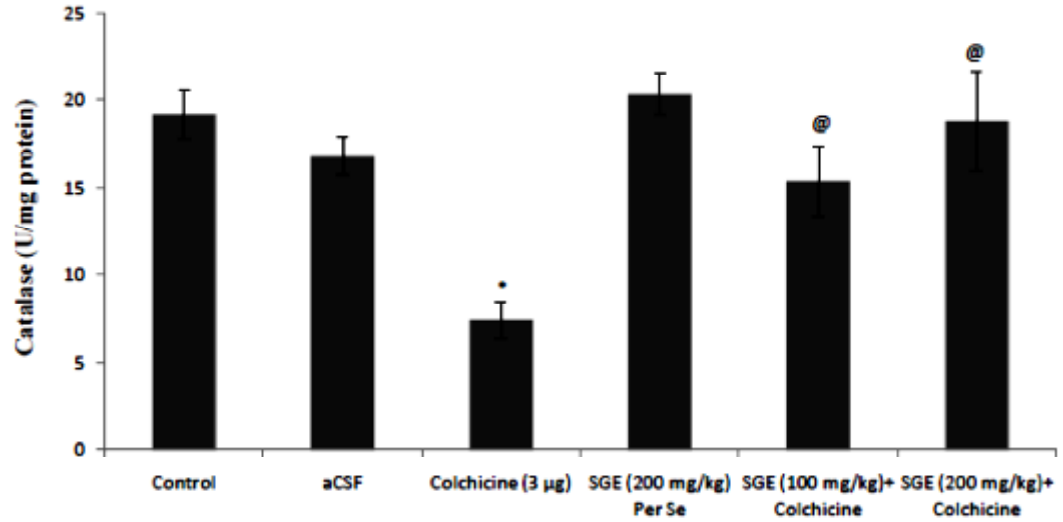

Fig. 8: Effect of SGE treatment on Catalase level, significant decrease ($^{*}P < 0.001$) vs. control and aCSF group and significant increase ($^{@}P < 0.01$) vs. Colchicine (i.c.) treated group.

Acetylcholinesterase (AChE) activity

Acetylcholine esterase activity (micromole/min/mg protein) was estimated on day 21 after the Intracerebral (IC) administration of Colchicine. There was a significant rise in enzyme activity in the Colchicine treated group ($P < 0.001$) as compared to the aCSF (IC) treated group

(0.051±0.007µmol/min/mg protein), SGE (200 mg/kg) Per se group (0.048±0.011µmol/min/mg protein) and control group (0.040±0.006µmol/min/mg protein). Acetylcholine esterase activity in Colchicine treated group that received SGE (100 mg/kg) (0.082±0.017µmol/min/mg protein, $P < 0.001$) and FPE (200 mg/kg) (0.043±0.019µmol/min/mg protein, $P < 0.001$) was significantly less than that of the IC Colchicine treated mice (0.159±0.015µmol/min/mg protein) (Fig. 9).

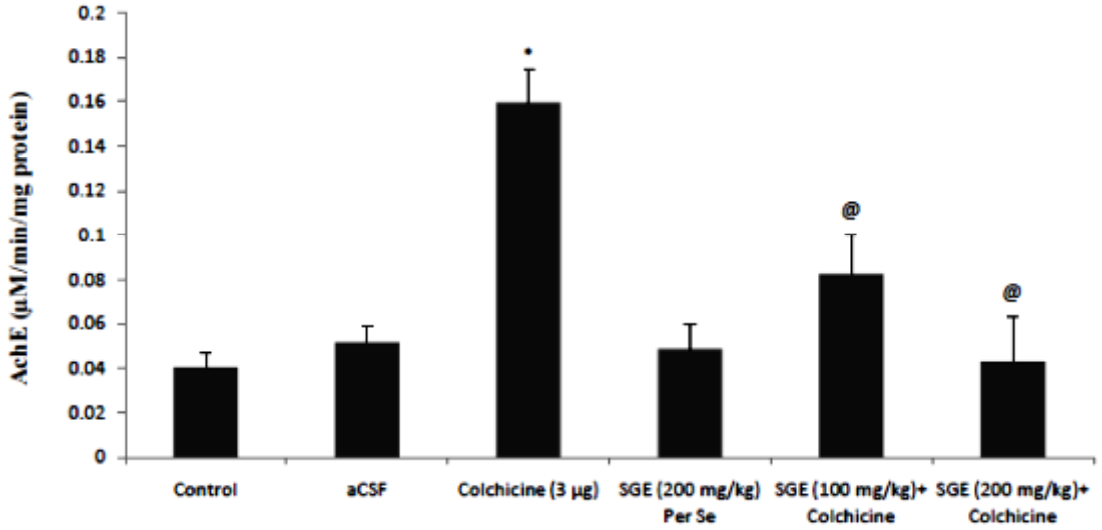

Fig. 9: Effect of SGE treatment on AChE level, significant increase ($^{*}P < 0.001$) vs. control and aCSF group and significant decrease ($^{@}P < 0.001$) vs. Colchicine (i.c.) treated group.

Discussion

The present study investigated the neuroprotective effect of *Stephania glabra* extract treatment on memory impairment, oxidative stress and cholinergic dysfunction in Intracerebral (IC) Colchicine induced model of cognitive dysfunction in mice. The intracerebroventricular (i.c.v.) Colchicine rat model is an appropriate animal model used for study of sporadic Alzheimer type dementia (SDAT) (Kumar et al., 2007). In the present study, colchicine at a dose of 3 µg/10 µL was used. Once administration of colchicine in mice by IC route showed a persistent memory deficit in Morris water maze which is evidenced by no significant reduction in escape latencies in Morris water maze and significantly reduced retention transfer latencies in passive avoidance test.

Intracerebral administration of Colchicine causes increase in free radical generation and the consequent oxidative stress leads to cognitive impairment (Kumar and Gupta, 2002). This is evidenced by increased lipid peroxidation, nitrite level, and decreased glutathione level, Superoxide dismutase and Catalase level. Treatment with *Stephania glabra* tubers extract increased GSH, SOD, Catalase level, decreased lipid peroxidation and Nitrite level.

Central administration of Colchicine induces a direct inflammatory response in the CNS which causes cholinotoxicity (Kumar et al., 2006). All these effects may contribute to the neuroprotective effect of *Stephania glabra* tubers extract. The cholinergic system is responsible for storage and retrieval of memory is the most affected in AD. Central administration of colchicine results in death of dentate granule cells and cerebellar granule cells by activation of caspase-3 (Bonfoco et al., 1995) and upregulation of cyclooxygenase-2 expression (Ho et al., 1998), thereby causing cholinergic deficit. Colchicine causes progressive deterioration of cognition, microtubule disruption and decrease in cholineacetyl transferase activity (Bensimon and Chermat., 1991). These effects of Colchicine are attributed to its ability to cause apoptosis in selected neuronal populations like cerebellar granule cells and basal forebrain cholinergic neurons by activating the c- Jun N-

terminal kinase (JNK) pathway (Muller et al., 2006). In the present study, Colchicine caused a significant decrease in the Cholineacetyl transferase activity which leads to memory deficits, which was ameliorated by treatment with *Stephania glabra* tubers extract.

Colchicine also causes an increase in expression of inducible nitric oxide synthase (Kumar et al., 2006, Laurence et al., 2000) resulting in increased nitric oxide levels which acts as a precursor for the peroxynitrite free radical (Beal, 1995). Overproduction of nitric oxide causes neurotoxicity to cholinergic neurons (Fass et al., 2003). This explains that centrally administered Colchicine caused a significant increase in nitrite levels in the brain and treatment with *Stephania glabra* tubers extract was able to decrease the nitrite levels.

There was no significant difference in locomotor activity of control, aCSF, Colchicine and *Stephania glabra* tubers extract treated Colchicine administered mice. This excludes the possibility that the locomotor activity per se may have contributed to the changes in Morris water maze and passive avoidance behavior.

The main findings of this study indicated that treatment with *Stephania glabra* tubers extract caused a significant enhancement in the memory performance tasks and depreciation of oxidative stress as marked by a decrease in the lipid peroxidation, nitrite levels, and restoration of the glutathione level, superoxide dismutase, catalase and Acetylcholinesterase level.

Conclusion

Our research findings confirm that *Stephania glabra* tubers extract has a protective effect against colchicine-induced cognitive impairment and oxidative stress in mice. This study suggests further investigation to confirm the neurochemical and molecular mechanisms involved in the neuroprotective effect of *Stephania glabra* tubers extract. May be it represents a valid rationale for the use of this plant in the prevention of memory dysfunction and related disorders in future.

References

1. Beal MF. Ageing, energy and oxidative stress in neurodegenerative disease. Ann Neurol 1995; 38: 357–66.
2. Bensimon G, Chermat R. Microtubule disruption and cognitive defects: effect of colchicine on learning behavior in rats. Pharmacol Biochem Behav 1991; 38:141–5.
3. Bonfoco E, Ceccatelli S, Manzo L, Nicotera P. Colchicine induces apoptosis in cerebellar granule cells. Exp Cell Res 1995; 218: 189–200.
4. Bruneton, J., Pharmacognosy Phytochemistry Medicinal Plants, second ed. Lavoisier Publishing Inc., New York, 1999.
5. Chopra, R.N., Chopra, I.C., Handa, K.L., Kapur, L.D., Chopra's Indigenous Drugs of India, 2nd ed. Char UN and Sons, Ltd., Calcutta, India, 1958: 412.
6. Colado MI, O'Shea E, Granados R, Misra A, Murray TK, Green AR. A study of the neurotoxic effect of MDMA ('ecstasy') on 5-HT neurones in the brains of mothers and neonates following administration of the drug during pregnancy. Br J Pharmacol 1997; 121: 827–33.
7. Dai J, Buijs RM, KamphorstW, Swaab DF. Impaired axonal transport of cortical neurons in Alzheimer's disease is associated with neuropathological changes. Brain Res 2002; 948:138–44.
8. Das B., Tandon V., Larisha M. L., Gray A.I., Ferro V. A., Phytochemicals from Flemingia vestita (Fabaceae) and Stephania glabra (Menispermeaceae) alter cGMP concentration in the cestode Raillietina echinobothrida. Comparative Biochemistry and Physiology, Part C, 2009: 149, 397–403.
9. Ellman GL, Courtney KD, Andres Jr V, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961; 7:88–95.

10. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82:70–7.
11. Fass U, Panickar K, Personett D, Bryan D, Williams K, Gonzales J, Differential vulnerability of primary cultured neurons to nitric oxide excess. Neuro Report 2003; 11: 931–6.
12. Fu TK, Matsuyama SS, Kesseler JO, Jarvick LF. Pilothermal response, microtubules and dementia. Neurobiol Aging 1986; 7: 41– 3.
13. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and nitrate in biological fluids. Analytical Biochemistry, 1982: 126 (1), 131–138.
14. Grundke-Iqbal I, Iqbal K, Quinian M, Tung YC, Zaidi MS, Wisniewski HM. Microtubule-associated protein tau: a component of Alzheimer paired helical filaments. J Biol Chem 1986; 261: 6084– 9.
15. Haley TJ, McCormick WG. Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. Br J Pharmacol Chemother 1957; 12:12–5.
16. Harborne, J.B., Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. Chapman and Hall, London, 1984.
17. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. Science, 1992; 256: 353–6.
18. Ingkaninan, K., Temkitthawon, P., Chuenchom, K., Yuyaem, T., Thongnoi, W, Screening for Acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. J. Ethnopharmacol, 2003: 89, 261–264.
19. Ji HF, Shen L. Berberine: a potential multipotent natural product to combat Alzheimer's disease. *Molecules* 2011; 16: 6732–6740.
20. Ji HF, Shen L. Molecular basis of inhibitory activities of berberine against pathogenic enzymes in Alzheimer's disease. *Sci World J* 2012; 2012: 823201.
21. Kevin PN, William RM, Hugh AT. Colchicine induced alterations of reference memory in rats: role of spatial versus non-spatial task components. Behav Brain Res 1989; 35: 45– 53.
22. Khan, N. A., Kumar, D., Bhat, Z. A., Kumar, V., Nagpal, N., Bhujbal, S. S., In vitro H_1-receptor antagonist activity of methanolic extract of tuber of Stephania glabra. Bangladesh J Pharmacol, 2010: 5, 89-91.
23. Khanna, N.K., Madan, B.R.,M ahatma, O.P., Surana, S.C., Some psychopharmacological actions of Stephania glabra (Roxb) Miers: an Indian indigenous herb. Indian Journal of Medical Research, 1972: 60, 472–480.
24. Kono Y. Generation of superoxide radical during auto-oxidation of hydroxylamine and an assay for Superoxide dismutase. Arch Biochem Biophys 1978; 186:189–95.
25. Kumar A, Naidu PS, Sehgal N, Padi SSV, Goyal R. Colchicines-induced neurotoxicity as an animal model of sporadic dementia of Alzheimer's type. Pharmacol Reports 2007; 59: 274–83.
26. Kumar A, Naidu PS, Sehgal N, Padi SSV, Goyal R. Colchicines-induced neurotoxicity as an animal model of sporadic dementia of Alzheimer's type. Pharmacol Reports 2007; 59: 274–83.
27. Kumar A, Sehgal N, Padi SSV, Naidu PS. Differential effects of cyclooxygenase inhibitors on intracerebroventricular colchicine-induced dysfunction and oxidative stress in rats. Eu J Pharmacol 2006; 551(1–3):58–66.
28. Kumar MHV, Gupta YK. Intracerebroventricular administration of colchicine produces cognitive impairment associated with oxidative stress in rats. Pharmacol Biochem Behav 2002; 73: 565–71.

29. Kumar MHV, Gupta YK. Intracerebroventricular administration of colchicine produces cognitive impairment associated with oxidative stress in rats. Pharmacol Biochem Behav 2002; 73: 565–71.
30. Laurence D, Daniele L, Maryvonne W. Differential effects of colchicine on the induction of nitric oxide synthase in neurons containing progesterone receptors of the guinea pig hypothalamus. Brain Res Bull 2000; 52(5):435–43.
31. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193:265–75.
32. Luck H. Catalase. In: Bergmeyer HU, editor. Methods of Enzymatic analysis. New York: Academic Press; 1971: 885–93.
33. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 1984; 11: 47–60.
34. Muller GJ, Geist MA, Veng LM, Willesen MG, Johansen FF, Leist M, et al. A role of mixed lineage kinases in granule cell apoptosis induced cytoskeletal disruption. J Neurochem 2006; 96:1242–52.
35. Musial A, Bajda M, Malawska B. Recent developments in cholinesterases inhibitors for Alzheimer's disease treatment. Curr Med Chem 2007; 14:2654–79.
36. N. Rajasekar, Subhash Dwivedi, Santosh kumar Tota, Pradeep Kumar Kamat, Kashif Hanif, Chandishwar Nath, Rakesh Shukla. Neuroprotective effect of curcumin on okadaic acid induced memory impairment in mice. European Journal of Pharmacology 2013; 715: 381–394
37. Reddy DS, Kulkarni SK. Possible role of nitric oxide in the nootropic and antiamnesic effects of neurosteroids an aging and dizocilpine-induced learning impairment. Brain Res 1998; 799: 215–29.
38. Semwal, D. K., Rawat U., Semwal, R. B., Semwal, R., Singh, R., Antihyperglycemic effect of stephania glabra tubers in alloxan induced diabetic mice. J Medicine, 2010a: 11, 17-19.
39. Semwal, D. K., Semwal, R. B., Semwal, R., Jacob, V., Singh, G., Analgesic and Antipyretic Activities of Gindarudine, a Morphine Alkaloid from Stephania glabra. Current Bioactive Compounds, 2011: 7, 214-217.
40. Semwal, D.K., Rawat, U., Antimicrobial hasubanalactam alkaloid from Stephania glabra. Planta Medica, 2009a: 75, 378–380.
41. Semwal, D.K., Rawat, U., Bamola, A., Semwal, R., Antimicrobial activity of Phoebe lanceolata and Stephania glabra; preliminary screening studies. Journal of Scientific Research, 2009: 1, 662–666.
42. Semwal, D.K., Rawat, U., Gindarudine, a novel morphine alkaloid from Stephania glabra. Chinese Chemical Letters 2009b: 20, 823–826.
43. Semwal, D.K., Rawat, U., Semwal, R., Singh, R., Singh, G.J.P., Antihyperglycemic effect of 11-hydroxypalmatine, a palmatine derivative from Stephania glabra tubers. Journal of Asian Natural Products Research 2010b: 12, 99–105.
44. Sivaprakasam K. Towards a unifying hypothesis of Alzheimer's disease: cholinergic system linked to plaques, tangles and neuroinflammation. Curr Med Chem 2006; 13:2179–88.
45. Slinkard, K., & Singleton, V. L. Total phenol analyses: Automation and comparison with manual methods. American Journal of Enology and Viticulture, 1977: 8, 49-55.
46. Tandon, V., Lyndem, L.M., Kar, P.K., Pal, P., Das, B., Rao, H.S.P., Anthelmintic efficacy of rhizome-pulp extract of Stephania glabra and aerial root extract of Trichosanthes multiloba in vitro: two indigenous plants in

Shillong, India. J. Parasit. Dis. 2004: 28, 37–44.
47. Tota S, Kamat PK, Awasthi H, Singh N, Raghubir R, Nath C. Candesartan improves memory decline in mice: involvement of AT1 receptors in memory deficit induced by intracerebral streptozotocin. Behav Brain Res 2009; 199: 235–40.
48. Trease GE, Evans WC. Pharmacognosy, 10th Ed., Balliere Tindal, London, 1972: 107-378.
49. Walsh TJ, Schulz DW, Tilson HA, Schmechel DE. Colchicine-induced granule cell loss in rat hippocampus: selective behavioral and histological alterations. Brain Res 1986; 398: 23–36.
50. Yu Z, Cheng G, Hu B. Mechanism of colchicine impairment on learning and memory, and protective effect of CGP36742 in mice. Brain Res 1997; 8:750-53.