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Roll of ethyl acetate soluble fraction of the methanol extract of *Plectranthus Vettiveroides* in cognitive enhancement and antioxidant activity on scopolamine-induced amnesia

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ABSTRACT

The intention of the current research was to appraise the cognitive enhancing and antioxidant bustle of *Plectranthus vettiveroides*. The learning and memory was impaired by administration of scopolamine (1 mg/kg, i.p.) in mice which is connected with distorted brain oxidative status. The object recognition test (ORT) and passive avoidance test (PAT) were used to appraise cognitive enhancing activity. Animals were treated with an ethyl acetate soluble fractions of the methanol extract of *P.vettiveroidesat* various doses (25, 50 and 100 mg/kg, p.o). The ethyl acetate soluble fraction of the methanol extract of *P.vettiveroides* (EASFPV) attenuated amnesia induced by scopolamine and aging. The discrimination index (DI) was drastically decreased in the aged and scopolamine group in ORT. Pretreatment with EASFPV significantly enlarged the DI. In PAT, scopolamine-treated mice exhibited significantly shorter step-down latencies (SDL). EASFPV treatment showed a major increase in SDL in young, aged as well as in scopolamine-treated animals. The biochemical analysis of brain exposed that scopolamine treatment increased lipid peroxidation and decreased levels of superoxide dismutase (SOD) and glutathione reductase (GSH). Administration of extract extensively reduced LPO and reversed the decrease in brain SOD and GSH levels. The administration of EASFPV improved memory in amnesic mice and prevented the oxidative stress associated with scopolamine. The mechanism of such protection of *P.vettiveroides* may be due to intensification of cellular antioxidants. The outcome of the present study suggested that *P.vettiveroides* had a shielding role against age and scopolamine-induced amnesia, representing its efficacy in management of cognitive disorders.

Keywords: *Plectranthus vettiveroides*, oxidative stress, scopolamine, Cognitive enhancing, passive avoidance test

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative brain disorder that is slow in onset but leads to dementia, unusual behavior, personality changes, and ultimately death [1]. AD is characterized by the presence of excessive amounts of neuritic plaques containing amyloid β protein and

abnormal tau protein filaments in the form of neurofibrillary tangles. Loss of cholinergic cells, particularly in the basal forebrain, is accompanied by loss of the neurotransmitter acetylcholine [2]. A decrease in acetyl choline in the brain of patients with AD appears to be a critical element in producing dementia [3]. AChE inhibitors from general chemical classes such as physostigmine, tacrine, galantamine, and heptylphysostigmine have been tested for the symptomatic treatment of AD [4]. However, nonselectivity of these drugs, their limited efficacy, poor bioavailability, adverse cholinergic side effects in the periphery, narrow therapeutic ranges, and hepatotoxicity are among the several limitations to their therapeutic success [5]. Therefore, it is worthwhile to explore the utility of other existing medicines for the treatment of various cognitive disorders [6].

Scopolamine, a muscarinic cholinergic receptor antagonist, has been widely adopted to study cognitive deficits in experimental animals. After intraperitoneal (i.p.) injection of scopolamine, the cholinergic neurotransmission was blockaded, leading to cholinergic dysfunction and impaired cognition in rats [7]. Recently, it has been reported that memory impairment induced by scopolamine in rats is associated with altered brain oxidative stress status [8]. Therefore, rats with scopolamine-induced memory deficits were used as an animal model for screening antidementia drugs [9].

Plectranthus vettiveroides is also known as *Coleus vettiveroides*, *Coleus zeylanicus*, *Plectranthus zeynanicus* (Lamiaceae). The main phytochemical components of Iris are diterpenoids, essential oils and phenols. About 140 diterpenes were identified from the colored leaf glands of Platycladus species. The main components of Jerusalem artichoke essential oil are mono and sesquiterpenes. Flavonoids seem to be rare in *Platycladus orientalis*, only two flavonoids have been identified, 4',7-dimethoxy-5,6-cone in *Platycladus orientalis*, thus obtaining viologen from *P.marruboides* And golden chicken essence. Traditionally, it has been used as an antibacterial, deodorant, and cooling agent. It has also been used to prevent headaches and fever from burning eyes. There is no major investigative reports available pertaining to its cognitive enhancing effect. The objective of the study was to investigate the cognitive enhancing and antioxidant potential of roots of *Plectranthus vettiveroides* in scopolamine-induced amnesic mice and in aged mice.

MATERIALS AND METHODS

Plant Identification and Collection

The plant was collected from Namakkal, Tamil Nadu, India in January 2015. The herbarium specimens of plants are stored in the Pharmacognosy Department. The plant was identified by Dr. G.V.S. Murthy, co-director of the Indian Botanical Survey in the South Ring of Coimbatore TNAU campus, who identified the plant with information he obtained from the literature.

Extraction

The roots were dried in the shade and then powdered and 100 g of the dried powder was extracted with methanol using a soxhlet apparatus. The solvent was removed under reduced pressure and controlled temperature using a rotary flash evaporator then the methanol extract of *Plectranthus vettiveroides* was exhaustively extracted with ethyl acetate to obtain ethyl acetate soluble (EASFPV, 1.7 w/w) and ethyl acetate insoluble fractions (EAISFPV, 1.3 w/w). The ethyl acetate soluble fraction was suspended in Tween 80 (0.2% v/v) in distilled water and administered per orally (p.o.).

Animals

Swiss albino mice of either sex (young, age 8 weeks, 18-20 g and aged, age 32 weeks, 35-40 g) were utilized for this work. Animals were housed in polypropylene cages and maintained under the standard laboratory environmental conditions; temperature 25 ± 2ºC, 12 h light: 12 h dark cycle and 50 ± 5% relative humidity with free access to food and water *ad libitum*. Animals were acclimatized to laboratory conditions before the test. Each group consisted of five (n = 5) animals. All the experiments were carried out during the light period (08:00-16:00 h).

Phytochemical screening of *Plectranthus vettiveroides*

Phytochemical screening of the EASF of the methanol extract of *Plectranthus vettiveroides* roots for the existence of flavonoids, glycosides, saponins, alkaloids, and sterols was carried out in accordance with procedures previously described.[10.11]

Treatment schedule

Animals (Young and aged mice) were separated into 16 groups having 5 animals in every group. EASFPV (25, 50 and 100 mg/kg, p.o.) was administered to young and aged mice of diverse groups. Amnesia was induced only in young mice by scopolamine. Groups I, II - Control (Young and aged, 0.2% v/v Tween 80, p.o respectively); Groups III, IV- Standard (Piracetam 200 mg/kg, i.p. in young and aged respectively); Group V- Scopolamine (1 mg/kg, i.p. in young); Group VI, VII, VIII-EASFPV (25, 50 and 100 mg/kg, p.o in young respectively); Groups IX, X, XI - EASFPV (25, 50 and 100 mg/kg, p.o in aged respectively); Groups XII, XIII, XIV- EASFPV (25, 50 and 100 mg/kg, p.o + Scopolamine in young respectively); Group XV- Piracetam (200 mg/kg, i.p. + Scopolamine in young); Group XVI- Donepezil (1 mg/kg, i.p. + Scopolamine in young).

EXPERIMENTAL METHODS

Object recognition test

The object recognition test (ORT) is a behavioral test that is extensively used to scrutinize animal's memory routine. Memory performance in the ORT is based on the natural tendency of animals to explore novel objects. The apparatus consists of an open white colored plywood box (70× 60× 30 cm) with a well-furnished floor. The apparatus is illuminated by a 60 W lamp suspended 50 cm above the box. The objects to be discriminated are made of plywood in two dissimilar shapes of 8 cm and colored black and white. The day before test, mice was given a habituation session where they were left to freely exploring the box for 2 min. No object was placed in the box during the habituation trial. On the day of test, two identical objects were presented in two opposite corner of the box during the first trial (T_1), and the amount of time taken by each mouse to complete 20 s of object exploration was recorded. Exploration was considered as directing the nose at a distance less than 2 cm to the object and/or touching it with nose or forepaw. Turning around or sitting on the object was not considered as an exploratory behavior. During the second trial (T_2, 90 min after T_1), one of the objects presented in T_1 (i.e., familiar object) was replaced by new object and mice was left in box for 5 min. The time spent (s) for exploration of the familiar (F) and new (N) object was recorded separately and the discrimination index (DI) was calculated. DI = N-F/ N + F, where DI= discrimination index, N = exploration of the new object, F= exploration of the familiar object. [12,13] Scopolamine (1 mg/kg) was injected i.p. after 45 min of administration of EASFPV (25, 50 and 100 mg/kg, p.o) or piracetam (200 mg/kg, i.p.) or donepezil (1 mg/kg, i.p.) or vehicle in young mice and first trial was given 45 min after injection of scopolamine. After 45 min of administration of EASFPV (25, 50 and 100 mg/kg, p.o), the first trial was given in aged mice.

Passive avoidance paradigm/test

Passive avoidance behavior based on negative reinforcement was used to examine the long-term memory. The apparatus consisted of a box (27 × 27 × 27 cm) having three walls of wood and one wall of Plexiglass, featuring a grid floor (3 mm stainless steel rods set 8 mm apart), with a wooden platform (10 ×7 ×1.7 cm) in the center of the grid floor. The box was illuminated with a 15 W bulb during the experimental period. Electric shock (20 V AC) was delivered to the grid floor. Training was carried out in two similar sessions. Each mouse was gently placed on the wooden platform set in the center of the grid floor. When the mouse stepped down and placed all its paws on the grid floor, shock was delivered for 15 s and the step-down latency (SDL) was recorded. SDL was defined as the time taken by the mouse to step down from wood platform to grid floor with all its paws. Animals showing SDL in the range (2-15 s) during the first test were used for the second session and the retention test. The second session was carried out 90 min after the first test. When the animals stepped down before 60 s, electric shock was delivered for 15 s. During the second test, animals were removed from the shock-free zone if they did not step down for a period of 60 s. Retention was tested after 24 h in a similar manner, except that the electric shocks were not applied to the grid floor. Each mouse was again placed on the platform, and the SDL was recorded, with an upper cut-off time of 300 s. EASFPV (25, 50 and 100 mg/kg, p.o), piracetam (200 mg/kg, i.p.) and donepezil (1 mg/kg, i.p.) or vehicle were administered orally for 8 days and (SDL) was noted after 45 min of administration of last dose on eighth day and again after 24 h i.e. on ninth day. In the scopolamine-treated group, scopolamine (1 mg/kg) was injected i.p. after 45 min of administration of EASFPV (25, 50 and 100 mg/kg) or piracetam or donepezil or vehicle and SDL was recorded after 45 min of injection of scopolamine on eighth day and after 24 h i.e. on ninth day. On the ninth day after measurement of SDL, the animals were sacrificed by cervical dislocation and

antioxidant parameters such as lipid peroxidation (LPO), superoxide dismutase activity (SOD), glutathione reductase (GSH) levels in the brain were measured.[14]

Dissection and Homogenization

At the end of experiment, the mice of groups I, V, XII, XIII, XIV, XV and XVI were sacrificed by cervical dislocation and brains were taken out. They were rinsed thoroughly with ice-chilled 0.9% NaCl and weighed. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The post nuclear fraction was obtained by centrifugation (Remi - C-30, Remi Industries Ltd, Mumbai, India) of the homogenate at 12000g for 60 min at 4ºC. A Shimadzu-160A spectrophotometer was used for subsequent assays. [15]

Biochemical analysis

Lipid peroxidation assay

The quantitative measurement of LPO in brain was done by the method of Wills (1966). The amount of malondialdehyde (MDA) formed was measured by reaction with thiobarbituric acid at 532 nm. The results were expressed as nanomole of MDA per milligram of protein, using the molar extension coefficient of chromophore (1.56×10^{5} M^{-1} cm^{-1}). [16]

Superoxide dismutase activity

Superoxide dismutase activity (SOD) was assayed according to the method of Kono (1978), wherein the reduction of nitroblue tetrazolium chloride (NBT) was inhibited by the superoxide dismutase which was measured at 560 nm spectrophotometrically. Briefly the reaction was initiated by the addition of hydroxylamine hydrochloride to the reaction mixture containing NBT and post nuclear fraction of brain homogenate. The results were expressed as % inhibition. [17]

Estimation of reduced glutathione

Reduced glutathione (GSH) in the brain was estimated according to the method of Ellman (1959). A 0.1 ml of sample of homogenate was precipitated with 0.75 ml of 4% sulfosalicylic acid. The assay mixture contained 0.5 ml of supernatant and 4.5 ml of DTNB in 0.1 M phosphate buffer, pH 8.0. The yellow color developed was read immediately at 412 nm. The results were expressed as nanomole of GSH per milligram of protein. [18]

Protein estimation

The protein content was measured according to the method of Lowery et al. (1951), using bovine serum albumin as standard and expressed as µg protein/mg of tissue. [19]

STATISTICAL ANALYSIS

Results are expressed as mean ± S.E.M., and the statistical analysis of data was done using one-way analysis of variance (ANOVA) followed by Dunnett's test. The probability level less than 0.05 was considered statistically significant.

RESULT

Phytochemical screening

The phytochemical screening of EASF of *Plectranthus vettiveroides* revealed the presence of glycosides, flavonoids, tannins and saponins.

Object Recognition Test

The discrimination index was radically ($P < 0.05$; $P < 0.01$) decreased in the aged and scopolamine group as compared to control indicating impairment of memory in object recognition test. Pretreatment with EASF of *Plectranthus vettiveroides* (25, 50 and 100 mg/kg) drastically improved the DI in the aged and scopolamine-treated group, representing development in short-term memory and turnaround of amnesia induced by scopolamine. Piracetam and donepezil also significantly improved short-term memory in the aged and scopolamine-treated group ($P < 0.01$). The results were summarized in Table 1.

Table 1 Effect of EASFPV on discrimination index in young and aged mice in object recognition test

TREATMENT	DISCRIMINATION INDEX
Control(young)	0.0415±0.03
Control(aged)	-0.0420±0.05$^{\$}$
Piracetam (200mg/kg, i.p. in young)	0.269±0.05**
Piracetam (200mg/kg, i.p.in aged)	0.91±0.08$^{@}$
Scopolamine(1mg/kg, i.p.in young)	-0.139±0.04**
EASFPV (25mg/kg, p.o.in young)	0.0381±0.09
EASFPV (50mg/kg, p.o.in young)	0.68±0.07
EASFPV (100mg/kg, p.o.in young)	0.0540±0.04
EASFPV (25mg/kg, p.o.in aged)	0.0304±0.09
EASFPV (50mg/kg, p.o.in aged)	0.0671±0.05
EASFPV (100mg/kg, p.o.in aged)	0.0380±0.05$^{@}$
EASFPV+ scopolamine(25mg/kg, p.o+1mg/kg, i.p. in young)	-0.0283±0.23
EASFPV+ scopolamine(50mg/kg, p.o+1mg/kg, i.p. in young)	-0.0183±0.06^{a}
EASFPV+ scopolamine(50mg/kg, p.o+1mg/kg, i.p. in young)	0.0689±0.05^{b}
Piracetam+scopolamine(200mg/kg, i.p+1mg/kg, i.p. in young)	0.08311±0.043^{b}
Donepezil+scopolamine(1mg/kg, i.p. in young)	0.0941±0.013^{b}

Values are expressed as mean ± SEM (n=5); *p<0.05; **<0.01 vs control group (in young group), $^{\$}$p<0.05; $^{@}$p<0.01vs control (aged control group); ^{a}p<0.05; ^{b}p<0.01 vs scopolamine-treated group; (one way ANOVA followed by dunnett's test).

Passive Avoidance Paradigm/Test

SDL of second day (ninth day of drug treatment) indicated the long-term memory of animals. The SDL was radically decreased (P < 0.01) in the aged and scopolamine-treated group as compared to the control group. EASFPV (50 and 100 mg/kg) administered to young and aged mice for 8 days showed an increase in SDL as compared to the respective control groups (P < 0.05; P < 0.01). Administration of EASFPV for 8 days overturned memory deficits due to scopolamine and aging-induced amnesia. Piracetam and donepezil also showed development in memory in the young, aged as well as scopolamine-treated groups (P < 0.01). The results were summarized in Figure:1.

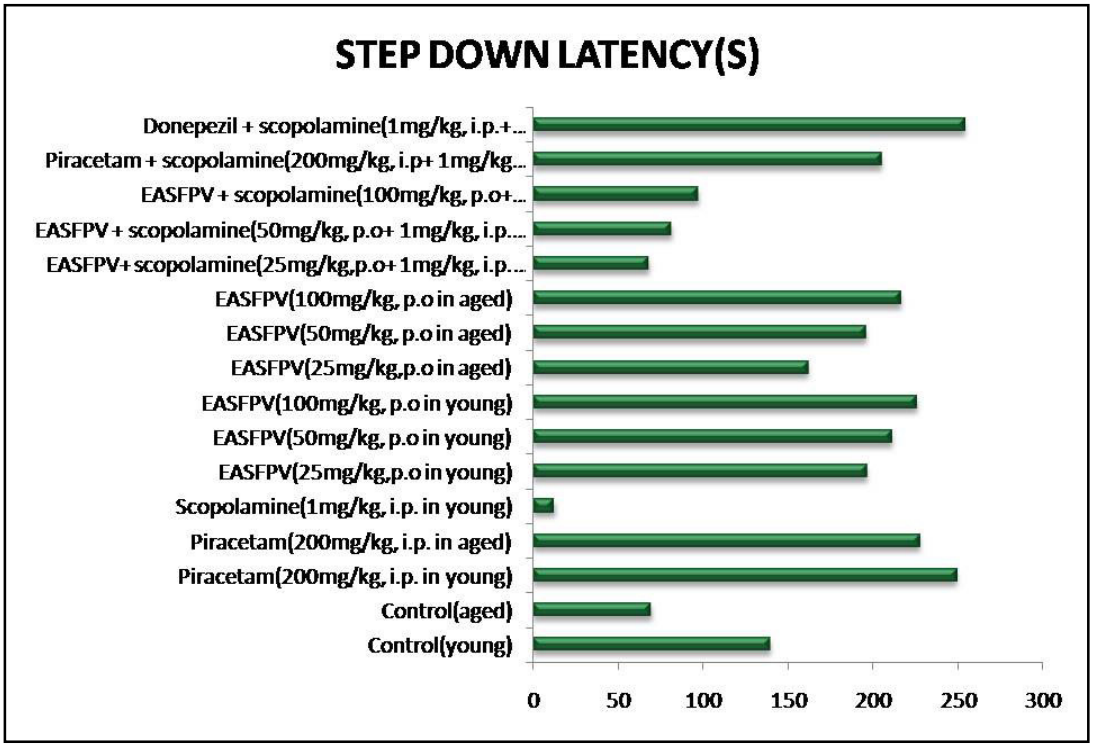

Figure 1: Effect of EASF of *Plectranthus vettiveroides* on transfer latencies of young and aged mice in passive avoidance test

Biochemical effect

Lipid peroxidation assay

On the ninth day after measurement of SLD, the level of MDA was investigated. The level of MDA was appreciably increased ($P < 0.01$) in the scopolamine group, as compared with the control group, while administration of EASF of *Plectranthus vettiveroides* (25, 50 and 100 mg/kg) significantly ($P < 0.01$) brought down the level of MDA, compared with the scopolamine group. The MDA level was also significantly decreased in the piracetam and donepezil group ($P < 0.01$). The results were summarized in Figure:2.

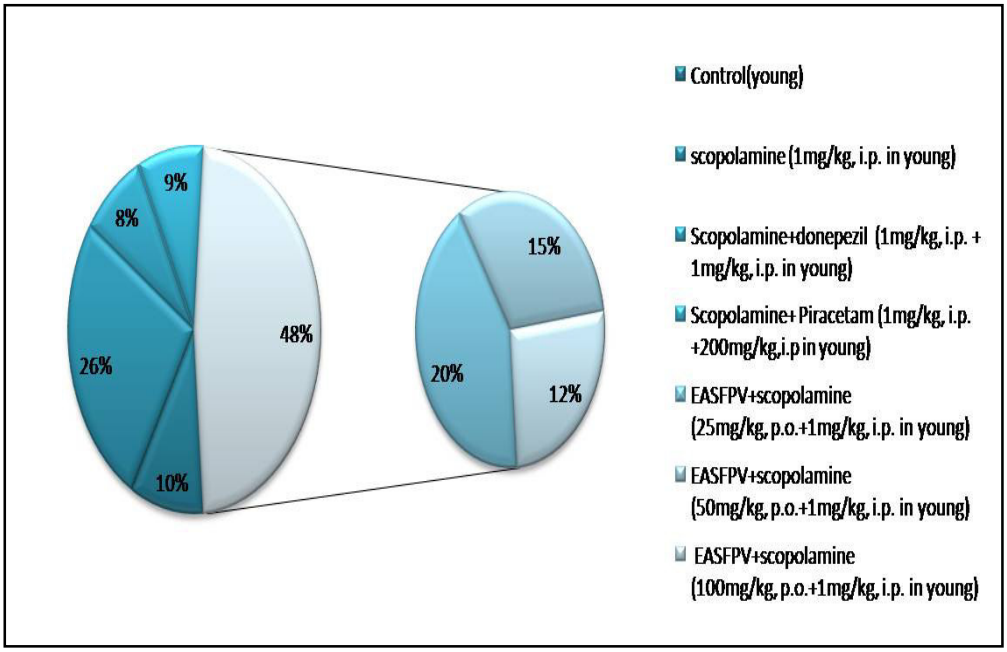

Figure 2: Effect of EASFPV on LPO in scopolamine-induced oxidative stress

Effect on brain GSH level

The content of GSH was depleted extensively ($P < 0.01$) in the scopolamine-treated group, as compared with the control group, indicating the neurotoxicity induced by scopolamine in mice. On the other hand, the GSH level was found to be eminent significantly ($P < 0.05$; $P < 0.01$) after administration of EASF of *Plectranthus vettiveroides* (50 and 100 mg/kg) as compared with the scopolamine-treated group. Piracetam and donepezil also significantly augmented the level of GSH. [Figure:3]

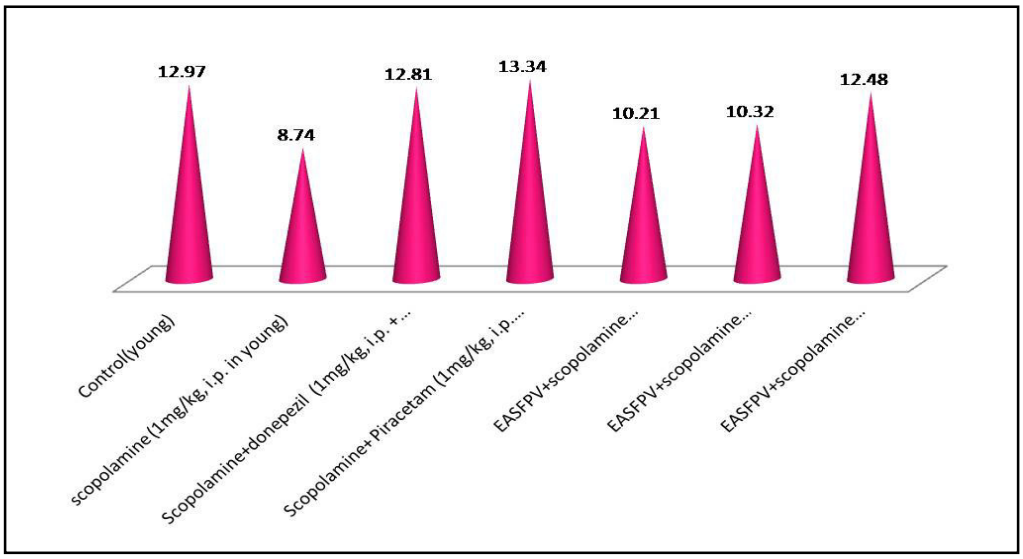

Figure 3: Effect of EASFPV on GSH in scopolamine-induced oxidative stress

Effect on brain SOD level

The level of the suspicious antioxidant enzyme SOD was drastically decreased in the scopolamine group ($P < 0.01$) as compared to the control group. Pretreatment with EASFPV (100 mg/kg) resulted in elevation of SOD ($P < 0.01$) as compared to the scopolamine-treated group. The SOD level was also significantly increased in the piracetam and donepezil group ($P < 0.05$). [Figure:4].

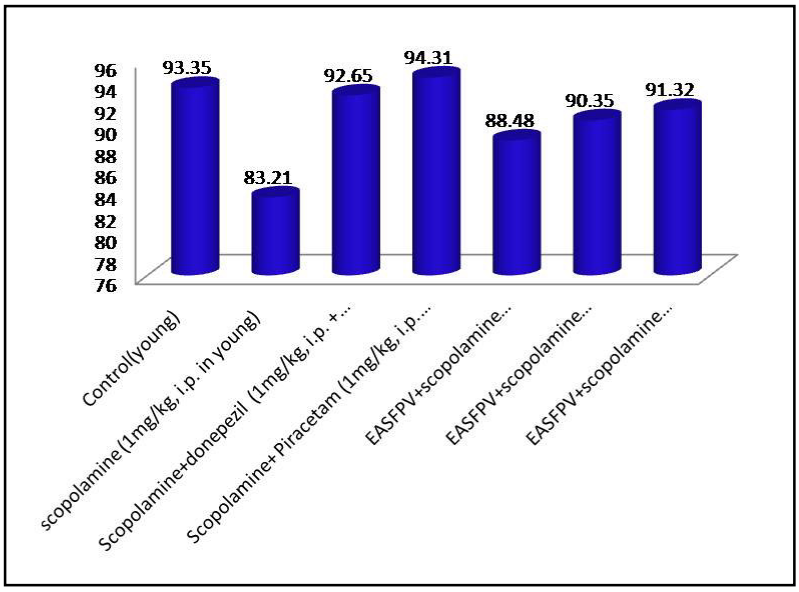

Figure 4: Effect of EASFPV on SOD in scopolamine-induced oxidative stress

DISCUSSION

In the current work, EASF of *Plectranthus vettiveroides* (25, 50 and 100 mg/kg) enhanced learning and memory of mice appreciably in interoceptive behavioral models employed. The concurrent analysis or a peculiarity between reference and working memory is well reputable through ORT and PAT. Scopolamine, a non-selective muscarinic antagonist blocks cholinergic signaling and produce memory shortfall that are similar to those found in age associated senile CNS dysfunction. Scopolamine interferes with memory and cognitive function and afterward causes impairment of reference (long term) and working (short term) memories. In this study, mice were given scopolamine to induce memory mutilation at a dose of 1 mg/kg.

Many clinical studies have reported strapping substantiation that oxidative stress is involved in the pathogenesis of Alzheimer's disease. The oxygen-free radicals are concerned in the process of age related decline in the cognitive performance may be responsible for the progress of Alzheimer's disease in elderly persons. [14] El-Sherbiny et al. (2003) reported that memory impairment in the scopolamine-induced animal model is associated with the increased oxidative stress within rat brain. An increased oxidation of lipids, proteins and deoxyribonucleic acid, alterations in mitochondrial function and a possible role of amyloid beta and its precursor protein in oxidative reaction in experimental models of Alzhei]mer's disease are demonstrated. Moreover, strong evidence supporting the involvement of oxidative damage in neurodegenerative disease has been suggested by various clinical studies. [20] The drugs with antioxidant effects might be valuable for preserving brain function. Antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase as well as glutathione reductase

(GSH) and ascorbate are involved in the lessening of oxidative stress. Antioxidant enzymes exhibit the abridged activities in the pretentious brain region of patients of Alzheimer's disease. Moreover, the lessening in the plane of intracellular oxidized protein under these circumstances has been allied with the perfection of cognitive and/or psychomotor functions. Augmentation of endogenous antioxidants by therapeutic substances has lately evoked scientific attention because any such a possessions of a therapeutic agent can be predictable to cause noteworthy upgrading in the endogenous argument against oxidative stress. These agents also reduce the oxidative damage and promote a functional upturn in degenerative disorders. In the course of searching natural products with memory ornamental activity using scopolamine-induced amnesic mouse as an experimental model for Alzheimer's disease, it was originate that the EASF of *Plectranthus vettiveroides* showed a momentous memory pretty activity in ORT and PAT. The memory destruction induced by acute administration of scopolamine is related with misrepresented level of SOD and GSH in the brain. More specifically, the entire brain of patients with Alzheimer's disease (AD) was shown to be subjected to an oxidative confront. Such a peroxidation process and the overproduction of free radicals may lead to utilization of detoxifying endogenous antioxidants such as SOD and GSH. The animals bare to conditioned fear, treated with scopolamine (1 mg/kg) exhibited lofty brain MDA level, while SOD and GSH levels were abridged.

In the present study, the effect of EASFPV was examined on the performance of mice in an entity recognition task that has been measured to be a pure working memory task. Mice are able to categorize stuck between a well-known object and a new object 1 h or less, but not 24 h, after the presentation of the familiar object. [21] The effect of EASFPV was investigated on the attainment of the information and on the consolidation of reminiscence that takes place shortly after the attainment and on the reimbursement of the information. The results indicated that mice spend more time in exploring a new object than a memorable object in the aged and scopolamine-treated group when pretreated with EASFPV (50 and 100 mg/kg). The DI was notably decreased in the aged and scopolamine-treated group. Pretreatment with EASFPV (50 and 100 mg/kg) significantly augmented the DI when compared with respective control. There was no significant effect on the DI in young mice treated with EASFPV.

Thus, the results demonstrates that EASFPV (50 and 100 mg/kg) enhanced maintenance in mice subjected to object gratitude task in the aged and scopolamine-treated group. EASFPV improves the consolidation and perhaps the acquisition phase of functioning memory that is misrepresented in interoceptive recollection discrepancy models, i.e. age and scopolamine. Piracetam and donepezil, the established nootropic agents used as a standard in the nearby study also appreciably superior the DI.

The ameliorative effects of EASFPV on learning and memory were investigated in the passive avoidance task. Scopolamine-treated mice exhibited drastically shorter step-down latencies. EASFPV (25, 50 and 100 mg/kg) treatment showed a momentous augment in SDL in young as well as aged animals. Pretreatment with EASFPV (25, 50, and 100 mg/kg) significantly decreased SDL in the scopolamine-treated group. Thus, EASFPV significantly reversed the deficit fashioned by scopolamine. Donepezil (1 mg/kg) and piracetam (200 mg/kg) used as positive control, also enlarged the SDL, which is dependable with previous reports.

The EASFPV meet the criteria for nootropic action. The pretreatment with EASFPV for 8 days sheltered the animals from memory deficit produced by scopolamine in PAT. Administration of scopolamine drastically enlarged the MDA level, an imperative marker for LPO and reduced both GSH and SOD activities in mice brain. Administration of EASFPV for 8 days shaped a significant fall in MDA and restored the GSH and SOD activities in mice brain. Extract may exert a defensive effect against oxidative damage induced by scopolamine by retreating the lessening in the activities of GSH and SOD in mice brain. The above behavioral and biochemical results propose that EASFPV has the capability to perk up or ameliorate spatial long term and working memory by the guideline of the antioxidant system. The observed helpful effects of *Plectranthus vettiveroides* may be accredited to its diversified chemical components namely glycosides, flavonoids, tannins and saponins.

CONCLUSION

Our exploration indicates that grouping of antioxidant and neuroprotective role could be accountable for a cognitive enhancing effect. Hence, *Plectranthus vettiveroides* may be useful in the treatment or preclusion of various cognitive disorders.

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