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Methanolic Extract of *Pimpinella anisum* L. Prevents Dementia by Reducing Oxidative Stress in Neuronal Pathways of Hypermnesic Mice

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ABSTRACT

Considering Alzheimer's disease a universal challenge, this study was planned to utilize the bio-constituents of *Pimpinella anisum* dried seeds to check the oxidative neuronal decay in brain parts. Methanolic extract of *Pimpinella anisum* (meP.a) dried seeds was explored qualitatively and quantitatively for analysis of phytoconstituents along with anti-oxidant activity. Anti-amnesic activity was found by behavioral and biochemical studies for which male albino mice were grouped into seven groups. Mice were subjected to elevated plus maze, hole board and light dark paradigm on (day 7th and 8th), for behavioral studies after which their brain were removed by decapitation for biochemical assessment. Results indicated the existence of total phenols (170.14 ± 1.16 mg of GAE/g of extract), flavonoids (87.67 ± 0.67 mg of RE/g of extract), tannins (194.04 ± 1.79 mg of TAE/g of extract) and anti-oxidants (IC_{50} value = 59.60 µg/ml found by DPPH method) in plant extract. Higher inflexion ratio (0.25 ± 0.05), most of time spent in dark area (158.34 ± 4.88 sec), increased no of hole pokings (34.16 ± 2.01), significant ($P < 0.001$) elevation in superoxide dismutase (17.36 ± 0.49 U/mg), catalase (1.42 ± 0.05 U/mg) glutathione (37.56 ± 1.89 µ mol/mg) and reduction in malondialdehyde (7.83 ± 0.21 n mol/mg) level was observed in group receiving 800mg/kg of mep.a as compared to amnesic group. *Pimpinella anisum* thus improved memory in mice by preventing oxidative stress in brain.

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Authors' Contribution
MA and RA conceived, designed and supervised the study. AM performed experiment work.

Key words
***Pimpinella anisum*, Alzheimer's disease, Elevated plus maze, Superoxide dismutase, Catalase**

INTRODUCTION

Progressive and irreversible neuronal damage in Alzheimer's disease (AD) (Joshi *et al.*, 2018) has not been overcome even by modern medicines, regardless of extensive research in the field of neuro-psychopharmacology. More than 46 million people, all over the World are suffering from AD and lack of effective treatment might tend to double this figure by 2030 (Prince *et al.*, 2015). Etiology of AD has been explained best by cholinergic hypothesis (Francis *et al.*, 1999), severe oxidative stress and misfolded protein accumulation in neuronal pathways (Omar *et al.*, 2017) which seeks the attention of researchers to explore patient friendly remedies of AD (Citron, 2010). To meet this challenge, WHO is promoting the attention of researchers towards the development of toxicity free herbal medicines with better therapeutic profile (Penumala *et al.*, 2018). There is a huge list of neuroprotective plants which are in practice in Ayurvedic system of medicines since 2000 BC (Rao *et al.*, 2012) and their pharmacological basis of therapeutics are needed to be explored scientifically. Considering this need a motivational challenge, the present study was planned to investigate the potential benefits of *Pimpinella anisum* dried seeds in managing AD associated dementias.

Pimpinella anisum (Umbelliferae), an annual herb native to India and Asia minor (Akhtar *et al.*, 2008) has been extensively used for the treatment of colic, nausea, constipation, duodenal ulcers, abdominal cramps, inflammation and dysmenorrhea (Shojaii and Fard, 2012). Moreover, its antiepileptic and neuro-protective role (Karimzadeh *et al.*, 2012) has also been suggested which provided the sense for its use in AD and current research is one of the initial findings of the project on AD.

MATERIALS AND METHODS

Extract preparation

Dried seeds were obtained from local market of Lahore, Pakistan and were identified by Prof. Dr. Zaheer ud Din Khan (Department of Botany Govt. College University Lahore) and sample was preserved in their

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herbarium (voucher no. GC.Herb.Bot.3385). Extract was made by simple maceration by soaking powdered material (1 kg) in methanol (5 L) in glass jar for 3 days. Filtration was done on 3rd day and material was re-soaked in methanol for another 3 days. Finally, filtrates of all five soakings were concentrated in rotary evaporator and semi solid, thick brownish extract was weigh down and preserved in air tight glass jar at 4 °C in refrigerator. Percentage yield was calculated as; %age yield = [weight of extract (g)/ weight of dried seeds powder (g)] x 100.

Phytochemical analysis of extract

Qualitative testing of phytoconstituents present in methanolic extract of *Pimpinella anisum* (meP.a.) responsible for biological activities was done by using simple identification tests for alkaloids, carbohydrates, proteins, flavonoids, glycosides, steroids, saponins, terpenoids, tannins, quinones, phenols, terpenes, phytosterols and fixed oils (Mushtaq *et al.*, 2018; Kumaran and Karunakaran, 2007; Polshettiwar *et al.*, 2007). Antioxidant activity was measured according to Blois (1958).

Animals

Swiss male albino mice (20–25 g) used in this research were maintained in the animal house of Punjab University College of Pharmacy. Permission regarding animals safety and ethics was granted by institutional research ethical committee of Punjab University College of Pharmacy vide letter no. AEC/PUCP/1072. Mice were kept in polycarbonate cages at standard humidity; 50–55%, temperature; 25 ± 2°C, equal light and dark periods, under full hygienic conditions and were given standard food and water *ad libitium*.

Study design

Mice were grouped into seven groups (n=6) i.e. G–I received 0.9% NaCl sol. 10 ml/kg/p.o (control), while G–II received 5% CMC 10 ml/kg/p.o (amnesic group). G–III and IV received Piracetam 200 mg/kg/p.o (standard A and B), G–V to G–VII received meP.a in doses 200, 400 and 800 mg/kg/p.o, respectively. Scopolamine (10 mg/kg/p.o) was administered to all groups except G–I and G–III, on 7th day after 45 min of last treatment. Then, behavioral studies were conducted by using following paradigms.

Behavioral studies

Elevated plus maze (EPM)

This paradigm was used for behavioral analysis which is considered the most reliable paradigm for evaluation of memory enhancement activity (Pahaye *et al.*, 2017). It was made up of polyacrylic sheets having two closed (15 x 5 x 12 cm) and two open (16 x 5 cm) arms joined in shape of plus sign (+) with a central platform (5 x 5 cm) and was fixed on wood stand (25 cm high from floor). Animal was put at the end of open arm with its face opposing to central area and was allowed to explore the apparatus and time taken to enter in any of closed arm with its full four legs was noted. Each animal was given maximum 90 sec to explore the apparatus and animal who failed to enter in closed arm was pushed with its tail into closed arm and assigned latency value 90 sec. Initial transfer latency (ITL) was observed after 45 min of last dose. Retention transfer latency (RTL) was noted on 2nd day (after 24 h of last dose) and inflexion ratio (IR) was found by using formula; IR = ITL – RTL)/ITL.

Light and dark paradigm

Based upon the principle that animals love to live in dark protected area, this paradigm was employed which consisted of light (30 x 30 x 35 cm) and dark (20 x 30 x 35 cm) compartments having shared opening (5 x 5 cm) at middle bottom for the entrance of mice. Animal was put into light compartment and time (sec) spent by it in light and dark compartment was noted. Maximum of five min were given to each mice to explore apparatus and observations were made on day 1st and day 2nd (Barry *et al.*, 1987).

Hole board paradigm

This was third model used to assess memory and learning behavior in mice which consisted of rectangle (35 x 45 x 45 cm) box made up of polyacrylic sheets. A sheet of same dimension but had 16 holes (2 cm diameter) was fixed at the corners just 5 cm above the bottom of box. Animal was put into the middle of hole board sheet and no of hole pokings per five minutes were noted for all the experimental groups on day 1 and 2 (Durcan and Lister, 1988).

Biochemical analysis

Each animal was given anesthesia using chloroform and brain was isolated after decapitation. It was rinsed with cold saline and 20 mg of it was homogenized with 1 ml cold phosphate buffer (pH 7.4) using tissue homogenizer. Mixture was centrifuged at 800 rpm (4 °C) for 5 min to separate the nuclear debris. Supernatant was re-centrifuged at 10000 rpm (4 °C) for 20 min and supernatant so obtained was assayed biochemically (Rajesh *et al.*, 2017).

Estimation of brain acetylcholinesterase (AChE)

Ellman's method was used to assess AChE in mice brain by mixing 0.4 ml of brain homogenate with 2.6

ml phosphate buffer and 100 µl of DTNB thoroughly, and absorbance was read at 412 nm. Acetyl thiocholine iodide (20 µl) was then added in mixture and absorbance was read at 2 min interval. Change in absorbance (per min) was found and AChE level was determined by using formula; [R = 5.74 x 10^{-4} x A/CO], where R is no of moles of substrate hydrolyzed/min/g of brain tissue, A is change in absorbance per min and CO is original concentration of tissue = 20 mg/ml (Ellman *et al.*, 1961).

Assessment of brain malondialdehyde (MDA)

According to **(**Xian *et al.*, 2011**)**, MDA was found by mixing 100 µl of brain homogenate with 200 µl of 8% w/v sodium dodecyl sulphate, 1.5 ml of 0.8% w/v DTNB and 1.5 ml of 20% v/v acetic acid. Mixture was heated for 1 h at 95 °C, mixed with 5 ml of butanol after cooling and was centrifuged for ten min (3000 rpm) and supernatant layer was removed. Absorbance was read for that layer at 510 nm and MDA level was found by using formula; [MDA (µM) = A (sample) x DF /I x ε], where I = light path = 1cm, ε = *molar absorptivity* = 1.56 X 10^5 M^{-1} cm^{-1} and DF = dilution factor = 21

Assessment of brain superoxide dismutase (SOD)

It was found by mixing 0.5 ml of brain homogenate with 1.5 ml chloroform, 2.5 ml chilled ethanol and 1 ml distilled water. Mixture was centrifuged and supernatant was mixed with 1.2 ml of phosphate buffer (0.025 M, pH 8.4), 0.1 ml PMS (186 µM), 0.3 ml NBT (30 µM), 0.2 ml of NADH (780 µM) and distilled water (3 ml). Mixture was incubated for 2 min and was first mixed with 1 ml of acetic acid and then with butanol. Butanol layer was then removed and its absorbance was read at 560 nm against blank and SOD level was found as unit/mg of protein by calibrating standard curve (Kakkar *et al.*, 1984).

Assessment of brain catalase (CAT)

Brain homogenate (0.1 ml) was mixed with 0.1 ml of phosphate buffer (0.01 M, pH 7) and 0.4 ml of H_2O_2 (2M) and then after some time, reaction was stopped by addition of dichromate acetic acid reagent (2 ml). Absorbance was read at 620 nm and CAT level was expressed as µM of H_2O_2 decomposed/min/mg of protein (Sinha, 1972).

Assessment of brain glutathione (GSH)

Mixture of brain homogenate (0.4 ml) and 20 % TCA (0.4 ml) was centrifuged at 10000 rpm (for 20 min at 4 °C) and 0.25 ml of supernatant was mixed with 2 ml of DTNB (0.6 M) and final volume of mixture was made 3 ml by adding phosphate buffer. Absorbance was measured against blank at 412 nm. Different concentrations of GSH (10–50 µM) dissolved in 0.4 ml TCA were used to take the absorbance for calibration curve and GSH level was expressed as µM/mg of protein (Moron *et al.*, 1979).

Statistical analysis

Values were expressed as mean ± SEM. Student's t-test was applied on data with paired comparisons and ANOVA followed by Dunnett's test was used for multiple comparisons by using GraphPad Prism–7. Value of $P < 0.05$ was marked as significant.

RESULTS

Pytochemical ingredients

Qualitative phytochemical studies indicated the presence of variety of constituents in methanolic extract of *Pimpinella anisum* (meP.a) dried seeds (Table I).

Table I.- Qualitative phytochemical analysis of methyl extract of *Pimpinella anisum.*

No	Phytochemical constituents	Tests	Presence
1	Alkaloids	Hagers's test	+++
		Wagner's test	+++
		Dragendroff's test	++
		Mayer's test	+++
2	Carbohydrates	Molish Test	-
3	Fixed oils	Spot test	+
4	Flavonoids	Alkaline reagent test	+
5	Glycosides	Killer Kiliani test	+
6	Phenols	FC method	++
7	Phytostyerol	Libermann Burchard test	++
8	Proteins	Ninhydrin test	++
9	Quinones		-
10	Saponins	Foam test	+
11	Steroids	Ring test	+
12	Tannins	Ferric chloride test	+++
13	Terpenes	Salkowski test	++
14	Terpenoids		++

, sign denotes absent; + denotes slightly present; ++ denotes moderately present and +++ denotes highly present

Quantitative screening of phytoconstituents

Table II shows quantitative presence of phenols (170.14 ± 1.16 mg of GAE/g), flavonoids (87.67 ± 0.67 mg of RE/g) and tannins (194.04 ± 1.79 mg of TAE/g) in dried plant extract.

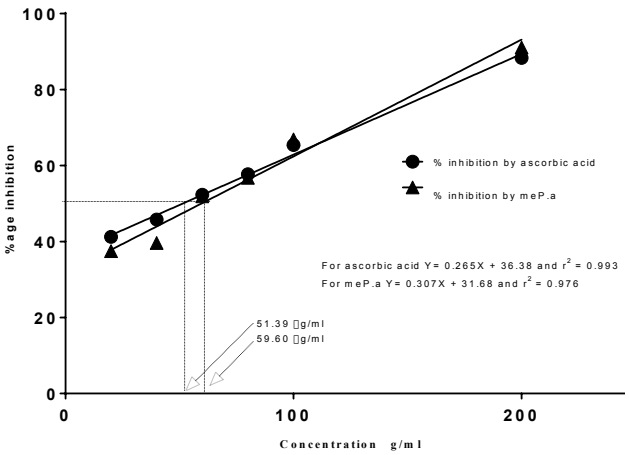

Fig. 1. Free radical scavenging activity of methyl extract of *Pimpinella anisum.*

Free radical scavenging by DPPH assay

This assay indicated that IC_{50} for methanolic extract of *Pimpinella anisum* (meP.a) was 59.60 µg/ml in comparison to ascorbic acid (IC_{50} = 51.39 µg/ml) which indicated strong antioxidant activity of extract (Fig. 1). Considering % age yield, it was found that 1 mg of dried seeds powder was equivalent to 197 µg of meP.a.

Effect of meP.a on transfer latencies in EPM paradigm

It was observed that initial transfer latency (ITL) values of standard control group were less than G–II (amnesic control), which indicated the loss of memory in G–II mice while animals treated with me.Pa 800 mg/kg/p.o, significantly *(P < 0.001)* improved the learning tasks. Similarly, RTL values were observed to be reduced in extract treated groups as compared to G–II animals which indicated the retention of memory in animals pretreated with plant extract. Inflexion ratio (a measure indicating improvement of memory) were significantly *(P < 0.001)* higher for all the groups except amnesic control group which indicated improvement of memory by the use of standard as well as plant extract (Table III).

Effect of me.Pa on relative time spent in light and dark paradigm

Relative time spent by mice in light and dark compartments (Table IV) indicated that animals treated with me.Pa 800 mg/kg/p.o spent less time in light compartment and lived most of the time in dark compartment on day 1st. Similarly, me.Pa 800 mg/kg/p.o significantly *(P < 0.001)* increased the time spent in dark compartment on day 2nd as compared to amnesic group which indicated improvement of memory. Plant extract in low doses produced non-significant results.

Effect of me.Pa on number of hole pokings in hole board model

Detailed results of this paradigm are shown in Table V which indicated that only meP.a 800 mg/kg/p.o increased the no of hole pokings on both day 1st and 2nd as compared to G–II animals. Plant extract in low doses (200 and 400 mg/kg/p.o) produced non-significant results.

Table II.-Total phenolic, flavonoid and tannin contents of methyl extract of *Pimpinella anisum.*

Extract	Phenols	Flavonoids	Tannins
Methanolic extract of *Pimpinella anisum* (meP.a)	170.14 ± 1.16 mg of GAE/g of extract	87.67 ± 0.67 mg of RE/g of extract	194.04 ± 1.79 mg of TAE/g of extract

Table III.-Effect of methyl extract of *Pimpinella anisum* on transfer latencies and IR in EPM paradigm.

	Initial transfer latency (ITL)	Retention transfer latency (RTL)	Inflexion ratio (IR)
G–I (normal)	21.83 ± 1.01	18.16 ± 1.30	0.16 ± 0.04
G–II (amnesic)	71.66 ± 2.73[a]	86.33 ± 3.08[a]	-0.20 ± 0.0[3]a
G–III (standard A)	18.83 ± 1.16[b]	16.33 ± 0.76[b]	0.19 ± 0.02[b]
G–IV (standard B)	45.17 ± 1.81[b]	25.17 ± 1.81[b]	0.44 ± 0.04[b]
G–V (meP.a 200 mg/kg)	68.83 ± 2.36[ns]	64.66 ± 2.88[b]	0.05 ± 0.09[b]
G–VI (meP.a 400 mg/kg)	65.16 ± 2.21[ns]	59.66 ± 1.99[b]	0.08 ± 0.03[b]
G–VII (meP.a 800 mg/kg)	55.16 ± 1.86[b]	40.50 ± 2.12[b]	0.25 ± 0.05[b]

Data were expressed as mean ± SEM (n=6). One way ANOVA followed by Dunnett's test was applied on data set and G-II was compared with G-I (shown by [a] corresponding $P \leq 0.001$) while other groups (G-III to G-VII) were compared with G-II (shown by [b] corresponding $P \leq 0.001$, [c] for $P \leq 0.01$, [d] for $P \leq 0.05$ or [ns] for $P \geq 0.05$).

Table IV.- Effect of methyl extract of *Pimpinella anisum* on time spent in light and dark compartments.

	Day 1st		Day 2nd	
	Time spent in light compartment (Sec)	Time spent in dark compartment (Sec)	Time spent in light compartment (Sec)	Time spent in dark compartment (Sec)
G–I (normal)	51.50 ± 2.63	248.50 ± 2.83	46.67 ± 1.64	253.33 ± 1.39
G–II (amnesic)	186.16 ± 5.8^{0}a	113.83 ± 5.51^{a}	205.16 ± 3.50^{a}	94.83 ± 3.39^{a}
G–III (standard A)	44.16 ± 5.39^{b}	255.84 ± 4.24^{b}	34.66 ± 2.45^{b}	265.34 ± 2.45^{b}
G–IV (standard B)	66.17 ± 2.8^{0}b	233.83 ± 4.41^{b}	53.67 ± 2.45^{b}	246.33 ± 2.67^{b}
G–V (meP.a 200 mg/kg)	175.66 ± 6.80^{ns}	124.34 ± 6.84^{ns}	199.83 ± 5.26^{ns}	100.17 ± 5.26^{ns}
G–VI (meP.a 400 mg/kg)	171.50 ± 5.08^{ns}	128.00 ± 4.27^{ns}	189.16 ± 4.05^{ns}	110.84 ± 4.05^{ns}
G–VII (meP.a 800 mg/kg)	159.33 ± 5.23^{c}	140.67 ± 6.72^{d}	141.66 ± 3.55^{b}	158.34 ± 4.88^{b}

Data were expressed as mean ± SEM (n=6). One way ANOVA followed by Dunnett's test was applied on data set and G-II was compared with G-I (shown by ^{a} corresponding $P \leq 0.001$) while other groups (G-III to G-VII) were compared with G-II (shown by ^{b} corresponding $P \leq 0.001$, ^{c} for $P \leq 0.01$, ^{d} for $P \leq 0.05$ or ^{ns} for $P \geq 0.05$).

Table V.- Effect of methyl extract of *Pimpinella anisum* on no of hole pokings in mice.

	No of pokings on day 1st	No of pokings on day 2nd
G–I (normal)	46.83 ± 1.49	41.33 ± 1.28
G–II (amnesic)	22.66 ± 1.76^{a}	25.83 ± 1.55^{a}
G–III (standard A)	51.16 ± 2.34^{b}	44.66 ± 1.77^{b}
G–IV (standard B)	41.83 ± 1.47^{b}	40.33 ± 1.72^{b}
G–V (meP.a 200 mg/kg)	23.33 ± 2.10^{ns}	24.16 ± 1.81^{ns}
G–VI (meP.a 400 mg/kg)	26.83 ± 1.70^{ns}	27.66 ± 2.10^{ns}
G–VII (meP.a 800 mg/kg)	34.33 ± 1.72^{c}	34.16 ± 2.01^{d}

Data were expressed as mean ± SEM (n=6). One way ANOVA followed by Dunnett's test was applied on data set and G-II was compared with G-I (shown by ^{a} corresponding $P \leq 0.001$) while other groups (G-III to G-VII) were compared with G-II (shown by ^{b} corresponding $P \leq 0.001$, ^{c} for $P \leq 0.01$, ^{d} for $P \leq 0.05$ or ^{ns} for $P \geq 0.05$).

Table VI.- Effect of methyl extract of *Pimpinella anisum* on brain biochemical markers (AChE, MDA, SOD, CAT and GSH).

	AchE µmol/min/mg	**MDA** nmol/hr/g	**SOD** U/mg of homogenate	**Catalase** U/mg of homogenate	**GSH** µ mol/mg
G–I (normal)	4.01 ± 0.26	1.43 ± 0.12	25.15± 0.58	1.97 ± 0.05	44.11 ± 1.50
G–II (amnesic)	8.94 ± 0.21^{a}	8.96 ± 0.18^{a}	7.94 ± 0.31^{a}	0.56 ± 0.0^{4}a	18.90 ± 0.9^{0}a
G–III (standard A)	3.53 ± 0.22^{b}	1.19 ± 0.08^{b}	26.16 ± 0.52^{b}	2.10 ± 0.04^{b}	46.84 ± 1.17^{b}
G–IV (standard B)	4.49 ± 0.19^{b}	2.47 ± 0.13^{b}	21.69 ± 0.31^{b}	1.44 ± 0.06^{b}	39.52 ± 1.49^{b}
G–V (meP.a 200 mg/kg)	8.91 ± 0.39^{ns}	8.39 ± 0.17^{ns}	11.03 ± 0.40^{c}	0.91 ± 0.06^{c}	28.80 ± 0.76^{b}
G–VI (meP.a 400 mg/kg)	8.51 ± 0.29^{ns}	8.30 ± 0.12^{ns}	12.01 ± 0.64^{b}	1.13 ± 0.06^{b}	32.68 ± 0.52^{b}
G–VII (meP.a 800 mg/kg)	8.29 ± 0.27^{ns}	7.83 ± 0.21^{b}	17.36 ± 0.49^{b}	1.42 ± 0.05^{b}	37.56 ± 1.89^{b}

Data were expressed as mean ± SEM (n=6). One way ANOVA followed by Dunnett's test was applied on data set and G-II was compared with G-I (shown by ^{a} corresponding $P \leq 0.001$) while other groups (G-III to G-VII) were compared with G-II (shown by ^{b} corresponding $P \leq 0.001$, ^{c} for $P \leq 0.01$, ^{d} for $P \leq 0.05$ or ^{ns} for $P \geq 0.05$).

Effect of me.Pa on brain biochemical markers (AChE, MDA, SOD, CAT and GSH)

It was observed that scopolamine increased the level of AChE and MDA levels in brain while standard drug (piracetam), significantly *(P < 0.001)* reduced the level of both (Table VI). Plant extract did not reduce the level of AChE in mice brain however, meP.a 800 mg/kg/p.o, significantly *(P < 0.001)* lowered the MDA contents of brain as compared to G–II animals. It was observed that meP.a 200 mg/kg/p.o, significantly *(P < 0.01)* improved SOD and CAT levels while meP.a 400 and 800 mg/kg/p.o produced highly significant *(P < 0.001)* elevation in SOD and CAT. Level of GSH was promoted, significantly *(P < 0.001)* by G–V to G–VII as compared to G–II animals (Table VI).

DISCUSSION

Severe oxidative damage produced by the imbalance of antioxidants and pro-oxidants along with deposition of carbohydrates, lipids, proteins and nucleic acid in neuronal circuits of brain are predisposing causes of dementias and related cognitive disorders (Butterfield *et al.*, 2007; Mariani *et al.*, 2005). Natural substances enriched with antioxidants prevent the ageing associated biological destructions in body by terminating reactive oxygen and other free radicals (Bravo, 1998).

It has been observed that extract treated animals showed significant reduction *(P < 0.001)* in initial and retention transfer latencies along with elevation of inflexion ratio in EPM paradigm, which indicate the enhancement of memory (Table III). Moreover, EPM is proved to be most effective paradigm for evaluation of memory as proposed by (Barez-Lopez *et al.*, 2017) in comparison to light dark test and hole board paradigms which produced less reliable results.

Biochemical assessment indicated that plant extract did not reduce the level of AChE in mice and hence having no any role in providing the defense against cholinergic hypothesis. But plant extract (400 and 800 mg/kg/p.o) and standard drug piracetam prevented scopolamine induced amnesia (as observed by behavioral studies as well) by elevating SOD, CAT and GSH levels in mice brain (Table VI). Glutathione is directly linked with removal of free oxygen radicals (Fang *et al.*, 2002) while SOD and CAT collectively convert superoxide into molecular oxygen and H_2O_2 into H_2O (Scandalios, 1993). Thus it has been proved that supplementation of mice for seven days with plant extract built up sufficient level of antioxidant enzymes which provided the defense against scopolamine induced structural damages in mice (Table VI). Results indicated that pretreatment with plant extract significantly *(P < 0.001)* reduced the level of MDA in mice brain as compared to negative control animals. Malondialdehyde is a lipid peroxidation product of polyunsaturated fats, formed by the reaction of free radicals (Gawel *et al.*, 2004). Higher level of MDA are built up in hippocampus and cerebral cortex of brain in AD (Singh *et al.*, 2010) while antioxidants successfully reduce its burden by reducing free radicals (Valko *et al.*, 2007).

Phytochemical studies indicated (Table II) that *Pimpinella anisum* exhibited strong antioxidant activity by the presence of total phenols, flavonoids and tannins. They protected the mice brain from oxidative stress by scavenging free radicals. Phenolic compounds have significant emerging role in management of AD and other cognitive disorders (Ramassamy, 2006). Current investigation further suggested that presence of alkaloids in *Pimpinella anisum* (Table I) also provided barrier against oxidative stress in mice brain as proposed by (Chonpathompikunlert *et al.*, 2010). The whole discussion is thus concluded that natural antioxidants prevent neuronal decay in brain by downgrading the pro-oxidants levels.

CONCLUSION

Bioactive phytoconstituents of *Pimpinella anisum* L. being strong antioxidants, improved the memory in mice by preventing neuronal damages associated with oxidative stress.

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Statement of conflict of interest

There is no conflict of interests regarding the publication of this article.

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