MEMORY ENHANCING EFFECT OF ANISE (*PIMPINELLA ANISUM)* WITH RESPECT TO ITS ANTIOXIDANT ACTIVITY IN ALBINO MICE

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ABSTRACT

The study was conducted to explore memory enhancing effect of Anise *(Pimpinella anisum)* to substantiate the scientific basis of its traditional use as a neurotic remedy. Aqueous (aqP.a) and *n*-hexane (nhP.a) fractions of crude Anise extracts were 1st investigated for phytochemical and antioxidant analysis and then administered to mice for behavioral (by using elevated plus maze (EPM), light dark test and hole board paradigms) and biochemical investigations. Flavonoids, tannins and phenols were found to be 127.43 ± 1.94, 201.04 ± 1.57 and 209.83 ± 1.30 mg/g, respectively for aqP.a and 125.33 ± 2.18, 74.66 ± 1.20 and 42.87 ± 1.65 mg/g, respectively for nhP.a. Values of IC_{50} were recorded as 37.48 and 166.53 µg/ml for aqP.a and nhP.a respectively. Moreover, aqP.a significantly *($P < 0.001$)* improved inflexion ratios in EPM test and biochemical parameters like glutathiones, catalases and superoxide dismutases. The levels of acetylcholinesterase (AChE) and malondialdehyde (MDA) were significantly *($P < 0.001$)* reduced by aqP.a as compared to nhP.a. The findings thus suggested that only aqueous extract of Anise improves memory both by preventing oxidative stress and by providing neuro protection to cholinergic pathways against AChE.

Keywords: Antioxidant, AChE, MDA, Dementia, Anise.

INTRODUCTION

Anise (*Pimpinella anisum)* belongs to family Apiaceae and is native to Mediterranean regions, while this is widely cultivated in India, Pakistan, Iran, Syria, Sri Lanka, Spain, Egypt, Turkey and in Middle East. This is commonly known as *"Soye"* in india and Pakistan (Nadkarni, 1996).

Anise seeds have been used extensively as a carminative substance since ancient times in Egypt (Kosalec *et al.*, 2005). Locally, it has been used as an expectorant, laxative, antiseptic, diuretic, antispasmodic and anti-parasitic agent by traditional healers (Andallu and Rajeshwari, 2011; Marino *et al.*, 2001). Moreover, it is also used in gynecology for the treatment of menstruation disorders and as a milk secretagogue in young mothers (Kosalec *et al.*, 2005). Cosmetics industry of Iran had used aniseed extract in preparation of whitening creams and lotions (Shojaii and Abdollahi Fard, 2012). It has also found its application in bakery products as well as a flavoring agent (Andallu and Rajeshwari, 2011). Past studies have scientifically proved its antimicrobial and antifungal activities along with strong antioxidant action (Rajeshwari *et al.*, 2011). Moreover, pharmacological studies have proved anti-diabetic (Rajeshwari *et al.*, 2011), anti-ulcer (Picon *et al.*, 2010), anti-inflammatory, analgesic (Tas *et al.*, 2006), muscle relaxant (Boskabady and Ramazani-Assari, 2001) and estrogenic (Czygan, 1992) potentials of Anise dried seeds. Dried seeds have been investigated for the prevention of brain disorders like epilepsy and partial seizures as well (Pourgholami *et al.*, 1999). Traditional healers (Hakeems), used to use this plant for the treatment of different forms of dementias, the scientific basis of which were needed to be investigated (Hakeem *et al.*, 1991). So the aim and scope of this study was to elaborate the pharmacological basis of action of Anise for the treatment of dementias associated with Alzheimer's disease (AD). Dementia is a group of symptoms characterized by progressive loss of memory, cognition impairment and decline in overall routine performance (Dubois *et al.*, 2010). It is caused by neuronal degeneration in cholinergic pathways of hippocampus and cingulated gyrus of the brain (Mazza *et al.*, 2006).

MATERIALS AND METHODS

Plant materials and extraction: Dried Anise seeds were procured from local market of Lahore and were identified by department of Botany, GC University Lahore. Specimen of it was preserved in herbarium of GC University by assigning voucher no: GC-HERB-BOT-3385. First methanolic extract was prepared by using simple maceration technique (Mushtaq *et al.*, 2017) and then aqueous and *n*-hexane fractions of it were obtained by simple liquid liquid extraction procedures. Finally extracts were separately concentrated by evaporating the solvents by using rotary evaporator and percentage yields of extracts were found by using simple formula:

Percentage Yield = Weight of Extract (g)/ Weight of dried seeds (g) x 100.

Both aqueous and *n*-hexane fractions of Anise extract were labeled as aqP.a and nhP.a, respectively, preserved in air tight closed glass jars and were put in refrigerator at 4 °C.

Phytochemical studies: Initially, phytochemical constituents (carbohydrates, proteins, alkaloids, flavonoids, phenols, glycosides, terpenoids, saponins, tannins, steroids, terpenes, phytosterols, quinines and fixed oils) present in both extracts were found qualitatively (Mushtaq *et al.*, 2013). Total phenols were quantitatively found by using Folin Ciocalteu's reagent (FCR) method while flavonoids and tannins were estimated by using aluminium chloride (Kumaran and Karunakaran, 2007) and modified FCR method (Polshettiwar *et al.*, 2007), respectively.

***In vitro* Antioxidant activity:** *In vitro* antioxidant activity of both fractions was found by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. Reagent stock solution was prepared by dissolving 1 mmol/l of DPPH in methanol and was covered with aluminium foil. Standard and sample solution was made by dissolving different concentrations (20-200 µg/ml) of ascorbic acid and plant fractions, respectively in methanol. Reagent solution (2 ml) was added in each test tube containing either sample or standard solution and incubated for half an hour in dark room. Absorbance of each sample and standard solution was measured at 527 nm against blank and following formula was used to find antioxidant activity (Blois, 1958).

% age scavenging = [Absorbance of blank – Absorbance of sample / Absorbance of blank] x 100

Animals: *In vivo* studies were conducted on male Swiss albino mice (25-30 g) which were kept in animal house of Punjab University College of Pharmacy, PU Lahore. Regarding use of animals, special permission was obtained from research ethics committee of college with diary number AEC/PUCP/1072. Animals were kept at standard living conditions i.e. temperature = 25 ± 2 °C, humidity = 50 ± 5%, light span = 12 h and were provided with food and water *ad libitium*. Animals were acclimatized for one week with lab environment and were given training before performance of behavioral studies.

Study design: Animals were arranged in ten groups i.e. GI- to G-X with n = 6. They were treated in the following way; G-I = 10 ml/kg/p.o, normal saline, G-II = 10 ml/kg/p.o, 5% CMC, G-III and IV = 200 mg/kg/p.o, piracetam, G-V to VII = aqP.a in doses 200, 400 and 800 mg/kg/p.o, respectively and G-VIII to G-X = nhP.a doses 200, 400 and 800 mg/kg/p.o, respectively, for seven days consecutively. After 45 minutes of last treatment, amnesia was induced in all groups except G-I and G-III, by administration of scopolamine (10 mg/kg/p.o). Forty five minutes after induction of amnesia, behavioral studies were performed in sound proof room on same day and also on 8th day of treatment (Devi, 2001). Biochemical studies were performed on 8th day after performance of behavioral studies.

Behavioral studies

Elevated Plus Maze (EPM): The EPM apparatus was specially designed in the shape of plus sign with the help of poly acrylic sheets. Two arms of it were closed (having dimensions 16 x 5 x 12 cm) and two were open (16 x 5 cm), with all arms sharing a central platform having dimensions (5 x 5 cm). This apparatus was adjusted on a stand made of wood having height 10 inches from the ground. Studies were performed by putting each mouse at the end of open arm in such a way that it faced opposite to the central platform. It was then given maximum 90 sec to explore the apparatus and time taken by it to enter into any of the closed arm with all its four legs was noted. Animal failed to enter in closed arm was pulled from its tail to the closed arm and were assigned latency as 90 sec. Studies performed on 7th day of treatment were termed as initial transfer latencies (ITL), while those conducted on 8th day were assigned as retention transfer latencies (RTL). From these transfer latencies, inflexion ratio (IR) was found by using formula: [IR= ITL– RTL/ITL] (Pahaye *et al.*, 2017).

Hole Board Paradigm: Apparatus consisted of rectangle box (35 x 45 x 45 cm) made up of clear poly acrylic sheets. A black colored sheet of same dimensions and containing sixteen holes was placed at level of 5 cm above the floor of box. Animals from all the groups were individually put in the center of sheet and were observed carefully for five minutes to record number of hole pokings. Observations were made both on 7th and 8th day of treatment (Durcan and Lister, 1988).

Light Dark Test: A rectangle box made up of poly acrylic sheets and having two compartments (large = 30 x 30 x 35 cm and small = 20 x 30 x 35 cm) was used as light dark paradigm. Both compartments had opening (5 x 5 cm) at bottom of separating wall for entry of mice. Experiment was started by putting each animal from all groups in the light area and was given five minutes to explore the apparatus. Time spent in light and dark compartments was recorded on day 7th and 8th, respectively (Barry *et al.*, 1987).

Biochemical studies: Biochemical studies were conducted on mice brains which were separated out by decapitation. They were rinsed with ice cold saline and 20 mg from each brain was mixed with 1 ml of phosphate buffer for tissue homogenization carried out in homogenizer. Homogenate was first centrifuged at 800 rpm (at 4 °C). Supernatant so obtained was again subjected to centrifugation at 10 kilo rpm (at 4 °C) and

very fine supernatant was obtained which was used for biochemical studies (Rajesh *et al.*, 2017).

Assessment of Acetylcholinesterase: Acetylcholinesterase in brain homogenates was found by Ellman's method by mixing brain homogenate (0.4 ml) with phosphate buffer (2.6 ml) and 5,5-dithiobis-2-nitrobenzoic acid (100 µl). The absorbance of the mixture was read at 412 nm by using UV-visible spectrophotometer. After that reaction mixture was added up acetylthiocholine iodide (20 µl) and absorbance was read many times at interval of 2 min to find the change in absorbance per min. values were put into formula: $R = 5.74 \times 10^{-4} \times A/CO$ to find level of AChE. R represents rate of hydrolysis of substrate /min/g of brain tissue, A is change in absorbance per min and CO is original concentration (20 mg/ml) of tissue (Ellman *et al.*, 1961).

Assessment of Malondialdehyde (MDA): The level of MDA in brain was found by mixing brain homogenate (100 µl) with sodium dodecyl sulfate (200 µl), acetic acid (1.5 ml) and thiobarbituric acid (1.5 ml). Mixture was heated (at 95 ℃ for 1 h) and then cooled at room temperature. It was mixed with *n*-butanol (5 ml) and centrifuged for ten min at 3000 rpm to separate the organic layer. Absorbance of the organic layer was read at 532 nm and MDA was found as (Xian *et al.*, 2011): MDA (µM) = [A (sample) x DF /I x ε] where, I = Light path = 1cm, ε = Molar absorptivity = 1.56×10^5 M^{-1} cm^{-1} and DF = Dilution factor = 21.

Assessment of Superoxide Dismutase (SOD): Brain homogenate (0.5 ml) was diluted with 1 ml distilled water and then mixed with chloroform (1.5 ml) and chilled ethanol (2.5 ml) and centrifuged. Supernatant was added up sodium pyrophosphate buffer (1.2 ml), nitro blue tetrazolium (0.3 ml), phenyl methanesulfonate (0.1 ml), reduced nicotinamide adenine dinucleotide (0.2 ml) and distilled water (3ml). After incubation at room temperature 1 ml of glacial acetic acid was mixed in it, agitated vigorously and added up with few ml of *n*-butanol. Butanol layer was removed to read the absorbance at 560 nm against butanol blank and SOD level was expressed as unit/mg of protein (Kakkar *et al.*, 1984).

Assessment of Catalase (CAT): Tissue homogenate (0.1 ml) was mixed with phosphate buffer (1 ml) and hydrogen peroxide (0.4 ml) and later on mixed with dichromate acetic acid reagent (2 ml). Absorbance was read at 620 nm and CAT activity was expressed as µM of H_2O_2 decomposed/min/mg of protein (Sinha, 1972).

Assessment of Glutathione (GSH): Brain homogenate (0.4 ml) was added with 0.4 ml of trichloracetic acid (TCA) and mixture was centrifuged at 10,000 rpm for half an hour. Supernatant was then mixed with 2 ml of 5,5-dithiobis-2-nitrobenzoic acid and final volume was made 3 ml by addition of phosphate buffer. Absorbance was read at 412 nm against blank and GSH was used in different concentrations (10-50 µM) after dissolving it in 0.4 ml of TCA. The absorbance of which was used to draw calibration curve and GSH in brain was expressed as µM/mg of tissue protein (Moron *et al.*, 1979).

Statistics: Values were expressed as mean ± SEM (n=6). Student's t-test analysis was applied on data with paired comparisons and multiple comparisons were made by ANOVA followed by Dunnett's test by using GraphPad Prism software (version 7). Value of $P < 0.05$ was marked as significant.

RESULTS

Percentage yield and phytoanalysis: Aqueous and *n*-hexane fractions of Anise crude extract gave yield as14 and 1.31 %, respectively. Results of qualitative and quantitative photochemical constituents of both of fractions of Anise are given in Table 1 and 2, respectively.

Antioxidant activity: The IC_{50} values of aqueous and *n*-hexane fractions of Anise were observed as 37.48 and 166.53 µg/ml, respectively in comparison to standard ascorbic acid (IC_{50} = 51.39 µg/ml), the details of which are given in Fig 1.

Behavioral studies: It was observed that animals treated with aqueous fraction of Anise significantly *($P < 0.001$)* reduced both initial and retention transfer latencies in comparison to animals treated with *n*-hexane fraction. They also exhibited higher inflexion ratios as shown in Table 3. Similarly, it was observed that only aqP.a treated mice showed significant *($P < 0.001$)* increase in time spent in dark area in light dark test apparatus and they also showed significant *($P < 0.001$)* increase in no of hole pokings in hole board paradigm as compared to nhP.a treated animals which produced non-significant results as shown in Table 4 and 5, respectively.

Biochemical findings: Animals treated with aqueous fraction of Anise significantly *($P < 0.001$)* reduced the levels of both AChE and MDA in brain homogenates as compared to animals treated with *n*-hexane fraction and those of amnesic control animals. Similarly, only aqueous fraction of Anise improved the levels of CAT, GSH and SOD with highest significant *($P < 0.001$)* difference from that of animals treated with *n*-hexane fraction as shown in Table 6.

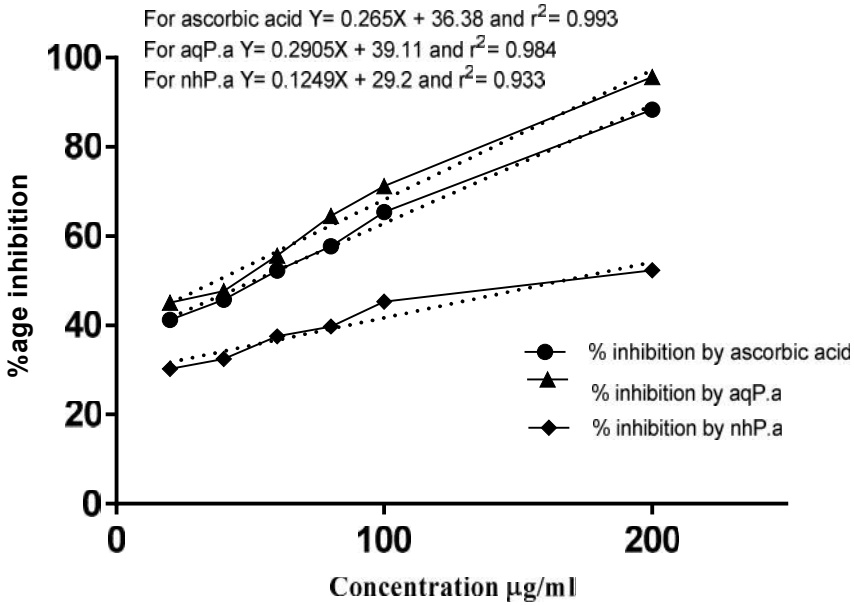

Figure 1. Free radical scavenging activity of aqP.a and nhP.a

Table 1. Qualitative phytochemical analysis of aqueous and *n*-hexane fractions of Anise dried seeds.

Phytochemical Constituents	Tests	aqP.a	nhP.a
Carbohydrates	Molish Test	-	-
Alkaloids	Dragendroff's test	++	++
	Wagner's test	++	+
	Mayer's test	+++	+
	Hagers's test	+	+
Flavonoids	Alkaline reagent test	++	++
Fixed oils	Spot test	+	++
Phenols	FC method	+++	+
Glycosides	Killer Kiliani test	+	+
Phytostyerol	Libermann Burchard test	++	+
Quinones		-	-
Terpenoids		++	+
Saponins	Foam test	++	+
Terpenes	Salkowski test	++	+
Tannins	Ferric chloride test	+++	++
Proteins	Ninhydrin test	++	+
Steroids	Ring test	+	+

+++ = highly present, ++ = moderately present, + = merely present, - = absent

Table 2. Total phenolic, flavonoid and tannin contents found in aqueous and *n*-hexane fractions of Anise extract.

Extract	Phenols (mg of GAE/g)	Flavonoids (mg of RE/g)	Tannins (mg of TAE/g)
Aqueous fraction of Anise (aqP.a)	209.83 ± 1.30	127.43 ± 1.94	201.04 ± 1.57
n-hexane fraction of Anise (nhP.a)	42.87 ± 1.65	125.33 ± 2.18	74.66 ± 1.20

Table 3. Effect of aqueous and *n*-hexane fractions of Anise on transfer latency (TL) in EPM paradigm.

	Initial Transfer Latency (Sec)	Retention Transfer Latency (Sec)	Inflexion Ratio (IR)
G-I	21.83 ± 1.01	18.16 ± 1.30	0.16 ± 0.04
G-II	71.66 ± 2.73[a]	86.33 ± 3.08[a]	-0.20 ± 0.03[a]
G-III	18.83 ± 1.16[b]	16.33 ± 0.76[b]	0.19 ± 0.02[b]
G-IV	45.17 ± 1.81[b]	25.17 ± 1.81[b]	0.44 ± 0.04[b]
G-V	61.50 ± 1.94[c]	52.66 ± 2.39[b]	0.13 ± 0.02[b]
G-VI	57.83 ± 1.85[b]	41.33 ± 1.35[b]	0.27 ± 0.02[b]
G-VII	51.16 ± 1.79[b]	35.50 ± 1.78[b]	0.29 ± 0.05[b]
G-VIII	69.66 ± 1.68[ns]	73.50 ± 2.54[d]	-0.05 ± 0.03[ns]
G-IX	70.33 ± 0.98[ns]	73.51 ± 2.26[c]	-0.05 ± 0.01[ns]
G-X	64.16 ± 2.34[ns]	65.50 ± 2.72[b]	-0.02 ± 0.02[b]

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

Table 4. Effect of aqueous and *n*-hexane fractions of Anise on number of hole pokings in hole board paradigm.

Groups	Day 1st	Day 2nd
	No of hole pokings/5 min	No of hole pokings/5 min
G-I	46.83 ± 1.49	41.33 ± 1.28
G-II	22.66 ± 1.76[a]	25.83 ± 1.55[a]
G-III	51.16 ± 2.34[b]	44.66 ± 1.77[b]
G-IV	41.83 ± 1.47[b]	40.33 ± 1.72[b]
G-V	31.16 ± 1.57[ns]	32.16 ± 1.70[ns]
G-VI	37.66 ± 1.83[b]	33.83 ± 1.88[ns]
G-VII	39.83 ± 1.83[b]	35.16 ± 1.56[c]
G-VIII	20.16 ± 1.75[ns]	26.83 ± 2.02[ns]
G-IX	19.66 ± 1.68[ns]	27.66 ± 1.49[ns]
G-X	19.83 ± 1.32[ns]	26.67 ± 1.28[ns]

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

Table 5. Effect of aqueous and *n*-hexane fractions of Anise on time spent in light and dark compartments.

Groups	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	51.50 ± 2.63	248.50 ± 2.83	46.67 ± 1.64	253.33 ± 1.39
G-II	186.16 ± 5.80[a]	113.83 ± 5.51[a]	205.16 ± 3.50[a]	94.83 ± 3.39[a]
G-III	44.16 ± 5.39[b]	255.84 ± 4.24[b]	34.66 ± 2.45[b]	265.34 ± 2.45[b]
G-IV	66.17 ± 2.80[b]	233.83 ± 4.41[b]	53.67 ± 2.45[b]	246.33 ± 2.67[b]
G-V	132.33 ± 4.37[b]	167.67 ± 4.37[b]	103.16 ± 5.24[b]	196.84 ± 5.24[b]
G-VI	119.50 ± 6.41[b]	180.50 ± 6.41[b]	75.83 ± 4.27[b]	224.17 ± 4.27[b]
G-VII	101.66 ± 5.33[b]	198.34 ± 5.33[b]	59.83 ± 3.60[b]	240.17 ± 3.60[b]
G-VIII	189.16 ± 7.08[ns]	110.84 ± 7.08[ns]	213.33 ± 6.52[ns]	88.33 ± 6.64[ns]
G-IX	188.50 ± 4.60[ns]	111.50 ± 5.83[ns]	207.50 ± 5.72[ns]	92.50 ± 5.72[ns]
G-X	196.50 ± 5.83[ns]	103.50 ± 5.83[ns]	208.33 ± 6.14[ns]	91.66 ± 6.14[ns]

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

Table 6. Effect of aqueous and *n*-hexane fractions of Anise on concentration of AChE, MDA, SOD, CAT and GSH in mice brain.

	AChE µmol/min/mg	MDA nmol/h/g	SOD U/mg of homogenate	Catalase U/mg of homogenate	GSH µmol/mg
G-I	4.01 ± 0.21	1.43 ± 0.12	25.15± 0.58	1.97 ± 0.05	44.11 ± 1.50
G-II	8.94 ± 0.26[a]	8.96 ± 0.18[a]	7.94 ± 0.31[a]	0.56 ± 0.04[a]	18.90 ± 0.90[a]
G-III	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	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	7.90 ± 0.14[d]	7.70 ± 0.20[b]	15.71 ± 0.26[b]	1.18 ± 0.07[b]	32.47 ± 0.24[b]
G-VI	7.56 ± 0.26[c]	6.49 ± 0.28[b]	17.94 ± 0.31[b]	1.34 ± 0.07[b]	34.25 ± 0.60[b]
G-VII	6.27 ± 0.20[b]	5.12 ± 0.24[b]	20.17 ± 0.28[b]	1.43 ± 0.08[b]	38.59 ± 0.88[b]
G-VIII	8.81 ± 0.23[ns]	7.67 ± 0.27[c]	7.93 ± 0.31[ns]	0.63 ± 0.04[ns]	18.88 ± 0.57[ns]
G-IX	8.35 ± 0.27[ns]	7.54 ± 0.26[b]	8.07 ± 0.24[ns]	0.64 ± 0.05[ns]	17.86 ± 0.75[ns]
G-X	7.99 ± 0.30[ns]	7.45 ± 0.24[b]	8.20 ± 0.22[ns]	0.58 ± 0.05[ns]	19.11 ± 0.62[ns]

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

DISCUSSION

Phytochemical studies conducted on anise extracts indicated the presence of various phyto constituents i.e. proteins, tannins, glycosides and fixed oils, which are actually responsible for pharmacological action of the plant. Free radical scavenging activity found by DPPH method indicated that plant has potential to prevent the damage caused by oxidizing agents produced in body. Findings suggested that low IC_{50} value was recorded (37.48 µg/ml) with the use of aqueous extract of Anise which was due to the presence of different bimolecular species. Past studies indicated the presence of anethol (Andarwulan and Shetty, 1999), anisaldehyde (Reichling *et al.*, 1995), coumarins (Kartnig *et al.*, 1975), eugenol (Al Mofleh *et al.*, 2007) and terpenes (Burkhardt *et al.*, 1986) as abundant constituents among others which are actually responsible for scavenging of free radicals. Thus strong antioxidant activity of anise is subjected to these molecules. Results indicated that flavonoids were abundantly present in aqP.a which is considered a powerful source of antioxidant activity. They are supposed to protect the DNA from the damage of oxidizing substances (Zhou *et al.*, 2001).

Behavioral studies conducted by EPM paradigm (Table 3), one of widely used model (Chauhan and Chaudhary, 2012) in mice indicated that aqueous extract of anise reduced the transfer latencies on both day 1st and 2nd, which is indication of improvement in learned activity. Similarly, higher inflexion ratio (a parameter used to find the retention of learned skills) was recorded in aqP.a treated mice as compared to nhP.a and amnesic control mice.

Findings of light dark paradigm (Table 5) suggested that animals treated with aqP.a spent most of the time in dark compartment on both days which is due to the fact that aqueous extract prevented the mice brain from potential damages of scopolamine while nhP.a failed to do so.

The findings of hole board model indicated that only aqueous Anise extract improved the memory of mice by preventing them from the damages of scopolamine while *n*-hexane extract of Anise failed to do so. Hole board apparatus is based upon theory that increase in no of hole poking by animal is a natural behavior of rodents and scopolamine reduces this act, but retention of the exploration behavior in treated animals is indication of enhancement of memory (Durcan and Lister, 1988).

The findings suggested that aqP.a increased the memory and learning tasks by significantly *($P < 0.001$)* reducing the level of AChE in mice brain (Table 6). Acetyl cholinesterase is an enzyme responsible for breakdown of acetylcholine at synaptic clef in brain (Ballard *et al.*, 2005). Hence it is obvious that plant extract can also be used for the prevention of Alzheimer's disease (AD) which is supposed to be caused by loss of cholinergic innervations in hippocampus of the brain (Terry and Buccafusco, 2003). Scopolamine is a potent anticholinergic drug which impairs memory by blocking cholinergic neurotransmission in certain brain parts. Short term amnesia is associated with the use of scopolamine and hence this is used to induced amnesia in rodents to assume memory enhancing potentials of test substances (Spignoli and Pepeu, 1987). In contrast to scopolamine, cholinergic drugs i.e rivastigmine, donepezil and piracetam prevent the cholinergic neurons from the damages of AChE and hence can be used to prevent AD (Birks *et al.*, 2000). Moreover, possibly it is believed that aqueous extract of Anise being strong antioxidant prevented the brain cholinergic neurons from the potential damages of AChE. The activity of AChE is enhanced by oxidizing agents in brain (Rosemberg *et al.*, 2010) and antioxidants prevent the loss of memory both

by reducing the level of AChE and by preventing accumulation of toxic protein metabolites in brain tissues (Frank and Gupta, 2005). Phytochemical investigation of aqP.a suggested the presence of alkaloids and flavonoids which are hallmark of anti AChE activity (Oboh *et al.*, 2012).

It has been reported that severe oxidative stress in brain causes lipid peroxidation which in turn increases the level of MDA in brain homogenates (Placer *et al.*, 1966). Thus the level of MDA in brain homogenates was observed to be significantly *($P < 0.001$)* reduced by the use of aqP.a which is indication of strong antioxidant potential of Anise aqueous extract. Similarly, it also increased the level of endogenous antioxidants like SOD, GSH and CAT in brain homogenates of treated mice (Table 6). These enzymes are responsible to prevent the lipid peroxidation and to maintain the structure of neurons (Koruk *et al.*, 2004). The antioxidant mechanism of SOD suggested that it converts free nascent oxygen into molecular oxygen and H_2O_2 (Gough and Cotter, 2011) by the presence of zinc, manganese and copper, and within peroxisome, CAT converts hydrogen peroxide into H_2O (Zhan *et al.*, 2004).

Conclusion: Thus it is concluded that Anise aqueous extract possess strong antiamnesic action by involving one or more mechanisms including scavenging of free radicals, neuroprotection and by inhibition of level of acetylcholinesterase in brain cells.

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