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# Reversal of repeated noise stress-induced behavioral, cognitive, neuroendocrine response and oxidative parameters by *Abelmoschus esculentus* (Okra) root powder in rats

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**Abstract**: Noise is an environmental stressor that may leads to unfavorable effects on the quality of life and behavior. Consuming medicinal plants can be considered for the relief of adverse effects produced by noise exposure. *Abelmoschus esculentus* (AE) is widely consumed plant, however least scientific work has been done on its roots. The current project is intended to appraise the properties of AE root powder on noise stress (NS)-induced behavioral and memory impairment, alteration in stress hormone release and oxidative stress. AE (1600mg/kg b.w) was given orally once a day for 2 weeks before experience to NS. Animals were experienced to NS for 4 h post treatment of AE for 2 weeks. After treatment period behavioral test were performed. Then, rats were sacrificed, brain and plasma samples were collected for biochemical analysis. Repeated NS-induced anxiety and depression, impaired learning and memory, increased glucocorticoid levels and produced oxidative stress. AE inhibited NS-induced behavioral deficits, improved cognitive abilities, enhanced antioxidant mechanism. Decreased brain acetylcholinesterase and plasma corticosterone levels in AE+NS treated rats. Administration of AE induced cognitive improvement, anti-stress effect and enhancing antioxidant defense mechanism. Thus, it may signify a potential remedial alternative against NS-induced neurological disorders which deserves consideration and further assessment.

**Keywords**: Noise stress, *Abelmoschus esculentus* root powder, anxiety, depression, memory, oxidative stress, glucocorticoid, antioxidant enzymes.

## INTRODUCTION

Noise is one of the prevailed environmental stressors that is one of the most health hazards globally. It is not only a great problem for human kind but also for animal life and influenced physiological, psychological and other behavioral activities (Naqvi *et al.,* 2012). Noisy condition is harmful and caused behavioral impairment and delayed communication skills (Cohen, 1980). Loud environmental noise is very harmful for health and produce cognitive malfunction in children (Matheson *et al.,* 2003; Stansfeld *et al.,* 2005). Repeated experience to unavoidable noise stress induced fatigue, social-interaction, depression and anxiety-like symptoms (Rylander, 2004; Evan & Johnson, 2000). Furthermore cognitive function was reported to be spoiled following chronic loud noise (Haider *et al*., 2012). Various stressors are involved in activation of neuroendocrine system to help maintaining the homeostasis (Figueiredo *et al*., 2003). It is well documented that stress responses involved in upregulation of hypothalamic-pituitary-adrenocortical (HPA) axis which control its activation by its end product (cortisol in human and corticosterone in animal) by using negative feedback mechanism (Herman & Cullinan, 1997). Psychological stressors such as noise stress via HPA axis produce its effects on limbic system that have association with acoustic system (Turner *et al*., 2005). Stress-induced stimulation of HPA-axis, elevates concentration of stress hormones i.e. glucocorticoid (Joels *et al*., 2006; Fukui *et al*., 2001). Glucocorticoid has high affinity for mineralocorticoid receptor (MR) while low-affinity for glucocorticoid receptor (GR) which is involved in controlling HPA-axis. In mammals, exposure to stress stimulates HPA axis and reduces expression of MR. Therefore, alteration in expression of MR is vital in normalizing behavioral deficits and neuroprotection (Krause *et al.,* 2015). Repeated stress decreased the neural growth and enhanced oxidative stress that altered brain functions (Borji *et al*., 2017). It is reported earlier that stressful situation enhanced the activity of mitochondrial enzymes and increased the activity of cytosolic NADPH oxidase that caused over production of reactive oxygen species (Fukui *et al.,* 2001). Increased glucocorticoid levels increased generation of NADPH oxidase-dependent free radicals in hippocampus that involved in alteration of behavioral responses and cognitive functions. Repeated noise stress increased the free radical generation and produced oxidative stress that deteriorated antioxidant mechanism (Sikandaner *et al.,* 2017), and associated with behavioral deficits such as anxiety and depression-like behaviors (Badache *et al*., 2017) and memory impairment (Sikandaner *et al.,* 2017; Badache *et al*., 2017). In addition, metabolism of biogenic amines is also suffered

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badly due to noise stress (Haider *et al*., 2012).

Extensive scientific reports have shown that plants play important role in the treatment of several diseases that are linked with oxidative stress such as neurological diseases. The consumption of antioxidant compounds from medicinal plants may be minimizing the risk of rising chronic diseases. It is reported earlier that *Indigofera tinctorial* and *Scoparia dulcis* are immunoprotective and prevented noise stress (Madakkannu & Ravichandran, 2017). Other studies found that *Acorus calamus* via its antioxidant potential attenuate noise stress induced memory impairment (Sundaramahalingam *et al.,* 2013) and normalized antioxidant status (Manikandan *et al.,* 2005).

Okra (*Abelmoschus esculentus* (AE) Linn) is annually grown plant in different countries around the world. Various bioactive compounds such as quercetin (Pankajmani & Seshadri, 1953), 3',4'-di-O-methylquercetin, proanthocyanidins (Danie, 1989), gossypin (Seshadri & Viswanathan, 1947), (_)-epigallocatechin (Maganha *et al*., 2010), 5-hydroxytryptamine (Sinha *et al.,* 1961), rhamno galacturonans (Deters *et al.,* 2005), lutein and zeaxanthin (Aruna *et al*., 2009; Murillo *et al.,* 2010) etc. are present in AE plant. Various *In-vitro* and *In-vivo* studies have been conducted with Okra plant. Some *In-vitro* studies indicated that seeds of Okra contain antitrypsin effect on chymotrypsin from bovine (Ogata *et al.,* 1986), while mucilage of okra plant exhibited anti-complementary effect (Tomada *et al.,* 1989). Furthermore, lectin from Okra contains hemagglutinating activity (Soares *et al*. 2012) whereas polysaccharide fraction of AE showed anti-fatigue activity (Gao *et al.,* 2018). Anti-bacterial effects of fresh water extract of pods (de Carvalho *et al.* 2011) and gold nanoparticles synthesized by using pulp extract ((Mollick *et al.,* 2014) have also been reported. *In-vivo* studies showed that AE exhibited anti-inflammatory responses in mice by increasing secretion of interleukin-12 and interferon-gamma while decreasing interleukin-10 (Ortac *et al.,* 2018) Anti-diabetic effects of fruit extract (Chen *et al.,* 2016) peel and seed powder of AE (Saha *et al.,* 2011) in rats have also been reported. Neuroprotective effect of AE fruit in dexamethasone-treated mice (Tongjaroenbuangam *et al.,* 2011) and aqueous and methanolic extract of seed (Doreddula *et al.,* 2014) prevented scopolamine induced memory impairment and exerted anti-stress effects in rats.

Based on the previously reported neuroprotective effects of fruit and seed of AE plant, although the mechanism of neuroprotective effect of AE is still unknown. The present study is aimed to evaluate the effect of AE root powder on repeated noise stress-induced behavioral and cognitive alterations, changes in the release of stress hormone and antioxidant defense mechanism.

## MATERIALS AND METHODS

### *Animals*

In this study Male (Sprague-Dawley) rats weighing 170-190 gram (8 weeks by age) procured from NIH, Islamabad were utilized. The animals were caged independently to keep away from social contact effect because social contact can influence behavioral analysis of animals. All the experiments were performed under 12 h light-dark cycle (light on at 6:00 h) and illicit animals treatment area's temperature (23 ± 3 °C) with unrestricted contact to standard rodent diet (Bocarsly *et al.,* 2012) and drinking water. Before start of experimental work, rats were experienced to 7 days of acclimation time and different conduct measures in order to diminish the stress of newness and treatment. All experiments were approved by Departmental Bio-ethical Committee (D-1891/1-Biochem; Dated: April 19, 2018) and performed with guidelines of NIH for care and use of laboratory animals.

### *Chemicals and reagents*

Acetylthiocholine Iodide, Sodium Azide, EDTA, Thio-Barbituric Acid, Hydrogen peroxide stock solution, Nitroblue Tetrazolium, Tri-chloroacetic Acid and Dithio-Bis-nitrobenzoic Acid and etc. were purchased from Sigma Chemicals.

### *Preparation of AE (Okra) root powder*

AE was collected from the nearby area of Multan City, Pakistan. The plant material was recognized and authenticated by the taxonomist (Dr. Zafar Ullah Zafar, Department of Botany, Bahauddin Zakariya University, Multan) and voucher (rjp-454) was retained in an herbarium. The AE root was separated and dried under shade. The dried material was made into fine powder using a blender, and was then stored in air tight containers until the completion of the experiments. Required amount of powder was weighed and mixed well in deionized water (3 ml) and fed to animal by gavage technique. Every time fresh drug was prepared for oral administration.

### *Acute toxicity test*

Toxicity procedure was performed as per reported previously (Saher *et al.,* 2015). Two sets of rats (n=4) were accommodated overnight in fasting condition with tape water and food was withdrawn for 4-5 hrs after oral intake of AE root powder. Starting dose 100mg/kg body weight was given to one set and maximum dose 1600 mg/kg body weight was given to set two. Third set was drinking tape water and all the rodents were observed individually. Clinical signs were examined. Body posture, tremors, locomotion, pain response, vocalization, body weight, water intake etc. were also observed. No death was seen with given doses. According to Economic Co-operation and Development (OECD) guidelines the $LD_{50}$ value considered above 100 and 1600 mg/kg body weight.

***Investigation 1: Assessment of dose associated effect of AE root powder on home cage and open field activity***

Effect of Doses (0, 100, 200, 400, 800 and 1600 mg/kg/day) of AE on exploratory activity in open field and home cage were assesses to select a potential dose that produce protective effect against repeated noise stress induced adverse effects. Animals were treated with AE for 15 days (1 group=5 rats). The activity was conducted for 300 sec post 15 days treatment of AE.

***Investigation 2: Assessment of the effect of selected dose (1600 mg/kg/day) of AE on repeated noise stress-induced behavioral alteration, memory impairment, increased stress hormone release and oxidative stress***

Twenty rats arbitrarily alienated into 4 equal groups of 05 each and treated for 2 weeks. (i) water+unstressed; (ii) water+stressed; (iii) AE+unstressed (1600mg/kg/day; oral administration); (iv) AE+stressed (1600 mg/kg/day; oral administration) and 20 minutes post treatment animals were subjected to 4-h noise stress.

After 2 weeks (on day 15) behavioral activities {Forced swim test [FST], light dark box activity [LDA], elevated plus maze [EPM] and Morris water maze [MWM] test (training session and short-term memory)} of all animals were monitored.

On day 16, 1-h after the assessment of long-term memory in MWM, animals were deeply anesthetized by Isoflurane (Induction dose 4%) and decapitated by using guillotine as reported earlier (Saleem *et al.,* 2013). After decapitation, cranium was incised and covering of brain membrane was detached with aid of forceps. The brain was taken out using spatula and cleared with cold isotonic solution. Blood sample was also taken for extraction of plasma. All samples were immediately kept freeze at −70ºC for biochemical estimations.

***Noise stress (NS)***

Noise was chronicled from the generator that was augmented by speakers in an isolated room. Speakers were positioned 32 cm above the cages. The level of noise was adjusted at 100 dB as it could be stressful for animal and intensity was measured by a sound level meter DS102 (range: 80–130 dB, accuracy: ± 1.5 dB), made in Taiwan (Naqvi *et al.,* 2012).

***Behavioral tests***

*Open field test (OFT)*

An open arena with the dimension of 80 x 80 x 42 (L x W X H in cm) was used for the evaluation of locomotor activity in rodents. The apparatus was placed in the isolated sound proof/illuminated room and all experimental animals were acclimatized with the environment for at least 2 hours before the start of experiments. During the test session of 5 minutes, the animal was placed in the center of novel restricted environment and number of crossings were recorded on the top mounted camera coupled with laptop **(**Haider *et al,* 2011). The apparatus employed in the experiment was manually marked with permanent marker and length x width was divided in to 25 equal squares (the dimension of each square is 256 cm).

*Home cage activity (HCA)*

A customized made transparent apparatus with the dimension of 25 x 25 x 20 cm was used for the measurement of locomotor activity in experimental animals. The animals were provided a familiar condition to home cage i.e. a sawdust covered floor. Experimental protocol was adopted from our previous experiment settings reported in (Haider *et al.,* 2011). Briefly each animal was recorded for the duration of 5 minutes in which the number of passages and falling/rearing were observed by the simultaneously recording system.

*Light-dark activity (LDA) test*

A customized made apparatus of acrylic glass was used for the measurement of animal's anxiety behavior in a two compartmental system of light and dark environment. The dimension of the apparatus is 40 x 30 x35 cm where light and dark compartments are equally divided i.e. 20 cm each and central door of 10x 10 cm allows free passage of animal between the compartments. Total duration of test is 5 minutes in which duration of animal's time spent in each compartment was noted with lateral camera recording system. Subsequently the recorded videos were analyzed by blinded observer to the experiments with the help of stop watch.

*Elevated plus maze (EPM) test*

Plus maze is a gold standard for the measurement of anxiolytic activity of test compounds and for the said purpose we used the apparatus of + shape with the total dimensions of 110 x 10 x15 cm. The closed and open arms are distinguished with central 10cm arena. For the testing purpose, we used the protocol previously established by (Samad & Haleem, 2009) and in summary the animal was allowed to stay on the evaluated platform for the duration of 5 minutes and behavioral assessment was done by its time and frequency spent in open arm vs closed arm. Top mounted camera system (Logitech C-310, Switzerland) recorded the videos. Between each trial, the apparatus was cleaned with 70% isopropyl alcohol to wash out the smell of previous trial.

*Forced swimming test (FST)*

FST is one of most widely employed method for the evaluation of depressant/antidepressant behavior of the rodents in a restraining environment (Porsolt, 1981). In our experimental setting, we used a glass tank with the dimension of 45 cm height and 30 cm radius for the assessment of antidepressant activity of the test substances. The apparatus was filled with water (25◦C) up to certain level where the feet of animal should not get the

support from the basement and simultaneously inescapable from the tank. In a trial session of 5 minutes each, all animals were allowed to forced swim. In experimental session, the treated rats were challenged to the environment of inescapability and difference between the states of mobility vs immobility was observed by lateral recording which subsequently were analyzed by blinded observer to the experiments. The rat will be considered in a phase of depression where it spent most of the time in a phase of immobility and makes virtually no efforts to escape and merely tries to keep its head above the water.

*Morris water maze (MWM) test*

In this test we assessed the impact of okra powder on the retention of spatial memory in rats. For the purpose, we used customized made circular tank of grey color with dimension of 150 cm in radius and 50 cm in height. The tank was filled with water and temperature was maintained at 25°C throughout the experiment. The tank was equally divided into quadrants i.e. NE, SE, NW and SW and an opaque color platform made of acrylic glass was placed in the SW quadrant of tank. The distal cues with distinct shapes were placed in the all 4 quadrants of tank walls which was helpful for the animal to navigate in the tank and direction. Before starting the experiment, the color of the water was changed to murky with the addition of nonhazardous white ink and platform was submerged 1-2 cm in water. The test was performed in two session (1) the training session and (2) test phase which comprises of retention of short (60 minutes after training) and long (24 h after training) term retention of memory. The animal was placed all the time from the same quadrant i.e. NE and headed for the wall of tank. In the training phase, each rat was allowed 2 minutes time to navigate and find the hidden platform and if located within the cut off time, the animal was allowed to stay at platform for 10-20 seconds to navigate the surroundings otherwise after the span of 2 minutes the animal was directed to the platform by manually guiding it to the platform. 60 minutes later to training phase, short term retention of memory was performed on the trained rats and latency to reach the platform was noted. The videos were recorded on the top mounted camera and subsequently analyzed by Any-Maze (V 6.01) video tracking software.

***Biochemical parameters***

Decapitation of all animals was done on same day after behavioral tests. Brain samples were detached and washed with 0.9% saline solution. A brain homogenate 10 % (wt./vol.) was prepared with phosphate buffer (0.1 M, pH 7.4) and got by centrifugation (12000xg) for 20 min at 4° Celsius for all malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) estimations.

*Levels of MDA*

The method of Chow and Tappel (1972) was used for analysis of MDA with some changes in the procedure. 0.3 ml brain homogenate was added in 2 ml TCA-TBA solution and then boiled in a water bath for 20 min. The mixture was then cooled down and centrifuged (35000 RPM) for 10 min. Light pink colored supernatant was collected and 532 nm absorbance was used to note down the absorbance.

*Activity of catalase (CAT)*

100 µl brain supernatants mixed with 1.4 ml solution of comprised of 400 µl of hydrogen peroxide and 1 ml of phosphate buffer (0.01 M, pH 7.0). The reaction was stopped 1 min after by adding 2 ml of reagent (dichromate acetic acid). To calculate activity of CAT absorbance was taken at 620 nm as described by previously (Pari *et al.,* 2001).

*% inhibition of SOD*

Previously reported method was used for analysis of SOD (Naskar *et al.,* 2011). 300 µl of brain homogenate was centrifuged with 0.75 ml ethanol and 0.15 ml of chloroform. After centrifugation 500 µl supernatant was taken and then 0.5 ml EDTA and1.0 ml of 0.1 M carbonate-bicarbonate buffer (10.2 pH) were added. By adding 0.5 ml epinephrine reaction became started, absorbance was taken at 480 nm and % inhibition of SOD was calculated.

*Activity of GPx*

The method of Flohe & Gunzler (1984) was used to measure activity of GPx as µmol/min/g of the brain. 1.0 ml of reaction solution that contained 0.3 ml phosphate buffer (0.1M, pH7.4), 0.2 ml of glutathione reduced, 0.1 ml of sodium azide, 0.1 ml of hydrogen peroxide and 0.3 ml of brain homogenate was prepared. The reaction solution was incubated for 15 min at 37°C, by adding 0.5 ml TCA the reaction was terminated and the solution was centrifuged (1500xg) for 15 min and filtrate was removed. 0.2 ml phosphate buffer and 0.7 ml 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) were mixed with 0.1 ml filtrate. Absorbance of solution was recorded at 420 nm.

*Activity of AChE*

The method of Ellman *et al.* (1961) was used to estimate activity of AChE. 0.4 ml brain homogenate, 2.6 ml phosphate buffer, 0.1 ml DTNB were mixed by bubbling air and put it down into spectrophotometer. When reaction solution was steady, basal reading followed by addition of 5.2 µl of acetylthiocholine iodide in a cuvette contained reaction solution. Absorbance was recorded at 412 nm. Activity of AChE was mentioned as µmol/min/g of brain tissue.

*Levels of Plasma corticosterone*

Blood was collected in the heparinized centrifuged tubes for the estimation of plasma corticosterone as describe

earlier (Samad *et al.* 2006; Haider *et al.* 2013)

## STATISTICAL ANALYSIS

Results are represented as ± SD. All the behavioral and biochemical data were analyzed by Tukey's test following two-way ANOVA (2- way analysis of variance) using SPSS version 20.0 and *P* less than 0.05 was taken as significant.

## RESULTS

Effect of AE doses on exploratory activites in an open field and home cage tests shown in fig 1. Data on activity in an open field test was analyzed by ANOVA (one way) [$F_{(5,24)}$=88.55, *p=0.014*] showed substantial effect of AE. It is observed by Tukey's test that activity of AE at doses 100.0, 200.0, 400.0, 800.0 and 1600.0 mg/kg substantially increased.

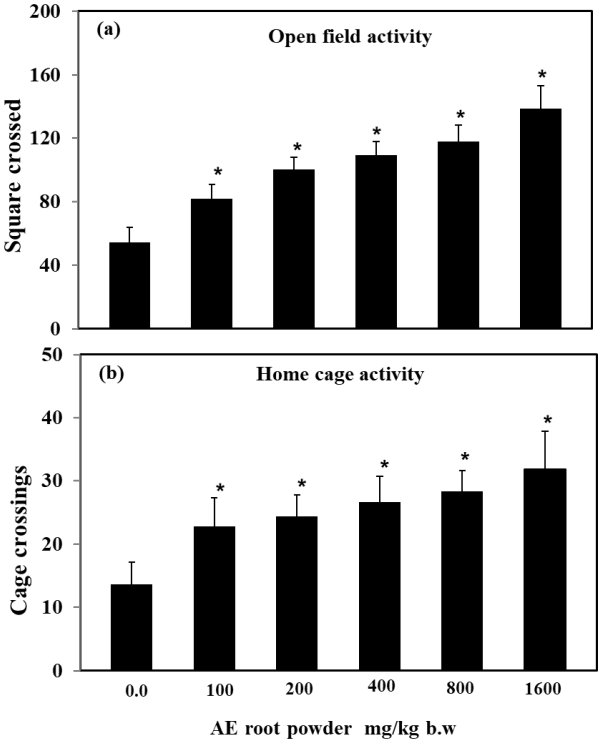


**Fig. 1**: Various doses of AE root powder were used to evaluate activity in open filed and home cage.
Mean values ± S.D. (n=5) 15 days post treatment of AE. Substantial changes by Tukey's test **P<0.05* from water treated rats ensuing 1-way Anova.

Data on activity in home cage was analyzed by ANOVA (one way) [$F_{(5,24)}$=52.82, *p=0.001*] showed substantial effect. It is observed by Tukey's test that activity of AE at doses 100.0, 200.0, 400.0, 800.0 and 1600.0 mg/kg substantially increased.

Fig. 2 shows the effects of NS induce anxiety-like symptoms in AE and water treated rats conducted in the light dark compartment. Data for time spent in light compartment analyzed by ANOVA (two way) exhibited that substantial effect of NS [$F_{(1,16)}$=53.10 *p=0.000*], AE [$F_{(1,16)}$=131.66 *p=0.000*], and interaction of NS x AE [$F_{(1,16)}$=4.00 *p=0.001*]. Tukey's test exhibited that repeated NS substantially reduced time spent in a light compartment in water treated than water+unstressed animals. A substantial increase was observed in the time spent of light box in AE treated rats than control.

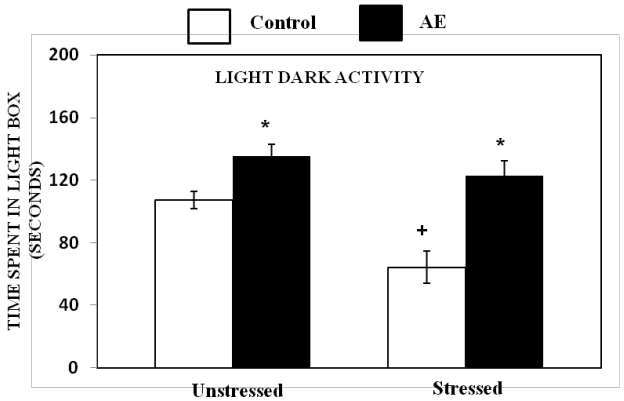


**Fig. 2**: Treatment of AE on anxiety like behavior in unstressed and noise stressed animals' pragmatic in LDA.
Mean values ± S.D. (n=5). Substantial changes by Tukey's test **P<0.05 vs.* corresponding control and *+P<0.05 vs.* unstressed animals ensuing 2-way Anova.

Fig. 3 shows the effects of NS induce anxiety-like symptoms in AE and water treated rats conducted in the EPM. Data for time spent in open arm analyzed by ANOVA (two way) exhibited insubstantial effect of NS [$F_{(1,16)}$=3.25, *p=0.063*]. Effect of AE [$F_{(1,16)}$=67.13 *p=0.001*] and interaction of NS×AE [$F_{(1,16)}$= 30.11 *p=0.000*] were substantial. Tukey's test exhibited that 4 h repeated NS substantially reduced time spent in open arm in water treated than water+unstressed controls. Time spent in open arm substantially increased in AE treated NS rats than controls.

Fig. 4 shows the effects of NS induced depression like behavior in AE and water treated rats. Data on immobility time in FST showed effects of NS [$F_{(1,16)}$=209.8 *p=0.000*], AE [$F_{(1,16)}$=39.15 *p=0.005*] and AE x NS interaction [$F_{(1,16)}$= 62.06 *p=0.002*] were substantial following ANOVA (two-way). Tukey's test revealed reduced immobility time in AE treated stressed animals. On the other hand, increased immobility time was observed in NS treated control one.

Fig. 5 shows the effects of NS -induced altered memory function in AE and water treated rats conducted in MWM. Data on time to reach the platform immediately post training (acquisition), 1-h (short term memory) and 24-h (long term memory) was used and analyzed by ANOVA (two-way). Data on acquisition revealed significant effect

of AE [$F_{(1,16)}$=5.24 *p=0.020*]. Effect of NS [$F_{(1,16)}$ = 0.68 *p=0.089*] and interaction [$F_{(1,16)}$=0.78 *p=0.093*] were insignificant. Data on short term memory revealed insignificant effects of NS [$F_{(1,16)}$=0.54 *p=0.102*]. Effect of AE [$F_{(1,16)}$=122.83 *p=0.000*] and interaction [$F_{(1,16)}$=6.47, *p=0.012*] were substantial. Data on long term memory revealed substantial effects of NS [$F_{(1,16)}$=73.88 *p=0.003*] and AE [$F_{(1,16)}$=20.89 *p=0.001*], while the interaction [$F_{(1,16)}$=2.68 *p=0.063*] was insubstantial. Tukey's test revealed short- and long-term memory enhanced by the treatment of AE in both sub-groups. While long term memory impaired following NS.

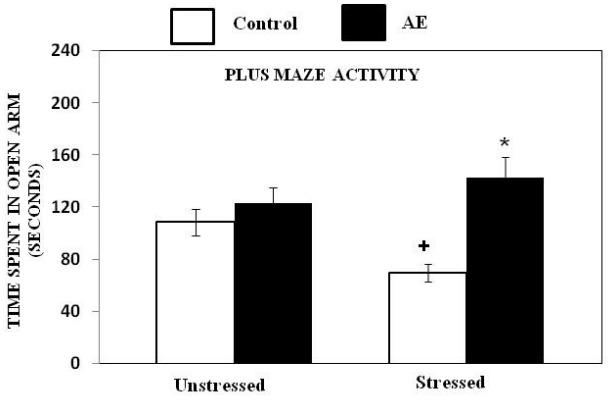


**Fig. 3**: Treatment of AE on anxiety like behavior in unstressed and noise stressed animals' pragmatic in EPM. Mean values ± S.D. (n=5). Substantial changes by Tukey's test **P<0.05 vs.* corresponding control and *+P<0.05 vs.* unstressed animals ensuing 2-way Anova.

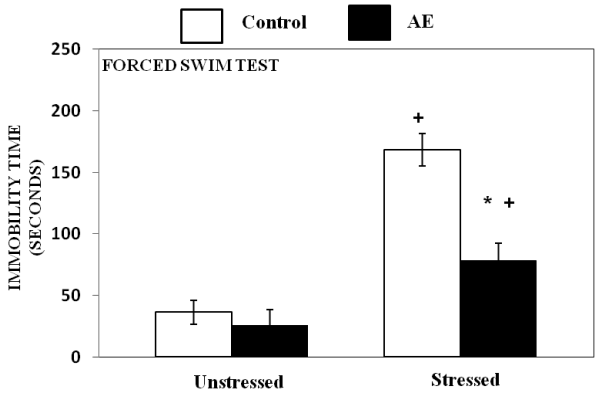


**Fig. 4**: Treatment of AE on depression like behavior in unstressed and noise stressed animals' pragmatic in FST. Mean values ± S.D. (n=5). Substantial changes by Tukey's test **P<0.05 vs.* corresponding control and *+P<0.05 vs.* unstressed animals ensuing 2-way Anova.

Fig. 6 shows the effects of NS on lipid peroxidation in AE and water treated rats. ANOVA (two-way) revealed effects of AE [$F_{(1,16)}$=53.75 *p=0.001*], NS [$F_{(1,16)}$=13.85 *p=0.006*] and interaction between NS and AE [$F_{(1,16)}$=31.2 *p=0.004*] were substantial. Tukey's test showed that increased MDA levels following NS in water treated animals. Conversely, reduced MDA level was estimated in AE treated animals.

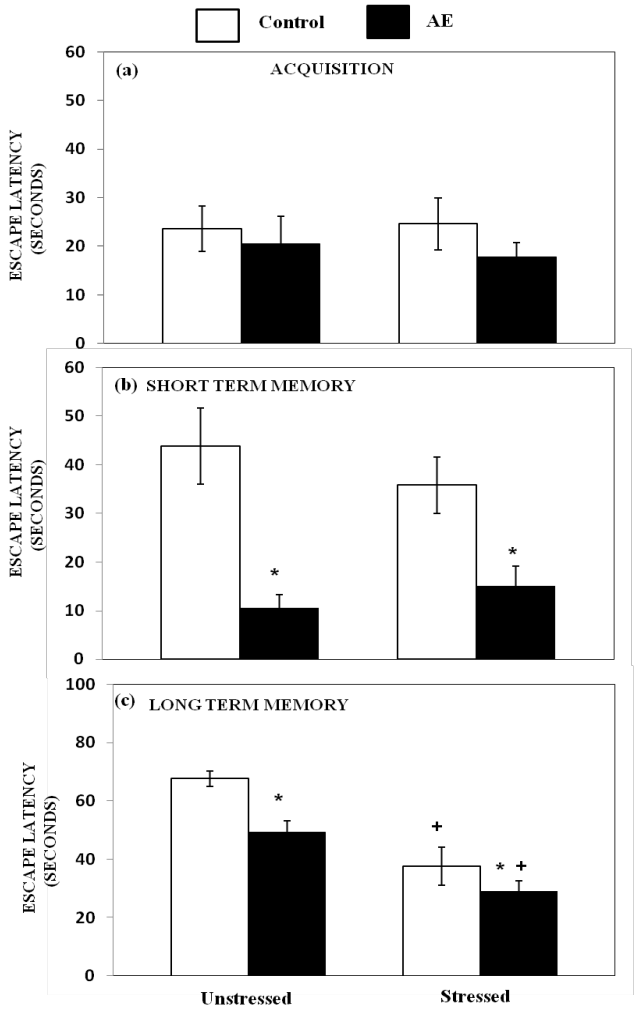


**Fig. 5**: Treatment of AE on memory function [acquisition (a) STM (b) and LTM (c)] as escape latency measured in MWM. Depression like behavior in unstressed and noise stressed animals' pragmatic in FST.
Mean values ± S.D. (n=5). Substantial changes by Tukey's test **P<0.05 vs.* corresponding control and *+P<0.05 vs.* unstressed animals ensuing 2-way Anova.

Fig. 7 shows effects of NS on activity of antioxidant enzyme in AE and water treated animals. ANOVA (two way) used to analyzed the activity of enzymes. Data on the activity of SOD revealed effects of AE [$F_{(1,16)}$=842.8 *p=0.000*], NS [$F_{(1,16)}$=114.45 *p=0.000*] and interaction between NS and AE [$F_{(1,16)}$=336.36 *p=0.000*] were substantial. Tukey's test revealed reduced activity of SOD in water+stressed rats. Conversely, increased activity of SOD observed in AE treated both sub-groups. The activity of SOD increased in AE+stressed than AE+unstressed rats.

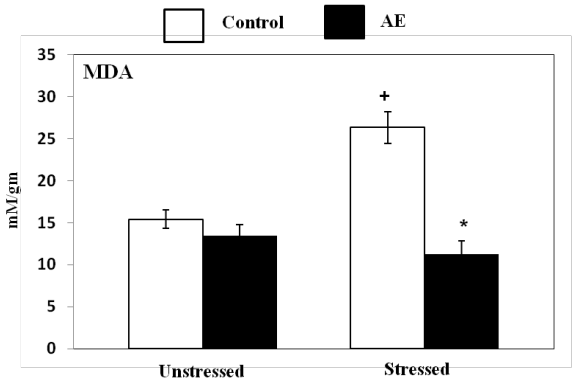


**Fig. 6**: Treatment of AE on brain MDA levels in unstressed and noise stressed animals.
Mean values ± S.D. (n=5). Substantial changes by Tukey's test **P<0.05 vs.* corresponding control and *+P<0.05 vs.* unstressed animals ensuing 2-way Anova.

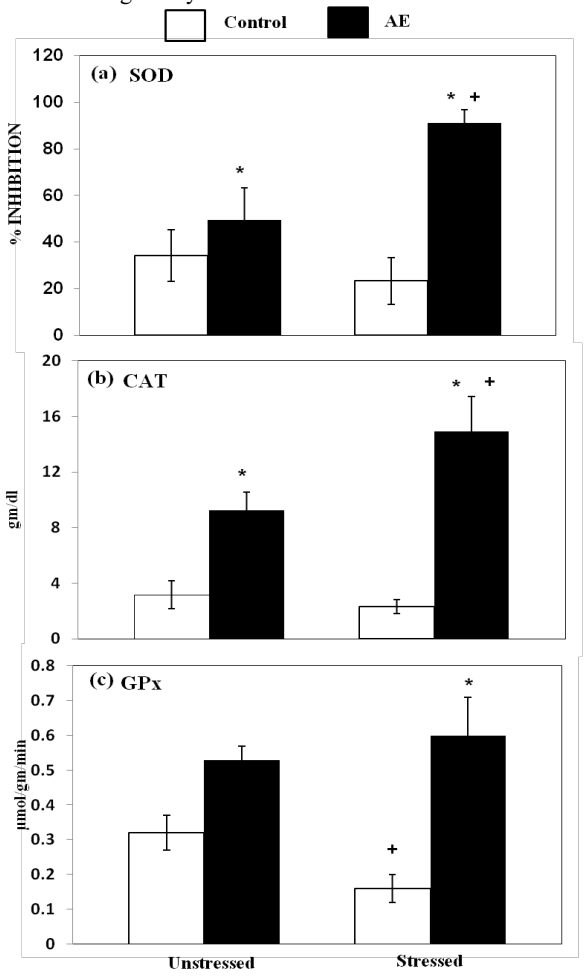


**Fig. 7**: Treatment of AE on brain SOD (a), CAT (b) and GPx(c) activity in unstressed and noise stressed animals.
Mean values ± S.D. (n=5). Substantial changes by Tukey's test **P<0.05 vs.* corresponding control and *+P<0.05 vs.* unstressed animals ensuing 2-way Anova.

Data on the activity of CAT revealed effects of AE [$F_{(1,16)}$=434.6, *p=0.000*], NS [$F_{(1,16)}$=118.48 *p=0.000*] and interaction between the two factors [$F_{(1,16)}$=145.29 *p=0.000*] were substantial. Tukey's test revealed increased activity of CAT in AE treated animals than their counterparts. Whereas, activity of CAT increased in AE+stressed than AE+unstressed animals.

Data on the activity of GPx revealed effect of AE [$F_{(1,16)}$=107.42, *p=0.000*] and interaction between AExNS [$F_{(1,16)}$=13.43 *p=0.002*] were substantial. Effect of NS [$F_{(1,16)}$=0.16 *p=0.165*] was insubstantial. Tukey's test showed reduced activity of GPx in water+stressed than water+unstressed rats. On the other hand, increased activity of GPx observed in AE+stressed than AE+unstressed rats.

Fig. 8 shows the effect of NS on activity of AChE in the brain in AE and water treated rats. ANOVA (two-way) revealed effects of AE [$F_{(1,16)}$=143.30 p=0.000], NS [$F_{(1,16)}$=6.94 p=0.003] and interaction between NSxAE [$F_{(1,16)}$=16.47 p=0.001] were substantial. Tukey's test revealed activity of AChE in the brain reduced following administration of AE. The levels of AChE activity also decreased in AE+stressed than water+stressed animals.

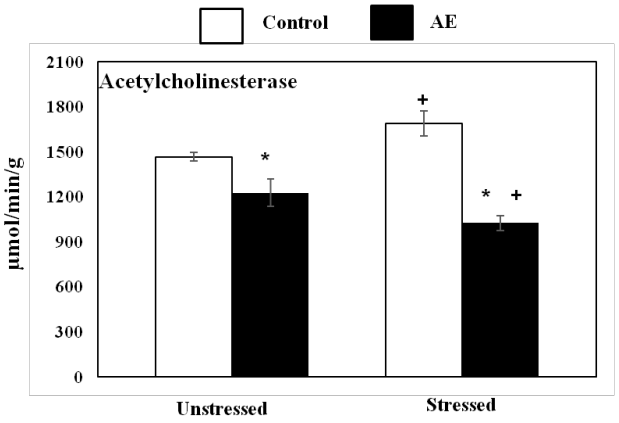


**Fig. 8**: Treatment of AE on brain AChE activity in unstressed and noise stressed animals.
Mean values ± S.D. (n=5). Substantial changes by Tukey's test **P<0.05 vs.* corresponding control and *+P<0.05 vs.* unstressed animals ensuing 2-way Anova.

Fig. 9 shows the effect of NS on plasma corticosterone levels in AE and water treated rats. ANOVA (two-way) showed effects of AE [$F_{(1,16)}$=19.29 *p=0.003*], NS [$F_{(1,16)}$=33.44, *p=0.000*] and interaction between NSxAE [$F_{(1,16)}$=5.61 *p=0.000*] were substantial . Tukey's test revealed levels of plasma corticosterone elevated in following NS in control rats. Levels of corticosterone decreased in AE treated NS animals than their counterpart.

## DISCUSSION

The present results showed that repeated NS (daily for 4 - h) decreased the time spent in light compartment (fig. 2)

and open arm (fig. 3) of light dark activity box and elevated plus maze respectively, enhanced immobility time in forced swim test (fig. 4) and prolonged latency to escape in Morris water maze test (fig. 5). These results suggest that repeated NS produced anxiety- and depression-like behaviors and impaired memory (figs. 2-4) and consistent with previous reports (Sikandaner *et al.,* 2017; Badache *et al.,* 2017). Administration of AE root powder (1600 mg/kg/day) increased the time spent in the light compartment and open arm as well (figs. 2 & 3) suggesting anxiolytic effects of AE root. The immobility time was also decreased by administration of AE root powder, indicating antidepressant effects. Furthermore, latency escape was also diminished following repeated administration of AE powder, results suggesting that AE powder also involved in enhancement of learning and memory function. These behavioral studies revealed that administration of AE root powder produced anxiolytic-antidepressant and memory enhancing effects following repeated NS (daily for 4 hrs).

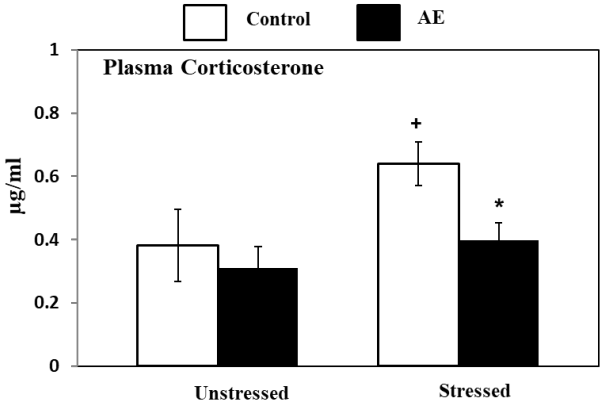


**Fig. 9**: Treatment of AE on plasma corticosterone levels in unstressed and noise stressed animals.

Mean values ± S.D. (n=5). Substantial changes by Tukey's test **P<0.05 vs.* corresponding control and *+P<0.05 vs.* unstressed animals ensuing 2-way Anova.

Previously it is understood that stress induced stimulation of HPA-axis and oxidative stress produce behavioral deficits (Lucca *et al.,* 2009). In the present study levels of plasma corticosterone were increased following repeated noise stress (fig. 9). Stress related hormone increased the metabolism to aid the accessibility of glucose which causes increased free radicals' production. It is reported earlier that stress exposure induced oxidative stress causes activation of glucocorticoid-dependent transcription of pro-oxidant factors and decreases antioxidant defense system (Ceriello *et al.,* 1996). The mitochondrial respiration plays important role in oxidative stress at cellular level. It is known that oxidative stress due to administration of glucocorticoid enhanced oxidative phosphorylation and mitochondrial respiration (Russell *et al.,* 2002). *In vitro* culturing of cortical and hippocampal neurons with glucocorticoid caused greater production of free radicals, suppressed the gene expression of antioxidant enzymes and supported NADPH-oxidase expression contributing towards oxidative stress (Behl *et al.,* 1997). Enzymatic and non-enzymatic antioxidants also diminished by administration of glucocorticoid and repeated stress in the rodent brain sample. In addition, the treatment of antioxidants prevented the free radical production induced by glucocorticoid (Manikandan *et al.,* 2006). Studies have also mentioned that expression and activity of antioxidant enzymes were reduced due to increased levels of glucocorticoid which involved in inhibition of Nrf2 gene expression (Ki *et al.,* 2005). Likewise, free radical production involves in stimulation of ACTH hormone production in that way it hyperactivate the HPA-axis using decrease feedback inhibition which may promote glucocorticoid induced oxidative stress (Lacoste *et al.,* 2001). In the present study levels of MDA become increased due to repeated NS (Fig. 6) in rat brain. Moreover, GPx activity was substantially decreased, while the activity of SOD and CAT comparably reduced than control (fig. 7). Increased free radical production may decrease the antioxidant enzyme activity that contributes in enhancing oxidative stress. The experiential NS induced oxidative stress can be associated with increased glucocorticoid levels in rats and may be elucidate the basis for behavioral deficits (anxiety and depression) and altered memory functions in this study. Increased AChE activity is also inked with altered memory function inclusive of increased plasma corticosterone levels and oxidative stress in brain samples of NS rodents. It is reported that decreased synaptic plasticity and hippocampal-based memory alteration occurred due to experience of repeated stress or administration of glucocorticoid (Evanson *et al.,* 2010). Treatment of glucocorticoid and chronic stress are also involved in hippocampal cholinergic dysfunction (Finsterwald & Alberini 2014). Hence, increased activity of AChE and corticosterone levels in NS animal reinforces the premise that the cholinergic dysfunction and stimulation of HPA-axis following NS may be liable for altered memory function in behavioral test.

AE root powder has proved to prevent NS associated behavioral alterations (figs. 2-4). It has observed that AE consists of a variety of components that acquire antioxidant activity, i.e. quercetin and (-)-epigallo-catechin (Shui & Peng, 2004). Quercetin exhibit neuroprotective properties in animal models, i.e. it improves memory and learning abilities (Nassiri-Asl *et al.,* 2013) and have antidepressant and anti-stress (Herrera-Ruiz *et al.,* 2011) actions as a compound of extracts from herbs (Maganha *et al.,* 2010). Epigallo-catechin is also found to have antioxidant, anti-inflammatory, antiapoptotic (Khalatbary & Khadimi, 2020) and anti-stress (Khomsug *et al.,* 2010) effects. Furthermore, it is also found that high contents of a constituent are present in AE that have potent antioxidant effects by using DPPH free radical capturing activity

(Khomsug *et al.,* 2010). Previously it has reported that repeated stress-induced impairment of hippocampal neurogenesis due to a reduction in DCX hippocampal expression is reversed by repeated administration of quercetin (Mehta *et al.,* 2017), suggesting that quercetin involves in neurogenesis particularly in the hippocampus and replaces the damaged cell that is hinder signaling in the brain and influence with various behavioral activities and cognitive abilities. The newly synthesized nerve cells enhance the integrity of hippocampus and improve hippocampus-dependent functions. The components present in AE such as lutein enhances the neurogenesis by increasing expression of various neural growth factors (Crupi *et al.,* 2016). In this study treatment with AE alongwith exposure of repeated NS significantly exhibited anxiolytic and antidepressant effects, improved the acquisition as wellshort-term and long-term memory which was connectedwith reduced glucocorticoid concentration in plasma and diminished lipid peroxidation in the brain. Moreover, the stress-instigated impaired activity of antioxidantenzymeswas also regularized due to consumption of AE root powder. The hypoactivity of HPA-axis is may be due to the contents of AE that associated with mineralocorticoid- and glucocorticoid-receptor in the hippocampus which is liable for enhanced negative feedback inhibition of HPA-axis following stress exposure. It is also recognized that uniform signaling of mineralocorticoid- and glucocorticoid-receptor have a positive response to behavioral response (De Kloet *et al.,* 1998). Thus the reduced corticosterone levels in AE+NS group in this study can be ascribed to the reduced hyperactivity of HPA-axis and may associate with reduced oxidative stress and increased activity of antioxidant enzymes. The present results are also in agreement with previous studies (Samad & Saleem, 2018) as we observed the significant attenuation of stress-induced altered activity of SOD, CAT and GPx thus normalizing the oxidative stress. The regulated inhibition of HPA-axis in AE+NS rats may also be responsible for anxiolytic and antidepressant responses and reduced activity of AChE via diminished oxidative stress. The decrease in activity of AChE may involve in increased availability of acetylcholine at synapse resulting in improved cognitive function regardless of the experience of the repeated stressful situation.

## CONCLUSION

Supplementation/administration of AE root powder normalized NS-induced behavior deficits, elevated corticosterone levels, oxidative stress and AChE activity and, as a result, produced anti-anxiety and antidepressant-like behaviors and improved memory function inthe present study. Further, in the presented work we did not compare the bioactive compounds present in AE root powder such as Quercetin, Lutein, Epigallo-catechin and others, while all possess potential antioxidant effects, as produced by AE root powder in the present study on stress-induced behavioral and biochemical alteration.

Hence, it can be recommended that intake of AE root powder may provide curative effects against stress-induced unpleasant effects on the activity of the brain by enhancing the firmness and adjustment to HPA-axis following stress exposure.

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