

# D-galactose induced dysfunction in mice hippocampus and the possible antioxidant and neuro-modulatory effects of selenium

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## Research Article

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# Abstract

Aging is an ultimate reality that everyone has to face. D-galactose (D-gal) has been used extensively to develop aging model. Trace elements such as selenium (Sel) have been used as a potential antioxidant for neuro-protection. The present work aims to develop therapeutic agents such as Sel for the treatment of aging-induced neurological ailments such as anxiety, depression, and memory impairment. For this purpose, mice were treated with D-gal at a dose of 300 mg/ml/kg and various doses of Sel (0.175 and 0.35mg/ml/kg) for 28 days. Behavioural tests were monitored after treatment days. After the behavioural assessment mice were decapitated and their brains were collected. Hippocampi were removed from the brain for biochemical and neurochemical analysis. The present findings of behavioural analysis showed that D-gal induced anxiety and depression-like symptoms were inhibited by both doses of Sel. D-gal induced memory alteration was also prevented by repeated doses (0.175 and 0.35mg/ml/kg) of Sel. Biochemical analysis showed that D-gal induced increase of oxidative stress marker and decrease of antioxidant enzymes in the hippocampus was prevented by Sel administration. An increase in the activity of acetylcholinesterase was also diminished by Sel. The neurochemical assessment showed that D-gal induced increased serotonin metabolism and decreased acetylcholine levels in the hippocampus were restored by repeated treatment of Sel. It is concluded that D-gal induced dysfunction in mice hippocampus caused anxiety, depression, memory impairment, oxidative stress that were mitigated by Sel via its antioxidant potential and modulating capability of serotonergic and cholinergic functions.

## Introduction

Aging is a gradual functional impairment at the organismal and cellular levels (Wagner et al. 2016) These physiological dysfunctions are attributed to increased genomic instability, altered metabolism, and the loss of regenerative potential (Baker et al. 2016). The process of aging is associated with a variety of disorders that cause tissue homeostasis imbalance, memory, and learning impairments (Bonfilii et al. 2020) depression, and anxiety-like behavior (Samad et al. 2019). Extensive evidence shows that aging is progressed by oxidative stress which is induced by a discrepancy between reactive oxygen species formation and antioxidant system (Cabello-Verrugio et al. 2016). Moreover, a decrease in oxidative stress and its associated homeostatic imbalance is a valuable measure to increase life expectancy (Pérez et al. 2009). The process of aging is subordinate with pathophysiology by declining redox status and is an inordinate challenge for therapeutic gerontology (Franco et al 2018).

D-Galactose (D-gal) is a reducing sugar (Timal et al 2012) which is mostly present in dairy foods, some vegetables, certain fruit pectin, chestnuts, and in few herbs (Fernandes et al. 2006). Extensive studies show that the accumulation of Dgal alters the antioxidant system and speed up the process of aging (Du et al. 2019). Excessive D-gal may also cause rapid reactive oxygen species formation which hinders the cellular antioxidant defense system and (Samad et al. 2019).

Furthermore, it is revealed that intoxication of D-gal responsible for neurochemical and behavioral alterations (Samad et al. 2019). Prolonged D-gal treatment mimics the consequence of natural aging in

numerous tissues of a pre-therapeutic animal model (Ho et al. 2003). It is reported that the intake of D-gal induces anxiety (Kaviani et al. 2017) depression (Samad et al. 2019) and memory impairment (Guo et al. 2018). Oxidative stress induced by chronic administration of D-gal was mitigated by blueberry (Çoban et al. 2015), ascorbic acid (Nam et al. 2019) quercetin (Dong et al. 2017) lithium chloride (Samad et al. 2019), n-3 polyunsaturated fatty acid (Guo et al. 2018), and caffeine (Ullah et al. 2015) to ameliorate the D-gal-induced behavioral and cognitive deficits.

Trace elements and minerals are important micronutrients (Qu et al. 2016) that can regulate the aging-associated alternations in physiological, homeostatic, and metabolic pathways (Méplan et al. 2011). Selenium (Sel) is a well-known mineral due to its therapeutic potential as an antioxidant (Oliveira et al. 2012). Sel is a novel and probable remedy for aging and aging-associated diseases such as tumours, cardiovascular syndromes, and skin aging, etc (Cai et al. 2019). It carried out its most biological roles via seleno-proteins (Kryukov et al. 2003). Sel exerts neuroprotective effects by homeostasis regulations through several mechanisms such as alleviation of oxidative stress (Cosín-Tomás 2019), inflammatory pathways regulation (Ataizi et al. 2019) decrease in DNA destruction (Kryukov et al. 2003), increasing telomere length and activity (Cao et al. 2017), and modulation of numerous neurotransmitter (Yan et al. 2019). Moreover, Sel intricates in the physiopathology of various psychiatric illnesses (Nogueira et al. 2011) by modulating serotonergic systems (Besckow et al. 2020).

Several shreds of evidence suggest that besides antioxidant effects, Sel plays a vital role in the treatment of various psychiatric ailments. Considering its therapeutic role, it is assumed that Sel may mitigate Dgal induced dysfunction in mice hippocampus resulting in anti-anxiety and/or depression-like behaviour and improvement in memory functions. For the said purpose low (0.175mg/ml/kg) and high (0.35mg/ml/kg) doses of sodium selenite was selected and used for the possible mitigation of aging induced psychiatric illnesses in mice.

## Materials And Methods

### Animals

Thirty-six male adult albino mice weighing 25-30g; 12 months old; were taken to conduct the present study. Animals with free access to water and food were housed individually at  $27\pm 1^{\circ}\text{C}$  on 12 hours day and night cycle. The experiment was carried out thoroughly in an optimal setting to overcome the effect of time and order. All experimental protocols were carried out after approval by the Departmental Bioethical Committee (Ref No# Biochem/D/162/2020; Dated: January 2, 2020).

### Experimental Schedule

Thirty-six mice were randomly separate in six groups (mice in each group); (i) Control (ii) Sel (0.175mg/ml/kg) (iii) Sel (0.35mg/ml/kg) (iv) D-gal; 100mg/ml/kg (v) D-gal plus Sel (0.175mg/ml/kg) (vi) D-gal plus Sel (0.35mg/ml/kg). The control animals given distilled water (1ml/kg), while D-gal and Sel dissolved in distilled water and injected intraperitoneally per day for 28 days. Behavioural activity was

performed after 28 days include; light-dark box activity and elevated-plus-maze activity (intended for anxiety), Morris-water-maze (for memory), forced-swim-test (intended for depressive symptoms). After behavioural tests mice were decapitated, their brains were taken out within 30 s and dipped in ice-cold saline. Afterward, instantly placed in brain slicer with ventral side up to dissect out the hippocampus by inserting the blade at into the slots of the brain slicer just above and below the hypothalamus, to cut the brain into three slices which were then shifted to a petri dish placed on ice, moistened with chilled saline (0.9% NaCl). The middle slice was used to dissect out hippocampus bilaterally with the help of sharp scalpel blade. All the hippocampus samples were stored at -40C° immediately for bio- and neuro-chemical estimations.

### ***Evaluation of various behaviours***

#### **Elevated Plus Maze (Epm)**

Epm consists of a plus sign maze having four arms with a central stage (a) in which two are open (b) and two are close (c) thru dimensions 5x9x5 cm (a), 16x9x5cm (b), and 16x9x5x9x15 cm (c) respectively, while the height of maze is 26 cm from the floor. Open arm and height are used to induce anxiety in rodents by Epm. 1-h after the last treatment every mouse separately hold at the central stage of Epm and time duration spent in open arm recorded for 5 minutes (Samad et al. 2018; 2019)

#### **Light–dark activity (Lda)**

Lda consists of a quadrilateral case separated by an impervious divided into two compartments; (a) dark compartment (b) light compartment. The magnitude of two chambers was 26x26x26 cm with a transportation hole of 12x12 cm in-between partition. Light compartment made up of transparent plastic while dark box made up of translucent plastic. Mice were placed to lightbox 1-h after the treatment of drugs and time duration spent in lightbox recorded for 5 minutes (Samad et al. 2018; 2019).

#### **Forced Swim Test (Fst)**

FST is one of the most widely employed methods for the evaluation of depressant/antidepressant behaviour of the rodents in a restraining environment. In our experimental setting, we used a glass tank with a dimension of 45 cm height and a 30 cm radius for the assessment of the antidepressant activity of the test substances. The apparatus was filled with water (25°C) up to a certain level where the feet of the 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 the experimental session, the treated mice were challenged to the environment of inescapability and the difference between the states of mobility vs immobility was observed by lateral recording which subsequently was analysed by the blinded observer to the experiments. The mice 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 (Samad et al. 2018).

#### **Morris Water Test (Mwm)**

This test was conducted to assess spatial memory in mice/rats. The reported procedure of Samad et al. (Samad et al. 2019) was used. A circular pool with 45cm in diameter, 37cm in height and 12 cm in depth was used. Inner side of pool was painted with white colour and a stage of 8cm in diameter was used to place below (2cm) of water thru water maze training. To unclear the stage, milky water ( $24\pm 2$ ) was filled in the pool. Acquisition, short term- and long-term memory were assessed in the experiment in term of latency to reach the stage. The test includes two phases; the training phase and the test phase with same starting positions. 2minutes were fixed as cut off times for every session. The retention latency was recorded to assess memory function of the animal. In the start of training stage each mice was placed into the water for movement in the pool. After placing, 2 minutes were allotted to each mice to reach and stand onto the unseen stage. If the animal reached on the stage it was permitted to stand there for 10 seconds. If it unsuccessful to reach the stage within given time then it was directed to the stage and that session repeated again. The evaluation of memory was conducted immediately after acquisition, 60 minute (Short term memory) and 24-h (long term memory) following training session during which escape latency was monitored.

### **Analysis of biochemicals in the hippocampus**

Hippocampus washed with 0.9% saline solution and weighted (averagely 35 mg). A 10% w/v tissue homogenate was prepared with 0.1M phosphate buffer (pH 7.4) and centrifuged at 10,000xg for 10 minutes at 4C. The supernatant was used for following biochemical analysis.

### **Malondialdehyde (MDA)**

MDA act as a biomarker of oxidative stress and is formed as a consequence of lipid peroxidation. In the case of oxidative stress, the level of MDA increases. MDA levels were assessed by the method of Chow and Tappel (1972). Centrifugation was performed at 3500-rpm and a light pink supernatant was obtained then absorbent 532nm was recorded. The level of MDA in a sample of the brain was described as "mM/g".

### **Superoxide dismutase (SOD)**

The activity of the SOD enzyme was calculated through the previously reported procedures (Sinha 1972). The Homogenate of tissue was made with phosphate buffer. Tissue homogenate used from each sample is 0.5 ml to form a reaction mixture. The reaction mixture also includes sodium bicarbonate (1ml), EDTA (0.2 ml), and NBT (0.4 ml). Hydroxylamine hydrochloride (0.4ml) was then added to the reaction mixture to direct the reaction. An extra test tube was used containing a mixture of all reaction components but without homogenate of tissue, known as control. The absorbance of each sample was measured at 570nm.

### **Catalase (CAT)**

The activity of the CAT enzyme was calculated through the previously reported procedure (Pari and Latha 2011). The homogenate of tissue was prepared through a 0.1 M phosphate buffer (pH 7.0). The reaction

mixture for the CAT activity analysis was mixed in test tubes separately having tissue homogenate (0.1 ml) with 1 ml of phosphate buffer (pH 7.4). Then H<sub>2</sub>O<sub>2</sub> (0.4ml) was added. Incubation was then performed at 37 ° C for 90 sec. To prevent the reaction, potassium dichromate / acetic acid (2 ml) was added in all test tubes, and after adding the reagent, the reaction mixture was turned blue. Now at 100°C, incubate again for 15 minutes then the color of the mixture turned solid blue to green. This change of color was due to the chromic acetate synthesis. The control test tube contained no tissue homogeneous, while the test tube having distilled water in place of a homogenate of tissue is called a blank. With the help of a spectrophotometer, absorbance was noted at 570nm. The activity of CAT in a sample of the brain was described as "mg/dl".

### **Glutathione peroxidase (GPx)**

GPx activity was assessed via chemicals and methods given by Flohe and Gunzle (1984). A reaction mixture was formed in each test tube by combining 0.1ml of sodium azide, 0.2ml of brain supernatant, 0.2ml of glutathione, and 0.3 ml of phosphate buffer. The reaction mixture was incubated for fifteen minutes at 37°C. To stop the reaction, TCA was inserted in all test tubes having each tissue sample reaction mixture. Again, the centrifugation was done at 15000-rpm for five minutes. The further reaction was performed by collecting the supernatant after centrifugation. Disodium hydrogen phosphate buffer (0.2ml), DTNB (0.7ml), and supernatant of each tissue sample (0.1ml) were assorted in each tube. Absorbance was noted at 420nm. The activity of GPx in a sample of the brain was described as "µmol/g/min".

### **Acetylcholinesterase (AChE)**

AChE activity was assessed via chemicals and methods given by Ellman et al (1961). The reaction mixture was formulated by adding acetylcholine-iodide as enzyme substrate. Homogenate of tissue (0.4 ml) was added in 2.6 ml phosphate buffer having 8.0 pH and DTNB (100µl) was mixed separately and now mix well with aerated air. Absorbance was noted at 420nm when the reaction mixture was stabilized with the use of a spectrophotometer. The acetylcholine-iodide (5.2µl) was then inserted in every test tube and then the absorption was noted after every 2min from 0 to 10 minutes for each sample.

The activity AChE in a sample of the brain was described as "µmol/g/min".

### ***Analysis of neurochemicals in the hippocampus***

#### **Analysis of 5-HT and its metabolite**

5-hydroxy indole acetic acid (5-HIAA) and 5-hydroxytryptamine (5-HT) levels in the hippocampus were estimated using the method described by Samad et al. (Samad et al. 2019). Reversed-phase High Performance Liquid Chromatography (HPLC) with an electrochemical detector (Shimadzu LEC 6A detector) was performed to detect levels of biogenic amines in brain samples. The electro-chemical (EC) detector was operated at a potential of +0.8 V. The stationary phase used for separation is a 5-µ Shim-

pack octadecyl silane (ODS) column having an internal diameter of 4.0 mm and a length of 150 mm. The mobile phase that passes through a column with a pump pressure of 2000–3000 psi contains octyl sodium sulfate (0.023%) in 0.1 M phosphate buffer at pH 2.9.

### **Analysis of Acetylcholine (ACh)**

The level of ACh concentration was determined as described by Liaquat et al (2019) and was presented as  $\mu\text{mol/g}$  of the hippocampus. The tissue sample was boiled to release the bound ACh and inactivate the enzyme. The reaction mixture was then mixed with ferric chloride 1% (1000  $\mu\text{L}$ ) to form a brown color complex and read at 540 nm against the reagent blank

### **Statistical analysis**

The findings are presented (mean  $\pm$  SD) for 6 mice/group. The statistically important differences were estimated by Tukey's test proceeding 2-way ANOVA using SPSS V. 21.  $p < 0.05$  was taken as statically substantial.

## **Results**

**Figure 1** exhibits the influence of Sel on anxiety-like symptoms in control and D-gal treated mice assessed in EPM and LDA. Data for time consumed in open arm (1a, EPM) evaluated by two-way ANOVA show that the effects of D-gal [(F1, 30) = 49.595,  $p < 0.01$ ], Sel [(F2, 30) = 86.140,  $p < 0.01$ ], and interaction of D-gal \* Sel [(F2, 30) = 3.802,  $p > 0.01$ ] were significant. According to Tukey's test, D-gal treatment caused a significant decrease in the time spent in the open arm as compared to control animals. The time expended in open arm significantly improved in Sel treated (at both doses) than water and D-gal treated mice.

Evaluation of data for time expended in light-box (1b, LDA) by Two-way ANOVA revealed that the influence of D-gal [(F1, 30) = 225.69,  $p < 0.01$ ], Sel [(F2, 30) = 169.52,  $p < 0.01$ ], and D-gal \* Sel [(F2, 30) = 31.59,  $p < 0.01$ ] were significant. According to Tukey's test, D-gal caused a significant reduction in the time spent in light-box as compared to control animals. The time spent in light-box was significantly higher in Se (at both doses) than control and D-gal treated groups.

**Figure 2** elaborates on the influence of Sel on the immobility time during FST in D-gal treated mice. Substantial effect of D-gal [(F1, 30) = 211.342,  $p < 0.01$ ], Sel [(F2, 30) = 155.99,  $p < 0.01$ ] and D-gal \* Sel interaction [(F2, 30) = 22.63,  $p < 0.01$ ] were found by Two-way ANOVA. According to Tukey's test immobility time during FST was substantially elevated by D-gal as compared to control mice. The immobility period was decreased significantly at both doses of Sel as compared to control and D-gal treated mice. Immobility time was greater in both doses of Sel and D-gal groups than both doses of Sel+water treated groups.

**Figure 3** represents the influence of drugs on cognitive function during MWM. Data on Acquisition shows substantial effect of D-gal [(F1, 30) = 39.25, p< 0.01], Sel [(F2, 30) = 55.76, p< 0.01] and D-gal \* Sel [(F2, 30) = 8.504, p< 0.01] by Two-way ANOVA. Tukey's test showed that time to reach the platform increased following D-gal administration. Time to reach the platform was reduced in Sel treated than water and D-gal treated mice.

Data on STM assessed by Two-way ANOVA exhibits the substantial effects for STM of D-gal [(F1, 30) = 26.64, p< 0.01], Sel [(F2, 30) = 95.64, p<0.01] and D-gal \* Sel [(F2, 30) = 15.76, p< 0.01].

Tukey's test showed that time to reach the platform increased following D-gal administration. Time to reach the platform was reduced in Sel treated than water and D-gal treated mice.

Data on LTM evaluated by Two-way ANOVA shows substantial effect of D-gal [(F1, 30) = 9.35, p< 0.01], Sel [(F2, 30) = 13.36, p< 0.01] while interaction between D-gal \* Sel interaction [(F2, 30) =

0.063, p > 0.01] was non-substantial. Tukey's test showed that time to reach the platform increased following D-gal administration. Time to reach the platform was reduced in Sel treated than water and D-gal treated mice.

**Figure 4** represents the influence of Se treatment on the level of MDA in water and D-gal treated mice. Substantial effects of D-gal [(F1, 30) = 79.77, p<0.01], Sel [(F2, 30) = 156.08, p<0.01] and D-gal \* Sel [(F2, 30) =36.09 p< 0.01] found by Two-way ANOVA. The results of Tukey's test indicated that the D-gal administration elevated the level of MDA than the control group. Both doses of Sel decreased the levels of MDA in water and D-gal treated groups than their respective control.

**Figure 5** shows the effect of Sel on the antioxidant enzyme. Data on SOD evaluated by Two-way ANOVA showed substantial effect of D-gal [(F1, 30) = 65.75, p< 0.01], Sel [(F2, 30) = 117.62, p< 0.01] and D-gal \* Sel [(F2, 30) = 6.61, p< 0.01]. Tukey's test exhibited that D-gal treatment reduced the activity of SOD. While Sel administration at both doses elevated the SOD activity in water as well as D-gal treated mice.

Data of CAT activity evaluated by two-way ANOVA represents the substantial influence of D-gal [(F1, 30) = 11.93, p<0.01], Sel [(F2, 30) = 65.084, p<0.01] and D-gal \* Sel [(F2, 30) = 7.094, p<0.01]. Results of Tukey's test displayed that Sel treatment at both doses enhanced the activity of CAT in both water and D-gal treated groups than their respective groups.

Analysis of data for GPx activity by two-way ANOVA represents the substantial effects of D-gal [(F1, 30) =7.92, p< 0.01] and Sel [(F2, 30) = 22.26, p< 0.01], while non-substantial effect of D-gal \* Sel interaction [(F2, 30) = 2.28, p> 0.05]. The results of Tukey's test exhibited the reduction of GPx activity by D-gal than the water treated group. Sel treatment at both doses improved the GPx activity in D-gal as well as in water treated mice.

**Figure 6** shows the effect of Sel on hippocampal AChE activity in D-gal treated and control mice.

Substantial effects of D-gal [(F1, 30) = 23.52, p< 0.01], Sel [(F2, 30) = 223.46, p< 0.01] and D-gal \*

Sel [(F2, 30) = 33.71, p< 0.01] were revealed by Two-way ANOVA. Tukey's test revealed, increased activity of AChE by D-gal treatment. Sel administration at both doses diminished the AChE activity in control as well as in D-gal treated group.

**Figure 7** shows the effect of Sel treatment on hippocampal serotonin metabolism in D-gal and water treated mice. Data on ACh levels revealed substantial effects of D-gal [(F1, 30) = 45.43, p< 0.01], Sel [(F2, 30) = 153.96, p< 0.01] and D-gal \* Sel [(F2, 30) = 0.092, p> 0.05] which were analyzed by Two-way ANOVA. Tukey's test revealed a decreased level of ACh in D-gal than control animals. Levels of ACh were enhanced in Sel+water and Sel+D-gal treated animals.

**Figure 8** shows the effect of Sel treatment on hippocampal serotonin metabolism in D-gal and water treated mice. Data on 5-HT levels revealed substantial effects of D-gal [(F1, 30) = 94.40, p< 0.01], Sel [(F2, 30) = 88.64, p< 0.01] and D-gal \* Sel [(F2, 30) = 15.78, p< 0.01] which were analyzed by Two-way ANOVA. Tukey's test revealed a decreased level of 5-HT in D-gal than control animals. Levels of 5-HT were enhanced in Sel+water and Sel+D-gal treated animals. Levels of 5-HT were decreased in D-gal+Sel (0.175mg/ml/kg) than water+Sel (0.175mg/ml/kg) treated animals.

Data on 5-HIAA levels revealed substantial effects of D-gal [(F1, 30) = 8.53, p< 0.01], Sel [(F2, 30) = 97.23, p< 0.01] and D-gal \* Sel [(F2, 30) = 2.937, p> 0.05] which were analyzed by Two-way ANOVA. Tukey's test revealed decreased level of 5-HIAA in D-gal than control animals. Levels of 5-HIAA were enhanced in Sel+water and Sel+D-gal treated animals.

## Discussion

D-gal is known for the induction of aging in animals (Wang et al. 2018). The present research work is aimed to evaluate the effects of Sel on D-gal induced aging-associated neurological ailments i.e. anxiety, depression, and cognitive abnormality. It was observed that repeated treatment with D-gal at a dose of 300 mg/ml/kg produced behavioural deficits that were similar to anxiety and depression-like behaviour, and memory impairment as well. Furthermore, un-balancing in oxidant and antioxidant enzyme levels indicating oxidative stress reduced cholinergic and serotonergic metabolism. Nevertheless, Se inhibited above mentioned negative effects of D-gal at both (0.175 and 0.35 mg/ml/kg) doses.

D-gal is widely used to prompt neurotoxicity in aging-related therapeutic research (Kumar et al. 2011). In experimental studies, an exogenous dose of D-gal raised the oxidative stress and influences the typical physiological process which ultimately elevates the process of aging in the brain (Ullah et al. 2015) which may lead various neurological disorders. Results of the present study showed that D-gal prolonged immobility time in FST suggesting depression-like behaviour (Figure 3) with decreased serotonin metabolism (Figure 8). Conversely, anxiety-like behaviour with decreased time spent in a light box and open arm of LDA and EPM (Figure 1) respectively with reduced serotonin metabolism following D-gal administration could not be impregnable. However, anxiety situations increased 5-HT metabolism due to

dysfunction/downregulation of 5-HT-1A receptor (Zhu et al. 2020) and stimulation of 5-HT-2C receptor at the dentate gyrus of the hippocampus (Sant'Ana et al. 2019). It might be possible that increased serotonin in the brain cause upregulation of 5-HT-2C and downregulation of 5-HT-1A receptor and produced anxiety condition. It is also notable that un-balancing of oxidant and antioxidant levels leads to increased oxidative stress which could be a reason for anxiety. Besides, it is examined that Se at both low (0.175 mg/ml/kg) and high (0.35 mg/ml/kg) inhibits anxiety and depression-like behaviours and produce anxiolytic and antidepressant effects.

Sel is a trace element and a potential antioxidant having neuroprotective effects observed in various clinical (Vinceti et al. 2018) and pre-clinical studies (Wang et al. 2017). It was evaluated from current research work that treatment of Sel increased 5-HT and its metabolite 5-HIAA (Figure 8) and decreased immobility time in FST in water and D-gal treated animals, suggesting the antidepressant effect of Sel. It was extensively reported that low levels of serum Sel levels produce depressive behaviours (Samad et al, 2019). While administration Sel increased serotonin metabolism and produced antidepressant effects (Nogueira and Rocha 2011). It is reported that Sel modulates serotonergic neurotransmission as an inhibitor of monoamine oxidase A, a degradative enzyme of serotonin, subsequent an increase in the synaptic availability of serotonin (Brüning et al. 2019). It is indicated that Sel as a powerful antioxidant can inhibit D-gal instigated un-balancing of oxidant and antioxidant system and modulates serotonergic mechanism.

Aging is characterized by alteration in brain anatomy and physiology which causes various neurological diseases (Frazzini et al. 2006). Aberrations in the antioxidant system can initiate aging and aging linked pathological conditions (Jadeja et al 2020). In the present work acquisition, short- and long-term memory alternation in D-gal treated mice evaluated during MWM activity (Figure 4) indicated that D-gal can impair learning & memory behaviours. Acetylcholine (ACh), a prototype neurotransmitter associated with cognitive functions (Papandreou et al. 2011). Acetylcholinesterase (AChE), a degradative enzyme, hydrolyzed ACh and alters the transmission of ACh at cellular and molecular level. Aging induces an increase in the activity of AChE (Daskalova et al. 2019). Previously it is reported that D-gal impaired the process of normal signalling pathways such as the decreased concentration of ACh by stimulating the activity of AChE (Kumar et al. 2011), which is also consistent with our results (Figure 6). The recent study elaborated that this D-gal elevate the activity of AChE and decrease the level of ACh may be responsible for D-gal induced memory impairment (Figure 7). Previous reports showed that other trace elements such as manganese (Biswas et al. 2019), zinc (Mecocci et al. 2018) improved cognition with the reduction of oxidative stress and inflammatory markers. At the central levels, Sel enhanced cholinergic function with the improvement of neuronal plasticity (Demirci et al. 2017) and by inhibition of inflammatory cytokines (Tang et al. 2019), which could increase the availability of ACh at the synaptic level and contend with the AChE activity. In the present study, Sel (0.175 & 0.35 mg/ml/kg) administration reduced the activity of AChE and enhanced acetylcholine with improved memory. It is suggested that as an antioxidant and modulator of cholinergic transmission Sel improves cognitive functions.

Oxidative stress impairs normal physiological and neurological functions (Grimm et al. 2016) and induce aging (Barnham et al. 2004). An important antioxidant enzyme SOD reacted with superoxide radical and changed it into water and H<sub>2</sub>O<sub>2</sub> that is detoxified by CAT and GPx enzymes (Saso et al. 2014). Previous studies revealed that chronic administration of D-gal can boost oxidative deterioration by diminishing the contents of antioxidant enzyme which alters the homeostasis and impairs many physiological processes (Benn et al. 2004). The reactive oxygen species generation by peroxidation of membrane lipids (Mladenov et al. 2006) can be disintegrated into different carbonyl compounds like MDA, so, the level of MDA is considered as oxidative stress biomarker. Like previous findings, recent study also revealed that repeated D-gal treatment cause reduction in activities of SOD, CAT and GPx (Figure 5) and boost the brain content of MDA (Figure 4) of mice as compared to control. It is reported that Sel supplementation in rats can decrease oxidative stress by increasing the activity of antioxidant enzymes (Aydođan et al. 2013). Similarly, our study hypothesized that D-gal induced oxidative stress can be barred by supplementation of Sel at (0.35 mg/ml/kg) & (0.175 mg/ml/kg) doses. Previously reported that Sel possess antioxidant properties (Huang et al. 2012) and the above-mentioned results of our study suggested that this antioxidant property of Sel may be responsible for its antidepressant, memory improving, and anxiolytic effects. It is concluded that Sel as a powerful antioxidant and neurotransmitter modulator can inhibit D-gal instigated anxiety, depression, and memory impairment. The present study confirms the neuroprotective role of Se and proves it as an anxiolytic, antidepressant, memory enhancer, antioxidant, and neurotransmitter (serotonin and acetylcholine) modulator.

## Declarations

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**Conflict of interest:** The authors declare that they have no conflict of interest

**Ethical Approval:** Institutional ethical approval (Ref No# Biochem/D/162/2020; Dated: January 2, 2020) was received for the animal experiment from the Department of Biochemistry, Bahauddin Zakariya University, Multan, Pakistan

**Consent to participate:** Not Applicable

**Consent to Publish:** Not Applicable

**Authors Contribution:** NS conceived and design research. FH conducted experiments. II provided laboratory for the conduction of experiments. NS wrote the manuscript. All author read and approved the manuscript.

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## References

- Ataizi ZS, Ertlav K, Nazıroğlu M (2019). [Mitochondrial oxidative stress-induced brain and hippocampus apoptosis decrease through modulation of caspase activity, Ca\(2+\) influx and inflammatory cytokine molecular pathways in the docetaxel-treated mice by melatonin and selenium treatments](#). *Metab Brain Dis* 34:1077-1089.
- Aydoğan F, Taştan E, Aydın E, Şenes M, Akgedik Ş, Berkem R, Üstün H (2013). Antioxidant role of selenium in rats with experimental acute otitis media. *Indian J Otolaryngol Head Neck Surgery* 65:541-547.
- Baker DJ, Childs BG, Durik M, Wijers ME, Sieben CJ, Zhong J, Pezeshki A (2016). Naturally occurring p16 Ink4a-positive cells shorten healthy lifespan. *Nature* 530:7589-184.
- Barnham KJ, Masters CL, Bush AI (2004). Neurodegenerative diseases and oxidative stress. *J Nature Reviews Drug Discovery* 3:205.
- Benn SC, Woolf CJ (2004). Adult neuron survival strategies—slamming on the brakes. *J Nature Reviews Neurosci* 5:9-686.
- Besckow EM, Nonemacher NT, Garcia CS, da Silva Espíndola CN, Balbom ÉB, Gritzenco F, Savegnago L, Godoi B, Bortolatto CF, Brüning CA (2020). Antidepressant-like effect of a selenopropargylicbenzamide in mice: involvement of the serotonergic system. *J Psychopharmacology (Berl)* 237:3149-3159.
- Biswas S, Anjum A, Banna HU, Rahman M, Siddique AE, Karim Y, Nikkon F, Haque A, Hossain K, Saud ZA (2019). [Manganese attenuates the effects of arsenic on neurobehavioral and biochemical changes in mice co-exposed to arsenic and manganese](#). *J Environ Sci Pollut Res Int* 26:29257-29266.
- Bonfili L, Cekarini V, Gogoi O, Berardi S, Scarpona S, Angeletti M, Rossi G, Eleuteri AM (2020) [Gut microbiota manipulation through probiotics oral administration restores glucose homeostasis in a mouse model of Alzheimer's disease](#). *Neurobiol Aging* 87:35-43.
- Brüning CA, Prigol M, Roehrs JA, Nogueira CW, Zeni G (2019). Involvement of the serotonergic system in the anxiolytic-like effect caused by m-trifluoromethyl-diphenyl diselenide in mice. *J Behav. Brain Res* 205:511–517.
- Cabello-Verrugio C, Simon F, Trollet C, Santibañez JF (2016). Oxidative stress in disease and aging: mechanisms and therapies. *J Oxidative Medicine and Cellular Longevity* 2017:4310469.
- Cai Z, Zhang J, Li H (2019). Selenium, aging and aging-related diseases. *Aging Clin Exp Res* 31:1035-1047.
- Cao L, Zhang L, Zeng H, Wu RT, Wu TL, Cheng WH (2017). [Analyses of Selenotranscriptomes and Selenium Concentrations in Response to Dietary Selenium Deficiency and Age Reveal Common and Distinct Patterns by Tissue and Sex in Telomere-Dysfunctional Mice](#). *J Nutr* 147:1858-1866.

- Chow C, Tappel AL (1972). An enzymatic protective mechanism against lipid peroxidation damage to lungs of ozone-exposed rats. *J Lipids* 7:518-524.
- Çoban J, Doğan-Ekici I, Aydın AF, Betül-Kalaz E, Doğru-Abbasoğlu S, Uysal M (2015). Blueberry treatment decreased D-galactose-induced oxidative stress and brain damage in rats. *Metab Brain Dis* 30:3-793-802.
- Cosín-Tomàs M, Senserrich J, Arumí-Planas M, Alquézar C, Pallàs M, Martín-Requero Á, Daskalova E, Delchev S, Topolov M, Dimitrova S, Uzunova Y, Valcheva-Kuzmanova S, Kratchanova M, Vladimirova-Kitova L, Denev P (2019). [Aroniamelano carpa \(Michx.\) Elliot fruit juice reveals neuroprotective effect and improves cognitive and locomotor functions of aged rats.](#) *J Food Chem Toxicol* 132:110674
- Daskalova E, Delchev S, Topolov M, Dimitrova S, Uzunova Y, Valcheva-Kuzmanova S, Kratchanova M, Vladimirova-Kitova L, Denev P (2019). [Aronia melanocarpa \(Michx.\) Elliot fruit juice reveals neuroprotective effect and improves cognitive and locomotor functions of aged rats.](#) *J Food Chem Toxicol* 132:110674
- Demirci K, Nazıroğlu M, Övey İS, Balaban H (2017). [Selenium attenuates apoptosis, inflammation and oxidative stress in the blood and brain of aged rats with scopolamine-induced dementia.](#) *Metab Brain Dis* 32:321-329
- Dong F, Wang S, Wang Y, Yang X, Jiang J, Wu D, Qu X, Fan H, Yao R. (2017). [Quercetin ameliorates learning and memory via the Nrf2-ARE signalling pathway in d galactose-induced neurotoxicity in mice.](#) *J Biochem Biophys Res Commun* 491:3-636-641.
- Du HM, Wang YJ, Liu X, Wang SL, Wu SM, Yuan Z, Zhu XK. (2019). Defective Central Immune Tolerance Induced by High-Dose D-Galactose Resembles Aging. *J Biochem (Mosc)* 84:6-:617-626.
- Ellman GL, Courtney KD, Andres Jr V, Featherstone RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacol* 7:88-95.
- Fernandes J, Saudubray JM, Van den Berghe G, Walter JH (2006). *Inborn metabolic diseases: diagnosis and treatment: Springer Science & Business Media.*
- Flohe L, Gunzler WA (1984). Assays of glutathione peroxidase. *J Methods Enzymol* 105:114– 121.
- Franco R, Vargas MR (2018) Redox Biology in Neurological Function, Dysfunction, and Aging. *J Antioxid Redox Signal* 28:18-1583-1586.
- Frazzini V, Rockabrand E, Mocchegiani E, Sensi S (2006). Oxidative stress and brain aging: is zinc the link? *J Biogerontol* 7:307-314.
- Huang Z, Rose AH, Hoffmann PR (2012). The role of selenium in inflammation and immunity: from molecular mechanisms to therapeutic opportunities. *J Antioxidants Redox Signaling* 16:705-743.

Grimm M, Regner L, Mett J, Stahlmann C, Schorr P, Nelke C, Zaidan S (2016). Tocotrienol affects oxidative stress, cholesterol homeostasis and the amyloidogenic pathway in neuroblastoma cells: consequences for Alzheimer's disease. *J Mol Sci Intl* 17:1809.

Guo YR, Lee HC, Lo YC, Yu SC, Huang SY. (2018). [n-3 polyunsaturated fatty acids prevent d-galactose-induced cognitive deficits in prediabetic rats.](#) *J Food Funct* 9:4-2228-2239.

Ho SC, Liu JH, Wu RY (2003). Establishment of the mimetic aging effect in mice caused by D-galactose. *J Biogerontol* 4:1- 15-18.

Huang Z, Rose AH, Hoffmann PR (2012). The role of selenium in inflammation and immunity: [Implication in anxiety behaviors.](#) *J CNS Neurosci Ther* 26:453-464.

Jadeja RN, Jones MA, Abdelrahman AA, Powell FL, Thounaojam MC, Gutsaeva D, Bartoli M, Martin PM. (2020). [Inhibiting microRNA-144 potentiates Nrf2-dependent antioxidant signalling in RPE and protects against oxidative stress-induced outer retinal degeneration.](#) *J Redox Biol* 28:101336.

Kaviani E, Rahmani M, Kaeidi A, Shamsizadeh A, Allahtavakoli M, Mozafari N, Fatemi I. (2017). [Protective effect of atorvastatin on d-galactose-induced aging model in mice.](#) *J Behav Brain Res* 15:334-55-60.

Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O, Guigó R, Gladyshev VN (2003). Characterization of mammalian selenoproteomes. *Science* 300:1439-1443.

Kumar A, Prakash A, Dogra S (2011). Centella asiatica attenuates D-galactose-induced cognitive impairment, oxidative and mitochondrial dysfunction in mice. *J Alzheimer's disease* 2011:347569.

Liaquat L, Sadir S, Batool Z, Tabassum S, Shahzad S, Afzal A, Haider S (2019). [Acute aluminum chloride toxicity revisited: Study on DNA damage and histopathological, biochemical and neurochemical alterations in rat brain.](#) *Life Sci* 217:202-211.

Mecocci P, Boccardi V, Cecchetti R, Bastiani P, Scamosci M, Ruggiero C, Baroni M (2018). [A Long Journey into Aging, Brain Aging, and Alzheimer's Disease Following the Oxidative Stress Tracks.](#) *J Alzheimer's Dis* 62:1319-1335.

Méplan C (2011). Trace elements and ageing, a genomic perspective using selenium as an example. *J Trace Elem Medicine Bio* 25: S11-S16.

Mladenov M, Gjorgoski I, Stafilov T, Duridanova D (2006). Effect of vitamin C on lipid hydroperoxides and carbonyl groups content of rat plasma depending on age and acute heat exposure. *J Thermal Biol* 31:588-593.

Nam SM, Seo M, Seo JS, Rhim H, Nahm SS, Cho IH, Chang BJ, Kim HJ, Choi SH, Nah SY. (2019). [Ascorbic Acid Mitigates D-galactose-Induced Brain Aging by Increasing Hippocampal Neurogenesis and Improving Memory Function.](#) *J Nutrients* 11:171-176.

- Nogueira CW, Rocha JB (2011). Toxicology and pharmacology of selenium: emphasis on synthetic organoselenium compounds. Arch Toxicol 85:1313-1359.
- Oliveira CES, Gai BM, Godoi B, Zeni G, Nogueira CW (2012). The antidepressant-like action of a simple selenium-containing molecule, methyl phenyl selenide, in mice. Eur J Pharm 690:13-119-123.
- Papandreou MA, Tsachaki M, Efthimiopoulos S, Cordopatis P, Lamari FN, Margarity M (2011). Memory enhancing effects of saffron in aged mice are correlated with antioxidant protection. Behav Brain Res 219:197-204.
- Pari L, Latha M (2001). Protective role of *Scorparia dulcis* plant extract on brain antioxidant status and lipid peroxidation in STZ diabetic male Wistar rats. BMC Complement Alternat Med 6:16
- Pérez VI, Buffenstein R, Masamsetti V, Leonard S, Salmon AB, Mele J, Friguet B (2009). Protein stability and resistance to oxidative stress are determinants of longevity in the longestliving rodent, the naked mole-rat. Proceedings of the National Academy of Sciences, 106:93059-3064.
- Qu Z, Zhang J, Yang H, Huo L, Gao J, Chen H, Gao W (2016). Protective effect of tetrahydropalmatine against d-galactose induced memory impairment in rat. J Physiol Behav 154:114-125.
- Samad N, Yasmin F, Manzoor N (2019). Biomarkers in Drug Free Subjects with Depression: Correlation with Tryptophan. Psychiatry Investig 16:12-948-953.
- Samad N, Imran I, Zulfiqar I, Bilal K. (2019) [Ameliorative effect of lithium chloride against d galactose induced behavioral and memory impairment, oxidative stress and alteration in serotonin function in rats.](#) Pharmacol Rep 71:5-909-916.
- Samad N, Jabeen S, Imran I, Zulfiqar I, Bilal K (2019). [Protective effect of gallic acid against arsenic-induced anxiety-/depression- like behaviors and memory impairment in male rats.](#) Metab Brain Dis 34:1091-1102.
- Samad N, Saleem A (2018). [Administration of Allium cepa L. bulb attenuates stress-produced anxiety and depression and improves memory in male mice.](#) Metab Brain Dis 33:271-281.
- Samad N, Saleem A, Yasmin F, Shehzad MA (2018). [Quercetin protects against stress-induced anxiety- and depression-like behaviour and improves memory in male mice.](#) J Physiol Res 67:795-808.
- Sant'Ana AB, Vilela-Costa HH, Vicente MA, Hernandez PM, de Andrade TGCS, Zangrossi H Jr (2019). Role of 5-HT<sub>2C</sub> receptors of the dorsal hippocampus in the modulation of anxiety- and panic-related defensive responses in rats. J Neuropharmacol 148:311-319.
- Saso L, Firuzi O (2014). Pharmacological applications of antioxidants: lights and shadows. J Current Drug Targets 15:1177-1199.

Sinha AK (1972). Colorimetric assay of catalase. *J Anal Biochem* 47:389-394.

Suñol C, Kaliman P, Sanfeliu C (2019). Role of Resveratrol and Selenium on Oxidative Stress and Expression of Antioxidant and Anti-Aging Genes in Immortalized Lymphocytes from Alzheimer's Disease Patients. *J Nutrients* 11:8-1764.

Tang KK, Li HQ, Qu KC, Fan RF(2019). Selenium alleviates cadmium-induced inflammation and meat quality degradation via antioxidant and anti-inflammation in chicken breast muscles. *J Environ Sci Pollut Res Int* 26:23453-23459.

Timal S, Hoischen A, Lehle L, Adamowicz M, Huijben K, Sykut-Cegielska J, Körner C. (2012). Gene identification in the congenital disorders of glycosylation type I by whole-exome sequencing. *J Human Mol Genetics* 21:19- 4151-4161.

Ullah F, Ali T, Ullah N, Kim MO (2015). Caffeine prevents d-galactose-induced cognitive deficits, oxidative stress, neuroinflammation and neurodegeneration in the adult rat brain. *J Neurochem Int* 90:114-124.

Vinceti M, Filippini T, Wise LA (2018). [Environmental Selenium and Human Health: an Update](#). *J Curr Environ Health Rep* 5:464-485.

Wagner KH, Cameron-Smith D, Wessner B, Franzke B. (2016) [Biomarkers of Aging: From Function to Molecular Biology](#). *Nutrients* 8:6-338.

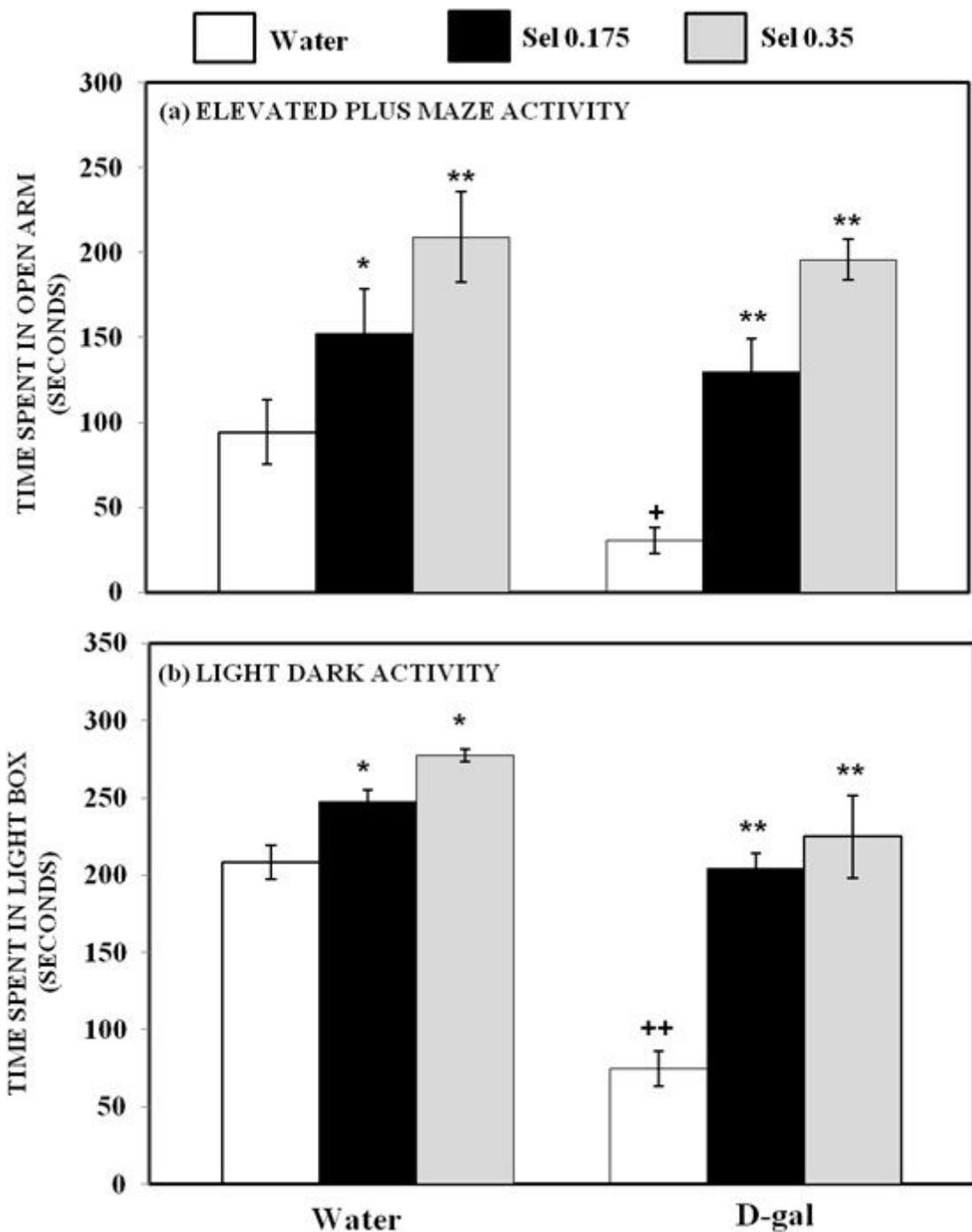
Wang N, Tan HY, Li S, Xu Y, Guo W, Feng Y (2017). [Supplementation of Micronutrient Selenium in Metabolic Diseases: Its Role as an Antioxidant](#). *J Oxid Med Cell Longev* 2017:7478523.

Wang ZL, Chen LB, Qiu Z, Chen XB, Liu Y, Li J, Wang L, Wang YP (2018). [Ginsenoside Rg1 ameliorates testicular senescence changes in D-gal-induced aging mice via antiinflammatory and antioxidative mechanisms](#). *Mol Med Rep*17:6269-6276.

Yan J, Pang Y, Zhuang J, Lin H, Zhang Q, Han L, Ke P, Zhuang J, Huang X (2019). [Selenepzil, a Selenium-Containing Compound, Exerts Neuroprotective Effect via Modulation of the Keap1-Nrf2-ARE Pathway and Attenuates A \$\beta\$ -Induced Cognitive Impairment in Vivo](#). *J ACS Chem Neurosci* 10:2903-2914.

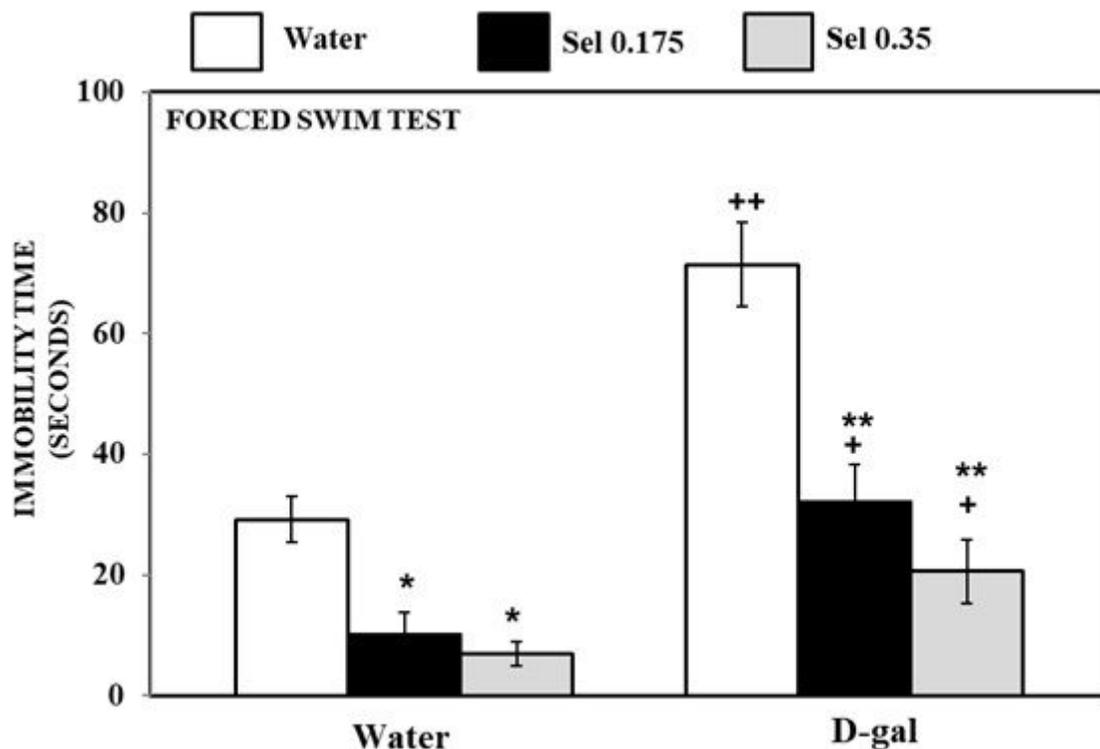
Zhu LJ, Xu C, Ren J, Chang L, Zhu XH, Sun N, Meng GL, Liu MY, Zhang J, Li YY, Tang YL, Zhou QG. (2020). [Dentate nNOS accounts for stress-induced 5HT\(1A\) receptor deficiency: Implication in anxiety behaviors](#). *J CNS Neurosci Ther* 26:453-464.

## Figures



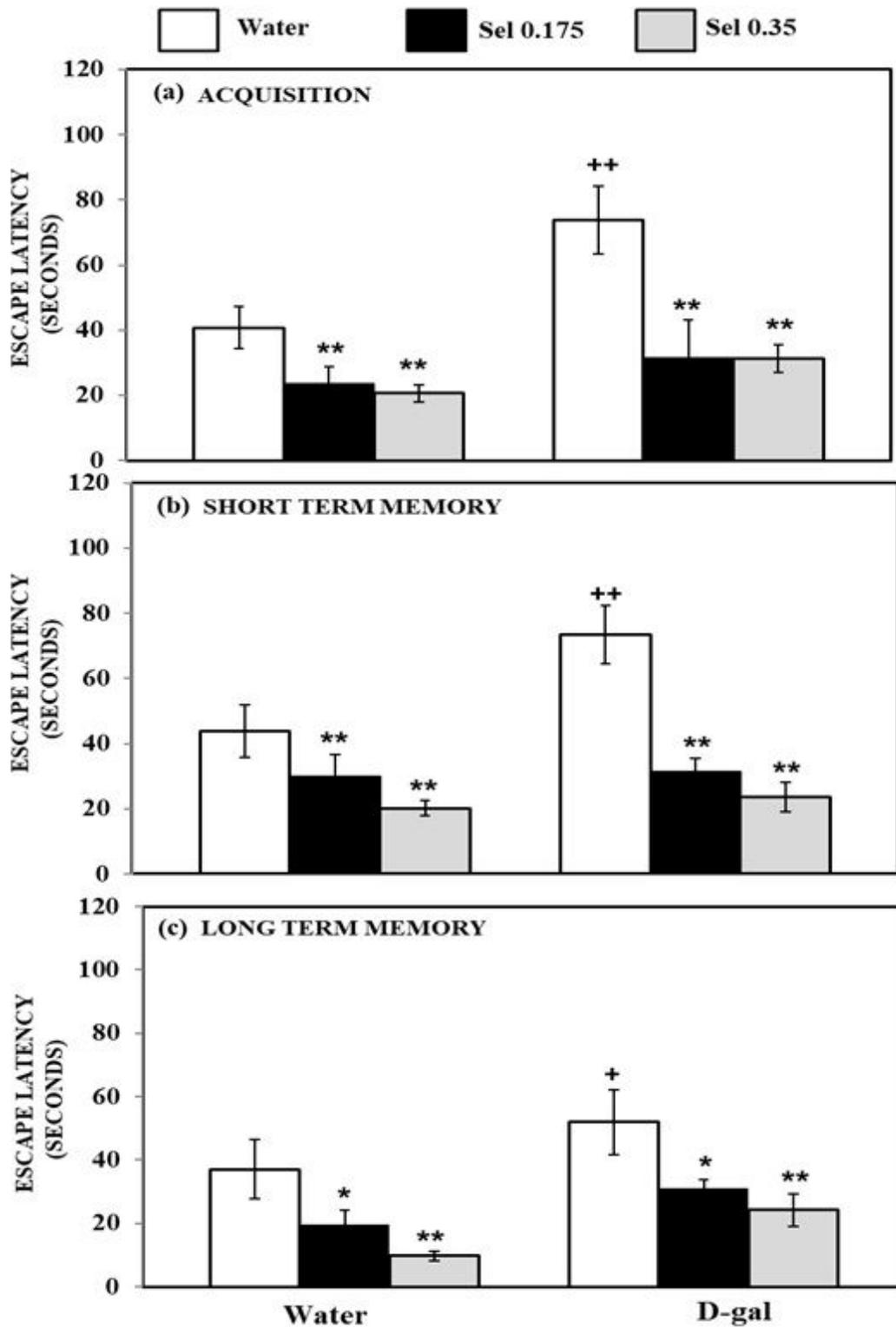
**Figure 1**

Time spent in open arm (A) and light box (B) in water, D-gal and Sel (0.175 and 0.35mg/ml/kg) administered mice [mean  $\pm$  SD (n = 5)]. Data was analyzed by Tukey's test. \*\*p<0.01 and \*p<0.05 when compared with respective control group and ++p<0.01 and +p<0.05 when compared with water+water and water+Sel administered animals.



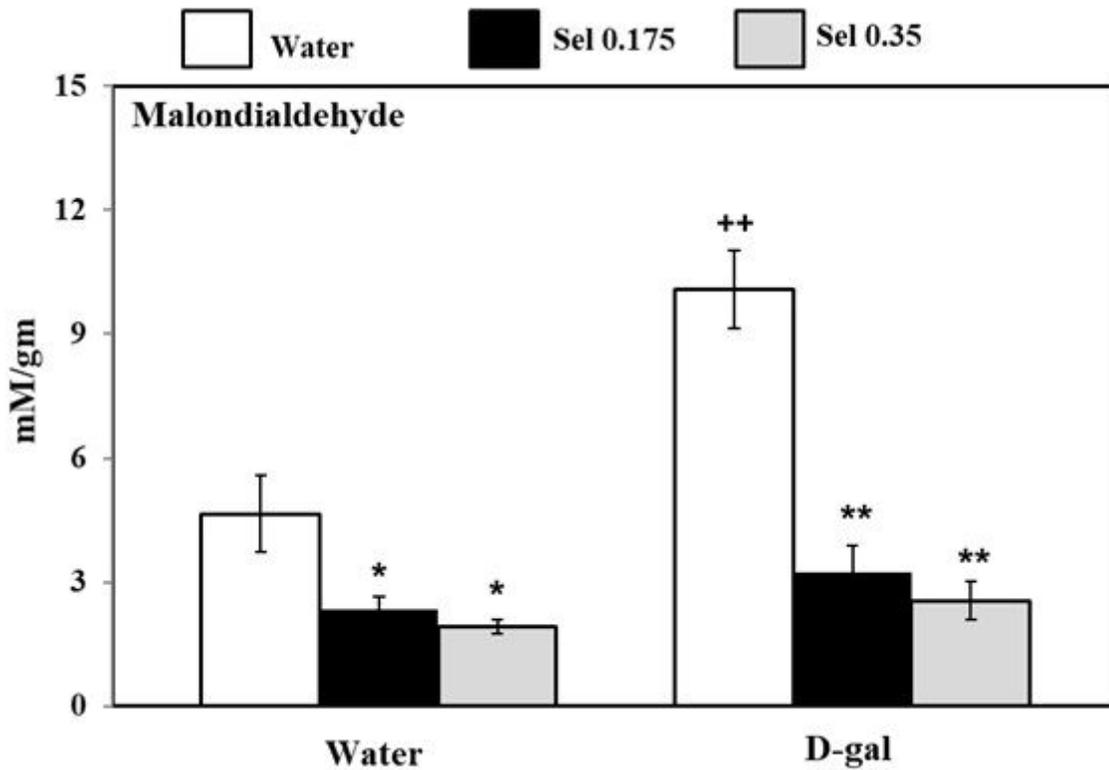
**Figure 2**

Immobility time monitored in forced swim test apparatus in water, D-gal and Sel (0.175 and 0.35mg/ml/kg) administered mice [mean  $\pm$  SD (n = 5)]. Data was analyzed by Tukey's test. \*\*p<0.01 and \*p<0.05 when compared with respective control group and ++p<0.01 and +p<0.05 when compared with water+water and water+Sel administered animals.



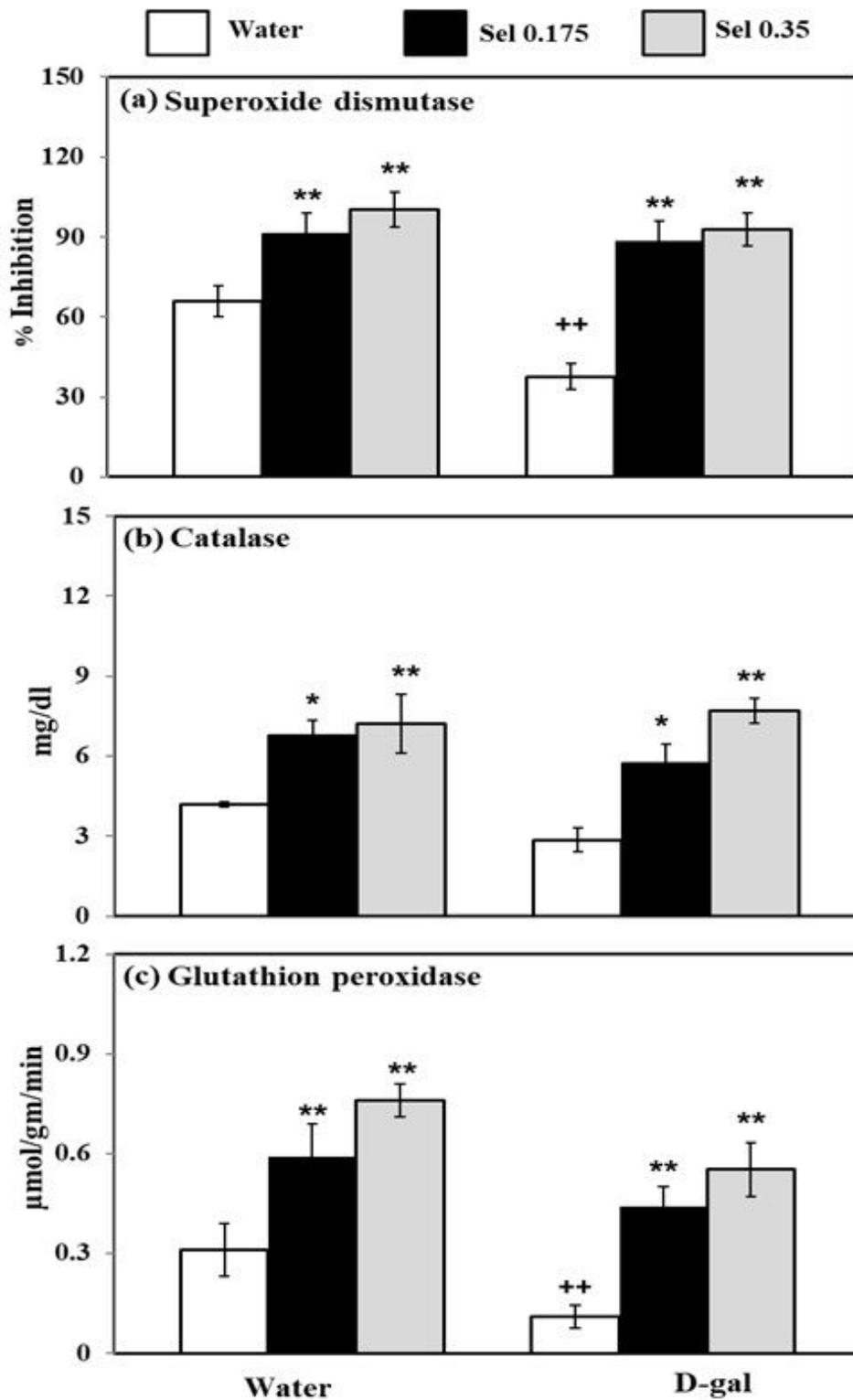
**Figure 3**

Evaluation of cognitive function in Morris Water maze test in water, D-gal and Sel (0.175 and 0.35mg/ml/kg) administered mice [mean  $\pm$  SD (n = 5)]. Data was analyzed by Tukey's test. \*\*p<0.01 and \*p<0.05 when compared with respective control group and ++p<0.01 and +p<0.05 when compared with water+water and water+Sel administered animals.



**Figure 4**

Oxidative stress marker in mice hippocampus in water, D-gal and Sel (0.175 and 0.35mg/ml/kg) administered mice [mean  $\pm$  SD (n = 5)]. Data was analyzed by Tukey's test. \*\*p<0.01 and \*p<0.05 when compared with respective control group and ++p<0.01 when compared with water+water and water+Sel administered animals.



**Figure 5**

Hippocampal antioxidant enzymes evaluation in water, D-gal and Sel (0.175 and 0.35mg/ml/kg) administered mice [mean  $\pm$  SD (n = 5)]. Data was analyzed by Tukey's test. \*\*p<0.01 and \*p<0.05 when compared with respective control group and ++p<0.01 when compared with water+water and water+Sel administered animals.

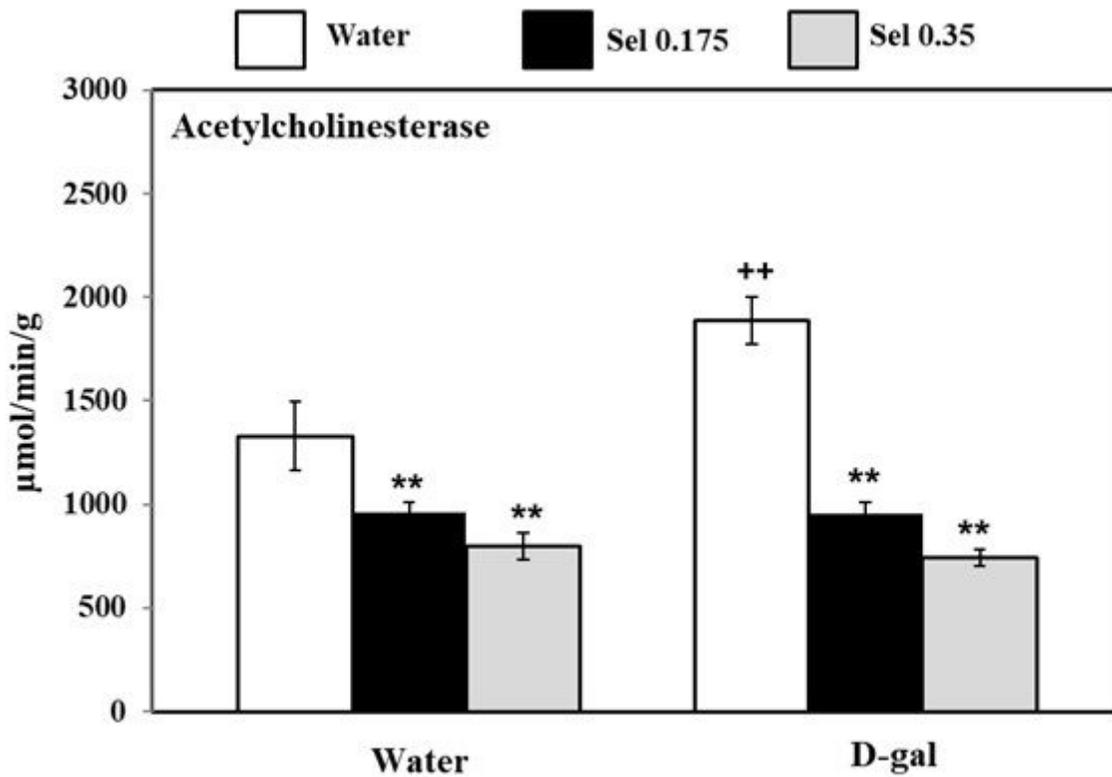
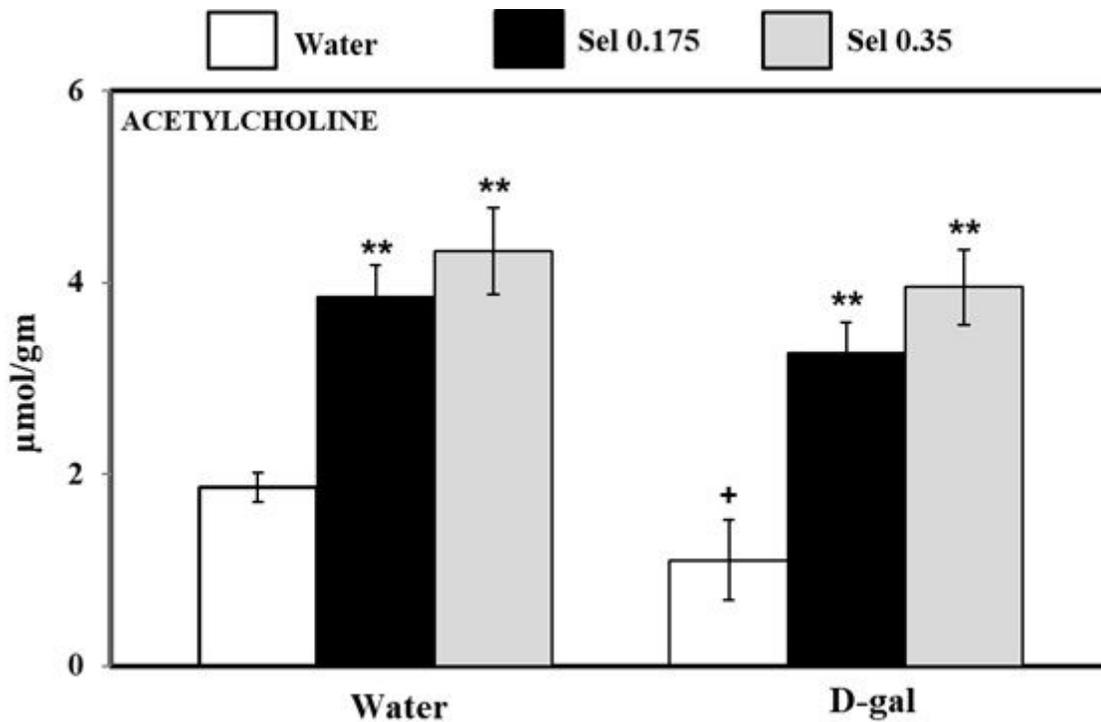


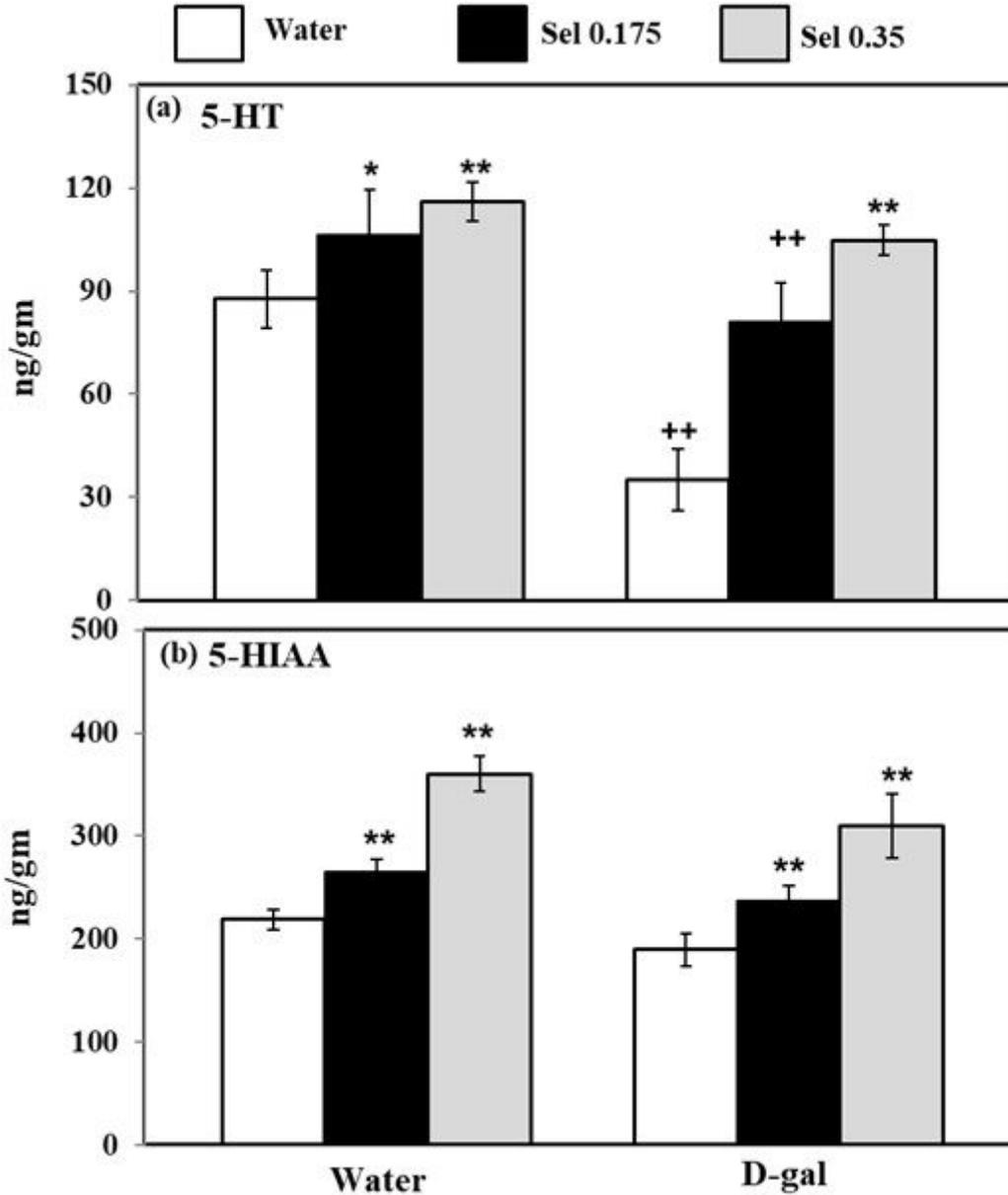
Figure 6

Hippocampal acetylcholinesterase activity in water, D-gal and Sel (0.175 and 0.35mg/ml/kg) administered mice [mean  $\pm$  SD (n = 5)]. Data was analyzed by Tukey's test. \*\*p<0.01 when compared with respective control group and ++p<0.01 and +p<0.05 when compared with water+water and water+Sel administered animals.



**Figure 7**

Hippocampal Acethcholine levels in water, D-gal and Sel (0.175 and 0.35mg/ml/kg) administered mice [mean  $\pm$  SD (n = 5)]. Data was analyzed by Tukey's test. \*\*p<0.01 and \*p<0.05 when compared with respective control group and +p<0.05 when compared with water+water and water+Sel administered animals.



**Figure 8**

Hippocampal serotonin metabolism in water, D-gal and Sel (0.175 and 0.35mg/ml/kg) administered mice [mean  $\pm$  SD (n = 5)]. Data was analyzed by Tukey's test. \*\*p<0.01 and \*p<0.05 when compared with respective control group and +p<0.05 when compared with water+water and water+Sel administered animals.