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Tryptophan lessens reserpine induced anxiety, depression and memory impairment by modulating oxidative stress and serotonergic activity

Noreen Samad[1]*, Saima Khaliq[2], Mehtab Alam[3], Farzana Yasmin[4], Saara Ahmad[5] Sana Mustafa[6], Azizuddin[6] and Uzma Raza[7]

[1]Department of Biochemistry, Bahauddin Zakariya University, Multan, Pakistan
[2]Department of Biochemistry, Federal Urdu University of Science and Technology, Karachi, Pakistan
[3]Department of Biochemistry, Azad Jammu Kashmir Medical College, Muzaffarabad, Azad Kashmir, Pakistan
[4]Department of Food Engineering NED University of Engineering and Technology
[5]Department of Biological and Biomedical Sciences, The Aga Khan University, Karachi, Pakistan
[6]Department of Chemistry, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan
[7]Department of Biochemistry, Hamdard Medical College, Hamdard University Karachi, Pakistan

Abstract: Reserpine (Res)-induced depletion of monoamines and altered neurotransmission and produces oxidative stress. Tryptophan (TRP) regulated the serotonin neurotransmission. Because systemically injected Res induced behavioral deficits and oxidative stress, while, dietary components prevented these adverse effects, we used TRP a pharmacological tool to prevent Res- induced changes in behavior, memory impairments, oxidative stress and regulation of serotonin neurotransmission in rats. Anxiolytic, antidepressant, cognitive functions, lipid peroxidation, antioxidant enzymes serotonin metabolism were studied in Res and vehicle treated animals following administration of 50 and 100 mg/ml/kg of tryptophan. Following administration of TRP [50 and 100mg/ml/kg], Res induced anxiety-and/or depression like behaviors normalized. Res-induced impaired cognitive function and increased acetylcholinesterase activity also improved following administration of TRP at both doses. Res induced increased brains' malondialdehyde (MDA) and decreased antioxidant enzymes activity also normalized by TRP. Res-induced decreased 5-HT metabolism also regulated by administration of TRP at both doses. In conclusion it can be recommended that administration/supplementation of TRP in daily life can aid in battling the anxiety, depression, modulating serotonergic activity and oxidative stress. Study also exhibits the anti-acetylcholinesterase role of TRP which may be possible reason for improved cognition following stress situation.

Keywords: Depression, anxiety, memory, oxidative stress, antioxidant enzyme, serotonergic activity, tryptophan, acetylcholinesterase activity, reserpine.

INTRODUCTION

Psychiatric disorders such as anxiety and depression are articulated by interaction of diverse pathogenic mechanism (Saki *et al.,* 2014) which is becoming an important well-being concern throughout the world. Depression is a major cause of suicide (Phillips *et al.*, 2009). According to World Health Organization (WHO), depression is the top fourth contributor prevails during the life cycle and cause major disabilities. It is thought that depression will become the major threat of various diseases worldwide by the year 2020 (Kessar and Bromet, 2013). It is also reported that 500 million peoples are affected to anxiety up till now and their numbers are increasing day by day (Saki *et al.,* 2014).

Clinical and experimental studies have shown that the serotonin is involved in the intonation of various processes (Dannlowski *et al.,* 2014; Tang *et al.,* 2013; 2014). Particularly, the pathophysiology of depression is associated to alteration in serotonin (5-HT) mechanism (Yu *et al.,* 2019; Khnychenko *et al.,* 2017; Sivolap, 2017). The release of 5-HT from serotonergic terminal is obsessed from the synaptic cleft in to these terminals via specific serotonin transporter (Hansson *et al.,* 1998), demonstrating that specific serotonin transporter may act as a physiologic arbitrator in depression.

Reserpine (Res; indole alkaloid) had been used for the treatment of psychiatric illnesses and hypertension (Davies and Shepherd, 1955; Duralch, 1956). Previously in experimental models a link was established between Res and development of depression by decreasing struggling duration in forced swimming test and behavior of anxiety by decreasing the time expended in light compartment (Chang *et al.,* 2015). This connection is established due to depletion of monoamines by disruption of vesicular storage of transmitters (indolamine and catecholamines) (Ikram and Haleem, 2017; 2019). Furthermore, Res is also involved in autoxidation of monoamines and oxidative catabolism by monoamine oxidase (MAO) these events are associated with oxidative stress in the brain (Colpaert, 1987; Fuentes *et al.,* 2007). Accordingly, these events are overturned by various antidepressant such as desipramine (Luo *et al.,* 2016), fluoxetine (Ruiz *et al.,* 2018) etc. and antioxidants such as

**Corresponding author:* e-mail: noreen.samad@bzu.edu.pk

agmatine (Cunhe *et al.* 2016), L-Theanine (Soung *et al.,* 2018), *Gyejibokryeong-hwan* (Park *et al.,* 2018), *Syndrella nodiflora* (Amoateng *et al.,* 2018), Melatonin (Favero *et al.,* 2017) etc.

Diet contributes significant role in psychological health and performance. Variety of dietary components has importance in the growth, organization and handling of mental ailments such as anxiety and depression (Hopkins and Cole, 1901). Tryptophan (TRP) is an important amino-acid; a predecessor of serotonin. It is well documented that TRP and other essential, amino acid cannot cross blood brain barrier directly, for this purpose they shared a common transport mechanism. Many studies have reported pharmacological properties of TRP such as; anxiolytic; antidepressant, hypotensive; anti-obesity and mood enhancer etc. (Lindseth *et al.,* 2015; Saleem *et al.,* 2018; Gul *et al.,* 2019; Bravo *et al.,* 2013). Furthermore, TRP also serve as antioxidant by scavenging free radicals and enhancing antioxidant mechanism (Bravo *et al.,* 2013). It has also been prophesied to have neuroprotective effects to cure deficits in behavior (Saleem *et al.,* 2018) and memory impairment (Haider *et al.,* 2007). However, report concerning its effect on Res-induced behavioral deficits and memory impairment is still inadequate. Because it has antioxidative potential and role in normalization of neurotransmission, we hypothesized that TRP rectify the increased oxidative deterioration, decreased antioxidant mechanism and altered serotonin neurotransmission which are induced by Res, and consecutively disrupt behavioral and cognitive activities. We studied the therapeutic outcome of various doses of TRP (50 and 100mg/ml/kg) to prevent the Res-instigated anxiety and/or depressive symptoms, and memory alteration in rats.

MATERIALS AND METHODS

Animals

Male rats (Sprague Dawley, 170-10g by weight) positioned alone 1 week before the start of experimental work. Animals were imprisoned in plastic made cages with standard laboratory condition and has admittance to standard rodent diet and water. All the experimental work permitted by departmental research ethical committee.

Chemicals

Reserpine (Res), Tryptophan (TRP), Di-thio-bis-nitro-benzoic acid (DTNB), Nitro-blue-tetrazolium (NBT), Thio-barbituric-acid (TBA), H_2O_2 and other chemicals purchased from Sigma.

Experimental Protocol

36 rats were arbitrary separated into 6 groups (n=6). (i) Vehicle+vehicle (ii)Vehicle+TRP (50mg/ml/kg) (iii) Vehicle+TRP (100mg/ml/kg) (iv) Res+Vehicle (v) Res+TRP (50mg/ml/kg) (vi) Res+TRP (100 mg/ml/kg). Res (0.2mg/ml/kg) and TRP (50 and 100 mg/ml/kg) dissolved in ethanol (25%) while the control group was treated with 'vehicle' (ethanol 25%) (1ml/kg) as documented (Samad *et al.,* 2018). All drugs were injected intraperitoneally (*ip*). The animal received this treatment for 14 days. Behavioral tests (Light dark activity box, elevated plus maze, forced swim test, tail suspension test and Morris water maze) were performed after 14 days prior the administration of treatment. Animals were sacrificed very next day after the behavioral analysis and whole brain were collected as described previously (Samad and Saleem, 2018) and placed at -20°C until biochemical estimations such as Malondialdehyde [MDA; lipid peroxidation] acetylcholinesterase (AChE) and antioxidant enzyme activity i.e. superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). All the behavioral and biochemical analysis were done by blinded researchers.

Behavioral assessment

Light dark activity box and elevated plus maze activity performed to evaluate the anxiety level as reported earlier (Samad *et al.,* 2018; Samad and Saleem, 2018). Depression-like behavior evaluated by monitoring immobility time in forced swim and tail suspension test (Samad *et al.,* 2018; Samad and Saleem, 2018). Cognitive function evaluated by Morris water maze test as reported previously (Samad *et al.,* 2018; Samad and Saleem, 2018).

Biochemical assays

Samples of brain washed away with isotonic solution. Brain homogenate (10% weight/volume) ready with phosphate buffer (0.1M, 7.4pH) and centrifuged at 10,000xg for 10-minutes at 4°C. The supernatant of rat brain was collected (Samad *et al.* 2018) and then used to quantify malondialdehyde (MDA; Chow and Tappel, 1972) and antioxidant enzyme activity, like, superoxide dismutase (SOD; Samad *et al.,* 2018), catalase (CAT; Pari and Latha, 2004) and glutathione peroxidase (GPx; Flohe and Gunzler, 1984). Activity of acetylcholinesterase (AChE) in brain sample determined by previous method (Ellman *et al.,* 1961).

Neurochemical analysis

The levels of 5-HT and 5-HIAA estimated as reported previously (Samad *et al.,* 2018).

STATISTICAL ANALYSIS

All the data expressed as $\pm$ Std. Dev. (n=6). ANOVA (Two-way) using SPSS software version 20.0 utilized to evaluate statistical difference among the groups. $P<0.05$ was taken as substantial.

RESULTS

Fig. 1 illustrates the effects of TRP to evaluate the anxiety in rats injected with Res. Data of light dark activity test

evaluated by *2-way anova* for time consumed in light compartment revealed that effect of Res [$F_{(1,30)}$ = 24.12, p<0.01], TRP [$F_{(2,30)}$ =624.85, p<0.01], interaction of ResxTRP [$F_{(2,30)}$ =20.68, p<0.01] were substantial. Test by Tukey showed that repeated treatment with Res reduced period consumed in light compartment as compare to vehicle treated animals. Administration of TRP (50 and 100mg/ml/kg)+vehicle and TRP(50 and 100 mg/ml/kg)+Res increased the time consumed in light box than vehicle treated animals.

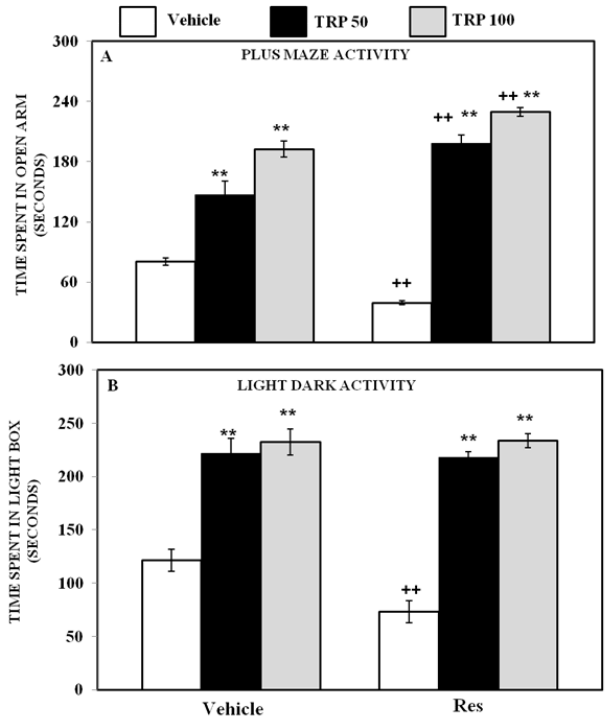

Fig. 1: Time spent in open arm (A) and light box (B) in light dark activity test and elevated plus maze activity respectively for the vehicle+vehicle, vehicle+TRP, Res+ vehicle and Res+TRP treated rats. Values are mean ± SD (n=6). Statistical analysis was done by Post hoc test following two-way ANOVA. Statistical difference is represented as ***p*<0.01 versus respective control and ++*p*<0.01 versus vehicle+vehicle and vehicle+TRP treated animals.

2-way anova implemented on the statistics for time consumed in open arm of elevated plus maze analyzed exposed substantial effect of TRP [$F_{(2,30)}$ = 1274.42, p< 0.01], Res ($F_{(1,30)}$=37.44 p<0.01) and ResxTRP [$F_{(2,30)}$ = 127.04, p<0.01]. Test by Tukey exhibited that Res markedly reduced time spent in open arm than vehicle treated rats. Treatment with TRP (50 and 100 mg/ml/kg)+vehicle and TRP (50 and 100 mg/ml/kg)+Res increased period consumed in open arm than control animals Time consumed in open arm was improved in TRP (both doses)+Res than TRP (both doses)+vehicle treated animals.

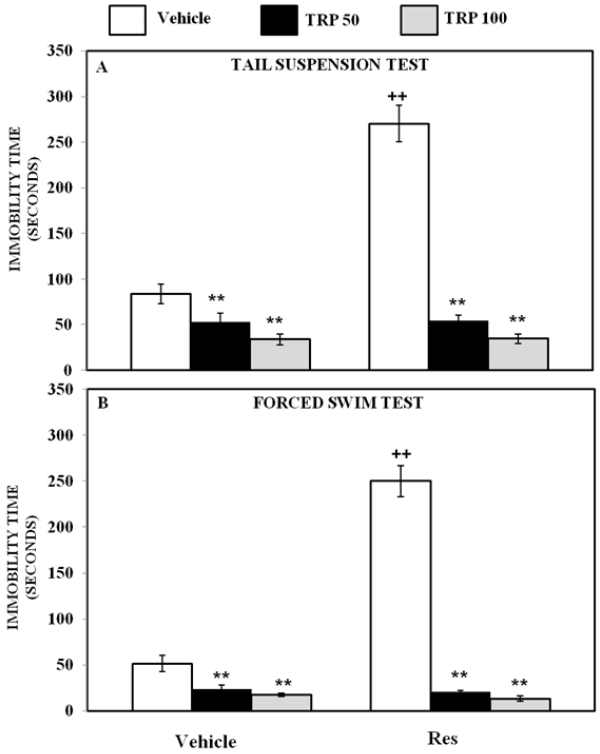

Fig. 2: Immobility time observed in tail suspension test (A) and forced swim test (B) to evaluate depression like behavior in vehicle+vehicle, vehicle+TRP, Res+ vehicle and Res+TRP treated rats. Values are mean ± SD (n = 6). Statistical analysis was done by Post hoc test following two-way ANOVA. Statistical difference is represented as ***p*<0.01versus respective control and ++*p*<0.01 versus vehicle+vehicle and vehicle+TRP treated animals.

Fig. 2 illustrates the effects of TRP to evaluate the depression -like behavior, immobility time in forced swim test and tail suspension test monitored in Res and vehicle treated rats. Data analyzed by *2-way anova* for immobility time in forced swim test revealed significant effect of Res [$F_{(1,30)}$ =560.60, p<0.01], TRP [$F_{(2,30)}$ =1076.82, p< 0.01], and TRPxRes [$F_{(2,30)}$ =628.66, p<0.01]. Test by Tukey exhibited that treatment with Res significantly enhanced immobility time than control. TRP (50 and 100 mg/ml/kg)+vehicle and TRP (50 and 100mg/ml/kg)+ Res decreased immobility time than vehicle .

2-way anova performed on Data for immobility time in tail suspension test exhibited substantial effect of Res [$F_{(1,30)}$ = 296.69, p<0.01], TRP [$F_{(2,30)}$ =607.25, p<0.01], and interaction between TRPxRes [$F_{(2,30)}$ =288.84, p< 0.01]. Tukey's test exhibited that administration of Res markedly increased immobility time than their respective control. TRP (50 and 100mg/ml/kg)+vehicle and TRP (50 and 100mg/ml/kg)+ Res injected rodents reduced immobility time than vehicle + vehicle and vehicle + Res treated rats.

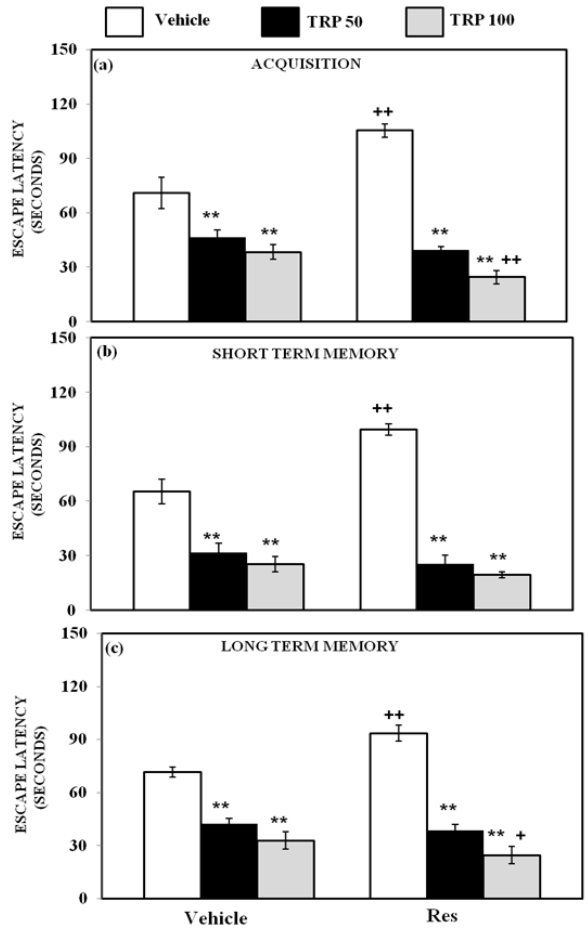

Fig. 3: Effect of TRP on Res induced impaired acquisition (A) short term memory (B) and long term memory (C) in terms of escape latency (s) assessed by Morris water maze. Values are mean ± SD (n = 6). Statistical analysis was done by Post hoc test following two-way ANOVA. Statistical difference is represented as ***p*< 0.01 versus respective control and ++*p*< 0.01, +*p*< 0.05 versus vehicle+vehicle and vehicle+TRP treated animals.

Fig. 3 illustrates the effect of TRP administration to evaluate the memory functions in Res treated rats. Latency escape in MWM activity is led instantly after training (acquisition, fig. 3a), 1-h (STM, fig. 3b) and 24-h (LTM, fig. 3c). All the data analyzed by *2-way anova*. Data for acquisition revealed significant effect of Res [$F_{(1,30)}$ =8.03, p<0.01], TRP [$F_{(2,30)}$ = 463.46, p<0.01], and TRPxRes [$F_{(2,30)}$ =88.26, p<0.01]. Data for STM revealed significant effect of Res [$F_{(1,30)}$ =23.08, p<0.01], TRP [$F_{(2,30)}$ =614.94, p<0.01] and TRPxRes [$F_{(2,30)}$ =74.87, p<0.01]. Data for LTM revealed significant effect of Res [$F_{(1,30)}$ =5.96, p<0.01], TRP [$F_{(2,30)}$ =593.26, p<0.01], and interaction between TRPxRes [$F_{(2,30)}$ =49.60, p<0.01]. Test by Tukey showed that Latency escape improved in all sessions in Res treated animals. TRP (50 and 100mg/ml/kg) +vehicle and TRP (50 and 100 mg/ml/kg)+ Res treated rats decreased latency escape than their counterparts. Latency escape of acquisition (p<0.01) and LTM (p<0.05) were greater in TRP (100 mg/ml/kg)+Res than TRP (100mg/ml/kg)+Vehicle treated animals.

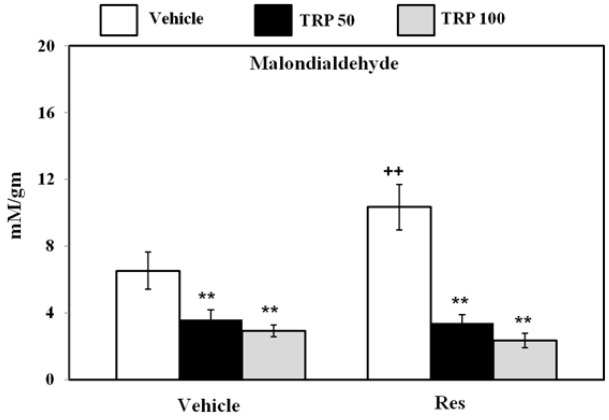

Fig. 4: Effect of TRP on Res induced oxidative stress. Values are mean ± SD (n = 6). Statistical analysis was done by Post hoc test following two-way ANOVA. Statistical difference is represented as ***p*<0.01, **p*<0.05 versus respective control and ++*p*<0.05 versus vehicle+vehicle and vehicle+TRP treated animals.

Fig. 4 illustrates the effects of TRP on level of oxidative stress marker (MDA) in the brain of Res and vehicle treated rats. *2-way anova* performed on the data revealed significant effect of Res [$F_{(1,30)}$= 13.70, p<0.01], TRP [$F_{(2,30)}$ =175.77, p<0.01], and interaction between TRPxRes [$F_{(2,30)}$ =26.64, p<0.01]. Test by Tukey exhibited that administration of Res substantially elevated MDA levels. Administration of TRP at both doses decreased MDA levels in Res and vehicle treated animal than their respective control.

Fig. 5 illustrates the effects of TRP on antioxidant enzymes in Res treated rats. All the data analyzed by *2-way anova*. Data on SOD revealed insignificant effect of Res [$F_{(1,30)}$ =2.18, p<0.01]. Effects of TRP [$F_{(2,30)}$ = 589.83, p<0.01], and TRPxRes [$F_{(2,30)}$ =13.58, p<0.01] were substantial. Test by Tukey exhibited that administration of Res substantially reduced activity of SOD. TRP (50 and 100 mg/ml/kg) increased activity of SOD in both Res and Vehicle treated animal than their respective control.

Data on CAT revealed that significant effect of Res [$F_{(1,30)}$ = 4.34, p<0.01], TRP [$F_{(2,30)}$ =122.20, p<0.01], and interaction between TRPxRes [$F_{(2,30)}$ =3.59, p<0.01]. Test by Tukey exhibited activity of CAT decreased by the treatment of Res. Administration of TRP (50 and 100 mg/ml/kg) increased CATs' activity in Res and vehicle treated rats than their counterparts.

Data on GPx revealed that significant effect of Res [$F_{(1,30)}$ = 17.21, p < 0.01], and TRP [$F_{(2,30)}$ =194.92, p<0.01]. The interaction between TRPxRes [$F_{(2,30)}$ = 0.199, p>0.05]

was not substantial. Test by Tukey exhibited that TRP (50 and 100 mg/ml/kg) increased activity of GPx of both Res and vehicle treated rats than their respective control.

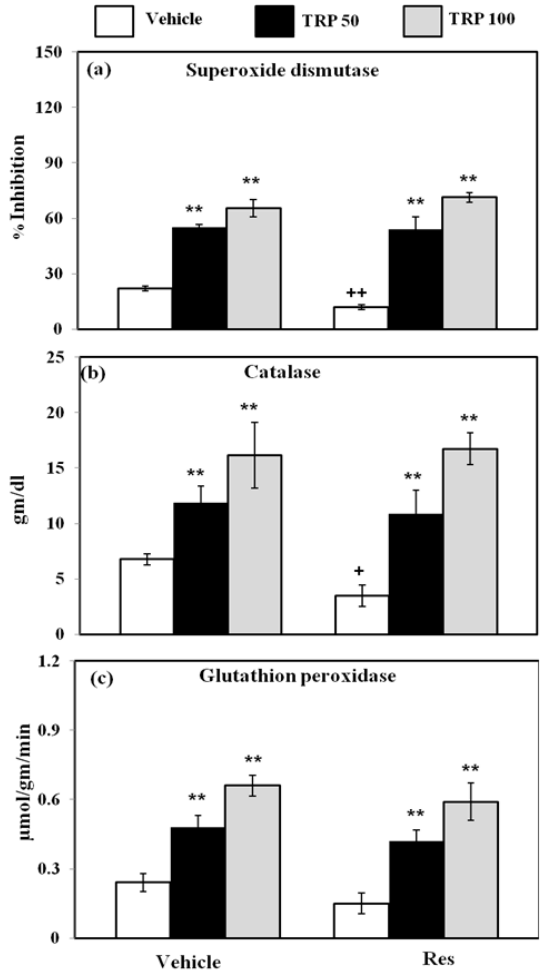

Fig. 5: Effect of TRP on Res induced altered brain antioxidant enzyme activity such as SOD (A), CAT (B) and GPx (C) activity. Values are mean ± SD (n=6). Statistical analysis was done by Post hoc test following two-way ANOVA. Statistical difference is represented as ***p*<0.01, versus respective control and ++*p*<0.01, +*p*< 0.05 versus vehicle+vehicle and vehicle+TRP treated animals.

Fig. 6 illustrates the effects of TRP on brains' AChE activity in Res treated rats. *2-way anova* performed on the data revealed significant effect of Res [$F_{(1,30)}$ =21.95, p< 0.01], TRP [$F_{(2,30)}$ =358.65, p<0.01], and interaction between TRPxRes [$F_{(2,30)}$ =56.94, p<0.01]. Tukey's test exhibited increased activity of AChE activity in Res injected animals. TRP (50 and 100 mg/ml/kg) decreased AChE activity of Res and vehicle treated animals than their respective control.

Fig. 7 illustrates the effects of TRP on brains'5-HT and 5-HIAA levels in Res treated rats. All data analyzed by *2-way anova*. Data on 5-HT revealed significant effect of Res [$F_{(1,30)}$=63.75, p<0.01], TRP [$F_{(2,30)}$ =59.43, p<0.01] and TRPxRes [$F_{(2,30)}$ =11.86, p<0.01]. Test by Tukey exhibited that levels of brain 5-HT decreased in Res treated animals. TRP (50 and 100 mg/ml/kg)+Res treated rats show exhibit increased levels of 5-HT than their respective controls. TRP (100mg/ml/kg)+vehicle showed an increased levels of 5-HT than vehicle+vehicle injected. The increases of 5-HT (p<0.01) was smaller in TRP (50 mg/ml/kg)+Res than TRP (50 mg/ml/kg)+ vehicle treated animals.

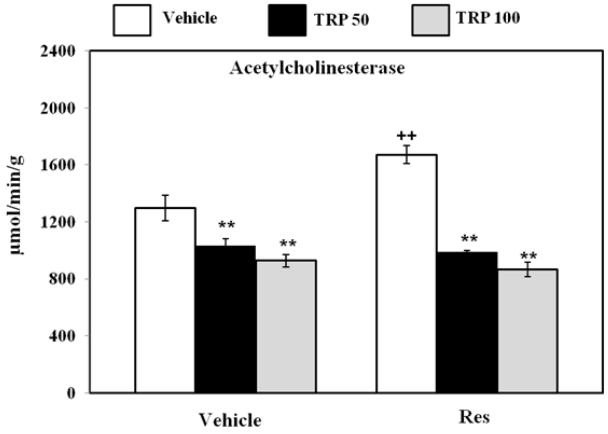

Fig. 6: Effect of TRP on Res induced impaired brain acetylcholinesterase activity. Values are mean ± SD (n= 6). Statistical analysis was done by Post hoc test following two-way ANOVA. Statistical difference is represented as ***p*<0.01versus respective control and ++*p*<0.01 versus vehicle+vehicle and vehicle+TRP treated animals.

Data on 5-HIAA revealed that significant effect of Res [$F_{(1,30)}$ =16.58, p<0.01], TRP [$F_{(2,30)}$ =130.21, p<0.01] and interaction between TRPxRes [$F_{(2,30)}$ =5.62, p<0.01]. Test by Tukey showed that Res substantially decreased the levels of 5-HIAA. TRP (50 and 100mg/ml/kg)+Res and TRP (50 and 100mg/ml/kg)+vehicle treated rodents showed improved levels of 5-HIAA than their respective controls. The increases of 5-HIAA was smaller (p<0.05) in TRP (50mg/ml/kg)+Res than TRP (50mg/ml/kg)+ vehicle treated animals.

DISCUSSION

Res-induced behavioral deficits are strongly resembled to the symptoms of depression, furthermore it is also associated with human depression progression (Leal *et al*., 2019). Our result shows that administration of Res (0.2 mg/ml/kg/day) decreased the time spent in light box and open arm which is similar with the earlier studies (Chang *et al*., 2015), suggesting anxiety like effect caused by Res. In addition, increased immobility duration in forced swim and tail suspension tests as observed in the present study are also in agreement with the previous

report (Chang *et al.,* 2015) indicating Res produces depression like symptoms. Studies have also shown the involvement of Res in memory alteration (Ullah *et al.,* 2019; Kronbauer *et al.,* 2015) and consistent with our study in term of alteration in short- and long-term memories and increased AChE activity. Our data also showed that Res increased MDA level which is an indication of enhanced generation of free radical with reduction in antioxidant enzyme activity that may somewhat trigger both anxiety and depression like behaviors. Similarly, dysregulation of serotonergic neurotransmission by Res is also a cause of behavioral deficits (anxiety and depression). Extensive research works indicated that Res induced behavioral deficits (anxiety and depression) are strongly linked with induction of oxidative stress in the brain (Fuentes *et al.,* 2007). It is observed in current work that repeated administration of TRP at doses 50 and 100mg/ml/kg; inhibited/prevented Res induced adverse effects.

Res causes distraction in neuronal firing by disturbing the vesicular monoamine transporter (VMT) to confines the storing of 5-HT in cleft of synapse, consequential the greater oxidative metabolism of 5-HT by monoamine oxidase (MAO) (Badawy *et al.,* 2015). It was reported that Res is also involved in autoxidation of 5-HT and oxidation of MAO (Fuentes *et al.,* 2007) which may lead enhanced generation of free radicals, the resultant augmentation of oxidative stress may deteriorate 5-HT neurons (Kronbauer *et al.,* 2015). The generation of free radical is involved in neurodegeneration (Datta *et al.,* 2016). Since monoamines are plentiful in brain areas and very much susceptible to free radical and damage to enhance oxidative stress would be expected (Delgado *et al.,* 2000). Furthermore, decreased 5-HT turnover (fig. 7) after Res administration more increased free radicals' production to potentiate oxidative damage. Res enhanced oxidative stress, behavioral deficit as well as other biochemical changes within the brain (Cunha *et al.,* 2016; Jamwal *et al.,* 2017). Excess oxidative stress often stimulates the inflammatory procedure and secretes inflammatory intermediaries, such as IL-6 and TNF-α to pledge the apoptosis, which is one of the important mechanisms for neuronal cell decease (Daglia *et al.,* 2017). Although in the present study we did not evaluate the inflammatory markers but definitely they played major role in progression of oxidative stress and simultaneously behavioral deficits and memory alteration. In addition, changes in brain neurotransmission, such as deficiency of monoamines, including dopamine (DA), noradrenaline (NE) and 5-HT, has also been observed in depression (Liu *et al.,* 2015): our results also in agreement with reduced concentration of 5-HT and 5-HIAA after Res administration (fig. 7). These results are consistent with the previous works, demonstrated that monoamine deficiency in the rat brain by Res-induced anatomical damage, resulting in the progress of behavioral deficits (anxiety and depression) (Chang *et al.,* 2015; Park *et al.,* 2018) and cognitive dysfunction. 5-HT, together with other neurotransmitters, is crucial in various psychiatric illness. The altered 5-HT concentration by Res was linked with commotion of the balance with other neurotransmitters, such as DA and NE (Samad *et al.,* 2017). These outcomes fixed with our experimental behavioral changes after Res administration.

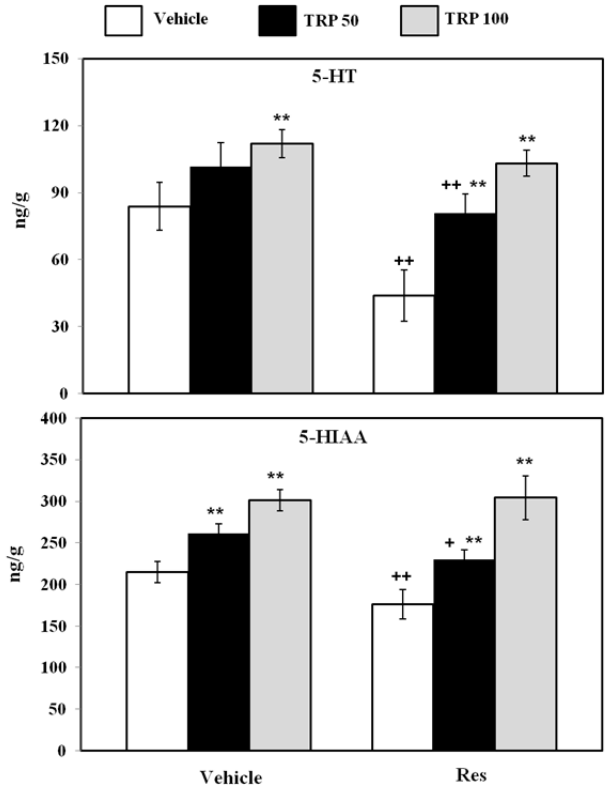

Fig. 7: Effect of TRP on Res induced changes in 5-HT (A) and 5-HIAA (B) levels. Values are mean ± SD (n = 6). Statistical analysis was done by Post hoc test following two-way ANOVA. Statistical difference is represented as $^{**}p<0.01$ versus respective control and $^{++}p<0.05$, $^{+}p<0.05$ versus vehicle+vehicle and vehicle+TRP treated animals.

It has reported that oxidative stress biomarkers and antioxidant become imbalanced in anxiety and depression conditions. Anxiety and depression diminished levels of antioxidant enzymes in human (Zhang *et al.,* 2019; Haleem *et al.,* 1998) and animals (Saleem *et al.,* 2018). Furthermore, antidepressant (Haleem *et al.,* 1998), anxiolytic (Haider *et al.,* 2007), dietary components (Saleem *et al.,* 2018), plant extract (Samad *et al.,* 2017) and their active compounds (Samad *et al.,* 2018) can normalize the mechanism of antioxidant enzymes, inhibit inflammatory markers and oxidative stress. The role of TRP is well documented as antidepressant (Carneiro *et al.,* 2018), anxiolytic (Gul *et al.,* 2019) and memory enhancer (Haider *et al.,* 2007) by its potential as precursor of 5-HT (Ikram *et al.,* 2014) and antioxidant capability (Ingawale *et al.,* 2014). In the present work Res induced

depression-/anxiety like behaviors and memory impairment rescued by co-administration of TRP (50 and 100 mg/kg).

The precursor of 5-HT, TRP (Ikram *et al.,* 2014) a recognized mood sustainer and/or antidepressant which has already documented neuroprotective role in experimental models (Saleem *et al.,* 2018; Gul *et al.,* 2019) and in human (Manna *et al.,* 2008). Administration of TRP increased 5-HT and 5-HIAA levels (fig. 7) in present study and produced antidepressant effects in control and Res treated animals. TRP dose dependently enhanced 5-HT and 5-HIAA contents in the brain (Calyniuk *et al.,* 2016). However, TRP induced serotonin synthesis and its release in raphe region may arouse the 5-HT release via somato-dendritic 5-HT receptors in rat hippocampus triggering the regulation of 5-HT firing. TRP is also reported as antioxidant (Ingawale *et al.,* 2014) and inhibited Res induced oxidative stress and involved in the normalization of 5-HT function.

Oxidative stress has a significant importance in Res induced adverse effects. Due to large production of RNS and ROS by oxidation of MAO the production of cellular inflammatory markers such as IL-6, IL-1, TNF-α (McCord *et al.,* 1976) become increased which also impair cellular integrity (Gutteridge, 1995). MDA, the final product of lipid peroxidation and an indicative of oxidative stress (Flora, 1999) become elevated due to enhanced production of free radical by repeated Res treatment (Bravo *et al.,* 2013) which is consistent with the present study (fig. 4) and confirmed oxidative stress. Antioxidant enzymes are also badly deteriorated by Res-induced adverse/toxic effects (Soung *et al.,* 2018). An antioxidant enzyme SOD, the first line of cellular defence against oxidative destruction, converts superoxide radicals into hydrogen peroxide (Kang *et al.,* 2018). CAT, a hemoprotein reacts with hydrogen peroxide and converts it into water and molecular oxygen (Wang *et al.,* 2015). Similarly, GPx also diminishes hydrogen peroxide (Haverkamp *et al.,* 2016). Repeated administration of Res has reported to increased superoxide radicles due to reduced activity of SOD (Tseng *et al.,* 2015) in the brain. Autoxidation of cellular molecules by Res also reduced the CAT and GPx levels in the brain as observed in the animal studies (Kruk-Slomka *et al.,* 2016). Bulk generation of free radicals is also connected with the progressive increase of inflammatory markers as a result antioxidant mechanism become weakens. The activity of SOD and CAT was decreased following administration of Res (fig. 5), indicates that repeated Res administration is involved in increasing free radical generation and reducing antioxidant enzymes mechanism and produced oxidative stress.

It is demonstrated that, TRP exhibited its antioxidant activity (Ingwale *et al.,* 2014) by scavenging free radicals, declining the inflammatory activity (Khadrawy *et al.,* 2017) and correcting the antioxidant enzyme activity (Kronbauer *et al.,* 2015). TRP recovers the Res-induced behavioral turmoil (fig. 1, 2) by normalizing the commotion of SOD and diminishing the destructive possessions of Res induced adverse effects on CAT, consequential in systematic commotion of GPx (fig. 5). The results showed that TRP at both doses lessened the oxidative stress (fig. 4) with augmentation in the activity of antioxidant enzymes (fig. 5). These possessions may be attributed to minimizing oxidative stress subsequently in declining of adverse effects of Res induced behavioral alteration. It is reported earlier that lessening of oxidative stress (Całyniuk *et al.,* 2016) may also overturn inflammatory reactions (Khadrawy *et al.,* 2017) by TRP. It is showed that anxiolytic/antidepressant effects of TRP against Res are due to its antioxidant potential.

Studies have been documented the involvement of Res in cognitive function (Ullah *et al.,* 2019). It is extensively reported that alteration in antioxidant defence system (Khadrawy *et al.,* 2017) and 5-HT neurotransmission (Haider *et al.,* 2007) play a crucial role to impair memory functions. Res-induced alteration in memory function (fig. 3). fig. 4 indicates that short term as well as long term memory negatively affected by the repeated treatment of Res when observed in MWM. AChE activity is reported as biomarkers for the determination of cholinergic function (Samad *et al.,* 2018). Studies has not been yet identified the accurate link between memory and AChE activity. Previously, Res treatment increased the activity of AChE and decreased memory function (Sarfaty *et al.,* 2005). Results (fig. 3 & 6) are also reliable with the previous report (Sarfaty *et al.,* 2005). However, reduced 5-HT turnover and antioxidant defence mechanism may be factors which influence normal cognitive functions. TRP decreased the overall activity performed in the MWM which may be attributed by lessened AChE activity in the rat brain (fig. 6). Taken into consideration, we assume that TRP has crucial role in neuronal circuit and plasticity (Perez-Gonzalez *et al.,* 2014), which in turn effect the level of acetylcholine (a neurotransmitter involves in memory function) in the brain. It is assumed that increase level of acetylcholine serves as a reactant for the enzyme AChE and could be the reason of diminished AChE activity as witnessed in our study (fig. 6). Though, it is also recommended that enhanced 5-HT function (fig. 7) and antioxidant enzyme activity (fig. 5) may also have a part in the enhancement of cognition because improved 5-HT function and antioxidant defence system are also intricate in development of memory function (Samad *et al.,* 2018; Samad and Saleem, 2018).

CONCLUSION

In conclusion our findings suggest that strong contribution of antioxidant effect and regularization of 5-HT neurotransmission underlying the neuroprotective role of TRP and provide proves of TRP's therapeutic value at pharmacological doses in treating psychiatric illness

(Anxiety and depression) and cognitive functions in an animal model, probably may be useful effect in treating clinically relevant human psychiatric and cognitive disorders.

REFERENCES

Amoateng P, Kukuia KKE, Mensah JA, Osei-Safo D, Adjei S, Eklemet A A, Vinyo EA and Karikari TK (2018). An extract of *Synedrella nodiflora* (L) Gaertn exhibits antidepressant properties through monoaminergic mechanisms. *Metab. Brain Dis.*, **33**(4): 1359-1368.

Badawy EA, Rasheed WI, Elias TR, Hussein J, Harvi M, Morsy S and Mahmoud YE (2015). Flaxseed oil reduces oxidative stress and enhances brain monoamines release in streptozotocin-induced diabetic rats. *Hum. Exp. Toxicol.*, **34**(11): 1133-1138

Bravo R, Matito S, Cubero J, Paredes SD, Franco L, Rivero M, Rodríguez AB and Barriga C (2013). Tryptophan-enriched cereal intake improves nocturnal sleep, melatonin, serotonin and total antioxidant capacity levels and mood in elderly humans. *Age*., **35**(4): 1277-1285.

Całyniuk B, Grochowska-Niedworok E, Walkiewicz K, Kawecka S, Popiołek E and Fatyga E (2016). Malondialdehyde (MDA)-product of lipid peroxidation as marker of homeostasis disorders and aging. *Ann. Acad. Med. Silesiensis*., **70**: 224-228

Carneiro IB, Toscano AE, Lacerda DC, da Cunha MD, De Castro RM, de Jesus Deiro TC and Medeiros JM (2018). L-tryptophan administration and increase in cerebral serotonin levels: Systematic review. *Eur. J. Pharmacol*., **836**: 129-135.

Chang CY, Guo HR, Tsai WC, Yang KL, Lin LC, Cheng TJ and Chuu JJ (2015). Subchronic arsenic exposure induces anxiety-like behaviors in normal mice and enhances depression-like behaviors in the chemically induced mouse model of depression. *BioMed. Res. Int*., p.159015.

Chow CK and Tappel AL (1972). An enzymatic protective mechanism against lipid peroxidation damage to lungs of ozone-exposed rats. *Lipids*, **7**(8): 518-524.

Colpaert FC (1987). Pharmacological characteristics of tremor, rigidity and hypokinesia induced by reserpine in rats. *Neuropharmacol*., **26**(9): 1431-40.

Cunha AS, Matheus FC, Moretti M, Sampaio TB, Poli A, Santos DB, Colle D, Cunha MP, Blum-Silva CH, Sandjo LP and Reginatto FH (2016). Agmatine attenuates reserpine-induced oral dyskinesia in mice: Role of oxidative stress, nitric oxide and glutamate NMDA receptors. *Behav Brain Res*., **312**: 64-76.

Daglia M, Di Lorenzo A, Nabavi SF, Sureda A, Khanjani S, Moghaddam AH, Braidy N and Nabavi SM (2017). Improvement of antioxidant defences and mood status by oral GABA tea administration in a mouse model of post-stroke depression. *Nutrients*, **9**(5): 446.

Dannlowski U, Kugel H, Redlich R, Halik A, Schneider I, Opel N, Grotegerd D, Schwarte K, Schettler C, Ambrée O and Rust S (2014). Serotonin transporter gene methylation is associated with hippocampal gray matter volume. *Hum. Brain Mapp*., **35**(11): 5356-5367.

Datta S, Jamwal S, Deshmukh R and Kumar P (2016). Beneficial effects of lycopene against haloperidol induced orofacial dyskinesia in rats: Possible neurotransmitters and neuro-inflammation modulation. *Eur. J. Pharmacol*., **771**: 229-235.

Davies DL and Shepherd M (1955). Reserpine in the treatment of anxious and depressed patients. *Lancet.*, **269**(6881): 117-120.

Delgado PL (2000). Depression: The case for a monoamine deficiency. *J. Clinic Psychiatry*, **61**(Suppl 6): 7-11.

Durlach J (1956). Treatment of emaciation with reserpine. *Concurs Med.*, **78**(7): 725-728.

Ellman GL, Courtney KD, Andres Jr V and Featherstone RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.*, **7**(2): 88-95.

Favero G, Trapletti V, Bonomini F, Stacchiotti A, Lavazza A, Rodella L and Rezzani R (2017). Oral supplementation of melatonin protects against fibromyalgia-related skeletal muscle alterations in reserpine-induced myalgia rats. *Int. J. Mol. Sci.*, **18**(7): 1389.

Flohe L and Gunzler WA (1984). Assays of glutathione peroxidase. *Methods Enzymol.*, **105**: 114-120.

Flora SJS (1999). Arsenic-induced oxidative stress and its reversibility following combined administration of N-acetylcysteine and meso 2,3-dimercaptosuccinic acid in rats. *Clin. Exp. Pharmacol. Physiol.*, **26**(11): 865-869.

Fuentes P, Paris I, Nassif M, Caviedes P and Segura-Aguilar J (2007). Inhibition of VMAT-2 and DT-diaphorase induce cell death in a substantia nigra-derived cell line an experimental cell model for dopamine toxicity studies. *Chem. Res. Toxicol*., **20**(5): 776-83.

Gul S, Saleem D, Haleem MA and Haleem DJ (2019). Inhibition of hormonal and behavioral effects of stress by tryptophan in rats. *Nut. Neurosci.*, **22**(6): 409-417.

Gutteridge JM (1995). Lipid peroxidation and antioxidants as biomarkers of tissue damage. *Clin. Chem.*, **41**(12 Pt 2): 1819-1828.

Haider S, Khaliq S and Haleem DJ (2007). Enhanced serotonergic neurotransmission in the hippocampus following tryptophan administration improves learning acquisition and memory consolidation in rats. *Pharmacol. Rep.*, **59**(1): 53-57.

Haleem DJ, Jabeen B and Parveen T (1998). Inhibition of restraint-induced anorexia by injected tryptophan. *Life Sci.*, **63**(14): PL205-212.

Hansson SR, Mezey E and Hoffman BJ (1998). Serotonin transporter messenger RNA in the developing rat brain: early expression in serotonergic neurons and transient

expression in non-serotonergic neurons. *Neurosci.*, **83**(4): 1185-1201.

Haverkamp GL, Loosman WL, Franssen CF, Kema IP, Van Diepen M, Dekker FW, Honig A and Siegert CE (2016). The role of tryptophan degradation in the association between inflammatory markers and depressive symptoms in chronic dialysis patients. *Nephrol. Dial. Transplant.*, **32**(6): 1040-1047

Hopkins FG and Cole SW (1901). A contribution to the chemistry of proteids: Part I. A preliminary study of a hitherto undescribed product of tryptic digestion. *J. Physiol*., **27**(4-5): 418-428.

Ikram H and Haleem DJ (2017). Repeated treatment with reserpine as a progressive animal model of depression. *Pak. J. Pharm. Sci.*, **30**(3): 897-902.

Ikram H and Haleem DJ (2019). Repeated treatment with a low dose of reserpine as a progressive model of Parkinson's dementia. *Pak. J. Pharm. Sci*., **32**(2): 555-62.

Ikram H, Mushtaq F and Haleem DJ (2014). Dose-dependent effects of tryptophan on learning and memory. *Pak. J. Pharm. Sci.*, **27**(5): 1131-1135.

Ingawale DK, Mandlik SK and Naik SR (2014). Models of hepatotoxicity and the underlying cellular, biochemical and immunological mechanism (s): A critical discussion. *Environ Toxicol. Pharmacol.*, **37**(1): 118-133.

Jamwal S and Kumar P (2017). L-theanine, a component of green tea prevents 3-nitropropionic acid (3-NP)-induced striatal toxicity by modulating nitric oxide pathway. *Mole Neurobiol*., **54**(3): 2327-2337.

Kang KT, Sullivan JC and Pollock JS (2018). Superoxide dismutase activity in small mesenteric arteries is downregulated by angiotensin II but not by hypertension. *Toxicolo Res.,* **34**(4): 363.

Kessler CK and Bromet EJ (2013). The epidemiology of depression across cultures. *Ann. Rev. Public Health*, **34**: 119-138.

Khadrawy YA, Sawie HG, Abdel-Salam OM and Hosny EN (2017). Cannabis exacerbates depressive symptoms in rat model induced by reserpine. *Behav. Brain Res.*, **324**: 41-50.

Khnychenko LK, Yakovleva EE, Bychkov ER and Shabanov PD (2017). Effects of fluorencarbonic acid derivative on the levels of monoamines and their metabolites in brain structures of rats with modeled depression-like state. *Bull Exp Biol Med*., **163**(5): 632-634.

Kronbauer M, Segat HJ, Antoniazzi CT, Roversi K, Roversi K, Pase CS, Barcelos RC and Burger ME (2015). Magnesium supplementation prevents and reverses experimentally induced movement disturbances in rats: Biochemical and behavioral parameters. *Biol Trace Elem Res.,* **166**(2): 163-172.

Kruk-Slomka M, Boguszewska-Czubara A, Slomka T, Budzynska B and Biala G (2016). Correlations between the memory-related behavior and the level of oxidative stress biomarkers in the mice brain, provoked by an acute administration of CB receptor ligands. *Neural Plasticity*, **2016**: 9815092.

Leal PC, Bispo JM, Engelberth RC, Silva KD, Meurer YR, Ribeiro AM, Silva RH, Marchioro M and Santos JR (2019). Serotonergic dysfunction in a model of parkinsonism induced by reserpine. *J. Chem. Neuroanat*., **96**: 73-78.

Lindseth G, Helland B and Caspers J (2015). The effects of dietary tryptophan on affective disorders. *Arch Psychiat Nursing.,* **29**(2): 102-107.

Liu J, Zhang Y, Li Y, Yan H and Zhang H (2019). L-Tryptophan enhances intestinal integrity in diquat-challenged piglets associated with improvement of redox status and mitochondrial function. *Animals*, **9**(5): 266

Liu T, Zhong S, Liao X, Chen J, He T, Lai S and Jia Y (2015). A meta-analysis of oxidative stress markers in depression. *PloS One.,* **10**(10): e0138904

Luo Y, Li Z, Peng X, Xia L and Ma L (2017). Desipramine rescues emotional memory deficit induced by low doses of reserpine. *Behav. Pharmacol*., **28**(6): 441-449.

Manna P, Sinha M and Sil PC (2008). Arsenic-induced oxidative myocardial injury: Protective role of arjunolic acid. *Arch Toxicol.,* **82**(3): 137-149.

McCord JM, Keele BB and Fridovich I (1976). An enzyme-based theory of obligate anaerobis: The physiological functions of superoxide dismutase. *Proc. Natl. Acad. Sci. USA*., **68**(5): 1024-1031.

Pari L and Latha M (2004). Protective role of *Scoparia dulcis* plant extract on brain antioxidant status and lipid peroxidation in STZ diabetic male Wistar rats. *BMC Complementary Altern Med*., **4**: 16.

Park BK, Kim YR, Kim YH, Yang C, Seo CS, Jung IC, Jang IS, Kim SH and Lee MY (2018). Antidepressant-like effects of gyejibokryeong-hwan in a mouse model of reserpine-induced depression. *BioMed Res. Int.*, **2018**: 5845491.

Penedo LA, Oliveira-Silva P, Gonzalez EM, Maciel R, Jurgilas PB, Melibeu AD, Campello-Costa P and Serfaty CA (2009). Nutritional tryptophan restriction impairs plasticity of retinotectal axons during the critical period. *Exp. Neurol.,* **217**(1): 108-115.

Perez-Gonzalez A, Munoz-Rugeles L, Alvarez-Idaboy JR (2014). Tryptophan: Antioxidant or target of oxidative stress? A quantum chemistry elucidation. *RSC Advances*, **4**(99): 56128-56131

Phillips MR, Zhang J, Shi Q, Song Z, Ding Z, Pang S, Li X, Zhang Y and Wang Z (2009). Prevalence, treatment, and associated disability of mental disorders in four provinces in China during 2001-2005: An epidemiological survey. *Lancet.,* **373**(9680): 2041-2053.

Saki K, Bahmani M and Rafieian-Kopaei M (2014). The effect of most important medicinal plants on two important psychiatric disorders (anxiety and

depression)-A review. *Asian Pac. J. Trop. Med*., **7**S1: S34-342.

Saleem DM, Mehboob S, Khan MM, Samad N, Zafar A and Haleem DJ (2018). Inhibition of diet-restriction-induced behavioral deficits by tryptophan administration in rats. *Pak. J. Pharm. Sci.*, **31**(3 Suppl): 1021-1029.

Samad N and 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**(1): 271-281.

Samad N, Muneer A, Zaman A, Ayaz MM and Ahmad I (2017). Banana fruit pulp and peel involved in antianxiety and antidepressant effects while invigorate memory performance in male mice: Possible role of potential antioxidants. *Pak. J. Pharm. Sci.*, **30**(3 Suppl): 989-995.

Samad N, Saleem A, Yasmin F and Shehzad MA (2018). Quercetin protects against stress-induced anxiety-and depression-like behavior and improves memory in male mice. *Physiol. Res.*, **67**(5): 795-808.

Serfaty CA, Oliveira-Silva P, Melibeu AD and Campello-Costa P (2005). Nutritional tryptophan restriction and the role of serotonin in development and plasticity of central visual connections. *Neuroimmunomodul*., **15**(3): 170-175.

Sivolap YP (2017). Serotonin neurotrasmission and treatment options for depression. *Zh Nevrol Psikhiatr Im. S. S. Korsakova*., **117**(11): 174–177.

Soung HS, Wang MH, Chang KC, Chen CN, Chang Y, Yang CC and Tseng HC (2018). L-theanine decreases orofacial dyskinesia induced by reserpine in rats. *Neurotoxicity Res.*, **34**(3): 375-387.

Tang M, He T, Sun X, Meng QY, Diao Y, Lei JY, He XJ, Chen L, Sang XB and Zhao S (2014). Subregion-specific decreases in hippocampal serotonin transporter protein expression and function associated with endophenotypes of depression. *Hippocampus*, **24**(4): 493-501.

Tang M, Lei J, Sun X, Liu G and Zhao S (2013). Stress-induced anhedonia correlates with lower hippocampal serotonin transporter protein expression. *Brain. Res.*, **1513**: 127-134.

Tseng HC, Wang MH, Soung HS, Chang Y and Chang KC (2015). (-) Epigallocatechin-3-gallate prevents the reserpine-induced impairment of short-term social memory in rats. *Behav. Pharmacol*., **26**(8: 741-747.

Ullah R, Khan M, Shah SA, Saeed K and Kim MO (2019). Natural antioxidant anthocyanins A hidden therapeutic candidate in metabolic disorders with major focus in neurodegeneration. *Nutrients*, **11**(6): 1195.

Wang MH, Lin RF, Tseng HC, Soung HS, Chang KC and Tsai CC (2015). (-) Epigallocatechin-3-gallate attenuates reserpine-induced orofacial dyskinesia and oxidative stress in rat striatum. *Pharmacol. Biochem. Behav*., **131**(6): 71-76.

Yu H, Lv D, Shen M, Zhang Y, Zhou D, Chen Z and Wang C (2019). BDNF mediates the protective effects of scopolamine in reserpine-induced depression-like behaviors via up-regulation of 5-HTT and TPH1. *Psychiat. Res.*, **271**: 328-334.

Zhang WY, Guo YJ, Han WX, Yang MQ, Wen LP, Wang KY and Jiang P (2019). Curcumin relieves depressive-like behaviors via inhibition of the NLRP3 inflammasome and kynurenine pathway in rats suffering from chronic unpredictable mild stress. *Int. Immunopharmacol.*, **67**: 138-144.