Inhibition of diet-restriction-induced behavioral deficits by tryptophan administration in rats

Darakhshan M Saleem[1], Shafaque Mehboob[2], Moona Mehboob Khan[3], Noreen Samad[4], Afshan Zafar[5]and Darakhshan J Haleem[6]

[1]Biomedical Engineering Department, Sir Syed University of Engineering & Technology, Karachi, Karachi, Pakistan
[2]Department of Pharmacology, Jinnah Sindh Medical University, Karachi, Pakistan
[3]Dow College of Pharmacy, Department of Pharmaceutical Chemistry, Dow University of Health Sciences, Karachi, Pakistan
[4]Department of Biochemistry, Bahauddin Zakariya University, Multan, Pakistan
[5]Department of Physiology, Jinnah Medical and Dental College, Karachi, Pakistan
[6]Dr. Panjwani Center for Molecular Medicine and Drug Research International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan

---

Abstract: Diet has a great impact on brain health and function. It plays an important role to improve and control a number of psychiatric disorders such as depression, anxiety, hyperactivity and behavioral impulsivity. Anorexia Nervosa (AN) is one of the psychiatric disorder which is associated with diet. In AN, patients show extreme dieting, weight loss, hyperactivity, depression/anxiety, self-control and behavioral impulsivity. Previous studies showed that during diet restriction, tryptophan decreases serotonin (5-hydroxytryptamine; 5-HT) metabolism in the brain due to its less availability and contributes psychiatric problems associated with AN. The present study is designed to investigate the effects of tryptophan administration on 5-HT metabolism in diet-restricted rats. Tryptophan at a dose of 50 or 100mg/kg was given orally to respective freely fed (FF) or diet restricted (DR) animals daily for five weeks. Behavioral activities were also monitored weekly. The results show significant effect ($p<0.05$) on behavior in activity box, open field and in light/dark transition test by tryptophan administration in diet-restricted rats. This may be associated with the increased in serum tryptophan and brain 5-HT metabolism. Therefore, it is concluded that diet-restriction-induced behavioral changes might be reverted back with the administration of tryptophan and may be helpful to improve psychological problems in AN.

Keywords: Tryptophan, serotonin, hyper activity, behavioral deficits, diet-restriction.

INTRODUCTION

Tryptophan is the precursor of 5-HT and an essential amino acid therefore, only available in the diet. The tryptophan is transported to the brain via common carrier transport mechanism shared by all large neutral amino acids (LNAAs). Therefore; an increase in the concentration of one amino acid decreases the transport of others. The enzyme tryptophan hydroxylase is the rate-limiting enzyme of 5-HT biosynthesis and remains unsaturated with its substrate under normal physiological conditions (Eccleston *et al*., 1965; Lovenberg *et al*., 1968). Diet restriction and malnutrition can alter 5-HTneurotransmission through a number of pathways (Hasan and Hasan, 2011; Rodgers *et al*., 2011). A role of 5-HT in Anorexia Nervosa (AN) largely emerged from pharmacological evidence linking the serotonergic system with the regulation of appetite (Sugrue, 1987; Haleem and Haider, 1996; Darakhshan *et al*., 2015). Evidences suggest that exogenous tryptophan not only increases tryptophan concentrations in the plasma but also increases 5-HT metabolism in the brain (Fernstrom *et al*., 1972; Young, 1996; Haleem *et al*., 1998). Studies show that diet restriction-induced exaggerated feedback control over 5-HT synthesis and the smaller availability of tryptophan decreases serotonin neurotransmission at postsynaptic sites, leading to hyperactivity, depression and behavioral impulsivity. A compensatory up regulation of postsynaptic 5-HT-1A receptors and hypophagic serotonin receptors may be involved in anxiety and suppression of appetite (Lovenberg *et al*., 1968; Gelegen *et al*., 2007; Haleem, 2009).

In our previous studies (Darakhshan *et al*., 2015) it was observed that DR for 5 weeks decreases the levels of tryptophan in serum and hence, 5-HT metabolism in the brain. The present experiment is designed to investigate the effects of oral tryptophan administration on 5-HT metabolism in diet restricted rats. It was hypothesized that long term tryptophan administration for 5 weeks may improve behavioral symptoms associated with diet restriction induced AN.

MATERIALS AND METHODS

Animals

Locally bred female Sprague-dawley rats of weight 150-160g were housed individually under 12 hour light dark cycle and controlled room temperature (22±2ºC) with the access of cubes of standard rodent diet and water for at

---

**Corresponding author:* e-mail: darakhshansaleem@yahoo.com

least three days before experimentation. Animals were cared according to a protocol approved locally which is consistent with the NIH guidelines for the care and use of laboratory animals.

Drug

Freshly prepared suspension of tryptophan (Merk, Germany) in tap water at dose of 50 and 100mg/kg body weight was used.

Experimental protocol

One week after arrival 36 female Sprague-Dawley rats weighing 150.4±8.6g were randomly divided into six groups, each containing six animals:(i) FF-water (ii) FF-Tryptophan (50.0mg/kg), (iii) FF- Tryptophan (100.0mg/kg) (iv) DR-water, (v) DR- Tryptophan (50.0mg/kg), and (vi) DR- Tryptophan (100.0mg/kg). Water or tryptophan was given orally to respective animals daily for five weeks. Food was available for 24 h to FF group while animals of DR group were given access to food daily for 2h. Food intake was monitored daily and body weights were monitored weekly. Behavioral activities were also monitored weekly. The animals were decapitated after 5 weeks between 10:00 and 11:00h. Blood was allowed to clot at 4°C and serum was separated and stored at -70°C for estimation of tryptophan. Brains removed rapidly were dipped in ice cold saline. Brain dissected out as described previously (Darakhshan *et al*., 2013) were stored at -70ºC until analysis by HPLC–EC.

Behavioral assessment

Activity monitoring activity cage

Transparent, Perspex activity cages (26× 26× 26cm) with a saw-dust covered floor were used to monitor activity. Experiment was conducted in a separate quite room. Locomotor activity was observed in activity boxes for 15 minutes in terms of numbers of cage crossings in all groups in a balanced design as described earlier (Khalid *et al*., 2017; Batool *et al*., 2009).

Open field test

The open field used in the present investigation was a square area (76×76 cm) with walls 42 cm high. The floor of apparatus was divided by lines into 25 squares of equal size. To monitor activity, an animal was placed in the central square of the open field. The number of squares crossed with all four paws was counted for 5 min starting immediately after exposure to the open field (Haleem, 2009).

Light dark activity test

The apparatus used in the present investigation was a two compartment light dark box. Both the light (made up transparent plastic) and the dark (made up of black translucent plastic) compartments measured 26x26x26 cm access between the compartments was provided by a 12x12 cm passageway. The experiment was performed in a quiet, air-conditioned room and the apparatus placed under white light. An animal was introduced to the apparatus by placing in dark compartment (Darakhshan *et al*., 2015). Time spent in the light compartment and the entries in the light compartment were monitored for a cut off time of 5 minutes.

Neurochemical Analysis

TRP, 5-HT and 5-HIAA were determined by HPLC-EC as described before (Tahira *et al*., 2012). A5II Shim-Pack ODS separation columns of 4.0 mm internal diameter and 15 cm length were used. Separation was achieved by a mobile phase containing methanol (14%), Octyl sodium sulphate (0.023%) and EDTA (0.0035%) in 0.1 M phosphate buffer of pH 2.9 at an operating pressure of 2000 -3000 psi on Schimadzu HPLC-EC 6A detector at an operating potential of 1.0 mV for TRP and of 0.8 mV for 5-HT.

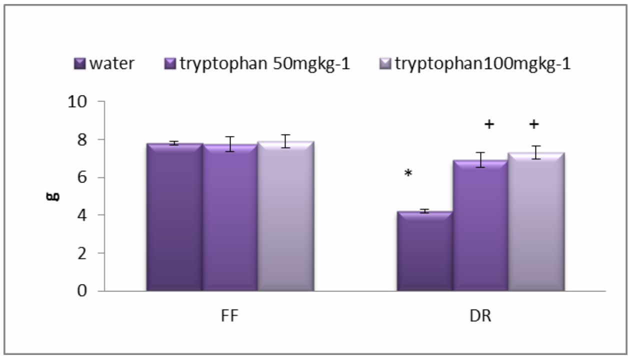

Fig. 1: Effects of tryptophan administration on food intake in Freely Feeding (FF) and Diet Restriction (DR) group of rats. Values are means ±SD (n=6) .Significant differences by Tukey's test.*p<0.01 from respective FF controls, +p<0.01 from respective water treated group following three way ANOVA (repeated measure design).

STATISTICAL ANALYSIS

Results are represented as means ± SD. Behavioral data were analyzed by three-way ANOVA (repeated measure design). Neurochemical and Biochemical data were analyzed by three-way ANOVA. *Post- hoc* comparisons done by Tukey's test. *p* values <0.05 were taken significant.

RESULTS

Food intakes of rats fed on DR schedule of 2 h/day for five week and FF rats are shown in fig. 1. Three-way ANOVA (df. 4, 30) (repeated measure design) showed significant effect of DR (F=19.903; $p<0.01$), week (df 4,30;F=31.76, $p<0.01$), week × group (df 16,120;F=1.367, $p>0.01$) insignificant, interaction (df 4,30;F=885.9, $p<0.01$), and group (df 4,30; F=19.525, $p<0.01$) were also significant. *Post- hoc* test showed that food intakes were significantly ($p<0.01$) decreased from week 1 to week 5 in water treated DR group than respective FF group. The significant ($p<0.01$) treatment effect of tryptophan at a dose of 50mg/kg and 100mg/kg in increased in food intake were observed after week 3 to week 5 and week 2 to week 5 respectively. The increases were insignificant ($p>0.05$) from week 1 to 2 and week 1 at low and high dose respectively. The effect of tryptophan after week 5 at a dose of 50mg/kg and 100mg/kg revealed no difference in food intake in FF group when compared with water treated rats.

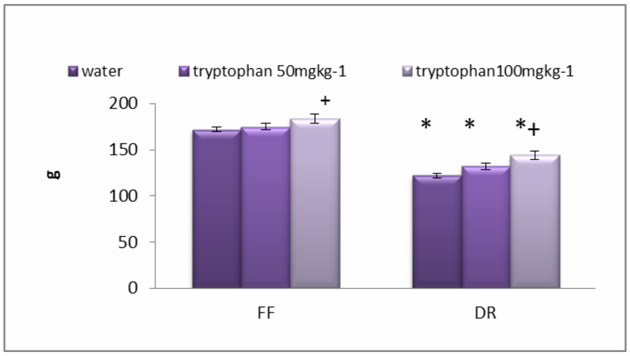

Fig. 2: Effects of tryptophan administration on body weight in Freely Feeding (FF) and Diet Restriction (DR) group of rats. Values are means ±SD (n=6) .Significant differences by Tukey's test.*p<0.01 from respective FF controls, +p<0.01 from respective water treated group following three way ANOVA (repeated measure design).

Body weight changes in FF and DR groups during five week treatment are shown in fig. 2. Three-way ANOVA (df 4, 30) showed significant treatment effect of tryptophan (F=80.553; $p<0.01$), week (df4,30;F=11.146, $p<0.01$), week × group (df 16,120;F=3.691,$p<0.01$), interaction (df4,30;F=791.8,$p<0.01$), and group (df 4,30; F=15.987, $p<0.01$) were significant. Post hoc test showed the significant ($p<0.01$) treatment effect of tryptophan (at a dose 100mg/kg) in increased in body weights after week 3 to week 5 in FF and DR group with respective water treated group. The increases were insignificant ($p>0.05$) after week 1 and 2 both in FF and DR group with respective water treated group. The treatment effect of tryptophan (at a dose 50mg/kg) on body weights were insignificant ($p>0.05$) from week 1 to week 5 in FF and DR group with respective water treated group.

DR induced hyperactivity in FF and DR group are shown as in fig. 3. Three way ANOVA (df 4, 30) (repeated measures design) showed significant effect of DR (F= 17.25; p<0.01), week(df4,30;F=4.45,$p<0.05$), week× group (df16,120; F=6.31, $p<0.01$), interaction (df 4,30;F=148.0, $p<0.01$), and group (df 4,30; F=42.7, $p<0.01$) were significant.. Post hoc test showed that activity scores (number of cage crossings) were greater ($p < 0.01$) in DR group than FF group. The significant (p<0.01) treatment effect of tryptophan in decreased in hyperactivity was observed from week 1 to week 5 in Tryptophan at a dose of (50mg/kg) and Tryptophan at a dose of (100mg/kg) in DR group as compared to respective water treated DR group. The decrease in hyperactivity ($p<0.05$) were also observed from week1 to week 3 in Tryptophan at a dose of (50mg/kg) and Tryptophan at a dose of (100mg/kg) in FF group. The decreases were insignificant from week 4 to week 5 in tryptophan treated FF group.

Effect of DR in an open field activity in FF and DR group are shown as in fig. 4 Three way ANOVA (df 4, 30) (repeated measures design) showed significant effect of DR (F=39.07; $p<0.05$), week (df 4,30; F=24.1,$p<0.01$), week × group (df 16,120; F=6.78, $p<0.01$), interaction (df 4,30;F=158.2, $p<0.01$) and group (df4,30; F=70.214, $p<0.01$). *Post- hoc* test showed that number of square crossed was greater from week 1 to week 5 in water treated DR group than respective FF group. Data showed that the treatment of tryptophan at a dose of (50mg/kg) significantly ($p<0.01$) decreased number of square crossed in DR group after week 2, 4 and in week 5 than water treated DR group. The decreases were insignificant after week 1 and week 3. The significant ($p<0.01$) treatment of tryptophan at a dose of 100mg/kg in decreased number of square crossed was observed from week 2 to week 5 than water treated DR group. The decreases were insignificant after week 1. An increased in open field activity ($p<0.05$) was observed after week 3 and week 4 in Tryptophan (at a dose of 50mg/kg) and Tryptophan (at a dose of 100mg/kg) in FF group when compared to water treated FF group. The increases were insignificant after week 1, 2 and 5.

Effect of DR on light dark activity in FF and DR group are shown as in fig. 5 Three way ANOVA (df 4, 30) (repeated measures design) showed significant effect of DR (F=45.55; $p<0.01$), week (df4,30;F=12.1,$p<0.01$), week× group (df 16,120; F=5.018, $p<0.01$), interaction (df 4,30;F=234.7,$p<0.01$), and group (df 4,30;F=90.983, $p<0.01$) . Post hoc test showed that time spent in light box was decreased ($p<0.01$) from week 3 to week 5 in DR water treated group than respective FF group. Data showed that the treatment of tryptophan (at a dose of 50mg/kg) significantly ($p<0.05$) increased time spent in light box in DR group after week 1and ($p<0.01$) from week 3 to week 4 than water treated DR group .The increases were insignificant after week 1,2 and 5. The greater significant ($p<0.01$) treatment of tryptophan (at a dose of 100mg/kg) in time spent in light box were observed from week 2 to week5 than water treated DR group. The decreases were insignificant after week 1. An increased in time spent in light box ($p<0.01$) was observed in week 1and ($p<0.05$) week 4, 5 in Tryptophan (at a dose of 50mg/kg) in FF group when compared to water treated FF group. The increases were insignificant after week 2 and 3. Whereas, significant ($p<0.05$) increased in time spent in light box was observed from week 1 to week 5 in Tryptophan (at a dose of 100mg/kg) in FF group than respective water treated group.

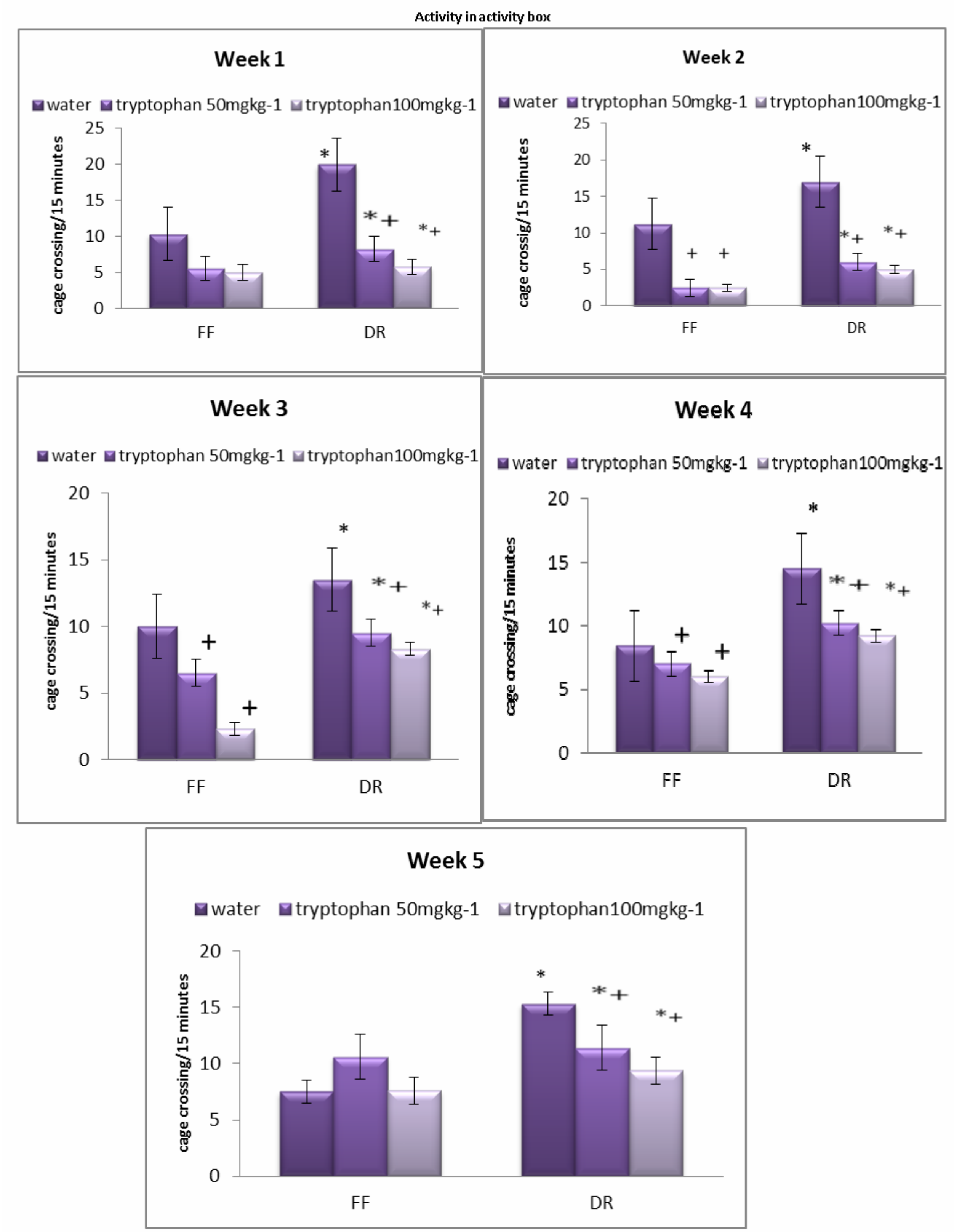

Fig. 3: Effects of tryptophan administration on activity in activity box in Freely Feeding (FF) and Diet Restriction (DR) group of rats. Values are means± SD (n=6). Significant differences by Tukey's test.*p<0.01 from respective FF controls, +p<0.01 from respective water treated group following three way ANOVA (repeated measure design).

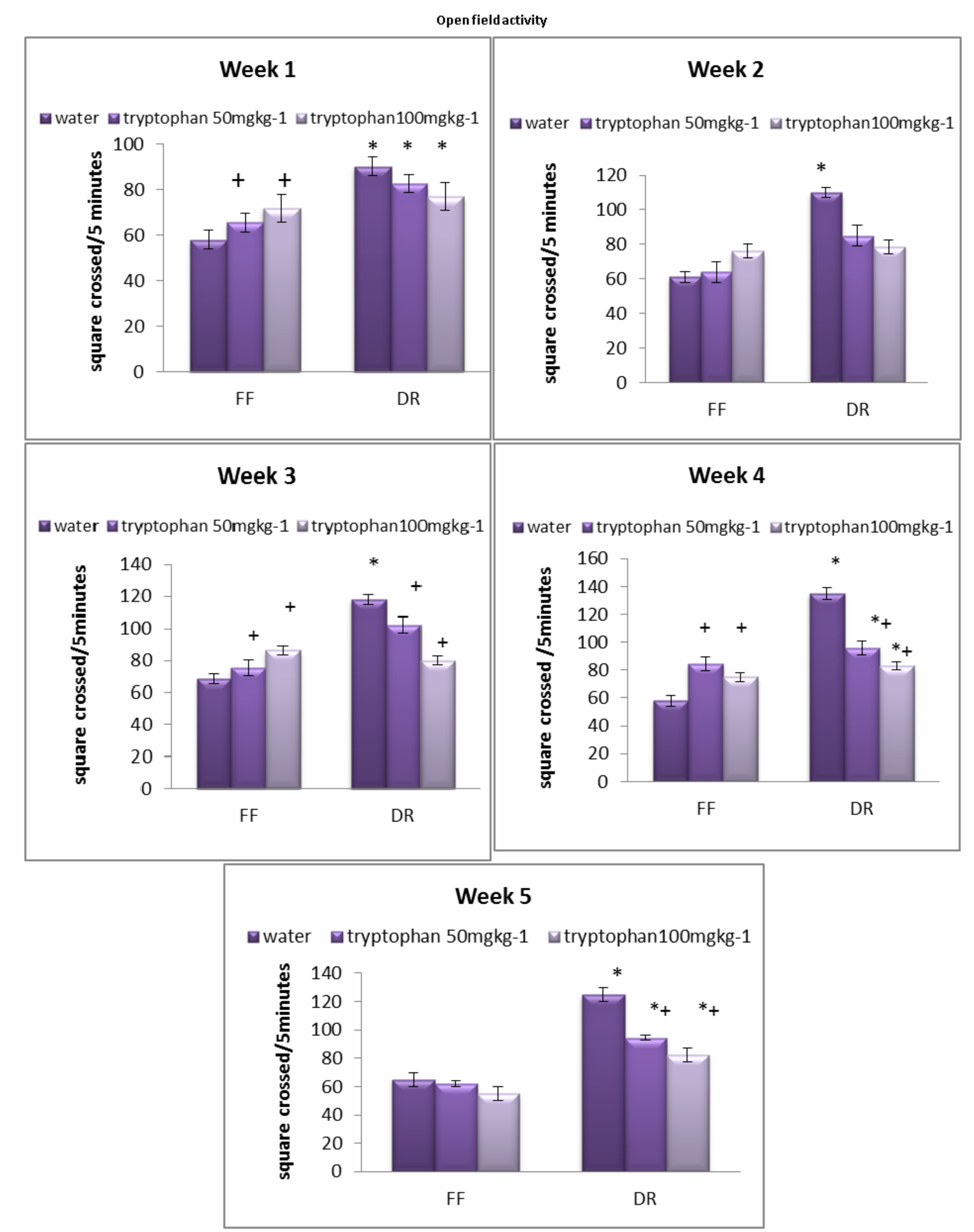

Fig. 4: Effects of tryptophan administration on open field activity in Freely Feeding (FF) and Diet Restriction (DR) group of rats. Values are means± SD (n=6). Significant differences by Tukey's test.*p<0.01 from respective FF controls, +p<0.01 from respective water treated group following three way ANOVA (repeated measure design).

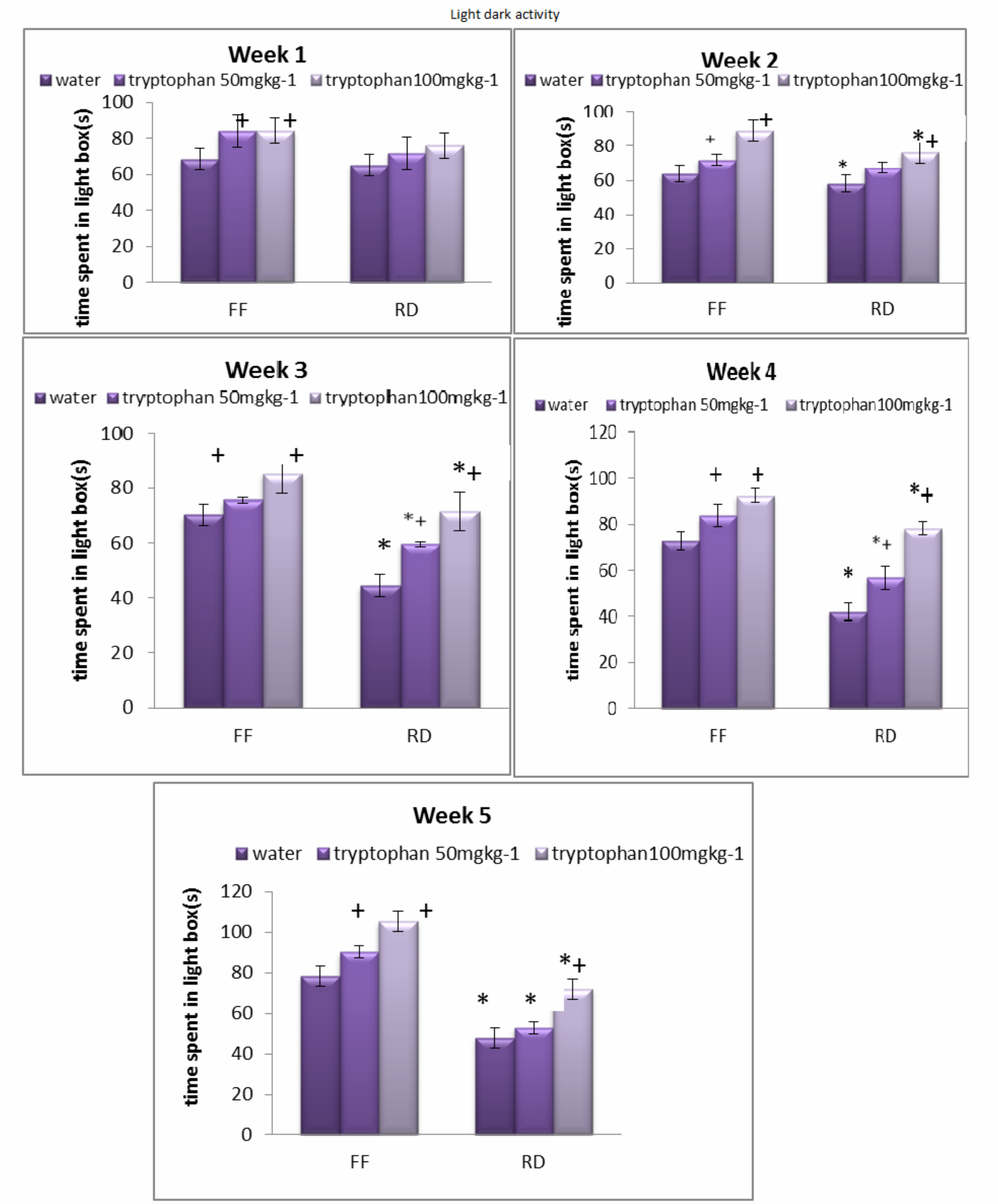

Fig. 5: Effects of tryptophan administration on light and dark activity in Freely Feeding (FF) and Diet Restriction (DR) group of rats. Values are means ± SD (n=6). Significant differences by Tukey's test.*$p<0.01$ from respective FF controls, +$p<0.01$ from respective water treated group following three way ANOVA (repeated measure design).

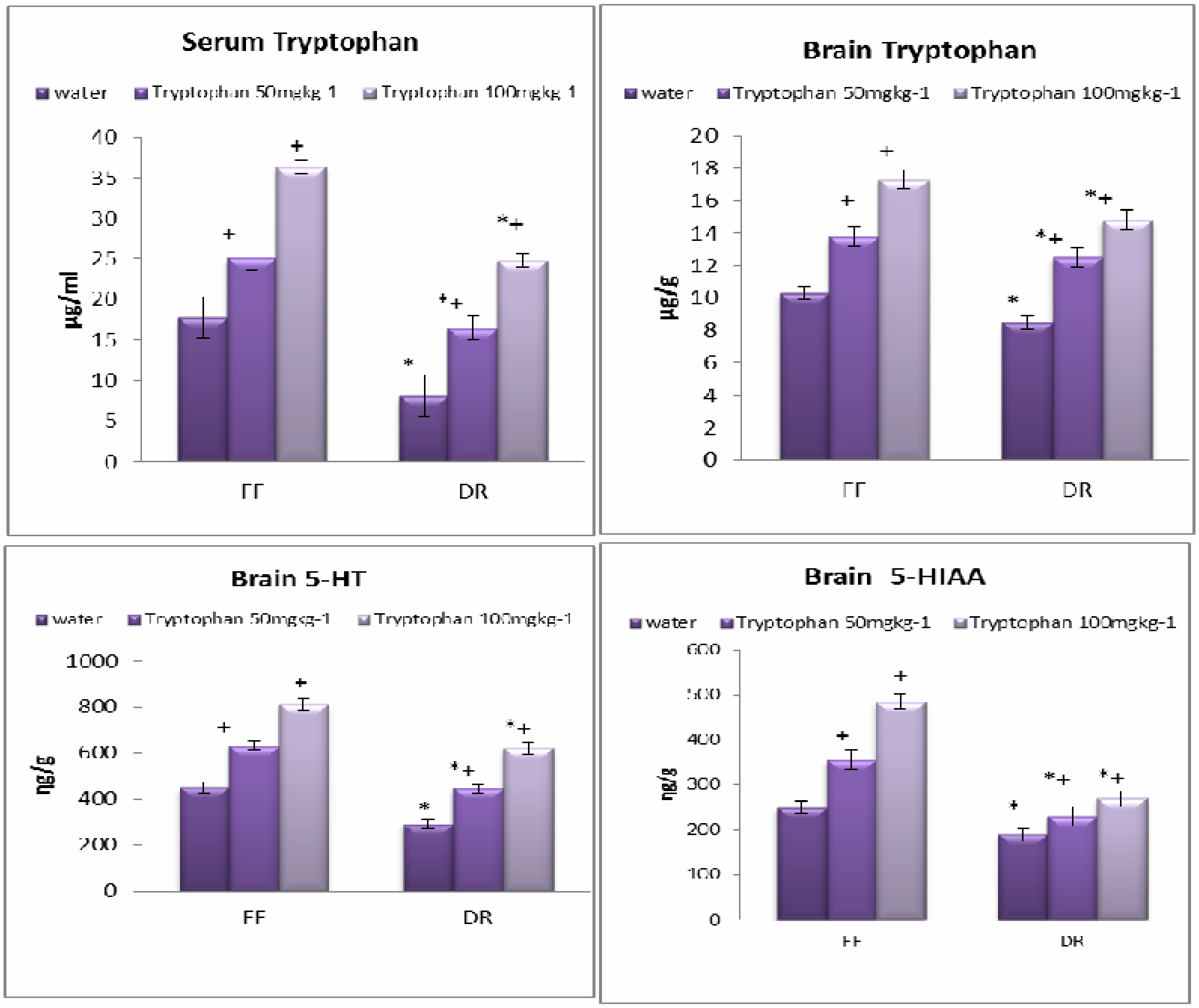

Fig. 6: Effects of tryptophan administration on serum tryptophan in Freely Feeding (FF) and Diet Restriction (DR) group of rats. Values are means± SD (n=6). Significant differences by Tukey's test.*p<0.01 from respective FF controls, +p<0.01 from respective water treated group following three way ANOVA.

Effects of DR on the levels of TRP in Serum and TRP, 5-HT and 5-HIAA in the brain of FF and DR rats are shown in figs. 6. Data analyzed by Three way ANOVA (df 5, 30) showed significantly increased treatment effect of tryptophan (F=65.26; *p*<0.01); interaction (df4, 30; F=195.9; *p*<0.01); group (df 4, 30; F=55.32; *p*<0.01). *Post- hoc* test showed increased levels of tryptophan 101.11% (*p*<0.01) in (50mg/kg) and 206.2% (*p*<0.01) in tryptophan (100mg/kg) in serum tryptophan in DR group. An increased treatment effect also produced 41.11% (*p*<0.01) in (50mg/kg) and 103% (*p*<0.01) in tryptophan (100mg/kg) in FF group. Data analyzed by Three way ANOVA (df4, 30) showed significantly increased treatment effect of tryptophan (F=37.52; *p*<0.01), interaction (df4, 30; F=533.3; *p*<0.01); group (df 5, 30; F=41.37; *p*<0.01) in the brain. *Post- hoc* test showed increased levels of tryptophan (at a dose of 50mg/kg and100mg/kg) 47.05% (*p*<0.01) and 74.1% (*p*<0.01) in brain in DR group than water treated DR group. An increased treatment effect of tryptophan also produced 33.59% (*p*<0.01) in (50mg/kg) and 67.96% (*p*<0.01) in (100mg/kg) in brain of FF group than water treated group. Data analyzed by Three way ANOVA (df 5,30) showed significantly increased treatment effect of 5-HT (F=602.9; *p*<0.01); interaction (df4,30; F=582.14; *p*<0.01);group (df 4, 30; F=614.72 ; *p*<0.01) in the brain. Increases of 5-HT was 53.72% (*p*<0.01) in (50mg/kg) and 113.7% (*p*<0.01) (100mg/kg) in DR group than water treated DR group. An increased treatment effect also produced 40% (p<0.01) in (50mg/kg) and 80% (*p*<0.01) (100mg/kg) in FF group. Data analyzed by Three way ANOVA (df 5, 30) showed significantly increased treatment effect of 5-HIAA (F=85.42; *p*<0.01), interaction (d.f.4, 30; F=225.65; *p*<0.01); group (df 4, 30; F=38.65; *p*<0.01). Increases of 5-HIAA levels 21% (*p*<0.01) in (50mg/kg) and 41.57% (*p*<0.01) (100mg/kg) in DR group than water treated DR group. An increased treatment effect also produced 42% (*p*<0.01) in (50mg/kg) and 94% (*p*<0.01) (100mg/kg) in FF group than respective water treated group.

DISCUSSION

Significant increased ($p<0.01$) was observed on food intake and body weight in DR rats after tryptophan administration. The effects of tryptophan on food intake and growth rate as observed in the present study agreed with a previous study when I.P administration for 12 days (Haleem *et al*., 1998) and for 4 weeks had no effect on food intake and bodyweight in FF group (Rodgers *et al*., 2011).

Changes ($p<0.05$) in open field activity were observed after tryptophan administration in freely feeding rats although depending on the time of measurement. However, there was a decreased activity ($p<0.01$) in the open field after the administration of tryptophan in DR rats. Similarly, the oral administration of tryptophan produced a dose-dependent anxiolytic-like effect in DR rats as measured in increased time spent in the light compartment of a light/dark box.

In our previous study (Darakhshan *et al*., 2015) it was observed that 2 hour DR-induced AN produces behavioral deficits when animals were subjected to light dark transition test, activity box and open field. The present results show that deficits of behavior in activity box, open field and in light dark transition test were attenuated by tryptophan administration. The evidence suggests that tryptophan exerts a potential antidepressant effect.

The present study shows that 5 weeks of oral TRP administration (at a low dose and high dose) daily increased TRP in serum, brain and also increased brain 5-HT metabolism in FF and DR group. The precursor relationship of the essential amino acid L-TRP to the synthesis of serotonin is well established (Fernstrom *et al*., 1985; Schaechter and Wurtman, 1990). Transport of TRP to the brain also increased as was indicated by an increase in the levels of TRP in the brain. It has been shown (Haleem *et al*., 1998; Haleem *et al*., 1990) that injected TRP increases levels of TRP and 5-HT in the brain. The present study also shows that ingestion of TRP increases 5-HT metabolism in the brain.

5-HT is involved in the control of appetite. Studies suggested that brain 5-HT is increased following the ingestion of meal (Blundell *et al*., 1995). Pharmacological manipulations that tend to increase 5-HT are anorexiogenic.

The present study shows that long-term oral TRP administration for 5 weeks increased body weights of TRP treated animals particularly during the 5th week of treatment. A previous study from our laboratory shows that injected TRP produced anti-depressant effects as it attenuated stress-induced hypophagia (Haleem *et al*., 1998). Taken together, these studies suggest that administration of TRP increases brain 5-HT synthesis and turnover, release and functional responses to 5-HT are not necessarily increased. The release of 5HT in TRP injected rats was increased more following electrical stimulation (Fernstrom *et al*., 1985) and antidepressant effects of TRP were observed in restrained but not in unrestrained rats (Haleem *et al*., 1998).

CONCLUSION

The studies presented here provide evidence that oral administration of tryptophan produced treatment effects on food intake and body weight in DR group. This could be because of greater incorporation of dietary TRP in various structural and functional proteins. Furthermore, behavioral changes elicited by diet restriction might be reverted back with the administration of TRP and may be helpful to improve psychological problems associated with AN.

REFERENCES

Batool F, Ahmed S and Haleem DJ (2009). Dietary supplementations of amino acids: Evidence for enhanced serotonergic functions following haloperidol withdrawal in rat medial prefrontal cortex. *J. Coll. Physicians Surg. Pak.*, **19**: 139-145.

Blundell JE, Lawton CL and Halford C (1995). Serotonin, eating behavior, and fat intake. *Obes Res.*, **3**(4): 471S-76S.

Darakhshan MS, ShafiaT, Ubaid N, Shafaque M.K , Haider S and Haleem DJ (2015). The interaction of serum leptin with brain serotonin in an animal model of diet-restriction-induced anorexia nervosa. *World J Pharmaceutical Sci.*, **3**(3): 430-37.

Darakhshan MS, Saida H, Haleem DJ, Shafaque M and Moona MK (2013). Altered Brain Monoamines Metabolism in Thioacetamide Induced Hepatic Encephalopathy in Rats. *International Journal of Toxicological and Pharmacological Research*, **5**(4): 96-101.

Khalid A, Darakhshan MS, Moona MK, ShafiaT and Haleem DJ (2017). Behavioral response to apomorphine in freely feeding and diet-restricted rats. *Nessa Journal of Pharmacy and Pharmacology*, **1**(2): 1-9

Eccleston D, Ashcroft GW and Crawford TB (1965). 5-Hydroxyindole acetic acid metabolism in rat brain: A study of intermediary metabolism using the technique of tryptophan loading II application and drug studies. *J Neurochem*., **12**: 493-493.

Fernstrom JD, Fernstrom MH, Grubb PE and Volk EA (1985). Absence of chronic effects of dietary protein content on brain tryptophan concentrations in rats. *J. Nutr.*, **115**: 1337-1344.

Fernstrom JD and Wurtman RJ (1972). Brain serotonin content: Physiological regulation by plasma neutral amino acids. *Science*, **178**: 414-416.

Gelegen C, Collier DA, Campbell IC, Oppelaar H, Heuvel J and Adan RA (2007). Difference in susceptibility to activity-based anorexia in two in bred strains of mice. *Eur. Neuropsychopharmacol.*, **17**: 199-205.

Haleem DJ and Haider S (1996). Food restriction decreases serotonin and its synthesis rate in the hypothalamus. *Neuroreport.*, **7**: 1153-1156.

Haleem DJ (2009). Exaggerated feedback control decreases brain serotonin concentration and elicits hyperactivity in a rat model of diet-restriction-induced anorexia nervosa. *Appetite.*, **52**: 44-50.

Haleem DJ, Jabeen B and Parveen T (1998). Inhibition of restraint-induced anorexia by injected tryptophan. *Life Sci.*, **63**: 205-212.

Haleem DJ, Kennett GA and Curzon G (1990). Hippocampal 5-HT synthesis is greater in females than in males and is more decreased by 5-HT-1A agonist 8-OH- DPAT. *J. Neural. Transm*., **79**: 93-01.

Hasan TF and Hasan H (2011). Anorexia nervosa: A unified neurological perspective. *Int. J. Med. Sci.*, **8**: 679-673.

Lovenberg WE, Jequier A and Sjoerdsma (1968). Tryptophan hydroxylation in mammalian systems. *Adv. Pharmacol.*, **6**: 21-26.

Rodgers GCI, Armstrong GA and Robertson RM (2011). Metabolic stress modulates motor patterning via AMP-activated protein kinase. *J. Neurosci.*, **31**: 3207-3216.

Schaechter JD and Wurtman RJ (1990). Serotonin release varies with brain tryptophan levels. *Brain Res*., **532**: 203-210.

Sugrue MF (1987). Neuropharmacology of drugs affecting food intake. *Pharmacol. Ther.*, **32**: 145-182.

Tahira Parveen, Naila Amin, Darakhshan Saleem, Faiza Razi, Saida Haider and Darakhshan Jabeen Haleem (2012). Antistress effect of *Mentha piperita* in rats and the role of brain serotonin and Dopamine. *Asian J. Pharm. Biol. Res.*, **2**(1): 73-78.

Young VR and S N (1996). Behavioral effects of dietary neurotransmitter precursors: Basic and clinical aspects. *Neurosci. and Biobehav. Rev.*, **20**: 313-323.