

REPORT

Protective effects of rice bran oil in haloperidol-induced tardive dyskinesia and serotonergic responses in rats

Noreen Samad¹, Muhammad Abdul Haleem² and Darakhshan Jabeen Haleem³

¹Department of Biochemistry, Bahauddin Zakariya University, Multan, Pakistan

²Department of Biomedical Engineering, Sir Syed University of Engineering and Technology, Karachi, Pakistan

³Neuroscience Research Laboratory, Dr. Panjwani Center for Molecular Medicine & Drug Research, UOK, Karachi, Pakistan

Abstract: Effect of administration of Rice bran oil (RBO) was evaluated on haloperidol elicited tardive dyskinesia in rats. Albino Wistar rats treated with haloperidol in drinking water at a dose of 0.2mg/kg/day and RBO by oral tubes at a dose of 0.4 mL/day for 5 weeks. Motor coordination, VCMs and 8-hydroxy-2-(di-n-propylamino) tetraline[8-OH-DPAT]_syndrome were monitored. Striatal serotonin (5-hydroxytryptamine; 5-HT) and 5-hydroxyindolacetic acid (5-HIAA) levels were determined by high performance liquid chromatography (HPLC-EC). Rats treated with haloperidol orally at a dose of for a period of 5 weeks developed VCMs, which increased progressively as the treatment continued for 5 weeks. Motor coordination impairment started after the 1st week and was maximally impaired after 3 weeks and gradually returned to the 1st week value. Co-administration of RBO prevented haloperidol-induced VCMs as well impairment of motor coordination. The intensity of 8-OH-DPAT-induced syndrome and decreased 5-HT metabolism were greater in water + haloperidol treated animals than RBO + haloperidol treated animals. The present study suggested that involvement of free radical in the development of TD and point to RBO as a possible therapeutic option to treat this hyperkinetic motor disorder.

Keywords: TD; RBO; 8-OH-DPAT; somatodendritic 5-HT-1A receptors.

INTRODUCTION

The brain is deficient in oxidative defense mechanism and hence at a great risk of damage mediated by reactive oxygen species, result molecular and cellular dysfunctioning (Sandberg *et al.*, 1988). Oxidative stress is an imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage (Sies, 1985).

The neurodegenerative hypothesis suggests that persistent tardive dyskinesia (TD) may be associated with, or induced by, neuronal damage somewhere in the basal ganglia, neuroleptic damage the γ -amino butyric acid (GABA) neurons in the basal ganglia. These damaged neurons in turn release the inhibitory effects that GABA neurons have on the dopaminergic output neurons in the nigrostriatal tract, possibly leading to a state of dopamine excess in the basal ganglia (Cadet and Lohr, 1989) suggested neuroleptic-induced increase of dopamine turnover could lead to excessive production of potentially damaging free radicals. Evidences have shown that immense oxidative stress is the main causes of neurodegenerative diseases (Jenner, 1996).

Haloperidol is an antipsychotic drug, which is used in the treatment of schizophrenia and other affective disorders (Rasheed *et al.*, 2010). It blocks dopaminergic action in the nigrostriatal pathway, which leads to extrapyramidal symptoms, such as Parkinsonism and tardive dyskinesia (Haleem *et al.*, 2007a, b). The use of haloperidol has been associated with an increased level of oxidative stress in the brain (Tewari *et al.*, 2000).

Rice bran oil (RBO) is an important derivative of rice, having very important substance such as α -oryzanol, β -sitosterol, unestrified fatty acid, tocopherol, tocotrienol (Tyagi *et al.*, 2012) with biologically active antioxidant properties (Rana *et al.*, 2004; Goufo *et al.*, 2014; Jun *et al.*, 2015). Animal studies have shown that RBO-based diet has an antioxidogenic potential and protect against free radical formation or oxidative stress (Samad, 2015).

The present study was designed to investigate the effects of RBO on haloperidol-induced orofacial dyskinesia, impaired motor coordination and super sensitivity at somatodendritic 5-HT-1A in a rat model of TD.

Materials and methods

Animals

Locally bred male albino Wistar rats (180-220g, 5-6 months old) purchased from HEJ Research Institute, University of Karachi, Pakistan. All animal experiments

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

were carried out according to an approved by institutional ethics committee.

Forty-eight animals were randomly divided to four equal groups (twelve animals in each group). (i) water + water (ii) water +haloperidol (iii) RBO + water (iv) RBO+ haloperidol. Haloperidol (Serenace, Searl; USA) purchased as oral drops of 2.0mg/mL was given orally by adding in drinking water at a dose of 0.2mg/kg/day. RBO extracted by the method of Tahir *et al.* (2007) and given orally by oral tubes at a dose of 0.4mL/day. The animals received the treatment daily at 9:00-9:30 for 5 weeks. Behavioral assessment of tar dive VCMs was carried out weekly at 8:00-8:30 h i.e. 1h before drug administration. Behavioral and neurochemical effects of 8-hydroxy-2-(di-n-propylamino) tetra line (8-OH-DPAT) were monitored after a drug washout period of 2 days so that the presence of drug may not interfere with the effects of drug.

Animals from each of the above four groups divided into saline or 8-OH-DPAT injected subgroups, were injected accordingly with saline (1mL/kg) or 8-OH-DPAT at a dose of 0.5 mg/kg selected on the basis of a previous study (Haleem & Khan, 2003). The drugs administered intraperitoneally (*i.p.*). Hyperlocomotion and forepaw treading elicited by the drug were scored for 25min starting 5 min post injection. Behavioral data were collected by a blind observer. Animals were killed 1 h after the drug or saline injection to collect the striatum as described before (Haleem & Khan, 2003). The samples were stored at a set of temperature of -70°C for the estimation of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA).

Behavioral analysis

Rota-rod activity

Motor coordination was assessed for all rats on a rota-rod, as described by Haleem *et al.* (2007a, b).

VCMs quantification

VCMs quantification done as described by Haleem *et al.* (2007a, b).

8-OH-DPAT_ elicited 5-HT syndrome

The animals were observed for a total scoring period of 25min starting 5min post injection. During the observation period number of cage crossing and forepaw treading were scored (Haleem & Khan 2003).

Neurochemical analysis

Dissection of striatum

The dissection procedure was essentially same as described by Haleem *et al.* (2004).

HPLC-EC determination of 5-HT and 5-HIAA

5-HT and 5-HIAA levels were determined by HPLC-EC as described (Haleem & Parveen 1994).

STATISTICAL ANALYSIS

Data on haloperidol-induced VCM, deficits of motor coordination were analyzed by Three-way ANOVA following Tukey's test. Data on 8-OH-DPAT-induced forepaw treading and hyperlocomotion in water + water, water + haloperidol, RBO + water and RBO + haloperidol treated animals were analyzed by TWO-way ANOVA followed by Tukey's test. Data on effects of 8-OH-DPAT on 5-HT and 5-HIAA levels in the striatum of rats pre-treated with water + water, water + haloperidol, RBO + water and RBO + haloperidol treated animals was analyzed by three-way ANOVA followed by Tukey's test. Statistical analysis was performed by using SPSS version 20.0. Statistical significance was accepted for *P* values of <0.05.

RESULTS

Fig. 1 shows the intensity of haloperidol-induced VCMs in animals treated with water and RBO. Data analyzed by three-way ANOVA showed significant effects of haloperidol ($F=25161.931$ $df= 1,220$ $p<0.05$), RBO ($F=14004.692$ $df=1,220$ $p<0.05$) and weeks ($F=140.187$ $df=4,220$ $p<0.05$). Interaction between haloperidol xweeks ($F=140.187$ $df=4,220$ $p<0.05$), haloperidol xRBO ($F=14004.692$ $df=1,220$ $p<0.05$), RBO xweeks ($F=109.736$ $df=4,220$ $p<0.05$) and haloperidol x weeks xRBO ($F=109.736$ $df=4,220$ $p<0.05$) was significant. *Post_hoc* analysis by Tukey's test showed that administration of haloperidol elicited VCMs for 2 weeks, which increased in a time dependent manner for 3-5 weeks. Administration of RBO prevented the induction of haloperidol-elicited VCMs.

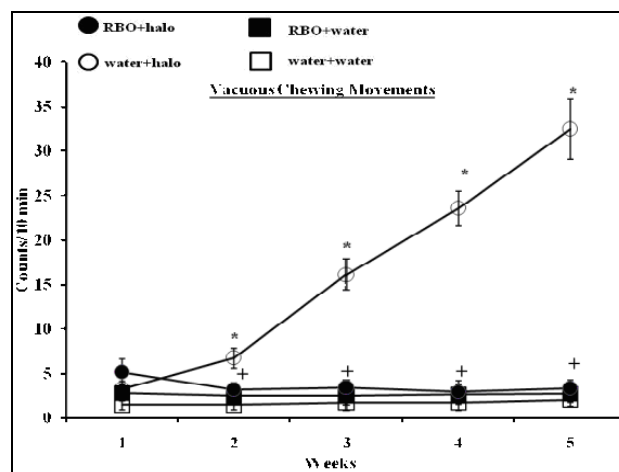


Fig. 1: Time course of the effects RBO on haloperidol-induced VCMs. Values are mean S.D. (n=12) 60 min post-injection. Significant differences by Tukey's test: * $P<0.05$ from water + water treated animals, + $P<0.05$ from water + haloperidol treated rats following three way ANOVA.

Fig. 2 shows the effect of administration of haloperidol on motor coordination in animals treated with water and RBO. Data analyzed by three-way ANOVA showed significant effects of haloperidol ($F=2157.605$ $df=1,220$ $p<0.05$), RBO ($F=1239.557$ $df=1,220$ $p<0.05$) and weeks ($F=242.512$ $df=4,220$ $p<0.05$). Interaction between haloperidol x weeks ($F=221.766$ $df=4,220$ $p<0.05$), haloperidol x RBO ($F=266.219$ $df=1,220$ $p<0.05$), RBO x weeks ($F=285.45$ $df=4,220$ $p<0.05$) and haloperidol x weeks x RBO ($F=1658.938$ $df=4,220$ $p<0.05$) were significant. *Post_hoc* analysis by Tukey's test showed that administration of haloperidol impaired motor coordination after 1st week. The impairment of motor coordination was maximum after 3rd week and gradually returned to 1st week value. Administration of RBO prevented the haloperidol-induced impairment of motor coordination.

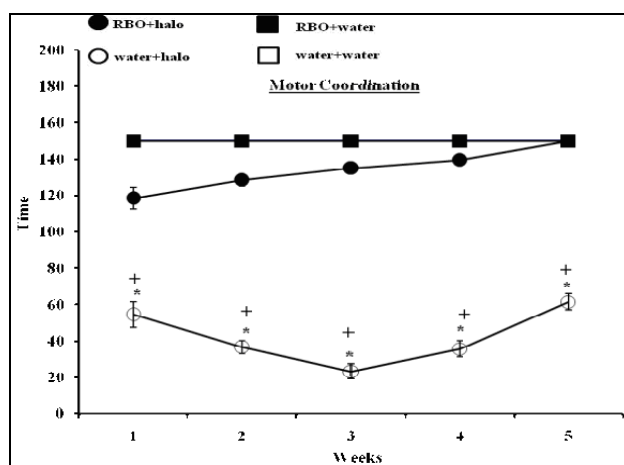


Fig. 2: Effects of RBO on haloperidol-induced deficits of motor coordination. Values are mean S.D. (n=12) 60 min post-injection. Significant differences by Tukey's test: * $P<0.05$ from water + water treated animals, + $P<0.05$ from water + haloperidol treated rats following three way ANOVA.

Fig. 3 shows 8-OH-DPAT induced hyperactivity and forepaw treading in water + water, water + haloperidol, RBO + water and RBO + haloperidol treated animals. Data on cage crossing analyzed by Two way ANOVA showed that significant effects of haloperidol ($F=17.762$ $df=1,20$ $p<0.05$), RBO ($F=18.890$ $df=1,20$ $p<0.05$) and interaction between haloperidol and RBO ($F=416.739$ $df=1,20$ $p<0.05$). Data on forepaw treading analyzed by Two way ANOVA showed that significant effects of haloperidol ($F=208.28$ $df=1,20$ $p<0.05$), RBO ($F=28.864$ $df=1,20$ $p<0.05$) and interaction between haloperidol and RBO ($F=52.835$ $df=1,20$ $p<0.05$). *Post_hoc* analysis by Tukey's test showed that 8-OH-DPAT induced cage crossing and forepaw treading were greater in water + haloperidol than water + water treated animals. Cage crossing and forepaw treading were smaller in RBO + haloperidol than water + haloperidol treated animals.

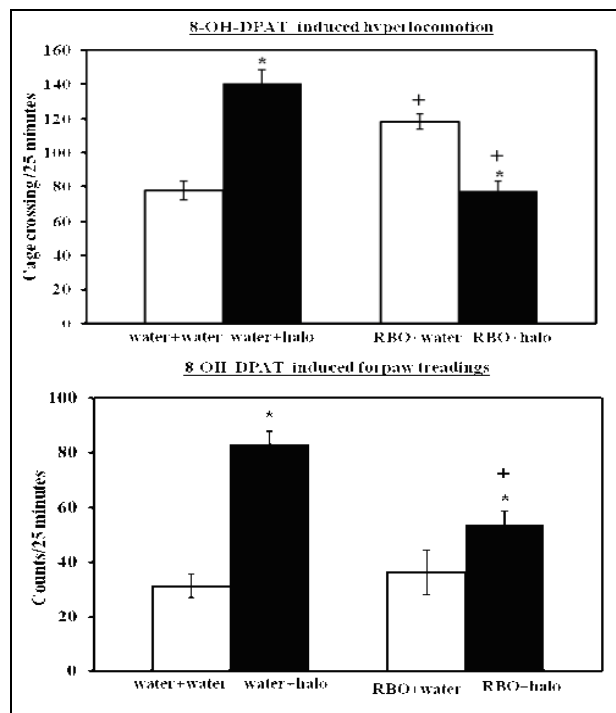


Fig. 3: 8-OH-DPAT induced hyperactivity and forepaw treading in water + water, water + haloperidol, RBO + water and RBO + haloperidol treated animals. Values are means S.D. (n=6) from 5-30min post 8-OH-DPAT injection and 48h after haloperidol or RBO administration. Significant differences by Tukey's test: * $P<0.05$ from water + water treated animals, + $P<0.05$ from water + haloperidol treated rats following two-way ANOVA.

Fig. 4 shows the effects of 8-OH-DPAT on 5-HT and 5-HIAA levels in the striatum of rats pre-treated with water + water, water + haloperidol, RBO + water and RBO + haloperidol treated animals. Data on 5-HT levels analyzed by three way ANOVA showed significant effects of haloperidol ($F=568.653$ $df=1,40$ $p<0.05$), RBO ($F=32.705$ $df=1,40$ $p<0.05$) and 8-OH-DPAT ($F=1489.429$ $df=1,40$ $p<0.05$). Interaction between 8-OH-DPAT x haloperidol ($F=6.521$ $df=1,40$ $p>0.05$) was not significant while interaction between 8-OH-DPAT x RBO ($F=27.199$ $df=1,40$ $p<0.05$) and 8-OH-DPAT x haloperidol x RBO ($F=64.332$ $df=1,40$ $p<0.05$) were significant. Data on 5-HIAA levels analyzed by three way ANOVA showed significant effects of haloperidol ($F=268.365$ $df=1,40$ $p<0.05$), RBO ($F=19.799$ $df=1,40$ $p<0.05$) and 8-OH-DPAT ($F=271.506$ $df=1,40$ $p<0.05$). Interaction between haloperidol x RBO ($F=7.048$ $df=1,40$ $p<0.05$) was significant. Interaction between 8-OH-DPAT x haloperidol ($F=0.159$ $df=1,40$ $p>0.05$), 8-OH-DPAT x RBO ($F=0.029$ $df=1,40$ $p>0.05$) and 8-OH-DPAT x haloperidol x RBO ($F=0.282$ $df=1,40$ $p>0.05$) were not significant. *Post_hoc* analysis by Tukey's test showed that administration of 8-OH-DPAT decreased 5-HT and 5-HIAA concentrations in water +

water as well as water + haloperidol treated animals. The decreases were smaller in water + haloperidol than water + water treated animals. Administration of 8-OH-DPAT decreased both 5-HT and 5-HIAA in RBO + water and RBO + haloperidol treated animals.

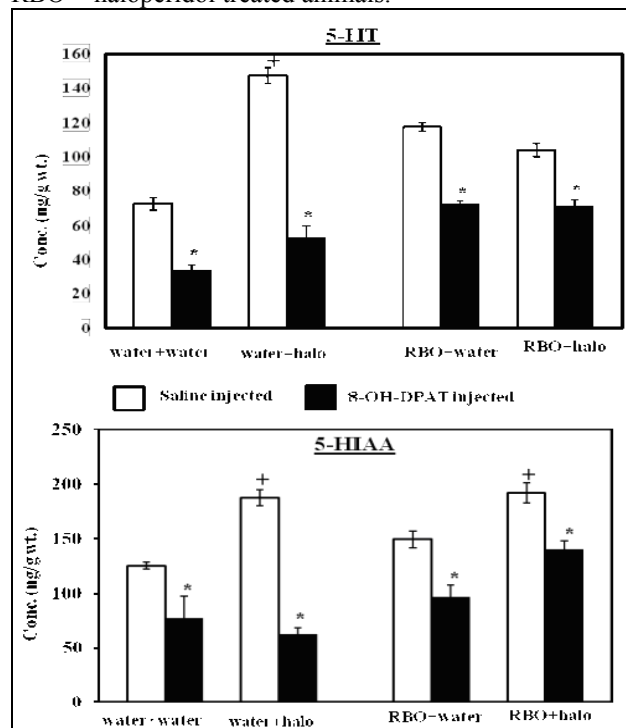


Fig. 4: 8-OH-DPAT induced decreases of 5-HT and 5-HIAA levels in the striatum of water + water, water + haloperidol, RBO + water and RBO + haloperidol treated animals. Values are means S.D. (n=6) 1h post 8-OH-DPAT injection and 48 h after haloperidol or RBO administration. Significant differences by Tukey's test: * $P < 0.05$ from water + water treated animals, + $P < 0.05$ from water + haloperidol treated rats following two-way ANOVA.

DISCUSSION

In the present study haloperidol-treated animals developed VCMs and impaired motor coordination. The co-administration of RBO showed a protective effect against haloperidol-induced orofacial dyskinesia (fig. 1) and deficits in motor coordination (fig. 2). Many studies have suggested that an imbalance in production and detoxification of free radicals may be associated with chronic neuroleptic use and it contributes to the extra pyramidal symptoms (Abdel-Salam *et al.*, 2013).

A role of somatodendritic 5-HT-1A receptors in the onset of VCMs was proposed (Haleem & Khan, 2003) because administration of haloperidol for 2 weeks elicited VCMs (Kulkarni & Naidu, 2001) and increased in the responsiveness of somatodendritic 5-HT-1A receptors in rats (Haleem & Khan, 2003). An important finding of the present study is that co-administration of RBO reverse the

haloperidol-induced VCMs (fig. 1) and impairment of motor coordination (fig. 2) and was associated, with reversal of haloperidol-induced increase in the responsiveness of somatodendritic 5-HT-1A receptor. Moreover study from our laboratory shown that long-term administration of stabilized rice bran (Mehdi *et al.*, 2014) decreased the efficacy of negative feedback control over the synthesis and release of 5-HT. So, the present finding suggests that somatodendritic 5-HT-1A receptors have an important role in the alleviation of haloperidol-induced VCMs and impairment of motor coordination.

RBO has been proven the neuroprotective effect and attenuate stress-induced behavioral and neurochemical deficits (Jabeen *et al.*, 2007). A decrease in 5-HT and 5-HIAA concentration has been reported to occur in the whole brain following the administration of RBO (Mehdi *et al.*, 2014) suggesting that RBO could preferentially stimulate somatodendritic 5-HT-1A receptors. In the present study RBO was also found to decrease 5-HT and 5-HIAA concentration in the striatum of both, RBO + water and RBO + haloperidol treated animals (fig. 4). The mechanism by which RBO could be reversed haloperidol induced super sensitivity of somatodendritic 5-HT-1A receptor (fig. 4) may involve a desensitization of the receptors by repeated administration of RBO (Jabeen *et al.*, 2007).

Administration of 8-OH-DPAT elicits a hyperactivity syndrome often described as serotonin syndrome (Haleem, 1992; Haleem & Khan, 2003). In the present study, administration of haloperidol for 5 weeks elicited a significant decrease in both hyperactivity and forepaw treading (fig. 3). In addition the present study shows that long-term administration of RBO for 5 weeks did not alter 8-OH-DPAT-induced hyperactivity or forepaw treading. The mechanism by which 8-OH-DPAT elicits hyperactivity may involve a stimulation of somatodendritic 5-HT-1A receptors resulting in a decrease in the inhibitory influence of 5-HT on dopamine neurotransmission (Haleem *et al.*, 2004). An increase in the sensitivity of dopamine D2 receptors has been shown to occur following long-term administration of haloperidol (Halperin *et al.*, 1989). The present study shows that an increase in the effectiveness of somatodendritic 5-HT-1A receptors in rats treated chronically with haloperidol (fig. 4.) resulting in an extra decrease in the inhibitory influence of 5-HT on motor activity may also contribute to the greater hyperactivity observed in these rats (fig. 3). Thus reversal of somatodendritic 5-HT-1A receptor dependent response in rats co-treated with RBO (fig. 4) resulted in the reversal of hyperactivity and forepaw treading to 8-OH-DPAT (fig. 3). The present results on the reversal of haloperidol-induced VCMs by RBO are largely explainable in term of the reversal of super sensitivity at somatodendritic receptors.

In conclusion, the present finding shows the beneficial effects of RBO on reversal of haloperidol-induced orofacial dyskinesia, impairment of motor coordination and super sensitivity of 5-HT_{1A} receptors occur due to the possible antioxidant activity. This activity appears particularly relevant for understanding the molecular mechanisms that underlie its multiple actions, particularly its usefulness in neurodegenerative disorders such as neuroleptic-induced TD and associated disorders.

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