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Decreases in plasma glucose, corticosterone and cholesterol levels following long-term consumption of stabilized rice bran in Albino Wistar rats

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Abstract: The present study was designed to investigate the effects of long term stabilized rice bran (SRB) rich diet consumption on neuroendocrine and behavioral responses in rats. Cubes of standard rodent diet were crushed and mixed with SRB in the ratio of 2:1 and 1:1(w/w) to prepare pellets of moderate and high amount of SRB rich diet respectively. Albino wistar rats randomly assigned as control, moderate and high amount of SRB groups were treated accordingly with the respective diet for 6 weeks. Weekly cumulative food intakes but not body weights were smaller in SRB rich diet treated during 1st to 5th week of treatment, which were normalized at the end of the treatment. Animals treated with high SRB rich diet exhibited a decrease in plasma glucose, corticosterone as well as cholesterol levels. Cholesterol levels were also decreased by moderate SRB rich diet. The results are discussed in context of antioxidant and antistress property of rice bran. It is suggested that presence of tocopherol, tocotrienol, γ-orynazol and other unsaponifiable compounds in rice bran may produce a reduction in the cholesterol and glucose levels. A decrease in corticosterone levels may be explained in terms of antistress effects of rice bran.

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INTRODUCTION

Rice bran (RB), the upper brown layer of brown rice is stabilized by heating promptly just after milling. When compared with other cereal grains the stabilized rice bran (SRB) has been found as a good source of complex carbohydrate, lipids, protein, dietary fiber, vitamins and minerals (Faria et al, 2012; Roth-Mater et al, 2002; Truswell, 2002; Nystrom et al, 2005). RB is composed of about 10% of rough rice grain and contains 12 to 23% oil (Friedman, 2013; Saunders, 1985). A range of human and animal studies have shown that rice bran oil (RBO) is an eatable oil of preference for bettering serum cholesterol (Renu Sharma et al, 2015; Sugano & Tsuji, 1997; Most et al., 2005) and blood glucose (Qureshi et al., 2002). These effects can be associated to the occurrence of specific compounds such as γ-orynazol (and its constitutes) β- sitosterol, tocotrienols (Yoon et al, 2014; Christine et al, 2010) and unsaturated fatty acids (Nantiyakul et al, 2012; Sharma & Rukmini, 1987) which make it antioxidant (Nantiyakul et al, 2012; Xu et al., 2001; Iqbal et al, 2005; Minhajuddin et al, 2005).

Studies show that the addition of orynazol to the diet containing RBO led to a further significant decrease in serum cholesterol in rats (Seetharamaiah & Chandrasekhara, 1989). Moreover it was reported that novel tocotrienols of RB overcome serum cholesterol levels in chickens (Qureshi et al., 2001). It is also known that RB itself exerts a cholesterol lowering action in hamsters (Kahola et al., 1992). Later on Most et al (2005) observed that instead of fiber, RBO lowers cholesterol in healthy, slightly hypercholesterol-emic adults (Sugano & Tsuji, 1997). The present study concerns the behavioural and neuroendocrine responses to long-term consumption of SRB rich diet in rats.

Previously it was noted that insertion of RBO in the diet improves the antioxygenic potential and protect against oxidative stress (Rana et al., 2004). Researchers have also found that RB fractions appear to have a beneficial dietary component that improves hypertension, hyperlipidimia and hyperglycemia (Ardiansyah, 2006) and prove useful as functional foods. So, it was hypothesized that SRB rich diet not only decreases the cholesterol levels in rats but also attenuates the circulating levels of corticosterone and glucose.

MATERIALS AND METHODS

Animals: Locally bred Albino Wistar rats weighing 180-200g were housed individually under a 12h light dark cycle (lights on at 6:00 h) with free access to tap water and standard rat food for at least

4 days before starting the experiment. All experimental animals were cared according to the approval of institutional ethics which is consistent with the National Institute of Health Guide for care and use of laboratory animals.

Preparation of Diet: Standard rodent diet available in the form of cubes was crushed finely. The crushed diet was mixed with SRB (microwave-treated RB just after milling) in the ratio of 2:1 and 1:1 (w/w) to prepare pellets of moderate and high amount of SRB rich diet, respectively. Control diet was prepared without mixing RB in it.

Experimental Protocol: Animals randomly assigned as control, moderate and high amount of SRB rich groups were treated accordingly with the respective diet for 6 weeks. Food intake and body weights were measured on week basis. Animals were sacrificed after 6 weeks to collect plasma sample. Samples were stored at -70º C for the estimation of glucose, cholesterol and corticosterone.

Biochemical Estimation:

Determination of Plasma Corticosterone, Glucose and Cholesterol: Just after decapitation, blood was collected in heparin containing tubes. It was centrifuged at 3000 rpm for 15-20 minutes to get plasma for the estimation of plasma corticosterone by the method of Mattingly (1962: Mattingly et al, 1989) as described by Haleem (1992; 1993). Plasma glucose and cholesterol levels were estimated by O-toluidine and Zlatkis methods respectively.

Statistical Analysis: Analysis was done by using SPSS version 13.0. Results are represented as mean±SD. All Data were analyzed by One-way ANOVA. Individual comparisons were made by Newman-Keuls test. Post hoc comparisons were made by Newman-Keuls test. P values $P<0.05$ were considered significant.

RESULTS

1. Effects of SRB Rich Diet on Food Intake and Growth Rate:

Figure 1A and Figure 1B shows the effects of SRB diet for 6 weeks on weekly food intake and body weight changes respectively. One- way ANOVA (df 2,15) performed on food intake data revealed a significant treatment effect on first week ($F=31.24$ $p<0.01$), second week ($F=14.89$ $p<0.01$), third week ($F=5.32$ $p<0.05$), fourth week ($F=4.92$ $p<0.05$) and fifth week ($F=4.02$ $p<0.05$) values. Effects of sixth week treatment ($F=0.65$ $p>0.05$) were not significant. Post hoc comparison showed that weekly cumulative food intakes of first and second week were smaller in SRB treated than normal diet treated rats. Animals treated with moderate SRB diet exhibited smaller food intake from 1 to 5 weeks and normal intake during sixth week.

Weight gain data showed significant treatment effect during 2nd ($F=3.46$ $P<0.05$), 3rd ($F=13.99$ $p<0.01$), 4th ($F=7.98$ $p<0.01$), 5th ($F=4.61$ $p<0.01$) and 6th ($F=23.02$ $p<0.01$) weeks. Effects during 1st week were not significant ($F=1.66$ $p>0.05$). Comparison made by Newman-Keuls test indicated that intake of moderate SRB diet decreased body weights after two weeks (i.e. from 3rd to 6th week), while intake of high SRB diet decreased body weight after one week (i.e. from 2nd to 6th week).

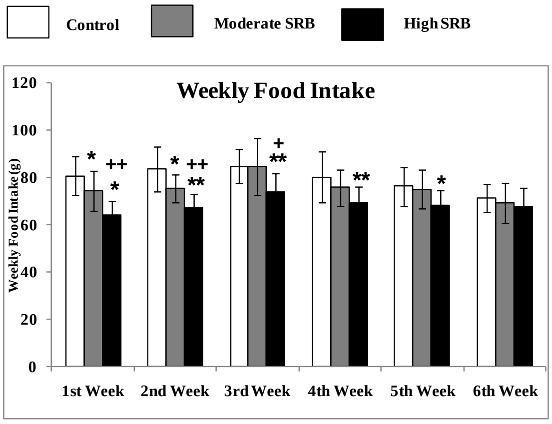

Figure 1A

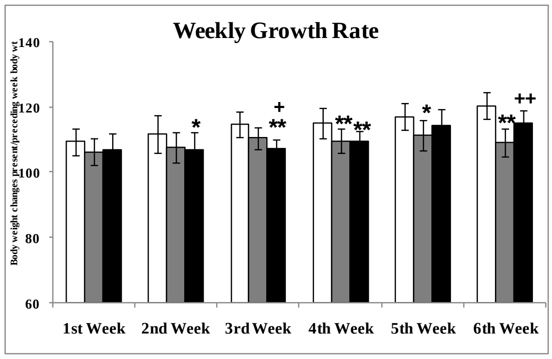

Figure 1B

Figure 1: Effects of 6 week SRB diet on weekly changes of food intake and weight. Values are represented as means ± S.D (n=12). Significant comparisons by post-hoc tests are shown as *$P<0.05$ **$P<0.01$ from respective week normal diet treated rats and +$P<0.01$; ++$P<0.01$ from respective week moderate RB treated rats.

2. Effects of feeding SRB-rich diet on plasma glucose levels:

Data on plasma glucose levels (Figure 2A) analyzed by one-way ANOVA (df 2,15) revealed a significant treatment effect ($F=5.761$ $p<0.05$). Post hoc comparison by Newman Keuls test showed that plasma glucose levels were significantly increased by high SRB rich diet treatment. Moderate SRB rich diet also decreased it but not significantly.

3. Effects of feeding SRB-rich diet on plasma corticosterone levels:

Figure 2B shows the effects of SRB rich diet treatment on plasma corticosterone levels. One-way ANOVA (df 2,15) revealed a significant ($F=9.28$ $p<0.01$) treatment effect. Comparison by post hoc showed that corticosterone was decreased in SRB rich diet than normal diet treated rats. These decreases were greater in high than moderate SRB diet treated rats.

4. Effects of feeding SRB-rich diet on plasma cholesterol levels:

Data analyzed by one-way ANOVA (df 2,15) revealed a significant treatment effect ($F=21.91$ $p<0.01$) on plasma cholesterol levels (Figure 2C). Comparison by Newman Keuls statistics showed that both moderate and high SRB treated rats exhibited decreased cholesterol levels as compared to normal diet treated controls. However these decreases were greater in high than moderate SRB diet treated rats.

Discussion:

The results of the present study confirm our hypothesis and clearly demonstrate that by giving SRB mixed diet for 6 weeks plasma cholesterol and corticosterone levels were attenuated in rats. Circulating levels of glucose were significantly smaller in these rats. In addition, the present study shows that following prolonged consumption of SRB mixed diet, rats exhibited decrease in food intake (Figure 1A) but not body weights (Figure 1B) from first to fifth week. This decrease was normalized at the end of the treatment. This was consistent to our previous report (Jabeen et al, 2007).

RB is composed of the aleurone layer of the rice kermel, endosperm and germ. These are rich sources of proteins, lipids, vitamins and trace minerals (Saunders, 1985). RB is unique because of the reports that the inclusion of oat bran in the diet lowers serum cholesterol (Demark-Wahnefried et al., 1990). In a 10-week controlled feeding trial RB was as effective as oat bran in lowering blood cholesterol levels of hypercholesterimic subjects (Hegsted et al., 1993). This fraction do have tocotrienols, γ-orynazol, β-sitosterol and unsaturated fatty acids (Oluremi et al, 2013), each one may contribute to cholesterol reduction (Sharma & Rukmini, 1987; Yoshino et al., 1989). Unique combinations of these components in RB are largely related to reports of lipid lowering effects and for cardiovascular disease (Most et al, 2005; Wilson et al, 2007; Arab et al, 2011).

Studies suggested that high content of these components of RBO exhibited significant antioxidant capacity in the inhibition of cholesterol oxidation (Sugano & Tsuji, 1997; Rana et al., 2004; Hegsted et al., 1993). Reports also showed that oryzanol and ferulic acid could reduce the risk of high-fat-induced hyperglycemia (Son et al, 2011). Kaup et al (2012) also investigated the antidiabetic effects of rice bran extract due to the presence of gamma-oryzanol. It was noted that soluble SRB and its fiber fraction significantly reduces hyperglycemia in insulin-dependent and non insulin dependent diabetes mellitus. This reduction of glycosylated hemoglobin is very useful for the control of blood glucose in these samples. A number of investigators have reported that these biological effects might be due to the synergic effects of microcomponents (antioxidant) present in these fractions of SRB (Rukmini, 2000; Gerhardt & Gallo, 1998) and might be able to maintain glucose levels.

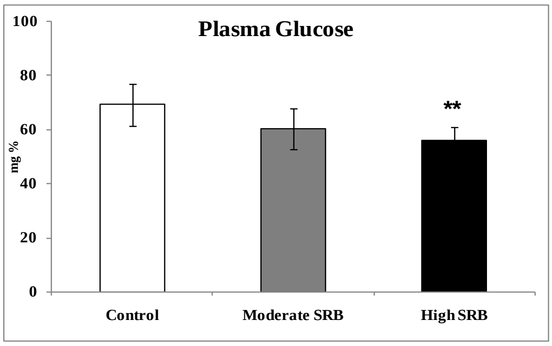

Figure 2A

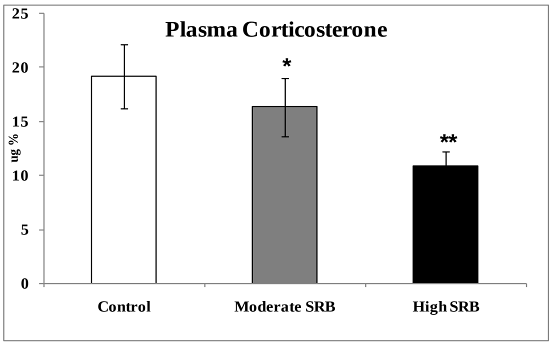

Figure 2B

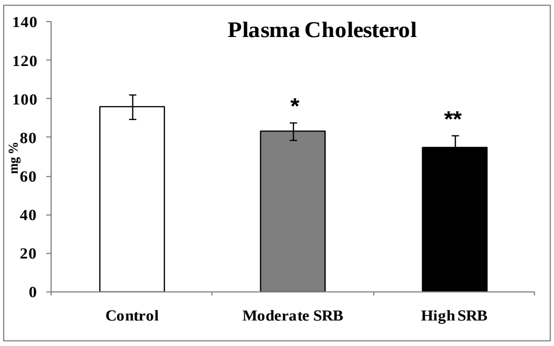

Figure 2C

Figure 2: Effects of 6 week SRB diet intake on plasma glucose **(A)**, corticosterone **(B)** and cholesterol **(C)** levels. Values are represented as means ± S.D. (n=12). Significant comparisons by post-hoc tests are shown as *P<0.01 from respective normal diet treated controls and +P<0.05 from moderate RB treated rats.

Therefore in the present report attenuation of plasma cholesterol and glucose levels by giving SRB mixed diet in rats can also be explained in terms of antioxidant profile of tocopherol, tocotrienol, γ-orynazol and other unsaponifiable components of RB containing oil (Moongngarm et al, 2012).

Exposure to stress-inducing situation increases the activity of hypothalamic pituitary adrenal axis (HPA) to increase circulating levels of catecholamine and glucocorticoid (DeBoer et al., 1991). An increase in the activity of HPA axis increases plasma levels of corticosterone (Cassano & D' mello, 2001). A role of life event stresses in the precipitation of depression has been described in clinical studies (Willner, 1997; Haleem & Perveen, 1994; Haleem et al., 2007; Farhan et al, 2014). Animal studies suggested that stress-induced repeated increases of plasma corticosterone may have a casual role in the precipitation of behavioural depression (Dunn & Berridge, 1990; Jacobs et al., 2000). Because the administration of antidepressant drugs attenuated restraint-induced behavioral deficits (Samad and Haleem, 2010; Perveen et al, 2013). As stated earlier that addition of RBO in the diet improves the antioxygenic potential and protect against oxidative stress (Rana et al., 2004). In view of the role of SRB rich diet as an antidepressant (Carlezon et al., 2005) and antioxidant (Minamiyama et al., 1994; Rana et al., 2004; Arab et al, 2011), the present report on the attenuation of corticosterone by SRB are explainable in terms of its antistress effect.

Previously it has also been reported that inclusion of SRB mixed diet attenuated the restraint-induced behavioural as well as serotonin responses in rats (Jabeen et al, 2007). Moreover, stress-induced increases of brain serotonin were also found to be attenuated by RBO treatment due to the presence of antioxidant compound in it (Mehdi et al, 2015). In a recent work from our laboratory long-term intake of RBO has been suggested to minimize the stress effects on behaviour by decreasing 5-HT1A auto receptor mediated feedback control over 5-HT synthesis and release (Mehdi and Haleem, 2018).

Since a long period RB and its oil have been used for treating high blood pressure, diabetes, high cholesterol, alcoholism, obesity and as an antioxidant (Ardiansyah et al, 2006; Kaup et al, 2012; Most el, 2005; Arab et al, 2011. Researchers have also been focused in using rice bran as an ingredient to find its beneficial effects. SRB has found to be a highly palatable ingredient, when added in an extruded dry dog diets without eliciting an effect on inflammatory immune mediations in healthy dogs (Spears et al, 2004). In another study feeding with stabilized full fat RB, defalted RB and RBO have been shown the pronounced effects to decrease serum lipid cholesterol and blood glucose levels in experimental animals (El-Hady and Saher, 2013).

In view of previous findings and our current study, inclusion of SRB in diet can be recommended not only for diseased but also for normal subjects to maintain health issues.

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