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# *IN VIVO* ANTIOXIDANT POTENTIAL OF RICE BRAN OIL (RBO) IN ALBINO RATS

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**Abstract :** Rice Bran Oil (RBO) has got many health benefits. RBO has been analyzed for physico-chemical characteristics and compared with those of groundnut oil (GNO). The two oils were similar in various physico-chemical characteristics. The major difference in the two oils lay in the amount of unsaponifiable matter, which was higher in the case of RBO. To find the *in vivo* antioxygenic potential of RBO, particularly its ability to protect against oxidative stress, rats were divided into two groups of 10 animals, each and were maintained on diets containing RBO or GNO for a period of 4 weeks. After which stress was induced to half the animals of each group by administering intraperitoneally N-nitrosodiethylamine (NDEA) (100 mg/kg) body weight and remaining half served as respective controls. Animals were sacrified 1 week after stress induction. Intraperitoneal administration of NDEA resulted in a significant reduction in body weight and feed intake, the effect being appreciably less in RBO fed group. NDEA toxicity was mainly reflected in liver as supported by increased activities of enzymes of liver function test (AST, ALT, ALP) on stress induction but the effect was appreciably of lesser degree in the group fed on RBO. The urea levels were also less in the group fed on RBO, The lipid peroxidation (LPO) increased on stress induction in erythrocytes and in all the tissues, the increase being less in RBO fed group except in kidneys. Stress induction resulted in decreased catalase (CAT) activity, the decrease being less in RBO fed group. The increase in peroxidase (Px) activity on stress induction was more in RBO fed group. Stress induction had no significant effect on superoxide-dismutase (SOD) activity except in liver and heart where it increased on stress induction. Thus, it appears that inclusion of RBO in the diet improves the antioxygenic potential and protect against oxidative stress.

**Key words :** antioxidants rice bran oil oryzanol defense enzymes nitroso compounds

## INTRODUCTION

Aerobic metabolism produces, as its byproducts, various highly reactive molecules collectively termed as “oxidants”. These oxidants include a variety of electron-stealing molecules known as free radicals, as well as highly reactive singlet form of oxygen (1). Some of these reactive molecules (e.g. superoxide anion, hydrogen peroxide and nitric oxide) are physiologically useful and, infact, are necessary for life, but can

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also be harmful if present in excess or in inappropriate amounts. All of these oxidants can react with various components of a living cell such as proteins, DNA or lipids, thus causing damage by changing the chemical structure of these components. Such damage has been linked to a number of pathological conditions. In addition to it, various environmental factors, such as air pollution, tobacco smoke, exposure to chemicals and exposure to ultraviolet light or other forms of ionizing radiation also bring about oxidative stress (2, 3).

The human body has evolved a large array of endogenous antioxidant defenses against oxidative stress, including antioxidant enzymes such as superoxide dismutase, catalase and various peroxidases, as well as ability to use small molecules with antioxidant activity such as glutathione, the hormone melatonin and uric acid. However, these endogenous antioxidants do not completely protect against the sum of oxidative stresses challenging the body, and thus there is net oxidative damage that in the long term contributes to aging and various diseases.

Hence it is important to identify natural antioxidants in the diets consumed by human population. There has been a constant search for dietary regimens with high antioxygenic potential. Important contribution of RBO, which has fatty acid composition similar to that of groundnut oil (GNO) (4) lies in its non fatty acid component which may give many health benefits. RBO, though contains a high content of free fatty acids, is rich in unsaponifiable matter (3-5%) especially natural antioxidants such as oryzanol, tocopherol, tocotrienols. This when consumed may add to the antioxygenic potential and hence may prove useful in protection against oxidative stress caused by a large number of xenobiotics.

Although the prospect of RBO is promising, the exploitation of this oil has not achieved the degree it should have attained due to technological problems. Rice bran contain lipases, which get activated in course of milling of rice and begin to split the oil present in bran at a very fast rate. Thus the oil obtained after solvent extraction contains a very high level of free fatty acids and becomes useless for edible purposes. As a result refined RBO is not coming up a big way in India, in spite of India being the second largest producer of rice in the world with high potential to produce RBO.Unlike other edible oils from oilseeds, the production of RBO requires no additional cultivated area, as it can be obtained from the already cultivated paddy for the production of rice grains. Moreover, RBO is cheaper in comparison to other edible oils.

Thus, in view of the abundance of rice bran in India and potential of its being recommended as dietary fat, it's in vivo antioxygenic potential has been carried in albino rats.

## METHODS

The disease free male albino rats (6–8 weeks old) were obtained from small animal colony, Punjab Agricultural University, Ludhiana. All chemicals were of analytical

grade. N-nitroso -diethyl amine (NDEA) used was procured from Sigma Chemical Company, St. Louis, Missouri, USA. Rice bran oil was received from AP Solvex Dhuri and groundnut oil was procured from market. The RBO and groundnut oils were evaluated for various physico-chemical characteristics viz. saponification value, iodine value, peroxide value, acid value, refractive index, fatty acid composition and unsaponifiable matter was done and compared to that of groundnut oil.

In order to determine the antioxygenic potential of RBO, rats were divided into 2 groups of 10 animals each and fed on diets containing starch (60%), oil (20%), casein (15%), salt mixture (4%) and yeast extract (1%). Group I was fed groundnut oil and served as control while animals of group II were fed on diets containing RBO and served as test. All the rats were fed on these diets for a period of 4 weeks before the administration of NDEA. Half the animals of each group were subjected to oxidative stress by injecting NDEA intraperitonially at the level of 100 mg/kg body weight in 0.5 ml normal saline and remaining half of each group were administered 0.5 ml of normal saline and served as respective controls without stress. All rats were housed individually at 23°C–25°C on 12:12 hour light dark cycle. Diet and water were supplied *ad libitum.* The weights of rats were recorded weekly and record of diet intake was kept. All the rats were sacrificed after 1 week of stress induction under light ether anesthesia and blood was removed by direct cardiac puncture into test tubes rinsed with saturated solution of ethylenediamine tetra acetate (EDTA). The organs such as liver, spleen, heart, lungs and kidneys were removed and washed with normal saline and weighed. A small portion of these tissues was fixed in 10% formalin (4% formaldehyde) for histopathological examinations and remaining part was stored at –20° for analysis.

The blood samples collected were centrifuged at 1000 xg for 10 min at 4°C to obtain plasma for biochemical analysis. The erythrocytes were washed three times with phosphate buffered saline (PBS, pH=7.4). After washing the packed cell volume (PCV) was adjusted to 5% for determining lipid peroxidation (LPO). Erythrocyte lysate was prepared by adding 0.8 ml of distilled water to 0.2 ml of 5% PCV. The lysed preparation was used as such for the estimation of antioxidant enzymes. A 10% tissue homogenate was prepared in 0.1 M potassium phosphate buffer, pH 7.4. The homogenate was centrifuged at 3000 xg for 15 minutes at 4°C and a clear supernatant obtained was used for determining LPO and activities of antioxidant enzymes.

Haemoglobin of erythrocytes was determined by the method of Dacie and Lewis (6) using Drabkins reagent. The osmotic fragility of erythrocytes was determined by hemolysis of erythrocytes in 0.5% saline (6). The blood glutathione was determined by the method of Beutler-et al (7). The *in vitro* lipid peroxidation in erythrocytes was determined by measuring malondialdehyde (MDA) produced using thiobarbituric (TBA) by the method of Stocks and Dormandy (8). The LPO of tissue homogenate was determined by the method of Ohkawa et al (9) as modified by Jamal

and Smith (10). Protein was estimated by the method of Lowry et al (11) using Folin phenol reagent. The activities of various antioxidant enzymes viz. catalase (12), superoxide dismutase (13), peroxidase (14) were determined in lysate and tissue homogenate. The alkaline phosphatase activity was determined by using p-nitrophenylphosphate as substrate (15). Aspartate transaminase (AST) and Alanine transaminase (ALT) activities was determined by the colorimetric method of Reitman and Frankel as described by Bergmeyer (16). The plasma urea levels were estimated by the method of Wootton (17). Statistical analysis was done by students 't' test.

## RESULTS

### Characteristics of oils

Saponification value of RBO (182) was relatively lower as compared to that of groundnut oil (196). The lower saponification value for RBO can be attributed to the presence of higher amount of unsaponifiable matter (4%) compared to GNO (0.1%). The lower iodine value in RBO (92) compared to GNO (98), indicate a slightly lower content of unsaturated fatty acids. This matches well with the observed fatty acid composition. The acid value and refractive index of RBO and GNO were comparable. No peroxides were detected in either of the oils. Two oils were also found to be similar with respect to fatty acid composition (Table I).

TABLE I: Characteristics of rice bran oil (RBO) vs. groundnut (GNO) Oils.

| *Characteristics* | *GNO* | *RBO* |
|---|---|---|
| Sponification value | 196 | 182 |
| Iodine value | 98 | 92 |
| Acid value | 0.45 | 0.50 |
| Peroxide value | – | – |
| Refractive Index | 1.48 | 1.46 |
| Unsapnifiable matter | 0.1% | 4.0% |
| Fatty acid composition: | | |
| 14:0 | – | 0.30 |
| 16:0 | 10.9 | 17.90 |
| 18:0 | 3.1 | 2.3 |
| 18:1 | 42.8 | 43.1 |
| 18:2 | 35.7 | 34.4 |
| 18:3 | 1.1 | 0.8 |
| 20:0 | 1.8 | 0.9 |
| 22:0 | 1.9 | – |
| 24:0 | 2.3 | – |

Not detected.

### Feeding Experiment

Feed intake and weight gains were

TABLE II: Effect of stress induction on food intake and body weight of rats fed on diets containing RBO.

| *Parameters* | *GNO* | | *RBO* | |
|---|---|---|---|---|
| | *Without stress* | *With stress* | *Without stress* | *With stress* |
| Feed intake g/day | 9.15±0.44 | 6.24±0.17[a] (31.80) | 9.38±0.03 | 7.97±0.28[ac] (15.03) |
| Change in body weight(g) | Nil | –(31.25±4.14) | Nil | –(22.66±6.12) |

Values are mean ± s.d. n = 5.
Effect of stress [a]P<0.01, [b]P<0.05 w.r.t. corresponding control without stress.
Effect of RBO [c]P<0.01, [d]P<0.05 w.r.t. corresponding GNO controls.
Values in parenthesis indicate percent change over corresponding control without stress.

almost similar with either of the dietary oil used during first 4 weeks of feeding when no stress was induced. However upon stress induction by i/p administration of NDEA resulted in a significant decrease in feed intake and body weight gain with both oils (Table II), but the effect was appreciably less in group fed on diets containing RBO. Organ weights were similar with either of the dietary oil used in the groups without stress. However, stress induction resulted in decrease in liver weight in GNO fed group. Little or no change in liver weight in RBO fed group was observed.

Activity of ALT was normal and almost same with either of the dietary oil used in groups without stress (Table III). NDEA administration resulted in substantial increase in ALT activity in GNO fed group but it remained almost unchanged in RBO fed group. Likewise AST activity increased significantly upon stress induction, the increase being appreciably less in RBO fed group. Plasma alkaline phosphatase activity was also lower in RBO fed group without stress, however, stress induction resulted in increase in alkaline phosphatase activities in both the groups almost to similar extent. Urea levels were almost similar in both the groups without stress. Moreover, stress induction had no effect on urea levels in either of the groups.

Haemoglobin content was similar under both the dietary conditions in respective controls and stress-induced groups (Table IV) Glutathione content was also similar with either of dietary oils used and stress induction did not cause any significant change in glutathione content. The *in vitro* osmotic fragility was, however, lower in RBO fed group compared to GNO fed group. The *in vitro* lipid peroxidation of erythrocytes was significantly lower in RBO fed group. Stress induction resulted in increased *in vitro* lipid peroxidation, the increase being more or less similar in both the groups.

TABLE III: Effect of stress induction on Plasma ASI, ALT, Alkaline phosphatase ($UL^{-1}$) and urea levels ($mgdl^{-1}$) in rats fed on diets containing RBO or GNO.

| *Parameters* | *GNO* | | *RBO* | |
|---|---|---|---|---|
| | *Without stress* | *With stress* | *Without stress* | *With stress* |
| ALT | 26.00±2.82 | 60.00±10.00[a] (130.77) | 25.20±2.40 | 27.66±5.18[c] |
| AST | 23.75±5.67 | 47.45±6.88[a] (99.79) | 16.20±2.04[d] | 3153±5.47[ac] (94.63) |
| Alkaline phosphatase | 583.30±33.00 | 787.50±58.00[a] (35.00) | 490.00±57.12 | 668.60±66.00[a] |
| Urea | 44.53±7.77 | 47.66±5.12 | 35.93±1.56 | 36.46±5.31[d] |

Values are mean ± s.d. n = 5.
Effect of stress [a]P<0.01, [b]P<0.05 w.r.t. corresponding control without stress.
Effect of RBO [c]P<0.01, [d]P<0.05 w.r.t. corresponding GNO controls.
Values in parenthesis indicate percent change over corresponding control without stress.

TABLE IV: Effect of stress induction on Hb, (GSH), Osmotic fragility in vitro lipid peroxidation (LPO) and activities* of antioxidant enzymes of erythrocytes in rats fed on diets containing RBO or GNO.

| *Parameters* | *GNO* | | *RBO* | |
|---|---|---|---|---|
| | *Without stress* | *With stress* | *Without stress* | *With stress* |
| Hb g/dL | 9.81±0.93 | 10.48±1.28 | 9.28±1.27 | 11.18±1.79 |
| Glutathione | 333.33±9.42 | 315±57.22 | 336.00±23.32 | 340.00±48.98 |
| Osomotic fragility | 29.30±1.66 | 32.15±2.41 | 25.16±2.81[d] | 29.16±0.68[d] |
| LPO (nanomoles of MDA formed/g Hb/h) | 547.39±11.82 | 565.67±36.79 | 411.55±55.41[c] | 470.68±26.993[c] |
| Catalase (Units $Mg^{-1}$ protein) | 78.01±8.53 | 48.00±7.34[a] (38.47) | 83.60±4.70 (18.09) | 68.48±1.36[a] |
| Peroxidase** Units $mg^{-1}$ protein | 2.10±0.32 | 2.60±0.36 | 3.60±0.90[d] | 4.10±0.73[d] |
| SOD*** Unit $mg^{-1}$ protein | 2.70±0.32 | 2.30±0.34 | 3.10±0.39 | 2.70±0.48 |

*Activities expressed as standard international units unless defined.
**1 unit = Increase in absorbance/min.
***1 unit = Amount of enzyme that inhibits 50% of auto oxidation of pyrogallol.
Values are mean ± s.d. n = 5.
Effect of stress [a]P<0.01, [b]P<0.05 w.r.t. corresponding control without stress.
Effect of RBO [c]P<0.01, [d]P<0.05 w.r.t. corresponding GNO controls.
Values in parenthesis indicate percent change over corresponding control without stress.

Almost similar catalase (CAT), peroxidase (Px) and superoxide dismutase (SOD) activities were observed in RBO and GNO fed groups without stress in erythrocytes. Stress induction, however, caused a decrease in CAT activity, the decrease being less in RBO fed group. The Px activity was higher in RBO fed group compared to GNO fed group. NDEA induced stress resulted in almost similar increase in Px activity. The SOD activity was almost similar in both the groups without stress and stress induction did not cause much change in its activity.

Lipid peroxidation in all the tissues (liver, heart, lungs and spleen) was appreciably less in the group fed on RBO as compared to GNO fed group (Table V). Stress induction resulted in further increase in lipid peroxidation in all the tissues through varying degrees, the increase being relatively less in RBO fed group. The CAT activity was also similar with either of dietary oil used and stress induction resulted in significant decrease in CAT activity in all the tissues, again the decrease was appreciably less in RBO fed group. The Px activity was also similar with either of dietary oil used in all the tissues except liver where Px activity was significantly higher in the group fed on RBO. The SOD activity was slightly higher in group fed on RBO than the group fed on GNO and stress induction resulted in increased SOD activity in liver and heart, with no changes in lung, spleen and kidney.

TABLE V: Effect of stress induction on lipid peroxidation (LPO) and activities* of antioxidant enzymes of liver, hearts, lungs, spleen and kidneys of rats fed on diets containing RBO or GNO.

| *Parameters* | *GNO* | | *RBO* | |
|---|---|---|---|---|
| | *Without stress* | *With stress* | *Without stress* | *With stress* |
| **Liver** | | | | |
| LPO (in moles of MDA formed/g tissue) | 115.33±8.22 | 128.00±7.34 | 88.66±8.37$^{c}$ | 102.00±2.83$^{c}$ |
| **Antioxidant enzymes** | | | | |
| Catalase (units mg$^{-1}$ protein) | 63.40±1.89 | 25.94±3.50$^{a}$ (59.08) | 68.16±4.00 | 42.84±8.14$^{a,c}$ (37.15) |
| Peroxidase** (units mg$^{-1}$ protein) | 0.187±0.035 | 0.175±0.0241 | 0.234±0.036 | 0.274±0.058$^{d}$ |
| SOD*** (units mg$^{-1}$ protein) | 1.55±0.37 | 2.05±0.46 | 2.52±0.36$^{c}$ | 3.91±0.92$^{b,c}$ (55.16) |
| **Heart** | | | | |
| LPO (in moles of MDA formed/g tissue) | 126.66±5.47 | 173.55±20.50$^{a}$ (37.02) | 105.56±13.02$^{d}$ | 142.27±8.32$^{a,d}$ (34.78) |
| **Antioxidant enzymes** | | | | |
| Catalase (units mg$^{-1}$ protein) | 73.70±9.65 | 51.16±6.26$^{a}$ (30.58) | 76.70±17.56 | 51.73±8.57$^{b}$ (32.56) |
| Peroxidase** (units mg$^{-1}$ protein) | 0.253±0.05 | 0.306±0.04 | 0.252±0.05 | 0.307±0.02 |
| SOD*** (units mg$^{-1}$ protein) | 2.01±0.15 | 2.51±0.60 | 2.52±0.56 | 3.58±0.14$^{a,c}$ (42.06) |
| **Lungs** | | | | |
| LPO (in moles of MDA formed/g tissue) | 111.07±11.31 | 123.25±13.76 | 93.33±7.80$^{d}$ | 99.73±10.62$^{d}$ |
| **Antioxidant enzymes** | | | | |
| Catalase (units mg$^{-1}$ protein) | 39.85±2.42 | 18.49±3.88$^{a}$ (53.60) | 47.71±8.42 | 24.54±1.56$^{a}$ (48.56) |
| Peroxidase** (units mg$^{-1}$ protein) | 0.61±0.01 | 0.429±0.09$^{a}$ (29.67) | 0.625±0.12 | 0.583±0.12 |
| SOD*** (units mg$^{-1}$ protein) | 3.11±0.72 | 2.44±0.41 | 3.17±0.27 | 2.45±0.55 |
| **Spleen** | | | | |
| LPO (in moles of MDA formed/g tissue) | 133.30±5.47 | 145.0±11.93 | 108.93±14.55$^{d}$ | 119.93±14.44$^{d}$ |
| **Antioxidant enzymes** | | | | |
| Catalase (units mg$^{-1}$ protein) | 13.16±0.57 | 13.87±3.00 | 15.08±3.24 | 32.62±4.94$^{a}$ (116.31) |
| Peroxidase** (units mg$^{-1}$ protein) | 0.277±0.03 | 0.446±0.07$^{a}$ (61.01) | 0.280±0.05 | 0.462±0.05$^{a}$ (65.00) |
| SOD*** (units mg$^{-1}$ protein) | 1.056±0.16 | 0.939±0.20 | 1.13±0.22 | 1.007±0.13 |
| **Kidneys** | | | | |
| LPO (in moles of MDA formed/g tissue) | 88.67±2.37 | 108.00±7.35$^{a+}$ (21.80) | 113.00±11.9$^{d}$ | 143.33±24.57$^{d}$ |
| **Antioxidant enzymes** | | | | |
| Catalase (units mg$^{-1}$ protein) | 80.17±16.05 | 49.61±6.69$^{a}$ (38.12) | 84.82±12.40 | 64.63±3.69$^{b}$ (23.80) |
| Peroxidase** (units mg$^{-1}$ protein) | 0.210±0.03 | 0.235±0.03 | 0.205±0.02 | 0.297±0.02$^{a,c}$ (44.88) |
| SOD*** (units mg$^{-1}$ protein) | 1.48±0.36 | 1.64±0.30 | 1.12±0.26 | 1.39±0.39 |

*Activities expressed as standard international units unless defined.
**1 unit = Increase in absorbance/min.
***1 unit = Amount of enzyme that inhibits 50% of auto oxidation of pyrogallol.
Values are mean ± s.d. n = 5.
Effect of stress $^{a}P<0.01$, $^{b}P<0.05$ w.r.t. corresponding control without stress.
Effect of RBO $^{c}P<0.01$, $^{d}P<0.05$ w.r.t. corresponding GNO controls.
Values in parenthesis indicate percent change over corresponding control without stress.

## DISCUSSION

Oil characteristics and fatty acid composition of RBO were found to be almost similar to those of GNO except for the unsaponifiable matter which was relatively higher in RBO and agrees well with that reported values in literature (18).

Inclusion of RBO in the diet did not affect feed intake or gain in body weight compared to those with GNO. However, stress induction by intraperitoneal administration of N-nitrosodiethylamine (NDEA), a N-nitroso compound present in environment and food chain, resulted in reduction of feed intake and body weight gain. This reduction was appreciably less in rats receiving RBO as compared to those receiving GNO. These results indicate the usefulness of RBO in protecting against NDEA induced stress. The reduction in feed intake can be attributed to decreased thyroid function as ingestion of nitroso compounds is known to impair thyroid function (19). Organ weights were not affected by stress induction in either of the groups except decrease in liver weight in GNO fed group, with little or no change in RBO fed group. These results further indicate protective role of RBO on stress induction.

Activities of amino tranferases increased substantially on stress induction in both the groups but the effect was appreciably of lower degree in RBO fed group. As amino transferases has been implicated as markers of liver damage (20), the increase in activities of plasma transaminases suggest hepato cellular damage. Hepatotoxic responses such as increased level of AST, ALT, Ketone bodies with nitroso compounds have also been reported earlier (21, 22). Brunning-Faun and Kanenna (19) also reported that N-nitrosodimethylamine (NDMA) causes hepatitis in cattle and sheep. Lower degree of effect observed in RBO fed group upon stress induction by NDEA indicate that RBO protects against stress induction.

NDEA administration resulted in increased *in vitro* lipid peroxidation (LPO) of erythrocytes and the effect was appreciably lower in RBO fed group. Moreover, osmotic fragility was lower in RBO fed group and can be attributed to the lower degree of increase in LPO. These results indicate a relatively higher degree of antioxygenic potential of erythrocytes of RBO fed rats. This, in fact, is supported by decreased *in vitro* lipid peroxidation with oryzanol, a unique component of RBO (23). Stress induction resulted in decreased catalase activity and decrease being less in RBO fed group. On the contrary, peroxidase activity increased to similar extent in both the groups. Increased peroxidase activity could be a result of adaptive response.

Lipid peroxidation of all the tissues increased appreciably on stress induction through varying degree. These results agree with the earlier reports of oxidative stress and cellular injury caused by N-nitroso compounds through the involvement of free radicals (24). *In vitro* studies in human erythrocytes and rat liver mitochondria have shown that NDEA exposure increased lipid peroxidation and decreased activities of antioxidant enzymes (25, 26). Appreciably less increase of LPO in RBO fed rats, indicates that RBO helps in improving antioxidant potential of tissues. Moreover,

stress induction affected activities of defense enzymes through varying degree in different tissues but the effect being relatively similar in both the groups. The increased activities of peroxidase observed on stress induction can be a result of adaptive response.

Thus, it appears that inclusion of RBO in diet improves antioxygenic potential and protect against oxidative stress to a reasonable extent.

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