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IN VITRO AND *IN VIVO* ANTIOXIDATIVE AND ANTIHYPERGLYCEMIC POTENTIALS OF BRAN AND BRAN OIL OF FARO 60 (JAMILA RICE)

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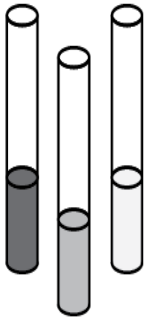
Graphical Abstract


PADDY RICE


RICE BRAN


POLISHED RICE


RICE BRAN OIL



In vitro Antidiabetic/Antioxidant

Antioxidant effects
Antidiabetic effects

Parameters	Rice Bran Oil	Rice Bran Extract	Ascorbic Acid
Hydrogen peroxide (H_2O_2)	95.26±0.07^a	82.77±0.42^b	70.17±0.06^c
DPPH Scavenging	90.62±1.61^a	87.01±0.44^b	81.35±0.02^c
Hydroxyl Radical (OH^-)	33.10±8.10^a	30.81±0.41^a	20.02±1.13^b

Antihyperglycemic/Antioxidant

Antihyperglycemic effects
Antioxidant effects

Groups	PLASMA	LIVER	KIDNEY
Normal Control	3.32±4.07^a	6.13±1.05^a	6.94±4.69^a
200mg/kg body weight Vitamin C	3.11±1.82^b	5.07±2.21^b	6.28±5.18^b
200mg/kg body weight Rice Bran Oil	3.22±3.70^a	4.87±2.43^a	4.88±3.61^a
400mg/kg body weight Rice Bran Oil	3.12±1.06^a	4.84±2.80^a	7.09±4.50^a

Abstract: Oxidative stress is a concept used to describe the condition of oxidative challenges resulting from the critical imbalance between free radical generation and antioxidant defences. The study was designed to evaluate the *in vitro* and *in vivo* antioxidative and anti-hyperglycemic potentials of bran and bran oil of Faro 60 (Jamila Rice). The rice bran and oil demonstrated a significant free radical scavenging activity as it scavenges hydrogen peroxide radical with values 82.77±0.42 mg/mL and 95.26±0.07 mg/mL respectively when compared with the standard vitamin C, which scavenges with 70.17±0.06 at 20 mg/mL. Furthermore, rice bran oil demonstrated anti-diabetic effects *in vitro,* inhibiting alpha-amylase activity at 35.65±10.10% when compared with acarbose at 20.83±2.71% at a concentration of 20 mg/mL. More so, rice bran oil was able to lower the effect of lipid peroxidation in the plasma, liver and kidney of diclofenac-induced oxidative stressed mice at 3.22±3.70, 4.87±2.43 and 4.88±3.61 µmole/mg protein when compared with the normal control at 3.32±4.07, 6.13±1.05 and 6.94±4.69 µmole/mg protein respectively. The rice bran oil significantly ($p<0.05$) lowered blood sugar levels during the oral glucose tolerance test. The rice bran and rice bran oil demonstrated significant free radical scavenging and anti-hyperglycemic activities both *in vitro* and *in vivo. It could* be utilized as a good source of natural antioxidants.

Keywords: Oxidative stress; bran oil of Faro 60; Jamila Rice; Lipid peroxidation; Acarbose

Introduction

Rice bran is a by-product produced during milling of rice; it is obtained from the outer layer of brown rice. In addition to phytonutrients, it contains nutritional dietary fiber, high valued protein and fat. It was reported that rice bran inherently contains high level of medicinally important antioxidant gamma oryzanol, a nutritional mixture of ferulic ester aside from a significant oil concentration it contains (Anwar *et al.*, 2005). Rice bran is chemically composed of protein, lipid, carbohydrate, crude fiber and vitamin B (Saunder, 1990). It was reported to be an excellent source of minerals and vitamins (Zullaikah *et al.*, 2005). The aim of the study is to evaluate the *in vitro* and *in vivo* antioxidative and anti-hyperglycemic potentials of rice bran oil of Faro 60 (Jamila rice) in diclofenac induced oxidative stressed mice.

Material and methods

Chemicals and reagents

α – amylase, α – glucosidase, metformin, *p*-Nitrophenyl-α-glucopyranoside (PNPG) and 3, 5-dinitrosalicylic acid (DNSA) were purchased from Sigma-Aldrich, Germany. All other reagents used were of analytical grade.

Rice Bran

Rice brans (Faro 60, Jamila) was collected from Dr. Waziri Milling Factory (Gouria Rice Factory) in Zigau, located at Latitude 58^{o}N and Longitude 9^{o}W, Shira Local Government, Bauchi State, Nigeria. The rice bran sample was stabilized with Microwave oven (MW489) for 30 seconds. The sample was then stored in a freezer at 0^{o}C until the time of analysis. The rice bran oil was extracted using soxlet extraction.

Experimental animals

Albino mice weighing between 20±2 g where purchase from the animal unit, University of Jos, Plateau state and acclimatized for 14 days, given standard mouse chow and water *ad libitum*. The animal care procedure was approved by the ethical review committee, University of Jos, with registration number UJ/FPS/F17-00379.

Material and methods Cont.

Assays

Antihyperglycemic assay was determined according to the method of Du Vigneaud and Karr (1925), while α-amylase and α-glucosidase inhibition assays was determine according to Andrade-Cetto *et al.* (2008) and Kuppusamy *et al.* (2011).

In vitro antioxidant analyses was performed according to the methods of McCune and Johns, (2002) and Ruch *et al.* (1989) for 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity, and hydrogen peroxide (H_2O_2) scavenging activity assays respectively. The total protein was measured according to the method of Bradford (1976). Nitric oxide (NO), and malondialdehyde (MDA) concentrations were determined according to the methods of Green *et al.* (1982), and Varshney and Kale (1990) respectively. The methods described by Misra and Fridovich (1972), and Sinha (1972) were used to determine superoxide dismutase (SOD) and catalase (CAT) respectively.

Analysis of data

Data was expressed as Mean ± Standard Error of Mean (SEM) and was subjected to Analysis of Variance (ANOVA) followed by Dunnett's test using SPSS version 20, PSS Inc., Chicago. IL, USA. Significance was considered at $p<0.05$.

Results and discussion

Table 1: Radical scavenging activities of rice bran oil of Faro 60 (Jamila Rice)

Parameters	Rice Bran Oil	Rice Bran Extract	Ascorbic Acid
Hydrogen peroxide (H_2O_2)	95.26 ± 0.07^{a}	82.77 ± 0.42^{b}	70.17 ± 0.06^{c}
DPPH Scavenging	90.62 ± 1.61^{a}	87.01 ± 0.44^{b}	81.35 ± 0.02^{c}
Hydroxyl Radical (OH^{-})	33.10 ± 8.10^{a}	30.81 ± 0.41^{a}	20.02 ± 1.13^{b}

Values are expressed as the mean ± SEM for triplicate determination. Values with different superscript were significantly different at $p<0.05$.

Results and discussion Cont.

Table 2: *In vitro* antidiabetic activities of rice bran oil of Faro 60 (Jamila Rice)

Parameters	Rice Bran Oil	ACARBOSE
alpha amylase	35.65 ± 10.10^{a}	20.83 ± 2.71^{b}
alpha glucosidase	82.53 ± 3.34^{a}	38.83 ± 3.85^{b}

Values are expressed as the mean ± SEM for triplicate determination. Values with different superscript were significantly different at $p<0.05$.

Results and discussion Cont.

Table 3: Total protein concentration in diclofenac oxidative stressed induced mice treated with rice bran oil of Faro 60 (Jamila Rice)

Groups	PLASMA	LIVER	KIDNEY
Normal Control	637.58±3.31^{a}	20.42±0.28^{a}	84.5±0.53^{a}
200mg/kg body weight Vitamin C	625.50±5.69^{b}	20.10±0.29^{b}	81.25±0.75^{b}
200mg/kg body weight Rice Bran Oil	656.01±28.30^{b}	19.29±0.83^{a}	71.16±5.11^{a}
400mg/kg body weight Rice Bran Oil	643.08±15.21^{b}	18.36±0.80^{a}	84.17±0.30^{a}

Values are expressed as the mean ± SEM for five (n=5) determinations. Values with different superscript were significantly different at $p<0.05$.

Results and discussion Cont.

Table 4: Lipid peroxidation concentration in diclofenac oxidative stressed induced mice treated with rice bran oil of Faro 60 (Jamila Rice)

Groups	PLASMA	LIVER	KIDNEY
Normal Control	3.32±4.07^{a}	6.13±1.05^{a}	6.94±4.69^{a}
200mg/kg body weight Vitamin C	3.11±1.82^{b}	5.07±2.21^{b}	6.28±5.18^{b}
200mg/kg body weight Rice Bran Oil	3.22±3.70^{a}	4.87±2.43^{a}	4.88±3.61^{a}
400mg/kg body weight Rice Bran Oil	3.12±1.06^{a}	4.84±2.80^{a}	7.09±4.50^{a}

Values are expressed as the mean ± SEM for five (n=5) determinations. Values with different superscript were significantly different at $p<0.05$.

Results and discussion Cont.

Table 5: Nitric oxide concentration in diclofenac oxidative stressed induced mice treated with rice bran oil of Faro 60 (Jamila Rice)

Groups	PLASMA	LIVER	KIDNEY
Normal Control	0.63 ± 0.03^{a}	0.68 ± 0.09^{a}	0.65 ± 0.06^{a}
200mg/kg body weight Vitamin C	0.64 ± 0.05^{b}	0.69 ± 0.01^{b}	0.67 ± 0.01^{b}
200mg/kg body weight Rice Bran Oil	0.61 ± 0.02^{ab}	0.72 ± 0.03^{b}	0.78 ± 0.05^{a}
400mg/kg body weight Rice Bran Oil	0.62 ± 0.01^{a}	0.72 ± 0.03^{a}	0.65 ± 0.05^{a}

Values are expressed as the mean ± SEM for five (n=5) determinations. Values with different superscript were significantly different at $p<0.05$.

Results and discussion Cont.

Table 6: Catalase activities in diclofenac oxidative stressed induced mice treated with rice bran oil of Faro 60 (Jamila Rice)

Groups	PLASMA	LIVER	KIDNEY
Normal control	2.94±1.17^{a}	3.35.6±1.87^{a}	1.97±4.88^{a}
200mg/kg body weight Vitamin C	3.57±5.27^{b}	5.32±1.31^{b}	4.23±1.31^{b}
200mg/kg body weight Rice Bran Oil	2.44±3.26^{a}	1.50±1.10^{a}	4.24±1.00^{a}
400mg/kg body weight Rice Bran Oil	3.19±1.20^{a}	9.24±6.74^{a}	2.23±4.18^{a}

Values are expressed as the mean ± SEM for five (n=5) determinations. Values with different superscript were significantly different at $p<0.05$.

Results and discussion Cont.

Table 7: Superoxide dismutase activities in diclofenac oxidative stressed induced mice treated with rice bran oil of Faro 60 (Jamila Rice)

Groups	PLASMA	LIVER	KIDNEY
Normal Control	51.51±9.04^{a}	236.36±51.52^{a}	18.18±8.85^{a}
200mg/kg body weight Vitamin C	49.74±2.67^{b}	285.85±21.74^{b}	4.54±1.46^{b}
200mg/kg body weight Rice Bran Oil	53.78±8.17^{a}	247.34±36.24^{b}	16.66±8.59^{ab}
400mg/kg body weight Rice Bran Oil	50.00±2.27^{a}	186.36±18.21^{a}	24.49±3.97^{a}

Values are expressed as the mean ± SEM for five (n=5) determinations. Values with different superscript were significantly different at $p<0.05$.

Results and discussion Cont.

Table 8: Oral glucose tolerance test (mmol/L) in glucose induced hyperglycemic mice treated with rice bran oil of Faro 60 (Jamila Rice)

GROUPS	Base line	30 Minutes	1 hour	2 hours	4 hours
Normal control	8.40±0.14	9.60±0.42	8.40±0.03	8.10±0.56	6.65±0.12
Negative control	10.20±0.10	13.85±0.72	10.60±0.31	9.00±0.24	8.70±0.42
Metformin (12.4 mg/kg)	7.35±0.01	13.55±1.50	10.00±0.81	8.35±0.01	8.10±0.28
Rice Bran Oil (100 mg/kg)	7.80±0.14	11.90±0.03	6.60±0.70	7.45±0.33	7.15±0.22
Rice Bran Oil (200 mg/kg)	8.55±0.15	12.20±1.02	7.95±0.47	7.85±0.19	7.30±0.00
Rice Bran Oil (400 mg/kg)	7.40±0.49	7.80±0.17	7.10±0.14	6.50±0.00	6.75±0.26

Values are presented as mean ± SEM of triplicates determinations

Conclusions

The rice bran and rice bran oil demonstrated significant free radical scavenging and anti-hyperglycemic activities both *in vitro* and *in vivo*. *It could* be utilized as a good source of natural antioxidants.

Acknowledgments

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