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Original article

Hypercholesterolemia ameliorating effect of bangladeshi high yield variety rice bran oil

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A R T I C L E I N F O

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A B S T R A C T

Background: Hypercholesterolemia led cardiovascular disease (CVD) complications are plaguing the globe. In the present study, Bangladesh Rice Research Institute (BRRI) high yield variety (HYV) rice named as BR5 had been selected for extracting bran oil followed by fatty acid analysis and in vivo hypocholesterolemic effect of the extracted rice bran oil (RBO) for the sake of ameliorating CVD. Method: RBO had been extracted using n-Hexane followed by fatty acid analysis and anti-hypocholesterolemic study on rats against soybean oil (SBO), mustard oil (MBO) and butter oil (BTO) followed by organ function tests. Study period was four weeks. Then the rats had been killed, their blood had been collected and serum biochemical parameters had been analyzed using semi-auto analyzer. Results: Fatty acid analyses revealed BR5 had been the best among the six HYVs of rice. Compared with the other edible oils, RBO derived from BR5 showed significantly increasing effect upon plasma total cholesterol and low density lipoprotein cholesterol lowering while high density lipoprotein cholesterol increasing. Toxicological studies showed no detrimental effect of BR5 derived RBO on cardiac, renal and hepatic functions. Conclusion: BR5 is a rich source of RBO that provides balanced fatty acid support as well as exerts hypocholesterolemic effects without any detectable side effects. Consumption of BR5 derived RBO would aid in CVD amelioration.

1. Background

Cardiovascular diseases (CVD) stand among the leading causes of death globally (WHO, CVD FACT SHEET, 2020). Current dietary pattern and sedentary lifestyle have been blamed for accruing CVD . Thus, CVD management guidelines recommend lifestyle modification. Among them, amelioration of hypercholesterolemia and attenuation of oxidative stress (OS) seem apt. Multiple strategies have been formulated worldwide in managing hypercholesterolemia and OS. Though therapeutic approaches have shown positive results, they are expensive and render side effects and not easy to reach to the common mass, especially of the developing nation . Besides, interest of the consumers towards natural items seems higher than those of the chemically synthesized drugs. Thus, search for cost-effective, easily available, safe and natural CVD modifying agents has got momentum. In this context, edible oils of different origin have attracted scientific notion .

Rice bran oil (RBO) has been gaining popularity in different cuisine for the last few years. RBO possesses relatively higher antioxidants and balanced fatty acid composition . RBO has been hailed over other edible oils owing to its richer content of bio-components such as antioxidants: γ-oryzanol, tocopherols and tocotrienols . γ-oryzanol has been regarded as the most active bio-component providing multitude of health promoting effects .

Though studies in different countries on non-human primates and on humans have shown promising effect of RBO in amelioration of CVD complications, there is hardly any study performed in Bangladesh . Thus, the current experiment had been designed to evaluate the efficacy of RBO derived from Bangladeshi rice (DHAAN, in Bengali), in ameliorating experimentally induced hypercholesterolemia in animal models.

Rice is the staple food in Bangladesh and accounts about 80% of the cropped area (KNOWLEDGE BANK BRRI, 2020). About three million tons of rice bran (at 10 percent of the weight of clean rice) could be produced from about 34.75 million tons of clean rice in Bangladesh annually (KNOWLEDGE BANK BRRI, 2020). Total estimated bran production by the auto and semi-auto rice mill is about 0.9 million ton which could produce 0.20 million ton rice bran oil . Bangladesh consumes about 1.3 million tons of edible oil per year most of which is imported by private refiners. Locally produced oil can hardly meet 30 percent of the country's total requirement and thus Bangladesh imports edible oil every year. Rice bran oil produced from rice mills could substitute for a huge quantity of the imported edible oil every year. Fulfilling the

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country's demand of edible oil, quality RBO could be exported that could benefit national economy highly. Quality of RBO would be determined based on their capability on health promotion and disease modification. Thus, the present study aimed at comparing the fatty acid content of the selected [previously selected based on higher antioxidative potentialities, data not shown in this study] high yield varieties (HYV) of rice (named as BR5, BR 11, BR 28, BR29, BR48 and BR 49) discovered by Bangladesh rice research institute (BRRI) followed by extraction of RBO from the best variety and determination of the best variety's effect on lipid profile modification along with its toxicological studies on rats.

Materials and Methods

Selection and extraction of RBO

Among six HYV discovered by BRRI, BR5 had been chosen based on in vitro antioxidative potentiality [data not shown in this article] for RBO extraction. RBO had been extracted using n-Hexane followed by de-gumming, de-waxing, bleaching and de-deodorizing. Fatty acid profiling of RBO had been followed by determination of its antihypercholesterolemic effect on rat models against three edible oils as standards: soybean oil (SBO), mustard oil (MBO) and butter oil (BTO).

Fatty acid analysis

Fatty acid analyses of the selected RBO were performed following the modified method of Deshpande et al. (2016). Gas chromatographic (GC) analysis of fatty acid methyl esters were carried out using a Nucon 5700 gas chromatograph (India). Stainless steel 10% Silar 7C column packed with 60-120 mesh Gas Chrom Q was used. Maintaining both injector and detector temperature at 240°C, column temperature was initially set at 160°C for 5 min followed by ramping a rate of 5 °C per min to the final temperature of 220°C for 20 minutes.

Identification of individual fatty acids had been performed based on the received peak area followed by the quantification against external calibration curve.

In vivo experiments

Twenty five male Long-Evans rats (12 weeks old), weighing 150 ± 2g were used in this study. Animals had been housed individually in cages in a room at animal house of Bangladesh Rice Research Institute (BRRI). Animals had been maintained at 22–24°C with a controlled 12 hrs light–dark cycle and had free access to tap water and commercial rat feed. Rats were randomly divided into five groups namely control, RBO, SBO, MTO, BTO, each group containing five rats. Each rat was given 15g feed per day along with 10 g respective oil per rat per day for 28 days. Rats had been anaesthesized using diethyl ether and blood collected from left jugular vein. Blood samples had been centrifuged at 6000 rpm for 15 minutes, serum collected and stored at 4°C in a refrigerator. Biochemical parameters including liver function tests such as Cholesterol, TG, AST, ALT, ALP, Kidney function tests such as Urea, Creatinine, Uric Acid, Total protein, Albumin, A:G ratio and thyroid hormones such as TSH, FT4 were measured for all groups using commercially available reagent kits following manufacturer's instructions and a semi-auto biochemical analyzer. All the experimental procedure had been approved by the ethical committee of BRRI.

Statistical analyses

Data of triplicate studies had been presented as mean±SEM. One-way ANOVA followed by Duncan's multiple range test (DMRT) had been performed using SPSS version 20.0.

Results and Discussion

Fatty acid profiling

Fatty acid profiling of popular high yielding rice varieties (named as BR5, BR 11, BR 28, BR29, BR48 and BR 49) indicate the range of saturated fatty acids from 16.59 to 21.97 %, mono unsaturated fatty acids from 39.82 to 49.95% and unsaturated fatty acids from 78.03 to 83.88%. RBO possesses the highest level of monounsaturated fatty acid (MUFA), oleic Acid (C18:1) (48.03%) (Table 1 and Table 2). Our findings are in close proximity with a previous study .

Table 1. Fatty acid profiling of popular high yielding rice varieties in Bangladesh

Fatty acid composition of rice bran oils

Fatty acid %	BR11	BRRI dhan28	BRRI dhan29	BRRI dhan48	BRRI dhan49
Lauric acid	ND	0.66	ND	ND	ND
Myristic acid	0.24	0.61	ND	0.44	1.00
Palmiticacid	16.43	18.39	14.10	16.74	17.44
Stearic acid	1.52	1.78	1.74	1.91	2.18
Arachidic acid	0.70	0.54	0.75	0.83	0.97
Behenic acid	0.33	ND	ND	0.38	ND
SFA%	**19.21**	**21.97**	**16.59**	**20.29**	**21.60**
Oleic acid	43.16	44.79	49.95	45.36	39.39
Eicosenoic acid	0.44	ND	ND	0.4637	0.4356
MUFA%	**43.60**	**44.79**	**49.95**	**45.82**	**39.82**
Linoleic acid	35.98	32.13	32.58	32.66	37.26
Linolenic acid	1.21	1.11	0.88	1.22	1.32
PUFA%	**37.19**	**33.24**	**33.46**	**33.88**	**38.58**
UFA%	**80.79**	**78.03**	**83.41**	**79.71**	**78.40**

Table 2. Fatty acid profiling of BR5 derived RBO and standard edible oils

Table 2. Fatty acid profiling of BR5 derived RBO and standard edible oils

Fatty acid profiling of edible oils	RBO (BR5)	MTO	SBO	BTO
SFA%	16.62	15	12	22
a) Myristic Acid (C14:0)	00.22	ND	00.5	ND
b) Palmitic Acid (C16:0)	12.95	96	11	35
c) Stearic Acid (C18:0)	3.02	00.4	3.5	10
d) Arachidic Acid(C20:0)	00.43	ND	ND	ND
e) Behenic (C22:0)	ND	2	ND	ND
2. UFA(%)	83.88	88	85	23
i) MUFA (%)	48.32	60	37	35
a) Oleic Acid (C18:1)	48.03	13	37	20
b) Eicosenoic Acid (C20:1)	00.29	ND	ND	ND
c) Erucic Acid(C22:0)	ND	47	ND	ND
ii) PUFA (%)	35.06	28	48	25
a)Linoleic Acid (C18:2)	33.98	21.2	43	23
a)Linoleic Acid (C1 Feed 8:3)	01.07	6.8	05.0	1.9

Effect of RBO feeding on body weight change of the rats

During the twenty eight day long observation, gradual increase in body weight (ranging from 140 ± 5 g up to 218 ±2 g) of the rats of all the groups was noticed (Figure. 1). However, there was distinct body weight change among different groups. The BTO group gained the maximum weight and their rate of becoming weighty surpassed that of the others. Compared with other groups, increased body weight of the BTO group was statistically significant ($P < 0.05$).

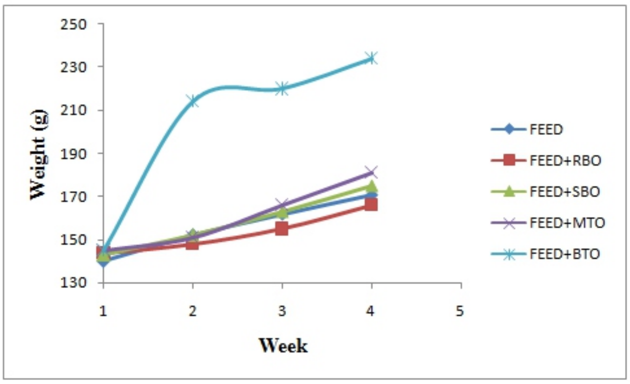

Figure 1. Body weight change of the rats fed RBO and standard edible oils. Where, FEED=commercially available rat food pellet; RBO=rice bran oil; SBO=soybean oil; MTO=mustard oil; BTO=butter oil.

Biochemical parameters

Effect of RBO feeding on lipid profile of the rats

Antihypercholesterolemic and other health promoting effect of RBO were evaluated comparing with those of SBO, MTO and BTO (Table 3). Obtained data reveals that consuming RBO for 28 days caused significant reduction of plasma total cholesterol level than those of SBO, MTO and BTO fed animals. Compared with the SBO, MTO and BTO fed animals; there was significant reduction in the plasma total cholesterol (TC) level of the RBO fed animals. Though there was a little increase in TC level of the RBO group than those of the normocholesterolemic control (feed) group, this increment was statistically insignificant (Table 3). On the contrary, all the SBO, MTO and BTO fed animals showed significantly increased level of plasma TC levels than those of the control group. Similar trend had been observed in case of plasma triacylglycerol levels. RBO showed plasma HDL-C increasing while LDL-C and VLDL-C lowering effects (Table 3). However, this effect was not uniformly significant in all groups (Table 3).

Our findings are in concordance with those of Al-Okbi et al. (2020). Poly unsaturated fatty acid (PUFA, 35.06%) composition, especially linoleic acid (33.98%), along with non-fatty acid components such as the micronutrients (triterpene alcohols, phytosterols, γ- oryzanol and tocotrienols) present in the unsaponifiable fraction of RBO might had synergistically lowered rat serum cholesterol level (Al-Okbi et al., 2019). Hypocholesterolemic effect of RBO might be attributed to fecal excretion of bile acids (Lei et al., 2018; Sunitha et al., 1997).

Triacylglycero (TG) level increased insignificantly in the RBO fed animals. Earlier studies on rats indicate that rats fed diets containing 10% RBO for 8 weeks lowered plasma TC, LDL-C and VLDL-C and increased HDL-C levels, while no changes in TG level on cholesterol-containing or cholesterol-free diets had been observed (Sunitha et al., 1997).

RBO caused significant increment in plasm HDL-C level and decrement in LDL-C and VLDL-C levels. Similar trend was observed for SBO and MTO while BTO had opposite effects. The reduction of TG and TC associated with LDL-C suggest that cholesterol lowering activity of RBO might result from the enhanced catabolism of LDL-C through hepatic receptors for final elimination in the form of bile acids (Khanna et al., 2002). There exists a positive correlation between an increased LDL-C/HDL-C ratio and the development of atherosclerosis. Increased level of HDL-C in the RBO fed rats indicates its potent protective action against atherogenesis since an independent inverse relationship exists between plasma HDL-C levels and cardiovascular risk. Underlying mechanism may be attributed to the enhancement of lecithin cholesteryl acyl transferase (LCAT), that had incorporated the free cholesterol into HDL and transferred back to VLDL or intermediate density lipoproteins (IDL), which had been taken back by the liver cells (Devi & Sharma, 2004). Additional mechanism may involve an inhibition of hepatic triglyceride lipase (HTL) on HDL which may lead to a rapid catabolism of blood lipids through extrahepatic tissues (Anila &Vijayalakshmi, 2002). Thus, the current findings confirm the anti-hyperlipidaemic properties of BR5 derived RBO and its overall impact to lower the CVD risk.

Table 3. Plasma lipid profile of rats

Treatments	Total cholesterol (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Feed	45.68± 0.12^{a}	61.81± 0.48^{a}	29.06 ± 0.42^{a}	36.97 ± 0.58^{a}	24.54 ± 0.13^{a}
RBO+ Feed	47.73± 0.16^{a}	64.36± 0.34^{a}	36.47 ± 0.55^{b}	35.44 ± 0.58^{a}	20.78 ± 0.17^{b}
SBO+ Feed	58.79± 0.22^{b}	92.39± 0.44^{b}	31.94 ± 0.58^{a}	55.97 ± 0.58^{b}	34.40 ± 0.18^{c}
MTO+ Feed	67.71± 0.27^{c}	109.41± 0.54^{c}	30.33 ± 0.40^{a}	66.97 ± 0.58^{c}	33.47 ± 0.18^{d}
BTO+ Feed	86.23± 0.29^{d}	161.16± 0.52^{d}	28.73 ± 0.40^{a}	70.97 ± 0.58^{d}	36.67 ± 0.10^{e}

Results have been expressed as mean±SEM of triplicate determination (n=5). Data in the same column having different superscripts are significantly different at P< 0.05, as measured by the DMRT Test.

Cardiac and liver function tests

Enzymatic tests had been performed to assess the safety issue of RBO and other edible oils on the experimental subjects. Compared with the control group, significantly increased levels of serum AST/GOT, ALT/GPT and ALP enzymes (P ≤0.05) were observed in the SBO, MTO and BTO rats; however, there was little and insignificant increment in case of RBO rats (Table 4). Obtained results suggest that RBO had less toxic effect on rats' cardiac and hepatic tissues than those of the SBO, MTO and BTO. Our findings are in concordance with those of Araghi et al. (2016), Al-Okabi et al. (2019, 2020) and . Neither any rat died nor they showed any behavioral abnormalities during the experimental period. Thus, BR5 derived RBO had no detectable detrimental effect on cardiac and renal functions.

Table 4. Cardiac and liver function tests

Treatments	AST (U/L)	ALT (U/L)	ALP (U/L)
Feed	229.27± 0.59^{a}	504.12± 0.67^{a}	35.33± 0.34^{a}
RBO+ Feed	233.16± 0.64^{a}	506.16± 0.45^{a}	37.11± 0.30^{a}
SBO+ Feed	300.37± 0.44^{b}	514.10± 0.18^{a}	45.33± 0.48^{b}
MTO+ Feed	325.36± 0.74^{c}	528.17± 0.33^{a}	57.23± 0.23^{c}
BTO+ Feed	357.66± 0.29^{d}	533.13± 0.39^{a}	82.17± 0.27^{d}

Results have been expressed as mean±SEM of triplicate determination (n=5). Data in the same column having different superscripts are significantly different at P< 0.05, as measured by the DMRT Test.

Kidney function tests

Compared with those of the SBO, MTO and BTO groups, feeding of RBO caused very little increase in serum levels of creatinine, urea and uric acid (Table 5). Our findings are in concordance with that of Al-Okabi et al. (2019). Thus, from the toxicological point of view, BR5 derived RBO could be considered as safe.

Table 5. Kidney function tests

Treatments	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
Feed	0.52± 0.02^{a}	35.1± 0.29^{a}	1.48± 0.12^{a}
RBO+ Feed	0.53± 0.05^{a}	37.66± 0.22^{a}	1.61± 0.14^{a}
SBO+ Feed	0.59± 0.09^{b}	44.33± 0.19^{b}	2.23± 0.09^{b}
MTO+ Feed	0.58± 0.04^{b}	46.66± 0.12^{b}	2.39± 0.11^{b}
BTO+ Feed	0.65± 0.07^{b}	47.66± 0.26^{b}	2.80± 0.20^{b}

Results have been expressed as mean±SEM of triplicate determination (n=5). Data in the same column having different superscripts are significantly different at P< 0.05, as measured by the DMRT Test.

Protein and hormonal levels

Plasma levels of total protein, globulin, albumin/globulin ratio, free T4 (FT4; thyroxine) and thyroid stimulating hormone (TSH) had been measured in all the experimental animals (Table 6). There was discrepancy among the groups regarding each of the parameter; however, this was statistically insignificant. Previous studies suggest TSH lowering effect of γ-oryzanol extracted from rice bran oil . Thus, our findings suggest no detrimental effect of BR5 derived RBO on blood biochemistry profile of the experimental animals.

Table 6. Plasma protein and hormone level

Treatments	Total Protein (g/dl)	Globulin (g/dl)	A/G Ratio	FT4 (mg/dL)	TSH (mIU/L)
Feed	7.15± 0.15^{a}	2.11± 0.13^{a}	2.40± 0.25^{a}	1.46± 0.08^{a}	<0.06
RBO+ Feed	6.66± 0.11^{a}	1.65± 0.05^{a}	3.07± 0.09^{a}	1.26± 0.11^{a}	<0.06
SBO+ Feed	7.00± 0.05^{a}	1.37± 0.09^{a}	4.12± 0.25^{a}	1.47± 0.07^{a}	<0.06
MTO+ Feed	6.89± 0.09^{a}	1.66± 0.05^{a}	3.15± 0.30^{a}	1.82± 0.08^{a}	<0.06
BTO+ Feed	7.42± 0.06^{a}	2.20± 0.08^{a}	2.36± 0.15^{a}	1.93± 0.03^{a}	<0.06

Results have been expressed as mean±SEM of triplicate determination (n=5). Data in the same column having different superscripts are significantly different at P< 0.05, as measured by the DMRT Test.

Conclusion

Plasma cholesterol lowering effect of RBO derived from the high yield rice variety BR5 surpasses those of SBO, MTO and BTO. Toxicological studies suggest this RBO to be safe. Thus, BR5 derived RBO could replace the other edible oils usually utilized in our daily cooking and dressing. This would aid in modification of current dietary pattern towards healthy life style practicing for the sake of overcoming CVD complications. However, large scale studies of BR5 derived RBO on human sample is warranted. Meanwhile, in countries, where production cost of RBO is higher, combination of RBO with low cost edible oils could be considered.

Conclusion

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Abbreviations

A:G ratio: Albumin/Globulin ratio; ALP: Alanine phosphatase; ALT: Alanine amino transferase; ANOVA: Analysis of variance; AST: Aspartate amino transferase; BRRI: Bangladesh rice research institute; BTO: Butter oil; CVD: Cardiovascular diseases; GC: Gas chromatographic analysis; HDL-C:High density lipoprotein-cholesterol; LDL-C:Low density lipoprotein-cholesterol; VLDL-C:Very low-density lipoprotein cholesterol; MTO: Mustard oil; RBO: MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; Rice bran oil; SBO: Soybean oil.

Conflict of interest

All authors declare that they have no conflict of interest/All authors declare no conflict of interest.

Consent for publications

All authors have read and approve the final manuscript for publication/We have read and approve the final manuscript for publication.

Availability of data and material

We have embedded all data in the manuscript.

Authors' contributions

Study design, supervision, analysis of data and manuscript writing had been performed by Mohammad Azizur Rahman and Habibul Bari Shozjib. Animal handling and data collection had been performed by Jannatul Ferdous and Shakir Hossen. Mahmudul Hasan and Afroza Parvin edited the manuscript.

Ethics approval and consent to participate

Prior approval from animal experimentation ethics committee of Bangladesh Rice Research Institute had been collected.

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