

-BIOEFFECT OF SEMI-PURIFIED UNSAPONIFIABLE COMPONENTS OF RICE BRAN OIL ON PLASMA LIPID LEVELS IN MODERATELY HYPERCHOLESTEROLEMIC RATS

Ghanem, K. Z.; M. H. Mahmoud; Sahar A. Abdel-aziz and M. A. Mohammad

Dept. of Food Science and Nutrition, National Res. Center, Giza, Egypt

ABSTRACT

Number of studies on humans and animals showed lowering effect of rice bran oil (RBO) as well as other vegetable oils in plasma cholesterol levels. The aim of this study was undertaken to compare this effect of two concentrations (1%) and (2%) of Egyptian-RBO active compounds on hypercholesterolaemia in rats. Four groups of six rats were used, the first group was fed on basal diet (control), while the second group was fed on basal diet plus 1% cholesterol. Groups 3 and 4 were fed basal diet +1 % cholesterol + RBO active compounds (1 and 2 % respectively). Non significant differences were noticed in the mean values of body weights, organs weights and relative organs weights (organ wt/body wt) between different experimental groups. The results obtained showed no significant changes in transaminase (AST & ALT) activities between different treated groups. No significant effect was observed in plasma urea and creatinine or hemoglobin in different treated groups compared to control group. On the other hand total cholesterol and low density lipoprotein cholesterol data showed a significant increase in hypercholesterolemic groups (50 % and 168 %) compared to control group. Supplementation with rice bran active compounds in groups 4 decreased its level compared to cholesterol group 2. The data obtained revealed that triacylglycerol was significantly decreased due to supplementation with rice bran oil of groups 3 (by 27.64 %) and 4 (by 15.07%) compared to hypercholesterolemia in rats of group 2.

Conclusion: Rice bran oil active compounds seem to be a very promising phytochemical alternative to classic lipid-lowering agents.

Keywords: Rice bran oil, Hypercholesterolemia, Plasma lipids

INTRODUCTION

Hypercholesterolemia is an established major risk factor for coronary heart disease. Lifestyle modification is the preferable form of treatment for most types of hyperlipidemia [National Cholesterol Education Program 1993]. The most potent drugs that are currently used to lower elevated (LDL-C) levels are the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) [Gould et al., 1995; Law et al., 2003]. Because of patient reluctance to be treated with chemically derived drugs, especially for primary

prevention which may contribute to the above discrepancy, there is a need for effective, safe and ideally naturally derived drugs. A number of studies in humans and animals have shown that rice bran oil (RBO) is as effective as other vegetable oils in lowering plasma cholesterol levels [Lichtenstein *et al.*, 1994]. In some cases, RBO lowered plasma cholesterol more effectively than other commonly used vegetable oils rich in linoleic acid [Rukmini & Raghuram, 1991], this effect can be attributed to the occurrence of specific components in RBO, γ -oryzanol and perhaps tocotrienols [Nicolosi *et al.*, 1991; Rukmini & Raghuram 1991; Juliano & Cossu., 2005]. The amount of linoleic acid in RBO is rather moderate among the vegetable oils (~ 40 % of total fatty acids), but is still considered a rich source of this acid [Edwards & Radcliff., 1994].

MATERIALS AND METHODS

Extraction of crude oil:

To extract crude rice bran oil, 100 g of rice bran was extracted with 1 L of n-hexane on a horizontal shaker for 12 hours at 300 oscillations per minute and filtered through fiberglass filter paper. After repeating the extraction procedure, extracts were combined, and n-hexane was evaporated under vacuum at 30 °C [Ha *et al.*, 2005].

Semi purification of bioactive component (γ -oryzanol) using low-pressure Silica column. :

A glass column (2.5 cm x 25 cm) packed with 20 g of silica (grade 60) (Merck Company) was used to remove the triglycerides and other lipids. Initially, the crude oil was solubilized in 50 ml of the solvent (hexane/ethyl acetate = 9:1) for flushing through the column. Then 50 ml of solvent (hexane/ethyl acetate = 7:3) was allowed to flow through the column, and the eluant was collected. The column was then washed with 50 ml of hexane/ethyl acetate (1:1), and the semipurified bioactive components were obtained after the solvent was evaporated [Xu & Godber., 1999].

Animals and diets:

Basal diet was provided in accordance with AIN-93 formulation [Reeves *et al.*, 1993], as shown in Table 1. Forty eight male albino rats with an average body weight 82 ± 4 g were used in this study individual housed in stainless steel cages. The rats were fed basal diet for one week; water was allowed ad-libitum. The rats were divided into 4 groups, 8 rats for each group.

Groups	120	Group (1)	Group (2)	Group (3)	Group (4)
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Experiment I:

The first group was fed on basal diet (control) .The second group was fed on basal diet +1 % cholesterol. Group 3 and 4 were fed on basal diet +1 % cholesterol + semi purification of active compounds of rice bran oil (1 and 2 g /100 diet, respectively). After 6 weeks the animals were fasted overnight, blood samples were withdrawn by a fine capillary glass tube from the orbital plexus vein. The blood was collected in heparin containing tubes and which were centrifuged at 3000 rpm for 15 min. and stored at -20°C until analysis.

The plasma enzyme activities of aspartate trans aminos (AST) and alanine trans aminase (ALT) were determined according to the method of: Reitman & Frankel [1957]. Plasma urea was determined by the method of Patton & Grouch [1977]. Plasma total cholesterol, triglycerides, HDL-C and LDL-C were determined using the respectively enzymatic methods: Allain *et al.* [1974], Fossati &Prencipe [1982], Arcol [1989], and Sharf *et al.* [1985]. Creatinine was determined according to the methods of Bartles *et al.* [1972]. Blood hemoglobin was measured using the method of Wintrobe [1956].

Table 1: Composition of the diets (g/kg diet). Reeves *et al.*, 1993

Vitamin	50	50	50	50	Salt mixture	100	100	100	100	Sucrose	
						Corn	10	10	10	10	mix

oil
Rice bran active compound
compound

Statistical analysis:

Results were expressed using student's t test according to Statistical Graphic System V statistically significant if the p

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Significant increase active compounds) compar significant decrease was c compared to hypercholester /HDL-C was lowered from 3. 4. It also improves the ris commonly used as an index

Table 2: Initial, terminal bo among different t

Group	Group (1)	Group (2)
weight (g)		
weight(g)		
Gain(g)		
(g)		
(g)		
weight %		
(g)		

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weight %

(g) 1.61±0.12 1.31±0.50 1.32±0.12

weight % 0.88±0.04 0.88±0.04 0.88±0.04

(g) 1.27±0.14 1.17±0.06 0.97±0.06

weight % 0.69±0.06 0.70±0.04 0.70±0.04

The results show that sup (levels 1 and 2) for 45 d induced in rats by a chole diminished the increase in p moreover level of HDL-C w accordance with those of Ge

Table 3: Plasma, liver, different treatment groups.

Group	Group (1)	Group
SE	Hemoglobin g/dl	15.20±3.7
Unit/L	22.63±5.80	31.20±3.7
81	43.75±6.54	40.59±5.27
1.57±3.99	39.46±3.23	Crea
0.41	(p<0.05)	

Rice bran oil and its main alcohols, phytosterols, tocot ability to improve the plas primates and human, red concentration and increas [Cicero & Gaddi 2001]. Yet rice bran oil typically approximately equal amou

Rajharam, 1991]. Previous saturated fatty acids on total cholesterol and triglyceride levels in rats. Rice bran oil lowers cholesterol and triglyceride levels in rats. This suggests that rice bran oil may be beneficial for cardiovascular health by its unsaponifiable components.

[Sugano & Tsuji 1997; Wilson et al. 2000].

beginning to focus on the composition of the sterol fraction. Triterpene alcohols, tocopherols, and other minor components. The major sterol component is γ -oryzanol, a sterol with a unique structure (Tsuji 1997). Major components of the sterol fraction include campesterol and β -sitosterol. When the plant sterols fraction was provided at 2.1 g/d to mice, total cholesterol was decreased by 5% and LDL cholesterol was decreased (Wilson et al. 2000). The investigators proposed that the sterol fraction and other 4-dsmethylsterols, such as cycloartenol, sitosterol structure is more similar to the structure of the 4-dsmethylsterols, and it may be inhibiting cholesterol absorption.

Vitamin 50 50 50 50 Salt mixture 100 100 100 100 Sucrose
 Corn 10 10 10 10 mix

oil
 Rice bran active compound
 compound

Statistical analysis:
 Results were expressed as mean \pm standard error of the mean (SEM) using student's t test according to the Statistical Graphic System V. Differences were considered statistically significant if the p value was less than 0.05.

RESULTS

The body weight gain, feed intake, and relative organs weights (heart, lung) and relative organs weights were shown in Table 2. Non significant differences were observed between the groups.

of body weight, organs weight, and body weight of experimental groups. Table 3 shows the effect of (ALT) in plasma of male albino rats of the different experimental groups. No significant changes in transaminase activity were observed in the treated groups. No significant changes in creatinine in the different treatment groups. Table 4 illustrates the total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) /HDL-C. The Total cholesterol (TC) showed significant increase (158% and 168 %) compared to control group. The active compounds in both groups (group 2 and 3) lowered cholesterol group 2. Thus, compound (group 4), induced a significant increase (42.10 %) in plasma, compared to control group.

Significant increase in body weight (active compounds) compared to control group. A significant decrease was observed in body weight compared to hypercholesterolemia group. The TC /HDL-C was lowered from 3.5 to 2.5 in group 4. It also improves the risk factor commonly used as an index of atherosclerosis.

Table 2: Initial, terminal body weight and weight gain among different treatment groups

Group
weight (g)
weight(g)
Gain(g)
(g)

(g)
weight %
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(g) 1.61±0.12 1.31±0.50 1.3
weight % 0.88±0.04 0.
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Table 3: Plasma, liver, different treatment groups.

Group	Group (1)	Group
SE Hemoglobin g/dl	15.20	
Unit/L	22.63±5.80	31.20±3.81
	43.75±6.54	40.59±5.27
	1.57±3.99	39.46±3.23
	0.41	(p<0.05)

Rice bran oil and its main alcohols, phytosterols, tocotrienes, and other compounds have the ability to improve the plasma lipid profile in primates and human, reduce LDL-C concentration and increasing HDL-C [Cicero & Gaddi 2001]. Yet rice bran oil typically contains approximately equal amount of saturated and unsaturated fatty acids [Rajharam, 1991]. Previous studies have shown that rice bran oil lowers cholesterol levels in rats, suggesting that rice bran oil may be beneficial by its unsaponifiable components [Sugano & Tsuji 1997; Wilson

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Table 2: Initial, terminal body weight and relative organ weight among different treatment groups

Group	Initial weight (g)	Terminal weight (g)	Gain (g)	Gain (%)
Control	1.61±0.12	1.31±0.50	1.33±0.12	82.5%
Hypercholesterolemia	1.61±0.12	1.31±0.50	1.33±0.12	82.5%
Compound 1	1.61±0.12	1.31±0.50	1.33±0.12	82.5%
Compound 2	1.61±0.12	1.31±0.50	1.33±0.12	82.5%
Compound 3	1.61±0.12	1.31±0.50	1.33±0.12	82.5%
Compound 4	1.61±0.12	1.31±0.50	1.33±0.12	82.5%

(g)
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The results show (levels 1 and 2) for 45 d induced in rats by a chole diminished the increase in p moreover level of HDL-C w accordance with those of Ge

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suggests that rice bran oil is characterized by its unsaponifiable compounds [Sugano & Tsuji 1997; Wilson et al. 2000]. Research has begun to focus on the composition of these compounds, including triterpene alcohols, tocopherols, and sterols. One of the major compounds is γ -oryzanol, a triterpene alcohol (Tsuji 1997). Major components include γ -oryzanol, β -sitosterol, and 24-methylene cycloartenol. Other components include campesterol and β -sitosterol. When the plant sterols from rice bran oil were provided at 2.1 g/d to mice, the total cholesterol was decreased by 5% and LDL cholesterol was decreased (Sugano et al. 2000). The investigators proposed that the sterols and other 4-dsmethylsterols, such as cycloartenol, β -sitosterol, and other 4-dsmethylsterols, such as cycloartenol, β -sitosterol structure is more similar to the structure of the 4-dsmethylsterols, and it may be inhibiting cholesterol absorption.

Vitamin 50 50 50 50 **Salt mixture** 100 100 100 100 **cr**
Corn 10 10 10 10 **oil**
Rice bran active compound
compound

Statistical analysis:

Results were expressed as mean \pm SD. Statistical analysis was performed using student's t test according to the Statistical Graphic System V. Results were considered statistically significant if the p value was less than 0.05.

RESULTS

The body weight gain, food intake, and relative organs weight (heart, liver, lung) and relative organs weight (heart, liver, lung) are shown in Table 2. Non significant differences were observed in the body weight, organs weight, and relative organs weight of experimental groups. Table 3 shows the levels of ALT (ALT) in plasma of male albino mice.

of the different experimental groups. No significant changes in transaminase activities were observed in the treated groups. No significant changes in creatinine in the different treatment groups were observed. Table 1 illustrates the total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and the ratio of TC /HDL-C. The Total cholesterol showed significant increase (168% and 168 %) compared to control group. Significant increase in cholesterol was observed in both groups 2 and 4. Thus, compound (group 4), induced a significant increase (42.10 %) in plasma, compared to control group.

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Table 2: Initial, terminal body weight (g) and weight gain (g) among different treatment groups

Group
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weight(g)
Gain(g)
(g)
(g)
weight %

(g)
 weight % 0.46±0.04 0.4
 (g) 1.61±0.12 1.31±0.50 1.3
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Table 2: Initial, terminal body weight and weight gain among different treatment groups

Group	Initial weight (g)	Terminal weight (g)	Gain (g)	Gain (%)
Control	161±0.12	131±0.50	1.31±0.50	0.46±0.04
Hypercholesterolemia	127±0.14	117±0.06	0.90±0.06	0.70±0.04
Compound 1	161±0.12	131±0.50	1.31±0.50	0.46±0.04
Compound 2	161±0.12	131±0.50	1.31±0.50	0.46±0.04
Compound 3	161±0.12	131±0.50	1.31±0.50	0.46±0.04
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Table 2: Initial, terminal body weight (g) among different treatment groups

Group	Initial weight (g)	Terminal weight (g)	Gain (g)	Gain (%)
1	100	146	46	46
2	100	146	46	46
3	100	146	46	46
4	100	146	46	46

(g) 1.61±0.12 1.31±0.50 1.3
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Table 2: Initial, terminal body weights and weight gain among different treatment groups

Group	Initial weight (g)	Terminal weight (g)	Gain(g)	Weight gain (%)
Control	1.61±0.12	1.31±0.50	1.33±0.12	0.46±0.04
Group 1	1.61±0.12	1.31±0.50	1.33±0.12	0.46±0.04
Group 2	1.61±0.12	1.31±0.50	1.33±0.12	0.46±0.04
Group 3	1.61±0.12	1.31±0.50	1.33±0.12	0.46±0.04
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42.10 %) in plasma, compar
Significant increase
active compounds) compar
significant decrease was c
compared to hypercholester
/HDL-C was lowered from 3.
4. It also improves the ris
commonly used as an index

**Table 2: Initial, terminal bo
among different t**

Group	weight (g)	weight(g)	Gain(g)	(g)
(g)	weight %	(g)	weight	% 0.46±0.04 0.4
(g)	weight %	(g)	weight	% 1.61±0.12 1.31±0.50 1.3
(g)	weight %	(g)	weight	% 0.88±0.04 0.

(g)
weight %

The results show (levels 1 and 2) for 45 d induced in rats by a cholesterol diet. Rice bran oil diminished the increase in plasma cholesterol. Moreover, level of HDL-C was higher in accordance with those of Ge...

Table 3: Plasma, liver, and kidney cholesterol in different treatment groups

Group

Rice bran oil and other components like triterpene alcohols, phytosterols, tocotrienols, and tocopherols have the ability to improve the plasma lipid profile in primates and human, reduce LDL-C concentration and increasing HDL-C concentration [Cicero & Gaddi 2001].

Yet rice bran oil type contains approximately equal amount of these components [Rajharam, 1991]. Previous studies have shown that saturated fatty acids on total cholesterol and rice bran oil lowers cholesterol levels. This suggests that rice bran oil may be beneficial by its unsaponifiable components [Sugano & Tsuji 1997; Wilson et al. 2001]. Research has begun to focus on the components of rice bran oil: triterpene alcohols, tocopherols, and tocotrienols. One compound is γ -oryzanol, a sterol compound [Tsuji 1997]. Major components include β -sitosterol and 24-methylene cycloartenol, campesterol and β -sitosterol.

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Vitamin	50	50	50	50	Salt mixture	100	100	100	100
					Corn	10	10	10	10 mix

When the plant sterols f
and"provided at 2.1\$g/d to n
decreased by 5% and LDL
2000). The investigators pos
and other 4-dsmethylsterols
dimethylsterols, such as cyc
sitosterol structure is more s
dimethylsterols, and it may b
inhibiting cholesterol absorpt

oil
**Rice bran active compound
compound**

Statistical analysis:

Results were expres
using student's t test acco
Statistical Graphic System V
statistically significant if the p

RESU

The body weight gain,
lung) and relative organs v
shown in Table 2. Non signifi
of body weight, organs weig
experimental groups. Table 3
ALT) in plasma of male albi

diminished the increase in p

moreover level of HDL-C w

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accordance with those of Ge

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Table 3: Plasma, liver,

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Group

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alcohols, phytosterols, tocot

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ability to improve the plasme

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primates and human, red

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[Cicero & Gaddi 2001].

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approximately equal amount

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saturated fatty acids on total

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suggests that rice bran oil □

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by its unsaponifiable compo

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[Sugano & Tsuji 1997; Wilso

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begun to focus on the com

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triterpene alcohols, tocopher

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compound is γ -oryzanol, a

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Tsuji 1997). Major!compon

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100	100	Sucrose			
100	100	ucrose			
100	100	crose			
100	100	rose			
100	100	ose	100		
100	100	se			
100	100	e			
100	100				
100	100				

	Salt	mixture	50	50	50	50	Vitamin
mix	10	10	10	10	10	10	Corn
oil	60	60	60	60	60	60	Cellulose
holesterol	0	10	10	10	10	10	Rice bran active
compound	0	0	10	20	20	20	Starch
590	Total	1000	1000	1000	1000	1000	

Statistical analysis:

Results were expressed as mean ±SD. Statistical significance was calculated using student's t test according to the method of Statgraphics Program Statistical Graphic System Version 2.6 [1987]. Differences were considered statistically significant if the p value < 0.05.

RESULTS AND DISCUSSION

The body weight gain, organs weight (liver, spleen, kidney, heart and lung) and relative organs weight (organ weight / body weight x 100) are shown in Table 2. Non significant differences were noticed in the mean value of body weight, organs weight and relative organs weight between different experimental groups. Table 3 illustrates the activities of transaminase (AST & ALT) in plasma of male albino rats in addition to plasma creatinine and urea of the different experimental groups. The results obtained showed no significant changes in transaminase (AST & ALT) activities between different treated groups. No significant effect was observed in plasma urea and creatinine in the different treated groups compared to control group. Table 4 illustrates the total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triacylglycerols and risk ratio TC /HDL-C. The Total cholesterol and low density lipoprotein cholesterol showed significant increase in hypercholesterolemic rats of group 2 (49 % and 168 %) compared to control. The results showed that supplementation of active compounds in both groups 3 and 4 decreased its level compared with cholesterol group 2. Thus, rats fed diet containing high level of active compound (group 4), induced decrease in TC (by 14.24 %) and LDL-C (by 42.10 %) in plasma, compared to hypercholesterolaemic rats (group 2).

Significant increase was observed in HDL-C in group 4 (high level of active compounds) compared to group 2 (hypercholesterolaemic rats). Non significant decrease was observed in triacylglycerol of groups 3 and 4 compared to hypercholesterolemic rats of group 2 (table 4). The risk ratio TC /HDL-C was lowered from 3.16 in group (2) to 2.56 and 1.82 in groups 3 and 4. It also improves the risk ratio by decreasing its value; such ratio is commonly used as an index of coronary heart disease.

Table 2: Initial, terminal body weights (g) and relative organs weight (%) among different treatment groups.

Group	Group (1)	Group (2)	Group (3)	Group
-------	-----------	-----------	-----------	-------

and 24-methylene cycloartanol. Also it was notable that phytosterols campesterol and β-sitosterol, are found at relatively high amounts in RBO. When the plant sterols from RBO were incorporated into margarine and provided at 2.1\$g/d to normolipidemic men and women, total cholesterol decreased by 5% and LDL cholesterol decreased by 9% (Vissers *et al.* 2000). The investigators postulated that the effect was due to the β-sitosterol and other 4-dsmethylsterols and other 4-desmethylsterols and not to the 4,4-dimethylsterols, such as cycloartenol and 24-methylene cycloartanol. The β-sitosterol structure is more similar to that of cholesterol than is that of the 4,4-dimethylsterols, and it may be more effective than the 4,4-dimethylsterols in inhibiting cholesterol absorption in the small intestine.

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