

STUDIES ON HYPOCHOLESTEROLEMIC ACTIVITY OF STABILIZED RICE BRAN OIL.

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ABSTRACT

The present investigation was carried out to study the effect of stabilized rice bran oil (SRBO) on hypocholesterolemic activity of experimental rats, and also the effect of feeding with stabilization rice bran oil (SRBO) and blend oil on the growth and lipid parameters of serum and liver of rats. Fatty acids composition and unsaponifiable matter of stabilization rice bran oil and blend oil were determined, Results showed that, stabilization rice bran oil had a higher content of saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA) than that of blend oil. stabilization Rice bran oil contained relatively higher concentration of campesterol, stigmasterol, β -sitoisterol, cycloartanol, cycloartenol 24-Methylene cycloartanol and oryzanol compared to the blend oil. The serum total and LDL-cholesterol level of rats that maintained on stabilization rice bran oil diet was significantly lower than those fed on blend oil diet. HDL-cholesterol showed a tendency to be higher. Liver lipids of rats fed on stabilization rice bran oil were also markedly lower than their blend oil fed counterparts. Finally, it can be concluded that, using stabilization rice bran oil had the pronounced effects for lowering cholesterol levels of the blood in experimental rats. The cholesterol lowering ability stabilization of rice bran oil appears to be due to unsaponifiable matter and a higher content of polyunsaturated fatty acids.

Keywords: rice bran oil, cholesterol level, polyunsaturated fatty acids.

INTRODUCTION

Rice bran, a by-product of milled rice, and its oil may have cardiovascular health benefits. Human consumption of rice bran has been limited primarily because of the rapid onset of rancidity in rice bran, but methods used to stabilize rice bran and to extract its oil have been developed (Demark-Wahnefried *et al.*, 1990).

Rice bran oil (RBO) is not a traditional oil worldwide, but it is in steady demand as a so-called "healthy oil". Approximately 80 thousand tons of RBO, corresponding to only 3.5% of total edible oils, is consumed annually in Japan (Sugano and Tsugi 1997).

A number of studies on humans and animals have shown that RBO is as effective as other vegetable oils in lowering plasma cholesterol levels (Lichtenstein *et al.*, 1994, Rukmini and Raghuram, 1991).

Compared with other vegetable oils or oil blends of similar fatty acid composition, rice bran oil has been reported to have a hypocholesterolemic effect when fed to rodents and nonhuman primates (Seetharamaiah and Chandrasekhara, 1989). In some cases this diet-induced hypercholesterolemia resulted from a selective decrease in the low-density lipoprotein (LDL-C) cholesterol fraction (Kahlon *et al.*, 1996 and Sunitha *et*

al., 1997). In addition the hypocholesterolemic effect of rice bran oil was greater than predicted from the fatty acid composition of the oil itself (Nicolosi *et al.*, 1991). This discrepancy between predicted and observed changes in plasma cholesterol levels at the end of the rice bran oil consumption period has been attributed to the oil's relatively high concentration of unsaponifiable compounds, including plant sterols, oryzanols, and tocotrienols relative to other vegetable oils generally available for human consumption (Nicolosi *et al.*, 1991 and Rukmini and Raghuram, 1991).

Hypercholesterolemia is an established major risk factor for coronary artery disease. Lifestyle modification is the preferable form of treatment for most types of hyperlipidemia (National Center for Health Statistics, 1993).

Plant sterols are structurally similar to cholesterol and compete with cholesterol for incorporation into micelles in the intestine. Usual levels of phytosterol consumption do not significantly affect cholesterol absorption. When consumed at higher levels, however, these compounds inhibit absorption of exogenous and endogenous cholesterol in the gastrointestinal tract (Sugano and Tsuji, 1997).

The present study was aimed to assess the effect of rice bran oil (RBO) on hypocholesterolemic activity of rats.

MATERIALS AND METHODS

Materials:

1. Rice bran sample was obtained from El-Obbour Mill company, Kafr El-Sheikh, Egypt. Then sample was packed in suitable bags and kept in freezer until used.
2. Blend oil (75% sunflower oil and 25% soybean oil) was obtained from Tanta Company for Oil and Soaps, Tanta, Egypt.

Methods:

Rice bran stabilization:

Microwave stabilized rice bran (MW-RB):

A microwave oven with 550 W output power was used for the stabilization of rice bran. The moisture content of raw rice bran was adjusted to 21% before treatment. One hundred gram of sample was packed in a microwave-safe polyethylene bag and subjected to microwave heating for 3 min at 120 °C and then cooled at room temperature and collected in polyethylene bags according to (Ramezanzadeh *et al.*, 2000).

Stabilization rice bran oil extraction:

Stabilization rice bran oil was extracted from rice bran with n-hexane (B.P 60-80°C) according to Kahlon *et al.* (1992). A weight of dry full fat stabilization rice bran was soaked in n-hexane solvent for 24 hr. at room temperature then the obtained solution was filtered and the solvent was removed by rotary evaporator. The crude oil, which obtained have a dark greenish color.

Determination of fatty acids:

Fatty acids were determined by using a Pye-Unicam IPU 4550 Gas liquid chromatography (GLC) according to the method of A.O.A.C. (1995).

Analysis of unsaponifiable matter:

The unsaponifiable matter were separated from stabilization rice bran oil and blend oil at room temperature according to the method of **A.O.A.C. (1995)**. The hydrocarbons and sterols compounds were identified by using a gas liquid chromatography/pye unicam/PU4550/packed.

Biological evaluation:

Experimental animals and diets:

Twenty four rats of young male Albino rats, with average weight of 58-67 gm were used. All animals were housed individually in cages with screen bottoms and fed on a basal diet for 7 days under laboratory conditions. Rats were given free access to food and water throughout the experimental period of 8 weeks.

After acclimation, rats were randomly divided into 4 groups (each of 6 rats) as shown in Table (1). The animals were weight every week. Although fed intake was closely monitored, an exact record of feed spillage was impossible to make due to the rats constant digging and scattering of the food. At the end of the experimental, weight gain and food efficiency ratio (calculated as gm of weight gain/gm of foods intake) were calculated for each rats.

Table (1): Composition the experimental diets (prepared and mixed according to Purushothama *et al*, .1995).

Dietary component (g/kg)	Diet groups			
	G1. Control blend oil	G2. Control (SRBO)	G3. (blend) + 1% cholesterol + 0.15% bile salt	G4. (SRBO) + 1% cholesterol + 0.15% bile salt
Starch	650.0	650.0	638.5	6385
Casein	120.0	120.0	120.0	120.0
Sugar	100.0	100.0	100.0	100.0
Blend oil	100.0	-	100.0	-
Rice bran oil	-	100.0	-	100.0
Salt mixture	20.0	20.0	20.0	20.0
Vitamin mixture	10.0	10.0	10.0	10.0
Cholesterol	-	-	10.0	10.0
Bile salt	-	-	1.5	1.5

G1 – Rats fed on diet contain blend oil.

G2 – Rats fed on diet contain stabilized rice bran oil.

G3 – Rats fed on diet contain blend oil + 1% cholesterol + 0.15% bile sal .

G4– Rats fed on diet contain stabilized rice bran oil + 1% cholesterol + 0.15% bile salt.

Blood sampling:

In all the previously mentioned groups blood samples were taken at the end of the experiment. The blood samples were collected after 12 hours fasting from Vein plexus eye into dry clean centrifuge tubes and left to clot. The blood was centrifuged for 10 minutes at 3000 r.p.m. to separate the serum, which was carefully aspirated and transferred into clean quite plastic tubes and kept frozen at $-18 \pm 2^{\circ}\text{C}$ until biochemical analysis (El-Khamissy, 2005).

Collection of organs:

All rats were scarified, the abdomen was opened, and the organs were separated by carefully dissection, cleaned from the adhesive matter, and washed with running water, then weighted. The relative weight of the organs was calculated following the next equation:

$$\text{Relative weight} = \frac{\text{Organ weight}}{\text{Animal weight}} \times 100$$

Determination of serum lipids:

Triglycerides, total cholesterol, high density lipoprotein cholesterol (HDL-C) concentrations were measured by enzymatic-colorimetc procedures using commercial available kits. Triglycerides was determined according to the method of Fossati and Prancipe (1982). Total cholesterol (TC) was carried out following the method of Richmond (1973). High-density lipoprotein cholesterol (HDL-C) was performed using precipitating reagent according to the method described by Richmond (1973). Low-density lipoprotein cholesterol concentration was calculated as the difference between total and HDL-cholesterol according to the method of Freidwald *et al.* (1972). Phospholipids content was estimated by the method of Zilveramit and Davis (1950).

Determination of liver lipids:

Liver lipids were determined according to Kim and Shin (1998). Liver samples were extracted with solvents before subjecting to aforementioned analysis according to Folch *et al.* (1957). A solvent system composed of chloroform : methanol, 2:1 (v/v) was used.

Statistical analysis:

Most of the obtained data were analyzed statistically using the analysis of variance and means were further tested using the least significant difference test (LSD) as outlined by **Steel and Torrie (1980)**.

RESULTS AND DISCUSSION

Fatty acids composition of Stabilization rice bran and blend oils

Data presented in Table (2) indicated that, **Stabilization** rice bran oil has a higher proportion of saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) especially palmitic acid (16:0) and linoleic acid (18:2), respectively than oil blend that used as control diet.

Table (2): Fatty acids composition of used oils.

Fatty acids (g/100 g)	Stabilized Rice bran	Blend
14:0 myristic	0.46	0.38
16:0 palmitic	19.15	16.10
18:0 stearic	1.97	2.93
20:0 arachidic	0.50	0.31
Total SFA	22.08	19.72
18:1 oleic	39.26	40.10
20:1 eicosaenoic	0.42	0.30
Total MUFA	39.68	40.40
18:2 linoleic	37.77	36.10
18:3 linolenic	1.81	1.40
Total PUFA	39.58	37.50

SAFA indicate saturated fatty acids; MUFA, monounsaturated fatty acids; and PUFA, polyunsaturated fatty acids.

Blend oil was relatively high in (MUFA) monounsaturated fatty acids especially oleic acid (18: 1) than stabilized rice bran oil. Similar results were mentioned by Grundy (1994), Edwards and Radcliff (1994), and Wilson *et al.* (2000), who indicated that, among the various components of the diet, fat influences plasma cholesterol levels more than others. It very well established that polyunsaturated fatty acids lower plasma cholesterol level whereas, saturated fatty acids increase it.

Sterol and triterpene contents in different edible oils (mg/100 g oil):

Data in Table (3) indicate that, Stabilization rice bran oil contained the relatively high concentration of campesterol, stigmasterol, β -sitosterol, cycloartanol, cycloartenol and 24 methylene-cycloartanol as the blend oil. These results were in agreement with the findings of Hood and Sidhu (1992) and Warkins *et al.* (1993), who reported that cycloartenol and 24-methylene cycloartanol are the major component terpene alcohols, followed by cycloartanol.

The results in the same Table show the high content of the Stabilization rice bran oil in oryzanol against blend oil. The aforementioned results coincide with those obtained by Shinomiya *et al.* (1983), Sharma and Rukmini (1987), Kanbara *et al.*, (1992) and Sugano and Tsuji (1997) who demonstrated that oryzanol and ferulic acid esters of plant sterols, such as triterpene alcohols and 4-methyl sterols, have been reported to exert a hypocholesterolemic effect by decreasing cholesterol absorption and inhibiting hepatic cholesterol synthesis.

Table (3):Sterol and triterpene contents in different edible oils(mg/100g oil).

Oil	Camp-esterol	Stigma-sterol	β -sito-sterol	Cyclo-Artanol	24 methylene-cycloartanol	Cyclo-artenol	Oryza-nol
Stabilizati on Rice bran	403	202	1159	101	452	431	0.6
Blend	41	32	248	1	59	14	0.08

Body weight gain, food intake and food efficiency ratio (FER) of rats fed on various diets contain blend oil and stabilization rice bran oil:

Data in Table (4) indicate that, at the end of 8 weeks, the body weight gain showed no significant difference between group 1, 2 and 4 while, the gain in body weight of the group 3 was more than that observed in other groups, this may be due to the higher polyunsaturated/saturated fatty acids ratio in the diet. These results are in agreement with these of Beynen and Kritchevsky (1986) who reported that high P/S ratio (polyunsaturated fatty acids/saturated fatty acids) in the diet increases the body weight of rats.

Table (4):Body weight gain, food intake and food efficiency ratio (FER) of rats fed on various diets contain blend oil and stabilization rice bran oil.

Dietary groups	Initial weight (gm)	Final weight (gm)	Body weight gain in 8 weeks		Food intake 8 weeks	Food efficiency ratio (FER)
			(gm)	(%)		
G1	62.70 a	165.2 a	102.5 a	62.46	684 a	0.1498 b
G2	62.85 a	167.05 ab	104.2 a	62.38	720 b	0.1447 ab
G3	63.01 a	173.31 b	110.3 b	63.64	756 c	0.1470 ab
G4	63.58 a	167.88 ab	104.3 a	62.13	750 c	0.1379 a

Each value is an average of sex determination.

Values followed by the same letter in column are not significantly different $P < 0.05$.

G1, G2 ... etc. were as given in Table (1).

Furthermore, Most *et al.* (2005) suggested that the body weight gain of the experimental rats depends on the content of protein and fat of their diets.

Appeared also from the Table (4) that, food efficiency ratio (FER) showed no significant difference at ($P \leq 0.05$) between groups 1, 2 and 3, also between groups 2, 3 and 4.

Organ weights (liver, kidney and spleen) of rats fed on various diets contain blend and stabilization rice bran oils:

The weight of liver, spleen and kidney of groups of rats were determined and the results are recorded in Table (5). The relative ratio between organ and body weight was also calculated. From the obtained data, it is clear that the liver weight of rats fed on diets containing blend oil and stabilized rice bran oil (SRBO) free from cholesterol and bile salt (G1 and G2) were relatively lower than that of others treatments, indicating thereby that the decrease in liver weight is proportionate to the body weight decrease.

Concerning kidney and spleen weights, no significant difference among all of the tested groups. These results are in accordance with those obtained by Purushothama *et al.* (1995), they found that, no significant difference with respect to the organ weights between the control animals were fed on synthetic diets containing 5 and 20% peanut oil (PNO) and the experiential groups were fed on similar diets, containing the same level of Stabilization rice bran oil (SRBO).

Table (5): Organ weights (Liver, kidney and spleen) of rats fed on various diets contain blend and stabilization rice bran oils.

Dietary groups	Final weight Of rats	Liver weight		Kidney weight		Spleen weight	
		gm	R.W	gm	R.W	gm	R.W
G1	165.2 a	3.10 a	0.019	0.62 a	0.0037	0.16 a	0.0010
G2	167.05 b	3.19 a	0.019	0.63 a	0.0038	0.17 a	0.0010
G3	173.31 b	4.42 b	0.026	0.65 a	0.0038	0.19 a	0.0011
G4	167.88 ab	4.22 b	0.025	0.64 a	0.0038	0.18 a	0.0011

Each value is an average of sex determination.

Values followed by the same letter in column are not significantly different $P \leq 0.05$.

G₁, G₂ ... etc. were as given in Table (1).

R.W : relative weight

Serum lipids of rats fed on various diets contain blend and stabilization rice bran oils:

The results of analysis regarding total cholesterol, phospholipids, triglycerides and lipoproteins are presented in Table (6). It is evident from the Table that, there were differences between groups fed on blend oil and rice bran oil (group 1 and 2) with serum total, LDL cholesterol level and triglycerides showed a tendency to decrease. While, HDL-cholesterol was increased in rats fed on stabilized rice bran oil (group 2) than that fed on blend oil (group 1).

These results are supported by those reported by Wilson *et al*, (2000), Cicero and Gaddi (2001), Berger *et al*. (2004) and Frank *et al*. (2005).

Furthermore, Orthoefer (1996) and Ordovas *et al*. (2007) reported that, hypocholesterolemic activity of (SRBO) can not entirely be due to its linoleic acid content but due to the unsaponifiable matter.

In rats fed on cholesterol along with blend oil group (3) the total cholesterol was elevated significantly. Substitution of SRBO for blend oil in diet containing the cholesterol, group (4) significantly reduced total cholesterol level.

Table (6): Serum lipids of rats fed on various diets contain blend and stabilization rice bran oils.

Dietary groups	Total cholesterol (mg/dl)	Cholesterol (mg/dl)		Ratio LDL/HDL cholesterol	Triglycerides mg/dl	Phospholipids mg/dl
		HDL	LDL			
G1	66.87 b	32.10 c	34.77 b	1.08 a	115.55 d	159.6 a
G2	62.10 a	36.31 d	25.79 a	0.71 a	98.30 c	153.2 a
G3	217.30 d	12.6 a	204.7 d	16.25 c	80.10 a	168.8 a
G4	136.12 c	15.9 b	120.22 c	7.56 b	90.31 b	157.4 a

Each value is an average of sex determination.

Values followed by the same letter in column are not significantly different $P \leq 0.05$.

G₁, G₂ ... etc. were as given in Table (1).

Addition of cholesterol to the diet of rats fed on blend oil (group 3) decreased the HDL-cholesterol but increased LDL-cholesterol. This trend was observed also with rats fed on rice bran oil (group 4).

The ratio of LDL-to HDL-cholesterol which was elevated to 16.25 in rats fed on blend oil was brought down to 7.56 (50% or more reduction) in rats fed on rice bran oil (RBO).

The aforementioned results coincide with those obtained by Rong *et al.* (1999), they found that oryzanol has been shown to decrease absorption of cholesterol and inhibit aortic fatty streak formation in hamsters.

Liver lipids of rats fed on various diets contain blend and Stabilization rice bran oils.

According to the results given in Table (7), liver lipids also showed a tendency to fall in rats fed on Stabilization rice bran oil (SRBO). Liver cholesterol and triglycerides were significantly elevated in rats fed on cholesterol and blend oil (group 3), while, markedly lower in rats fed on cholesterol and rice bran oil (group 4). These results are in line with the results of Kahlon *et al.* (1992) and Kahlon *et al.* (1996).

Table (7): Liver lipids of rats fed on various diets contain blend and Stabilization rice bran oils.

Dietary groups	Total cholesterol (mg/g)	Triglycerides (mg/g)	Phospholipids (mg/g)
G1	4.9 a	10.3 b	20.8 a
G2	4.6 a	7.5 a	20.1 a
G3	65.2 c	23.2 d	30.3 a
G4	46.0 b	18.8 c	29.35 a

Each value is an average of sex determination.

Values followed by the same letter in column are not significantly different $P \leq 0.05$.

G₁, G₂ ... etc. were as given in Table (1).

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دراسات على النشاط الخافض للكوليسترول لزيت رגיע الأرز المثبت.

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أجريت هذه الدراسة بهدف توضيح الأثر الفعال لزيت رגיע الأرز المثبت بالميكروويف لخفض الكوليسترول في فئران التجارب. تم دراسة تأثير التغذية بزيت رגיע الأرز المثبت والزيت الخليط على نمو الفئران وكذلك مؤشرات الليبيدات في السيرم والكبد في الفئران كما تم أيضا تقدير الأحماض الدهنية والمواد الغير متصينة في كل من زيت رגיע الأرز والزيت الخليط.

وكانت النتائج المتحصل عليها:

- زيت رגיע الأرز المثبت يحتوى على أحماض دهنية مشبعة وغير مشبعة بكمية اكبر من الزيت الخليط كما يحتوى على نسبة عالية من المواد الغير متصينة مقارنة بالزيت الخليط.
- كانت نسبة الكوليسترولات الكلية والكوليسترول منخفض الكثافة اقل في الفئران المغذاة على زيت رגיע الأرز المثبت ، بينما زادت نسبة الكوليسترول عالي الكثافة في الفئران المغذاة على زيت رגיע الأرز المثبت مقارنة بالزيت الخليط.
- كانت نسبة الليبيدات الكبد منخفضة في الفئران المغذاة على زيت رגיע الأرز المثبت مقارنة بالزيت الخليط.

مما سبق نستنتج أن :

استخدام زيت رגיע الأرز المثبت يعمل على خفض الكوليسترول في فئران التجارب نظراً لاحتوائه على مواد غير متصينه بنسبة كبيرة وكذلك ارتفاع الأحماض الدهنية الغير مشبعة بزيت رגיע الأرز.