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EFFECTS OF STABILIZED (RICE BRAN , DEFATTED RICE BRAN AND RICE BRAN OIL) ON SERUM LIPID PARAMETERS AND BLOOD GLUCOSE LEVELS IN RATS

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ABSTRACT

This study was performed to investigate the effect of feeding with stabilized (full-fat rice bran (FFRB) , Defatted rice bran (DFRB) and rice bran oil (RBO)) on growth, serum lipid parameters and blood glucose levels of rats . Results showed that, (DFRB) contain protein content, ash, fiber and carbohydrates significantly higher than that of (FFRB) on contrast they contain significantly lower lipids. (RBO) has a higher proportion of saturated fatty acids (SFA) especially palmitic acid (16:0), and monounsaturated fatty acids (MUFA) than corn oil. But, corn oil was relatively high in polyunsaturated fatty acids (PUFA) especially linoleic acid (18: 2) than (RBO). Furthermore, (RBO) contained relatively higher concentration of compesterol, stigmasterol, β-sitoisterol, cycloartanol, cycloartenol 24-Methylenecy cloartanol and oryzanol compared to the corn oil. The highest gain in body weight was observed in rats fed on diets containing(RBO) followed by control and (FFRB), In addition, no significant difference with the organ weights among all of the tested groups. The serum total cholesterol level and triglyceride of rats that maintained on full-fat rice bran diet was significantly lower than those fed on different diet , while Lowest glucose was observed in rats fed on defatted rice bran. non-significant variations for HDL-cholesterol among the experimental diets as compared to control. It is apparent from the results that ALT (Alanine amino transferase) and AST (Aspartate amino transferase) activities were slightly reduced with full-fat rice bran, defatted rice bran and rice bran oil as compared to normal basal diet. Finally, it can be concluded that, using stabilization (FFRB), (DFRB) and (RBO) had the pronounced effects for lowering serum lipid cholesterol and blood glucose levels in experimental rats.

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 contains large amounts of bioactive phytochemicals, such as tocopherols, tocotrienols, oryzanols, and phenolic compounds (Zhang, *et al .,* 2010).Furthermore, rice bran oil (RBO) is not a popular oil worldwide, but it is in steady demand as a so-called ‘‘healthy oil’’ not only in Japan but also in Asian countries, particularly India. Approximately 80 thousand tons of RBO, corresponding to only 3.5% of total vegetable oils, is consumed annually in Japan. (Ghosh, 2007). 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).

A number of studies on humans and animals have shown that the unsaponifiables of RBO lowered serum TC and LDL-C and raised high-density lipoprotein cholesterol (HDL-C), and these alterations in lipoprotein cholesterol were associated with increased fecal excretion of neutral sterols and total bile acids (Lichtenstein *et al.*,1994, Ha *et al* ., 2005, Wilson, *et al.,* 2007). In addition, 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). Other fractions of rice showing hypocholesterolemic effects in rats include rice protein , hemicelluloses from defatted rice bran, rice bran oil and unsaponifiable matter from rice bran oil (Kahlon *et al.,* 1992). The present study was aimed to assess the effect of stabilized (rice bran , Defatted rice bran and rice bran oil) on serum lipid parameters and blood glucose levels in rats .

MATERIALS AND METHODS

Materials:

The samples of rice bran were obtained from the milling of two varieties of rice (Oryza sativa L.) namely Sakha 104 and Sakha 105 a popular short grain Japonica cultivar for consumption in the Egypt were obtained from Rice Research and Training Center (RRTC) at Sakha, Kafr El-Sheikh Governorate, Egypt during the season of 2010. Other chemicals and solvents used were of analytical reagent grade.

Methods:

Microwave stabilized rice bran (MW-RB): A microwave oven with 550 W output power was used for the stabilization of 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 120ºC, then cooled at room temperature (Ramezanzadeh *et al*., 2000).

Rice bran oil extraction:

A weight of microwave rice bran (MWRB) was soaked in n-hexane solvent (B.P 60 - 80 ^{o}C) at room temperature for 24 hr., then obtained solution was filtered and the solvent was removed by rotary evaporator according to Kahlon *et al.* (1992). The recovered crude oil, which obtained have a dark greenish color. The defatted microwave rice bran meal was milled using a laboratory scale hammer mill. The resulting flour was sieved through a 60-mesh screen, then kept in polyethylene bags and stored at 4 ^{o}C until used .

Fatty Acid Composition:

Fatty acid composition was determined by using a Pye-Unicam IPU 4550 Gas liquid chromatography **(**GLC**)** according to the method of A.O.A.C. (2005).

Analysis of unsaponifiable matter:

The unsaponifiable matter were separated from microwave rice bran oil (MWRB) and corn oil at room temperature according to the method of **A.O**.A**.**C. (**2005**). The hydrocarbons and sterols compounds were identified by using a gas liquid chromatography/pye unicam/PU4550/packed.

Chemical composition of rice bran samples:

Rice bran samples were analyzed for their chemical composition after subjecting to stabilization by microwave process and defatted microwave rice bran . Moisture, ash, crude protein, ether extract and total dietary fiber contents were determined according to the methods of **A**.**O**.**A**.**C**. (**2005**). Total carbohydrates content was calculated by difference.

Biological evaluation:

Experimental animals and diets:

Twenty four rats of young male Albino rats, with average weight of 60.12-61.70 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 7 weeks.

The animal 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. After acclimation, rats were randomly divided into 4 groups (each of 6 rats) as shown in Table (1).

Table (1): Composition the experimental diets (prepared and mixed according to A.O.A.C. (2005).

Dietary component (g/100g)	Diet groups			
	G1. Control	G2. RBO	G3. FFRB	G4. DFRB
Full fat RB	-	-	48.28	-
Defatted RB	-	-	-	41.70
Rice bran oil	-	10	-	-
Corn Oil	10	-	-	9.75
Corn Starch	65	65	43.91	41.16
Casein	10	10	2.81	2.39
Cellulose	10	10	-	-
Salt mixture	4	4	4	4
Vitamin	1	1	1	1

G1 – Rats fed on diet contend Corn oil/ starch.
G2 – Rats fed on diet contend microwave rice bran oil (RBO).
G3 – Rats fed on diet contend microwave Full-fat rice bran (FFRB).
G4 – Rats fed on diet contend microwave Defatted rice bran (DFRB).

Blood sampling:

Blood samples of the previously mentioned groups blood samples were taken at the end of the experiment. Blood samples were collected after 12 hours fasting from Vein plexus eye into dry clean centrifuge tubes and left to colt. The blood was centrifuged for 10 minutes at 3000 rpm to separate

the serum, which was carefully aspirated and transferred into clean quite plastic tubes and kept frozen at -18± 2°C until biochemical analysis (El-Khamissy, 2005).

Collection of organs:

Rats were scarified, the abdomen was opened, where 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 blood glucose:

Blood glucose was measured according to the method described by Alles *et al.* (1999) using blood glucose meter (free style TM). A drop of blood was taken from tail of the rats, placed on a test strip and blood glucose was measured immediately with the blood glucose meter.

Determination of serum lipids:

Triglycerides, total cholesterol, high density lipoprotein cholesterol (HDL-C) concentrations and low Density Lipoprotein Cholesterol (LDL-C) were measured by enzymatic-colorimetc procedures using commercial available kits. Triglycerides (TG) 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 (LDL-C) was carried out as described by McNamara *et al.*, (1990) . Phospholipids content was estimated by the method of Zilversmit and Davis (1950).

Liver function tests:

Serum was analyzed to estimate activities of liver functioning enzymes such as ALT (Alanine amino transferase), ALP (Alkaline Phosphatase) and AST (Aspartate amino transferase) by using their commercial kits (Tolman and Rej, 1999).

Renal function tests:

The extracted serum was analyzed for urea and creatinine by using their commercial kits (Newman and Price, 1999)

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

Proximate chemical composition (%) of Microwave full fat rice bran (FFRB) and defatted Microwave rice bran (DFRB).

The chemical composition of Microwave full fat rice bran (FFRB) and defatted Microwave rice bran (DFRB) were given in Table (2). The results revealed that, defatted Microwave rice bran contain protein content, ash, fiber and carbohydrates significantly higher than that of Microwave full fat rice

bran. In contrast they contain significantly lower lipids. These results are in line with those found by Farrell (1994), Amarasinghe and Gangodavilage, (2004) and Sharif *et al.,* (2005) .

Table (2): Proximate chemical composition (%) of Microwave full fat rice bran (FFRB) and Microwave defatted rice bran (DFRB).

Parameter %	Microwave rice bran	Defatted Microwave rice bran
Moisture	7.20 a	7.39 a
Crude protein	14.91 b	18.25 a
Lipids	20.71a	0.60 b
Ash	9.51 b	11.64 a
Crude fiber	10.75 b	13.15 a
Total carbohydrates*	54.87 b	69.51 a

Values followed by the same letter in row are not significantly different P ≤ 0.05
*** calculated by difference**

Fatty acids composition of microwave rice bran and corn oils:

Data presented in Table (3) indicated that, microwave rice bran oil has a higher proportion of saturated fatty acids (SFA) especially palmitic acid (16:0), and monounsaturated fatty acids (MUFA) . than corn oil that used for preparing control diet. Corn oil was relatively high in polyunsaturated fatty acids (PUFA) especially linoleic acid (18: 2) than microwave rice bran oil. Similar results were received by Terpstra, *et al.* (1991) and Lichtenstein *et al.* (2004), who indicated that, Despite of rice bran oil (RBO) containing higher amounts of saturated fat and lower amounts of mono- and polyunsaturated fats than other often-utilized hypocholesterolemic vegetable oils, the reductions in plasma non-HDL-Cholesterol concentrations observed in the current study were comparable to those of other studies when RBO replaces other oils .

Table (3): Fatty acids composition of used oils.

Fatty acids (g/100 g)	Microwave rice bran	Corn
14: 0 myristic	0.80	0.20
16: 0 palmitic	18.35	10.60
18: 0 stearic	2.11	1.21
Total SFA	21.26	12.01
18: 1 oleic	43.47	27.88
Total MUFA	43.47	27.88
18: 2 linoleic	32.85	58.87
18: 3 linolenic	2.40	4.7
Total PUFA	35.25	60.69

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

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

Data in Table (3) indicate that, microwave rice bran oil contained the relatively high concentration of campesterol, stigmasterol, β-sitosterol, cycloartanol, cycloartenol and 24 methylene-cycloartanol compared with

corn oil. These results were in agreement with the findings of Kiribuch *et al.,* 1983 and Sugano and Tsuji (1997), who indicated that the unsaponifiable component of RBO, that is, the plant sterols and oryzanol, are major cholesterol-lowering factors in RBO . In addition, RBO also contains tocotrienols, which were reported to inhibit cholesterol synthesis (Qureshi *et al.,* 1991). Thus, it is impossible to state with any certainty which unsaponifiable component or a combination of all three is the contributing component to the hypocholesterolemic response of RBO in a human study by Lichtenstein *et al. (*1994).

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

Oil	Camp-esterol	Stigma-sterol	β-sito-sterol	Cyclo-artanol	24 methylene-Cycloartanol	Cyclo-artenol	Oryzanol
Rice bran	345	170	1109	100	445	421	0.6
Corn	182	82	560	6	11	10	0.18

The results in the same table show the high content of oryzanol in the rice bran oil against corn oil. The aforementioned results concide with those obtained by Sharma and Rukmini (1987) and Sugano and Tsuji (1997), who demonstrated that orzanol 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.

Body weight gain, food intake and food efficiency ratio (FER) for rats fed on different diets:

Data in Table (4) indicate that, at the end of 7 weeks, the body weight gain showed significant difference between groups, the highest gain in body weight was observed in rats fed on diets containing RBO followed by control and FFRB . while the lowest gain was observed in DFRB group. These results might be due to low feed intake from the DFRB group (Ahmed *et al.,* 2007). It has already been recognized from various studies that dietary fiber may have some potential in the management of weight loss. This effect is derived from the potential influence of fiber on several aspects of food intake and nutrient availability (Vahouny, 1982). The effects on weight loss are often deduced from decreased caloric intake, satiety and increased fecal excretion of energy in the form of fat and nitrogen (Leeds, 1985 and Wisker *et al.,* 1985).

Table (4): Body weight gain, food intake and food efficiency ratio (FER) of different groups of rat.

Dietary groups	Initial weight (gm)	Final weight (gm)	Body weight gain in 7 weeks		Food intake 7weeks	Food efficiency ratio (FER)
			(gm)	(%)		
G1. Control	60.12 a	131.10 ab	70.98 ab	54.14	803.25 b	0.1498 b
G2. RBO	61.35 a	134.78 a	73.43 a	54.48	864.45 a	0.1447 ab
G3. FFRB	60.80 a	129.82 b	69.02 b	53.17	759.60 c	0.1470 ab
G4. DFRB	61.70 a	124.63 c	62.93 c	50.49	652.50 d	0.1379 a

Each value is an average of sex determinations.
Values followed by the same letter in column are not significantly different at P < 0.05.
G1, G2 … etc. were as given in Table (1).

Rats fed on full fat and defatted rice bran showed less increase in body weight; where defatted rice bran was found to be more effective in weight loss programs. Appeared also from the same table that, rats fed on RBO diet had the highest feed intake followed by rats feed on control diet and FFRB diet while the lowest consumption was observed in rats fed on diet containing DFRB .

Organs (liver, kidney and **spleen) weight of rats fed on various diets:**

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, no significant difference among all of the tested groups.

Table (5): Effect of diets on organs weight (g/100g) of different groups of rats.

Dietary groups	Final weight Of rats	Liver weight		Kidney weight		Spleen weight	
		Gm	R.W	Gm	R.W	Gm	R.W
G1. Control	131.10 ab	4.11 a	3.14	0.68 a	0.52	0.35 a	0.27
G2. RBO	134.78 a	4.05 a	2.98	0.65 a	0.51	0.33 a	0.22
G3. FFRB	129.82 b	4.02 a	3.12	0.66 a	0.50	0.29 a	0.25
G4. DFRB	124.63 c	4.01 a	3.23	0.69 a	0.53	0.30 a	0.23

Each value is an average of sex determinations.
Values followed by the same letter in column are not significantly different at P < 0.05.
G1, G2 … etc. were as given in Table (1). R.W : relative weight .

These results are in accordance with those obtained by Purushothama *et al.* (1995), who 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 rice bran oil (RBO).

Effect of feeding different diets on serum lipid profile and glucose (mg/dL) levels in different groups of rats:

Results given in Table (6), show a significant decreased in serum total cholesterol (TC), LDL-cholesterol , total triglycerids and glucose level among the experimental diets as compared with control at the end of the feeding period (7weeks). Maximum serum total cholesterol was found to be (92.70 mg/dL) in control group while, the lowest serum cholesterol was observed in rats fed on fullfat rice bran (84.81 mg/dL). The results indicated that, fullfat rice bran is more effective in cholesterol lowering than either rice bran oil or defatted rice bran, certainly due to the presence of comparatively high levels of tocopherol, tocotrienol and oryzanol as well as unsaponifiables. These results are supported by those of Minhajuddin *et al.,* (2005) , Wilson *et al.,* (2007), Zigoneanu *et al.,* (2008).

There are several cholesterol lowering mechanisms coupled with rice bran. It has been observed that rice bran lowers the cholesterol by increasing short chain fatty acid production in the cecum by hindering cholesterol absorption due to a change in intestinal fluid viscosity or by directly inhibiting cholesterol synthesis in the liver (Fukushima *et al.,* 1999). It is apparent from

table (8) that LDL-cholesterol were significantly varied among the rat groups fed on different diets. Maximum LDL was 37.30 mg/dL in control group followed by 32.90, 30.80 and 31.11 mg/dL in groups fed on DFRB, RBO and FFRB, respectively. aforementioned results coincide with those obtained by (Purushothama *et al.*, 1995).

Table (6): Effect of feeding on different diets on serum lipid profile and glucose (mg/dL) levels in different groups of rats

Dietary groups	Total cholesterol (mg/dl)	Cholesterol (mg/dl)		Ratio TC/HDL cholesterol	Triglycerides mg/dl	Glucose mg/dl
		HDL	LDL			
G1.Control	92.70 a	39.30 a	37.30a	2.36 a	80.50 a	114.15 a
G2. RBO	85.63 b	41.60 a	30.80 c	2.06 b	68.9 bc	113.40. a
G3. FFRB	84.81 b	40.57 a	31.11 bc	2.09 b	65.95 c	108.0 ab
G4.D FRB	88.10 b	40.66 a	32.90 b	2.17 b	70.60 b	105.60 b

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).

Apparent also from the same table that HDL-cholesterol were non-significant variations for HDL-cholesterol among the experimental diets as compared to control. Furthermore, it was observed that, FFRB showed slight improvement in serum HDL-cholesterol of rats followed by RBO and DFRB. These results are in agreement with those of Khosla *et al.,* (1995) and Rehman *et al.,* (2001). Furthermore, significant variations were observed among different groups of rats fed on various diets from glucose concentration. Maximum glucose concentration was found in control group followed by groups fed on rice bran oil and fullfat rice bran, respectively. Lowest glucose was observed in rats fed on defatted rice bran. appears to improve insulin utilization resulting in decreased fasting glucose levels. This appears to be the result of phytonutrients, antioxidants, vitamins and minerals in diabetic subjects. Therefore this natural product can be used as a diet therapy in diabetic subjects to regulate glucose metabolism. (Lai *et al.,* 2001 and McPeak *et al.,* 2001).

Effect of feeding on different diets on serum kidney and liver function tests in different groups of rats:

Serum was separated and analyzed for liver and renal functioning tests using commercial kits. for serum urea and creatinine (Table 7) Means for serum urea and creatinine concentration ranged from 14.41 to 14.55 and 0.60 to 0.63mg/dL, respectively, in rats fed on experimental diets for a period of 7 days.

Means for ALP (Alkaline phosphatase), ALT (Alanine amino transferase) and AST (Aspartate amino transferase) ranged from 257.50 to 266.30, 105.00 to 106.10 and 79.50 to 83.0 U/L, respectively. It is apparent from the results that AST and ALT activities were slightly reduced with fullfat rice bran, defatted rice bran and rice bran oil as compared to normal basal diet. These treatments can alleviate the damage induced by serum cholesterol (Ha *et al.,* 2005). However, toxicological studies of rice bran oil by the food safety evaluation protocol of WHO/FDA carried out in rats; indicated the safety of RBO for human consumption (Rukmini, 1988). Finally, it can be

concluded that, using stabilization (FFRB), (DFRB) and (RBO) had the pronounced effects for lowering serum lipid cholesterol and blood glucose levels in the experimental rats.

Table (7): Effect of diets on serum kidney and liver function tests in different groups of rats.

Dietary groups	Urea (mg/dL)	Creatinine (mg/dL)	ALP (U/L)	ALT (U/L)	AST (U/L)
G1. Control	14.41 a	0.60	257.50	106.10	83.0
G2. RBO	14.50 a	0.61	262.10	105.0	81.10
G3. FFRB	14.47 a	0.63	263.35	106.3	81.20
G4. DFRB	14.55 a	0.62	266.30	105.20	79.50

ALT: Alanine amino transferase ALP: Alkaline Phosphatase AST: Aspartate amino transferase
Each value is an average of sex determinations.
Values followed by the same letter in column are not significantly different at P < 0.05.
G1, G2 … etc. were as given in Table (1).

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تاثير رجيع الارز المثبت و رجيع الارز المثبت منزوع الدهن وزيت رجيع الارز المثبت على مستويات ليبيدات السيرم و الجلوكوز فى الفئران .

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تم إجراء هذه الدراسة بهدف توضيح الاثر الفعال لرجيع الارز المثبت و رجيع الارز المثبت منزوع الدهن وزيت رجيع الارز المثبت على ليبيدات السيرم ومستوى الجلوكوز فى فئران التجارب .

ويمكن تلخيص النتائج المتحصل عليها في الآتي:

١- رجيع الارز المثبت منزوع الدهن يحتوى على كمية عالية من البروتين و الرماد والألياف و الكربوهيدرات وعلى كمية اقل من الليبيدات بالمقارنة برجيع الارز المثبت.

٢- زيت رجيع الارز المثبت يحتوي علي كمية عالية من الاحماض الدهنية المشبعة وخاصتا البالمتيك مقارنتا بزيت الذرة ويحتوى زيت الذرة علي كمية عالية من الاحماض الدهنية الغيرالمشبعة وخاصة حامض اللينوليك مقارنتا بزيت رجيع الارز المثبت.

٣- زيت رجيع الارز المثبت يحتوي علي كمية عالية من الاستيرولات والمواد التربينية مقارنة بزيت الذرة.

٤- لوحظ زيادة فى وزن الفئران المغذاة على زيت رجيع الارز المثبت يلية المغذاة على وجبة الكنترول ثم المغذاة على رجيع الارز المثبت.

٥- لا يوجد اختلافات معنوية بين وزن الاعضاء للفئران المغذاة على المجاميع المختلفة.

٦- نسبة الكوليسترول الكلية والتراى جليسرايد فى الفئران المغذاة على زيت رجيع الارز المثبت اقل من المغذاة على باقى المجاميع بينما المستوى المنخفض من الجلوكوز لوحظ فى الفئران المغذاة على رجيع الارز المثبت منزوع الدهن.

٧- لايوجد اختلافات معنوية فى الكوليسترول عالى الكثافة بين المجاميع المختلفة.

مما سبق نستنتج أن :

استخدام رجيع الارز المثبت و رجيع الارز المثبت منزوع الدهن وزيت رجيع الارز يعمل على خفض الكوليسترول وكذلك مستوى الجلوكوز فى فئران التجارب نظراً لاحتوائه على مواد غير متصبنه بنسبة كبيرة

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