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

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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)	120	Group (2)	120	Group (3)	120	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 peurification 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 aminas (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:

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

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0.41 **(p<0.05)**

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weight	**%**	0.88±0.04	0.		

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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)		1.61±0.12	1.31±0.50	1.3
weight	**%**	0.88±0.04	0.	

(g)
weight %

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

Table 3: Plasma, liver,
different treatm

Group

Rice bran oil and
alcohols, phytosterols, tocotr
ability to improve the plasm
primates and human, redu
concentration and increasin
[Cicero & Gaddi 2001].

Yet rice bran oil typ
approximately equal amoun
Rajhuram, 1991]. Previous
saturaôed fatty akids on tota
bran oim lowers cholestero
suggests thaT rice bran oil
by its unsaponifiable compo
[Sugano & Tsuji 1997; Wilso
begun to focus on the com
triterpene alcohols, tocopher
compound is γ-oryzanol, a
Tsuji 1997). Major!compone
and 24-methylene cycloart
campesterol and β-sitospero

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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

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

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

RESUL

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

of the different experimen
significant changes in transa
treated groups. No signific
creatinine in the different tre
illustrates the total cholesterc
C), low density lipoprotein c
TC /HDL-C. The Total cho
showed significant increase
and 168 %) compared to co
active compounds in both gr
cholesterol group 2. Thus,
compound (group 4), induc
42.10 %) in plasma, compare

Significant increase
active compounds) compare
significant decrease was
compared to hypercholesterc
/HDL-C was lowered from 3.
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Table 2: Initial, terminal bo
among different t

Group			
weight (g)			
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Gain(g)			
(g)			
(g)			
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(g)			
weight %	0.46±0.04	0.4	
(g)	1.61±0.12	1.31±0.50	1.3
weight %	0.88±0.04	0.	
(g)	1.27±0.14	1.17±0.06	0.9
weight %	0.690.06	0.70±.04	

The results show that sup
(levels 1 and 2) for 45 c
induced in rats by a chole

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diminished the increase in p

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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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Rice bran oil and

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alcohols, phytosterols, tocot

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Yet rice bran oil ty

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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!compone

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

Salt mixture 50 50 50 50 **Vitamin mix** 10 10 10 10 **Corn oil** 60 60 60 60 **Cellulose** 40 40 40 40 **C holesterol** 0 10 10 10 **Rice bran active compound** 0 0 10 20 **Starch** 620 610 600 590 **Total** 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 (80 % 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 triacylglycrol 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-sitosperol, 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 choìesterol decreased by 5% and LDL cholesterol decreasmd by 9% (Vissers *et al.* 2000). The investigators postulated that the effect was due to the β-sitosterol and other 4-dsmethylsterols and othur 4-desmethylsterols anl not to the ´,4-dimethylsterols, such as cycloartenol and 24-methylene"cycloartanol.

The β-sitosterol structure is more similar to that of chole{terol than is thát 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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