EXTRACTION AND SEPARATION OF γ-ORYZANOL FROM RICE BRAN OIL BY HPLC AND STUDYING ITS EFFECT ON RATS FED A HIGH-CHOLESTEROL DIET

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Abstract: This study investigated the effect of γ-oryzanol extracted from Rice Bran Oil (RBO) on rats fed a high-cholesterol diet. γ-oryzanol content in RBO were determined and separated by HPLC. γ-oryzanol was administered at different doses (250, 500 and 750 mg/100 g diet) for 50 days. It was found that serum Total Cholesterol (TC) concentration significantly decreased compared with the control. In addition, serum High Density Lipoprotein Cholesterol (HDL-C) levels, total protein and albumin were significantly increased and the concentration of Triglyceride (TG), Total Lipids (TL), Low-Density Lipoprotein Cholesterol (LDL-C), Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), urea and creatinine were significantly decreased after 50 days of the administration. Also, risk ratio and A/G ratio were calculated. The obtained results indicated that using 500 mg and 750 mg from γ-oryzanol were more beneficial than using 250 mg for lowering cholesterol in hyper-cholesterolemic rats. It could be recommended to use γ-oryzanol as natural source for cholesterol-lowering and effectiveness of doses used is due to 750 mg/100 g.

Keywords: rice bran oil; γ-oryzanol; feed efficiency; total cholesterol; high density lipoprotein; low density lipoprotein; risk ratio; total lipids; triglycerides; total protein; HPLC.

INTRODUCTION

Developing countries are experiencing dramatic changes in the health needs of their populations. Although many countries currently face a double burden of infectious diseases and Non-Communicable Diseases (NCDs), the latter, including cancer, diabetes and Cardiovascular Diseases (CVDs), are fast replacing the traditional enemies of infectious diseases and malnutrition as the leading causes of disability and premature death. This trend will continue and by the year 2020, NCDs are expected to account for seven out of every ten deaths in the developing regions, compared with less than half today (Murray and Lopez, 1996). With increasing consumption of dietary fat and animal-based food products, coronary

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heart disease is the leading cause of death (National Statistical Office, 2004; World Health Organization, 2002). Various risk factors of coronary heart disease have been elucidated for the treatment and prevention of the disease. Among them, abnormal lipid metabolism, such as an increase in blood cholesterol, triacylglycerol (TG) and lipoproteins, is considered to be a major factor (Jeppesen et al., 1998; Kestin et al., 1990; Stamler et al., 1986). It is well known that chronic diseases represent a major problem and public health burden in developing countries. It represents 73% of mortality and 60% of global morbidity burden. There is emerging evidence that diabetes mellitus, obesity, hypertension and hyperlipidemia contribute to national morbidity and mortality in Egypt as it represents about 26% of all deaths related to chronic diseases (Ministry of Health and population, Egypt, 2006). Phytosterols carry out functions in plants equivalent to those of cholesterol in animals, being thus required as necessary components of cell membranes and as precursors of important biomolecules, including sex hormones and vitamins. There are about 44 phytosterols known to exist in plants, the most abundant being β-sitosterol, campesterol and stigmsasterol. The food sources with the highest total phytosterol contents, as the sum of these three compounds (in mg/100 g), are the oils of rice bran (1055), corn (952), wheat germ (553), flax seed (338), cottonseed (327), soybean (221), peanut (206) and olive (176) (Kritchevsky, 1997). Rice bran is a by-product produced in the rice milling industry. It possesses approximately 10% weight of the total rice grain. Rice bran is an excellent source of lipids, containing from 18% to 22% oil, especially unsaturated fatty acids (McCaskill and Zhang, 1999; Orthoefer, 1996; Tanaka et al., 1973). Rice Bran Oil (RBO) (20–25 wt% in rice bran) is a unique rich source of commercially-important bioactive phytochemicals, most of them of interest in nutrition, pharmacy and cosmetics. Most research has been conducted to take advantage of the high contents in RBO of phytosteryl transferulates. The production of both RBO and RBO fractions enriched in γ-oryzanol, as well as the health properties of γ-oryzanol, were reviewed a few years ago (Patel and Naik, 2004). Since then, the interest in the production and applications of RBO, including fractions enriched in phytosteryl transferulates, has remarkably increased. It is a mixture of 10 ferulate esters of triterpene alcohol (Zhimin et al., 2001) and can be used to reduce blood cholesterol levels, treat nerve imbalance, as well as an antioxidants and preservation (Murase and Iishima, 1963; Rong et al., 1994; Sasaki et al., 1990). The unsaponifiable constituents of RBO include mainly tocols (vitamin E, 0.10–0.14%) and γ-oryzanol (esters of transferulic acid with sterols and triterpenic alcohols, 0.9–2.9%) (Lerma-Garcĭa et al., 2009). The concentrations of tocols and γ-oryzanol in RBO depend largely on genetic and environmental factors, being within the 0.10–0.14% and 0.9–2.9% ranges, respectively (Diack and Saska, 1994; Lloyd et al., 2000; Patel and Naik, 2004). γ-Oryzanol is mainly composed of esters of transferulic acid (trans-hydroxycinnamic acid) with phytosterols (sterols and triterpenic alcohols). Among these, cycloartenol, β-sitosterol, 24-methylenecycloartenol and campesterol predominate (Lloyd et al., 2000; Xu and Godber, 1999). γ-Oryzanol also contains lower concentrations of esters of the trans-ferulic acid with Δ^7-stigmsasterol, stigmsasterol, Δ^7-campesterol, Δ^7-sitostenol, campestenol and sitostenol (Xu and Godber, 1999), as well as esters of cis-ferulic (Akihisa et al., 2000) and caffeic acids (Fang et al., 2003). The major components of γ-oryzanol in rice bran are cycloartenyl ferulate, and campestanyl ferulate (Zhimin et al., 2001). The γ-oryzanol was first extracted from

RBO and presumed to be a single component (Rogers et al., 1993). Physiological effects that have been shown to be associated with oryzanol intake are decreasing plasma cholesterol (Yoshion et al., 1989), decreasing platelet aggregation, decreasing hepatic cholesterol biosynthesis, increasing fecal bile acid excretion and decreasing cholesterol absorption. γ-oryzanol has also been used to treat nerve imbalance and disorders of menopause (Rogers et al., 1993). So, we present methodology for extraction γ-oryzanol from RBO, simultaneous determination of RBO γ-oryzanol by HPLC and study is to know whether γ-oryzanol a major unsaponifiable components of RBO, have a greater cholesterol-lowering activity and possibly different mechanism(s) of action with regard to their potential antiatherosclerotic property.

MATERIALS AND METHODS

Sample and chemicals

Rice bran was obtained from Rice Breeding Section, Field Crops Research Institute, Agriculture Research Center, Egypt. kits of TC; HDL-C; TG; TL; ALT; AST; total protein; albumin; globulin; urea; creatinine; were obtained from Biodiagnostic Co., 29 El-Tahreer St., Dokki-Giza, Egypt, E-mail Biodiagonstic eka @ Lycos. Com. Pure cholesterol was obtained from Sigma Chemical Co., Saint Louis, Missouri, USA.

Extraction of γ-oryzanol

Rice bran (50 g) was allowed to stand overnight at room temperature (25°C) with chloroform-methanol (2:1, 300 ml). After filtration the sample residue was reextracted with acetone (2 × 100 ml). The chloroform-methanol and acetone extracts were combined and reduced to a freely flowing oil by rotary evaporation. The total extract volume was adjusted to 10 ml with acetone. After addition of an equal volume of 4 M sodium hydroxide the neutral lipids were extracted with light petroleum (boiling range 40–60°C, 3 × 20 ml). The light petroleum was washed with 4 M sodium hydroxide (20 ml) and the combined aqueous alkali extracts adjusted to pH 7.0 with concentrated aqueous hydrochloric acid and then extracted with diethyl ether (3 × 20 ml) to give the phenolic materials. The solvent yielded was evaporated (Evershed et al., 1988).

High-performance liquid chromatography

Reversed-phase HPLC was used to separate acetylated steryl ferulates bearing differing sterol moieties. Solvent delivery was by a Kontron LC414-T pump (Kontron Analytical. St. Albans, UK). Detection was at 280 nm with a Kontron UVIKON 740LC fixed-wavelength spectrophotometric detector (Kontron Analytical). Samples were introduced with a Rheodyne (Cotati, CA, USA) 7125 injector with a 20-μl loop. Separation was performed on a 15 cm × 4.6 mm S3 ODS Spherisorb (3-μm) column (Phase Separations. Queensferry, UK), with isocratic elution (0.5 ml min^{-1}) of acetonitrile-methanol (1:1). The steryl ferulates were acetylated by treatment with acetic anhydride-pyridine (1:1, 100 μl, 50°C, 30 min) (Evershed et al., 1988).

Biological methods

Male albino adult rats (30 animals weighing 140 g ± 2) were obtained from the private market, Helwan, Giza, Egypt, then transported to Animal House of Ophthalmology Research Institute, Giza, Egypt.

Rats were housed in individual cages with screen bottoms and fed on basal diet (corn starch 70%, casein 10%, corn seed oil 10%,

cellulose 5%, salt mixture 4% and vitamins mixture 1%) for seven days. After equilibration, rats were weighted and divided into five groups (six animals per each) every one was assigned to one of the five diet groups (Negative Control (NC), hypercholesterolmic (Positive Control (PC)) and three hypercholesterolmic groups given γ-oryzanol (250, 500, 750mg/100g diets).

Total feed consumption was weighted, fresh feed was provided every day and total body weight of the animals was recorded at the beginning and during the experimental period. Blood samples were collected from the orbital plexus by mean of heparinized capillary glass tubes according Schermer (1967). Each sample was placed into a dry clean centrifuge tube and centrifuged 1500 × g for 30 min. at 4°C to obtain serum. Total Cholesterol (TC) was determined according to the method described Allain et al. (1974), Total Lipids (TL) were determined according to the method described Kinght et al. (1972) and triglycerides were determined according to the method described Fossati and Prencipe Precipe (1982).

High Density Lipoprotein Cholesterol (HDL-C) was determined according to the method described Lopez-virella et al. (1977) and Low-Density Lipoprotein Cholesterol (LDL-C) levels and risk ratio were calculated for serum samples using the formula of Friedewald et al. (1972) using the following equations:

$$\text{LDL-C} = \text{T.C} - \left[\frac{\text{T.G}}{5+\text{HDL-C}}\right]$$

$$\text{Risk ratio} = \frac{\text{LDL-C}}{\text{HDL-C}}$$

Serum transaminases sAST and sALT (Aspartate transferase and Alanine transferase) were measured colorimetrically according to the method described Reitaman and Frankel (1957). Serum urea was determined according to Fawcett and Soctt (1960) and creatinine was determined according to the method of Barthes et al. (1972). The protein and albumin contents in serum were determined according to the methods of Lowry et al. (1977) and Doumas and Biggs (1971).

Statistical analysis of data

Data collected from biological evaluation were statistically analysed using one-way ANOVA with post hoc Newman Keuls, test. $P < 0.05$ was considered significant. All data are expressed as mean ± S.D.

RESULTS

The result in Table 1 shows that γ-oryzanol was 1.88 mg/100 g RBO.

Figure 1 shows the analysis of γ-oryzanol mixture by HPLC.

Effect of feeding rats for 50 days on different diets contained basal (NC), hypercholesterolemic (PC) and 250, 500, 750 mg γ-oryzanol are recorded in Table 2. All rats significantly increased in their weight but the maximum increase was found in rats fed on NC. The rats fed on 500 and 750 mg γ-oryzanol diets can be grow in high rate than rats fed on 250 mg γ-oryzanol and PC diets. In the same Table feed intake show that rats fed on NC gave the highest body weight gain consumed the highest amount of their diet which reflected on their weight

Table 1 γ-oryzanol content in RBO

Constituent	mg/100 g
γ-oryzanol	1.88

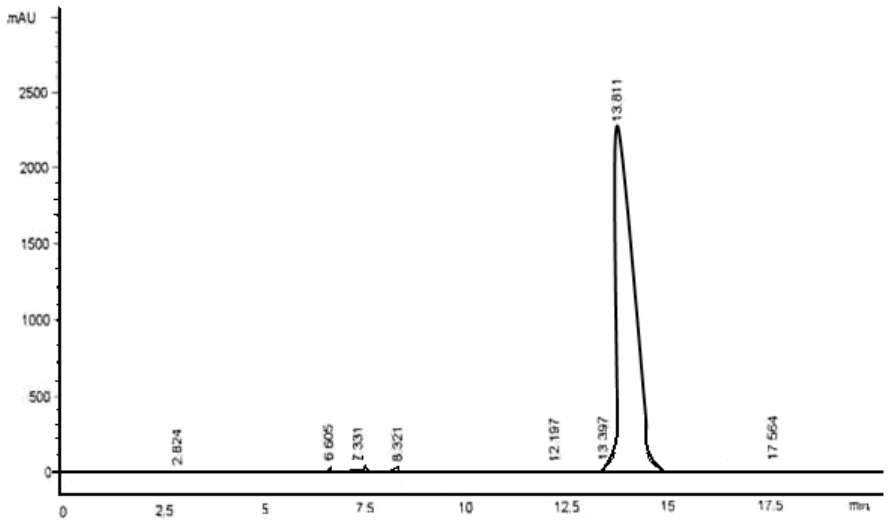

Figure 1 Analysis of γ-oryzanol mixture by HPLC

Table 2 Body weight, feed intake and feed efficiency of rats

Treatments	Initial body weight	Final body weight	Body weight gain	Daily body weight increase	Feed intake	Daily feed intake	Feed efficiency	Feed efficiency rate
NC	140.32 ± 7.39	275.01 ± 16.18	134.69 ± 7.92	2.7487 ± 0.15	918.92 ± 48.36	18.754 ± 1.04	0.1465 ± 0.01	14.65 ± 0.76
PC	141.83 ± 8.34	193.70 ± 10.76	51.87 ± 2.88	1.0586 ± 0.01	583.12 ± 32.40	11.900 ± 0.66	0.0890 ± 0.01	8.90 ± 0.49
Oryzanol 250 mg	139.92 ± 7.77	257.30 ± 13.54	117.38 ± 6.18	2.3955 ± 0.06	879.35 ± 46.28	17.946 ± 0.95	0.1335 ± 0.01	13.35 ± 0.79
Oryzanol 500 mg	140.82 ± 7.41	262.37 ± 13.81	120.73 ± 6.71	2.4639 ± 0.06	896.21 ± 48.29	18.290 ± 1.08	0.1317 ± 0.01	13.17 ± 0.73
Oryzanol 750 mg	142.00 ± 8.35	268.89 ± 14.94	126.89 ± 6.68	2.5895 ± 0.07	902.57 ± 53.09	18.420 ± 0.97	0.1406 ± 0.01	14.06 ± 0.83

Note: NC = Negative Control (basal diet); PC = Positive Control (hypercholesterolemic). Each value is mean ± SD for six rats in each group.

followed by rats fed on 750 mg γ-oryzanol. Daily feed intake followed the same trend. For feed efficiency and feed efficiency rate results indicate that NC and 750 mg γ-oryzanol had maximum feed efficiency and feed efficiency rate as compared with rats fed on 250 and 500 mg γ-oryzanol. On the other hand, the rats feed on PC obtained the lowest feed efficiency and feed efficiency rate.

Table 3 Serum TC levels (mg/dl) in normal and hypercholesterolimic rats

Treatments	T.C (mg /dl)								
	Zero time	After 1 week	After 2 weeks	After 3 weeks		After 4 weeks	After 5 weeks	After 6 weeks	After 7 weeks
NC	94.36 ± 4.97	96.21 ± 5.35	94.99 ± 4.10	96.11 ± 5.06	NC	95.56 ± 5.31	96.37 ± 5.35	96.00 ± 5.33	96.32 ± 5.07
					PC	264.99 ± 13.95	273.14 ± 16.07	286.01 ± 15.05	287.35 ± 6.05
PC	94.36 ± 4.97	140.10 ± 7.78	198.30 ± 10.44	254.01 ± 14.94	Oryzanol 250 mg/ 100 g diet	201.70 ± 11.21	159.30 ± 10.85	132.68 ± 6.98	108.81 ± 6.05
					Oryzanol 500 mg/ 100 g diet	191.21 ± 10.62	143.00 ± 8.41	127.88 ± 6.73	103.00 ± 6.06
					Oryzanol 750 mg/ 100 g diet	182.10 ± 10.12	139.00 ± 7.32	123.78 ± 6.88	101.61 ± 5.35

Note: NC = Negative Control (basal diet); PC = Positive Control (hypercholesterolemic). Each value is mean ± SD for six rats in each group.

Data in Table 3 indicates that feeding rats on 250, 500 and 750 mg γ-oryzanol decreased TC in their serum (108.81, 103.00 and 101.61 mg/dl, respectively). Significant differences were found between all the used treatments (diet). On the other hand, TC increased form 94.36 mg/dl in NC to 287.35 mg/dl in PC for 50 days.

Data in Table 4 summarises that HDL followed opposite trend (51.32, 54.03, 57.12 mg/dl) in rat fed on 250, 500 and 750 mg γ-oryzanol, respectively. The level of HDL in rats serum fed on PC diet is the lowest one (38.01 mg/dl). Moreover, the effect of the studied diets significantly differed on risk ratio (LDL value/HDL value) of blood serum of used rats. This ratio was in the maximum level in rats fed on PC diet (5.586), while this ratio was in ascending order ranged from 0.682 to 0.972 for 750, 500 and 250 mg γ-oryzanol, respectively. Rats fed on NC diet significantly had the lowest risk ratio as compared with the other used diets. The decrease in LDL and increase in HDL may be due to the increase in hepatic LDL receptor activity. From the same Table the results of serum TL and triglycerides of rats fed on the different γ-oryzanol diet are shown. Serum TL and triglycerides significantly increased from 418.13 and 143.21 mg/dl in NC diet to 889.51 and 342.00 in PC diet and to 487.00, 454.11, 439.72, 162.31, 159.01 and 154.11 mg/dl for rats fed on 250, 500 and 750 mg γ-oryzanol, respectively.

With respect to serum total protein, albumin, globulin and A/G ratio, recorded in Table 5. Data show the amounts of these contents, the increase and decrease in protein, albumin, globulin and A/G ratio in rats fed NC and PC diets or in rats fed on γ-oryzanol. Serum total protein and albumin decreased in rats fed PC diet from 7.23

Table 4 Serum total lipid, triglycerides, HDL, LDL and risk ratio levels (mg/dl) in normal and hypercholesterolimic rats

Treatments	Total lipid mg/dl	Triglycerides mg/dl	HDL mg/dl	LDL mg/dl	Risk ratio LDL-C/ HDL-C
NC	418.13 ± 23.23	143.21 ± 7.54	62.05 ± 3.27	32.08 ± 1.89	0.517 ± 0.03
PC	889.51 ± 49.42	342.00 ± 19.00	38.01 ± 2.78	212.31 ± 11.74	5.586 ± 0.31
Oryzanol 250 mg/100 g diet	487.00 ± 25.63	162.31 ± 9.02	51.32 ± 2.85	49.88 ± 2.77	0.972 ± 0.06
Oryzanol 500 mg/100 g diet	454.11 ± 25.23	159.01 ± 9.35	54.03 ± 3.00	42.11 ± 2.43	0.779 ± 0.04
Oryzanol 750 mg/100 g diet	439.72 ± 25.87	154.11 ± 8.56	57.12 ± 3.17	38.93 ± 2.29	0.682 ± 0.04

Note: NC = Negative Control (basal diet); PC = Positive Control (hypercholesterolemic). Each value is mean ± SD for six rats in each group.

Table 5 Serum total protein, albumin, globulin and A/G ratio levels (mg/dl) in normal and hypercholesterolimic rats

Treatments	Total protein mg/dl	Albumin (A) mg/dl	Globulin (G) mg/dl	A/G ratio
NC	7.23 ± 0.38	3.87 ± 0.22	3.11 ± 0.16	1.24 ± 0.07
PC	4.88 ± 0.27	0.99 ± 0.05	3.69 ± 0.21	0.27 ± 0.01
Oryzanol 250 mg/100 g diet	7.88 ± 0.42	4.01 ± 0.22	3.22 ± 0.17	1.26 ± 0.07
Oryzanol 500 mg/100 g diet	7.66 ± 0.45	4.00 ± 0.24	3.51 ± 0.21	1.14 ± 0.07
Oryzanol 750 mg/100 g diet	7.53 ± 0.40	3.92 ± 0.22	3.48 ± 0.18	1.13 ± 0.06

Note: NC = Negative Control (basal diet); PC = Positive Control (hypercholesterolemic). Each value is mean ± SD for six rats in each group.

mg/dl and 3.87 mg/dl to 4.88 mg/dl and 0.99 mg/dl, respectively compared with fed NC diet. It clear from the previous data that rats of PC obtained the lowest content of serum protein and albumin, while rats fed on 250 mg γ-oryzanol had the highest level.

Table 6 Serum sALT (U/dl), sAST (U/dl), urea and Creatinine levels (mg/dl) in normal and hypercholesterolimic rats

Treatments	sALT U/dl	sAST U/dl	Urea mg/dl	Creatinine mg/dl
NC	18.02 ± 1.06	31.18 ± 1.83	17.32 ± 1.02	0.63 ± 0.04
PC	69.13 ± 3.64	92.52 ± 4.87	68.81 ± 3.82	4.01 ± 0.21
Oryzanol 250 mg/100 g diet	24.41 ± 1.29	40.00 ± 2.22	24.01 ± 1.26	0.78 ± 0.05
Oryzanol 500 mg/100 g diet	20.88 ± 1.23	38.10 ± 2.01	21.51 ± 1.20	0.69 ± 0.04
Oryzanol 750 mg/100 g diet	19.28 ± 1.02	36.33 ± 1.91	19.99 ± 1.18	0.67 ± 0.04

Note: NC = Negative Control (basal diet); PC = Positive Control. Each value is mean ± SD for six rats in each group.

Rats fed on 500 and 750 mg γ-oryzanol lay in between. In the same table the highest globulin content was in rats fed on PC diet followed by rats fed on γ-oryzanol diets. The decrease in serum albumin may be due to the effect of hypercholesterolemic diets on RNA, protein synthesis and increased protein losses.

The data presented in Table 6 shows the level of sALT, sAST, urea and creatinine in the serum of albino rats as administrated NC diet (18.02 U/dl, 31.18 U/dl, 17.32 mg/dl and 0.63 mg/dl), respectively, while rats of PC gave the high levels (69.13, 92.52 U/dl, 68.81 and 4.01 mg/dl), respectively of this parameters. γ-oryzanol as an individually diet resulted in increasing the level of the previous components from (19.28 to 24.41 U/dl), (36.33 to 40.00 mg/dl), (19.99 to 24.01 mg/dl) and (0.67 to 0.78 mg/dl) for sALT, sAST, urea and creatinine, respectively.

DISCUSSION

It is well known that chronic diseases represent a major problem and public health burden in developing countries. It represents 73% of mortality and 60% of global morbidity burden. There is emerging evidence that diabetes mellitus, obesity, hypertension and hyperlipidemia contribute to national morbidity and mortality in Egypt as it represents about 26% of all deaths related to chronic diseases (Ministry of Health and population, Egypt 2006).

The concentrations of tocols and γ-oryzanol in RBO depend largely on

Table 7 Cholesterol level of Egyptian population stratified by gender

Cholesterol level	Male (%)	Female (%)	Both (%)
≥5.2 mmol/L	15.7	23.1	19.4
≥6.5 mmol/L	2	4.4	3.3

Source: Ministry of Health and population, Egypt (2006).

Table 8 Rate of hypercholesterolemia stratified by age group and gender, Egypt, 2005
The prevalence of high serum cholesterol (≥ 5.2 mmol/L) was 19.4% being higher in females (23.1%) than males (15.7%)

Age group	Total cholesterol ≥5.2 mmol/L or ≥200 mg/dl			Total cholesterol ≥5.2 mmol/L or ≥200 mg/dl		
	Men (%)	Women (%)	Both sexes (%)	Men (%)	Women (%)	Both sexes (%)
15–24 years	7.9	11.4	9.7	0.3	2.1	1.2
25–34 years	15.7	18.5	17.0	0.7	3.6	2.1
35–44 years	21.6	28.5	25.2	4.7	3.0	3.8
45–54 years	22.4	35.2	29.1	4.3	8.5	6.5
55–65 years	20.4	41.7	31.1	2.7	10.4	6.6
15–65 years	15.7	23.1	19.4	2.0	4.4	3.3

Source: Ministry of Health and population, Egypt (2006).

genetic and environmental factors, being within the 0.10–0.14% and 0.9–2.9% ranges, respectively (Diack and Saska, 1994; Lloyd et al., 2000; Patel and Naik, 2004). The unsaponifiable constituents of RBO include mainly tocols (vitamin E, 0.10–0.14%) and γ-oryzanol (esters of trans-ferulic acid with sterols and triterpenic alcohols, 0.9–2.9%) (Lerma-Garcĭa et al., 2009). The chromatogram of total γ-oryzanol was similar to those of Evershed et al. (1988), Rogers et al. (1993), Xu and Godber (1999) and Chen and Bergman (2005). γ-Oryzanol in RBO was preseparated and quantified by HPLC-UV, and its profile was established online by GC (Miller et al., 2003). Identification of 24-methylenecycloartenyl trans-ferulate, campesteryl trans-ferulate, b-sitosteryl transferulate and campestanyl trans-ferulate was confirmed by off-line GC–MS. Fang et al. (2003) have used direct RBO analysis by RP-HPLC–MS/MS to address inconsistencies in the reported number and structures of ferulates. Nine novel phytosteryl esters were characterised by their chromatographic retention and both negative- and positive-ion mode ESI-MS. Evidence for the presence of hydroxylated ferulate esters and caffeate esters as part of γ-oryzanol was obtained. It has been found that γ-oryzanol exhibits antioxidant properties in many types of in vitro model systems, such as cholesterol oxidation acceterated by 2,2'-azobis 2-methylpropionamidine (Xu et al., 2001). Cholesterol is transported in the blood plasma of all animals by lipoproteins, which have a wide range of molecular sizes, including VLDL, IDL, LDL and HDL. Hyperlipoproteinaemias are heritable disorders associated with increased plasma concentrations of cholesterol, higher LDL-cholesterol than normal, implying a high risk of CVD. The first (and sometimes the only) therapeutic approach to hyperlipoproteinaemias is diet. In this concern, RBO and its main components have demonstrated an ability to improve the plasma lipid pattern of rodents, rabbits, non-human primates and humans, reducing total plasma cholesterol and triglyceride concentrations, and increasing the HDL cholesterol level. γ-oryzanol was shown to be able to reduce cholesterol absorption (Rong et al., 1997). It was appropriate for the treatment of the inflammatory process (Akihisa

et al., 2000) and it could inhibit linoleic acid and cholesterol oxidation (Xu and Godber, 2001; Xu et al., 2001). The oryzanol-fed hamsters excreted significantly more coprostenol and cholesterol in their feces than the ferulic acid (127% and 120%, respectively) diet (Wilson et al., 2007). Tsuji et al. (2003) studied the effects of different contents of γ-oryzanol in RBO on serum cholesterol levels of rats fed with a Hypocholesterolaemic Diet (HCD). Dietary lipid sources were lard in the control group, and normal RBO, with either low or high levels increased in the control group, and clearly decreased in the RBO-fed groups. Wilson et al. (2007) also conducted a study using hamsters, to separately establish the relative cholesterol-lowering activities of RBO, γ-oryzanol and trans-ferulic acid. They found that plasma TC and VLDL + LDL cholesterol concentrations, were significantly lower in the group fed with the RBO (–64% and –70%, respectively), *trans*-ferulic acid (–22% and –24%, respectively) and γ-oryzanol (–70% and –77%, respectively) diets, compared to the control. The levels of plasma triglycerides, lipid hydroperoxides and aortic cholesterol ester accumulation were also much lower in the groups fed with the RBO and γ-oryzanol diets. In the current study, we observed that both 0.5% oryzanol and 0.5% ferulic acid lowered plasma TC and non-HDL-C compared to the control hamsters; however, the hamsters fed the oryzanol diet exhibited significantly lowered plasma TC and non-HDL-C than did ferulic acid. Also, the amount of lowering by oryzanol was similar to RBO in the current study. The cholesterol-lowering activity of oryzanol has been demonstrated by other investigators (Berger et al., 2005; Nakamura, 1966; Shinomiya et al., 1983; Yoshino et al., 1989). Shinomiya et al. (1983) showed that when 0.5% oryzanol was fed to rats on a high cholesterol diet, plasma TC was significantly decreased after eight weeks on the dietary treatment. In a study reported by Seetharamaiah and Chandrasekhara (1988), plasma non-HDLC was significantly reduced in rats fed 0.2% or more oryzanol after seven weeks of dietary treatment. They also showed that when oryzanol was added to a cholesterol-free diet, no differences in plasma lipids were observed, suggesting that oryzanol may affect cholesterol metabolism by altering dietary cholesterol absorption. In the current study, we observed that hamsters fed 0.5% oryzanol increased their fecal excretion of coprostanol and cholesterol, thus, TC excretion, compared to the hamsters fed the ferulic acid diet but not to the control. The hamsters fed the RBO diet excreted more fecal cholesterol than the hamsters fed the ferulic acid diet. Thus, it appears that a major mechanism by which oryzanol and possibly RBO lower blood cholesterol concentrations is via increased fecal excretion of cholesterol and its metabolic products (Wilson et al., 2007). The abnormal higher level of serum AST and ALT observed in ethanol treated mice is the consequence of daily dose of ethanol. *Trans*-ferulic acid and γ-oryzanol gave a high hepatoprotective effect of reducing the increased AST and ALT activities by ethanol. The observed significant decreases in the activities of these enzymes suggest that *trans*-ferulic acid and γ-oryzanol preserve the liver injury by the toxic effects of daily dose of ethanol such as curcumin (Chotimarkorn and Ushio, 2008). The activities of sALT and sAST were determined in serum to evaluate the role of full fat rice bran, crude RBO, refined RBO and defatted rice bran on liver functions. All the rice diets decreased sALT with the following decreasing order; full fat rice bran 96.85 > crude RBO 97.12 > refined RBO 102.53 > defatted rice bran 107.31%. While

sAST was also deceased in the same order; full fat rice bran 100.96 > crude RBO 102.64 > refined RBO 102.82 > defatted rice bran 107.48% as compared with NC (100%) (Soheir, 2000). Soheir et al. (2004) also conducted the effect of unextruded and extruded bran of rice, barley, wheat and corn on Glutamic Oxaloacetic Transaminases (GOT), Glutamic Pyrovic Transaminases (GPT), urea and creatinine. Rats fed on NC showed the lowest levels (39.10, 16.34, 15.61, 0.55), while rats of PC gave the highest levels (82.65, 58.81, 62.84, 3.24) of these paramiters, respectively. It is evident that all value were significantly followed the same trend. Unextruded and extruded bran of rice, barley, wheat and corn as an individually diet resulted in increasing the level of the previous components from (40.38 to 72.10); (17.33 to 42.24); (16.34 to 48.44) and (0.59 to 2.85) for GOT; GPT; urea and creatinine, respectively. Total protein, albumin, globulin and A/G ratio were determined in serum to evaluate the role of full fat rice bran, crude RBO, refined RBO and defatted rice bran on their metabolism. All the rice bran diets increased protein in the serum with the following decreasing order; full fat rice bran 97.44 > crude RBO 95.08 > defatted rice bran 95.55 > refined RBO 93.39%. While albumin was decreased in the same order; full fat rice bran 98.55 > crude RBO and defatted rice bran 93.60 > refined RBO 87.79%. Globulin in different groups did not varied so much, it could be said, that in hypercholesterolemic rats serum globulin increased but this increase was not significant (Soheir, 2000). Serum total protein and albumin decreased in rats fed on NC and PC diets from 6.96 mg/dl and 3.44 mg/dl to 4.0 mg/dl and 0.95 mg/dl, respectively. Rats of PC obtained the lowest content of serum protein and albumin, while rats fed on unextruded or extruded barley or rice bran had the highest levels (Soheir et al., 2004).

CONCLUSION

The results indicated that γ-oryzanol was 1.88 mg/100 g RBO. Separation and analysis of γ-oryzanol mixture is successfully performed by using HPLC. Rats fed γ-oryzanol 750 mg had maximum feed efficiency and feed efficiency rate as compared with rats fed on 250 and 500 mg γ-oryzanol. Feeding rats on 250, 500 and 750 mg γ-oryzanol decreased TC in their serum (108.81, 103.00 and 101.61 mg/dl, respectively). Significant differences were found between all the used treatments (diet). Risk ratio was in the maximum level in rats fed on PC diet (5.586), while this ratio was in ascending order ranged from 0.682 to 0.972 for 750, 500 and 250 mg γ-oryzanol, respectively. Serum TL and triglycerides significantly increased from 418.13 and 143.21 mg/dl in NC diet to 889.51 and 342.00 in PC diet and to 487.00, 454.11, 439.72, 162.31, 159.01 and 154.11 mg/dl for rats fed on 250, 500 and 750 mg γ-oryzanol, respectively. γ-oryzanol as an individually diet resulted in increasing the level of the previous components from (19.28 to 24.41 U/dl), (36.33 to 40.00 mg/dl), (19.99 to 24.01 mg/dl) and (0.67 to 0.78 mg/dl) for sALT, sAST, urea and creatinine, respectively.

BIOGRAPHY

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