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Biological Evaluation of Microwave Defatted Black Rice Bran in Hypercholsterolemic Rats.

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ABSTRACT

This study was performed to investigate the effect of feeding on Microwave defatted black rice bran (MDBLRB) in hypercholesterolemic rats . Chemical composition and total phenolic compounds (TPC) of microwave defatted black rice bran (MDBLRB) and microwave full fat black rice bran (MFBLRB) were determined **.** Results revealed that (MDBLRB) protein, ash, fiber and carbohydrates contents of (18.20, 0.70, 10.1, 13.7 and 71.00) were higher than those of MFBLRB. Also, total phenolic compounds (TPC) being 561 as mg Tannic acid equivalent/kg for (MDBLRB) in compare with 486 as mg Tannic acid equivalent/kg for MFBLRB, obtained results illustrated that substitution of feeding hypercholesterolemic diet with MDBLRB led to improvement the High lipoprotein cholesterol HDL-C. Furthermore, hypercholesterolemic diet with (MDBLRB) replacement at 75% and 100% also recorded the best and nearest of HDL-C to the negative control. It was observed also that LDL- cholesterol value of negative control diets (G1and G2) were 46.78 and 45.15 mg/dl, but also the value of the hypercholesterolemic in control (G3) was 198.77mg/dl. On the other hand, the LDL cholesterol of rats fed on hypercholesterolemic diet substitution with MDBLRB 25,50.75 and 100% (G4, G5, G6 and G7) being 50.67, 47.85, 46.28 and 41.73 mg/dl, respectively. At the final of experiment (10 weeks), ALT was significantly increased for the hypercholesterolemic control (G3) was 50.30 U/L, while negative control (G1 and G2) were 25.80 and 24.20 U/L respectively. Feeding on hypercholesterolemic diets substituted with MDBLRB (G4, G5, G6 and G7) led to a more reduction at level 25, 50, 75 and 100% were 38.52, 36.61, 34.34 and 32.55 U/L, respectively comparing with hypercholesterolemic control (G3). Briefly it be could conclude that MDBLRB has pronounced effect in lowering cholesterol serum levels and may be useful for patients suffering from liver and cholesterol diseases

Keywords: Black rice bran, Hypercholesterolemic

INTRODUCTION

Rice bran is the outer layer of raw rice that is obtained as a by-product of paddy milling include protein, fat, carbohydrate, dietary fiber, ash, vitamin, mineral and antioxidant compounds (Saenjum *et al.,* 2012 and Chinma *et al.,* 2015).

Recently, the use of rice bran is gaining importance in many research due to the reality that, during the processing of whole rice, large amounts of the grain's outer layers are eliminated, raising the concentration of nutrients in the bran and rendering it an important source of nutrients for the food industry(Imsanguan *et al*., 2008 and Faria *et al.,* 2012)

Direct use of the whole rice bran is problematic, because it rapidly turns into rancid due to the hydrolysis of neutral fatby using quite energetic lipase enzymes right after the milling (Sibakov *et al.,* 2013). Therefore, the rice bran needs to be either stabilized or further processed into different fractions for food application. After oil extraction, the residual defatted rice bran can be further valorised for dietary fiber production (Nandi and Ghosh, 2015). Rice bran dietary fiber (RBDF) is in particular composed of cellulose, hemicellulose, lignin, and pectin substances (Chinma *et al.,* 2015). Most dietary fiber from rice bran is insoluble and called insoluble dietary fiber (IDF); only a small part is soluble and called soluble dietary fiber (SDF). IDF has proven the physiological functionalities of helping the growth of intestinal microflora, increasing the faecal bulk, decreasing the intestinal transit, and inhibiting the pancreatic lipase activity (Foschia, *et al.,* 2013 and He *et al.,* 2015).

Rice bran also include phytochemical compounds in significant amount and these compounds have been taken into consideration as natural antioxidants (Gullón *et al.,* 2014). Furthermore, Anthocyanin pigments have noticeably powerful in lowering cholesterol levels within the human body, reduce the dangers of cardiovascular diseases and assist lower cholesterol levels (Thounaojam *et al.,* 2012).

Several research has verified that elevation in serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) and reduction in high-density lipoprotein cholesterol (HDL-C) augmentation the danger of atherosclerosis and coronary heart disease (Ausman *et al.,* 2005). Oxidative damage initiated by free radicals is a primary contributor to CVD development (Revilla *et al.,* 2005)

MATERIALS AND METHODS

Materials:

Black Rice bran *(Oryzasativa L.)* was obtained during the season of 2015 from Rice Research and Training Center (RRTC) at Sakha,Kafr El-Sheikh Governorate, Egypt. Other chemicals substances had been of analytical reagent grade were used.

Methods:

Microwave stabilized black rice bran: MBLRB was treated in a microwave oven (LG – 550W) set at level 5 for 3 minutes and manually mixed every minute. All collected and prepared samples were kept at room temperature to cool after which sealed in plastic bags (polypropylene) and stored in a freezer at -12ºC for much less than 24hr., until analyses. (Faria *et al.,* 2012).

Extraction of rice bran oil: -

Rice bran oil were extracted according to the method described by Kahlon et al. (1992)

Gross chemical composition of black rice bran:

Chemical composition analysis based on the Official Methods of Analysis of A.O.A.C. (2005), including: moisture , ash, crude protein, fat and dietary fiber contents. Total carbohydrates was calculated based on the difference .Phenolics compounds were

determined by the method described by Nara *et al.,* (2006).

Total phenolic compounds of the extract were performed according to the method of Bonoli *et al.,* (2004). Phenolics- acid was predestined by a standard curve prepared using Tannic acid.

Biological assay :

Animal and experimental design:

Seventy animals of adult male albino rats (173-175g) were taken from Food Technol. Res. Instit. Agric. Res. Giza, Egypt. Animals have been housed in cages with screen bottoms and fed on a basal diet for ten days underneath laboratory conditions. Rats had been given free access to food and water throughout the experimental period of 10 weeks after the adaptation period, 70 rats were randomly assigned to the control and hypercholesterolemic groups. Rats were divided into seven groups each ten, with similar body weight . Rats were feed according to the following schema:

Group1(G1): Fed on the basal diet (negative control).

Group2(G2): Fed on the basal diet substituted 100% (MDBLRB) (negative control).

Group3(G3): Fed on Hypercholesterolemic diet (Positive control).

Group4(G4): Fed on Hypercholesterolemic diet substituted 25% (MDBLRB)

Group5(G5): Fed on Hypercholesterolemic diet substituted 50% (MDBLRB).

Group6(G6): Fed on Hypercholesterolemic diet substituted 75% (MDBLRB).

Group7(G7): Fed on Hypercholesterolemic diet substituted 100% (MDBLRB)

The composition of different experimental diets illustrated in Table (1). At the end of experimental period (10 weeks), rats were fasted over night before sacrificing. Blood was collected and centrifuged. Serum was separated for analysis as reported by LanePeter and Person, 1971

Table 1. Composition of different experimental hypercholesterolemic diets.

Groups / Ingredients	G1	G2	G3	G4	G5	G6	G7
MDBLRB	0	778.21	0	194.55	389.10	583.65	778.21
Corn starch	710.5	128.13	598.0	440.96	331.1	173.73	15.63
Casein	140	0	140	105	70	35	0
Corn oil	50	44.16	50	48.54	47.08	45.62	44.16
Cellulose	50	0	50	24.41	0	0	0
Mineral mixture	35	35	35	35	35	35	35
Vitamin mixture	10	10	10	10	10	10	10
Cholesterol	0	0	10	10	10	10	10
Beef tallow	0	0	100	100	100	100	100
Bile salt	0	0	2.5	2.5	2.5	2.5	2.5
L-cystine	2	2	2	2	2	2	2
Choline chloride	2.5	2.5	2.5	2.5	2.5	2.5	2.5

MDBLRB: Microwave defatted black rice bran
G1: Feed on the basal diet (negative control).
G2: Feed on the basal diet (negative control substituted 100% (MDBLRB)).
G3: Feed on Hypercholesterolemic diet (Positive control).
G4: Feed on Hypercholesterolemic diet substituted 25% (MDBLRB) for casein.
G5: Feed on Hypercholesterolemic diet substituted 50% (MDBLRB) for casein.
G6: Feed on Hypercholesterolemic diet substituted 75% (MDBLRB) for casein.
G7: Feed on Hypercholesterolemic diet substituted 100% (MDBLRB) for casein.

All rats were weighed weekly so as food intake throughout the experimental period. The determination of body weight gain (B.W.G.) and food efficiency ratio (F.E.R.) according to the method of Chapman *et al.* (1959) using the following equation:

B.W.G.% = [(Final Weight) - (Initial Weight)] / [(Initial Weight)] x 100

F.E.R. = [Gain in body(g/day)/ Feed intake (g/day)]x 100.

Blood sampling

Blood samples were taken from all experimental groups at the end of the experiment. Blood samples were collected from Vein plexus eye after 12 hours fasting, then centrifuged at 3000 rpm for 15 min to separate the serum which was carefully aspirated and transferred into clean quite plastic tubes and kept frozen at -18 ^{0}C until biochemical analysis using the procedure of (El-Khamissy, 2005).

Collection of organs:

Liver, kidney and heart of each animal were removed and weighed immediately.

The relative weight of the organs (R.O.W) was calculated following the next equation:

(R.O.W) = (Organ weight / Animal weight) x 100

Biochemical Analysis and Enzymes Assays: -

Triglycerides were performed following the method of Fossati and Prancipe (1982). Total cholesterol (T.C.) and High density lipoprotein cholesterol H.D.L.-C had been carried out in step with the method of Richmond (1973). Low-density lipoprotein cholesterol concentration and very low - density lipoprotein cholesterol (VL.D.L-c) were calculated mathematical according to the method of Friedwald, *et al.,* 1972

L.D.L. cholesterol = total cholesterol – H.D.L - cholesterol – triglycerides/5

$$\textbf{VL.D.L.- c} = \frac{\text{Triglycerides}}{5}$$

The activity of serum glutathione peroxidase (G.P.x), superoxide dismutase (S.O.D.) and catalase (C.A.T.) were measured according to Oyanatui (1984).

Liver function tests:

The activity of serum alanine transaminase (ALT) and serum aspartate transaminase (AST) were estimated according to the method described by Varley *et al.,* (1980) using commercial kits produced by Pasteur

Lab,Paris, France.and alkaline phosphatase enzymes (ALP) as outlined by King, (1965).

Determination of kidneys functions:

Urea was determined by using a commercial kit (Biomed Company, Germany) according to the method described byChaney and Marbach, (1962).

Uric acid was estimated by using a commercial kit (Biomed Company, Germany) according to the method described by Trinder, (1969).

Creatinine concentrations in the plasma were determined using enzymatic colorimetric kit (Biolabo , Maizy, France) according to the method described byFabiny and Ertingshausen , (1971)

Statistical analysis:

Data was analyzed by one way analysis of variance (ANOVA) were done using the program Spss (Version 22,2013 for Windows), at 5% ($p<0.05$) probability

RESULTS AND DISCUSSION

Proximate chemical composition of Microwave fullfat black rice bran and Microwave defatted black rice bran.

Chemical composition of microwave defatted black rice bran (MDBLRB) and Microwave fullfat black rice bran (MFBLRB) were given in Table (1).

Results reveal that MDBLRB contain protein, ash, fiber and carbohydrates were significantly higher than those of MFBLRB. These results are going in the same line with that reported by Nordin *et al*., (2014) and Patil *et al*., (2016).

Results also in the same Table show the total phenolic compounds of microwave full fat black rice bran (MFBLRB) and (MDBLRB).there was no significant difference detected. These were nearly in the same content by Mariod *et al.,(* 2010).

Table 2. Proximate chemical composition of Microwave fullfat black rice bran and Microwave defatted black rice bran (on dry weight basis).

Parameter%	Microwave fullfat Black rice bran	Microwave Defatted Black rice bran
Moisture	9.50[b]	9.10[a]
Crude protein	15.80[c]	18.20[a]
Lipids	17.80[b]	0.70[a]
Ash	7.80[b]	10.1 [a]
Crude fiber	11.50[c]	13.7 [a]
Total carbohydrates*	58.60[b]	71.00[a]
Total phenolic compounds (mg Tannic acid equivalent/kg)	486 [b]	561 [a]

***Total carbohydrates was calculated by difference.**
Each amount is an average of three replicates.
Values observed through the same letter in row are not significantly different at LSD at $p \leq 0.05$

Effect of feeding at different levels of MDBLRB on feeding and growth parameters of the hypercholesterolemic rats:

The effect of MDBLRB on feed intake (FI), body weight gain (BWG) and feed efficiency ratio (FER) of hypercholesterolemic rats for 10weeks is shown in Table (3). Values of feed intake were not differing by substitution of MDBLRB fed. (B.W.G.) indicated that the positive group (G3) had a higher weight gain as compared to (G1 and G2). Feed efficiency ratio of other tested groups showed a significant decrease in compared to hypercholesterolemic positive control (G3).

Table 3. Effect of feeding at different levels of MDBLRB on feeding and growth parameters of hypercholesterolemic rats.

Groups	Initial weight (g)	Final weight (g)	Body weight gain (BWG)		Feed Intake	FER
			G	%		
G1	173.00[a]	200.11[a]	26.60 [f]	15.33	980 [a]	2.71[f]
G2	173.90[a]	198.80[a]	24.90 [g]	14.31	973 [a]	2.56 [f]
G3	174.40[a]	232.90[a]	58.50 [a]	33.5	1057 [a]	5.53 [a]
G4	175.10[a]	218.80[a]	43.70 [b]	24.95	1029 [a]	4.24 [b]
G5	175.21[a]	216.41[a]	41.20 [c]	23.51	994 [a]	4.14c [cd]
G6	174.10[a]	212.93[a]	38.83 [d]	22.30	969.5 [a]	4.00 [d]
G7	175.42[a]	210.92 [a]	35.50 [e]	20.23	959 [a]	3.70 [e]

Each amount is an average of three replicates.
Values observed through the same letter in columns are not significantly different at LSD at $p \leq 0.05$
G1, G2 … etc. were as given in Table (1).

Effect of feeding at different levels of MDBLRB on the relative organs weight in hypercholesterolemic rats:

Liver, kidney and heart of rats fed on basal diet and other treatments, as well, were weight at the end of experimental period (10 weeks) and the ratio of each organ to final body weight of rats was calculated. Results presented in Table (4) reveal that all treatments showed no significant changes in the weight of kidney and heart of all tested experimental rats. On the other hand, the liver of positive control group (G3) had the highest liver weight (6.10 gm) and relatively liver weight (2.62) among all groups. This may be due to high cholesterol as declared by Metwalli, (2005), who reported that the increase of liver weight may be due to the accumulation of fat in liver tissues. Regarding to data in the same table negative control(G1 and G2) had the lowest value in both of liver weight and. In addition, the liver weight of rats fed with substitution of MDBLRB for casein in the diet after hypercholesterolemic were lower than positive control. The results revealed that there were no significant difference between kidney and heart weights, of neither control –ve nor (control +ve) and all tested groups. Feeding on MDBLRB at levels (25, 50, 75 and100%) produce no significant differences between weight gain of kidneys and heart as compared to the control groups, in well agreement with the results obtained by Ronis *et al.,* (2010) and Wang *et al.,(*2014).

Table 4. Effect of feeding at different levels of MDBLRB on the relative organs weight in hypercholesterolemic rats:

Group	Relative weight (gm %) of						Final body weight (g)
	Liver		Heart		Kidney		
	Weight (g)	R.O.W	Weight (g)	R.O.W	Weight (g)	R.O.W	
G1	5.31 [d]	2.65	0.89 [a]	0.45	1.35[a]	0.67	200.11 [a]
G2	5.30 [d]	2.67	0.88 [a]	0.44	1.33[a]	0.67	198.80 [a]
G3	6.10 [a]	2.62	0.89 [a]	0.28	1.69[a]	0.73	232.90 [a]
G4	5.42 [c]	2.48	0.86 [a]	0.39	1.53[ab]	0.70	218.80 [a]
G5	5.57 [b]	2.57	0.93 [a]	0.42	1.53[a]	0.71	216.41 [a]
G6	5.60 [b]	2.62	0.88 [a]	0.41	1.59[a]	0.75	212.93 [a]
G7	5.40 [c]	2.56	0.89 [a]	0.42	1.60[a]	0.76	210.92 [a]

Each amount is an average of three replicates.
Values observed through the same letter in columns are not significantly different at LSD at p ≤ 0.05
G1, G2 … etc. were as given in Table (1). R.O.W: relative weight.

Effect of feeding on MDBLRB bran on serum lipids profile.

Obtained results in Table (5) indicate that total cholesterol content at the end of experimental period for both the negative control (G1 and G2) were131.66 and 130.76 mg/dl respectively, while total cholesterol contents of positive control (G3) was 288.53 mg/dl. On the other hand G4, G5, G6 and G7 which fed on hypercholesterolemic diet substitution with of MDBLRB(25 ,50, 75 and 100% of diets)showed values of 134.53, 132.00, 131.86, and 128.93 mg/dl respectively. It could be noted that, hypercholesterolemic rats fed on (MDBLRB) had a significantly at (P≤0.05) were lowered in serum cholesterol in compared with positive control (G3).

The obtained results illustrated that total triglycerides of serum at the end of experimental period for negative control (G1 and G2) were 106.17 and 107 mg /dl and increased to 230.60 mg/dl in hypercholesterolemic rats which fed hypercholesterolemic diet while, the total triglycerides contents for G4, G5, G6 and G7 fed on hypercholesterolemic diet substitution with MDBLRB (25, 50, 75 and 100%), showed values of 155.20, 147.62, 141,21,and 137.50 mg/dl respectively.

Table 5. Effect of feeding on MDBLRB on serum lipids parameter in rats.

Groups	T. cholesterol mg/dl	Triglyceride mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
G1	131.66[bc]	106.17 [e]	63.55 [a]	46.78[c]	21.23 [d]
G2	130.76[bc]	107[e]	64.21 [a]	45.15[c]	21.40 [d]
G3	288.53[a]	230.60 [a]	43.61 [e]	198.77[a]	46.12 [a]
G4	134.53[b]	155.20 [b]	52.82 [d]	50.67[b]	31.04 [b]
G5	132.00[bc]	147.62[c]	54.90[cd]	47.85[c]	29.25[bc]
G6	131.86[bc]	141.21 [d]	57.34[c]	46.28[c]	28.24 [c]
G7	128.93[d]	137.50[d]	59.70[b]	41.73[d]	27.50 [c]

Each amount is an average of three replicates.
Values observed through the same letter in columns are not significantly different at LSD at p ≤ 0.05
G1, G2 … etc. were as given in Table (1).

Results showed that, supplementation of feeding hypercholesterolemic diet with (MDBLRB) led to improvement the HDL-C. Furthermore, hypercholesterolemic diet with (MDBLRB) replacement at 75% and 100% also recorded the best and nearest of HDL-C to the negative control. These values were significantly different comparing with that recorded in positive control.

It was observed also that LDL- cholesterol value of negative control diets (G1and G2) were 46.78 and 45.15 mg/dl, but the value of the hypercholesterolemic control (G3) was 198.77mg/dl. On the other hand, the LDL cholesterol of rats fed on (HD) diet substitution with (MDBLRB) 25,50.75 and 100% (G4, G5, G6 and G7) being 50.67, 47.85, 46.28 and 41.73 mg/dl, respectively.Data of vLDL cholesterol for fed rats on basal, hypercholesterolemic diets summarized in Table (5) was observed that vLDL cholesterol value of negative control (G1 and G2) were 21.23 and 21.40 mg/dl, while the value of positive control (G3) was 46.12 mg/dl. On the other hand, the vLDL cholesterol of rats fed on hypercholesterolemic diet substitution with MDBLRB 25,50,75 and 100% (G4,G5,G6andG7) produced 31.04, 29.25, 28.24 and 27.50mg/dl , respectively.

It could be observed that, hypercholesterolemic rats fed on hypercholesterolemic diet substitution with (MDBLRB) 25,50,75 and 100% relative to basal diets had a significant lower serum total cholesterol, total triglycerides, LDL cholesterol and VLDL cholesterol compared with hypercholesterolemic positive control (G3).

In contrary, these groups had significantly high level HDL-C at (P≤0.05). While, negative control or other group fed on basal diet had a significantly lower mean values for TC, TG and LDL-C. These results were in agreement with Zawistowski *et al., (*2009); Ronis *et al.,* (2010) and Wang *et al*, (2014) and Karthika, D. and Devi, P.N. (2016)

Effect of feeding on MDBLRB on Liver function activities (ALT), (AST) and ALP (U/L) in hypercholesterolemic rats.

The effect of feeding on (MDBLRB) at the level of, alanine amino transferase (ALT), aspartate aminotransferase (AST) and (ALP) enzymes in serum of hypercholesterolemic rats for 10 weeks recorded in Table (6).At the final of experiment (10 weeks), the concentration value of ALT (GPT) was significantly increased for the hypercholesterolemic control (G3).The concentration value of (ALT) enzyme in hypercholesterolemic rats (G3) was 50.30 U/L, while negative control (G1 and G2) were 25.80 and 24.20U/L, respectively. Data in the same table showed that, the feeding on hypercholesterolemic diets substitution with MDBLRB (G4,G5,G6 and G7) led to a more reduction at level 25, 50,75 and 100% were 38.52, 36.61, 34.34 and 32.55 U/L, respectively comparing with hypercholesterolemic control (G3).

Table 6. Effect of feeding on MDBLRB on Liver function activities (ALT), (AST) and ALP (U/L) in hypercholesterolemic rats.

Group	ALT U/L	AST U/L	ALP U/L
G1	25.80 ^{e}	53.30 ^{c}	94.30 ^{d}
G2	24.20 ^{e}	52.65 ^{c}	92.48 ^{d}
G3	50.30 ^{a}	84.61 ^{a}	128.80 ^{a}
G4	38.52 ^{b}	59.40 ^{b}	114.36 ^{b}
G5	36.61 ^{bc}	57.51 ^{bc}	112.54 ^{bc}
G6	34.34 ^{d}	55.43 ^{c}	110.05 ^{c}
G7	32.55 ^{d}	53.30 ^{c}	108.14 ^{c}

Each amount is an average of three replicates.
Values observed through the same letter in columns are not significantly different at LSD at p ≤ 0.05
G1, G2 … etc. were as given in Table (1).

AST activity was significantly increased for hypercholesterolemic control (G3). The liver AST activity of hypercholesterolemic rats was 84.61 U/L relative to negative control (G1 and G2) were 53.30 and 52.65 U/L respectively, Data in the same Table showed that , the rats fed on substitution of MDBLRB for casein at 25,50,75and100% were 59.40, 57.51, 55.43 and 53.30 U/L, respectively for (G4, G5, G6 and G7). The results were in a good agreement with those many authors Ha *et al*, (2005) and *Wang et al.,* (2014).

In addition to (David, 2001) reported that the decreased of alanine amino transferase (ALT) and aspartate aminotransferase (AST) in rats may be referred to feeding dietary fibers including natural antioxidant. Furthermore, these treatments can alleviate the damage induced by serum cholesterol On the other hand, (ALP) activity was the negative control (G1 and G2) were 94.30 and 92.48 U/L while, Alkaline phosphatase (ALP) activity of hypercholesterolemic diets positive control (G3) was 128.80 U/L. Hypercholesterolemic rats fed on MDBLRB which substitutes 25, 50, 75 and 100% for casein showed significant decreases comparing with hypercholesterolemic control (G3).

Effect of feeding on MDBLRB on kidney functions (urea, uric acid and creatinine) in hypercholesterolemic rats.

The results of urea, uric acid and creatinine in serum of negative control (G1 and G2) and hypercholesterolemic positive control (G3), at the end of experimental period after feeding for 10 weeks are reported in Table (7) . Obtained results illustrated that urea at the end of experimental period for (G1andG2) were 42.71 and 42.30 mg/dl, respectively.

The same table presented that urea content of hypercholesterolemic positive control (G3) showed that value of 58.00 mg/dl in serum, while the hypercholesterolemic rats fed on (MDBLRB) at 25, 50, 75 and 100 % (G4, G5, G6 and G7) gave 49.61, 47.55, 45.41 and 43.35mg/dl respectively. The results showed that, the urea contents was decreased in rats fed on hypercholesterolemic diets substitution with (MDBLRB) 25,50,75and100 % compared to hypercholesterolemic control (G3) .The obtained results (Table 7) illustrated that uric acid contents at the end of experimental period for negative control (G1 and G2) were 1.70 and 1.57 mg/dl, respectively . The same Table presented that uric acid contents of hypercholesterolemic positive control (G3) showed a value 3.51mg/dl. while the hypercholesterolemic diets of G4,G5,G6 andG7 substitution with (MDBLRB) 25, 50, 75 and 100 % were 2.10, 1.89 1.72 and 1.57 mg/dl, respectively. The obtained results illustrated that creatinine contents at the end of experimental period for negative control were 0.345 and 0321 mg/dl, respectively the same Table presented that creatinine contents of hypercholesterolemic positive control showed that a value of 0.881mg/dl, while hypercholesteramia diets G4, G5, G6 and G7 fed on (MDBLRB) 25, 50, 75 and 100 % were 0.63, 0.589, 0.590 and 0.550mg/dl, respectively. It could be seen from the data present in Table (7) illustrated that hypercholesterolemic rats fed (MDBLRB) 25,50,75 and 100% had significantly lower serum urea ,uric acid and creatinine compared with hypercholesterolemic group G3 (P≤0.05). Mean while, negative group G1 and G2 fed on basal diet had a significantly lower mean value for urea, uric acid and creatinine

Table 7. Effect of feeding on MDBLRB on kidney functions (urea, uric acid and creatinine) in rats.

Group	Urea mg/dl	Uric acid mg/dl	Creatinine mg/dl
G1	42.71 ^{a}	1.70 ^{c}	0.345 ^{e}
G2	42.30 ^{a}	1.57 ^{d}	0.321 ^{e}
G3	58.0 ^{f}	3.51 ^{a}	0.881 ^{a}
G4	49.61 ^{b}	2.10 ^{b}	0.630 ^{b}
G5	47.55 ^{c}	1.89 ^{bc}	0.589 ^{c}
G6	45.41 ^{d}	1.72 ^{c}	0.590 ^{c}
G7	43.35 ^{d}	1.57 ^{c}	0.550 ^{d}

Each amount is an average of three replicates.
Values observed through the same letter in columns are not significantly different at LSD at p ≤ 0.05
G1, G2 … etc. were as given in Table (1).

Effect of MDBLRB on glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT).

Obtained results in Table (8) indicate that shows that hypercholesterolemic rats had significantly lower levels of glutathione peroxidase (GPX),superoxide dismutase (SOD) and catalase (CAT) antioxidant enzymes activity in compared with (G1 and G2). The data in the aforementioned Table clearly show that substitution of MDBLRB at level(25,50,75and100%) in hypercholesterolemic diet improved the activity levels of (GPX), (SOD) and (CAT) antioxidant enzymes in compared with positive group.This finding is agreed and supported with reported by Hsu *et al*., (2010) and Houa *el al*., (2013).

Table 8. Effect of MDBLRB on (GPX), (SOD) and (CAT) enzymes in serum.

Group	GPX	SOD	CAT
G1	18.70^{a}	91.10^{a}	66.40^{a}
G2	18.20^{a}	90.87^{a}	66.21^{a}
G3	5.60^{d}	54.60^{e}	35.61^{e}
G4	8.70^{c}	61.70^{d}	41.40^{d}
G5	11.40^{bc}	79.50^{c}	45.20^{c}
G6	12.30^{bc}	82.40^{b}	47.40^{bc}
G7	14.40 ^{b}	84.60^{b}	53.70 ^{b}

Each amount is an average of three replicates.
Values observed through the same letter in columns are not significantly different at LSD at p ≤ 0.05.
G1, G2 … etc. were as given in Table (1).

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التقييم البيولوجي لرجيع الأرز الأسود منزوع الدهن المعامل بالميكروويف على الفئران المصابة بالكولسترول

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تم إجراء هذا البحث بغرض دراسة تأثير رجيع الأرز الأسود منزوع الدهن المعامل بالميكروويف علي فئران التجارب المصابة بالكولسترول.وتم تقدير التركيب الكيميائي والمركبات الفينولية الكلية لرجيع الأرز الأسود منزوع الدهن المعامل بالميكروويف وكذلك رجيع الأرز الأسود كامل الدهون المعامل بالميكروويف وأظهرت نتائج التركيب الكيميائي لرجيع الأرز الأسود منزوع الدهن المعامل بالميكروويف احتوائه على نسبة من البروتين والرماد والألياف والكربوهيدرات تقدرب (١٨.٢٠ و ٠.٧٠ و ١٠.١ و ١٣.٧ و ٧١ %) وكانت أعلى من رجيع الأرز الأسود كامل الدهون المعامل بالميكروويف .وكذلك أوضحت النتائج ارتفاع محتوي رجيع الأرز الأسود منزوع الدهن المعامل بالميكروويف من المركبات الفينولية الكلية حيث وصلت الي (٥٦١) مقارنة برجيع الأرز الأسود كامل الدهون المعامل بالميكروويف ٤٨٦ (مجم ما يعادل حمض التانيك / كجم). وتم دراسة تأثير استبدال رجيع الأرز الأسود منزوع الدهن المعامل بالميكروويف بنسبة من الكازين على كلا من وزن الجسم – معدل لتمثيل الغذائي–وظائف الكبد و إنزيمات الاكسده وكذلك مؤشر الليبيدات فى السيرم وكذلك وزن الأعضاء الداخلية للفئران المصابة بالكولسترول. وأظهرت النتائج ان استبدال رجيع الأرز الأسود منزوع الدهن المعامل بالميكروويف بنسبه من الكازين أدى الى تحسن فى نسب الكولسترول عالى الكثافة علاوة على ذلك، فان استبدال رجيع الأرز الأسود منزوع الدهن المعامل بالميكروويف بالكازين بنسبة ٧٥ و ١٠٠٪ في الفئران بالكولسترول سجلت أفضل وأقرب النتائج بالنسبة للمجموعات غير المصابة بالكولسترول .وأوضحت النتائج أيضا أن نسبة الكولسترول منخفض الكثافة للمجموعة الأولى والثانية (السالبة)من الفئران غير المصابة بالكولسترول (G_1 and G_2) كانت ٤٦.٧٨و٤٥.١٥ وكذلك فى المجموعة الثالثة المصابة بالكولسترول (الموجبة) كانت١٨٩.٧٧ بينما فى المجموعات الأخرى الموجبة المصابة بالكولسترول والتى تغذت على رجيع الأرز الأسود منزوع الدهن المعامل بالميكروويف المستبدل بنسبه من الكازين٢٥, ٥٠, ٧٥ و ١٠٠% (G4, G5, G6 and G7)كانت ٥٠.٦٧ , ٤٧.٨٥, ٤٦.٢٨ و٤١.٧٣. من هذه النتائج يمكن أن نستنتج أن رجيع الأرز الأسود منزوع الدهن المعامل بالميكروويف له تأثير واضح وفعال في خفض نسبة كولسترول السيرم كما أنه مفيد للمرضى الذين يعانون من أمراض الكبد و الكولسترول.

الكلمات الدالة : رجيع الأرز الأسود ، الكولسترول