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BIOCHEMICAL EVALUATION OF RICE GERM

2- PHYSIOLOGICAL RESPONSES OF BISCUIT DIETS FORTIFIED WITH RICE GERM TO DIABETIC RATS

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ABSTRACT

The present study was performed to evaluate the biological effects of feeding biscuit fortified with rice germ, or rice germ powder on serum glucose levels, total triglycerides, total cholesterol, HDL-, LDL-cholesterol, liver and kidney functions for alloxan-induced diabetic rats.

The obtained results indicated that the biscuit fortified with rice germ, or rice germ powder improved the body weight gain, food intake and feed efficiency of alloxan-induced diabetic rats relative to control. The liver, kidney, heart, spleen and brain weights of rats fed biscuit supplemented with rice germ or rice germ powder had similar mean values and lower values than that of rats fed basal diet and hyperglycemic diet. Also, the results indicated that the rats fed biscuit fortified with rice germ or rice germ powder had significantly decreased the level of serum glucose after 5 weeks when compared with alloxan-induced diabetic rats. A significant decrements were observed in triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol with rats fed on different diets relative to diabetic rats. On the other hand, alloxan-induced diabetic rats had a higher values of ALP, AST and ALT activities than that of diabetic rats fed on basal diet and biscuit fortified with rice germ or rice germ powder. Also, the obtained results showed that improvement of kidney functions in alloxan-induced diabetic rats.

Key words: Biscuit - Alloxan-induced diabetic rats - Biological evaluation.

INTRODUCTION

Anderson *et al.* (1995) studied the effects of high-fiber (HF) and low-fiber (LF) meals on postprandial serum glucose, insulin, lipid, lipoprotein, and apolipoprotein concentrations of 10 hypercholesterolemic men. Responses over a 15-hour period after multiple meals (MM) and over a 10-hour period after a single meal (SM) were compared. HF meals were associated with a significant reduction in postprandial serum glucose and insulin. Serum free fatty acids (FFA) levels decreased significantly after MM and SM, but differences between HF and LF meals were insignificant. Although, serum triglycerides responses did not differ significantly between HF and LF meals.

Hozumi *et al.* (1995) studied the effects of the dietary fiber supplementation on serum levels of glucose and lipoproteins in cholesterol-fed diabetic rats feeding a diet containing 1.5% cholesterol and 0.37% cholic acid for 18 weeks to rats made diabetic by streptozotocin (35 mg/Kg body weight) produced moderate hyperglycemia and moderate hypercholesterolemia, the latter being characterized by high concentrations not only of low density lipoproteins but also, intermediate density lipoproteins and very low density lipoproteins. These changes in serum lipoproteins and hyperglycemia were substantially reduced after 18 weeks of supplementation with glucomannan but high density lipoprotein cholesterol and triglycerides levels did not change after feeding a cholesterol-rich diet in the presence or absence of glucomannan supplementation.

Sidky *et al.* (1996) studied the effect of high fiber biscuits on some special diseases by using the potato peels as a new source of dietary fiber. Biscuits were made with supplementation of 5, 10, and 15% potato peels. The results showed that 15% potato peel biscuit had the highest content of fiber (10.2%) and scored a good grade. Weight gain, cholesterol and triglycerides were reduced comparable to control.

Kawabata *et al.* (1999) investigated the modifying effect of dietary administration of defatted rice germ and gamma-aminobutyric acid (GABA)-enriched defatted rice germ on azoxymethane (AOM)-induced colon carcinogenesis with male rats. They found that dietary exposure to rice germ during the initiation phase (5 weeks)

significantly reduced the incidence of colonic adenocarcinoma (71 versus 29%). GABA-enriched defatted rice germ or rice germ during the postinitiation phase (30 weeks) also decreased the frequency of colonic adenocarcinoma (71 vs. 20% GABA-enriched defatted rice germ feeding and 27% rice germ feeding).

Mori *et al.* (1999) reported that defatted rice or rice germ powder decreased incidences of azoxymethane (AOM)-induced formation of aberrant crypt foci (ACF) and intestinal carcinogenesis in rats.

Lee *et al.* (2003) investigated the effects of rice germ oil supplement on lipid metabolism of insulin-dependent diabetic mice. They found that no significant difference in diet intake, body weight and organ weight among the experimental groups. But, the concentrations of serum triglycerides and total cholesterol in the rice germ oil groups were significantly lower than those of insulin dependent diabetic mice.

Tag El-Din *et al.* (2006) investigated the effect of some plant extracts on serum glucose, urea and creatinine levels in alloxan-diabetic and non-diabetic rats. They found that plant extracts under investigation had significant decrements in serum glucose, urea and creatinine levels in alloxan-diabetic rats when compared with control group after 30 days. Also, the examined extracts had hypoglycemic effects and improved the histopathological changes in kidney and pancreas tissues of alloxan-diabetic rats.

The present study aims to investigate the biological effects of rice germ as by-product on glycemic responses in alloxan-induced diabetic rats.

MATERIALS AND METHODS

1. Materials:

Rice (*Oryza sativa*) germ was obtained from El-Obour Mill, Desoug, Kafr El-Sheikh, Egypt. Commercial wheat flour (72% ext.) was obtained from South Cairo Mills Comp., Fysal, Giza, Egypt.

2. Biological experiment:

The rats were obtained from the Farm of Central Organization of Serum and Vaccine (Abasia Farm), Egypt. A total of twenty four male

albino rats weighing 140-190 g were used. Rats were allowed to be acclimatized to laboratory conditions for two weeks prior to the experiment and fed on basal diet as control. After the adaptation period, the rats were injected by alloxan solution 150 mg/kg body weight of recrystallized alloxan (Buko *et al.*, 1996) to induce hyperglycemia.

Basal diet was fed for 72 hr where hyperglycemia was developed. To ensure occurrence of diabetes in rats, blood samples were withdrawn after 72 hr of alloxan injection.

→ The diabetic rats were divided into three groups (G_1, G_2 and G_3), 6 rats for each. All groups were fed on experimental diets as shown in (Table, 1). During the whole experiment, rats were kept separately in well aerated cages, diet and water were supplied *ad-libitum*. Each rat was weighed every week and food intake was also daily recorded. At the end of experimental period (5 weeks), rats were killed by decapitation after an overnight fast, and the blood of each rat collected in tubes and centrifuged at 3000 rpm for 20 min to obtain the serum which was kept in the deep-freezer until analysis. The liver, kidney, heart, splin and brain were removed from each rat and weighed.

Table (1): Composition of different experimental diets (g/100 g).

Diet	Casein	Corn oil	Salt mix.	Vitamin mix.	Cellulose	Starch	Biscuit	Rice germ
G1	10.00	10.00	4.00	1.00	5.00	70.00	-	-
G2	10.00	10.00	4.00	1.00	5.00	70.00	-	-
G3	-	-	1.85	1.00	0.43	-	96.72	-
G4	-	-	0.62	1.00	-	48.09	-	50.29

G1: Basal diet

G2: Hyperglycemic

G3: Biscuit with rice germ powder

G4: Rice germ powder

3. Determination of biological parameters:

Scrum total cholesterol was determined by the method of (Allain *et al.*, 1974) and total triglycerides was determined by the method of Fossati and Prencipe (1982) by using kits. Serum HDL-cholesterol

was determined by the method of Lopes-Virella *et al.* (1977), while LDL-cholesterol was determined by the method of Steinberg (1981). Blood glucose was determined according to Tietz (1986).

Enzymes activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically according to the method described by Reitman and Frankel (1957). Alkaline phosphatase (ALP) enzyme activity was determined according to Hausamen *et al.* (1967). Urea in the serum was determined according to Fawcett and Soctt (1960) and creatinine was estimated according to the method described by Bartles *et al.* (1972).

Uric acid in the serum was determined according to the method described by Haisman and Muller (1977).

Total protein and albumin in serum were determined according to the method described by Doumas *et al.* (1971), but serum globulin was calculated by subtracting the amount of albumin from total protein.

4. Statistical analysis:

Statistical analysis of the obtained data was done by using the procedure outlined by Gomez and Gomez (1984). The treatment means were compared using the least significant difference test (LSD) at the 5% level of probability as outline by Waller and Duncan (1969) using SAS institute program (SAS, 1996).

RESULTS AND DISCUSSION

Effect of biscuit supplemented with rice germ diets on biological parameters of diabetic rats:

The biological effects of biscuit which was fortified by using rice germ, or rice germ powder diets on alloxan-induced diabetic rats at the end of experimental period (5 weeks) were evaluated.

Effect of experimental diets on body weight gain, food intake and feed efficiency of diabetic rats:

The results cited in Table (2) shows body weight gain, food intake, feed efficiency and feed efficiency ratios of diabetic rats after 5 weeks. From the obtained results, it could be observed that rats fed

Table (2): Effect of biscuit fortified with rice germ on body weight gain, food intake and feed efficiency ratio of alloxan-induced diabetic rats at the end of experimental period (5 weeks).

Groups	Initial body weight (g)	Final body weight (g)	Body weight gain (A) (g)	Daily food intake (g)	Consumption food intake (B) (g)	Feed efficiency A/B	F.E.R.
G1	172.20±6.41	236.40±5.22	64.20±2.30	10.53±0.54	368.50±19.03	0.174±0.008	17.4
G2	176.00±1.78	119.60±7.86	-56.40±7.12	10.45±0.64	365.75±22.49	-0.154±0.018	-15.4
G3	164.00±1.00	172.00±2.91	8.00±2.12	11.35±1.02	397.40±36.01	0.021±0.008	2.0
G4	147.80±1.92	140.60±2.70	-7.20±0.83	9.25±0.57	323.90±20.21	-0.022±0.004	-2.2
L.S.D. at 0.05	3.978	5.949	5.108	0.785	27.481	0.0140	

G1: Basal diet (control)

G2: Alloxan diabetic rats.

G3: Alloxan diabetic rats + biscuit with rice germ

G4: Alloxan diabetic rats + rice germ powder

Feed efficiency = Body weight gain/feed intake

F.E.R. = Feed efficiency x 100

Table (3): Effect of biscuit fortified with rice germ on weight organs of alloxan-induced diabetic rats at the end of experimental period (5 weeks).

Groups	Final body weight (g)	Liver (g)	Kidneys (g)	Heart (g)	Spleen (g)	Brain (g)
G1	236.40±5.22	7.86±1.33	2.43±0.19	1.18±0.27	0.92±0.21	2.19±0.35
G2	119.60±7.86	4.56±0.90	1.16±0.26	0.52±0.05	0.67±0.22	1.63±0.22
G3	172.00±2.91	5.20±0.12	0.96±0.10	0.60±0.04	0.53±0.06	1.21±0.17
G4	140.60±2.70	4.06±0.20	0.76±0.10	0.47±0.03	0.40±0.09	1.00±0.06
L.S.D. at 0.05	5.949	0.950	0.209	0.150	0.191	0.320

G1: Basal diet (control)

G2: Alloxan diabetic rats.

G3: Alloxan diabetic rats + biscuit with rice germ

G4: Alloxan diabetic rats + rice germ powder

basal diet had the highest body weight gain and feed efficiency ratio, these mean values were 64.2 ± 2.30 g and 17.4%, respectively. While, rats with hyperglycemic effect had very lowest mean values which recorded (-56.4 ± 7.12 g) and (-15.4%). On the other hand, rats fed biscuit with rice germ had improvement of body weight gain from (-56.4 ± 7.12 g) to ($+8.00 \pm 2.12$ g). But, the mean values of body weight gain and feed efficiency ratio were (-7.20 ± 0.83 g) and (-2.2%) for rats fed rice germ powder.

From the above results, it could be observed that the addition of rice germ as a by-products to wheat flour for making biscuit had improved the total body weight and feed efficiency ratio relative to hyperglycemic rats. Such results are in agreement with those reported by Anderson *et al.* (1995), Sidky *et al.* (1996) and Lee *et al.* (2003).

Effect of experimental diets on organ weights of diabetic rats:

The weights of liver, kidney, heart, spleen and brain of diabetic rats fed biscuit supplemented with rice germ and rice germ powder are presented in Table (3). From the obtained results, it could be observed that the liver, kidney, heart, spleen and brain of hyperglycemic rats had lower mean values than that of rats fed basal diet. While, rats fed biscuit with rice germ, or rice germ powder had the similar trend. These results are in agreement with those reported by Sidky *et al.* (1996), Lee *et al.* (2003) and Tag El-Din *et al.* (2006).

Effect of experimental diets on serum glucose levels of diabetic rats during experimental period:

Table (4) shows the glucose levels of rats fed basal diet, hyperglycemic rats and biscuit fortified by using rice germ replacing with wheat flour and rice germ powder.

From these results, the mean values of serum glucose contents were found to be 99.81, 262.30, 218.24 and 200.68 mg/100 ml serum at zero time (after 72 h) for rats fed basal diet, hyperglycemic rats, biscuit with rice germ and rice germ powder, respectively. However, after the first week the level of glucose was decreased from 218.24 to 208.32 mg/100 ml with rats fed biscuit containing rice germ. While, after two weeks the glucose content was decreased from 200.68 to 174.79 mg/100 ml for rats fed rice germ powder. On the other hand, the mean values of serum glucose levels after the end of experimental

period (5 weeks) were 95.30, 243.80, 167.62 and 164.86 mg/100 ml serum for rats fed basal diet, hyperglycemic rats, biscuit with rice germ and rice germ powder, respectively. From the above results, it could be observed that the decrement percentage of serum glucose levels were 31.25 and 32.38% for rats fed biscuit containing rice germ and rice germ powder, respectively when compared with rats hyperglycemic effect. These observations may be due to the dietary fiber specially β -glucan polymer as suggested by Hozumi *et al.* (1995). These results are in agreement with these reported by Lee *et al.* (2003) and Tag El-Din *et al.* (2006).

Effect of experimental diets on serum triglycerides, total cholesterol, HDL-, LDL-cholesterol and risk ratio of diabetic rats after 5 weeks:

After the end of experimental period (5 weeks) serum triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol and risk ratio of alloxan-induced diabetic rats were measured and presented in Table (5).

From the obtained results, it could be observed that rats with hyperglycemic effect had the highest value of triglycerides, total cholesterol, LDL-cholesterol and risk ratio. But, HDL-cholesterol was found the lowest value when compared with rats fed other diets.

However, rats fed diet containing biscuit fortified with rice germ or rice germ powder diet had lower mean values of triglycerides, total cholesterol, LDL-cholesterol and risk ratio than that rats with hyperglycemic effect. From the above-mentioned results, it could be said that rats fed diets containing rice germ (biscuit or powder) improved the serum triglycerides, total cholesterol and LDL-cholesterol relative to alloxan-induced diabetic rats. The obtained results indicated that biscuit fortified with rice germ and rice germ powder reduced the levels of total cholesterol concentrations in the serum of insulin-dependent diabetic mice and that the hypolipidaemic effect of rice germ may be due to increasing fecal lipid excretion and decreasing lipid absorption, as reported by Lee *et al.* (2003). These results are in agreement with those reported by Anderson *et al.* (1995) and Kawabata *et al.* (1999).

Table (4): Effect of experimental diets on serum glucose content (mg/100 ml) of alloxan-induced diabetic rats after 72 h (zero time) and during 5 weeks of experimental period.

Groups	Zero time	After 1 week	After 2 week	After 3 week	After 4 week	After 5 week
G1	99.81±12.03	116.30±14.73	98.95±12.84	106.41±14.81	96.72±4.46	95.30±7.81
G2	262.30±14.47	258.50±22.98	256.72±15.68	246.32±13.01	245.83±11.42	243.80±12.21
G3	218.24±10.01	208.32±13.64	201.42±14.88	193.30±16.03	185.28±11.99	167.62±13.33
G4	200.68±15.91	198.97±16.89	174.79±18.49	167.89±9.82	168.06±10.05	164.36±10.88
L.S.D. at 0.05	21.009	21.692	20.384	16.931	13.581	13.582

G1: Basal diet (control)

G2: Alloxan diabetic rats.

G3: Alloxan diabetic rats + biscuit with rice germ

G4: Alloxan diabetic rats + rice germ powder

Table (5): Effect of biscuits diets containing different levels of rice germ on serum total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol and risk ratio of diabetic rats after 5 weeks.

Groups	Triglycerides (mg/100 ml)	Total cholesterol (mg/100 ml)	HDL-cholesterol (mg/100 ml)	LDL-cholesterol (mg/100 ml)	T. chol/HDL-cholesterol	Risk ratio
G1	121.36±3.95	117.50±7.73	65.43±4.71	27.50±5.95	1.79	1.79
G2	176.41±3.85	211.30±10.61	37.65±3.77	138.37±9.75	5.61	5.61
G3	144.38±2.61	161.72±6.14	45.58±3.60	87.26±8.62	3.55	3.55
G4	130.74±3.49	138.90±2.57	58.54±2.97	54.21±4.96	2.37	2.37
L.S.D. at 0.05	4.150	7.335	4.452	7.810		

G1: Basal diet (control)

G2: Alloxan diabetic rats.

G3: Alloxan diabetic rats + biscuit with rice germ

G4: Alloxan diabetic rats + rice germ powder

Effect of experimental diets on total protein, albumin, globulin, alkaline phosphatase (ALP) and aminotransaminase enzymes (AST and ALT) activity of diabetic rats.

Total protein, albumin, globulin, alkaline phosphatase (ALP) and aminotransferase enzymes of experimental rats were determined and illustrated in Table (6). From the obtained results, total protein, albumin and globulin contents were (6.88, 3.14 and 2.65 g/100 ml) and (7.12, 4.56 and 2.48 g/100 ml) for rats fed basal diet and alloxan-induced diabetic rats, respectively. However, these parameters were found to be lower values which recorded (5.88, 3.44 and 2.44 g/100 ml) and (6.44, 3.62 and 2.82 g/100 ml) for rats fed diet containing biscuit with rice germ and rice germ powder, respectively.

On the other hand, alkaline phosphatase activity was found to be 81.17 and 132.50 U/ml for rats fed basal diet and hyperglycemic effect, respectively. However, rats fed biscuit with rice germ or rice germ powder had lower mean values (99.26 and 90.50 U/L) of alkaline phosphatase (ALP) activity if compared with alloxan-induced diabetic rats. Also, the activities of aminotransferase (AST and ALT) had the highest values (61.18 and 37.13 U/L) for rats of hyperglycemic effect than that of rats on basal diet (40.15 and 21.10 U/L) and biscuit containing rice germ (51.55 and 29.87 U/L) or rice germ powder (44.81 and 21.60 U/L), respectively. From the obtained results, it could be observed that rats fed diet containing rice germ improved the enzymes of liver functions.

These results are in coincident with those reported with Hozumi *et al.* (1995) and Lee *et al.* (2003).

Effect of experimental diets on serum urea, uric acid and creatinine levels of diabetic rats:

These parameters were assayed as induction for the function of kidney and recorded in Table (7). From the obtained results, it could be seen that alloxan-induced diabetic rats had the higher mean values of urea, uric acid and creatinine (70.21, 7.12 and 1.85 mg/100 ml, respectively) than that of rats fed basal diet (44.32, 3.25 and 0.94 mg/100 ml). On the other hand, rats fed diets containing biscuit fortified with rice germ, or rice germ powder had the lower values of urea, uric acid and creatinine than that of hyperglycemic rats.

Table (6): Effect of experimental diets on serum total protein, albumin, globulin, alkaline phosphatase (ALP) and transaminase enzymes (AST and ALT) of diabetic rats at the end of experimental period (5 weeks).

Groups	Total protein (g/100 ml)	Albumin (g/100 ml)	Globulin (g/100 ml)	ALP U/L	AST U/L	ALT U/L
G1	6.88±0.58	3.14±0.14	2.65±0.53	81.17±6.47	40.15±2.59	21.10±2.00
G2	7.12±0.56	4.56±0.59	2.48±0.99	132.50±3.56	61.18±2.73	37.13±1.74
G3	5.88±0.79	3.44±0.27	2.44±0.60	99.26±2.54	51.55±2.49	29.87±2.53
G4	6.44±0.70	3.62±0.08	2.82±0.72	90.50±1.11	44.81±0.92	21.60±3.49
L.S.D. at 0.05	0.906	0.378	0.917	4.436	3.796	3.254

G1: Basal diet (control)

G2: Alloxan diabetic rats.

G3: Alloxan diabetic rats + biscuit with rice germ

G4: Alloxan diabetic rats + rice germ powder

Table (7): Effect of experimental diets on serum urea, uric acid and creatinine contents of diabetic rats after 5 weeks.

Groups	Urea (mg/100 ml)	Uric acid (mg/100 ml)	Creatinine (mg/100 ml)
G1	44.32±4.05	3.25±0.53	0.94±0.12
G2	70.21±5.98	7.12±0.97	1.85±0.19
G3	61.15±3.02	5.23±0.32	1.35±0.13
G4	56.17±6.04	5.65±0.55	1.28±0.05
L.S.D. at 0.05	6.452	0.760	0.166

G1: Basal diet (control)

G2: Alloxan diabetic rats.

G3: Alloxan diabetic rats + biscuit with rice germ

G4: Alloxan diabetic rats + rice germ powder

The obtained data are in agreement with those reported by Tag El-Din *et al.* (2006).

From the above-mentioned results, it could be concluded that the diets containing rice germ reduced serum glucose levels, triglycerides, total cholesterol and LDL-cholesterol in the hyperglycemic rats. Also, the obtained results indicated that there is an improvement in liver and kidney functions as a result of these additives.

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التقييم الكيميائي الحيوي لجنين الأرز
٢- الاستجابة الفسيولوجية للفئران المصابة بمرض السكر لطبقة
البسكويت المدعومة بمسحوق جنين الأرز
فرحات فودة على فودة
قسم الكيمياء الزراعية- كلية الزراعة - جامعة بنها

يهدف هذا البحث لدراسة التأثيرات البيولوجية لمنتجات البسكويت المدعوم بجنين الأرز على فئران التجارب المصابة بمرض السكر عن طريق التغذية بمنئج البسكويت المضاف إليه مسحوق جنين الأرز، والتغذية بمسحوق جنين الأرز بمفرده على مستوى السكر في الدم وكذا مستوى الكوليستيرول ووظائف الكبد والكلى.

أظهرت النتائج المتحصل عليها أن إضافة جنين الأرز للبسكويت أو التغذية بمسحوق جنين الأرز بمفرده أدت إلى زيادة معنوية في وزن الفئران والغذاء المتناول وكفاءة الغذاء وذلك عند مقارنتها بأوزان الفئران التي تم حقنها بالأوكسان (المصابة بمرض السكر) وتتغذى على الوجبة القياسية وهذا يعطى دلالة على القيمة الغذائية لجنين الأرز. كما أحدثت التغذية زيادة في وزن الكليتين والمخ والقلب والطحال لمجموعة الفئران المحقونة بالأوكسان ومقارنتها بالمجموعة الضابطة. كان هناك تأثير خافض لجلوكوز الدم نتيجة التغذية على منتجات البسكويت المضاف إليها مسحوق جنين الأرز أو مسحوق جنين الأرز بمفرده حيث حدث انخفاض معلوق في جلوكوز سيرم الدم بعد ٥ أسابيع من التغذية. سجلت النتائج أيضا إنخفاضا في نسب الجليسريدات الكلية والكوليستيرول الكلى والـ LDL-كوليستيرول في سيرم الدم مع تحسين في نسبة HDL-كوليستيرول وذلك في مجاميع الفئران المغذاة على منتجات البسكويت المضاف إليه مسحوق جنين الأرز، مسحوق جنين الأرز بدون أى إضافات.

كما سجلت النتائج أيضا أن الفئران المصابة بمرض السكر والمغذاة على العليقة الأساسية أعطت أعلى إرتفاع في نشاط أنزيمات الكبد عن الفئران المصابة المغذاة على العليقة المحتوية على مسحوق جنين الأرز. كما حدث إنخفاض في نسب اليوريا واليوريك والكرياتينين في سيرم دم الفئران المصابة المغذاة على عليقة البسكويت المضاف إليه مسحوق جنين الأرز أو مسحوق الجنين فقط وذلك مقارنة مع الفئران المصابة بمرض السكر والمغذاة على العليقة الأساسية.