

Effect of White, Black Rice and their Mixtures on Induced-Hypercholesterolemia in Rats

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Abstract

Effect of different concentrations 5% and 10% of white, black rice (*Oryza sativa*, *L. indica*) and their mixtures as powder on biological and biochemical changes of hypercholesterolemic rats were investigated. Forty-eight white male albino rats weighing 140 ± 10 g were used and divided to 8 groups, each group (6) rats. Rats infected of hypercholesterolemic by injected with Triton-X-100 (100mg/ kg body weight). Glucose, serum liver functions (GOT GPT and ALP), T.G, T.C, LDL-c, HDL-c, VIDL-c, AIT, kidney functions (uric acid, urea and creatinine) were determined. Also, identification of phenolic compounds were determined using HPLC technique. The obtained results of hypercholesterolemic rats revealed that white, black rice as powder improve serum glucose level, liver functions, kidney functions and lipid profile in rats especially 10% black rice powder. The HPLC results showed that the black rice had higher bioactive compounds than white rice, besides the fact that it has so many health benefits.

Key words: Rice powder - Rats - Hypercholesterolemic – Bioactive compounds - Biochemical analysis.

INTRODUCTION

Hyperlipidemia is a medical disorder characterized by a rise in one or several lipids, such as cholesterol, cholesterol esters, triglycerides phospholipids and maybe even plasma lipoproteins like very density lipoprotein and low density lipoprotein while caused a reduced in high density lipoprotein levels. This raising of plasma lipids is causing risk factors related with cardiovascular diseases. On the other hand, statins and fibrates appear to be the primary anti-hyperlipidemic agents for treating elevated plasma triglycerides and cholesterol, with the price of harmful side effects on the liver and muscles, respectively (**Shattat, 2014**). Hyperlipidemia is becoming one of the most risk factors causing cardiovascular diseases (CVDs). It is one third of the world's

total deaths, believing that CVDs will prove to be the world's leading cause of death and disability by 2020 (Jorgensen *et al.*, 2013).

Bailey, (2000) stated that there are several major types of lipids can be recognized from metabolic overview, such as free fatty acids, triacylglycerol, phospholipids, and cholesterol and its esters. The main functions of lipids are to know as energy stores and to provide as essential structural component of cells. To identify these functions, lipids have to be moved in plasma from a tissue to another, from the liver or intestine to some tissues such as muscular, adipose tissue or from the other tissues to the liver. Xenoulis and Steiner, (2010) reported that lipids are water insoluble organic compounds, that are necessary for many functions of living cells, such as they are important cell membranes components, they are used to store energy, and play an important role as co-factors of enzymes, hormones and intracellular messengers. As for lipids, they are water-insoluble molecules; they cannot be transported in any aqueous solutions, such as plasma. Behind this reason, lipids are transported in plasma as lipoproteins known as macromolecular complexes. There are normally no clear signs of hyperlipidemia but they are generally identified throughout routine monitoring and when it has become the danger stage of a heart attack or a stroke.

Rice (*Oryza sativa*, L.) is the most widely cultivated cereal food crop in the world especially in most developing countries. Most of the world population depends on rice as their main source of food especially carbohydrates source. About 95% of the rice production was recorded in Asian countries (Bhattacharjee *et al.*, 2002). Chaudhary, (2003) reported that there are a common white-rice variety. There are some especially colored rice's such as (black, purple, brown and red). Colors in the rice's are due to contains of a large amounts of anthocyanin pigment in the rice coat. Black rice is some cereal rich in fiber and polyphenols, especially anthocyanins. Many studies have shown that certain polyphenols, such as anthocyanins, can be good for cardiovascular health (Xia *et al.*, 2006). Nutritional value of black rice is superior to any other kind of rice; even this black rice is free of gluten, cholesterol, and low sugar, salt and fat content. It is a super nutritious whole grain rice type which is high content in fiber, anthocyanin, antioxidants, vitamins B complex and E, iron, thiamine, magnesium, niacin and phosphorous.

Also, it is reported that 50 g of black rice provides 35% of RDA of selenium, copper, zinc and magnesium per day quality and quantity of protein is higher than any other rice varieties (Ujjawak, 2016). Black rice is a species of rice called *Oryza sativa*, L. indica, filled with high nutrients and primarily grown in the pericarp (outer part) of the kernel of this rice color is black due to

the pigment known as anthocyanin. Antioxidant black rice is also known as violet rice, forbidden rice, heavenly rice, imperial rice, king's rice and precious rice (Kong *et al.*, 2008). Many studies have shown that fruits and cereals contain some substances that are able to reduce or control the levels of blood cholesterol. Among the substances investigated are fiber, vitamins with antioxidant action, such as vitamins E, C, and A, carotenoid pigments, such as b-carotene, lycopene, and lutein, and phenolic compounds with antioxidant activity, such as catechins, anthocyanidins, quercetin, limonoids, and kaempferol (Colli *et al.*, 2002). Salgado *et al.*, (2010) reported that diet containing black rice reduced the level of plasma cholesterol, triglycerides, and low-density lipoprotein. The diet that provided an increase in the levels was black rice for the high-density lipoprotein values. Compared with the whole rice diet, the diet containing black rice was more effective in regulating lipidemia in rats. Several investigators have recently written about the health benefits of black glutinous rice. A recent report showed that anthocyanin supplementation in humans improves LDL and HDL levels (Qin *et al.*, 2009) and can delay cancer development in rodent's models of carcinogenesis (Thomasset *et al.*, 2009). Black rice may have anti-atherogenic activity and may improve certain metabolic pathways associated with diets high in fructose (Guo *et al.*, 2007).

This study was conducted to investigate the effect of white and black rice (*Oryza sativa*, *L. indica*) and their mixture on hypercholesterolemic rats.

Material and Methods

Materials

Black rice

Black rice (*Oryza sativa*, *L. indica*) was obtained from Agriculture Research Center, Kefir El-sheikh Governorate, Egypt.

Triton- X-100

The chemical Triton X-100 used to induce the hypercholesterolemia obtained from Morgan Chemical Factory, Cairo, Egypt.

Chemical and chemical kits:

Casein, cellulose, choline chloride powder, and DL methionine powder, were obtained from El-Gomhoria Company for chemical, Drugs and Medical Instruments, Cairo, Egypt. Oil and corn starch were obtained from local market in Menoufia, Egypt. The kits were supplied by Bio Diagnostics Company Cairo, Egypt.

Experimental animals

A total of 48 adult normal male albino rats Sprague Dawley strain weighing 140 ± 10 g were obtained from Vaccine and Immunity Organization, Ministry of Health, Helwan Farm, Cairo, Egypt.

Methods

Preparation of black rice

Black rice obtained from the Agricultural Research Center in Kafr El-Sheikh then removed the impurities from it and then black the black rice grains in the blender to get the powder form.

Identification of phenolic compounds:

HPLC analysis of extracts was performed using an Agilent 1200 chromatograph equipped with a PDA model G1315B, a Bin pump model G1312A, an auto-sampler model G1313A and a RR Zorbax Eclipse Plus C18 column ($1.8\ \mu\text{m}$, $150\ \text{mm} \times 4.6\ \text{mm}$). The mobile phase A was 0.2 % formic acid in water and the mobile phase B was acetonitrile. Elution was performed at $0.95\ \text{ml min}^{-1}$ with the following gradient program of solvent B: 0–20 min, 5–16 %; 20–28 min, 16–40 %; 28–32 min, 40–70 %; 32–36 min, 70–99 %; 36–45 min, 99 % and 45–46, min. 99–95 %. The injection volume was $10\ \mu\text{l}$. Wavelengths of 280 nm (for flavan-3-ols and derivatives of benzoic acid) and 360 nm (for flavonols and derivatives of cinnamic acid) were selected for detection; quantification of the compounds was realized using calibration curves obtained by HPLC of pure standards. The HPLC method was used according to **Radovanović *et al.*, (2010)** with some modification (elution gradient and flow rate).

Experimental design and animal group

Forty-eight male albino rats, weighing 140 ± 10 g were used in the study. The animals were obtained from Vaccine and Immunity Organization, Ministry of Health, Egypt. Rats were housed in individual stainless steel cages under controlled environmental conditions, in the animal house and fed 7 days on basal diet (casein diet) prepared according to **AIN, (1993)**, period to start feeding on experimental diet for acclimatization. Rats are divided into 8 groups, each group which consists of six rats as follows: Group 1 (-ve): fed on basal diet only, as negative control. Group 2 (+ve): fed on basal diet and injected by a single dose of freshly prepared solution of Triton-X-100 (100mg/kg)

and was used as a positive control group. Group 3: A group infected by hypercholesterolemia fed on basal diet with 5% white rice as powder of the weight of the diet. Group 4: A group infected by hypercholesterolemia fed on basal diet with 10% white rice as powder of the weight of the diet. Group 5: A group infected by hypercholesterolemia fed on basal diet with 5% black rice as powder of the weight of the diet. Group 6: A group infected by hypercholesterolemia fed on basal diet 10% black rice as powder of the weight of the diet. Group 7: A group infected by hypercholesterolemia fed on basal diet with 5% mixture of white black rice (1:1) as powder of the weight of the diet. Group 8: A group infected by hypercholesterolemia fed on basal diet with 10% mixture of white black rice (1:1) as powder of the weight of the diet. All rice put on the expense of starch. The experiment period was take 28 days, at the end of the experimental period each rat weight separately then, rats are slaughtered and collect blood samples. Blood samples were centrifuged at 4000 rpm for ten minute to separate blood serum, and then kept in deep freezer till using.

Biochemical analysis

Lipids profile

Determination of total cholesterol

Serum total cholesterol was determined according to the colorimetric method described by **Thomas (1992)**.

Determination of serum triglycerides

Serum triglyceride was determined by enzymatic method using kits according to the **Young, (1975) and Fossati & Principe, (1982)**.

Determination of high density lipoprotein (HDL-c):

HDL-c was determined according to the method described by **Friedewaid (1972) and Grodon & Amer (1977)**.

Calculation of very low density lipoprotein cholesterol (VLDL-c)

VLDL-c was calculated in mg/dl according to **Lee and Nieman (1996)** was using the following formula:

$$\text{VLDL-c (mg/dl)} = \text{Triglycerides} / 5$$

Calculation of low density lipoprotein cholesterol (LDL-c)

LDL-c was calculated in mg/dl according to **Lee and Nieman (1996)** as follows:

$$\text{LDL-c (mg/dl)} = \text{Total cholesterol} - \text{HDL-c} - \text{VLDL-c}$$

Calculation of atherogenic index (AI):

$$\text{Calculation of atherogenic index} = (\text{VLDL-c} + \text{LDL-c}) / \text{HDL-c}$$

This index was calculated as the (VLDL-c+ LDL-c/HDL-c ratio according to the formula of **Kikuchi-Hayakawa *et al.*, (1998)**.

Liver functions

Determination of serum alanine amino transferase (ALT), serum aspartate amino transferase (AST), serum alkaline phosphatase (ALP) were carried out according to the method of **Hafkenschied (1979); Clinica Chimica Acta (1980) and Moss (1982)**, respectively.

Determination of low density lipoprotein cholesterol (LDL-c)

Serum low density lipoprotein cholesterol (LDL-c) was calculated as mg/dl according to **Castelli *et al.*, (1977)** equation:

$$\text{LDL Concentration mg/dl} = \text{Total Cholesterol} - \text{HDL-c} - \text{VLDL-c}$$

Kidney functions

Determination of serum urea and creatinine

Serum urea and serum creatinine were determined by enzymatic method according to **Henry (1974)** and **Patton & Crouch (1977)**.

Determination of glucose level

Serum glucose was measured using the modified kinetic method according to **Kaplan, (1984)** by using kit supplied by spin react. Spain.

Statistical analysis

The data were analyzed using a completely randomized factorial design (**SAS, 1988**) when a significant main effect was detected; the means were separated with the Student-Newman-Keuls Test. Differences between treatments of ($P \leq 0.05$) were considered significant using Costat Program. Biological results were analyzed by One Way ANOVA.

RESULTS AND DISCUSSION

Data tabulated in Table (1) show the identification of phenolic compounds of white and black rice. In case of black rice, it is clear to notice that the highest phenolic compounds recorded for ferulic acid and *p*-coumaric acid, which were 19.93 and 9.13 $\mu\text{g}/\text{mg}/\text{g}$ DW, respectively. While, the lowest value recorded for syringic acid and gallic acid, which were 0.39 and 0.60 $\mu\text{g}/\text{mg}/\text{g}$ DW, respectively, while, chlorogenic acid and *p*-hydroxybenzoic acid did not detect at these conditions.

On the other hand, the highest phenolic compounds recorded for caffeic acid and *p*-coumaric acid, which were 4.10 and 3.36 $\mu\text{g}/\text{mg}/\text{g}$ DW, respectively. While, the lowest value recorded for *p*-hydroxybenzoic acid and protocatechuic acid, which were 0.25 and 0.45 $\mu\text{g}/\text{mg}/\text{g}$ DW, respectively. While, gallic acid did not detect at these conditions. From obtained results it could be concluded that the black rice had higher bioactive compounds than white rice, besides the fact that it has so many health benefits. These results are in agreement with **Shen *et al.*, (2009)** also reported the variations in phenolics content, flavonoid and antioxidant properties among the cereal grains with special emphasis on black rice, brown rice, white rice and red sorghum. Also, **Zhang *et al.*, (2015)** reported that many phenolic compounds such as ferulic, *p*-coumaric, isoferulic, syringic, vanillic, sinapic, caffeic, *p*-hydroxybenzoic, and protocatechuic acid are found in the whole rice grain, of which ferulic acid is the most abundant phenolic acid.

Data presented in Table (2) show the effect of white, black rice and their mixture on serum glucose level in hypercholesterolemic rats. It is clear to notice that, the mean value of serum glucose level of positive control group was significantly higher than negative control group, which were 157 and 97.50 mg/dl, respectively. Also, the mean value of treated groups (hypercholesterolemic rats) G3, G4, G5, G6, G7 and G8 recorded a significant decreased in serum glucose, which were 138, 129, 110, 99.50, 112.50 and 104.00 mg/dl, respectively, with significant difference when compared with positive control. The best results were recorded for G6 which fed on 10% black rice; the mean value was 99.50 mg/dl. These results are in agreement with **Apichai *et al.*, (2012)**, they reported that a diet containing 5% purple rice bran (black rice) improved the diabetic conditions in streptozotocin-induced diabetic rats by 8-week ingestion, the diet decreased fasting blood glucose and triglyceride, and enhanced glucose transporter 4 (Glut4) level in the soleus muscle. Also, **Torimitsu *et al.*, (2010)** reported that chronic hyperglycemia is an indicator of T2DM, which can be

described by determining the glucose tolerance. The pre-germinated brown rice slightly reduces the plasma adipocytokines, HbA1c level, and insulin resistance in the rat, by which it prevents the development of T2DM. Also, **Tabas *et al.*, (2008)** demonstrated that C3G and Cyanidin-3-galactoside in black rice can stimulate insulin secretion directly from the pancreas. Flavonoids also work by improving pancreatic beta cells.

Data given in Table (3) show the effect of white, black rice and their mixture on liver functions (ALP, GOT and GPT) of hypercholesterolemic rats. Concerning GPT (ALT) enzyme, results indicated that the mean value of positive control group was significantly higher than that of negative control group (healthy rats), which were 47.00 and 20.00 (U/L), respectively with significant difference. On the other hand, the mean values of treated groups (hypercholesterolemic rats) G3, G4, G5, G6, G7 and G8 were lower than positive control group. Group rats fed on 10 % black rice (G6) showed the highest reduction of GPT enzyme with non-significant difference as compared with negative control group.

As for GOT (AST) enzyme, it could be noticed that the mean value of positive control group was significantly higher than that of negative control group (healthy rats), which were 57.0 and 29.5 (U/L), respectively with significant difference. While, the mean values of treated groups (hypercholesterolemic rats) G3, G4, G5, G6, G7 and G8 were lower than positive control group, which were 48.20, 40.50, 38.60, 31.50, 40.10 and 34.0 (U/L), respectively. Rats fed on (G6) 10% black rice showed non-significant difference as compared with negative control group and recorded the best treatment.

As for ALP enzyme, it could be noticed that the mean value of positive control group was significantly higher than that of negative control group, which were 135.0 and 102.5 (U/L), respectively with significant difference. The mean value of treated groups (hypercholesterolemic rats) G3, G4, G5, G6, G7 and G8 were lower than positive control group, which were 125.0, 120.0, 112.0, 104.50, 115.0 and 109.0 (U/L), respectively. The best result was recorded for (G6) rats fed on 10% black rice. These results are supported by published by **Zhaohua *et al.*, (2010)** indicated that chronic ethanol consumption caused a significant increase in the activities of AST, ALT and GGT which could cause severe damage to tissue membrane. The

decreased activities of these enzymes on black rice extract administrated rats indicate the hepatic protective effect.

The effect of white, black rice and their mixture on the serum cholesterol and triglyceride of hypercholesterolemic rats are shown in Table (4). The obtained results showed that the mean value of T.C. of positive control group was significantly higher than negative control group, which were 245.0 and 102.0 mg/dl, respectively. The mean values of treated groups (hypercholesterolemic rats) G3, G4, G5, G6 and G8 was 176.0, 157.0, 115.0, 105.0, 135.0 and 123.0 mg/dl, respectively and showed a significant difference when compared with positive control group, while, G6 when compared with negative control group showed non-significant difference between them. These results are in agreement with **Um *et al.*, (2013)**, who found also that the black rice intake is associated with reduced levels of plasma cholesterol. Similarly, **Um *et al.*, (2013)**, who found also that the black rice intake is associated with reduced levels of plasma cholesterol. **Park *et al.*, (2014)** demonstrated that consumption of red or black rice rich in polyphenols caused a decrease in serum and hepatic level of LDL-cholesterol in mice fed a high cholesterol diet, which was due to inhibition of hepatic cholesterol synthesis by the decreased expression of HMG-CoA reductase, ACAT-2 and SREBP-2 expression, and the increased degradation of cholesterol by the increased expression of CYP7a1 and CYP8b1.

Concerning triglycerides, results showed that the mean value of serum triglycerides of positive control group) was significantly higher than negative control group, it was 155.0 and 75.0 mg/dl, respectively. The mean values of treated groups (hypercholesterolemic rats) G3, G4, G5, G6 and G8 was 126.0, 109.0, 96.0, 81.0, 103.0 and 88.0 mg/dl, respectively and showed a significant difference when compared with positive control group. The best result was recorded for group (6). These results are in agreement with **Soheir *et al.*, (2016)**, they indicated that the rats fed on black rice reduce the levels of serum triglycerides (T.G.). Also, **Wang *et al.*, (2006)** found that the used of rice as a supplement in the diet dishes and the animals that fed that diet recorded a significantly lower levels of cholesterol and triglycerides at the end of the experiment.

Data presented in Table (5) show the effect of white, black rice and their mixture on high density lipoprotein (HDL-c), Low density lipoprotein (LDL-c) and very Low density lipoprotein (VLDL-c) of hypercholesterolemic rats. Results showed that the mean value of HDL-c of positive control group was significantly lower than negative control group; it was 54.50 and 25.30 mg/dl, respectively. The mean value of

treated groups (hypercholesterolemic rats) G3, G4, G5, G6, G7 and G8 were 37.27, 41.30, 46.67, 51.90, 40.97 and 46.50 mg/dl and showed a significant difference when compared with positive control group.

As for LDL-c results showed that the mean value for positive control group was significantly higher than negative control group, which was 159.50 and 32.50 mg/dl, respectively. The mean value of treated groups (hypercholesterolemic rats) G3, G4, G5, G6, G7 and G8 were 113.53, 93.90, 49.13, 36.90, 73.43 and 58.90 mg/dl and showed a significant difference when compared with positive control group.

Concerning VLDL-c, results indicated that the mean value of positive control group was significantly higher than negative control group. The mean values were 31.0 and 15.0 mg/dl, respectively. The mean value of treated groups (hypercholesterolemic rats) G3, G4, G5, G6, G7 and G8 were 25.20, 21.80, 19.20, 16.20, 20.60 and 17.60 mg/dl and showed a significant difference when compared with positive control group.

On the other hand, the mean value of atherogenic index (AI) for hypercholesterolemic rats fed on various diets was observed. It could be noticed that the mean value of atherogenic index (AI) ratio level of control (+) group was higher than control (-) group, it was being 6.0 ± 0.13 and 0.65 ± 0.10 mg/dl, respectively with a significant difference. All hypercholesterolemic rats fed on various diets, showed significant differences in mean values as compared to control (+) group. The values were 1.30, 0.89, 0.10, 1.93, 1.78 and 3.13 mg/dl, respectively. The obtained results from Table (4) showed that group 6 which fed on 10 % black rice recorded the best result for lipid profile. These results are in agreement with **Mishra et al., (2011)**, they reported that hyperlipidemia is an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters and phospholipids and/or plasma lipoproteins including very low density lipoprotein and low-density lipoprotein, and reduced high-density lipoprotein levels. Also, **Brouwers et al., (2012)** reported that hypercholesterolemia and hypertriglyceridemia are the main cause of atherosclerosis which is strongly related to ischemic heart disease (IHD). There is a strong relation between IHD and the high mortality rate. Furthermore, elevated plasma cholesterol levels cause more than four million deaths in a year. **Suh et al., (2005)**, indicated that the diet contained black rice decreases levels of VLDL. **Chutipaijit et al., (2011)** showed that black rice is considered to be a healthy food because of its antioxidant content that

are able to prevent oxidative stress. Oxidative modification of low-density lipoprotein (LDL) may play an important role in the development of atherosclerosis. **Guo et al., (2007)** showed that black rice compounds can reduce low-density lipoprotein cholesterol (LDL), improve lipid profiles.

Data tabulated in Table (6) show the effect of white, black rice and their mixture on kidney function of hypercholesterolemic rats. Concerning uric acid (mg/dl), data revealed that the mean value of positive control group was significantly higher than that of negative control group, which were 6.80 and 2.40 mg/dl, respectively. The mean value of G3, G4, G5, G6, G7 and G8 indicated a significant difference; it was 4.90, 4.50, 3.90, 2.60, 4.10 and 2.90 (mg/dl), respectively when compared with positive control group.

As for urea, it could be noticed that the mean value of positive control group was significantly higher than that of negative control group, which were 59.0 and 23.0 mg/dl, respectively. The mean value of treated groups (hypercholesterolemic rats) G3, G4, G5, G6, G7 and G8 indicated non-significant differences between them and a significant difference when compared with positive control group., which were 42.0., 39.0, 36.0, 29.0, 40.0 and 35.0 (mg/dl), respectively.

As for creatinine, results indicated that the mean value of positive control group was significantly higher than that of negative control group, which were 1.75 and 0.73 (mg/dl), respectively. The mean value of treated groups (hypercholesterolemic rats) G3, G4, G5, G6, G7 and G8 indicated non-significant differences between them and with positive control group., which were 1.65, 1.60, 0.92, 0.84, 1.25 and 1.01 (mg/dl), respectively. These results are in agreement with **Missoun et al., (2010)** who found also that the black rice reduced levels of serum urea. Also, **Heba et al., (2018)**, found that the activities of uric acid in senescence accelerated mice treated with black rice extract showed a marked decrease. The obtained results from Table (5) showed that group 6 which fed on 10 % black rice recorded the best result for uric acid, urea and creatinine levels.

Finally it could be observed that the higher the level of white or black rice in diets the more the desirable action on biological and biochemical parameters evaluated for hypercholesterolemic rats. All treatment groups revealed improvement of mentioned parameters, provided that the best group was that of 10% black rice diet. Over and above, no synergistic action, occurred when combining both plants

together, provided that the mix black rice diet revealed also some improvement.

Table (1): Identification of phenolic compounds of white and black rice

Active compounds of black rice	Concentration $\mu\text{g}/\text{mg}$	Active compounds of white rice	Concentration $\mu\text{g}/\text{mg}$
Chlorogenic acid	ND	Chlorogenic acid	1.72
Sinapic acid	4.18	Sinapic acid	1.05
Vanillic acid	5.35	Vanillic acid	1.40
Syringic acid	0.39	Syringic acid	0.55
Gallic acid	0.61	Gallic acid	ND
<i>p</i> -Coumaric acid	9.13	<i>p</i> -Coumaric acid	3.36
<i>p</i> -Hydroxybenzoic acid	2.12	<i>p</i> -Hydroxybenzoic acid	0.25
Ferulic acid	19.93	Ferulic acid	1.41
Protocatechuic acid	4.76	Protocatechuic acid	0.45
Caffeic acid	ND	Caffeic acid	4.10

Table (2): Effect of white, black rice and their mixture on glucose level of hypercholesterolemic rats

Parameters Groups	Glucose (mg/dl)
	Mean \pm SD
Control (-Ve)	97.50 ^f \pm 2.08
Control (+Ve)	157.00 ^a \pm 2.00
(5% White rice powder)	138.00 ^b \pm 2.15
(10% White rice powder)	129.00 ^c \pm 1.52
(5% Black rice powder)	110.00 ^d \pm 1.50
(10% Black rice powder)	99.50 ^f \pm 1.00
(5% Mixture of white and black rice)	112.50 ^d \pm 2.50
(10% Mixture of white and black rice)	104.00 ^e \pm 2.00
LSD	4.85

Each value is represented as mean \pm standard deviation ($n = 3$).

Mean under the same column bearing different superscript letters are different significantly ($p \leq 0.05$).

Table (3): Effect of white, black rice and their mixture on liver functions of hypercholesterolemic rats

Parameters Groups	(GPT) U/L	(GOT) U/L	(ALP) U/L
	Mean±SD	Mean±SD	Mean±SD
Control (-Ve)	20.00 ^f ±1.30	29.50 ^e ±1.60	102.5 ^e ±0.16
Control (+Ve)	47.00 ^a ±1.21	57.00 ^a ±1.86	135.0 ^a ±0.10
WRP 5%	36.30 ^b ±1.43	48.20 ^b ±1.25	125.0 ^b ±0.14
WRP 10%	31.40 ^d ±1.58	40.50 ^c ±1.20	120.0 ^c ±0.10
BRP 5%	30.50 ^d ±1.70	38.60 ^c ±1.50	112.0 ^d ±0.15
BRP 10%	21.15 ^f ±0.87	31.50 ^e ±1.20	104.5 ^e ±0.12
MWBR 5%	33.60 ^c ±1.18	40.10 ^c ±1.98	115.0 ^d ±0.12
MWBR 10%	23.30 ^e ±0.99	34.00 ^d ±0.40	109.0 ^d ±0.13
LSD	2.36	2.17	3.37

Each value is represented as mean ± standard deviation (n = 3).

Mean with the same letters in the same horizontal column are not significantly different at (P≤0.05).

Table (4): Effect of white, black rice and their mixture on the serum cholesterol and triglyceride of hypercholesterolemic rats

Parameters Groups	T.C. (mg/dl)	T.G. (mg/dl)
	Mean±SD	Mean±SD
Control (-Ve)	102.0 ^g ±0.11	75.0 ^h ±0.10
Control (+Ve)	215.0 ^a ±0.10	155.0 ^a ±0.15
WRP 5%	176.0 ^b ±0.10	126.0 ^b ±0.11
WRP 10%	157.0 ^c ±0.12	109.0 ^c ±0.15
BRP 5%	115.0 ^f ±0.14	96.0 ^e ±0.12
BRP 10%	105.0 ^g ±0.12	81.0 ^g ±0.10
MWBR 5%	135.0 ^d ±0.15	103.0 ^d ±0.11
MWBR 10%	123.0 ^e ±0.13	88.0 ^f ±0.14
LSD	3.93	4.29

Each value is represented as mean ± standard deviation (n = 3).

Mean with the same letters in the same horizontal column are not significantly different at (P≤0.05).

Table (5): Effect of white, black rice and their mixture on serum lipid profiles of hypercholesterolemic rats

Parameters Groups	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)	AI level (mg/dl)
	Mean±SD	Mean ± SD	Mean ± SD	Mean ± SD
Control (-Ve)	54.50 ^a ±1.29	32.50 ^h ±2.29	15.00 ^h ±0.42	0.65 ^d ±0.10
Control (+Ve)	25.30 ^f ±1.78	159.00 ^a ±1.44	31.00 ^a ±0.31	6.00 ^a ±0.13
WRP 5%	37.27 ^c ±0.64	113.53 ^b ±2.41	25.20 ^b ±0.40	1.30 ^c ±0.16
WRP 10%	41.30 ^d ±0.98	93.90 ^c ±2.34	21.80 ^c ±0.53	0.89 ^d ±0.11
BRP 5%	46.67 ^c ±1.7	49.13 ^f ±1.0	19.20 ^e ±0.61	0.10 ^d ±0.10
BRP 10%	51.90 ^b ±1.15	36.90 ^g ±1.15	16.20 ^g ±0.50	1.93 ^c ±0.15
MWBR 5%	40.97 ^d ±0.95	73.43 ^d ±1.95	20.60 ^d ±1.56	1.78 ^c ±0.11
MWBR 10%	46.50 ^c ±1.5	58.90 ^e ±2.18	17.60 ^f ±0.50	3.13 ^b ±0.13
LSD	2.17	3.42	0.79	0.81

Each value is represented as mean ± standard deviation ($n = 3$).

Mean with the same letters in the same horizontal column are not significantly different at ($P \leq 0.05$).

Table (6): Effect of white, black rice and their mixture on kidney functions of hypercholesterolemic rats

Parameters Groups	Uric acid (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
	Mean ± SD	Mean ±SD	Mean ± SD
Control (-Ve)	2.40 ^c ±0.10	23.0 ^d ±0.10	0.73 ^a ±0.10
Control (+Ve)	6.80 ^a ±0.13	59.0 ^a ±0.13	1.75 ^a ±0.13
WRP 5%	4.90 ^b ±0.11	42.0 ^b ±0.11	1.65 ^a ±0.11
WRP 10%	4.50 ^b ±0.13	39.0 ^b ±0.10	1.60 ^a ±0.13
BRP 5%	3.90 ^b ±0.16	36.0 ^b ±0.15	0.92 ^a ±0.16
BRP 10%	2.60 ^c ±0.11	29.0 ^c ±0.11	0.84 ^a ±0.11
MWBR 5%	4.10 ^b ±0.15	40.0 ^b ±0.13	1.25 ^a ±0.10
MWBR 10%	2.90 ^c ±0.11	35.0 ^b ±0.16	1.01 ^a ±0.15
LSD	1.15	3.40	0.83

Each value is represented as mean ± standard deviation ($n = 3$).

Mean under the same column bearing different superscript letters are different significantly ($p \leq 0.05$).

REFERENCES

- AIN**, American Institute of Nutrition (1993): Purified diet for laboratory Rodent, Final Report. J. Nutrition, 123: 1939-1951.
- Apichai, S.; Pongchaidecha, A.; Kaeapai, W. and Jitprawet, N. (2012):** Beneficial effect of Thai purple sticky rice supplement in streptozotocin induced diabetic rats. CMU. J. Nat. Sci., Special Issue on Agricultural & Natural Resources, 11: 371- 381.
- Bailey, B.D. (2000):** Peroxisome proliferator activated receptors in the cardiovascular system. British journal of pharmacology, 129: 823-834.
- Bhattacharjee, P.; Singhal, R. S. and Kulkarni, P. R. (2002):** Basmati rice: A review. International Journal of Food Science & Technology, 37 (1): 1-12.
- Brouwers, M.C.; VanGreevenbroek, M.M.; Stehouwer, C.D.; de Graaf, J. and Stalenhoef, A.F. (2012):** Thegenetics of familial combined hyperlipidaemia. Nat. Rev. Endocrinol., 8 (6): 352-362.
- Castelli, W. P.; Doyle, J. T.; Gordon, T.; Hames, C. G.; Hjortland, M. C.; Halley, S. B.; Kagan, A. and Zuckel W. J. (1977):** HDL cholesterol and other lipids in coronary heandisease. The cooperative lipoprotein phenotyping study Circulation, 55: 767-772.
- Chaudhary, R. C. (2003):** Specialty rice's of the world: Effect of WTD and IPR on its production trend and marketing. Journal of Food Agric. and Environ. 1 (2): 34-41.
- Chutipajit, S.; Chaum, S. and Sompornpailin, K. (2011):** High contents of proline and anthocyanin increase protective response to salinity in *Oryza sativa*, L. spp: Aust. J. Crop Sci., 5: 1191-1198.
- Clinica Chimica Acta (1980):** 105: 147-172, (Chemical kits).
- Colli, C.; Sardinha, F. and Filisetti, T.M. (2002):** Alimentos funcionais. In: Guia de Nutricao: Nutricao Clinica no Adulto (Schor N, coord.; Cuppari L, ed.). Manole, Sao Paulo, pp. 55-70.
- Fossati, P. and Principe L. (1982):** Triglyceride enzymatic colorimetric method. J. Clin. Chem., 28 (10): 2077-2080.

Friedwaid, W.T. (1972): Determination of HDL. Clin. Chem., 18: 499. (Chemical Kits).

Grodon, T. and Amer, M. (1977): Determination of HDL. Clin. Chem., 18: 707.

Hafkenschied, J.C. (1979): Determination of GOT. Clin. Chem., 25:155.

Guo, H.; Ling, W.; Wang, Q.; Liu, C.; Hu, Y.; Xia, M.; Feng, X. and Xia, X. (2007): Effect of anthocyanin rich extract from black rice (*Oryza sativa*, L. indica) on hyperlipidemia and insulin resistance in fructose-fed rats. Plant Foods Human Nutr., 62: 1-6.

Heba, S. A.; Fatma, A. El. and Mai, M. (2018): Nutraceutical and Biochemical Studies on Black Rice (*Oryza sativa*, L) using Hepatic Albino Rats. Thesis, M.Sc., Faculty of Home Economics, Menoufia University, Egypt.

Henry, R.J. (1974): Clinical Chemist: principles and Techniques. 2nd , Edition, Hagerstown (MD), Harcer, ROW, P. 882.

Jorgensen, T.; Capewell, S.; Prescott, E.;Allender, S.; Sans, S. and Zdrojewski, T. (2013):Population-level changes to promote cardiovascular health. Eur. J. Prev. Cardiol., 20 (3): 409-421.

Kaplan, L.A.(1984): Clinical Chemistry. The C.V. Mosby Co., St Louis, Toronto. Princt., 1032-1036.

Kikuchi-Hayakawa; Onodera, N.; Matsubara, S.; Yasudo, E.; Chonan, O.; Takahashi, R. and Ishikawa, F. (1998): Effect of soymilk and bifidobacterium fermented soymilk on lipid metabolism in aged avariectomized rats. Bioscience Biotechnology and Biochemistry, 62 (9): 1688-1692.

Kong, L.; Wang, Y. and Cao, Y. (2008): Determination of Myoinositol and D-chiro-inositol in black rice bran by capillary electrophoresis with electrochemical detection. AGRAS, 21 (6): 501-504.

Lee, R. and Nieman, D. (1996): Nutrition Assessment. 2nd Ed. Mosby, Missouri, USA, pp., 591-594.

- Mishra, P. R.; Panda, P.K.; Apanna, K.C. and Panigrahi, S. (2011):** Evaluation of acute hypolipidemic activity of different plant extracts in Triton WR-1339 induced hyperlipidemia in albino rats. *Pharmacologyonline*, 3: 925-934.
- Missonu, F.; Slimani, M. and Aoues, A. (2010):** toxic effect of lead on kidney function in rat wistar, *African Journal of Biochemistry, Research*, 4: 21-27.
- Moss, D.W. (1982):** Alkaline Phosphates isoenzymes, *Clin. Chem.*, 28: 2007-2016.
- Most, M.M.; Tulley, R.; Morales, S and Lefevre, M. (2005):** Rice bran oil, not fiber, lowers cholesterol in humans. *Am. J. Clin. Nutr.*, 81: 64-68.
- Murata, K.; Kamei, T.; Torium, Y.; Kobayashi, Y.; Iwata, K.; Fukumoto, I. and Yoshioka, M. (2007):** Effect of processed rice with brown rice extracts on serum cholesterol level. *Clin. Exp. Pharmacol. Physiol.*, 34: 87-89.
- Park, Y.; Park, E.; Kim, E. and Chung, M. (2014):** Hypocholesterolemic metabolism of dietary red pericarp glutinous rice rich in phenolic compounds in mice fed a high cholesterol diet. *Nutrition Research and Practice*, 8 (6): 632-637.
- Patton, C.J. and Crouch, S.R. (1977):** Spectrophotometric and kinetics investigation of the Berthelot reaction for determination of ammonia. *Anal. Chem.*, 49: 464-469.
- Qin, Y.; Xia, M. and Ma, J. (2009):** Anthocyanin supplementation improves serum LDL and HDL-cholesterol concentrations associated with the inhibition of cholesteryl ester transfer protein in dyslipidemic subjects. *Am. J. Clin. Nutr.*, 90 (3): 485-492.
- Radovanović, B. and Radovanović, A. (2010):** Free radical scavenging activity and anthocyanin profile of Cabernet Sauvignon wines from the Balkan Region. *Molecules*, 15: 4213-4226.
- Salgado, J.M.; Oliveira, A.G.; Mansi, D.N.; Carlos, M. P.; Bastos, C.R. and Marcondes, F.K. (2010):** The role of black rice (*Oryza sativa*, L.) in the control of hypercholesterolemia in rats. *J. Med. Food*, 13 (6): 1355-1362.
- SAS (1988):** SAS Users Guide: Statistics version 5th Ed. SAS. Institute Inc., Cary N.C.

- Shattat, G. F. (2014):** A review article on hyperlipidemia: Types, treatments and new drug targets. *Biomedical & Pharmacology Journal* Vol. 7(2), 399-409.
- Shen, Y.; Jin, L.; Xiao, P.; Lu, Y. and Bao, J. (2009):** Total phenolics, flavonoids, antioxidant capacity in rice grain and their relations to grain color, size and weight. *Journal of Cereal Science*, 49 (1): 106-111.
- Soheir, N.A.; Saad, A.M.; Walid, M.S. and Afaf, A.A. (2016):** preventive effect of black rice ethanolic extract as natural antioxidants on some heavy metals induced disorders in experimental animal, *Int. J. Food, Nutrition and public Health*, 8 (1): 20-34.
- Suh, M.H.; Yoo, S.H.; Chang, P.S. and Lee, H.G. (2005):** Antioxidative activity of microencapsulated *c*-oryzanol on high cholesterol - fed rats. *J. Agric. Food Chem.*, 53: 9747-9750.
- Tapas, A.R.; Sakarkar, D. and Kakde, R.B. (2008):** Flavonoids as nutraceuticals: A review: *Trop. J. of Pharm. Res.*, 7 (3): 89-99.
- Thomas, L. (1992):** *Labor and Diagnose*, 4th Ed. Marburg: Die Medizinische Verlagsgesellschaft.
- Thomasset, S.; Berry, D.P. and Cai, H. (2009):** Pilot study of oral anthocyanins for colorectal cancer chemoprevention. *Cancer Prev. Res. (Phila)*, 2: 625-633.
- Torimitsu, M.; Nagase, R.; Yanagi, M.; Homma, M.; Sasai, Y.; Ito, Y.; Hayamizu, K.; Nonaka, S.; Hosono, T. and Kise, M. (2010):** Replacing White rice with pre-germinated brown rice mildly ameliorates hyperglycemia and imbalance of adipocytokine levels in type 2 diabetes model rats. *J. Nutr. Sci. Vitaminol.*, 56: 287-292.
- Tripathi, K. D. (2008):** *Essentials of Medical Pharmacology*, 6thedn, India: JP brothers medical publishers, pp613-614.
- Ujjawak K. S. K. (2016):** *Black rice Research, History and Development*, Springer International Publishing (publisher), e-book-ISBN: 978-3-319-3015.
- Um, M.Y.; Jiyun, A. and Tae, Y.H. (2013):** Hypolipidaemic effects of cyanidin 3-glucoside rich extract from black rice through regulating hepatic lipogenic enzyme activities. *J. Sci. Food Agric.*, 93 (12): 24-31.

Wang, J.J.; Pan, T.M.; Shieh, M.J. and Hsu, C.C. (2006): Effect of red mold rice supplements on serum and meat cholesterol levels of broilers chicken. Appl. Microbiol. Biotechnol., 71: 812-818.

Xia, X.; Ling, W.; Ma, J.; Xia, M.; Hou, M.; Wang, Q.; Zhu, H, and Tang, Z. (2006): An anthocyanin-rich extract from black rice enhances atherosclerotic plaque stabilization in apolipoprotein-deficient mice. J. Nutr., 136: 2220-2225.

Xenoulis, P.G. and Steiner, J.M. (2010): Lipid metabolism and hyperlipidemia in dogs. The Veterinary Journal, 183: 12-21.

Young, D. (1975): Effects of drugs on clinical laboratory tests. Pestaner, L. Clin. Chem., 21 (5): 1-432.

Zhang, H.; Shao, Y.; Bao, J. and Beta, T. (2015): Phenolic compounds and antioxidant properties of breeding lines between the white and black rice. Food Chem., 172: 630-639.

Zhaohua, H.; Peiyu, Q. and Guixing, R. (2010): Effect of anthocyanin-rich extract from black rice (*Oryza sativa*, L. Japonica) on chronically alcohol-induced liver damage in rats. J. Agric. Food Chem., 58: 3191-3196.

الملخص

تأثير الأرز الأبيض والأسود ومخلوتهما علي الجرذان المصابة بارتفاع مستوى الكوليستيرول في الدم

تم دراسة تأثير تركيبات مختلفة ٥٪، ١٠٪ من الأرز الأبيض والأسود ومخلوتهما كمسحوق على التغيرات البيولوجية والكيميائية الحيوية للجرذان المصابة بارتفاع مستوى الكوليستيرول. حيث تم استخدام ثمانية وأربعون من ذكور الجرذان البيضاء وزنها (٤٠ جم ± ١٠ جم) وقسمت إلى ٨ مجموعات، كل مجموعة بها (٦) جرذان. وتم اصابة الجرذان بارتفاع الكوليستيرول عن طريق الحقن بمادة تريبتون اكس ١٠٠ بجرعة (١٠٠مجم/كجم من وزن الجسم). تم تقدير كلا من مستوى الجلوكوز ووظائف الكبد في الدم ((GOT, GPT) و(الكوليستيرول الكلي والجليسيريدات الثلاثية والكوليستيرول عالي الكثافة و الكوليستيرول المنخفض الكثافة و الكوليستيرول المنخفض جدا الكثافة الدم، ألانين أمينو ترانسفيراز و الفوسفاتيز القلوي) وظائف الكلي (حمض البوليك ، اليوريا والكرياتينين) . كذلك تم تحديد التعرف على المركبات الفينولية في مسحوق الأرز الأبيض والأسود بواسطة جهاز الكروماتوجرافي الغازي عالي الأداء. وأظهرت النتائج المتحصل عليها من الفئران المصابة بارتفاع الكوليستيرول أن الأرز الأبيض والأسود كمسحوق يحسن مستوى الجلوكوز في الدم ووظائف الكبد والكلي والدهون في الجرذان التي تغذت على مسحوق الأرز الأسود بتركيز ١٠٪. كذلك اظهرت نتائج الكروماتوجرافي الغازي عالي الأداء أن الأرز الأسود يحتوي على تركيبات عالية من كل المركبات النشطة الفعالة أعلي من الأرز الأبيض، إلى جانب حقيقة أن له العديد من الفوائد الصحية.

الكلمات المفتاحية: مسحوق الأرز . الجرذان . ارتفاع مستوى الكوليستيرول في الدم - المركبات الفعالة . التحاليل الكيميائية الحيوية.