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BIOLOGICAL EVALUATION OF MICROWAVE DEFATTED BLACK RICE BRAN (MDBRB) IN CCL_4 INTOXICATED RATS

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ABSTRACT

The aim of the present study is to prepare Microwave defatted black rice bran (MDBRB) to study the effect of its substitution for casein on body weight, feed efficiency ratio, serum liver function enzymes, serum lipids profile and antioxidant enzymes in carbon tetrachloride (CCl_4) intoxicated rats. Results showed that substitution of MDBRB for casein especially at 75 and 100% in CCl_4 -intoxicated rats increased their feed intake and body weight gain. This substitution also decreased the levels of serum liver function enzymes, improved lipid profile and increased the activity levels of antioxidant enzymes in CCl_4 intoxicated rats. Histopathological examination revealed alleviation of hepatic lesions caused by CCl_4 by increasing the percentage of DBRB used. In conclusion, it was suggested that MDBRB could protect the liver cells from CCl_4 induced liver damages perhaps, by its antioxidative effect on hepatocytes, hence eliminating the deleterious effects of toxic metabolites from CCl_4. So the present study recommended that the use of MDBRB may be useful for patients suffering from liver diseases due to its hepatoprotective and hypolipidemic activities.
Keywords: Black rice bran, Liver function enzymes, Lipid profile.

INTRODUCTION

Rice bran as a waste product of paddy milling contain protein, carbohydrate, dietary fiber, ash, fat, vitamin, mineral and natural antioxidant compounds (Chen *et al*., 2008 and Saenjum *et al.*, 2012). Rice bran also contains phytochemical compounds in significant amount and these compounds have been considered as natural antioxidants (Xu and Godber, 1999).

The liver has a pivotal role in the metabolism and detoxification of the majority of substances entering human body. Many factors, such as toxic chemicals, excessive consumption of alcohol and virus infections, can cause liver injuries to different extent. Liver diseases have nowadays become one of the main concerns threatening human health at a high prevalence (He *et al*., 2011 and Tanaka *et al*., 2011). As a traditional medicinal food, black rice was recorded to have many health benefits such as invigorating spleen and warming liver in a well-known Chinese ancient pharmacopeia. Recent studies have shown that the main difference between black and white rice is that the bran of black rice is highly enriched with phytochemicals, especially anthocyanins (Zhang, *et al*., 2010).

Antioxidant properties of colored rice bran were better than that of non colored rice bran. The antioxidant properties of colored rice bran varieties is due to their pigment compounds of anthocyanin. Pigmented rice variety had a better scavenging activity than non pigmented rice variety because pigmented variety had a higher anthocyanin content which is a potent reducing agents and possesses strong radical scavenging activity (Nam *et al*., 2006).

Previous research about antioxidant properties in colored rice bran indicated that rice bran with certain color that contains anthocyanin has a reductase enzyme inhibitory and anti diabetic activity (Kim *et al.*, 2008 and Park *et al*., 2008). Furthermore, Anthocyanin pigments have highly effective in reducing cholesterol levels in the human body (Lee *et al*.,2008).

Anthocyanins, particularly cyanidin 3-glucosidase and peonidin 3-glucosidase, are responsible for the color of black rice, also exerted an inhibitory effect of cell invasion on various cancer cells and reduce the risks of cardiovascular diseases (Chen *et al.,* 2006). These bioactive compounds were reported to have strong free radical scavenging and antioxidant effects, (Ling *et al.,* 2002 and Zhang *et al.,* 2006) and help lower cholesterol levels, (Zawistowski *et al.,* 2009).

The present work aimed to study the possibility of using Microwave defatted black rice bran (MDBRB) on hepatic diseases, cholesterol and on the biological and histopathological effects in experimental rats which have hepatic injury induced by CCl_4.

MATERIALS AND METHODS

Materials:

Rice bran was obtained from the milling of black rice variety (*Oryza sativa* L.). The sample of rice bran obtained from Rice Research and Training Center (RRTC) at Sakha, Kafr El-Sheikh Governorate, Egypt during the season of 2013. Other chemicals and solvents used were of analytical reagent grade.

Methods:

Microwave stabilized black rice bran: A microwave oven with 550 W output power was used for the stabilization of bran. The moisture content of raw rice bran was adjusted to 21% before treatment. One hundred gram of sample was packed in a microwave-safe polyethylene bag and subjected to microwave heating for 3 min at 120 ºC and then cooled at room temperature (Ramezanzadeh *et al.,* 2000).

Defatted microwave black rice bran:

A weight of microwave black rice bran was soaked in n-hexane solvent (B.P 60 - 80 °C) at room temperature for 24 hr., then the obtained solution was filtered and the solvent was removed by rotary evaporator according to Kahlon *et al.* (1992). The defatted microwave rice bran meal was milled using a laboratory scale hammer mill. The resulting flour was sieved through a 60-mesh screen and was kept in polyethylene bags and stored at 4 °C until used.

Gross chemical composition of black rice bran:

In this study, black rice bran was analyzed for their chemical composition after subjecting to stabilization by microwave process and defatted microwave black rice bran. Moisture, ash, crude protein, ether extract and total dietary fiber contents were determined according to the methods of A.O.A.C. (2005). Total carbohydrates content was calculated by difference.

Phenolic compounds were extracted from rice bran samples twice using methanol 80% at a ratio of 1:20 (w/v). Each time, the mixture was shacked by a mechanical shaker (150 rpm) at room temperature for 16 h. After centrifugation at 4000 rpm for 5 min, the supernatants obtained from each time were combined and concentrated to dryness by a rotary evaporator at 35°C. The dried methanol extract was dissolved in 5 ml of methanol 50% and used as crude extracts according to the method described by Nara *et al.,* (2006). Total phenolic compounds of the extract were determined spectrophotometrically using Folin-ciocalteau reagent according to the method described by Bonoli *et al.,* (2004). Phenolics-acid content of phenolic compounds was estimated by a standard curve prepared using ferulic acid.

Biological assay:

Experimental design:

Forty two rats of young male Albino rats (153-155 gm) were obtained from Food Technology Research Institute, Agric. Research center, Giza, Egypt. All animals were housed individually in cages with screen bottoms and fed on a basal diet for 7 days under laboratory conditions. Rats were given free access to food and water throughout the experimental period of 8 weeks. After acclimation, rats were randomly divided into 6 groups (each of 7 rats) as shown in Table (A).

Table (A): Composition of experimental diets (as reported by LanePeter and Person, 1971).

Ingredient	Experimented diets					
	G1 Control-ve	G2 Control+ve	G3 MDBRB 25%	G4 MDBRB 50%	G5 MDBRB 75%	G6 MDBRB 100%
MDBRB	-	-	212	424	636	847.9
Starch	650	650	504.5	353.3	180	7.1
Casein	150	150	112.5	75	37.5	-
Oil	100	100	99	97.7	96.5	95
Cellulose	50	50	22	-	-	-
Mineral Mix	40	40	40	40	40	40
Vitamin Mix	10	10	10	10	10	10

G1– Control (-ve) non hepatotoxic- Rats was fed on basal diet.

G2– Control (+ve) hepatotoxic– Rats was fed on basal diet.

G3–hepatotoxic– Rats was fed on basal diet substituted 25 % (MDBRB) for casein.

G4–hepatotoxic– Rats was fed on basal diet substituted 50 % (MDBRB) for casein.

G5–hepatotoxic– Rats was fed on basal diet substituted 75 % (MDBRB) for casein.

G6–hepatotoxic– Rats was fed on basal diet substituted 100 % (MDBRB) for casein.

The first group was fed on the basal diet and served as a negative control (-Ve).The rest five groups were given carbon tetrachloride (CCl_4) for induction of acute liver damage. CCl_4 was diluted in an equal volume of paraffin oil as a vehicle and subcutaneously injected in the first and the second day of the experiment in a dose of 1 ml/kg body weight (Wilfried *et al.,* 1994). The first hepatotoxic group was fed basal diet and kept as a positive control (+Ve) while the other hepatotoxic groups were fed on basal diets that substitute 25, 50, 75 and 100% (DBRB) for casein. Feed intake (FI) and body weight were recorded weekly. And body weight gain (BWG) and feed efficiency ratio (FER) were calculated at the end of the experimental period according to the following equations: BWG (g) = final weight (g) – initial weight (g). FER = body weight gain (g)/feed intake (g).

Blood sampling:

In all the previously mentioned groups blood samples were taken at the end of the experiment. The blood samples were collected after 12 hours fasting from Vein plexus eye into dry clean centrifuge tubes and left to colt. The blood was centrifuged for 10 minutes at 3000 r.p.m. to separate the serum, which was carefully aspirated and transferred into clean quite plastic tubes and kept frozen at -18 ± 2°C until biochemical analysis (El-Khamissy, 2005).

Collection of organs:

All rats were scarified. The abdomen was opened, and the organs were separated by carefully dissection, cleaned from the adhesive matter. Then washed with running water, then weighted. The relative weight of the organs was calculated following the equation:

$$\text{Relative weight} = \frac{\text{Organ weight}}{\text{Animal weight}} \times 100$$

Biological analysis of Serum:

Triglycerides, total cholesterol and high density lipoprotein cholesterol (HDL-C) levels were measured by enzymic colorimetric procedures using commercial available kits. Triglycerides were carried out according to the method of Fossati and Principe (1982). Total cholesterol (TC) and HDL-C were carried out according to the methods of Richmond (1973). Low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) were calculated mathematical According to Friedwald's equations (Friedewald *et. al*., 1972). LDL-c = TC – [HDL-c+ (TG/5)] while, VLDL-c = Triglycerides/5. The activity of serum glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) were determined by Oyanatui (1984).

Liver function tests:

Serum was analyzed to estimate activities of liver functioning enzymes such as ALT (Alanine amino transferase) and AST (Aspartate amino transferase) by using their commercial kits Reitman and Frankel (1957). and alkaline phosphatase enzymes (ALP) according King, (1965).

Histopathological examinaton:

Livers of the scarified rats were dissected, removed, washed with normal saline and put in 10% formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. The tissue specimens were cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness, stained with Hematoxylen and Eosin (H and E) and then studied under an electronic microscope (Bancroft *et al.,* 1996).

Statistical analysis:

Most of the received data were analyzed statistically using the analysis of variance and the means were further tested using the least significant difference test (LSD) as outlined by Steell and Torrie (1980).

RESULTS AND DISCUSSION

Recently, increasing attention has been focused on the health-benefits of phenolic compounds. As an important subclass of phenolics, anthocyanins have been reported to have many bioactivities including antioxidant, anti-inflammatory and anti-carcinogenic properties. Some foods containing abundant anthocyanins, such as blueberry, are becoming extremely popular among ordinary consumers. Furthermore, they have been used as predominant materials for functional foods. As mentioned previously, black rice, whose bran fraction contains abundant anthocyanins is being favored by an increasing number of consumers (Zhang *et al.,* 2011).

Gross chemical composition of Microwave black rice bran and Microwave defatted black rice bran .

The chemical composition of Microwave full fat black rice bran and Microwave defatted black rice bran were given in Table (1).

Table (1): Proximate chemical composition of Microwave black rice bran and Microwave defatted black rice bran (MDBRB) .

Parameter %	Microwave black rice bran	Microwave Defatted black rice bran
Moisture	9.20 a	9.39 a
Crude protein	15.45 b	17.69 a
Lipids	18.85 a	0.55 b
Ash	8.70 b	10.65 a
Crude fiber	10.75 b	13.15 a
Total carbohydrates*	57.00 b	71.11 a
Total phenolic compounds (mg ferulic acid equivalent/kg)	477.6a	498.4a

***Total carbohydrates was calculated by difference.**
Each value is an average of three determinations.
Values followed by the same letter in row are not significantly different at P<0.05.

The results reveal that defatted Microwave black rice bran contain protein, ash, fiber and carbohydrates significantly higher than that of Microwave full fat black rice bran on contrast they contain significantly lower lipids. These results are in agreement with those found by (Amarasinghe and Gangodavilage, 2004, Sharif *et al.,* 2005 and Abd El-Hady, (2013) , Results for total phenolic compounds of Microwave black rice bran and Microwave defatted black rice bran there was no significant difference detected. This finding was in accordance with that reported by Scalbert and Williamson, (2000) and Abd El-Galeel and El Bana, (2012).

Effect of substituting Microwave defatted black rice bran (MDBRB) for casein on food intake (FI), body weight gain (BWG) and feed efficiency ratio (FER) in CCl_4 intoxicated rats.

The effect of Microwave defatted black rice bran (MDBRB) on FI, body weight gain % (BWG) and feed efficiency ratio (FER) of hepatotoxic rats for 8 weeks is shown in Table (2). Substitution of Microwave defatted black rice bran (MDBRB) for casein at 75 and 100% in the diet after CCl_4 intoxication significantly increased FI in CCl_4 intoxicated rats compared to negative control group. The body weight gain indicated that the CCl_4 treated groups had a lower weight gain as compared to the negative control group. The body weight gain observed in the 50, 75 and 100% defatted black rice bran fed groups, being more significantly pronounced than the CCl_4 treated control group. FER was not differing by substitution of Microwave defatted black rice bran (MDBRB) fed for casein. The body weight decrease as a result of CCl_4 injection was considered to be the result of direct toxicity of CCl_4 and/or indirect toxicity related to the liver damage. These results were in the same line with Bruckner *el al.,* (1986) and Pradeep *et al.,* (2005) they reported, Changes in the body weight after CCl_4 dosing have been used as a valuable index of CCl_4 related organ damage . On the other hand, no available literature could be found concerning the effect of (MDBRB) on FI, BWG and FER.

Table (2): Effect of feeding at different levels of Microwave defatted black rice bran (MDBRB) on body weight gain%, food intake and food efficiency ratio in hepatotoxic rats:

Treatments	Initial body weight (g)	Final body weight (g)	Body weight gain		Food intake (g)	FER
			g	%		
G1 (Control-ve)	153..31 a	179.80 a	26.50 a	17.285	678.16 a	0.0391 a
G2 (Control+ve)	154.20 a	171.50 c	17.30 c	11.219	504.56 e	0.0343 b
G3 (MDBRB 25%)	154.90 a	172.90 c	18.00 c	11.620	543.20 d	0.0331 b
G4 (MDBRB50%)	155.01 a	175.11 b	20.10 b	12.967	571.2 c	0.0352 b
G5 (MDBRB75%)	153.90 a	175.56 b	21.66 b	14.074	638.96 b	0.0339 b
G6 (MDBRB100%)	155.22 a	176.19 b	20.97 b	13.509	644.0 b	0.0326 b

Each value is an average of seven determinations.
Values followed by the same letter in column are not significantly different at P<0.05.
G1, G2 … etc. were as given in Table (A).

Effect of feeding on different levels of Microwave defatted black rice bran (MDBRB) on organs of rats in CCl_4 intoxicated rats.

The results presented in Table (3) revealed that all treatments showed significant changes in the weight of liver, kidney and spleen of all experimental rats. It could be noticed that the mean value of the weight of liver in control (-ve) G1 was 6.24g. The results obtained for the same parameters of hepatotoxic rats control (+ve) G2 was 6.35g. In addition, the liver weight of rats fed with substitution of Microwave defatted black rice bran (MDBRB) for casein in the diet after CCl_4 intoxication was lower than control groups.

Table (3): Effect of substituting Microwave defatted black rice bran (MDBRB) for casein on organs weight (liver, kidney and spleen) in hepatotoxic rats:

Treatments	Liver weight (g)	Kidneys weight (g)	Spleen weight (g)
G1 (Control-ve)	6.24 a	1.55 b	0.55 a
G2 (Control+ve)	6.35 a	1.70 a	0.55 a
G3(MDBRB 25%)	5.67 b	1.44 b	0.48 b
G4 (MDBRB50%)	5.53 b	1.62 a	0.54 a
G5 (MDBRB75%)	5.47 b	1.69 a	0.49 b
G6 (MDBRB100%)	5.14 c	1.53 b	0.55 a

Each value is an average of seven determinations.
Values followed by the same letter in column are not significantly different at P<0.05.
G1, G2 … etc. were as given in Table (A).

Effect of substituting Microwave defatted black rice bran (MDBRB) for casein on lipid profile in hepatotoxic rats:

It is clear from Table (4): that administration of CCl_4 caused significant elevation in serum lipids parameters compared to negative control group. CCl_4 intoxicated rats fed with Microwave defatted black rice bran which substitute 25, 50, 75 and 100% for casein showed significant decreases in serum levels of total cholesterol and triglycerides in comparison to positive control group.

Substitution of Microwave defatted black rice bran for casein at 25, 50, 75 and 100% in the diet of CCL_4 intoxicated rats caused a significant decrease in the serum level of LDL-c, while there were significant increase in levels of HDL-c in the serum, compared to the positive control group. These results are in agreement with those of (Gopal and Sengottuvelu 2008 and Houa *et. al.*, 2013). They reported that, CCl_4 intoxicated rats exhibited significant higher levels of TC and TG. This perhaps due to the presence of damage in the liver. The observed improvement in the levels of TC, TG, LDL-C and VLDL-C is probably indicative of hepato-protective effect of MDBRB in $CC1_4$ injected rats.

Table (4): Effect of substituting Microwave defatted black rice bran (MDBRB) for casein on lipid profile in CCl_4-hepatotoxic rats.

Treatments	Total cholesterol (TC) (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)	Total triglycerides (mg/dl)
G1 (Control-ve)	92.22 d	65.10 a	11.27 b	15.85 c	56.33 b
G2 (Control+ve)	221.28 a	37.20 b	15.10 a	168.98 a	75.50 a
G3(MDBRB 25%)	104.80 b	64.70 a	11.06 b	29.04 b	55.30 b
G4 (MDBRB50%)	103.50 b	63.80 a	10.73 c	28.97 b	53.66 b
G5 (MDBRB75%)	100.16 b	62,0b a	10.46 c	27.70 b	52.30 b
G6 (MDBRB100%)	98.50 c	61.50 a	10.22 c	26.78 b	51.10 b

Each value is an average of seven determinations.
Values followed by the same letter in column are not significantly different at $P \leq 0.05$.
G1, G2 … etc. were as given in Table (A).

Effect of substituting Microwave defatted black rice bran (MDBRB) for casein on (ALT), (AST) and (ALP) enzymes in serum of hepatotoxic rats:

The AST and ALT activity in each group are shown in Table (5). The AST and ALT activities in the model of CCl_4 induced hepatotoxicity in rats demonstrated that substitution of defatted black rice bran for casein at 75 and 100% caused significant inhibition of ALT and AST levels in serum compared to the positive control group. In addition, substitution of defatted black rice bran for casein especially at all percentages used caused significant inhibition of ALP level.

The reduced concentrations of ALT and AST as a result of MDBRB administration observed during the present study might probably be due in part to the presence of polyphenol. The tendency of these marker enzymes to return towards a near normalcy in MDBRB fed groups point towards an early improvement in the secretory mechanism of the hepatic cell and is a clear manifestation of antihepatotoxic effect of Microwave defatted black rice bran (MDBRB). This effect was similar to that reported by (Yawadio *et al.*, 2007; Kim *et al.*, 2008; Park *et al.*, 2008 and Houa *el al.*, 2013),they indicated that rice bran with certain color that contains anthocyanin has a reductase enzyme inhibitory and anti diabetic activity (Yawadio *et al.*, 2007; Kim *et al.*, 2008 and Park *et al.*, 2008).

Effect of substituting Microwave defatted black rice bran (MDBRB) for casein on (GPX), (SOD) and (CAT) enzymes in serum of hepatotoxic rats:

According to results given in Table (6), shows that CCl_4 injected rats had significantly lower levels of GPX, SOD and CAT antioxidant enzymes activity compared to negative control group. Substitution of Microwave defatted black rice bran (MDBRB) for casein at 25, 50, 75 and 100% in the diet of CCl_4 -

Intoxicated rats increased the activity levels of GPX, SOD and CAT antioxidant enzymes. Aforementioned results coincide with those obtained by (Purushothama *et al.,* 1995, Hsu *et al.,* 2010 and Houa *el al*, 2013).

Table (5): Effect of substituting Microwave defatted black rice bran (MDBRB) for casein on (ALT) , (AST) and (ALP) enzymes in serum of hepatotoxic rats :

Treatments	ALT	AST	ALP
G1 (Control-ve)	30.50 d	59.60 d	94.50 d
G2 (Control +ve)	61.70 a	101.60 a	128.91 a
G3 (MDBRB 25%)	50.30 b	88.40 b	114.50 b
G4 (MDBRB50%)	49.50 b	87.60 b	112.72 bc
G5 (MDBRB75%)	46.22 bc	83.0 c	110.20 bc
G6 (MDBRB100%)	42.30 c	80.45 c	108.31 c

Each value is an average of seven determinations.
Values followed by the same letter in column are not significantly different at P $\leq$ 0.05.
G1, G2 … etc. were as given in Table (A).

Table (6): Effect of substituting Microwave defatted black rice bran (MDBRB) for casein on (GPX), (SOD) and (CAT) enzymes in serum of hepatotoxic rats:

Treatments	GPX	SOD	CAT
G1 (Control-ve)	18.50 a	90.10 a	65.30 a
G2(Control+ve)	5.30 d	53.60 e	35.61 e
G3(DBRB 25%)	7.90 c	60.70 d	40.40 d
G4 (DBRB50%)	10.60 bc	78.50 c	46.30 c
G5 (DBRB75%)	11.10 bc	81.40 b	48.80 bc
G6 (DBRB100%)	13.20 b	83.60 b	52.60 b

Each value is an average of seven determinations.
Values followed by the same letter in column are not significantly different at P $\leq$ 0.05.

Histopathological examination:

Biological systems protect themselves against the damaging effects of activated species by several means. These include free radical scavengers and chain reaction terminators; enzymes that found in blood and liver such as SOD, CAT and GPX system (Lee *et al*., 2002).

Liver:

Microscopically, liver of control group 1 untreated rat revealed the normal histological structure of hepatic lobule (Fig. 1). Meanwhile, liver of rat from group 2 showed vacuolar degeneration of hepatocytes (Fig. 2) and edema in the portal triad (Fig. 3). Liver of rat from group 3 showed vacuolar degeneration of hepatocytes (Fig. 4). In addition, liver of rat from group 4 revealed vacuolar degeneration of hepatocytes as well as simusoidal leucocytosis (Fig. 5). However, no changes observed in liver from group 5 except vacuolar degeneration of sporadic hepatocytes (Fig. 6). No histopathological changes were noticed in liver of rats from groups 6 (Fig. 7).

Table (7): Histopathological changes liver of rats fed on different experimental diets .

Treatments	Degeneration of hepatocytes	Vascular degeneration of sporadic heaptocytes	Edema in the portal triod	Hepatocytes associated	Coagulative necrosis
G1 (Control-ve)	-	-	-	-	-
G2 (Control+ve)	+++	+++	++	+++	++
Hepoatotoxic rats fed on diet replaced by:					
G3(MDBRB 25%)	++	-	-	-	-
G4(MDBRB 50%)	++	+	-	-	-
G5(MDBRB 75%)	+	-	-	-	-
G6(MDBRB 100%)	--	-	-	-	-

(-) no change (+) very mild (++) mild (+++) severe
G1, G2 … etc. were as given in Table (A).

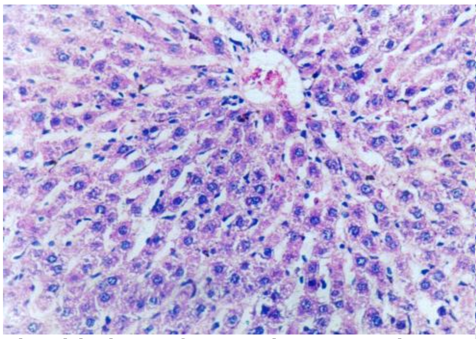

Fig. (1):Liver of control, untreated rat showing the normal histological structure of hepatic lobule (H and E x 200).

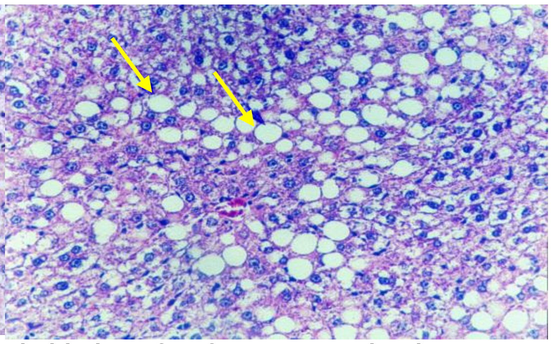

Fig.(2):Liver of rat from group 2 showing vacuolar degeneration of hepatocytes (H and E x 200).

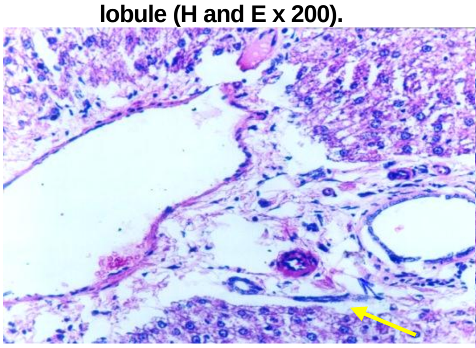

Fig.(3):Liver of rat from group 2 showing edema in the portal triad (H and E x 200).

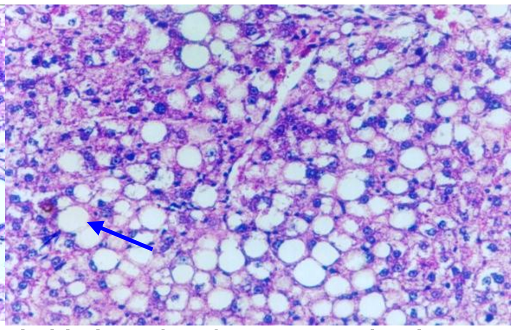

Fig.(4):Liver of rat from group3 showing vacuolar degeneration of hepatocytes (H and E x 200).

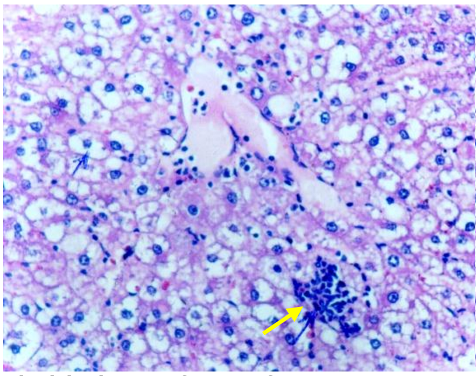

Fig.(5):Liver of rat from group 4 showing vacuolar degeneration of hepatocytes as well as sinusoidal leucocytosis(H and E x 200).

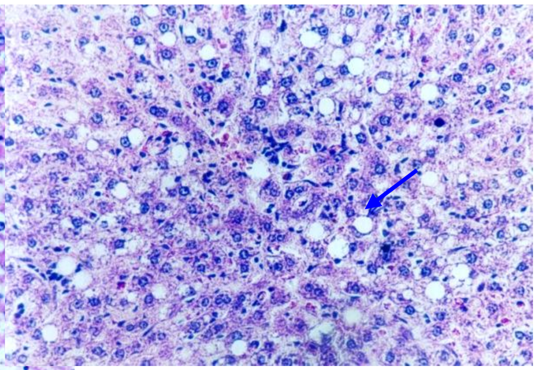

Fig.(6):Liver of rat from group 5 showing vacuolar degeneration of sporadic hepatocytes (H and E x 200).

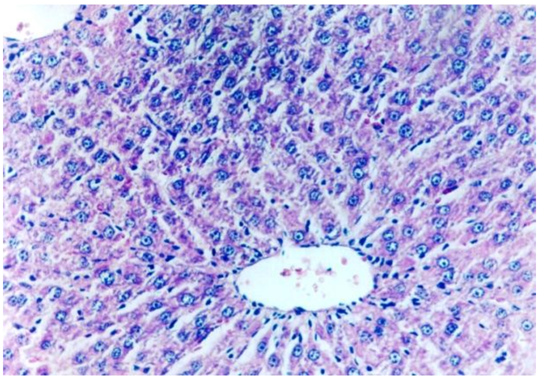

Fig. (7): Liver of rat from group 6 showing no histopathological changes (H and E x 200).

From these results, it was suggested that Microwave defatted black rice bran (MDBRB) could protect the liver cells from CCl_4 induced liver damages perhaps, by its antioxidative effect on hepatocytes, hence eliminating the deleterious effects toxic metabolites from CCl_4 . So the present study recommended that the use of Microwave defatted black rice bran (MDBRB) may be useful for patients suffering from liver diseases due to its hepatoprotective and hypolipidemic activities Further studies are required in this field.

REFERENCES

A.O.A.C., Association of Official Analytical Chemists (2005). Official Methods of Analysis of the Association of Official Analytical Chemists. 18th Ed. Washington, DC, USA.

Abd El-Galeel, M. A. and El-Bana, M. E. (2012) .Effect of milling degree on chemical composition and nutritional value of stabilized rice bran. J. Agric. Res. Kafrelsheikh Univ., 38 (4): 550-567.

Abd El-Hady, Sahar, R. (2013) . Effect of some thermal processing on stability of rice bran during storage at room temperature . J. Agric. Res., Kafrelsheikh Univ., 39 (1) : 92-106 .

Amarasinghe, B.M.W.P.K. and N.C. Gangodavilage. (2004). Rice bran oil extraction in Sri Lanka: Data for process equipment design. Food Bioprod. Process. 82(C1):54-59.

Bancroft, J.D.; Sterens, A. and Turnor, D.R. (1996). Theory and Practices of Histological Techniques. 4th Ed., Churchill Livingston, New York.

Bonoli, M.; Marconi, E. and Caboni, M. F. (2004). Free and bound phenolic compounds in barley (Hordeum vulgare L.) flours. Evaluation of the extraction capability of different solvent mixtures and pressurized liquid methods by micellar electrokinetic chromatography and spectrophotometry. J. of Chromatography A, 1057: 1-12.

Bruckner, J.V., W.F. MacKenzie, S. Muralidhara, R. Luthra, G.M. Kyle, D Acosta, (1986). Oral Toxicity of Carbon Tetrachloride: Acute, Subacute and Subchronic Studies in Rats. Fundam. Appl. Toxicol., 6: 16-34.

Chen, C.R., Wang, C.H., Wang, L.Y., Hong, Z.H., Chen, S.H. and Ho, W.J. (2008). Supercritical-carbon dioxide extraction and deacidification of rice bran oil. Journal Supercritical Fluids 45: 322-331.

Chen, P.-N., Kuo, W.-H., Chiang, C.-L., Chiou, H.-L., Hsieh, Y.-S. and Chu, S.-C. (2006). Black rice anthocyanins inhibit cancer cells invasion via repressions of MMPs and u-PA expression. Chemico-Biological Interactions, 163(3), 218–229.

El-Khamissy, A. (2005). Studies on Biological Effects of some Diabetes Foods. Ph. D. Thesis, Faculty of Specific Education. Home Economics, Tanta Univ.Food Science and Technology, 43(6), 1078–1082.

Fossati, P. and Prancipe, L. (1982). Triglycerides determination after enzymatic hydrolysis. Clin. Chem., 28: 2077.

Freidwald, W.T.; Levy, R.I. and Fredrickson, D.S. (1972). Estimation of the concentration of low-density lipoprotein separate by three different methods. Clin. Chem., 18: 499-502

Gopal, N. and Sengottuvelu, S. (2008). Hepatoprotective Activity of Clerodendrum Inerme Against CCL4 Induced Hepatic Injury in Rats Fitoterapia., 79: 24-26. 30.

He, S., Bao, W., Shao, M., Wang, W., Sun, J., Jiang, Y., Feng, X. and Niu, J. (2011). Risk factors for non-alcoholic fatty liver disease in a Chinese population. Acta Gastro-Enterologica Belgica, 74, 503–508.

Houa, F., Zhanga, R., Zhanga, M., Zhencheng Weia, D., Denga, Y., Zhanga, Y., Chia, J., Tanga,x.. (2013). Hepatoprotective and antioxidant activity of anthocyanins in black rice bran on carbon tetrachloride-induced liver injury in mice. J . O F Functional F oods, (5) 1 7 0 5 –1 7 1 3.

Hsu, Y.W., Tsai, C. F., Chuang, W. C., Chen,W. K., Ho, Y. C., and Lu, F.J. (2010). Protective effects of silica hydride against carbon tetrachloride-induced hepatotoxicity in mice. Food and Chem. Toxicology, 48, 1644–1653.

Kahlon, T.; Chow, F.; Sayre, R. and Betschart, A. (1992). Cholesterol-lowering in hamsters fed rice bran at various levels, defatted rice bran and rice bran oil. J. Nutr. 122: 513-519.

Kim, M.K., Kim, H., Koh, K., Kim, H.S., Lee, Y.S. and Kim, Y.H. (2008). Identification and quantification of anthocyanin pigments in colored rice. Nutrition Research and Practice 2(1): 46-49.

Kim, M.K., Kim, H., Koh, K., Kim, H.S., Lee, Y.S. and Kim, Y.H. (2008). Identification and quantification of anthocyanin pigments in colored rice. Nutrition Research and Practice 2(1): 46-49.

King, J. (1965). The phosphohydrolases acid and alkaline phosphatases. In: Practical and Clinical Enzymology, Eds., King, J. Van Nostrand Co. Ltd., London, pp: 191-208.

Lanepeter, W. and Person, A.E.G. (1971). Dietary require. In the laboratory animal principal and practice. Academic Press, London and New York.

Lee, J. C.; Kim, J. D.; Hsieh, F. H. and Eun, J. B. (2008). Production of black rice cake using ground black rice and medium-grain brown rice. International Journal of

Lee, Mi-K.; Bok, S.H.; Jeong, T.S.; Moon, S.S.; Lee, S.E.; Yong, B.P. and Choi, M.S. (2002). Supplementation of Naringenin and its Synthetic Derivative Alters Antioxidant Enzyme Activities of Erythrocyte and Liver in High Cholesterol-Fed Rats. Bioorganic and Medicinal Chem., 10, 2239-2244.

Ling, W.H.; Wang, L.L.; Ma, J. (2002). Supplementation of the black rice outer fraction to rabbitsdecrease atherosclerotic plaque formation and increased antioxidant status. Journal of Nutrition 132, 20–26.

Nam, S.H., Choi, S.P., Kang, M.Y., Kho, H.J., Kozukue, N. and Griedman, M. (2006). Antioxidative activities of bran extracts from twenty one pigmented rice cultivars. Food Chemistry 101: 947-954.

Nara, K.; Miyoshi, T.; Honma, T. and Koga, H. (2006). Antioxidant activity of bound from phenolics in potato peel. Bioscience Biotechnology and Biochemistry, 70, 1489-1491.

Oyanatui, Y.(1984). Reevaluation of essay methods and establishment of kit for superoxide dismutase activity. Anal. Bio.,142,290-296.

Park, Y.S., Kim, S.J. and Chang, H.I. (2008). Isolation of anthocyanin from black rice (Heugjinjubyeo) and screening of its antioxidant activities. Journal of Microbiology Biotechnology 36(1): 55-60.

Pradeep, K., C.V. Mohan and K.G. Karthikeyan, . (2005). Effect of Pretreatment of Cassia fistula Linn. Leaf Extract Against Subacute Ccl4 Induced Hepatotoxicity In Rats. Indian J. Exp. Biol., 43: 526-530.

Purushothama, S. , Raina, P.L. and Hariharan, K. (1995). Effect of long term feeding of rice bran oil upon lipids and lipoproteins in rats. Mol. Cell. Biochem. 146:63-69.

Ramezanzadeh, F.M., Rao, R.M., Prinyawiwatkul, W., Marshall, W.E. and Windhauser, M., (2000). Effects of microwave heat, packaging and storage temperature on fatty acids and proximate compositions in rice bran. J. Agric., and Food Chem., 48 (2), 464-467.

Reitman, S. and Frankel, S. (1957). A colormetic method for the determination of serum glutamic oxaloacetic and glutamic pyrovic transaminase. Am. J. Clin. Path., 28: 56-66.

Richmond, W. (1973). Preparation and properties of cholesterol oxidase from Nocrdia spp. And its application to the enzymatic assay of total cholesterol in serum. Clin. Chem., 19: 1350-1356.

Saenjum, C., Chaiyasut, C., Chansakaow, S., Suttajit, M. and Sirithunyalug, B. (2012). Antioxidant and anti-inflammatory activities of gamma-oryzanol rich extracts from Thai purple rice bran. Journal Medical Plants Research 6: 1070-1077.

Scalbert, A. and Williamson, G., (2000). Dietary intake and bioavailability of polyphenols. J. of Nutrition, 2073S–2085S.

Sharif, K., M.S. Butt and N. Huma. (2005). Oil extraction from rice industrial waste and its effect on physico-chemical characteristics of cookies. Nutr. Food Sci. 35(6):416-427.

Steell, R.G. and Torrie, J.H. (1980). Principles and procedures of statistics. 2nd ed. 120. McGrow-Hill, New York. USA. Studies on the nutritional quality of some cucurbit kernel proteins. J. Sci. Food Agric, 37, 418-420.

Tanaka, M., Katayama, F., Kato, H., Tanaka, H.,Wang, J., Qiao, Y. L and Inoue, M. (2011). Hepatitis B and C virus infection and hepatocellular carcinoma in China: A review of epidemiology and control measures. Journal of Epidemiology, 21, 401–416.

Wilfried, F.; Anne Bosma, F.; Hendriks, J.; Rohol, E. and Dick, (1994). Vitamin A deficiency potentiates carbon tetrachloride induced liver fibrosis in rats, J. of Hepathology, 19(1): 193-201.

Xu, Z. and Godber, S. (1999). Purification and identification of components of γ-oryzanol in rice bran oil. Journal Agriculture Food Chemistry 47: 2724-2728.

Yawadio, R., Tanimori, S. and Morita, N. (2007). Identification of phenolic compounds isolated from pigmented rices and their aldose reductase inhibitory activities. Food Chemistry 101: 1616-1625.

Zawistowski, J.; Kopec, A. and Kitts, D.D. (2009). Effects of a black rice extract (*Oryza sativa* L. indica) on cholesterol levels and plasma lipid parameters in Wistar Kyoto rats. Journal of Functional Foods 1, 50–56.

Zhang, M.W.; Guo, B.J.; Zhang, R.F.; Chi, J.W.; Wei, Z.C.; Xu, Z.H.; Zhang, Y.; Tang, X.J. (2006). Separation, purification and identification of antioxidant compositions in black rice. Agricultural Sciences in China 5, 431–440.

Zhang, M.W.; Zhang, R. F., Zhang, F. X. and Liu, R. H. (2010). Phenolic profiles and antioxidant activity of black rice bran of different commercially available varieties. Journal of Agricultural and Food Chemistry, 58, 7580–7587.

Zhang, R. F., Zhang, F. X., Zhang, M. W., Wei, Z. C., Yang, C. Y., Zhang, Y., Tang, X. J., Deng, Y. Y., & Chi, J. W. (2011). Phenolic composition and antioxidant activity in seed coats of 60 Chinese black soybean (*Glycine max* L. Merr.) varieties. J. of Agric. and Food Chem., 59, 5935–5944.

التقييم البيولوجي لرجيع الأرز الأسود منزوع الدهن والمعامل بالميكروويف في الفئران المصابة بالتسمم الكبدي برابع كلوريد الكربون (CCl_4)

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أجريت هذه الدراسة بهدف إعداد رجيع من الأرز الأسود منزوع الدهن المعامل بالميكروويف (MDBRB)لدراسة تأثير استبداله بنسب للكازين على وزن الجسم - معدل التمثيل الغذائي- وظائف الكبد وإنزيمات الأكسدة وكذلك مؤشرات الليبيدات في السيرم في الفئران المصابة بالتسمم الكبدي برابع كلوريد الكربون (CCl_4). تم تقسيم اثنين وأربعين ذكور الفئران إلى ٦ مجموعات الأولى منها تمت تغذيتها على Basal diet العليقة الأساسية وكانت تمثل الكنترول الطبيعى (كنترول ١ سالب) أما المجموعات الأخرى فتم إحداث تسمم كبدي فيها معمليا وذلك بحقنها برابع كلوريد الكربون بجرعة ١ملجم/كجم من وزن الجسم عضل مرتين في الأسبوع لمدة أسبوعين لإحداث التسمم الكبدي (التليف الكبدي) وتركت منها مجموعة تتغذى على Basal diet وكانت هي المجموعة (كنترول موجبة) وتم تغذية المجاميع الأخرى على الوجبات التي تم استبدال رجيع من الأرز الأسود منزوع الدهن معامل بالميكروويف (MDBRB) بنسبة ٢٥ و ٥٠ و ٧٥ و ١٠٠ ٪ بدلا من الكازين وفي نهاية فترة التغذية (٨ أسابيع)،تم تجميع عينات الدم للتحاليل الكيميائية وتم دراسة التغيرات الهستوباثولوجيه في كبد الفئران .

وأظهرت النتائج أن إحلال MDBRB بدلا من الكازين لا سيما بنسبة ٧٥ و ١٠٠٪ في الفئران المصابة بتسمم كبدي أدى إلى زيادة استهلاك الغذاء المتناول و زيادة الوزن .وخفض هذا الاستبدال أيضا مستويات إنزيمات الكبد وتحسين مؤشرات الليبيدات في السيرم وزيادة مستويات نشاط الأنزيمات المضادة للأكسدة في الفئران المصابة بالتسمم الكبدي برابع كلوريد الكربون (CCl_4). وكشف فحص الأنسجة التخفيف من تسمم الكبد الناجم عن الإصابة برابع كلوريد الكربون (CCl_4) عن طريق زيادة نسبة MDBRBالمستخدمة.

بناءا علي النتائج المتحصل عليها وجد أن MDBRB يمكن أن تحمي خلايا الكبد من أضرار الكبد الناجم عن CCl_4 من خلال تأثير مضادات الأكسدة على خلايا الكبد، وبالتالي القضاء على الآثار الضارة من CCl_4 و توصي الدراسة بإستخدام MDBRBحيث أنها مفيدة للمرضى الذين يعانون من أمراض الكبد والكوليسترول.