BIOLOGICAL EVALUATION OF MICROWAVE DEFATTED BLACK RICE BRAN (MDBRB) IN CCL₄ 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 (CCL₄) intoxicated rats. Results showed that substitution of MDBRB for casein especially at 75 and 100% in CCL₄ 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 CCL₄ intoxicated rats. Histopathological examination revealed alleviation of hepatic lesions caused by CCL₄ by increasing the percentage of DBRB used. In conclusion, it was suggested that MDBRB could protect the liver cells from CCL₄ induced liver damages perhaps, by its antioxidative effect on hepatocytes, hence eliminating the deleterious effects of toxic metabolites from CCL₄. 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 CCl₄.

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 ºC) at room temperature for 24 hr., then the obtained solution was filtered and the solvent was removed by rotary evaporator according to Kahlol 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 ºC 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 Lane-Peter and Person, 1971).**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>G1 Control-ve</th>
<th>G2 Control+ve</th>
<th>G3 MDBRB 25%</th>
<th>G4 MDBRB 50%</th>
<th>G5 MDBRB 75%</th>
<th>G6 MDBRB 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDBRB</td>
<td>-</td>
<td>-</td>
<td>212</td>
<td>424</td>
<td>636</td>
<td>847.9</td>
</tr>
<tr>
<td>Starch</td>
<td>650</td>
<td>650</td>
<td>504.5</td>
<td>353.3</td>
<td>180</td>
<td>7.1</td>
</tr>
<tr>
<td>Casein</td>
<td>150</td>
<td>150</td>
<td>112.5</td>
<td>75</td>
<td>37.5</td>
<td>-</td>
</tr>
<tr>
<td>Oil</td>
<td>100</td>
<td>100</td>
<td>99</td>
<td>97.7</td>
<td>96.5</td>
<td>95</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>50</td>
<td>22</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mineral Mix</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Vitamin Mix</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

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 (CCl₄) for induction of acute liver damage. CCl₄ 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:

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 examination:**

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).**

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

*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 CCl₄ 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 CCl₄ intoxication significantly increased FI in CCl₄ intoxicated rats compared to negative control group. The body weight gain indicated that the CCl₄ 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 CCl₄ 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 CCl₄ injection was considered to be the result of direct toxicity of CCl₄ 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 CCl₄ dosing have been used as a valuable index of CCl₄ 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:

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Body weight gain g</th>
<th>%</th>
<th>Food intake (g)</th>
<th>FER</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (Control-ve)</td>
<td>153.31 a</td>
<td>179.80 a</td>
<td>26.50 a</td>
<td>17.285</td>
<td>678.16 a</td>
<td>0.0391 a</td>
</tr>
<tr>
<td>G2 (Control+ve)</td>
<td>154.20 a</td>
<td>171.50 c</td>
<td>17.30 c</td>
<td>11.219</td>
<td>504.56 e</td>
<td>0.0343 b</td>
</tr>
<tr>
<td>G3 (MDBRB 25%)</td>
<td>154.90 a</td>
<td>172.90 c</td>
<td>18.00 c</td>
<td>11.620</td>
<td>543.20 d</td>
<td>0.0331 b</td>
</tr>
<tr>
<td>G4 (MDBRB50%)</td>
<td>155.01 a</td>
<td>175.11 b</td>
<td>20.10 b</td>
<td>12.967</td>
<td>571.2 c</td>
<td>0.0352 b</td>
</tr>
<tr>
<td>G5 (MDBRB75%)</td>
<td>153.90 a</td>
<td>175.56 b</td>
<td>21.66 b</td>
<td>14.074</td>
<td>638.96 b</td>
<td>0.0339 b</td>
</tr>
<tr>
<td>G6 (MDBRB100%)</td>
<td>155.22 a</td>
<td>176.19 b</td>
<td>20.97 b</td>
<td>13.509</td>
<td>644.0 b</td>
<td>0.0326 b</td>
</tr>
</tbody>
</table>

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 CCl₄ 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 CCl₄ 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:

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

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 CCl₄ caused significant elevation in serum lipids parameters compared to negative control group. CCl₄ 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 CCl₄ 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, CCl₄ 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 CC₁₄ injected rats.
Table (4): Effect of substituting Microwave defatted black rice bran (MDBRB) for casein on lipid profile in CCl₄-hepatotoxic rats.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total cholesterol (TC) (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>Total triglycerides (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (Control-ve)</td>
<td>92.22 d</td>
<td>65.10 a</td>
<td>11.27 b</td>
<td>15.85 c</td>
<td>56.33 b</td>
</tr>
<tr>
<td>G2 (Control+ve)</td>
<td>221.28 a</td>
<td>37.20 b</td>
<td>15.10 a</td>
<td>168.98 a</td>
<td>75.50 a</td>
</tr>
<tr>
<td>G3 (MDBRB 25%)</td>
<td>104.80 b</td>
<td>64.70 a</td>
<td>11.06 b</td>
<td>29.04 b</td>
<td>55.30 b</td>
</tr>
<tr>
<td>G4 (MDBRB50%)</td>
<td>103.50 b</td>
<td>63.80 a</td>
<td>10.73 c</td>
<td>28.97 b</td>
<td>53.66 b</td>
</tr>
<tr>
<td>G5 (MDBRB75%)</td>
<td>100.16 b</td>
<td>62.0b a</td>
<td>10.46 c</td>
<td>27.70 b</td>
<td>52.30 b</td>
</tr>
<tr>
<td>G6 (MDBRB100%)</td>
<td>98.50 c</td>
<td>61.50 a</td>
<td>10.22 c</td>
<td>26.78 b</td>
<td>51.10 b</td>
</tr>
</tbody>
</table>

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 (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 CCl₄ 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 CCl₄ 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 CCl₄ -
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 et 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:

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

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).

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

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

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

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.
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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.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Degeneration of hepatocytes</th>
<th>Vascular degeneration of sporadic hepatocytes</th>
<th>Edema in the portal triod</th>
<th>Hepatocytes associated</th>
<th>Coagulative necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1  (Control-ve)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G2  (Control+ve)</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Hepatoxic rats fed on diet replaced by:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3(MDBRB 25%)</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G4(MDBRB 50%)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G5(MDBRB 75%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G6(MDBRB 100%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(-) 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 group 3 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 CCl₄ induced liver damages perhaps, by its antioxidative effect on hepatocytes, hence eliminating the deleterious effects toxic metabolites from CCl₄. 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


التقييم البيولوجي لرجيع الأرز الأسود منعزل الدهن والمعمل بالميكروويف في
الفرن المصباح بالتنسيم الكبدى براى كلوئيد الكرزون (CCl4)،
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أجريت هذه الدراسة يهدف إعداد رجيع من الأرز الأسود منعزل الدهن المعمل بالميكروويف
(MDBRB) لدراسة تأثير استبدال الدجاج في وزن الجسم - معدل التمثيل الغذائي - وظائف الكبد
وإنزيمات الأكمة وكذلك مؤشرات النسيجيات في السوم في الفرن المصباح بالتنسيم الكبدى براى كلوئيد
الكرزون (CCl4)، تم تقسيم اثنان وأربعين ذكر الفرار إلى 6 مجموعات أولية من نمت تغذىها على
الغذاء الطبيعي وتم تمثيل الكنللول الطبيعى (كنللول 10 سايب) أما المجموعات الأخرى فتم
إحداث تسخين كبيدي فيما معملى وذلك بِراى كلوئيد الكرزون بجرعة 15 مل/كجم من وزن الجسم عضل
مرتين في الأسبوع لمدة أسبوعين لإحداث التسخين الكبيدي (التيتيف الكبدى) وتم كشف منها مجموعة تغذى على
Basal diet وتلك المجموعة (كنللول موجه) وتم تغذية المجامع الأخرى على الوجبات التي تم
استبدال رجيع من الأرز الأسود منعزل الدهن معمل بالميكروويف (CCl4) بنسبة 25 و50 و
100 % بدلاً من الكرز. وفي نهاية فترة التغذية (8 أسابيع) تم تجميع عينات الدم والتحليل الكيميائي وتتم
دراسة التغيرات الهيستوبولوجية في كبد الفرار.

وأظهرت النتائج أن إجادل MDBRB بدلاً من الوادى لا سيما بنسبة 25 و 100 % في الفرار
المصاب بالتنسيم الكبدى أدى إلى زيادة استهلاك الغذاء المتراصة وزيد تىادة الوزن. وخفض هذا الاستبدال أيضاً
الميلات لزيادة الكرزون وتحسين مشاكل العظام وبدلاً من الفRAR المصباح بالتنسيم الكبدى براى كلوئيد الكرزون
( CC14). وكشف فحص الأنسجة المحيطة من تسمى الكرزون ينفع من الإصابات براى كلوئيد الكرزون (CC14)
على طرق زيادة نسبة MDBRB المستخدمة.

يمكن أن يُحضر الخضراوات للاستخدام مسبقاً على النتائج المتصور عليها وجذب أن
الكرزون الناجح عن CC14 من خلال تأثير مضادات الأكسدة على خلايا الكبد، وبالتالي القضاء على الآثار
الटى أضمها مظهرة للمرضى الذين يعانون من

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