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Protective role of MGN-3/Biobran against dimethylhydrazine-induced toxicity in rats

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Abstract: Dimethylhydrazine (DMH) is a potent colonic and hepatic carcinogen that is metabolized into oxyradicals, causing liver injury and DNA mutations. Arabinoxylan rice bran (MGN-3/Biobran) is a well-documented natural product that possesses anti-inflammatory, antioxidant and anti-carcinogenic properties. The main objective of the study is to determine the protective potential of MGN-3/Biobran against DMH-induced toxicity in vital organs of rats. The study was performed using Sprague Dawley rats allocated into four groups. The first group is the normal untreated control, 2nd group received MGN-3/Biobran only, 3rd group received subcutaneous injection of DMH-2HCL once per week for 4 consecutive weeks. Rats in the 4th group were pretreated with MGN-3/Biobran, 2 weeks prior to the injection with DMH-2HCL. Biobran treatment was continued throughout the experimental period. After 18 weeks of experimental period, the organs (liver, spleen and kidney) were excised and the blood samples were collected then the sera were separated. Serum was used for the determination of liver and kidney function biomarkers and lipid profile parameters. The results showed that DMH induced markedly decreased organ weights, increased total bilirubin, decreased albumin and total protein content, increased serum concentrations of urea, creatinine, cholesterol and triacylglycerol. Protective effects of Biobran were observed by maintaining normal liver, spleen and kidney weights, ameliorating all biochemical parameters within normal values. It can be concluded that MGN-3/Biobran may be a promising chemopreventive agent against DMH toxicity.

keywords: Dimethylhydrazine, MGN-3/Biobran, Sprague Dawley rats, toxicity.

1.Introduction

1, 2 Dimethyl hydrazine (DMH) is a potent colon carcinogen inducing colorectal tumors in experimental animals and is the most widely used model of chemically induced-colon carcinogenesis (1,2). DMH is a highly toxic chemical that can affect number of body organs including liver (3,4). It is metabolized in the liver (5,6) and produces highly reactive electrophiles as carbonium ions and alkyl free radicals, which cause severe hepatic damage of necrosis and fatty infiltration (7) as well as methylate nucleobases that disrupt the polysomal assembly (8). Oral administration alters the composition of the intestinal brush border membranes and their absorptive functions (9,10). Although such changes have not been studied in humans, the adverse effects of alkyl hydrazine derivatives cannot be overlooked owing to the direct exposure to them through chemical and pharmaceutical regimens (11,12). Dimethylhydrazine has been shown to be present in tobacco, commercial and wild mushrooms and other food items (13).

Drugs used to treat liver disease, at both traditional and synthetic levels, can have serious side effects, and there is an increasing global interest in using traditional medicinal plant products instead. The hepatotoxicity is a potentially serious side effect of the anti-tubercular drug isoniazid (alkylated hydrazine). The toxic doses have been shown to affect the liver, cell membranes and different organelles of animals (14,15).

MGN-3/Biobran arabinoxylan (MGN-3) is a natural blend of hemicelluloses derived from partially hydrolyzed rice bran with shiitake mushroom enzymes (*Lentinus edodes* mycelia extract). The main chemical structure of MGN-3 is an arabinoxylan with a xylose in its main chain and an arabinose polymer in its side chain (16). The purpose of the present study is to evaluate the potential protective role of MGN-3/Biobran against DMH-induced toxic damage in some vital organs of rats.

2. Materials and methods

2.1. Experimental animals

Male Sprague Dawley (SD) rats, weighing 80-100g, were obtained under hygienic conditions from animal house of the Biological Products & Vaccines (VACSERA), Cairo Egypt. Rats were placed into plastic cages covered with metal grids with wood chips for bedding and allowed to acclimate for one week in the animal facility of Faculty of science, Mansoura University and were housed 4 per cage. Animals were given standard laboratory pellets diet and drinking tap water *ad libitum*. All rats were accommodated for 1 week prior to the experiments under controlled conditions of 24 ± 2°C, 50 ± 10% relative humidity, and 12 h light/12 h dark cycle. This study was carried out in accordance with the approval of the Ethics Committee for Animal Experimentation, Mansoura University (Sci-Z-M-2020-17) and in compliance with the "Guide for the Care and Use of Laboratory Animals".

2.2. Chemicals

2.2.1. Dimethylhydrazine (DMH)

1, 2-dimethylhydrazine dihydrochloride (DMH-2HCL), purity 98% was purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, USA). DMH-2HCL was dissolved in 0.9% normal saline and administered subcutaneously (SC) with dosage of 40 mg/kg body weight once a week for 4 consecutive weeks according to Salim and Fukushima (17).

2.2.2. MGN-3/Biobran

MGN-3/Biobran is arabinoxylan extracted from rice bran treated with hydrolyzing enzymes from Shiitake mushroom. The main chemical structure of Biobran is arabinoxylan with xylose in its main chain and arabinose polymer in its side chain. Biobran was freshly prepared by dissolving in 0.9% saline solution and given intraperitoneally (IP) according to Badr El-Din *et al*. (18) for 18 weeks (all the study period). Biobran was kindly provided by Daiwa Pharmaceuticals Co. Ltd., Tokyo, Japan.

2.3. Experimental design

After one week of acclimation period in the animal facility conditions, rats were divided into four weight-matched groups, with five for each (n = 5), to minimize the standard errors as follows: Group-1(control): untreated rats serving as negative control group. Group-2 (Biobran): rats were intraperitoneally (IP) treated with MGN-3/Biobran 40 mg/kg B.W/day every other day for 18 weeks. Group-3 (DMH): rats were subcutaneously (SC) injected with 40 mg/kg DMH-2HCL once per week for 4 consecutive weeks (Carcinogen injection begins 2 weeks after the start of the experiment). Group-4 (Biobran + DMH): rats were pretreated with MGN-3/Biobran, 40 mg/kg BW, every other day, 2 weeks prior to the injection of DMH-2HCL. Biobran treatment was continued throughout the experimental period.

2.4. Sample collection

At the end of the experiment, after 18 weeks, animals were fasted overnight and anesthetized using diethyl ether. Blood was withdrawn from abdominal aorta using vacuum tubes, left to clot at room temperature. Serum was separated by centrifugation at 3000 rpm for 20 min and kept at -20ºC until further analysis. Serum was used for the determination of liver and kidney function parameters; total proteins, albumin, bilirubin, creatinine and urea levels in addition to lipid profile markers, cholesterol and triacylglycerol levels.

2.5. Measured parameters

2.5.1. Organ weights

At week 18 after sacrificing the animals, liver, spleen and kidney of the different groups were removed to determine the weight differences.

2.5.2. Liver function tests

Total proteins content was assayed by colorimetric method of Gornal *et al.* (19). Albumin content was assayed by colorimetric method of Doumas *et al*. (20). Bilirubin level was determined by colorimetric method of

Walter and Gerade (21). All liver function tests were performed using kits purchased from Biodiagnostic Co. Dokki, Giza, Egypt.

2.5.3. kidney function tests

The creatinine content was estimated according to the kinetic colorimetric method of Young *et al*. (22) using BioMed Diagnostic kit supplied by EGY-CHEM, Cairo, Egypt. The urea content was estimated according to the enzymatic colorimetric method of Tietz (23) using a kit supplied by LINEAR CHEMICALS, Spain.

2.5.4. Lipid profile

The total cholesterol content was estimated according to the enzymatic colorimetric method of Richmond (24) using a kit purchased from Biodiagnostic Co., Dokki, Giza, Egypt. Triacylglycerol content was estimated according to the enzymatic colorimetric method of Fossati and Prencipe (25) using a kit bought from Biodiagnostic Co., Dokki, Giza, Egypt.

2.5.5. Statistical analysis

Data were expressed as mean ± SD for organ weights and as mean ± SE for other results. The data were analyzed and the statistical differences were tested by One Way Analysis of Variance (ANOVA) test followed by post-hoc tests for multiple comparisons. All statistical analyses were performed using the SPSS 25 software. Differences were considered significant at $P < 0.01$ and $P < 0.05$.

3. Results

3.1. Organ weights changes

The data for rat liver, spleen and kidney weights of the different experimental groups are shown in figure (1). DMH treatment caused a significant decrease in liver, spleen and kidney weights as compared with normal control group. In contrast, Biobran pretreatment to Biobran + DMH group, prevented the decrease of the different organ weights that caused by DMH intake, and maintained the weights within normal values.

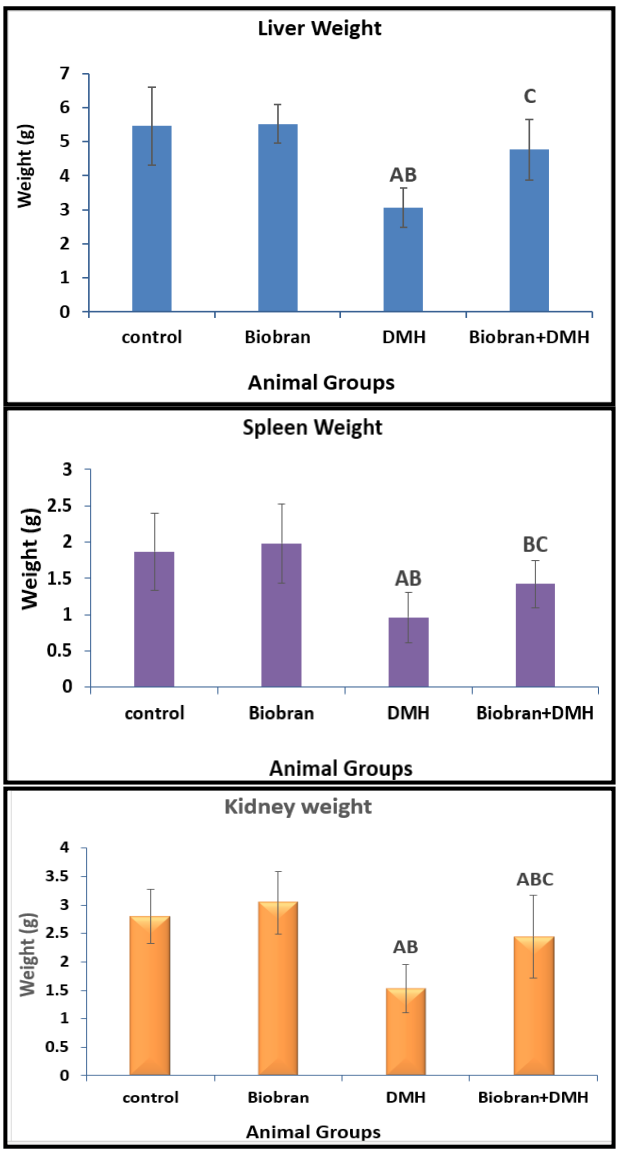

Fig. 1. Changes in liver, spleen and kidney weights in the different groups.

[A] significantly different from the normal control group at $p < 0.01$ level.

[B] significantly different from the Biobran group at $p < 0.05$ and $p < 0.01$ level respectively.

[C] significantly different from the DMH group at $p < 0.01$ level.

3.2. Liver function tests

Serum bilirubin, total protein and albumin levels were estimated as shown in figure (2). DMH treatment caused a marked increase in serum bilirubin level and marked decreases in albumin and total protein content versus the normal control group. On the other hand, Biobran pretreatment to the 4th group modulated the levels of liver markers to be slightly different from the normal control group.

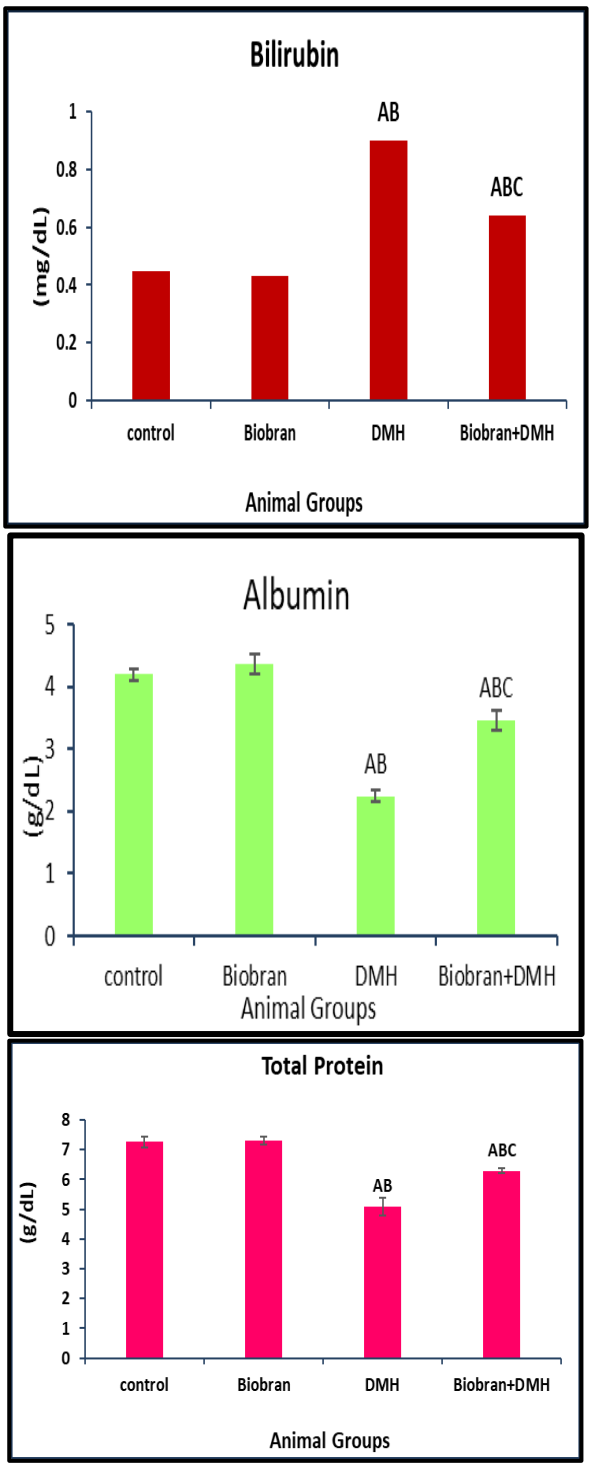

Fig. 2. Changes in serum bilirubin, albumin and total protein content in the different groups.

[A] significantly different from the normal control group at $p < 0.01$ level.

[B] significantly different from the Biobran group at $p < 0.05$ and $p < 0.01$ level respectively.

[C] significantly different from the DMH group at $p < 0.01$ level.

3.3. Kidney function tests

Serum creatinine and urea contents are represented in figure (3). DMH treatment caused a significant increase in creatinine and urea content when compared with the normal control group. In Biobran + DMH group, the level of creatinine significantly decreased in comparison with DMH group and urea level could be comparable with the normal control group.

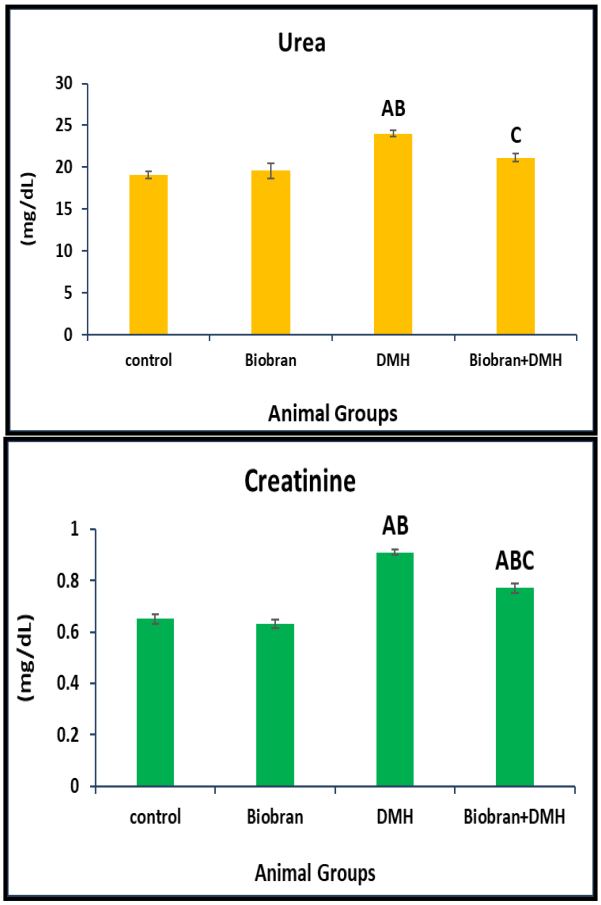

Fig. 3. Changes in serum urea and creatinine content in the different groups.

[A] significantly different from the normal control group at $p < 0.01$ level.

[B] significantly different from the Biobran group at $p < 0.05$ and $p < 0.01$ level respectively.

[C] significantly different from the DMH group at $p < 0.01$ level.

3.4.lipid profile

The data for serum triacylglycerol and total cholesterol level for rats in all groups are presented in table (1). DMH treatment caused highly significant increases in serum triacylglycerol and total cholesterol level when compared with the normal control group. In Biobran + DMH group, the treatment with Biobran caused highly significant decreases in triacylglycerol and total cholesterol levels versus DMH group.

Table 1. The levels of serum total cholesterol and triacylglycerol in the different groups.

GroupsParameters	Normal control	Biobran	DMH	Biobran + DMH
Total cholesterol (Tc) (mg/dL)	62.06± 0.53	64.44± 0.5	106.94^{AB} ± 1.27	82.28^{ABC} ± 0.73
% change from the normal control group	-	3.84	72.32	32.58
Triacylglycerol (TG) (mg/dL)	30.66 ± 0.55	31.87 ± 0.49	99.24^{AB}± 0.83	52.14^{ABC} ± 0.95
% change from the normal control group	-	3.95	223.68	70.07

[A] significantly different from the normal control group at $p < 0.01$ level.

[B] significantly different from the Biobran group at $p < 0.05$ and $p < 0.01$ level respectively.

[C] significantly different from the DMH group at $p < 0.01$ level.

Discussion

Dimethylhydrazine (DMH) is a potent colonic and hepatic carcinogen that is metabolized into oxyradicals causing liver injury and DNA mutations (26).

The liver is a vital organ with a wide range of metabolic, detoxification, and immune functions (27). Despite its considerable regenerative capacity, continuous and various exposures to xenobiotics, environmental pollutants, and chemotherapeutic agents could suppress and possibly overcome the natural protective mechanisms of the liver, leading to liver malfunction and later if it is not treated properly leads to injury (28). DMH treatment can lead to hepatic damage that histologically resembles carbon tetrachloride damage because both proceed through free radical mechanisms. Loss of metabolic enzymes that are located in intracellular structures results from changes in the endoplasmic reticulum (29). Free radicals produced as a result of oxidative stress initiate chain reactions that lead to the process of lipid peroxidation which causes damage to cell membranes. So any agent inhibiting free radical processes contributes to chemo-protection of cellular damage and also the carcinogenesis (30).

In the present study, liver, spleen and kidney weights showed significant decrease in DMH-treated animals versus the negative control group. Also, DMH-treated rats showed a marked increase in serum total bilirubin accompanied by a marked decrease in albumin and total protein contents as compared to the untreated normal controls. Serum bilirubin is the most sensitive test employed in the diagnosis of hepatic diseases. Elevated level of serum bilirubin is a very sensitive test to substantiate loss of functional integrity of liver and severity of necrosis (31,32).

On the other hand, albumin is a protein present in large amounts in plasma and constitutes about 60% of the total protein in plasma. Albumin is known to decrease in cases of renal disorder, hepatic disorder and inflammatory diseases, such as cancer (33). Similar findings were observed by Sharma and Sharma (34), who recorded high elevation of bilirubin in serum of mice treated with DMH, and attributed to the damage to mouse liver and biliary dysfunction caused by DMH treatment.

The hepatotoxic activity of DMH shown in the current study was probably due to its ability to generate free radicals (35). DMH is an alkylating agent that is widely used to induce tumours in the colon of rodents (36). Subcutaneously administered DMH released slowly into the circulation, reached the liver, and metabolized into various carcinogenic intermediates (37).

MGN-3/Biobran is a natural product extracted from rice bran whose effectiveness in the treatment of cancer has been widely examined in both animal models and humans with different types of malignancies (18). Results of the current study indicated that MGN-3/Biobran pretreatment to DMH injected rats (Biobran + DMH group) resulted in preventing the decrease of organs weight versus normal controls. In addition, MGN-3/Biobran treatment reduced the levels of serum total bilirubin, and elevated the level of serum albumin and total proteins to be within normal values. Such amelioration may be due to the ability to prevent DMH-induced toxicity that maintained normal organs weight and improved the rat liver function by stabilizing the biliary dysfunction due to DMH. DMH exhibited also significant increase in serum urea and creatinine in DMH treated group when compared with control group. Raised creatinine

concentration is an index of kidney dysfunction (38). These results are agreement with those reported by Abdel-Hamid *et al*. (39) who demonstrated that the levels of urea and creatinine in serum of rats treated with DMH were higher than that of control group. In contrast, pretreatment with MGN-3/Biobran to rats treated with DMH revealed a significant decrease in serum Urea and Creatinine levels versus DMH group and to reach nearly normal values when compared with the normal control group, which indicated the protective role of the natural agent MGN-3/ Biobran.

Similarly, lipid profile in our study showed an increase in serum total cholesterol, triacylglycerols concentration in DMH-treated rats when compared to control group. However, MGN-3/Biobran treatment caused a marked decrease in these markers as compared to DMH group (38). The correlation between carcinogen-related changes in lipid metabolism and oxidative stress has been of great interest. Earlier studies showed how changes of the lipid profile result in increased oxidative stress. Increased lipid content is thought to result in decreased antioxidant enzyme expression, increased NADPH oxidase expression, and increase ROS concentrations (40).

5. Conclusion

The present study has demonstrated the protective potential of MGN-3/Biobran against DMH induced toxicity in different vital organs of rats including liver. The protective effect of MGN-3/Biobran is due to stimulation of hepatic regeneration by preventing damage by alkyl free radicals as well as by neutralization and excretion of electrophiles generated due to DMH metabolism, by the active ingredients of MGN-3/Biobran.

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