INHIBITION OF EXPERIMENTAL CARCINOGENESIS BY THE BIOACTIVE NATURAL PRODUCT BIOBRAN.
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ABSTRACT

To investigate the protective effects of biobran against N-nitrosodiethyamine (NDEA) and carbon tetrachloride CCl₄-induced hepatocarcinogenesis in rats.

Hepatocarcinogenesis was induced in rats by a single intraperitoneal (i.p.) injection of N-nitrosodiethyamine (NDEA) at a dose of 200 mg/kg body weight followed by weekly subcutaneous injections of CCl₄ (3 ml/kg) for 6 weeks, as the promoter of carcinogenic effect. After administration of the carcinogen, 25 mg/kg/day of Biobran were administered i.p., five times a week throughout the study. At the end of 20 weeks, the body weight, liver weight were measured, blood samples were collected for liver function tests, liver biopsies were processed for histopathology examination.

Results demonstrated that biobran has significantly prevented the decrease of the body weight and the increase in the liver weight caused by NDEA. Liver function tests showed significant increase in serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and -glutamyl transpeptidase ( -GT) of untreated NDEA group, meanwhile treatment with Biobran to rats exposed to carcinogens, significantly minimized the elevation of the liver function enzymes level to be comparable with the normal control values. Histopathological examination of the liver sections of rats subjected to (DENA + CCl₄) treatment revealed fibrosis and fatty infiltration of hepatocytes, with inflammatory collection and loss of architecture Biobran treatment showed minimal changes in hepatocyte morphology and histology with no inflammation.

this study showed that Biobran has a protective effect against hepatocarcinogenesis induced by NDEA and CCl₄ in rats.

Keywords: N-nitrosodiethyamine; Carbon tetrachloride; Carcinogen; Biobran.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common form of liver cancer in adult, which account for about 75% of primary liver cancers. It is the 5th most liver common cancer worldwide and represents 83% of all cases (Ferlay et al., 2001). Liver cancers have different growth patterns; the first type begins as a single tumor that grows larger in hepatic tissue. The second type of is spread through the liver almost from the beginning and is not confined to a single tumor. This is seen most often in people with liver cirrhosis. Risk factors for HCC include hepatitis B virus (HBV), hepatitis C virus (HCV) and aflatoxins are assumed to play an important role in high incidence of HCC. HBV vaccination of children and high-risk population must be the priority in reducing the incidence of HCC. Measures to reduce food spoilage by fungi and the associated dietary exposure to aflatoxins are desirable public health goal (Wild and Hall, 2000). Liver carcinogenesis may also develop through progressive accumulation of different mutations (genetic) and/or genetic products (protein), which eventually lead to malignant transformation (Macphee, 1998 and Seufi et al., 2009).

N-nitrosodiethyamine, a potent hepatocarcinogenic dialkyl nitrosamine is present in tobacco smoke, water, cheese, cured and fried meats and in a number of beverages (Rajes kumar and kuttan., 2000). A review on NDEA reported that a number of species including mice, rats, guinea pigs, hamsters, rabbits, dogs and monkeys, (Verna et al., 1996) developed liver cancer on exposure. It is metabolized to its active ethyl radical(CH₂CH₂) by cytochromes and the reactive product interacts with DNA producing mutation and further oncogenesis.

Biobran is a compound made from breaking down rice bran with enzymes from the Shitake mushroom. Previous reports have shown Biobran to be a potent biological response modifier (BRM) that stimulates several different arms of the immune system including natural killer (NK) cells (Ghoneum and Brown., 1999). In addition, MGN-3 is capable of sensitizing human leukemic cell surface CD95 receptors that are involved in the triggering of apoptosis (Ghoneum and Gollapudi., 2003).

MATERIALS AND METHODS

Chemicals & drug:

N-nitrosodiethyamine, was purchased from sigma chemical company, USA. Carbon tetrachloride (CCl₄) was obtained from El-Gomhorya company, Cairo, Egypt. Biobran was kindly provided by Daiwa Pharmaceuticals Co Ltd., Tokyo Japan.

Animals:

Male albino rats weighing 120-140 g were used. Their age between 8-10 weeks old were procured from the animal house of the Nile Centre for experimental research, Mansura, Egypt. The rats were housed in groups in plastic cages with wood chips for bedding under controlled conditional of temperature (22 ± 3 °C) with a 12 h light/dark cycle respectively for one week before and during the experiment. Animals were allowed to access standard rodent pellets diet and drinking water.

Experimental design:

Adult male Wister albino rats, 120-140g, the rats were randomly assigned into five experimental groups, group 1 & 2 containing 15 rats, groups 3, 4 & 5 containing 20 rats.

- Group (1: Control): rats served as controls.
- Group (2:Biobran): rats were given 25 mg/kg/day of Biobran by i.p. injection five times a week throughout the study.
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- Group (3: Carcinogen): rats received single intraperitoneal injection of NDEA (200 mg/kg body weight) after one week they are received weekly subcutaneous injections of CCl4 (3ml/kg b.w) for 6 weeks (Sundaresan & Subramanian, 2003).
- Group (4: Biobran + Carcinogen): animals received Biobran as group 2 two weeks before the injection of carcinogens and continued for 20 weeks.
- Group (5: Carcinogen + Biobran): animals received the carcinogen as in group 3, then treated with Biobran starting from week 10 up to the end of the study.

Body and liver weight changes:

Body weight (BW/g) of the different experimental groups was measured weekly during the experiment time. At the end of experimental study after sacrificing the rats, liver of different groups were excised and weighed.

Histopathological examination:

The liver samples were preserved in phosphate-buffered 10% formalin for 24 hours, cut into small pieces. After fixation, the samples were dehydrated in ascending series of ethyl alcohol 70%, 80%, 90% and 95% for 30 minutes each, then into changes of absolute ethyl alcohol for 30 minutes each. Tissue were cleared in xylene for 20 min two changes), then embedded in paraffin wax. Sections 4 to 5 μm thick were cut using microtome, mounted on glass slide and stained according to the following histological method then examined by light microscope (Weenser, 1968).

Biochemical analysis:

At the end of the experimental period, all the animals were sacrificed. Blood samples were collected in heparinized tubes and centrifuged at (3000 rpm for 20 min) without hemolysis. The levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), were determined using an automatic biochemical analyser (BTS-370, BioSystems S.A., Barcelona, Spain) according to the instructions supplied with the commercial assay kits (Roche, Switzerland).

Statistical analysis

Results were expressed as means ± SE. Statistical significance was calculated using one-way analysis of variance (ANOVA) followed by post hoc tests for multiple comparisons. All the statistical analysis carried out with the use of SPSS 18 software. Differences were considered significantly at p < 0.05 level.

RESULTS

1. Effect of Biobran on body weight changes induced by NDEA.

Body weight (BW) of the different experimental groups was recorded weekly during the experiment time. Figure 1, shows the BW changes in rats. Initial BW without treatment was comparable between groups. On first week after NDEA treatment, the rats began to show a slow growth and continues gradually through injection of CCl4 for 6 weeks as compared to normal control group. Final body weight of rats showed increased in control group to record (318±7.65 g) and Biobran intake to normal rats recorded (300±6.11g). On the other hand untreated carcinogen group showed highly significant (p<0.01) BW loss as compared to the other groups to record (192±3.86 g, -39.54% BW) loss of control group. The body weight in pretreatment group (Biobran+Carcinogen) showed increase as compared to untreated carcinogen group to record (264±5.34 g), -17% BW, and decreased when compared to the normal control group. Posttreatment animals (Carcinogen+Biobran) significantly recovered the body weight gain of rats (243.5±4.51 g, -23.44% BW) compared to that of carcinogen untreated group.

![Figure 1: Effect of Biobran intake on rat BW/gm. The data of BW were presented as mean±SE. A Significantly different from control group at p < 0.01 level. B Significantly different from Biobran group at p < 0.01 level. C Significantly different from Carcinogen group at p < 0.01 level. D Significantly different from (Biobran+Carcinogen) at p<0.01](image-url)
2. Effect of Biobran on Liver weight

As shown in Figure 2, treatment with Biobran alone to normal animals showed comparable liver weight with the normal control animals and recorded (8.45±0.29 g, 8.56±0.25 g) respectively, liver weight of Carcinogen group animals recorded 10.38±0.34 g which represents a marked increase by 24.73%, p<0.01 of untreated normal control group. In the prevention animals by Biobran before induction of tumor (Biobran+Carcinogen) showed a moderate increase in liver weight to record (8.75±0.51 g, 3.57%, p<0.01) as compared to normal animals. Posttreatment animals (Carcinogen+Biobran) showed slight insignificant increase in liver weight to record 8.66±0.21 g, 2.57% when compared to untreated normal control group.

![Liver weight graph]

Figure (2): Effect of Biobran on liver weight. Each value represents the mean±SE.

A Significantly different from control group at p< 0.01 level.
B Significantly different from Biobran group at p<0.01 level.
C Significantly different from Carcinogen group at p< 0.01 level.

3. Histopathological study

Study of the liver tissue sections from rats in the normal and Biobran control groups revealed a normal hepatic lobular architecture and the presence of normal hepatocytes with granulated cytoplasm and small uniform nuclei and nucleolus. In contrast, the study of sections obtained from rats subjected to (DENA + CCl4) treatment revealed fibrosis and fatty infiltration of hepatocytes, with inflammatory collection and loss of architecture, necrosis and hepatocellular degeneration with frequent mitotic activity. Pretreatment animals with Biobran showed minimal changes in hepatocyte morphology and histology with no inflammation. Animals post-treated with Biobran showed lesser damage of hepatocytes and low index of necrosis, vacuolation of hepatocytes and scanty mitosis.
Figure (3): Histopathological effects of biobran treatment against hepatocarcinogenesis in rats. A (untreated), B (Biobran treated); normal control groups showing the normal histological structure of hepatic lobular with granulated cytoplasm and small uniform nucleus and nucleolus. C: (NDEA+CCL₄) showing fatty infiltration of hepatocytes, with inflammatory collection and loss of architecture, necrosis and fibrosis hepatocellular degeneration. D: pre-treatment group (Biobran+Carcinogen) showing preserved hepatic architecture, minimal nuclear changes and vacuolation of hepatocellular cytoplasm and no inflammation. E: animals post-treated (Carcinogen+Biobran) showed lesser damage of hepatocytes and low index of necrosis, vacuolation of hepatocytes. (H&E x400).

4. Effect of Biobran on liver function tests
Data in Figure 4, represent the activity levels of liver function enzymes AST, ALT, ALP and GGT in serum of rats under different experimental conditions. Animals that administrated of NDEA induced a significant increase (p<0.01) in serum levels of AST by 145%, ALT by 224% and 99.23% for ALP as compared with the normal control. Further, serum GGT level showed also a marked high elevation by 1584%, p<0.01 of normal values.

Pretreatment group by Biobran (Biobran+Carcinogen), significantly minimized the elevation of the liver function enzymes level to record 20%, 65.38% & 31.40% for AST, ALT and AL respectively, when compared to the normal control rats. On the other hand, GGT level showed a significant decrease in serum activity (p<0.01) and recorded 426% when compared to the normal control. Administration of Biobran to Carcinogen group (Carcinogen+Biobran) improved the liver function by inducing a remarkable reduction in the elevated AST, ALT & ALP levels in serum to reach 23.89%, 74.85%, 37.74% and 426% respectively, GGT level showed 637% with estimate to normal control values.
DISCUSSION

N-nitrosodimethylamine (NDEA) is a major environmental carcinogen suggested to increase the generation of reactive oxygen species (ROS) resulting in oxidative stress and cellular injury (Bartsch et al., 1989). Since liver is the main site of NDEA metabolism, the production of ROS in the liver may be responsible for its carcinogenic effects (Bansal et al., 2005). NDEA is known to cause perturbations in the nuclear enzymes involved in DNA repair/replication (Bhosale et al., 2002). Treatment with NDEA and CCl₄ has been shown to induce extensive necrosis and inflammatory infiltration, clusters of hepatocyte, necrosis, bile duct proliferation and marked atypia (Sundaresan & Subramanian, 2003, Al-Rejaie et al., 2009).

The results of the present study seem to provide support for the chemopreventive effects of Biobran against NDEA-induced hepatocarcinogenesis in rats. There is an appreciable reduction in body weight and increase in liver weight observed in carcinogen group rats as compared to control group rats. Decreased appetite and food intake contribute to the weight loss which could be an indication of the declining hepatic function, an increase in the liver weight of the animals. Sreepryia and Bali, 2005 have also reported marked loss of body weight and increase in liver weights. The steady increase in body weight during the course of the study for the animals pretreated or posttreated with Biobran, might indicate increase in the animal appetite that resulted in prevention of body weight loss. In addition, Biobran treatment maintained normal animal liver weight probably by preventing NDEA and CCl₄ induced hepatotoxicity.

Histopathological examination of the normal control groups showed normal hepatic lobular architecture with granulated cytoplasm and small uniform nucleus and nucleolus. Carcinogen group showed fatty infiltration of hepatocytes with inflammatory collection and loss of architecture, necrosis and hepatocellular degeneration (Ramakrishnan et al., 2006). On the other hand, pre-treated group showed preserved hepatic architecture, minimal nuclear changes and vacuolation of hepatocellular cytoplasm with no inflammation. Group post-treated (carcinogen+Biobran) showed lesser damage of hepatocytes and low index of necrosis, vacuolation of hepatocytes.

In the present study, NDEA and CCL₄ administration to rats led to marked increase in the levels of serum AST, ALT and ALP compared to the normal group, which indicating that NDEA could induce a liver damage in rats. These results are in agreement with Bansal et al (2005) who attributed the elevation of serum transaminases and alkaline phosphatase to the injured structural integrity of the liver as these enzymes released from the cytoplasm into the blood circulation after rupture of the plasma membrane and cellular damage. γ-GT is an enzyme embedded in the hepatocyte plasma membrane, mainly in the canicular domain and its liberation into serum indicates damage of the cells and thus injury to liver (Sivaramakrishnan et al., 2008). It is important to point out that serum γ-GT activity is considered to be one of the best indicators of liver damage (Jeena et al., 1999). These results are also in agreement with Mittal et al (2006) who found that activities of AST, ALT and ALP were increased significantly following nitroso compounds treatment in rats due to substantial liver damage. Pretreatment and posttreatment with Biobran significantly decreased the elevation in serum liver enzymes levels to a great extent suggesting that Biobran supplementation protected the hepatocytes from injuries and improves the liver functions of tumor-bearing mice due to its antioxidant potency (Noaman et al., 2008).

From these observations it can be concluded that Biobran is a potent natural agent that possesses chemopreventive action against NDEA and CCl₄ induced hepatocarcinogenesis.
REFERENCES


الذور الىقائي للواءاتي النباتي بنىعرى التسرطى التجربى

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ملاحظة: استخدمات مادة البيوران ماأدى إلى تواجدها في العالم، وقد نانى العديد من أنواع الفواكه منها الجرذان والعلاج

لكي يأتي إلى الوفاة في العالم. و هناك العديد من أنواع العلاج منها الجرذان والعلاج

الكيميائي والعلاج الأشعائي. في هذه الدراسة تم استخدام مادة طبيعية (بيوران) مشتقة من نبات الأرك الماء بذروات مشتقة من قشر شياياتي بهدلية الكبد من تأثير البكتيريا المائية ذات النسبة البدائية في مادتي الببتيدية، ايتي لين ورابع كوربين. في هذه الدراسة تم استخدام إصابات السرطانات الدراسة يوماً واحداً بعد خذ الاضاعات. الكوربس 200

ملجع/كجم من ورود المجموعة الببتيدية الريبوتروين ورادك كوربين مرسب عائلاً 6 أسابيع بجرعة مل/كم من وزن

المجموعة تحت الحنطة مادة سرطان وتم العلاج مادة البيوران. 25 مل/كم من وزن الجسم في التجريب البريئي.

تم تقسيم 90 جرذ إلى خمس مجموعات كالتالي:

- المجموعة الأولى (المجموعة الضامنة): 15 جرذ تم ملئها بالمواد السرطانية ولم تلقي أي مادة علاجية
- المجموعة الثانية (مادة البيوران): 15 جرذ تم معاملتهم بدقة البيوران بجرعة 30 مل/كم من وزن الجسم في التجريب البريئي.
- البريئي 5 أيام في الأسبوع أبناء من اليوس للتجربة (26 أسبوع).
- المجموعة الثالثة (المجموعة السرطانية): 20 جرذ تم تحققيهم بمادة الببتيد 200 مل/كم من وزن الجسم في التجريب البريئي.
- بعد أسبوع تم تحققيهم بمادة رابع كوربين 3 مل/كم من وزن الجسم ونكل تحت الجلدة مدة 6 أسابيع.
- المجموعة الرابعة (المجموعة المعروفة بالبيوران): تم معاملة هذه المجموعة ونكل تحت جلدة مدة 20 جرذ مادة البيوران لمدة أسبوعين قبل تحققيها بالمواد السرطانية والاستمارة بعد ذلك نهائية التجربة.
- المجموعة الخامسة (المجموعة السرطانية مع المعالجة بالبيوران): 20 جرذ تم تحققيهم بمادة البيوران أبناء من الأسبوع العاشر من

التجربة 25 مل/كم من وزن الجسم حتى نهاية التجربة.

ويمكن تخصيص النتائج التي حصلنا عليها كالاتي:

أظهرت نتائج الدراسة بأن معالجة الجرذان بدقة البيوران قد منع إلى حد كبير فقدان وزن الجسم بالمقارنة مع مجموعة الجرذان السرطانية. وذلك وجد أن المجموعة السرطانية أظهرت أداء ملحوظ في وزن الكبد بنسبة (73.2)٪ مقارنة بالمجموعة الضامنة، بينما وجد أن الجرذان المعالجة بدقة البيوران إلى حد ما إلى الحفاظ على الوزن الطبيعي بالمقارنة مع مجموعة السرطانية.

ما كان يسمى مستويات ضغط الكبد فعالة التي أن المجموعة التي تم تحققيهما السرطانية غير المعالجة أظهرت أرقاماً

ملحوظًا في مستويات ضغط الكبد (ALT) 23.42٪ بنسبة %، و(ALT) 21.27٪ بالنسبة %، و(ALT) 20.32٪ بنسبة %، و(ALT) 6.87٪ بنسبة %، و(ALT) 4.67٪ نسبة %، و(ALT) 13.27٪ بنسبة %، و(ALT) 5.67٪ نسبة %، و(ALT) 1.27٪ نسبة %، و(ALT) 0.32٪ نسبة %، و(ALT) 0.01٪ نسبة %.

وهي اختر تراقي وتباينها كبيرة في مستوى هذه الأحماض في النسبة السرطانية والبيوران أولاً ثم المواد السرطانية

الfrica اوثقية واسعة باكتريا في مستويات هذه الأحماض بالبيوران أولاً ثم المواد السرطانية

وكان نسبة (ALT) 76.5٪ و(ALT) 3.45٪ و(ALT) 4.57٪ و(ALT) 2.57٪ و(ALT) 1.45٪ و(ALT) 0.57٪ و(ALT) 0.25٪

الضائبة.

وؤثر أيضاً السحح المحرر باستخدام الميكروسكوب الضوئي light microscopy

جرز السرطانية في الجرذان الفي الحير معالجة

وجود الخلايا السرطانية المخلطة للنسيج الكبيدي وجود تجمعات فيية وهدنة. أما أنفسه الكبد الدائى في الجرذان المعالجة بدقة

البيوران فظهر التالق الواضح في عدد الخلايا الكبيدي السرطانية وجود عدد كبير من الحذاء النسيجية زياميمي مع ارتفاع نسبة

الموتو الخفوى بعد عدو انيب بعد العلاج.

استخلص من هذه الدراسة أن تناول بوران الطبيعية أدى إلى حفاظ على الكبد الدائى الكبد من تأثير المواد السرطانية بدرجة عالية كما مع

الي حذف كبير فقدان وزن الجسم حافظ على المستوى الطبيعى لإزيمات وظائف الكبد. مما سي حقق ان تؤدي نتائج هذه الدراسة إلى

إمكانية استخدام مادة بوران الطبيعية الأحماض كمادة واقية ضد الأحماض السرطانية.