The curative effect of a burdock plant against toxicity induced in rats by diethylnitrosamine

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Abstract

Aim: The present study was performed to evaluate the role of the medicinal plant burdock; Arctium lappa against toxicity in rats induced by the chemical carcinogen (Diethylnitrosamine). Method: Rats were divided into four groups (8 rats per group). The Group (A) served as a normal control. Group (B) was injected intraperitoneally with a single dose of diethylnitrosamine DENA. Group (C) was injected intraperitoneally with a single dose of DENA; then after one week treated with a methanolic extract of burdock. Rats belonging to Groups (D) were treated only with a methanolic extract of burdock. Hepatocellular carcinoma (HCC) was induced by a single dose of intraperitoneal injection of DENA (200mg/kg body weight) followed by phenobarbital of 0.05% mixed with drinking water for 14 weeks. After 14 weeks, rats belonging to all groups were sacrificed and blood and liver samples were collected. Liver weights and relative liver weights were measured; biochemical investigations and histopathological studies were performed. Results: The administration of DENA and promoted phenobarbital agent leads to different histopathological changes. Total proteins, albumin and globulin levels were decreased significantly when compared to control. While; the liver weights, relative liver weights, ALT, AST, ALP, GGT and AFP levels were elevated significantly compared to control values. Administration of a methanolic extract of burdock induced different marked ameliorations and chemopreventive effects in liver histology and different biochemical investigations.

Keywords: Diethylnitrosamine, Biochemical parameters, histopathology, Liver, Rats

1 Introduction

Hepatocellular carcinoma (HCC) is the most common predominant hepatic malignancy of adults. It is the sixth most common cancer worldwide and the is the third most common cause of cancer-related deaths worldwide (Liping et al., 2014). HCC incidence is higher in men than in women worldwide with a male to woman ratio of about 4:1 (Nordenstedt et al., 2010; Sherman, 2010; Carr, 2012). In Egypt, liver cancer forms 11.75% of the malignancies of all digestive organs and 1.68% of the complete malignancies. HCC contributes 70.48% of all liver tumors between Egyptians (Nanis et al., 2015).

Diethylnitrosamine (DENA) is a representative chemical carcinogen with the potential to cause tumors in various organs, including the liver, gastrointestinal tract, skin and respiratory system (Park et al., 2009). Phenobarbital is used as a promotor of DENA-induced hepatocarcinogenesis in rodents (Thiery et al., 1999).

Medicinal plants were classified as one of the natural products and the plant components such as flower, bark, leaves, berries, roots, or seeds can be used for medicinal and therapeutic requirements (Falodun, 2010).
Arctium, or burdock, is a two-yearly plant species native to parts of Northern Asia and Europe. It is a member of the Asteraceae family and burdock root is usually used as meals in Asia and is known in Japan as Gobo. Acetic acid, beta-carotene, butyric acid, caffeic acid, inulin, calcium, essential oils, and flavonoids, are among the most components of a burdock plant (Duke, 1992). Burdock was also used as a folk medicine, as a diuretic and antipyretic (Kan, 1981). Several studies have reported that the root of burdock possesses various pharmaceutical activities including antibacterial activity (Chow et al., 1997), desmutagenic activity (Morita et al., 1984), antioxidant activity (Lin et al., 1996), hepatoprotective efficacy (Lin et al., 2002), and anti-inflammatory activity (Lin et al., 1996), among which the hepatoprotective efficacy, anti-inflammatory activity, and antioxidant activity are associated with free radical scavenging activity. Therefore, free radical scavenging activity is an important biological activity of burdock. The present work was aimed to study the possible curative effect of burdock against toxicity induced in rats by DENA.

2 Materials and Methods

Chemicals

N-Nitrosodiethylamine (DENA) was purchased from Sigma Aldrich (St. Louis, MO, USA) and Phenobarbital (Pb) was purchased from a local pharmacy. It is a product of ALEX PHARM, Egypt.

Burdock extraction

The root of burdock (Arctium lappa); has been brought from a local herbalist market and authenticated by a college of pharmacy; 6 Oct University, Cairo, Egypt. About 200 g of a burdock root was dried in a laboratory at room temperature and then powdered in a mixer grinder. To prepare a methanolic alcohol extract; 100 g of the burdock plant powder was put in 1000 ml of 80 % methyl alcohol and then placed for 48 hours in a shaker incubator. The residue was removed by filtration. The combined filtrate was evaporated till dryness at 40 -80 °C under reduced pressure in a rotary evaporator. The extract was suspended in -80°C till used via oral administration.

Experimental Animals

The present study was carried on 32 healthy adult male rats (Rattus norvegicus) of an average body weight about 160 – 200 g. They were purchased from Egyptian Vaccine and Antibody Company (VACSERA, Giza, Egypt) and divided in equal group number then housed in cages. Animals were randomly selected and housed in designed cages with hard wood chips. They were kept in laboratory under constant condition of 25°C, and 12h light / dark cycle for two weeks before experimentation. They were fed on a standard rodent pellet diet manufactured by the Egyptian Company for Oil and Soap. Also, animals were supplied with tap water during the period of experiment. The experiments were approved by the state authorities and it followed the Egyptian rules on animal protection, as well as specific local institutional laws for protection of animals under the supervision of authorized investigators.

Experimental design

Animals were divided into four groups; each group contains eight rats. Group (A): Normal-control that received drinking water for 45 days. Group (B): Toxic-control that injected intraperitoneally on 1st day with a single dose of diethylenitrosamine DENA (200 mg/kg of body weight) followed by administration of PB for 45 days through drinking water in a dose of (60 mg/kg body weight/48h) from the 2nd week (Roy and Gadad, 2016). Group (C): The rats were also injected intraperitoneally on 1st day with a single dose of diethylenitrosamine DENA (200 mg/kg of body weight) followed by administration of PB for 45 days through drinking water in a dose of (60 mg/kg body weight/48h) from the 2nd week; After 10 days of DENA injection; burdock extract (100 mg/kg body weight) was given orally through drinking water for 45 days. Group (D): The rats of this group were given orally the burdock extract (150 mg/kg body weight) through drinking water for 45 days.

Sampling and Serum collection

After 45 days from the beginning of experimentation, the rats belonging to all groups were sacrificed under mild ether anesthesia and both of total body weight and liver weight has been weighed. Blood samples were collected by retro-orbital puncture in sterilized, heparinized tubes, which were then centrifuged using microcentrifuge. The sera were separated and used for the evaluation of biochemical parameters. After sacrificing of rats, liver was dissected out, ruined off blood, washed with saline and stored in 10% formalin, for histopathology study. A portion was taken out of the tissue to prepare the homogenate for the assay of various parameters. Blood samples was put at room temperature to be clotted then centrifuged at 3000 rpm for 15 minutes. Sera were then, separated and stored at -20 °C into aliquots for individual biochemical investigations.

Macroscopic Investigations
The body weight of rats of all groups from the first day of the experimentation till before sacrificing the rats was recorded. Also, the liver weight was taken at the end of study of every rat from each group. The relative liver weights can be calculated from the following equation: Relative Liver Weight = Absolute liver weight (g) / Body weight of rat on sacrifice day (g) X 100 (Stanley et al., 2005).

3 Results
Liver Weights and Relative Liver Weights

The data recorded in table (1) showed that the values of the liver weights in (Mean ± SD); was extremely significant elevated (P ≤ 0.001) in rats injected with DENA in relation to normal liver weights in control rats (group B). The methanolic extract of burdock induced a significant increase (P ≤ 0.05) in liver weights in rats induced with DENA in relation to normal liver weights (group C). While, the recorded liver weights in the group (D) which treated only with a burdock extract; showed no-significant changes (P > 0.05) in relation to control values.

Data in table (1) also, showed that the values of the relative liver weights in (Mean ± SD); was extremely significant elevated (P ≤ 0.001) in rats injected with DENA and induced with HCC in relation to normal relative liver weights in control rats (group B). In other groups, the medicinal plant extract; burdock induced restoration of the normal relative liver weights of rats as found in a (group C). In the other hand, rats treated with burdock extract only (group D); leads to a little non-significant increase (P > 0.05) in their relative liver weights.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver Weights (gm)</th>
<th>Relative Liver Weights (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (A)</td>
<td>6.500±0.4441</td>
<td>2.854± 0.3534</td>
</tr>
<tr>
<td>Group (B)</td>
<td>10.14±1.836***</td>
<td>5.234±0.8502***</td>
</tr>
<tr>
<td>Group (C)</td>
<td>7.051±0.3323*</td>
<td>3.036±0.1991ns</td>
</tr>
<tr>
<td>Group (D)</td>
<td>6.850±0.3834**</td>
<td>2.898±0.2713**</td>
</tr>
</tbody>
</table>

All data are expressed as mean ± SD; (***), extremely significant (P ≤ 0.001). (**) very significant (P ≤ 0.01). (*) significant (P ≤ 0.05) and (ns) not-significant (P > 0.05).

Biochemical Results

The Alanine aminotransferase (ALT) values was extremely significant elevated (P ≤ 0.001) in rats injected with DENA in relation to normal values recorded in control group. The methanolic extract of burdock in groups (C) induced a significant increase (P ≤ 0.05) in ALT values in rats given DENA in relation to normal ALT values. While, the recorded ALT values in group (D); treated with a burdock only showed a non-significant change (P > 0.05) in relation to control (table 2).

Data in table (2) also showed that the Aspartate aminotransferase (AST) values was extremely significant elevated (P ≤ 0.001) in rats injected with...
DENAl in relation to normal values recorded in control group. The methanolic extract of burdock (group C) induced a significant increase (P ≤ 0.05) in AST values in rats induced with DENA and have HCC compared to normal values of AST. While, the recorded AST values in the other group (D); treated with a burdock only, showed a non-significant change (P > 0.05) in AST values in relation to control.

In relation to Alkaline Phosphatase (ALP) values, the recorded data in table (3), showed that the ALP values was very significant increased (P ≤ 0.01) in rats injected with DENA compared to normal values. On the other hand, the extract of burdock when treated in in DENA rats or treated alone in normal rats induced a non-significant change (P > 0.05) in the ALP values compared to normal values.

Finally; table (3) also showed the activity of the recorded data of Gamma Glutamyl Transferase (GGT) in different rat groups. The GGT values recorded an extremely significant elevation (P ≤ 0.001) compared to control values. On the other hand, the burdock extract induced a non-significant elevation (P > 0.05) in the GGT values in relation to control in DENA-treated rats (group C) and in other group (group D) in which normal rats treated with the burdock only.

Table 2. Effect of a burdock extract on the levels of (ALT) and (AST) in different rat groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (A)</td>
<td>22.35±1.052</td>
<td>43.55±1.366</td>
</tr>
<tr>
<td>Group (B)</td>
<td>87.28±2.922***</td>
<td>110.0±9.805***</td>
</tr>
<tr>
<td>Group (C)</td>
<td>24.24±1.736*</td>
<td>47.84±3.994*</td>
</tr>
<tr>
<td>Group (D)</td>
<td>22.98±2.002ns</td>
<td>44.66±1.583ns</td>
</tr>
</tbody>
</table>

Table 3. Effect of a burdock extract on the levels of (ALP) and (GGT) in different rat groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALP (U/L)</th>
<th>GGT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (A)</td>
<td>19.41±4.872</td>
<td>11.71±3.893</td>
</tr>
<tr>
<td>Group (B)</td>
<td>26.38±3.223***</td>
<td>25.49±4.945***</td>
</tr>
<tr>
<td>Group (C)</td>
<td>22.03±3.886***</td>
<td>14.89±3.445***</td>
</tr>
<tr>
<td>Group (D)</td>
<td>18.50±5.280ms</td>
<td>12.67±3.840ms</td>
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On the other hand, the table (4) showed that the total protein values were very significant decreased (P ≤ 0.01) in rats injected with DENA compared to normal values. On the other hand, the extract of burdock extract in group (C) and group (D); induced a non-significant change (P > 0.05) in the total protein levels in relation to control.

Also, the albumin (table 4) and globulin (table 5) levels was decreased in an extremely significant manner (P ≤ 0.001) in rats injected with DENA compared to normal values. The burdock extract modulated the levels of both albumin and globulin in groups (C and D) to record normal values as found in control.

Alpha-fetoprotein (AFP) levels recorded in table (5) explained that in rats injected with DENA, the AFP level was elevated in an extremely significant manner (P ≤ 0.001) compared to control (group B). Burdock extract decreased the level of AFP in (group c) but the recorded data was still elevated in an extremely significant manner (P ≤ 0.001). When, normal rats treated with burdock extract (group D), the recorded data showed a significant increase (P ≤ 0.05) in AFP level compared to control.

Table 4. Effect of a burdock extract on the levels of total proteins and albumin in different rat groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Proteins (g/dl)</th>
<th>Albumin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (A)</td>
<td>6.645±1.697</td>
<td>4.088±0.3201</td>
</tr>
<tr>
<td>Group (B)</td>
<td>4.328±0.4160**</td>
<td>2.960±0.2415***</td>
</tr>
<tr>
<td>Group (C)</td>
<td>6.309±1.227m*</td>
<td>3.796±0.5912m*</td>
</tr>
<tr>
<td>Group (D)</td>
<td>6.330±1.456m*</td>
<td>4.306±0.612m*</td>
</tr>
</tbody>
</table>

Table 5. Effect of a burdock extract on the levels of globulin and alpha-fetoprotein (AFP) in different rat groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Globulin (g/dl)</th>
<th>AFP (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (A)</td>
<td>2.558±0.7111</td>
<td>4.698±0.6609</td>
</tr>
<tr>
<td>Group (B)</td>
<td>1.368±0.2346**</td>
<td>60.33±3.019***</td>
</tr>
<tr>
<td>Group (C)</td>
<td>2.513±0.2240m*</td>
<td>24.70±0.5577***</td>
</tr>
<tr>
<td>Group (D)</td>
<td>2.024±0.3129m*</td>
<td>5.323±0.4424*</td>
</tr>
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Histopathological Results
Liver tissue section of control group rats; showed a normal histological structure of hepatic lobules and organization of hepatic cords with prominent central hepatic vein. Polygonal hepatic cells were joined to one another in anastomosing plates, with borders that face either the sinusoids or adjacent hepatocytes (Fig.1 A).
Figure 1. Liver, rat: A) Control group: Showing normal histological architecture including central vein (CV), blood sinusoids (BS), hepatic cells (H) and Kupffer cells (KC) B) Burdock treated group: Showing normal histological appearance of rat liver C&D) DENA treated group: Showing enlarged and congested central vein (C) and abundant leucocytic infiltration (D) (H&E, X 400).
Liver tissue section of rats intoxicated with a diethylnitrosamine (DENA) induced different histopathological alterations in the hepatic tissue; enlarged and congested central vein (Fig.1C), abundant leucocytic infiltration (Fig.1D); cytoplasmic vacuolations of hepatocytes and fatty infiltration (Fig.2 A & B) are the predominant alterations.

Liver tissue section of rats intoxicated with a diethylnitrosamine (DENA) and then treated with a burdock extract showing normal histological architecture (Fig.2C); thus the burdock extract induced a complete recovery of the hepatic tissue. Finally, liver tissue section of rats treated with a burdock extract only exhibited a normal structure without any histopathological alterations (Fig.1B).
4 Discussion

Experimental liver cancer in rodents induced by DENA, has been considered as one of the best characterized experimental models of HCC, allowing the screening of potential anticancer compounds on various phases (Chakraborty et al., 2007). DENA-induced liver tumors in rodents closely resemble a subclass of human HCC, which allows to extrapolate potential chemopreventive effects of an applicant agent in clinical setting (Lee et al., 2004).

In the present study, we have investigated the curative effect of a burdock extract on body and liver weights, different biochemical studies and histological structures of rat livers in DENA-induced toxicity. The results of our study clearly indicate a beneficial effect of dietary burdock extract on chemically-induced liver toxicity. The results of this study revealed that the weights of rats were decreased significantly in DENA group and restored their normal weights when treated with a burdock extract and this is agreed with (Roy and Gadad, 2016) who mentioned the same finding. The liver weight in DEN + PB group significantly increased when compared to normal, whereas that in burdock extract-treated group significantly decreased when compared to DEN + PB group. This finding was approved with (Bishayee et al., 2011). It was demonstrated that DENA group showed a significant elevation in serum AST, ALT, ALP and γ-GT activity versus to the negative control group. This finding was in agreement with (Shahat et al., 2015). The current data indicated that treatment of DENA group with burdock extract caused significant reduction in serum AST, ALT, ALP and γ-GT activity relative to the untreated DENA group. These results are in agreement with (Saleem et al., 2014).

It has been recognized that exposure of rats to certain carcinogens like NDEA causes an elevation of circulating AFP levels and this finding was in agreement with (Sell et al., 1983). The AFP is a biomarker indicating the cancerous state of liver. The AFP is an onco-fetal protein that is abundantly synthesized in the fetal and newborn rat liver and it is absent in adult animals. The elevation of AFP in cancer may be due to either increased transcription of AFP gene affecting AFP production. The DEN-treated group rats exhibited the presence of this biomarker. However, in burdock extract-treated group, the AFP level was found to have normalized, possibly due to the inhibition of transcription of AFP gene by burdock (Roy and Gadad, 2016).

The results of this work showed that the many histopathological alterations were observed in DENA-treated rats. These findings were in concomitance with (Abdallah and Khattab, 2004). Burdock contains polyphenols such as chlorogenic acid, caffeic acid, isochlorogenic acid and dicaffeoylquinic acids which has been reported to have effect on anti-free radicals (Chen et al., 2004). The free radical scavenging activities of burdock were attributed to the presence of caffeoyl quinic acid derivatives. However, the lignans from burdock exerted antiproliferative and apoptotic effects for leukemic cells (Al-Snafi, 2014). Finally, the medicinal plant burdock has a curative effect against liver toxicity induced by DENA and this due to its antioxidant activity.

Conclusion

The results obtained from the present study demonstrate the potential curative activity of burdock extract in DEN-induced toxicity in rats. The administration of DENA leads to different histopathological changes. Total proteins, albumin and globulin levels were decreased significantly when compared to control. While; the liver weights, relative liver weights, ALT, AST, ALP, GGT and AFP levels were elevated significantly compared to control values. Administration of a methanolic extract of burdock induced different marked curative effects in liver histology and different biochemical investigations.

5 References


Scientific Publications.


