Hypolipidemic and Antioxidant Activities of Anethum Graveolens Against Acetaminophen Induced Liver Damage in Rats

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Abstract: Twenty eight rats classified into four groups (7 rats each). The first group kept as control (-ve) fed standard diet only. The rest of rats were administered a single dose of 2 g/kg of paracetamol by stomach tube to induce liver injury then classified into control (+ve) and treated groups which were dill powder (10% of dill powder in the diet) and dill extract 300 mg/kg body weight by stomach tube)groups. The experiment period was 45 days. Nutritional results revealed that rat groups which treated with dill powder and dill extract showed a significant increase in weight gain and feed efficiency ratio (FER) comparing to control (+ve) rat group. Biochemical results showed a significant decrease in liver function enzymes and some lipid parameters in serum and also liver malondialdehyde, cholesterol and total lipids. On the other side, they showed increase in high density lipoprotein cholesterol (CHO) in serum and glycogen, triglyceride, glutathione peroxidase and superoxide dismutase in liver comparing to control (+ve) rat group. It is concluded that dill powder and extract can increase the activity of antioxidant and liver function enzymes in paracetamol induced liver injury.

Key words: Anethum graveolens · Antioxidant activity · Feed intake · Feed efficiency ratio (FER) · Liver function enzymes

INTRODUCTION

Liver is a vital organ has a wide range of functions in the body, including biotransformation and detoxification of endogenous and exogenous harmful substances, plasma protein synthesis and glycogen storage. Liver disease is associated with distortion of various metabolic functions. Extensive studies reported that natural products with antioxidant activity are effective to prevent the oxidative stress-related liver pathologies due to particular interactions and synergisms. Acetaminophen (paracetamol) is used as analgesic and antipyretic drug but excessive usage of acetaminophen can damage liver. Liver damage induced by the acetaminophen is a classical model for screening the hepatoprotective activity [1]. Dill belongs to the Umbelliferae family to which belong the herbs bay, cumin and parsley. Its name is derived from the Nordic word ‘dilla’ pointing to its soothing properties and its botanical name is Anethum graveolens. Although native to the Mediterranean region, dill is cultivated across Europe and America. The plant is 40 to 120 cm tall. The stem is erect, round, smooth, dark-green and white-striped. The stem is branched above, with a bluish bloom. The leaves are double and more pinnate, feathery, white-tipped leaflets with a deep groove on the upper surface. Leaves have been used as a basic component in canning, soups and sauces and also flavouring salads and seafood while its seeds are used in tea, breads, soups, salads and preserves [2]. One of the most important nutrition benefits of dill leaves is its positive effect on blood sugar levels. Dill contains eugenol, an essential oil which is known to lower blood sugar levels in diabetes [3]. Dill leaves contains monoterpene which helps to protect the body from the harmful effects of free radicals. Dill has an antispasmodic effect on the smooth muscles of the gastrointestinal tract [4, 5]. Moreover, some pharmacological effects of dill have been reported, such as antimicrobial, antihyperlipidaemic, anticancer and antioxidant [6-8].

The present study was designed to investigate the effects of powder and extract of dill on paracetamol induced liver injury in rats.
MATERIALS AND METHODS

Materials: Paracetamol drug was obtained from Kahira Pharm. & Chem. Ind. Co. Cairo, Egypt. Kits for biochemical analysis were purchased from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt. Dill was obtained from the local market of Cairo city, Egypt. Twenty eight rats were purchased from Helwan Farm of Laboratory Animals. The initial weight was 180±7g. The standard diet comprised of casein (200 g/kg), corn starch (497 g/kg), sucrose (100 g/kg), cellulose (30 g/kg), corn oil (50 g/kg), mineral mixture (100 g/kg), vitamins mixture (20 g/kg) and DL-methionine (3 g/kg) according to NRC [9].

Methods: Leaves and steams of dill were dried at 60°C, then crushed into powder and added as 10% to the diet as fibers. The hydro alcoholic extract of dried dill was prepared in the food and drug research laboratory of EMRI using following procedure. For the preparation of the hydro alcoholic extract, 100 gram of the dried grounded plant was suspended in 400 mL double distilled water- ethanol (2:1, v/v). The extract was filtered and the filtrate was evaporated to dryness with a rotatory vacuum evaporator. Dill extract was administered at dose levels of 300 mg/kg body weight of rats orally by stomach tube. After five days of adaptation period, the rats were randomly classified into four groups (7 rats each). The first group kept as control (-ve) fed standard diet only. The rest of rats were administered paracetamol drug at a single dose of 2 g/kg by stomach tube to induce liver injury [10]. The liver injured rats were classified into control (+ve) group and the other two treated groups which were dill powder and dill extract groups. Feeding and growth performance were carried out by determination of daily food intake, body weight gain and feed efficiency ratio (FER) according to method of Chapman et al. [11]. The rats were sacrificed at the end of the experiment (45 days) for collection of blood samples which centrifuged at 3000 rpm/15 minutes to obtain serum. The livers of rats were also collected for biochemical analysis and histopathological examination.

Serum aspartate and alanine amino transferase, alkaline phosphatase and gamma glutamyl transferase (AST, ALT, ALP& γ GT) enzymes activity were estimated according to Reitman and Frankel [12], Draper and Hadley [13] and Kind and King [14], respectively. Serum cholesterol (CHO), triglycerides (TG) and high density lipoprotein cholesterol (HDL-c) were determined by using enzymatic colorimetric methods according to Abell et al. [15], Buccolo and David [16] and Kostener [17], respectively. Low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) were calculated according to Fruchart [18]. Liver cholesterol (CHO), total lipids, triglyceride and glycogen were determined according to Richmond [19], Folch et al. [20], Scheletter and Nussel [21] and Rerup and Lundquist [22], respectively. Liver glutathione S-transferase (GST), superoxide dismutase (SOD), glutathione peroxidase (GPX) and malondialdehyde (MDA) were estimated according to Ellman [23], Beuchamp and Fridovich [24], Weiss et al. [25] and Uchiyama and Mihara [26], respectively. The fixed samples of liver in 10 % neutral buffered formalin were cleared in xylol and embeded in paraffin. 4-5 µM thick sections were prepared and stained with Hematoxylin and Eosin (H&E) for subsequent histopathological examination [27].

Statistical Analysis: Collected data were statistically analyzed using one way analysis of variance (ANOVA). Student "t" test was used for significance. Differences were considered significant at p< 0.05 according to Artimage and Berry [28].

RESULTS AND DISCUSSION

The results presented in Table 1 revealed that control (+ve) and rat group which treated with dill powder showed a significant decrease in weight gain, food intake and FER at P<0.01,0.001&0.05 while rat group which treated with dill extract showed a non significant decrease comparing to control (-ve) rat group. Rat groups which treated with dill powder and dill extract showed a significant increase in weight gain and FER comparing to control (+ve) rat group. These results are in agreement with those obtained by Singh et al. [6] and Stavri and Gibbons [29], who reported that dill contains substantial amounts of iron, manganese and calcium. It also contains flavonoids which are powerful antioxidants with antiviral and anti-inflammatory properties. Dill also provides zinc, iron, vitamin A and vitamin C which helps to maintain good health. Dill contains essential oils which stimulate the peristaltic movements of the digestive system as well as the secretion of digestive juices and also fiber which aids digestion. Hence, it improves the overall functioning of the digestive system and can be used as an appetizer.

Data in Table 2 showed that control (+ve) showed a highly significant increase in ALT, AST, ALP and γ GT at P<0.001 while rat groups which treated with dill powder and dill extract showed a significant increase in these liver
The levels of these enzyme levels have been decreased by treatment with dill powder and dill extract indicating its hepatic treatment action. This may be due to dill contains vitamins and minerals. It also contains flavonoids which have antioxidant, anti-inflammatory and antimicrobial properties [5, 8].

Data in Table 3 showed that control (+ve) showed a highly significant increase in CHO, LDLc, T.G and VLDLc at P<0.001 and a highly significant decrease in HDLc at P<0.001 comparing to control (-ve) rat group. Rat group which treated with dill powder showed a significant increase in HDLc at P<0.05 and non significant increase in other lipids parameters while rat group which treated with dill extract showed a significant increase in CHO and LDLc at P<0.05 &0.01 comparing to control (-ve) rat group. On the other side, rat groups which treated with dill powder and dill extract showed a significant decrease in CHO, LDLc, T.G and VLDLc and increase in HDLc comparing to control (+ve) rat group. In recent years, applications of dietary plants with antioxidative property have been the center of focus for improving the life quality of patients with hypercholesterolaemia.

Yazdanparast and Alavi [31] reported that oral administration of dill leaf extract for 14 days reduced the levels of total cholesterol, triglycerides and low density lipoprotein (LDL) by a significant 20-50%. So, dill has shown promising results towards having a heart-protective function in rats. Valiollah and Naser [32] reported that daily oral administration of Anethum graveolens essential oil to rats at doses of 45, 90 and 180 mg/kg for 2 weeks significantly and in a dose-dependent manner reduced total cholesterol, triglyceride and LDLc and increased significantly HDLc. Anethum graveolens powder when added to the diet of animals showed similar effects on serum lipids.

The obtained results in Table 4 showed that control (+ve) showed a highly significant increase in liver CHO and total lipids at P<0.001 and a highly significant decrease in liver glycogen and liver triglyceride at P<0.001 comparing to control (-ve) rat group. Rat groups which treated with dill powder and dill extract showed a significant decrease in glycogen at P<0.05 comparing to control (-ve) rat group. On the other hand, rat groups which treated with dill powder and dill extract showed a significant decrease in liver CHO and total lipids and significant increase in both liver glycogen and triglyceride comparing to control (+ve) rat group. The obtained result may be due to antioxidant effects of dill. Previous studies have shown that dill’s protective ability is comparable to that of alpha-tocopherol, ascorbic acid and quercetin. Protection from free radicals helps prevent serious health problems like cancer, liver and heart disease among...
Table 5: Liver GPX, SOD and MDA of the experimental rat groups.

<table>
<thead>
<tr>
<th>Variables (µ/mg protein)</th>
<th>Control (-ve)</th>
<th>Control (+ve)</th>
<th>Dill powder</th>
<th>Dill Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST</td>
<td>10.17±1.71a</td>
<td>6.31±0.86c***</td>
<td>8.96±1.03ab</td>
<td>10.27±1.32a</td>
</tr>
<tr>
<td>GPX</td>
<td>49.60±4.18a</td>
<td>28.11±2.60c***</td>
<td>39.96±3.98b*</td>
<td>41.14±4.17b*</td>
</tr>
<tr>
<td>SOD</td>
<td>39.36±4.55a</td>
<td>19.99±1.21d***</td>
<td>29.71±2.14c*</td>
<td>33.68±3.11ab</td>
</tr>
<tr>
<td>MDA</td>
<td>10.21±1.24c</td>
<td>25.14±5.71a***</td>
<td>15.11±2.17b*</td>
<td>13.67±1.14b*</td>
</tr>
</tbody>
</table>

Significant with control group P<0.05  ** P<0.01  *** P<0.001.

Mean values in each raw having similar letters were not significantly different.

Fruits and vegetables with bright colors contain properties that protect the body from free radicals. Dill is green in color and it can activate processes in the body which seek out and eliminate free radicals [6, 33].

The obtained results in table 5 showed that control (+ve) showed a highly significant decrease in liver GST, GPX and SOD at P<0.001 and a highly significant increase in liver MDA at P<0.001 comparing to control (-ve) rat group. Rat groups which treated with dill powder and dill extract showed a significant decrease in liver GPX and SOD at P<0.05 and a significant increase in liver MDA at P<0.05 comparing to control (-ve) rat group but showed the opposite results comparing to control (+ve) rat group. These results are in line with the findings of Jaeschke et al. [34], who reported that in acetaminophen-induced hepatotoxicity, the balance between ROS generation and antioxidant defense mechanism may be lost thereby results in oxidative stress, which leads to hepatic necrosis. Increased levels of MDA in liver treated with acetaminophen suggest enhanced lipid peroxidation leading to tissue damage and failure to prevent formation of excess free radicals. Bahramikia and Yazdanparast [35] reported that treatment with different fractions of Anethum graveolens extract significantly increased hepatic antioxidant system activities such as SOD, catalase and GSH, along with decreased lipid peroxidation in feeding high-fat diet treated rats. Different fractions of Anethum graveolens extract especially different fractions diethyl ether besides its hypolipidaemic property could protect the liver against the feeding high-fat diet -induced oxidative damage in rats. Yazdanparast and Alavi [31] showed that Anethum graveolens crude extract can either increase the biosynthesis of glutathione or reduce the extent of oxidative stress leading to less glutathione degradation, or it may have both effects. In addition, the increase in glutathione peroxidase activities might be responsible for lowered hepatic MDA content. The elevated levels of both SOD and catalase with Anethum graveolens extract could be due to the influence of flavonoids and polyphenols. Anethum graveolens crude extract possesses in vivo antioxidant activity through decreasing the availability of lipid substances and increasing the activity of antioxidant enzymes.

Microscopical examination of liver of rat from control negative group revealed the normal histological structure of hepatic lobule (Picture 1). Conversely, liver of rat from control (+ve) group showed vacuolar degeneration of hepatocytes and focal area of hepatic necrosis replaced by mononuclear leucocytic cells (Picture 2). However, liver of rat from dill powder group showed no changes except kupffer cells activation (Picture 3). No histopathological changes were noticed in liver of rat from dill extract group (Picture 4). Histopathological findings of liver samples were in agreement with the results obtained in biochemical studies, indicating that dill powder and extract are able to inhibit acetaminophen induced hepatotoxicity.
REFERENCES


