The ameliorative effects of Spirulina platensis against Diethylnitrosamine induced hepatotoxicity in rats
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ABSTRACT

The preventive effect of Spirulina platensis on hepatotoxicity induced by diethylnitrosamine (DEN) in albino rats was evaluated. Forty male albino rats were divided into four equal groups: group (1): normal control, group (2): hepatotoxic rats administrated DEN orally (20 mg/kg b.wt/day), group (3): rats receive spirulina orally and group (4): Hepatotoxic rats treated with spirulina at a dose of (800 mg / kg bw/day). All animals were sacrificed at the end of experiment (12 weeks). The hepatotoxic effect of DEN was evidenced by elevation of serum Aspartate Aminotransferase, Alanine Aminotransferase, Alkaline Phosphatase, γ-Glutamyl transferase activities, blood Urea concentration and liver Malondialdehyde level. However serum albumin concentration and antioxidants biomarkers in liver tissue were markedly decreased. Thus, the present study indicated that, Spirulina platensis successfully prevented the hepatotoxic effects of DEN via enhancement of antioxidant defense system, suppression of oxidative stress and inflammation against oxygen free radicals.

Key words: Diethylnitrosoamine, hepatotoxicity, Spirulina, antioxidants.

1. INTRODUCTION

Hepatotoxicity refers to liver dysfunction or liver damage that is associated with an overload of drugs or xenobiotics (Navarro and Senior, 2006). The chemicals that cause liver injury are called hepatotoxins or hepatotoxicants.

Hepatotoxicants are exogenous compounds of clinical relevance and may include overdoses of certain medicinal drugs, industrial chemicals, natural chemicals like microcystins, herbal remedies and dietary supplements (Willett et al., 2004; Papay et al., 2009). Certain drugs may cause liver injury when introduced even within the therapeutic ranges. Hepatotoxicity may result not only from direct toxicity of the primary compound but also from a reactive metabolite or from an immunologically-mediated response affecting hepatocytes, biliary epithelial cells and/or liver vasculature (Saukkonen et al., 2006; Deng et al., 2009). The hepatotoxic response elicited by a chemical agent depends on the concentration of the toxicant which may be either
parent compound or toxic metabolite, differential expression of enzymes and concentration gradient of cofactors in blood across the acinus (Kedderis, 1996).

Hepatotoxicity related symptoms may include jaundice or icterus appearance causing yellowing of the skin, eyes and mucous membranes due to high level of bilirubin in the extracellular fluid, pruritus, severe abdominal pain, nausea or vomiting, weakness, severe fatigue, continuous bleeding, skin rashes, generalized itching, swelling of the feet and/or legs, abnormal and rapid weight gain in a short period of time, dark urine and light colored stool (Bleibel et al., 2007; Chang and Schaino, 2007). Diethylnitrosamine (DEN), a hepatocarcinogen and hepatotoxin, is synthesized endogenously and found in workplace, processed meats, tobacco smoke, soybean, cheese and wide variety of foods also it is produced from metabolism of some drugs (Verna et al., 1996). It is reported that DEN cause oxidative stress during the metabolism that lead to cytotoxicity, mutagenicity and carcinogenicity (Papay et al., 2009; Saukkonen et al., 2006). DEN is biotransformed by mixed-function cytochrome P450 dependent monooxidase systems and its metabolic activation is responsible for the onset of the toxic effects (Deng et al., 2009).

Spirulina platensis, a filamentous blue-green (cyanobacteria) alga, attracted the interest of researchers. The biochemical components provide the marketing value to spirulina platensis, it is one of the most promising microalgae for culture due to its high nutritional values (Baylan et al., 2012). It is also well known as a source of protein (60-70 g/100 g) of high biological value, since it is a rich source of vitamins, mainly vitamin B12 and pro-vitamin A, minerals, especially iron and -linolenic acid, an essential fatty acid precursor for prostaglandins (Simpore et al., 2006; Habib et al., 2008). Spirulina is blue green algae of the Oscillateriaceae family which grows naturally in countries which have a warm climate and has been considered as supplement in human and animal food. They have been found to be a rich source of vitamins, minerals, essential fatty acids and antioxidant pigments such as carotenoids (Tsai et al., 2016).

Hence, in the present study we investigated the possible protective effects of Spirulina administration against induced liver injury in male rats by DEN.

2. MATERIAL AND METHOD

2.1. Experimental animals: Forty white male albino rats of 4 - 6 weeks old and weighing (100-120) g were used in this study. Rats were obtained from "The Laboratory Animals Research Center", Faculty of Veterinary Medicine, Benha University. Animals were housed in separate metal cages, exposed to good ventilation, humidity and to a 12-hr light/dark cycle. All rats were acclimatized for a period of 15 days prior to the beginning of the experiment. The rats were fed on constant supplies of standard pellet diet, fresh and clean drinking water were supplied ad-libitum.

2.2. Chemicals and Spirulina:

a) Diethylnitrosamine (DEN) was purchased from Sigma chemical Co. (USA). DEN was prepared freshly in normal saline (20 mg/kg b.wt/ day). Rats were received (1 ml/kg b.wt) 5 days per week till the end of the experimental periods (12 weeks), according to (El-Shahat et al., 2012).

b) Spirulina: Spirulina was purchased from National Research Centre, Giza, Egypt, treated orally with a dose of (800 mg / 1 kg b.wt/ day) (Chamorro-Cevallos et al., 2008).
2.3. Experimental design and sample collection:
Rats were randomly divided into four groups each of them comprises of (10) rats:
Group (1): (Normal Control), rats received no drugs treatment during the experimental periods (12 weeks).
Group (2): (DEN-induced hepatotoxic group), rats were received DiethylNitrosamine (DEN) in normal saline at a dose level of (20 mg/kg b.wt/ day) orally. Rats were received (1 ml/kg b.wt) 5 days per week till the end of the experimental periods (12 weeks).
Group (3): (Spirulina treated), rats were administrated with spirulina orally at a dose of (800 mg /kg b.wt/ day). Rats were received (1 ml/kg b.wt) 5 days per week till the end of the experimental periods (12 weeks).
Group (4): (DEN+ Spirulina treated), rats were administrated with DiethylNitrosamine (DEN) in normal saline (20 mg/kg b.wt/ day) till the end of induction period of hepatotoxicity (8 weeks) then treated with spirulina orally at a dose of (800 mg / kg bw/day). Rats were received (1 ml/kg b.wt) of spirulina 5 days per week till the end of the treatment period (4 weeks).

2.4. Sampling:
2.4.1. Blood samples:
Twenty-four hours fasting after the last dose of the drugs treatment, rats were anaesthetized under diethyl ether anesthesia. Blood samples were collected by ocular vein puncture in dry, clean tubes and allowed to clot for 30 minutes and serum was separated by centrifugation at 3000 r.p.m for 15 minute. The serum was taken by automatic pipettes and collected in dry sterile tubes, then kept in deep freeze at -20 °C until use for assay of the liver biomarker. All sera were analyzed for determination of the following parameters: Urea, AST, ALT, albumin, GGT and Alkaline Phosphatase.

2.4.2. Liver tissue for biochemical analysis:
Briefly, liver tissues were cut, weighed and minced into small pieces, about 0.5 g of liver tissues were collected from all animals groups (control and experimental groups), put in Eppendorf tubes and were immediately kept at -20°C till for subsequent biochemical analyses.

2.4.3. Preparation of liver homogenates:
1. Prior to dissection, perfuse liver tissues with a PBS (phosphate buffered saline) solution, pH 7.4 containing 0.16 mg/ml heparin to remove any red blood cells and clots.
2. Liver tissues were divided into appropriate portions, and 0.5 gm from each were homogenized in 5 ml -10 % (w/v)- cold phosphate buffer saline (PBS) (i.e., 50 mM potassium phosphate, PH 7.5, 0.1mM EDTA) per gram tissue, using tissue homogenizer.
3. Centrifuged at 10,000 r.p.m for 20 minutes at 4°C, the resulting supernatant was assayed for: Oxidative stress and antioxidant biomarkers: L-MDA, reduced glutathione, Superoxide dismutase and Catalase.

2.5. Biochemical analysis
Serum aspartate aminotransferase (AST), Serum alanine aminotransferase (ALT), Serum albumin, Serum γ–GT, Serum Alkaline Phosphatase and Serum Urea levels were determined according to the methods described by (Murray, 1984), (Doumas et al., 1997), (Beleta and Gella, 1990), (Rosalki et al., 1993) and (Palton and Crouch, 1977) respectively.
Moreover, liver L-MDA, reduced glutathione, superoxide dismutase, Catalase levels were determined in liver tissue homogenate according to the method described by (Esterbauer et al., 1982), (Beutler et al., 1963), (Minami & Yoshikawa, 1979) and (Sinha, 1972).
3. Statistical analysis
Experimental data obtained were analyzed according to (Snedecor and Cochran, 1969) using the computer software program (SPSS, 2001).

4. RESULTS
The obtained results demonstrated in (table 1) revealed that, DEN hepatotoxicity in rats induced a significant increase in serum liver marker enzymes, GGT, Alkaline Phosphatase, Urea and liver L-MDA as compared to normal control group. Oral administration of spirulina caused a significant reduction in all of those parameters when compared with DEN exposed group with the ability to restore the enzymatic activities to the normal level of control group. On the other hand, Hepatotoxicity induced a significant decrease in serum albumin level, GSH, SOD and Catalase in liver tissue as compared with normal control group. Spirulina administration restored the DEN induced biochemical alterations in serum liver function tests and promoted oxidative stress and antioxidant defense in the liver tissue.

Table 1: Effect of Spirulina administration on Serum biochemical parameters in blood and liver tissue of DEN induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
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</thead>
<tbody>
<tr>
<td>AST (U/l)</td>
<td>50.83 ± 2.48b</td>
<td>101.00 ± 2.93a</td>
<td>60.50 ± 5.54ab</td>
<td>42.16 ± 0.91b</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>56.16 ± 1.75b</td>
<td>376.00 ± 14.36a</td>
<td>60.00 ± 4.54b</td>
<td>124.16 ± 11.33ab</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>21.58 ± 1.12b</td>
<td>65.68 ± 2.15a</td>
<td>27.08 ± 1.62ab</td>
<td>23.95 ± 0.86b</td>
</tr>
<tr>
<td>Albumin (gm/dl)</td>
<td>4.50 ± 0.33b</td>
<td>2.25 ± 0.60a</td>
<td>5.66 ± 0.18b</td>
<td>5.30 ± 0.23b</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/l)</td>
<td>644.83 ± 30.90b</td>
<td>1148.50 ± 40.10a</td>
<td>603.16 ± 71.89b</td>
<td>943.50 ± 40.51ab</td>
</tr>
<tr>
<td>γ–GT (U/l)</td>
<td>182.98 ± 4.50b</td>
<td>437.93 ± 9.45a</td>
<td>175.50 ± 5.28b</td>
<td>180.66 ± 4.15b</td>
</tr>
<tr>
<td>Catalase (U)</td>
<td>97.58 ± 1.51b</td>
<td>44.48 ± 1.84a</td>
<td>112.50 ± 5.58b</td>
<td>71.23 ± 13.08ab</td>
</tr>
<tr>
<td>L-Malondialdehyde (nmol)</td>
<td>12.46 ± 0.63b</td>
<td>67.95 ± 2.78a</td>
<td>16.65 ± 0.54b</td>
<td>20.26 ± 0.86ab</td>
</tr>
<tr>
<td>Superoxide Dismutase (U)</td>
<td>5.70 ± 0.21b</td>
<td>1.48 ± 0.15a</td>
<td>5.82 ± 0.27b</td>
<td>3.12 ± 0.07ab</td>
</tr>
<tr>
<td>Glutathione (mmol)</td>
<td>214.45 ± 4.06b</td>
<td>81.73 ± 2.50a</td>
<td>212.93 ± 4.37b</td>
<td>156.21 ± 6.07ab</td>
</tr>
</tbody>
</table>

Data are presented as Mean ± S.E.
S.E. : Standard Error
a, b, c: Mean values with different superscript letters in the same row are significantly different at (P ≤0.05).
G1: Normal control, G2: Hepatotoxic rats administrated DEN orally, G3: rats receive spirulina orally, G4: Hepatotoxic rats treated with spirulina orally

4. DISCUSSION
The present study evaluated the effects of spirulina treatment on hepatotoxicity resulting from DEN administration (Deng et al., 2009). Diethylnitrosamine (DEN) is known to induce damage in many enzymes involved in DNA repair and is normally used to induce liver cancer in experimental animal models (Bhosale et al., 2002). It is known to all that the activity of ALT,
AST, ALP and GGT are representative of liver function and their increased activity is sensitive indicators of hepatic injury (Singh et al., 2011). Generally, liver damage induced by DEN is related to the disruption of liver cell metabolism and membrane instability and subsequently causes distinctive changes in the activities of serum enzyme. The elevation of ALT activity is credited to hepatocellular damage and is usually accompanied by a rise in AST (Al-Rejaie et al., 2009). ALP as one of the liver function enzymes is closely connected with lipid membrane in the canalicular zone. Increased level in ALP reflects pathological alteration in biliary flow. Therefore, any interference with the bile flow, whether extra-hepatic or intra-hepatic leads to increased serum level of ALP activity (Nair et al., 1998). Upon liver injury, liver marker enzymes (AST, ALT, and ALP) enter into the circulatory system because of the altered permeability of the membrane (Sivaramakrishnan et al., 2008). GGT is a membrane-bound enzyme, mainly in the canalicular domain. GGT exhibits a tissue specific expression and modified under various physiologic and pathologic conditions, such as development and carcinogenesis (Yao et al., 2004). The significant elevation of GGT in rat sera may be attributed to the liberation of this enzyme from the plasma membrane into the circulation indicating damage of cell membrane as a result of carcinogenesis (Bulle et al., 1990).

Meanwhile, treatment with spirulina to DEN induced hepatotoxic rats caused a significant decrease in serum ALT, AST and ALP activities and increase in Albumin level when compared with DEN induced hepatotoxicity group. These results accordance with, (Bashandy et al., 2011), they have been reported that, spirulina possess strong antioxidant activity and provokes free radical scavenging enzyme system.

The current data run parallel to the study of (Ismail et al., 2010) who demonstrated the hepatoprotective of rat liver toxicity by Spirulina platensis. In addition, (Amin et al., 2006) reported the hepatoprotective effect of Spirulina against cadmium-induced hepatotoxicity. Moreover, (Kumar et al., 2005) stated that, consumption of Spirulina rich diets reversed DEN-hepatocellular carcinoma in rats.

Furthermore, Vandenberghe, (1996) reported that, hypoalbuminemia may result from liver disorders, which are accompanied by a reduction in albumin synthesis. Albumin is a key component of serum proteins. It is synthesized in the liver and is one factor used to monitor liver function (Moshage et al., 1987). Also, Studies showed that liver toxicity decreases serum albumin levels (Adams et al., 2005). The results of the present study are in agreement with this finding and demonstrate the decreased functional ability of CCl4-injected rat livers (Saravanan et al., 2006).

Diethylnitrosamine administration causes an increase in blood urea level. It has been suggested to cause the generation of ROS resulting in oxidative stress and cellular injury (Marzie et al., 2015). These reactive oxygen species can cause oxidative damage in DNA, proteins and lipids (Vitaglione et al., 2004). The elevation of serum urea level in the experimental groups was in accordance with the results obtained by (Ayla et al., 2011); Abou Seif, 2012) who showed that, DEN caused a marked rise in serum urea, creatinine, sodium and potassium levels. In another renal injury study, spirulina at dose of 1000 mg/kg elicited significant reno protective activity by decreasing urea level.
The current data revealed a significant increase of liver L-MDA level with a concomitant reduction of GSH level and activities of the antioxidant enzymes, SOD and CAT, in DEN intoxicated rats when compared with normal control rats at both experimental periods. Treatment of DEN administered rats with either spirulina produced marked reduction of the elevated L-MDA accompanied with improved antioxidant system.

GSH plays a crucial role in the detoxification process of majority of alkylating agents including DEN (Chan et al., 1986). It neutralizes the electrophilic site by providing a –SH group and renders the metabolite more water soluble (Conway et al., 1987). In agreement with the previous reports (Pradeep et al., 2007; Sehrawat and Sultana, 2006), the GSH level was found to decrease after DEN administration. The decrease may be due to its utilization in inactivating the free radicals generated during DEN metabolism.

We assumed that, the hepatoprotective effects of the tested algal extracts were due to their antioxidant properties and their ability to reduce lipid peroxidation. In this regard, (Evanprince et al., 2009) reported the hepatoprotective potential of spirulina in acetaminophen-induced hepatotoxicity in mice and attributed this finding to the antioxidant effects of Spirulina and to its ability to potentiate the antioxidant system. In addition, Spirulina supplementation has reduced lipid peroxidation and increased activity of the antioxidant system in the liver of cadmium-intoxicated rats as reported by (Amin et al., 2006).

Administration of spirulina increased the activities of hepatic SOD and CAT and GSH content when compared to rats treated with DEN. Moreover, it inhibits hepatic MDA production induced DEN. This could be attributed to the antioxidant properties of spirulina that have attracted the attention of many researchers due to its active ingredients, notably phycocyanin, β-carotene, tocopherol, selenium, and phenolic compounds that have operative antioxidant and anti-inflammatory activities. The antioxidant property of phycocyanin, a major water-soluble antioxidant constituent in Spirulina, is more efficient than vitamin C by 20 times (Ismail et al., 2015). The active principals of spirulina can act synergistically leading to intensive antioxidant effect.

5. CONCLUSION

Spirulina treatment ameliorates liver injuries, which induced by DEN by decreasing the values of biochemical parameters as ALT, AST, GGT, Alkaline Phosphatase, Urea and L-MDA and increasing the activities of antioxidant parameters as (CAT, GSH and SOD) and serum albumin restoring their alterations to near normal levels. From the obtained results, it could be concluded that, the hepatoprotective potential of spirulina as powerful natural agents and may be useful as an antioxidants in hepatotoxicity.

6. REFERENCES


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