Efficacy of Activated Charcoal in the Reduction of Hepatotoxic Effects of Verrucarin J in Mice

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ABSTRACT
To evaluate the effectiveness of activated charcoal in alleviating Verrucarin J, two treated groups of 10 male albino mice were treated with a sublethal dose (0.9mg/kg body weight orally) for two weeks in combination with or without activated charcoal. Highly significant increase in plasma level of lipid peroxidation (LPO), liver disease is often clinically assessed using serum enzyme activities such as glutamate oxaloacetate transaminase (AST), glutamate pyruvate transaminase (ALT), γ-glutamyl transferase (γ-GT), alkaline phosphatase (ALP), the level of these enzymes were increased significantly in serum of treated group with Verrucarin J and significant decrease of plasma level of antioxidants enzymes and total thiols. In tissue homogenate of liver, level of thiobarbituric acid (TBARs) were significantly increases while activities of superoxide dismutase (SOD) and catalase (CAT) and hepatic glutathione were significantly decreased. Addition of charcoal (1g/kg) to Verrucarin J contaminated diets appeared the adverse effect of toxin. The result indicates that charcoal may be used as antioxidant and antidote for Verrucarin J in male albino mice.

Keywords: Verrucarin J, charcoal, antioxidant, antidote

INTRODUCTION
The Trichotheccenes are a group of toxic secondary metabolites produced by several genera of Fungi such as Myrothecium, Fusarium, Trichotheccium and Stachybotrys [1] and Characterized by the tetracyclic 12, 13-epoxytrichotheccenes. They have been isolated primarily from in adequately stored agricultural product [2] and have been identified [3]. These species of fungi are well known as pathogenic microbes to plants which produced cereal grains and these cause alimentary toxicoses in man and animals [4] [5]. According to LD₅₀ of the trichotheccenes, Ueno [6] and Kravechenko et al [7] reported that T-2 toxin, Verrucarin J and Ocharatoxin A enhanced lipid peroxidation in liver. One of the most important trichotheccene is Verrucarin J, produced by several genera of fungi that may contaminate foods of both human and animal. Edrington and his workers [8] used activated charcoal to prevent toxicosis and death in rat given T-2 toxin.

The objective of this study is to describe biochemical changes of the liver as the result of administrating male mice from Verrucarin J and to evaluate activated charcoal as antidote to elucidate the probable changes in enzyme activities and lipid peroxides in serum and antioxidants enzyme activities in tissue homogenates of liver.
MATERIAL AND METHODS
Activated charcoal was obtained from BDH (England). Other chemicals were obtained from sigma (st.Louis, M.O.). Verrucarin J. (purity> 99% by HPLC, sigma company), was obtained from Prof. Bruce B. Jarvis (1996), department of Chemistry and Biochemistry, University of Maryland, College Park, Maryland, USA. The study was performed on 30 normal healthy mature male albino mice (30-35g) and purchased from the animal house of King Abdulaziz University - Saudi Arabia (2009). The animals were fed standard diets for about 2 weeks before administration of the toxin at room temperature for natural photobic periods. The mice were divided into three groups of 10 male albino mice, one group served as control (C), the second group (T1) was given Verrucarin J contaminated diets (0.9 mg/kg) according to LD50[9] for 2 weeks dissolved in 1% DMSO in sterile saline [10][11] using gastric tube and the addition of charcoal (1g/kg) to Verrucarin J contaminated diets was given to the third group. The control group was given 1% DMSO in sterile saline only. After 2 weeks, the mice were slaughtered under aseptic conditions. Blood sample from all groups were collected from the heart into heparinized tubes. Blood sample were centrifuged at 500 g for 10 min. for separation of plasma. Liver were excised immediately after mice were sacrificed, and homogenized in ice-cold 100 mM phosphate buffer solution comprising 10 mM Tris HC, 5mM EDTA, 10mM KCl, and 250 mM source (pH 7.5), using a potter-Elvehjem homogenizer fitted with a Teflon plunger. Homogenates were centrifuged at 11,000Xg for 20 min. and supernatants were stored at – 80°C. Lipid peroxidation was estimated in liver homogenate as thiobarbituric acid reactivity (TBARs) described by Thayer [12]. Superoxide dismutase (SOD) activity was determined according to its ability to autoxidation of epinephrine at alkaline medium by the method of Misra and Fridovich [13]. Catalase activity in tissue homogenate was determined according to the techniques described by Luck [14]. Also the supernatant of liver homogenate was also used for assay of reduced glutathione (GSH) and oxidized glutathione (GSSG) levels by enzymatic method of Griffith [15]. Glutamate pyruvate transaminase (ALT) and glutamate oxalocate transaminase (AST) were determined by method of Wilkinson et al [16]. The activity of alkaline phosphates activity (ALP) was estimated by the method of El- Aser and El-Merzabani[17] and γ glutamyl transeptidase (γGT) activity was assayed by the method of Tate and Meister [18] . Lipid peroxidase (LPO) levels were measured in plasma by Thyer [12] and also plasma total thiol were determined chemically as described by Ellman [19] were measured in all treated and control groups.

RESULTS
Rats which received Verrucarin J alone (T1) showed significance increase in liver enzymes ALP, γGT, AST and ALT respectively. By examining the effects of Verrucarine J on lipid peroxidation, we found that was elevation in its level and the total thiol was reduced by 50% by Verrucarine J (Table I). The TBARs showed significance elevated above normal values and significant decrease in SOD and CAT. Verrucarine J intoxication alone significantly produce a decrease in hepatic GSH level (27.3 to 17.4 m mol/ g protein), but it is significantly increased the GSSG level (3.4 to 6.42 m mol /g protein), resulting in the impairment in GSH redox status, as evidenced by the decline in GSH/ GSSG ratio compared with the control (Table II), decreasing GSH level and increasing the GSSH level. Mice receiving charcoal and Verrucarine J simultaneously (T2), showed decrease in the level of serum enzymes, and lipid peroxidation when compared to Verrucarine J intoxicated groups. The results in (Table II) reveal that the charcoal treatment elevate the level of SOD and Catalase to normal level and enhancement of hepatic GSH redox status in Verrucarine J intoxicated rats by increasing GSH level and decreasing the GSSH level and TBARs compared with Verrucarine J group only (T1), Table II.

DISCUSSION
After ingestion and absorption, poisons cycle between blood and bowel, forming an entrovascular circulation [20]. Some poisons undergo an enterohepatic circulation. Multiple doses of activated charcoal, even if given several hours after poison ingestion, have the potential to interrupt these circulations and increase the rate of poison elimination. The present study of Verrucarine J in male mice has received our attention for its effect on the liver function, renal function and lipid peroxidation parameters.
In the present study the level of some non-functional serum enzymes were measured, including alkaline phosphatase, γ-GT, and transaminases. Serum alkaline phosphatase is a membrane marker enzyme that is elevated after administration of Verrucarine J in mice. Alkaline phosphatase levels increase markedly in bone diseases, hepatocellular diseases, and in diseases that impair bile formation (cholestasis). Gamma-glutamyl transferase (γ-GT) is a membrane-bound enzyme with its active site oriented toward the outer surface of the cell. The results in Table I reveal that the level of γ-GT was in increased in treated group. The level of γ-GT is elevated in hepatobiliary and pancreatic diseases, but is normal in bone diseases. From the previous data for alkaline phosphatase and γ-GT, we can conclude that administration of Verrucarine J to mice may lead to hepatobiliary disease. The above results are in agreement with Helman et al [21], who reported that the trichothecenes have toxic effects in dogs including increased levels of serum alkaline phosphatase, AST and γ-GT. The level of transaminases (AST and ALT) was increased after administration of Verrucarine J to mice. Level of serum transaminases is low in normal subjects. Liver tissue is rich in both transaminases and after extensive liver tissue destruction; these enzymes are liberated into serum. Rodwell [22] reported that the presence of nonfunctional plasma enzymes at level elevated above normal values suggests an increased rate of tissue destruction. This implies that the mycotoxin Verrucarine J is highly cytotoxic compound, and it has a harmful effect on liver tissues. By examining the effect of Verrucarine J on lipid peroxidation, the data in Table I indicate that the plasma levels of lipid peroxides was significantly elevated in the Verrucarine J induced rats compared to control however, the plasma level of total thiols activity was significantly decreased. This suggests that Verrucarine J toxin leads to an increase of lipid peroxidation indicator parameters and this toxin alters free radicals metabolism in plasma and tissue [11]. This was also previously shown in trichoverrins (A&B) toxin as trichothecene [23]. In the present study, the level of thiobarbituric acid reactive substances (TBARs) which are marker for lipid peroxidation [24] has increased after administration of Verrucarine J toxin in mice. Segal et al [25] reported that the trichothecene T-2 toxin has a direct lysing effect on erythrocyte membranes mediated the generation of free radicals. Free radicals induced hemolysis is generally attributed to oxygen-containing free radicals which peroxidize the polyunsaturated fatty acids in the erythrocyte membrane [26]. The previous data indicated that Verrucarine J toxin may act as prooxidant (activates the peroxide oxidation). Also, the glutathione in liver is used as indicator for the oxidative toxicants in the organs and GSH is thus an important defense mechanism against certain toxic compounds such as some drugs and carcinogens [27]. The decreasing level of SOD and CAT activates in treated I could be due to the liver’s role in detoxification of Verrucarine J toxin and may reflect a body defense against oxidative toxicants.

For decades, activated charcoal has been used as a universal antidote for the majority of poisons because of its ability to prevent the absorption of most toxic agents from gastrointestinal tract such as pesticides, bacterial toxins and mycotoxins [28], and enhance the elimination of some agents already absorbed and is frequently recommended for the emergency management of ingested poisons [29]. Although, the metabolism of Verrucarine J is not known until now, In view of the longer terminal half-life of Verrucarin J (approximately four weeks) [11], we conclude that Verrucarin J may be converted into another form which is less cytotoxic metabolite by metabolism or due to the ability of body to adapt Verrucarin J effect via its detoxification in the liver or its excretion by kidney. Peng and his workers [30] reported that the charcoal is an effective method for the prevention of poisoning in rats from mycotoxin and in preventing death in acute T-2 toxicosis [31], the schedule for the charcoal administration in the present study was designed to maximize its effect on the bioavailability of Verrucarin J. The charcoal administration was timed to cover the period of maximum gastrointestinal turnover of Verrucarin J [11]. When activated charcoal administered during two weeks, an absolute bioavailability was obtained. This decrease in exposure can be explained by the adsorption of the Verrucarin J dose undergoing gastrointestinal recycling to the activated charcoal, thus preventing subsequent re-absorption. Since its antidotal efficacy is dependent upon when and for how long it is given after the dose of toxin, it is recommended that activated charcoal is administered as early as possible. Our results illustrate that charcoal may be considered as an option for the management of Verrucarin J toxin. Thus, concomitant administration of (1g/kg) activated charcoal leads to the almost total prevention of hepatotoxic effects of Verrucarin J in mice. It is concluded that Verrucarine J toxin affects liver tissue and activated charcoal is a potent antidote for Verrucarine J.
Table I

Effect of Verricarin J (0.9mg/kg body weight) without or with (1g/kg body weight) charcoal on some liver function parameters in male albino mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C</th>
<th>T₁</th>
<th>T₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alk (μl / l)</td>
<td>69.2±0.23</td>
<td>109±0.19</td>
<td>69.9±0.04</td>
</tr>
<tr>
<td>γGT (GGT) (IU / ml)</td>
<td>11.23±0.07</td>
<td>19.9±0.09</td>
<td>10.9±0.19</td>
</tr>
<tr>
<td>Alt (GPT) (IU/l)</td>
<td>30.6±0.22</td>
<td>51.8±0.14</td>
<td>30.4±0.08</td>
</tr>
<tr>
<td>Ast (GOT) (IU / l)</td>
<td>45.2±0.04</td>
<td>90.09±0.22</td>
<td>42.5±0.45</td>
</tr>
<tr>
<td>Lpo (η mol / ml)</td>
<td>1.11±0.01</td>
<td>2.5±0.003</td>
<td>1.9±0.14</td>
</tr>
<tr>
<td>Total thiols (η mol / mg protein)</td>
<td>0.96±0.003</td>
<td>0.44±0.006</td>
<td>0.88±0.009</td>
</tr>
</tbody>
</table>

Values are expressed as mean of 10 animals ± S.E.

C = control group without any toxin, T₁ = verricarin J, T₂ = verricarin J with charcoal.

a Means significantly different (P< 0.05) from control according to one way ANOVA with LSD test.
b Means significantly different (P< 0.05) from T₁ according to one way ANOVA with LSD test.

Table II

Effect of Verricarin J (0.9mg/kg body weight) without or with (1g/kg body weight) charcoal on liver tissue homogenate in male albino mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C</th>
<th>T₁</th>
<th>T₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARs mmol/ml</td>
<td>0.43±0.009</td>
<td>2.32±0.009</td>
<td>0.46±0.007</td>
</tr>
<tr>
<td>SOD mg/ml</td>
<td>2.45±0.95</td>
<td>0.7±0.004</td>
<td>1.19±0.004</td>
</tr>
<tr>
<td>Catalase units/mg protein</td>
<td>0.92±0.004</td>
<td>0.34±0.001</td>
<td>0.74±0.009</td>
</tr>
<tr>
<td>GSH mmol/g protein</td>
<td>27.3±0.05</td>
<td>17.4±0.0095</td>
<td>23.4±0.013</td>
</tr>
<tr>
<td>GSSH nmol/g protein</td>
<td>3.4±0.06</td>
<td>6.42±0.006</td>
<td>4.1±0.035</td>
</tr>
</tbody>
</table>

Values are expressed as mean of 10 animals ± S.E.

C = control group without any toxin, T₁ = Verricarin J, T₂ = Verricarin J with charcoal.
a Means significantly different (P< 0.05) from control according to one way ANOVA with LSD test.
b Means significantly different (P< 0.05) from T₁ according to one way ANOVA with LSD test.

REFERENCES