The effect of fucoidan on the changes of some biochemical parameters and protein electrophoresis in hepatotoxicity induced by carbon tetrachloride in rats

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ABSTRACT
Fucoidan is a sulfate polysaccharide extracted from brown algae. Fucoidan has various pharmacological properties, such as anti-tumor, anti-mutagenic, anti-inflammatory, antiviral, antioxidant, anti-fibrogenic activity and anti-complementary activities. Objective: This study was aimed to explore the effect of fucoidan on biochemical parameters (ALT, AST, GGT, total protein, albumin, globulin) and protein fractions in hepatotoxicity induced by CCl₄ in rats. Materials and Methods: The rats used in the study were randomly divided into four groups of 8 rats each: Control group, fucoidan group, fucoidan+CCl₄ group and CCl₄ group. After 24 hours from the process of an eight-day experiment, blood samples were taken. The analysis of ALT, AST, GGT activities and total protein, albumin, globulin levels were done by an autoanalyser and serum protein fractions (albumin, α₁-globulin, α₂-globulin β-globulin, γ-globulin and A/G ratio) were electrophoretically determined. Results: In the group of fucoidan+CCl₄, it was determined that the levels of AST (p<0.001), GGT (p<0.001), total protein (p<0.01), globulin (p<0.01), β-globulin % (p<0.01), γ-globulin % (p<0.001) statistically decreased compared to CCl₄ group. Conclusion: It can be said that fucoidan has the property of hepatoprotectant by looking to some biochemical parameters and changes in protein fractions that examined in hepatotoxicity induced by CCl₄.

Key words: CCl₄, Enzymes, Fucoidan, Hepatotoxicity, Protein electrophoresis.


INTRODUCTION
Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects (Kala et al., 2013). Many traditional remedies employ herbal drugs for the treatment of liver ailments (Mohan et al., 2005; Ayaz et al., 2017). Brown seaweed has been used as foodstuff in the Asian diet for centuries. Fucoidan is a sulfated polysaccharide from the cell wall of brown seaweed containing a substantial percentage of L-fucose and sulfate ester groups (Li et al., 2008). Sulphated polysaccharides extracted from marine algae represent a source of marine compounds with potential applications in medicine. Fucoidan has various pharmacological properties, such as anti-tumor, anti-mutagenic, anti-inflammatory, antiviral, antioxidant, anti-fibrogenic activity and anti-complementary activities (Rocha de Souza et al., 2007; Veena et al., 2007; Zhang et al., 2003; Wang et al., 2011; Hayashi et al., 2008; Hong et al., 2012; Feldman et al., 1999).

Fucoidan has also been reported to reduce liver fibrogenesis by protecting from hepatic cell death and inducing apoptosis of hepatic stellate cell, as well as showing anti-oxidative properties against carbon tetrachloride induced acute liver injury (Hayashi et al., 2008). It has recently been reported that hepatoprotective effects of fucoidan against acetaminophen-induced liver injury via anti-oxidant, anti-inflammatory, and anti-apoptotic mechanisms (Hong et al., 2012).

A number of drugs or chemicals have been used to prevent CCl₄-induced liver injury (Patel, 2010; Shi et al., 2014; Cao et al., 2014; Kober et al., 2015). To date there have been few studies of the protective effect of fucoidan on CCl₄-induced hepatotoxicity. In this study, we investigated the effects of fucoidan on the changes of some biochemical...
parameters and protein electrophoresis in hepatotoxicity induced by carbon tetrachloride in rats.

MATERIALS AND METHODS

Female Wistar Albino rats (aged 7-8 weeks) weighing 200-250 g were housed in a temperature-controlled (2±2°C) room in which a 12 h light: dark cycle was maintained. The animals were fed with standard diet and water ad libitum. All rats were acclimated to laboratory conditions for 7 days prior to the experiment. All experiments were performed in accordance with protocols approved by the Yuzuncu Yil University Animal Researches Local Ethic Committee (Turkey) (decision number: 2014/06).

Experimental procedure: Thirty two rats were randomly divided into four groups with 8 rats in each group: Control group, fucoidan group (fucoidan is sourced from Fucus vesiculosus (Sigma: F5631) was dissolved in saline solution and 100 mg/kg/day was given by intragastric gavage for 8 days), fucoidan+CCl4 group (fucoidan, 100 mg/kg/day was given by intragastric gavage for 8 days and injected i.p. single dose 1 ml/kg CCl4 on the eighth day), CCl4 group (injected i.p. with single dose 1 ml/kg CCl4 on the eighth day).

Biochemical analysis: Blood sample were taken and sera were obtained after 24 hours from the process of eight-days experiment (9th day). Biochemical parameters (ALT, AST, GGT activities and total protein, albumin, globulin) were analyzed with an automatic analyzer (Roche Modular P800), serum protein fractions (albumin, α1 globulin, α2 globulin β, globulin, γ globulin and A/G ratio) were measured by cellulose acetate electrophoresis (Helena Lab).

Statistical analysis: All data were expressed in the means ± S.D. Kruskal-Wallis Test was used to check differences among the groups. Dunnett’s test were used to determine different groups. p < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS v.13.0 software.

RESULTS AND DISCUSSION

The results of biochemical analyses were presented in Table 1 and 2. The highest ALT, AST, GGT activities and levels of total protein, globulin were found in the group of CCl4 (Table 1). In the group of fucoidan+CCl4 it was determined that the activity of AST (p<0.001), GGT (p<0.001) and total amounts of serum total proteins (p<0.01), globulin (p<0.01) statistically decreased compared to CCl4 group in Table 1. The levels of β globulin (%), γ globulin (%) were determined to be the maximum in the group of CCl4 (Table 2). There were statistical significance between other groups, respectively p<0.01, p<0.001. No significant differences were observed between groups in the levels of albumin (%), α1 globulin (%), α2 globulin (%) in protein electrophoresis. The lowest and the highest A/G ratios were estimated in CCl4 and fucoidan groups, respectively (p<0.01).

Carbon tetrachloride has been extensively studied as a liver toxicant and its metabolites such as trichloromethyl radical (CCl3) and trichloromethyl peroxyl radical (CCl3O•) were reported to be involved in the pathogenesis of liver damage (Kuriakose and Kurup, 2008). The free radicals after binding to lipoprotein lead to lipids peroxidation of endoplasmic reticulum (Boll et al., 2001). The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been disturbed by a hepatotoxin, is the index of its protective effects (Prakash et al., 2008; El Shahat et al., 2017).

Liver damage is detected by serum enzymes activities like ALT, AST and GGT, which has been released into the blood from damaged cell. They are also sensitive indicators of liver injury (Molander et al., 1955; Ozer et al., 2008; Ayaz et al., 2017). Injury to the hepatocytes alters their transport function and membrane permeability, leading to leakage of enzymes from the cells (Zimmerman and Seeff, 1970). Therefore, the marked release of ALT and AST into the circulation indicates severe damage to hepatic tissue membranes during CCl4 intoxication (Yang et al. 2008). The present study also revealed that the given dose of CCl4 (1 ml/kg, ip) produced significant elevation in ALT, AST and GGT indicating all impaired liver function. The obtained results are in accordance with those of the previous reports (Hayashi et al., 2008; Yang et al., 2008; Kang et al., 2008; Muriel, 1998). ALT, AST and GGT activities were elevated in the CCl4 group but all were decreased during the treatment with fucoidan. It might be able to protect liver from CCl4-induced injury, reducing enzyme activities

<table>
<thead>
<tr>
<th>Table 1. Changes in biochemical parameters</th>
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<tr>
<td><strong>Control</strong></td>
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</tr>
<tr>
<td>ALT (U/L)</td>
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<tr>
<td>AST (U/L)</td>
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<tr>
<td>GGT (U/L)</td>
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<tr>
<td>Total Protein (g/dl)</td>
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<tr>
<td>Albumin (g/dl)</td>
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<tr>
<td>Globulin (g/dl)</td>
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a, b, c: Different letters in the same row are statistically significant (*p<0.05, **p<0.01, ***p<0.001)
in blood. Similar results were also observed by Hayashi et al. (2008) and Kang et al. (2008).

All serum proteins except immunoglobulin are biosynthesized in liver cells. Severe liver damage induces the decrease in protein catabolism and the elongation of protein’s half time in order to supplement the decrease in the protein syntheses in the animal body. No decrease in serum total protein after single and multiple administrations of CCl₄ was observed (Okazaki, 1985). Shallan et al. (2008) said that total protein was decreased in serum because protein biosynthesis of CCl₄-intoxicated rats were inhibited (Rao et al., 2006). It is considered that severe liver damage induces the decrease in protein catabolism and the elongation of protein’s half time in order to supplement the decrease in the protein syntheses in the animal body (Okazaki et al., 1985). It is reported that hypergammaglobulinaemia is common in patients with liver disease (Kurahori et al., 1979; Hirayama et al., 1970). It is widely believed that many antigens are phagocytosed, and their antigenicity is lost by the Kupffer cells. However, in damaged liver, many antigens have a low chance of being phagocytosed by Kupffer cells because these cells must phagocyte many degraded cells. Therefore, foreign materials have a high chance of remaining in contact with antibody forming cells in blood, and a high gamma globulin content in serum is observed (Okazaki et al., 1985). In this study an elevation of total amounts of serum proteins after CCl₄ treatments was observed in Table 1. Because it could be a high beta and gamma globulin content in serum after administration of CCl₄, Okazaki et al. (1985) reported that after a single CCl₄ administration, the results of serum protein fractionation by electrophoresis showed a decrease in albumin, an elevation of beta and gamma-globulins and a decrease in A/G ratio.

Murali et al. (2012), examined the protective effect of Hemidesmus indicus pubescens leaves on the hepatotoxicity induced by orally given CCl₄. The mean total protein and albumin levels of CCl₄ group were low compared to the control group. Shallan et al. (2008), formed hepatotoxicity by CCl₄, they found that, the level of total protein significantly reduced in CCl₄ intoxication group. Similarly, the levels of globulin, albumin and A/G ratio were again lower in CCl₄ group compared with the controls.

In this study, in terms of serum albumin levels there weren’t any statistical importance between the groups (p>0.05) despite important reduction in albumin levels in experimental hepatotoxicity induced by CCl₄ were found (Shallan et al., 2008; Murali et al., 2012), in this study we didn’t observed important changes between the groups. Indeed the prolongation of the half life of serum albumin in acute hepatotoxicity and therefore may not detecting a change in its level. However, in this study serum globulin levels were identified as the highest in CCl₄ group. Compared to the other three groups with CCl₄ group, statistical significance was found (p<0.01). Because, hypergammaglobulinaemia is widely seen in patients with liver diseases (Kurahori et al., 1979; Hirayama et al., 1970), the level of serum globulin in CCl₄ group were higher. When given fucoidan, globulin levels were detected similar to the control group.

There was not any study on the effect of fucoidan in protein fractions obtained by electrophoresis, and also there was limited study on the protein fractions in CCl₄ induced liver injury (Okazaki et al., 1985). In addition to the enzymatic changes of liver disease, total protein and its fractions are important biochemical parameters during the course of the disease. In this study, important changes caused by the hepatotoxic effects of CCl₄ were observed and electrophoretic protein fractions patterns were affected. Especially, high level of gammaglobulin in CCl₄ group reveals the high liver injuries. It seems that the phagocytosis of Kupffer cells depends on the amounts of endogenous components and exogenous materials (Okazaki et al., 1985). Gamma-globulin (%) decrease in the fucoidan+CCl₄ group can be considered as an indication of the protection of rat liver. In addition, high level of A/G in the fucoidan + CCl₄ group is connected to the reduction in the globulin levels, we can understand that fucoidan has protective and the healing effects on liver injuries. It can be said that giving fucoidan before CCl₄ toxicity decreases the hepatic damage of CCl₄.

In conclusion, this study has a scientific importance due to the limited number of studies on liver damage by treating the seaweed extract. It can be said that giving fucoidan before CCl₄ damage occurs decreases the hepatic injury of CCl₄ and fucoidan has the property of liver protective by looking to some biochemical parameters (ALT, AST, GGT, total

Table 2. Changes in serum protein fractions

<table>
<thead>
<tr>
<th></th>
<th>Kontrol</th>
<th>Fucoidan</th>
<th>Fucoidan+CCl₄</th>
<th>CCl₄</th>
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<tbody>
<tr>
<td>Albumin (%)</td>
<td>3.13 ± 0.57</td>
<td>3.48 ± 0.26</td>
<td>3.16 ± 0.35</td>
<td>3.14 ± 0.53</td>
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<tr>
<td>α₁ globulin (%)</td>
<td>1.03 ± 0.21</td>
<td>0.96 ± 0.13</td>
<td>0.99 ± 0.21</td>
<td>1.05 ± 0.11</td>
</tr>
<tr>
<td>α₂ globulin (%)</td>
<td>0.69 ± 0.16</td>
<td>0.51 ± 0.12</td>
<td>0.65 ± 0.17</td>
<td>0.73 ± 0.15</td>
</tr>
<tr>
<td>β globulin (%)</td>
<td>1.02 ± 0.06b</td>
<td>0.94 ± 0.20b</td>
<td>1.06 ± 0.20b</td>
<td>1.41 ± 0.33b</td>
</tr>
<tr>
<td>γ globulin (%)</td>
<td>0.26 ± 0.08b</td>
<td>0.13 ± 0.07b</td>
<td>0.13 ± 0.04b</td>
<td>0.39 ± 0.23b</td>
</tr>
<tr>
<td>A/G</td>
<td>1.07 ± 0.29b</td>
<td>1.40 ± 0.26b</td>
<td>1.16 ± 0.30ab</td>
<td>0.89 ± 0.21b</td>
</tr>
</tbody>
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a, b: Different letters in the same row are statistically significant (**p<0.01, ***p<0.001)
protein) and changes in protein fractions that examined in hepatotoxicity induced by CCl\textsubscript{4}. However, there is a need for new studies to better understand therapeutic mechanism of fucoidan, obtained from brown algae which widely used in the Far East and also better to evaluate the effect of liver functions during the process of experimental treatment.

REFERENCES


