The immunomodulating and antioxidant activity of fucoidan on the splenic tissue of rats treated with cyclosporine A

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KEYWORDS
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Abstract  Cyclosporine A (CsA), a potent immunosuppressive agent, has several adverse effects on different organs including the spleen. Fucoidan is a sulfated polysaccharide extracted from marine algae and it has numerous biological activities. The present study aimed to investigate the adverse effects of CsA on the spleen of rats and to evaluate the role of fucoidan, as immune-modulator and antioxidant substance, in the treatment of the CsA adverse effects. CsA administration to rats caused some histological degenerated changes in lymphocytes, plasma cells and neutrophils as well as it caused a reduction in CD3+ T cell expression in the spleen and in IL-2, IL-6, IFN-γ, IgM and IgG levels in the plasma. The histological degeneration was ameliorated by fucoidan treatment in variable degrees. CD3+ T cell expression and the plasma levels of IL-2, IL-6 and IFN-γ were increased by fucoidan treatment. IgM had more inhibition by fucoidan but IgG was improved. Lipid peroxidation was increased by CsA and fucoidan failed to reverse this change. Nitric oxide was increased by the high dose of CsA and was decreased after treatment with fucoidan. Glutathione was increased by fucoidan treatment. Superoxide dismutase activity was inhibited by CsA and low dose of fucoidan improved this inhibition. In conclusion CsA exerted adverse effects on the
spleen tissue as shown by histological and biochemical changes. Fucoidan has immune-modulatory and antioxidant effects against CsA-induced adverse effects on the spleen tissue.

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Introduction

Cyclosporine A (CsA), a lipophilic drug, is a metabolic cyclic polypeptide of the fungus Tolypocladium inflatum and is widely used in allograft rejection and in the treatment of autoimmune diseases (Borel et al., 1976; Kapturczak et al., 2004). It has potent immunosuppressive properties through blockage of some cytokine gene transcription in activated T cells. CsA inhibits the phosphatase activity of calcineurin, thereby preventing the nuclear factor of activated T cell (NFAT) activation (Halloran, 1996; Allison, 2000; Gregory, 2000). NFAT activation and nuclear translocation are required for the transcription of many cytokines particularly interleukin-2 (IL-2), which is necessary for the proliferation and maturation of T cells. Moreover, CsA enhances the expression of transforming growth factor-β (TGF-β) (Salom et al., 1993; Halloran, 1996; Gregory, 2000) and blocks the activation of C-Jun N terminal kinases and p38 signaling pathways, that makes it a highly specific inhibitor of T cell activation (Allison, 2000). Despite the therapeutic benefits of CsA as immunosuppressant, it has adverse toxicity effects (De Mattos et al., 2000; Yoon and Yang, 2009) which are mediated by increases of the reactive oxygen species and a decrease of the activities of antioxidant enzymes (SOD, CAT and GPx) (Al-Malki, 2011). In addition, lymphoma and squamous cell carcinoma have been noted in CsA treated animal (Blackwood et al., 2004; Callan et al., 2005).

CsA binds to cyclophilin to form an active complex which inhibits calcineurin phosphatase enzyme and increase cytosol Ca²⁺. The higher cytosolic Ca²⁺ level in activated lymphocytes causes an increase in their susceptibility to oxidative stress-induced cell death (Degasperi et al., 2008). In addition, CsA has a direct oxidative effect on human B cells and these effects are blocked by the antioxidants (Chen et al., 2001). Thymus in CsA treated animal showed a very marked depletion of the mediullary zones as well as the histiocytes containing cellular debris appeared. However, the most prominent feature in the spleen of CsA treated animal was the loss of lymphocytes within the periarteriolar sheaths and marginal zones that resulted in marked reduction in these zones (Blair et al., 1982; Masri et al., 1988), whereas the reduction in cellular density of the periarteriolar sheaths was higher than the reduction in the marginal zones (Armas et al., 1989).

Fucoidan, a sulfated polysaccharide of brown algae, is potent anticoagulant, antitumor, antiviral and immunomodulator (Maruyama et al., 2003; Chabut et al., 2004; Lee et al., 2004). One mechanism by which the fucoidan exerts its immunomodulatory effect is provided by the abundant supply of fucose (http://www.agelsingapore.info, 2012). Fucoidan protects bone marrow cells from radiation (Byon et al., 2008), inhibits selectin-mediated adhesion of hematopoietic progenitor cells (Frenette and Weiss, 2000). Also, fucoidan modulates the function of various immune cells such as neutrophils (Zen et al., 2002), lymphocytes (Omizu et al., 2006), natural killer (NK) cells (Maruyama et al., 2006), and dendritic cells (DCs) (Kim and Joo, 2008). The antitumor activity of fucoidan is related to the activation of macrophage-mediated tumor cell killing as well as fucoidan interferes cancer cell metastasis by inhibiting the physical interaction between the tumor cell and basement membrane (Jiao et al., 2011). Moreover, fucoidan enhances the phagocytic activities of macrophages with significant increases in the production of NO, H₂O₂, TNF-α, and IL-6 (Choi et al., 2005). Also, fucoidan has a great potential in preventing free radical synthesis that mediated diseases and it can prevent the increase of lipid peroxide in the serum, liver and spleen of diabetic mice (Li et al., 2002). In light of the above, the present study was intended to investigate the adverse toxicity of CsA on the spleen of male albino rats and to evaluate the role of fucoidan as immunomodulator and antioxidant in the treatment of CsA adverse toxicities.

Material and methods

Experimental protocol

Animals

Forty adult male albino rats weighing 200–250 g were used in this study. Rats were purchased from the Animal House of the Faculty of Medicine, Assuit University. Rats were housed in cages and were kept in a room temperature (30 ± 5 °C) with normal 12 h light/12 h dark cycle. They had ad libitum access to pelleted diet and water throughout the study. The experiment was carried-out in accordance with the national regulations on animal welfare and Institutional Animal Ethical Committee.

Chemicals

Cyclosporine, Neoral® Oral Solution (cyclosporine oral solution, USP) MODIFIED. It is present as a clear, yellow liquid supplied in 50 ml bottles containing 100 mg/ml (NDC 0078-0274-22). It is distributed by Novartis Pharmaceuticals Corporation, East Hanover, New Jersey 07936. Fucoidan from Fucus vesiculosus, was purchased from Sigma Chemicals, St. Louis, MO, USA. Primary antibody for CD3+ and the secondary kit for the immunohistochemistry technique were obtained from Abcam Company (Abcam PLC, 204 and 330 Cambridge Science Park, UK). ELISA kits for quantitative measurements of Rat IL-2, IL-6, INF-γ, IgG and IgM levels in plasma in sandwich ELISA format were obtained from Koma Company (Koma Biotech INC., 1487, Gayang-don, Gансeo-gu, Seoul, South Korea). All others chemicals and reagents were of the highest purity commercially available.

Experimental protocol

The rats were randomly divided into five groups, eight rats each:

(1) Group I (Control) was treated with olive oil (2 ml/kg b.w.) and saline (2 ml/kg b.w.) for 20 days.

(2) Group II was treated orally with CsA (25 mg/kg b.w.), dissolved in olive oil, daily for 20 days according to Josephine et al. (2009).
(3) Group III was treated orally with CsA (25 mg/kg b.w.), dissolved in olive oil, concomitant with subcutaneous treatment with fucoidan (5 mg/kg b.w.), daily for 20 days according to Veena et al. (2006).

(4) Group IV was treated orally with CsA (50 mg/kg b.w.) according to Al-Malki and Moselhy (2010) for 10 days and left for recovery for another 10 days.

(5) Group V was treated orally with CsA (50 mg/kg b.w.) for 10 days then treated subcutaneously with fucoidan (10 mg/kg) according to Rhee and Lee (2011) daily for another 10 days.

Collecting of sample
After the experiment period (20 days) rats of different groups were anesthetized by ether and scarified by cervical dislocation. Blood was collected into a sterilized tube containing EDTA for ELISA study. For biochemical studies, spleen tissues were quickly removed, washed in (0.1 M) phosphate buffer (pH 7.4) and then stored at −20 °C. Pieces of spleen were quickly removed and fixed immediately in 10% neutral buffered formalin for histological and CD3 T cell immunohistochemical studies and some pieces were fixed in 4% cold glutaraldehyde for semithin and ultra structure studies.

Semithin and transmission electron microscopy
Spleen specimens fixed in glutaraldehyde (5%) were trained and approximately 1×1×1 mm blocks were prepared. These blocks were washed in cacodylate buffer (0.1 M, pH 7.2) for 1–3 h and then post fixed in 1% osmium tetroxide for 2 h. After repeated washing in cacodylate buffer and dehydration in ascending grades of ethyl alcohol up to 100%, the specimens were first placed in propylene oxide for 60 min, then in pure epon 812 and incubated in a special polymerization incubator (first day at 35 °C, second day at 45 °C and third day at 60 °C). The blocks were trimmed with LKB ultratom. Semithin sections were stained with toluidine blue (T.B.) for 2 min at 60°C and then stored at 4°C. The sections were photographed with light microscope. Representative fields of semithin sections were selected. Ultrathin sections (70 nm) were cut with a diamond knife using a Reichert OMVs ultramicrotome. They were mounted in copper grids and stained with uranyl acetate lead citrate stain. The ultrastructural investigation was carried out with Transmission electron microscope (TEM) (Joel CX II) according to Bancroft and Stevens (1982).

Immunohistochemical studies
For the immunohistochemical study, primary antibody for CD3+ and secondary kit for the immunohistochemistry technique were obtained from Abcam Company. Paraffin sections of 5 μm thickness were prepared and the staining was performed using labeled Streptavidin–Biotin immunoperoxidase technique according to the manufacturer’s instructions.

Spleenic tissue homogenate and plasma preparation
10% w/v homogenate of each spleen tissue was prepared by homogenized 500 mg of the spleen in 5 ml (0.1 M) phosphate buffer (pH 7.4) using homogenizer (IKA Yellow line DI 18 Disperser, Germany) and kept frozen at −20 °C for the subsequent biochemical assay. Blood samples were centrifuged at 4000 rpm for 10 min to separate the plasma and were kept frozen at −20 °C.

Cytokine and Immunoglobulin assay
The IL-2, IL-6, INF-γ, IgM and IgG levels in plasma were quantity measured by the ELISA method according to the manufacturer’s instructions. In brief, sandwich ELISAs utilize multiple-well microtiter plates, coated with capture antibodies, to capture soluble proteins. The bound proteins are then detected with a subsequent detection antibody, which is typically labeled with an enzyme or biotinylated, then followed with streptavidin-enzyme conjugate. A colorimetric substrate is then added, which results in a color change based on the amount of antigen captured. By using a plate reader and plotting resulting values on a standard curve, precise, quantitative values can be obtained.

Oxidative stress biomarker determination
Lipid peroxidation products as TBARS content were determined according to the method of Ohkawa et al. (1979). NO content was measured as nitrate concentration colorimetrically using the method of Ding et al. (1988). Glutathione (GSH) content was determined using the method of Beutler et al. (1963). Vitamin C content was measured as described by Jagota and Dani (1982). The activity of superoxide dismutase (SOD) was determined basing on its ability to inhibit the autoxidation of epinephrine at alkaline medium according to the method of Misra and Fridovich (1972). The activity of catalase (CAT) was determined basing on its ability to decompose H2O2 to H2O and O2 according to Beers and Sizer (1952). Glucose 6 phosphate dehydrogenase (G6PD) activity was determined basing on its ability to reduce NADP according to the method of Tsai and Chen (1998). Protein content in the spleen tissues was determined by the method of Lowry et al. (1951).

Statistical analysis
The results were analyzed by one way analysis of variance (ANOVA) followed by Newman–Keuls Multiple Comparison Test as post-Test by using Prism program for windows, version 3.0 (Graph pad software, Inc., San Diago CA, USA). The significance difference between groups was accepted at P < 0.05, <0.001 and <0.001.

Results
Semithin section observation
Pathological examination of a semithin section of the spleen in the control group showed a normal histological structure in the form of healthy lymphocytes, reticulum cells and splenic arteriole (Fig. 1A). In group II, many degenerated lymphocytes with pyknotic nuclei were noticed (Fig. 1B). In group III, a few degenerated lymphocytes and reticulum cells were seen (Fig. 1C). In group IV, the numbers of degenerated lymphocytes were numerous (Fig. 1D). In group V, the lymphocytes, reticulum cells and mast cells were more or less normal as well as a few of apoptotic and necrotic cells were observed (Fig. 1E).

Transmission electron microscope observation
With transmission electron microscopy the spleen in group I showed normal healthy looking lymphocytes, plasma cells...
and dendritic cells (Fig. 2A). In group II degenerated plasma cells were seen, some cells appeared as ghost and others showed a marked dilatation of RER (Fig. 2B). Also, some degenerated neutrophiles with rarified cytoplasm were seen and an apoptotic change was observed in some cells in which the nuclear chromatin became electron dense and took the halfmoon shaped nucleus (Fig. 2C). In group III mature lymphocytes with chromatin tended to be clumped around the periphery, as well as a healthy plasma cell with normal RER was noticed (Fig. 2D). In group IV, many degenerated splenocytes with multiple cytoplasmic vacuoles were seen. Apoptotic cells with condensed and shrinking nuclei were also observed (Fig. 2E). In group V, some mature and normal lymphocytes and a plasma cell were seen as well as some lymphocytes had electron dense nuclear chromatin (Fig. 2F).

**Immunohistochemical observation**

Immunohistochemical examination of CD3$^+$ T cells in the spleen of the different groups clarified that, CD3$^+$ T cells took
cytoplasmic blackish discoloration which appeared in high number in the periarterial sheath of the white pulp of group I (Fig. 3A). In group II, there were minimal number of CD3<sup>+</sup> T cells in the periarterial sheath (Fig. 3B), while in group III there are moderate number of CD3<sup>+</sup> T cells in the periarterial sheath (Fig. 3C). In group IV, the number of CD3<sup>+</sup> T cells was decreased (Fig. 3D), while in group V there is more density of CD3<sup>+</sup> T cells in the periarterial sheath of the white pulp (Fig. 3E).

**Fig. 2** Transmission electron photomicrograph of the spleen from rats in: (A) group I showing many healthy looking splenocyte: lymphocytes (asterisk), plasma cell (P) and part of dendritic cell (arrow) with cell surface processes elongated between splenocytes (headarrow). (B) Group II showing: two degenerated plasma cells, the left one appears as ghost (P1), the right one appears with marked dilatation of RER (P2). (C) Group II showing: degenerated neutrophil (arrow) with rarified cytoplasm. Apoptotic change is observed in one cell in the form of halfmoon shaped nucleus (arrowhead), (D) group III showing mature lymphocytes with chromatin tended to be clumped around the periphery (asterisk), small healthy plasma cell (P) with normal RER (arrowhead) and erythrocytes (R). Condensed nucleus is observed (arrow). (E) Group IV showing degenerated splenocyte (arrow) with multiple cytoplasmic vacuoles, some of them are large (v). Some apoptotic cells (arrowhead) with condensed and shrinking nuclei are observed, (F) group V showing mature lymphocyte (asterisk) and plasma cell (P). Lymphocyte with electron dense nuclear chromatin is observed (arrow). Urinyle citrate lead acetate stain.

**Cytokines and immunoglobulins**

The plasma level of IL-2 was significantly decreased ($P < 0.05$) in group II and III compared to the control group and significantly increased ($P < 0.05$) in rats of group III compared to the rats of group II (Fig. 4). Also, fig. 4 shows that, IL-6 was significantly decreased ($P < 0.05$) in rats of groups II and IV compared to the control group. However, it significantly increased ($P < 0.05$) in rats of group III. INF-$\gamma$ was
significantly decreased ($P > 0.05$) in all treated groups compared to the control group, however, it significantly increased ($P < 0.05$) in rats of group V compared to the rats of group IV (Fig. 4). Fig. 5 shows the levels of IgG and IgM in plasma of control and the different treated groups. It clearly shows that both IgG and IgM decreased in all CsA treated groups compared to the control group and fucoidan treatment botched to restore these decreases.

**Oxidative stress makers**

Table 1 shows the oxidative stress biomarkers in the spleen tissues of control and different treated groups. All the treated groups showed a significant increase in LPO compared to the untreated control group. Nitric oxide showed a non-significant change in rats of groups II and III compared to the control group. However, it was significantly increased ($P < 0.01$) in rats of group IV compared to the control group and significantly decreased ($P < 0.001$) in rats of group V compared to rats of group IV. GSH level was increased only in rats of groups II and III when it was compared to that of the control group. Vit C content and the activities of CAT and G6PDH did not show any significant changes between the treated groups and control and between the treated groups. However, SOD activity was significantly decreased ($P < 0.001$) in rats of group II compared to the control and increased ($P < 0.05$) in rats of group III and V compared to the group II.
In the present work, semithin and transmission electron microscopic studies of the spleen in rats treated with CsA revealed degeneration of lymphocytes, plasma cells and neutrophils. The observed degenerative changes in the neutrophils might reflect the suppressive effect that was exerted by an indirect effect of CsA through its effect on T cells. Several authors supported this observation and reported that, calcineurin might be involved in the inhibitory effect of CsA on neutrophils through the impairment of gene up-regulation which is regulated by nuclear factor of activated T cells (Greenblatt et al., 2010). In addition, CsA binds to all human leucocytes and might bind granulocytes and cells of the mononuclear phagocyte system more than lymphocytes (Garcia et al., 2000).

CsA is accumulated in cell membranes because it is a highly lipophilic drug (Kapturczak et al., 2004). Moreover, CsA induces ER stress and cell damage in tubular cells which can contribute to dedifferentiation and cell death in the kidney (Pallet et al., 2008a,b) through induction pro-apoptotic proteins (Han et al., 2008). Endoplasmic reticulum (ER) stress, which is induced by the accumulation of unfolded proteins in the ER under physiological and pathological conditions, triggers unfolded protein response to reestablish normal ER function. When adaptation fails and ER stress is prolonged, the cell triggers suicide programs (Dickhout and Krepinsky, 2009). So, the previous studies can explain the degenerated changes that occurred in RER of plasma cell in rats treated with CsA in the present study. On the other hand, the observed dilatations in RER of plasma cells may be explained by the immunoglobulin (Ig) accumulation inside RER and by an increase in their immunological activity (Sohal and Weidman, 1978). However, dilatation of the RER in several cell types indicates some defects in the progress of secretory material (Shiner, 1973).

In the present study, the degenerated changes, induced by CsA, in lymphocytes and other splenocytes were observed more than the apoptotic changes. In this aspect, maintenance
of mitochondrial integrity is critical in apoptosis inhibition. In addition, cytochrome-c is generally confined to mitochondria, however its release into the cytosol upon rupture of the mitochondrial membrane results in a cascade of caspase activation (Srinivasula et al., 2000). Doonan and Cotter (2004) confirmed the critical roles of CsA in preventing apoptosis by blocking the release of cytochrome-c. However, apoptosis in hippocampal neurons after CsA treatment was suggested as a result of calcium overload (Kaminska et al., 2001). Furthermore, higher cytosolic Ca²⁺ level in activated lymphocytes increases their susceptibility to oxidative stress which induced cell death in a mechanism involving the participation of mitochondrial permeability transition (Degasperi et al., 2008).

In the present study, fucoidan treatment ameliorated the degenerated changes in splenocytes induced by CsA. It is well known that, fucoidan enhances various immune responses in the spleen lymphocytes and peripheral macrophages and increased T and B lymphocytes during endotoxemia (Choi et al., 2005; Ko and Joo, 2011). Moreover, fucoidan encourages cell regeneration (Irhimeh et al., 2007) and apoptosis, and removes degenerated and abnormal cells (Kim et al., 2010).

The expression of CD3+ after CsA treatment was reduced in the present study. However, this reduction was ameliorated after fucoidan treatment. This decrease in CD3+ T cell by CsA is compatible with previous studies carried out by Fang et al. (2006) and Song et al. (2010) who found that, CsA significantly decreased the% of CD3+T cells in the spleen with a concomitant increase of CD19+B cell. Also, when Con A, lymphocyte proliferation inducer, was administered to rat injected with CsA, the spleen tissue showed a marked reduction of T cell proliferation (Varalakshmi et al., 2008). Moreover, T lymphocyte number in the dermis was decreased significantly by CsA treatment (Baker et al., 1989). Choi et al., 2005 found the cytotoxic effect of mouse spleen lymphocytes on tumor cells after culture with fucoidan. Moreover, fucoidan is highly effective in mobilizing stem cells into the peripheral circulation in baboons and mice (Irhimeh et al., 2007). The mechanism of stem cell mobilization is related with the inhibition of selectin binding and with the ability of fucoidan binding to stromal-derived factor-1 (SDF-1). So, fucoidan can increase the number of stem cells, blood cells and SDF-1 level in plasma (Sweeney et al., 2000; Ferreras et al., 2008).

CsA inhibits T-cell activation largely by blocked IL-2 production that is seen in the present study. Fitzpatrick et al. (1994) found the inhibitory effect of CsA on the secretion of IL-2 and other growth-promoting lymphokines prevent the activation and proliferation of lymphocytes and other immune cells. Also, CsA inhibited IL-2 responsiveness through inhibited IL-2R (Donnan and Cotter, 2004). However, IL-2 and IL-4 in combination were able to reverse the immunosuppressive activity of CsA (Fitzpatrick et al., 1994). Moreover, the inhibition of CsA calcineurin-NFAT pathway leads to toxic changes and immunosuppressive effects (Krejcia et al., 2010). Also, CsA preventive events occurring during the G1 phase of growth are necessary for non-motile lymphocytes to acquire locomotor capacity (Wilkinson and Higgins, 1987).

In the present study, IFN-γ level was significantly deceased in all CsA treated rats with or without fucoidan compared to control, whereas it was increased in rats treated with fucoidan in recovery period after CsA treatment. It has been found that, CsA significantly inhibited IFN-γ level which was enhanced in Con A stimulated spleen cells (Song et al., 2010). Also, the immunosuppressive effect of CsA on Mycobacterium bovis BCG-infected mice was expressed by inhibiting the development of effector T cells, which then caused a reduction in the production of IFN-γ (Takashima and Collins, 1987). Fucoidan significantly enhanced the cytolytic activity of NK cells and increased IFN-γ production by T cells (Maruyama et al., 2003). However, fucoidan suppressed TNF-α and IFN-γ-induced NO production and iNOS expression in neuro-degenerative disorders (Do et al., 2010). In the present study, IL-6 level was decreased in plasma of rats treated with CsA, whereas the treatment with fucoidan reversed this decrease. It is known that, IL-6 is secreted by monocytes, macrophages, lymphocytes and epithelial cells (Hirano et al., 1990). CsA inhibited IL-2, IL-6, and IFN-γ production by CD4+ T lymphocytes which stimulated via the T cell antigen receptor.

### Table 1 Oxidative stress parameters (M ± SE) in the spleen tissue of control and different treated groups.

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Groups</th>
<th>Group I (Control I)</th>
<th>Group II (CsA, 25 mg/kg b.w)</th>
<th>Group III (CsA, 25 mg/kg b.w plus fucoidan 5 mg/kg b.w)</th>
<th>Group IV (CsA, 50 mg/kg b.w) (CsA, 5 mg/kg b.w plus fucoidan 10 mg/kg b.w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid peroxidation (nmol/mg protein)</td>
<td></td>
<td>2.417 ± 0.018</td>
<td>3.245 ± 0.238</td>
<td>3.045 ± .139**</td>
<td>3.664 ± .139***</td>
</tr>
<tr>
<td>Nitric oxide (nmol/mg protein)</td>
<td></td>
<td>0.294 ± 0.017</td>
<td>0.266 ± 0.039</td>
<td>0.224 ± 0.012</td>
<td>0.410 ± 0.020 ***</td>
</tr>
<tr>
<td>Glutathione (µg/mg protein)</td>
<td></td>
<td>1.402 ± 0.059</td>
<td>1.340 ± 0.0565</td>
<td>1.814 ± 0.122</td>
<td>1.435 ± 0.074</td>
</tr>
<tr>
<td>Vitamin C (µg/mg protein)</td>
<td></td>
<td>2.105 ± 0.088</td>
<td>2.030 ± 0.117</td>
<td>2.166 ± 0.097</td>
<td>2.287 ± 0.148</td>
</tr>
<tr>
<td>Superoxide dismutase (U/min/mg protein)</td>
<td></td>
<td>0.917 ± 0.058</td>
<td>0.469 ± 0.053***</td>
<td>0.706 ± 0.025*</td>
<td>0.660 ± 0.106</td>
</tr>
<tr>
<td>Catalase (U/min/mg protein)</td>
<td></td>
<td>11.680 ± 0.839</td>
<td>8.889 ± 1.054</td>
<td>11.430 ± 0.645</td>
<td>12.030 ± 1.246</td>
</tr>
<tr>
<td>Glucose 6 phosphate dehydrogenase</td>
<td>(U/min/mg protein)</td>
<td>0.332 ± 0.081</td>
<td>0.318 ± 0.075</td>
<td>0.544 ± 0.101</td>
<td>0.231 ± 0.030</td>
</tr>
</tbody>
</table>

* a Significance difference between group I and different treated groups.
  b Significance difference between group II and other treated groups.
  c Significance difference between group IV and other treated groups.

** P < 0.05.
*** P < 0.001.
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pathway (Thomson, 1992). In macrophage, IL-6 production and other specific defense functions are more susceptible to the action of CsA, whereas the phagocytic activity of macrophage is resistant to CsA (Mrowietz and Christophers, 1988; Losa García, 1996). Fucoidan could activate murine peritoneal macrophages that resulted in an increase in the production of TNF-α and IL-6 (Choi et al., 2005). However, fucoidan improved murine chronic colitis by down-regulating the synthesis of IL-6 in the colonic epithelial cells (Matsumoto et al., 2004).

In the present study, the plasma levels of IgM and IgG were decreased in all treated groups compared with the control group. In this aspect, the growth and differentiation of B lymphocytes were decreased due to the inhibitory effect of CsA on some cytokine production by stimulated CD4+ T lymphocytes (Thomson, 1992). Furthermore, CsA inhibited IgG and IgA production in T depleted cultures, but had little effect on IgM or IgD (Pereira et al., 1983). Similar findings on T-independent B cell responses have been described in murine systems (Kunkl and Klaus, 1980). Moreover, CsA decreases Ig production in cultures from normal healthy adults, without prior immunity (Pereira et al., 1983). Rapamycin, a more potent in vitro immunosuppressant of B lymphocytes than CsA, did not stimulate either the proliferation or antibody production by pre-activated B cells (Kim et al., 1994) and did not prevent Ig isotype switching that was observed with CsA (Thomson et al., 1993). In the present study, fucoidan treatment decreased plasma levels of IgM and IgG in all treated groups, whereas low dose of CsA caused the most inhibition of IgG level. In this topic, Zhang et al. (2003) found that, fucoidan treatment reduced IgG and IgM levels in delayed-type hypersensitivity reaction and suppressed the increase of the total IgG through Ig class switching inhibition in culture of mouse splenocytes and B cells. Similarly, fucoidan suppressed IgE induction by inhibited Ig class-switching to IgE in human B cells (Iwamoto et al., 2011). Furthermore, fucoidan inhibited Ig class-switching to IgE in murine B cells through its inhibitory effect on NF-κB p52-mediated pathway (Oomizu et al., 2006).

Oxidative stress has been proposed as a causative factor in CsA-induced renal, hepatic and cardiovascular toxicity (Walker et al., 1990; Suleymanlar et al., 1994; Rezzani et al., 2004). In the present study, the plasma levels of IgM and IgG were decreased due to the inhibitory effect of CsA on some cytokine production by stimulated CD4+ T lymphocytes (Thomson, 1992). Furthermore, CsA inhibited IgG and IgA production in T depleted cultures, but had little effect on IgM or IgD (Pereira et al., 1983). Similar findings on T-independent B cell responses have been described in murine systems (Kunkl and Klaus, 1980). Moreover, CsA decreases Ig production in cultures from normal healthy adults, without prior immunity (Pereira et al., 1983). Rapamycin, a more potent in vitro immunosuppressant of B lymphocytes than CsA, did not stimulate either the proliferation or antibody production by pre-activated B cells (Kim et al., 1994) and did not prevent Ig isotype switching that was observed with CsA (Thomson et al., 1993). In the present study, fucoidan treatment decreased plasma levels of IgM and IgG in all treated groups, whereas low dose of CsA caused the most inhibition of IgG level. In this topic, Zhang et al. (2003) found that, fucoidan treatment reduced IgG and IgM levels in delayed-type hypersensitivity reaction and suppressed the increase of the total IgG through Ig class switching inhibition in culture of mouse splenocytes and B cells. Similarly, fucoidan suppressed IgE induction by inhibited Ig class-switching to IgE in human B cells (Iwamoto et al., 2011). Furthermore, fucoidan inhibited Ig class-switching to IgE in murine B cells through its inhibitory effect on NF-κB p52-mediated pathway (Oomizu et al., 2006).

The ability of CsA to inhibit macrophage-dependent NO synthesis involves both inhibition of transcription of the iNOS gene and the enzyme activity (Conde et al., 1995; Hattori and Nakanishi, 1995). In the present study, NO was significantly increased in the spleen tissue of rats treated with a high dose of CsA and not changed by low dose. In this topic, CsA exerted a moderate inhibition of NO synthesis (Liebmann et al., 1995; Wang et al., 1997). Hortalano et al. (1999) suggested that, CsA interacts with several pro-apoptotic as well as they suggested that, viability of activated macrophages is improved in the course of immunosuppressive therapy (Hortalano et al., 1999). Fucoidan treatment restored the increases in NO content in the spleen tissue. Similarly, apigenin inhibited the production of NO by suppressing the iNOS expression and inhibiting the iNOS activity (Ha et al., 2008).

The implication of oxidative stress in CsA spleen toxicity was strengthened by the marked observed histopathological changes in the spleen. Endogenous antioxidants are responsible for neutralizing the free radical-induced oxidative damage. In the present study, SOD activity and GSH content were altered by CsA treatment, however, CAT and G6PD did not show any significant changes in all the treated rats. In this aspect, CsA administration significantly decreased the activity of antioxidant enzymes in CsA-induced nephrotoxicity (Mohamadin et al., 2005). Moreover, antioxidant-rich diets protect from diseases involved in oxidative damage (Bordoni et al., 2002). In conclusion, modulating the antioxidants’ defense mechanisms by chemopreventive agents has become a part of many therapeutic strategies and in the present study; co-supplementation of fucoidan with CsA significantly restored the adverse toxicity in the spleen tissue of CsA-treated rats.

Acknowledgment

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