Chili pepper-induced injury to splenic tissue of rabbit

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Chili pepper is the most common species used in food throughout the world. In the present study, we examined the effects of chili pepper on rabbit's splenic tissue. Oral administration of chili pepper was carried out every day for 10 days at a dose of 2 g/kg rabbit. Chili pepper induced significant increase in spleen weight as well as the number of leukocytes. Spleen architecture was altered as indicated by the histological score. White and red pulps were enlarged and the splenic capsule became thinner after administration of chili pepper to rabbits. Histochemical studies revealed a decrease in both carbohydrates and protein contents in the spleen. Therefore, based on our findings the excessive consumption of chili pepper is capable of inducing spleen damage and should be used in proper amounts.

Key words: Chili pepper, rabbit spleen, histology, histochemistry.

INTRODUCTION

Chili pepper is the fruit of plants from the genus Capsicum, members of the family, Solanaceae (Borges, 2009). It originated in the Americas. After the Columbian exchange, many cultivars of chili pepper spread across the world, used in both food and medicine (Heiser, 1976). Chili peppers have been a part of the human diet in the Americas since at least 7500 BC (Perry et al., 2007).

Chili pepper is the most used spice in food throughout the world (Nopaintaya and Nye, 1974; Chukwu, 2006). Chili pepper is known to be harmful if consumed in excess (Nwaopara et al., 2004; Nwaopara et al., 2007). Chili pepper possesses some chemical and pharmacological properties similar to the classes of drugs that are capable of inducing tissue damage (Govindarajan and Sathyanarayana, 1991).

The active ingredient of pepper is called capsaicin. It has captured the attention of many investigators, because its biting and burning properties suggesting it could have physiological and pharmacological effect (Govindarajan and Sathyanarayana, 1991). Capsaicinoids, natural component of chili pepper and produced by glands in the peppers placenta range from 0.1 to 2.5 mg/g (Parrish, 1996). The range of capsicum spices consumption was reported to be 2.5 to 20 g/person/day in India, Thailand and Mexico (Monsereenusorn, 1983; Lopez-Carrillo et al., 1994). The maximum daily intake of capsaicin in USA and Europe from mild chili and paprika was roughly estimated to be 0.025 mg/Kg bw/day (Council of Europe, 2001).

The spleen is the largest secondary immune organ in the body and is responsible for initiating immune reactions to blood-borne antigens and for filtering the blood of foreign material and old or damaged red blood cells.

The two main compartments of the spleen, the white pulp (including the marginal zone) and the red pulp carry out these functions, which are vastly different in their architecture, vascular organization, and cellular composition (Dkhil, 2009). There was no study dealing with the effect of pepper on splenic histology. The current study aimed to investigate the histological alterations induced in splenic tissue of rabbit subjected to extensively administered chili pepper.
Experimantal animals

Twelve healthy male rabbits (Oryctolagus cuniculus) approximately 8 to 9 weeks old and weighed 2.1 ± 0.3 kg, were obtained from the animal facility of King Saud University and used in the present study. Rabbits were bred under specified pathogen-free conditions, and fed a standard diet and water ad libitum. Animals were divided into two groups. The first group was considered as the control group (6 non-treated rabbits). Animals in this group received only normal feed with carrot every day for 10 days. The second group was orally ingested 2 g/Kg of red pepper fruit with carrot every day for 10 days. The experiments were approved by State authorities and followed Saudi Arabian rules for animal protection for the project number RGP-VPP-002.

Splenic index

Rabbits were weighed before dissection. Spleens were removed, weighed and the ratio of spleen weight in mg/rabbit to body weight in g/rabbit was calculated and expressed as splenic index.

Hematological studies

Blood was collected into tubes containing ethylene diamine tetra acetic acid for the determination of some important hematological parameters (total erythrocytes count, total leucocytic count, percentage of neutrophils, percentage of lymphocytes, hemoglobin contents and percentage of hematocrit) using an automatic counter (VET-530 CA Medonic; Medonic, Stockholm, Sweden).

Histological and histochemical study

Animals from control and treated groups were sacrificed after 10 days of chili pepper inoculation. Small pieces of the spleen were quickly removed, then fixed in neutral buffered formalin. Following fixation, specimens were dehydrated, embedded in wax, and then sectioned to 5 microns thickness. For histological examinations, sections were stained with haematoxylin and eosin (Drury and Wallington, 1980). A semi-quantitative scoring system (Evangelos et al., 2006) was used. Segments of spleen was scored for the enlargement of B- and T-lymphocyte areas in red and white pulps (0, absent; 1, slight; 2, moderate; and 3, pronounced) and for the increased number of apoptotic cells, macrophages, necrotic cells and presence of pigments (0, absent; and 1, present). Scoring of each tissue sample represented the mean score of five different high microscopic power fields.

Results

Statistical analysis

Statistical analysis was performed by using an unpaired Student’s *t* test. The data were analyzed by using MS Excel 2003 (Microsoft, Town, State, USA) and SigmaPlot 2001 (Systat Software, Inc, Town, State, USA).

RESULTS

Extensive consumption of chili pepper (2 g/Kg) for 10 days induced a splenomegaly in rabbits as indicated by a significant increase in splenic index (Figure 1). Chili pepper has no significant effect on the number of erythrocyte of rabbits, while leucocytic counts were significantly increased (Table 1). The percentage of both neutrophils (72.6 ± 2.7) and lymphocytes (47.4 ± 2.4) in
Table 1. Hematological analysis of rabbits administered chili pepper.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Non-treated rabbit</th>
<th>Treated rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes (10^6/mm$^3$)</td>
<td>6.1 ± 0.4</td>
<td>6.3 ± 0.5</td>
</tr>
<tr>
<td>Leucocytes (10^3/mm$^3$)</td>
<td>6.9 ± 0.9</td>
<td>9.2 ± 1.1*</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>63 ± 1.8</td>
<td>72.6 ± 2.7*</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>37 ± 2.8</td>
<td>47.4 ± 2.4*</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>13.6 ± 0.3</td>
<td>13.9 ± 0.5</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>39.7 ± 3.2</td>
<td>40.3 ± 2.2</td>
</tr>
</tbody>
</table>

All values are means ± SD. *, significant data with respect to non-treated rabbits ($p < 0.01$).

Blood of rabbits administered with chili paper were significantly increased. In addition, the hemoglobin and hematocrit values were not significantly affected (Table 1).

The spleen of the non-treated rabbit (Figure 2A) is surrounded by a capsule composed of dense fibrous tissue, elastic fibers, and smooth muscle. The outermost layer of the splenic capsule is composed of mesothelial cells. Irregularly spaced trabeculae of smooth muscle and fibroelastic tissue emanate from the capsule into the splenic parenchyma. These trabeculae also contain blood and lymph vessels and nerves. The lymph vessels are efferent vessels through which lymphocytes migrate to the splenic lymph nodes. Administration of chili pepper increased the thickness of the splenic capsule (Figure 2D).

The structure of the non-treated spleen was composed of white and red pulps surrounded by a capsule of dense connective tissue (Figure 2A). The white pulp was composed of a central, T-cell rich zone, and a peri-arterial lymphoid sheath surrounded by B-cell-rich primary follicles. The white pulp was separated from the red pulp by the marginal sinus embedded in a layer of marginal zone lymphocytes. When rabbits administered chili pepper for 10 days, the white pulp enlarged due to increased follicle size. This reaction is due to increased hematopoietic support and increased numbers of macrophages (Tolosano et al., 2002). Because of these alterations, phagocytosis increased the number of effete erythrocytes. This may also indicate brown pigments detected in the splenic parenchyma. These pigments are haemosiderin, which resulted from the destruction of red blood cells in the phagocytosis actively carried out by macrophages and swollen reticuloendothelial cells in the spleen. Enlargement of the marginal zones may be attributed to the fact that it is the region of the red pulp which receives the incoming arterial blood; thus, it is the site where blood borne cells and particulate matter first contact the splenic parenchyma. These zones, also, trap circulating antigens and are important in the immunological activities of the spleen (Cesta, 2006).

Because spleen is the body's filter against any foreign materials from the blood stream, large number of neutrophils migrate from the peripheral blood into the splenic parenchyma to be participating in the humoral immunological responses (Fawcett, 1986). This may explain the detection of large number of neutrophils in both marginal zone and red pulps.

Control spleen sections stained with PAS method are shown in Figures 4A. Spleens of rabbits administered chili pepper had a slight decrease in carbohydrate content. Glycogen appeared around the cell membranes (Figure 4B).

Examination of spleen sections from the control group, stained by bromophenol blue method, showed normal protein content (Figure 5A). Protein content was moderately decreased in the splenic cells of rabbits administered with chili pepper (Figure 5B).

**DISCUSSION**

It was reported that splenic weight is a relatively insensitive indicator of immunotoxicity (Elmore, 2006). The splenomegaly of rabbit administered chili pepper was associated with expansion of both white and red pulp due to increased follicle size. This reaction is due to increased hematopoietic support and increased numbers of macrophages (Tolosano et al., 2002). Because of these alterations, phagocytosis increased the number of effete erythrocytes. This may also indicate brown pigments detected in the splenic parenchyma. These pigments are haemosiderin, which resulted from the destruction of red blood cells in the phagocytosis actively carried out by macrophages and swollen reticuloendothelial cells in the spleen. Enlargement of the marginal zones may be attributed to the fact that it is the region of the red pulp which receives the incoming arterial blood; thus, it is the site where blood borne cells and particulate matter first contact the splenic parenchyma. These zones, also, trap circulating antigens and are important in the immunological activities of the spleen (Cesta, 2006).

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Dietary capsaicin (the active component in chili pepper) in Balb/c mice had been shown to enhance lymphocyte proliferation and serum Ig levels (Yu et al., 1998). Abundance of leucocytes, in general, and lymphocytes, in particular, are a prominent response of body tissues facing any injurious impacts (Nwaopara et al., 2007). Leukocyte elevation and adherence to the vascular endothelium have been suggested by Miura et al. (1991) and McCafferty et al. (1995) to play an important role in the pathogenesis of drugs-associated injury. In addition,
Figure 2. Histological changes in spleen of rabbit. A, Control spleen with normal distribution of white pulps (WP) and red pulps (RP). B-D, Spleen sections of rabbits administered chili pepper with enlarged white pulps, thickened capsule, hemosidrin pigments (arrow heads) and apoptotic cells (arrow), respectively. Sections were stained with hematoxylin-eosin.

Figure 3. Histological score of the spleen. All values are means ± SD. *, Significant data with respect to non-treated rabbits ($p < 0.01$).
extensive consumption of red pepper could induce a toxic effects as indicated by a destruction of the absorptive cells of the gastrointestinal tract (Chukwu, 2006) and hepatocytes (Nwaopara et al., 2007).

Chili pepper induced disorganization of the marginal zone macrophage of the white pulp. This marginal zone is a unique region of the spleen situated at the interface of the red pulp with the PALS and follicles (Figure 3). Considered by many to be a separate compartment rather than part of the white pulp, it is designed to screen the systemic circulation for antigens and pathogens and plays an important role in antigen processing (Kuper et al., 2002; Mebius and Kraal, 2005). A band of macrophages, the marginal zone metallophilic macrophages, and the marginal sinus (Dijkstra and Veerman, 1990; Mebius and Kraal, 2005; Satodate et al., 1986), separate the marginal zone from the PALS and follicles.

Previous investigations showed that extensive consumption of hot red pepper reduce adiposity (Snitker et al., 2009), a phenomenon which can be explained partly by the enhancing effects of capsaicin on energy and lipid metabolism via catecholamine secretion from the adrenal medulla (Watanabe et al., 1987a, b; Kawada et al., 1988). The change in the metabolic status may affect the carbohydrate and protein levels inside splenic tissues as demonstrated histochemically.

Collectively, our data indicate that extensive consumption of chili pepper affects the spleen structure and function. Administration of chili pepper should be
used in proper amounts.

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REFERENCES