EVALUATION OF BLOOD AND BIOCHEMICAL PARAMETERS IN EXPERIMENTAL COLITIS TREATED WITH LYCOPENE

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In this study, possible protective effects of lycopene on 2,4,6-trinitrobenzene sulphonic acid induced-colitis was investigated using blood and biochemical parameters. Totally, 122 Sprague Dawley rats weighing 220–250 gr were used in this study. Laboratory animals were divided into 6 groups as control group, 2,4,6-trinitrobenzene sulphonic acid introduced group (120 mg/kg), L-nitroamine methylester supplemented group (40 mg/kg), olive oil supplemented group (1 ml), 5 mg/kg and 10 mg/kg lycopene supplemented groups. The day after 2,4,6-trinitrobenzene sulphonic acid introduction to induce experimental colitis, animals were treated with L-nitroamine methylester, olive oil and lycopene intraperitoneally for three days. Blood samples were taken under ether anesthesia. Blood and biochemical analyses were performed with blood counter and otoanalysis systems. Erythrocyte, leucocyte and platelet levels of the blood samples of L-nitroamine methylester treated group showed mild effect but in lycopene group levels were similar to the control group with increasing dosages. Biochemically, alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase were analysed in control and experimental groups. It was seen that alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase levels of 10 mg/kg and 5 mg/kg lycopene groups were decreased and approximated to control levels compared to the L-nitroamine methylester group. According to the parameters obtained from blood and biochemical analysis it can be concluded that lycopene treats better than L-nitroamine methylester in 2,4,6-trinitrobenzene sulphonic acid induced colitis.

Key words: colitis, lycopene, nitric oxide synthetase inhibitors, rat.

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disorder of the colon and rectum that is seen in almost every group of age. The etiology of this disorder is

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unknown, but genetic and environmental factors (1, 2), microorganisms (3, 4) and also immune system disorders (5, 6) are considered by the researchers (7, 8).

Several natural and artificial substances have been used for UC treatment in histological, genetic, immunologic, physiological and also biochemical studies on human and laboratory animals (9–12). Analysis of blood cells like neutrophils and macrophages and also epithelial cells demonstrated that the increase in nitric oxide (NO) level is a determining factor in the pathology of UC (13, 14). NO is produced not only by endothelial cells, but also by macrophages using the enzyme nitric oxide synthase (NOS). As known, there are three different types of NOS; endothelial (eNOS), neuronal (nNOS) and cytokine inducible (iNOS) (15, 16) NO derivated peroxinitride (ONOO) and oxygen radicals (O•) accelerate the devastation of cells (17, 18).

Earlier studies revealed that mercaptoethylguanidine, aminoguanidine and N-iminoethyl-L-Lysine like synthetic iNOS inhibitors display a mild effect on colitis (12, 19, 20). In recent studies it has been indicated that a synthetic iNOS inhibitor (1400W-N-(3[amino-methyl] benzyl) exhibits considerable effect on colitis (11, 21). In this study, L-NAME (N(G)-nitro- L-arginine-methyl-ester) was used as an iNOS inhibitor.

It was advanced that an important carotenoid, lycopene, is most found in tomatoes (Licopersicum esculentum) and other fruits and vegetables (22, 23). Lycopene is known to have the ability to quench singlet oxygen and this property served researchers to use lycopene as a therapeutic agent (24). Several studies were practised on damaged tissues and organs of gastrointestinal and urinary system using lycopene treatment. Especially in inflammatory diseases and cancer, organs like pharynx, esophagus, stomach and rectum (25), large intestine (26), urinary bladder (27) and prostatis (28) were treated with lycopene and effective results were obtained. In this study we aimed to display the protective effects of L-NAME and lycopene against the oxidative damage formed in TNBS (2,4,6-trinitrobenzene sulphonic acid) induced acute experimental colitis in rats using blood and biochemical parameters.

MATERIAL AND METHODS

ETHICAL APPROVAL

Institutional ethical approval for this experiment was granted by the Animal Experimentation Ethics Committee (2007/257) Osmangazi University, Eskisehir, Turkey.

ANIMALS AND EXPERIMENTAL PROTOCOL

Male Sprague Dawley rats weighing 220±30 g were obtained from Osmangazi University Medical and Surgical Experimental Research Center (TICAM), Eskisehir. All animals were housed in cages under 12/12 hr light and
dark cycle. They were fed with standard chow diet and water. Rats were divided into six groups: control \((n=7)\), TNBS \((120 \text{ mg/kg}) \ (n=21)\), L-NAME \((40 \text{ mg/kg}) \ (n=21)\), olive oil \((1 \text{ mg/kg}) \ (n=21)\), 5 mg/kg lycopene \((n=21)\) and 10 mg/kg lycopene \((n=21)\). All chemicals were obtained from Sigma Co., USA.

TNBS was prepared with 50% methanol. Acute experimental colitis was induced by applying TNBS from the rectum with a plastic canule \((29)\). Control rats received only physiologic solution intraperitoneally \((\text{ip})\). The day after induction of colitis L-NAME, olive oil and lycopene groups I and II received treatments daily for three days \(\text{ip}\) lycopene was administered 1:1 in oil.

**BLOOD SAMPLES**

Animals were anesthetized and blood samples were collected intracardiac by sodium citrate containing enjectors. Samples obtained from each group for three days were analysed on blood counter system \(\text{(Hemawet)}\). Blood samples of each group that were collected to tubes with heparin were centrifuged and ALT, AST, LDH levels were obtained from serum using biochemical analyser \(\text{(Crony)}\).

**STATISTICAL ANALYSIS**

ANOVA test was performed using SPSS programme \(9.05\) to analyse blood cell counting results and biochemical results obtained from control and experimental groups.

**RESULTS**

**PERIPHERIC BLOOD PARAMETERS**

Blood samples of control and experimental groups were analysed on blood counter system. Peripheric blood cell levels (erythrocyte, leucocyte and platelets) were shown in Table 1.

On the first day of experiments, results of blood cell levels were as follows: The TNBS group showed increasing rate of blood cells \((60\%, 72\%, 54\%) \ (p<0.001)\) compared with the control group. The L-NAME group showed an increasing rate of cells \((40–50\%)\) compared with the control group, but a decreasing rate when compared with TNBS group \((6–8\%) \ (p<0.001)\). The olive oil group revealed an increase of \(35–46\% \ (p<0.001)\) compared with the control group, but a decrease of \(15–16\% \ (p<0.001)\) compared with the TNBS group. The 5 mg/kg lycopene group displayed a rate of increase \(23–34\%) \ (p<0.001)\) compared with the control group, but a decrease \(15–23\%) \ (p<0.001)\) compared with the TNBS group. The 10 mg/kg lycopene group showed an increase of \(14–23\% \ (p<0.001)\) compared with the control group, but a decrease \(22–30\%) \ (p<0.001)\) compared with the TNBS group.
### Table 1

Peripheric blood levels of control and experimental groups

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<tr>
<th>Groups</th>
<th>Peripheric blood levels</th>
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<td>erythrocytes 10^6</td>
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<td>platelets 10^6</td>
<td>erythrocytes 10^6</td>
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<td>erythrocytes 10^6</td>
<td>leucocytes 10^3</td>
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<td>Control</td>
<td></td>
<td>8.6±0.6 9.7±1.8 961.2±143.2</td>
<td>8.6±0.6 9.7±1.8 961.2±143.2</td>
<td>8.6±0.6 9.7±1.8 961.2±143.2</td>
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<tr>
<td>120 mg/kg TNBS</td>
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<td>13.8±0.8 16.6±2.1 1472±181.5</td>
<td>13.0±1.7 15.6±1.7 1339.4±159</td>
<td>11.6±4.3 14.4±0.7 1292.7±156</td>
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<td>40 mg/kg L-NAME</td>
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<td>12.8±0.6 15.8±1.6 1372.3±74.4</td>
<td>11.8±0.5 14.0±5.5 1281.4±153</td>
<td>10.8±3.1 13.6±0.9 1230.1±98.8</td>
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<tr>
<td>TNBS + olive oil</td>
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<td>11.6±1.0 14.1±5.2 1372.3±88.8</td>
<td>11.0±1.3 13.3±2.3 1226.4±107</td>
<td>10.3±2.9 12.2±0.7 1130.4±88.4</td>
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<td>5 mg/kg Lycopene</td>
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<td>10.6±0.5 13.0±4.5 1250±51.2</td>
<td>9.8±0.9 12.2±1.7 1100.7±73.8</td>
<td>9.3±3.2 11.0±0.8 1050.4±89.4</td>
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<tr>
<td>10 mg/kg Lycopene</td>
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<td>9.8±1.5 12.0±2.4 1149.6±98.7</td>
<td>9.0±0.5 11.0±2.9 1028.7±129</td>
<td>8.8±0.5 10.4±0.9 980.9±94.9</td>
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### Table 2

LT, AST, LDH levels of control and experimental groups

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<th>Groups</th>
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<td>ALT U/L</td>
<td>AST U/L</td>
<td>LDH U/L</td>
<td>ALT U/L</td>
<td>AST U/L</td>
<td>LDH U/L</td>
<td>ALT U/L</td>
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<tr>
<td>Control</td>
<td></td>
<td>32.6±5.3 80.5±10.6 532±80.2</td>
<td>32.6±5.3 80.5±10.6 532±80.2</td>
<td>32.6±5.3 80.5±10.6 532±80.2</td>
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<tr>
<td>120 mg/kg TNBS</td>
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<td>75.8±7.2 154.3±9.5 1455.6±129.6</td>
<td>65.2±8.4 143.5±11.1 1341.3±80.5</td>
<td>60.1±8.5 132.9±6.6 1290.4±94.6</td>
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<tr>
<td>40 mg/kg L-NAME</td>
<td></td>
<td>70.1±8.6 143.1±13 1374.5±91.4</td>
<td>60.2±6.3 134.2±12.5 1267.3±109</td>
<td>51±5.5 120.4±15.5 1157.1±134</td>
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<tr>
<td>TNBS + olive oil</td>
<td></td>
<td>65.6±4.2 136.9±9 1352.3±69.1</td>
<td>58±6.2 131.2±18.4 1226.5±93.3</td>
<td>50±7.4 110.7±16.6 1135.2±134</td>
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<tr>
<td>5 mg/kg Lycopene</td>
<td></td>
<td>58.5±6.4 130.1±9 1278.9±109</td>
<td>54.7±8.5 114.5±14.1 1157.3±103</td>
<td>43.3±4.9 100.9±12.3 996.5±86.8</td>
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<tr>
<td>10 mg/kg Lycopene</td>
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<td>54.5±8.5 122.3±14 1101.5±123.</td>
<td>46±7.5 106.4±10.5 987.1±107</td>
<td>38.2±5.2 88.2±6.5 782.8±58.7</td>
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</table>
On the second day of experiments results of blood cell levels were as follows – The TNBS group showed increasing rate of blood cells (51%, 61%, 40%) (p<0.001) compared with the control group. The L-NAME group showed an increasing rate of cells (34–44%) (p<0.001) compared with the control group, but a decreasing rate when compared with the TNBS group (17%, 10%, 5%) (p<0.001). The olive oil group revealed an increase of 28–37% (p<0.001) compared with the control group, but a decrease (21%, 15%, 9%) (p<0.001) when compared with the TNBS group. The 5 mg/kg lycopene group displayed a rate of increase 14–26% (p<0.001) compared with the control group, but a decrease 25%, 23%, 18% (p<0.001) compared with the TNBS group. The 10 mg/kg lycopene group showed an increase of 5–13% (p<0.001) compared with the control group, but a decrease (32%, 29%, 24%) (p<0.001) compared with the TNBS group.

On the third day of experiments, results of blood cell levels were as follows – The TNBS group showed increasing rate of blood cells (35%, 48%, 34%) (p<0.001) compared with the control group. The L-NAME group showed an increasing rate of cells (26–40%) (p<0.001) compared with the control group, but a decreasing rate when compared with TNBS group (7%, 6%, 5%) (p<0.001). The olive oil group revealed a decrease (14%, 12%, 7%) (p<0.001) compared with the TNBS group. The 5 mg/kg lycopene group displayed a rate of decrease (8–14%) (p<0.001) compared with the control group, but a decrease (20%, 24%, 19%) (p<0.001) compared with the TNBS group. The 10 mg/kg lycopene group showed an increase of 2–7% (p<0.001) compared with the control group, but a decrease (25%, 28%, 24%) (p<0.001) compared with the TNBS group.

On the first day of experiments, results of ALT, AST, LDH levels of groups were as follows – The TNBS group showed an increasing rate (132%, 92%, 173%) (p<0.001) compared with the control group. The L-NAME group showed a decreasing rate when compared with TNBS group (8%, 7%, 6%) (p<0.001). The olive oil group revealed a decrease (14%, 12%, 7%) (p<0.001) compared with the TNBS group. The 5 mg/kg lycopene group displayed a rate of decrease (23%, 16%, 12%) (p<0.001) compared with the TNBS group. The 10 mg/kg lycopene group showed a decreasing rate (28%, 21%, 24%) (p<0.001) compared with the TNBS group.

On the second day of experiments, results of ALT, AST, LDH levels of groups were as follows – The TNBS group showed an increasing rate (100%, 78%, 152%) (p<0.001) compared with the control group. The L-NAME group showed a decreasing rate when compared with the TNBS group (8%, 6%, 6%) (p<0.001). The olive oil group revealed a decrease (11%, 9%, 9%) (p<0.001) compared with the TNBS group. The 5 mg/kg lycopene group displayed a rate of decrease
(16%, 20%, 14%) (p<0.001) compared with the TNBS group. The 10 mg/kg lycopene group showed a decreasing rate (30%, 26%, 27%) (p<0.001) compared with the TNBS group.

On the third day of experiments, results of ALT, AST, LDH levels of groups were as follows – The TNBS group showed an increasing rate (84%, 65%, 143%) (p<0.001) compared with the control group. The L-NAME group showed a decreasing rate when compared with the TNBS group (15%, 9%, 10%) (p<0.001). The olive oil group revealed a decrease (17%, 18%, 12%) (p<0.001) compared with the TNBS group. The 5 mg/kg lycopene group displayed a rate of decrease (28%, 24%, 23%) (p<0.001) compared with the TNBS group. The 10 mg/kg lycopene group showed a decreasing rate (37%, 34%, 39%) (p<0.001) compared with the TNBS group.

DISCUSSION

Nitric oxide is known to be produced in experimental colitis and NOS inhibitors are used to prevent the harmful effects of NO. Some NOS inhibitors are beneficial (14), some are effective (30) and the others are known to be ineffective (3). Jaiswal et al. (2001) suggested that the type of inflammation in colitis, whether acute or chronic, depends on the amount, type and relation with other compounds of secreted NO.

In the early stages of inflammation in TNBS induced experimental colitis leucocyte iNOS derivated NO has known to be effective; however, in later stages it appears to be ineffective. The reason of this action is said that NOS inhibitors have selective properties about the inflammation site. It is documented that in the first day of experimental acute colitis studies, an instant increase in the blood and biochemical parameters can be observed due to the severity of inflammation, but on the second and third days this increase lowers (11, 14, 20, 30).

An important carotenoid, lycopene was known to be a radical scavenger; therefore, in several studies it has been well demonstrated that it shows protective properties in serious diseases like cancer, heart and degenerative eye diseases (22, 23, 31, 32). In addition, it is reported that tomato and tomato products have opposite relationship with certain cancer types and plasma membrane lipid peroxidation, due to the antioxidant capacity of lycopene (33, 34).

In this study, peripheric blood parameters of control and experimental groups indicated that high dosages of TNBS (120 mg/kg) introduced to the colitis group showed a distinct increase in peripheric blood levels. L-NAME (40 mg/kg), an iNOS inhibitor used in the treatment of colitis for three days, approximated the levels of peripheric blood levels to control levels (p< 0.001). However, lycopene in the dosages of 5 and 10 mg/kg treated the colitis group better. This situation can arise from the structure of lycopene and its properties; so, with one or more mechanisms, lycopene acts on the bone marrow or activates stored blood cells in certain organs. By this way, peripheric blood levels may have been influenced. We
came to the conclusion that lycopene acts on the inflammation site effectively and immediately, and it makes a protective impact.

A study on mice, reported by Yaping et al. (2003), has indicated that lycopene has both anti-inflammatory and anticoagulant impacts. The data obtained in our study is parallel to the recent studies on the blood parameters with NOS inhibitors and anti-inflammatory (35, 36) and anticoagulant (23) impacts of lycopene.

It is known that ALT, AST and LDH parameters are evaluated in diagnosis and treatment of infections. In this study, daily serum samples of all groups were analysed and ALT, AST and LDH levels were statistically compared. In 5 mg/kg and 10 mg/kg lycopene groups, especially on the third day compared with the first day, these levels were decreased and approximated to control levels. The L-NAME group levels were decreased too but not much. It can be said that the L-NAME dosage introduced for three days showed little impact, but increasing lycopene dosages showed much more impact on the levels.

Kankuri et al. (2001) reported that non-selective NOS inhibitor L-NAME did no good for both colon tissue and biochemical parameters. Studies on certain NOS inhibitors, such as aminoguanidine or N-aminoethyl-L-lysine, have shown that NOS inhibitors had no protective effect on experimental colitis models (12, 37, 38). In recent years, studies on oxidative stress have documented that lycopene acts as antioxidant, anticarcinogen and that it also protects tissues. Organizing cyclooxygenase and lipoxygenase enzyme activities, lycopene inhibits prostaglandin, prostacycline and leucotrien synthesis, and prevents inflammation (36). Lycopene was used for treatment and protection of tissues and cells both in vivo and in vitro in recent years (39–41). Reifen et al. (2004) introduced rats 5-ASA plus lycopene in diet and indicated that lycopene approximates immunohistological and biochemical parameters to normal levels supporting our findings.

Similar results were also obtained in our histological studies in experimental colitis with lycopene treatment. It should be indicated that TNBS induced damage on colon tissues was not demonstrated here, but proved in this model with macroscopic and microscopic scores.

As this study provides evidence for therapeutic benefits of lycopene in experimental colitis, further research is needed to understand the action of carotenoids.

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REFERENCES


