

The healing effects of prp and thymoquinone on dss induced inflammatory bowel disease in rats

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Abstract

Aim: Crohn's disease and ulcerative colitis are included in inflammatory bowel disease (IBD). The study aimed to compare the healing effects of two promising therapeutic agents: Platelet Rich-Plasma and Thymoquinone (TQ) on DSS induced IBD.

Materials and Methods: The animals were divided into three groups as IBD (n=10), PRP treatment (n=10) and TQ treatment (n=10). Experimental IBD was initiated through the oral intake of 5% Dextran Sodium Sulfate (DSS) in drinking water. PRP (0.5 cc) and thymoquinone dissolved in distilled water (100mg/kg) were daily administered rectally to PRP treated group animals for seven days. All animals were sacrificed and rectal tissue calprotectin, lactoferrin and hydroxyproline levels were assessed on the seventh day. Total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) levels were statistically compared.

Results: Rectal tissue calprotectin, lactoferrin and hydroxyproline levels were higher in the IBD group ($p<0.001$, $p<0.001$ and $p<0.001$, respectively). TQ treated group animals had lower rectal tissue lactoferrin levels ($p<0.011$) and TOS and OSI levels ($p=0.001$ and $p=0.001$, respectively) than PRP group animals and also the highest antioxidant capacity.

Conclusion: Calprotectin, lactoferrin, and hydroxyproline levels are valuable markers in the presence of IBD and response to treatment. Oxidative stress is an important problem that cannot be avoided in unattended cases. Our experimental IBD model found TQ treatment to be more effective in healing than PRP treatment.

Keywords: Crohn's disease; inflammation; inflammatory bowel disease; platelet rich-plasma; thymoquinone; ulcerative colitis

INTRODUCTION

Crohn's disease and ulcerative colitis are included in inflammatory bowel disease (IBD) (1). Inflammatory bowel disease is a serious health problem due to chronicity, exacerbations, lack of definitive treatment, insufficient existing treatment methods and its negative effects on quality of life. IBD mainly includes two diseases: Crohn's disease (CD) and ulcerative colitis (UC). The frequency and pervasiveness of inflammatory bowel disease (IBD) continue to increase worldwide (2). Although the etiology of CD and UC remains vague, host genetic susceptibility, environmental factors, intestinal microbial flora and immune responses were found to affect the pathogenesis of IBD. The inflammatory response which cannot be controlled due to complicated interactions among these factors causes intestinal tissue damage associated with IBD (3-5).

Increased health costliness of IBD prove the importance of IBD related biomarkers. On another note, the follow-up of patients with IBD is a tough problem, so; the

follow-up strategy should be personalized. Recently, serum calprotectin was reported as a novel promising biomarker in monitoring IBD patients (6). Calprotectin and lactoferrin are the two most commonly used fecal biomarkers in clinical practice. Gut inflammation causes calprotectin release which is a calcium-binding protein from neutrophils into the intestinal lumen and discharged in the stool. Fecal calprotectin levels should be assessed for IBD monitoring, disease activity and response to therapy. Lactoferrin, which is an extensively utilized commercial biomarker similar to calprotectin, is an iron-binding glycoprotein. Buderus et al. found that increased fecal lactoferrin levels suggested intestinal inflammation even in patients with regular CRP levels (6-8).

Apart from the drugs that were used for a long time, various new drugs such as anti-TNF and Anti-Integrin agents for IBD therapy were recently introduced in clinical practice to decrease the usage of corticosteroid enforce response and remission rates. None of the current treatment options for IBD are entirely effective and each has various negative

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effects. Thereby, patients suffering from IBD constantly seek different treatment options (9,10).

Many studies showed that platelets are crucial in inflammation by creating numerous growth factors and cytokines (11). Additionally, experimental studies found that administering platelet-rich plasma (PRP) to intestinal anastomoses has positive effects on anastomosis healing (12). Thymoquinone (TQ) is the main component of the volatile oil of *Nigella sativa* and former studies reported TQ to have antioxidative, anti-inflammatory, immunomodulatory and antimicrobial properties (11,13-16).

This study aimed to compare the possible effects of PRP and TQ in the DSS-induced experimental colitis model.

MATERIALS and METHODS

Our experimental study was approved by Kirikkale University Animal Experiments Local Ethics Committee on 30.01.2014 with the number 14/34. The experiments were carried out in accordance with the Universal Declaration of Animal Rights. There were three groups including 35 Wistar Albino rats weighing between 220-250g: control (n=10), PRP(n=10) and TQ treatment (n=10) groups. Five animals were separated to obtain PRP. The colitis model was created as described by Wirtz (17). Experimental colitis was induced by adding 5% Dextran Sodium Sulfate (DSS) in drinking water of all animals for seven days. All animals were placed in collective cages under controlled temperature (24°C), 12 hours of daylight and dark

conditions with water and rat chow provided ad libitum. The TQ group was delivered 100 mg/kg and thymoquinone dissolved in 0.5 cc distilled water rectally as a daily dose. The PRP group was delivered 0.5 cc prp rectally as a daily dose. Both TQ and PRP groups were administrated the drugs for seven days. All animals were sacrificed and 5cm rectal tissue samples were obtained from all of them seven days later. Hydroxyproline (OHP), calprotectin and lactoferrin levels were biochemically assessed in the rectal tissue samples. Total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) were also evaluated.

Data were analyzed using the Statistical Packages for the Social Sciences for Windows version 20.0 (SPSS Inc, USA). The results were expressed as mean \pm standard error and, mean (SEM) and median (minimum-maximum). Kruskal-Wallis Test was used for the comparison of continuous and ordinal variables, Mann Whitney U Test for binary comparisons and $p < 0.05$ was accepted to be statistically significant. The number of animals in each group required non-parametric tests to be used. The difference in numbers was firstly checked with the Kruskal-Wallis Test and the Mann Whitney U test was used to analyze the differences between groups.

RESULTS

Rectal tissue calprotectin, lactoferrin and OHP levels of the groups are presented in Figure 1, respectively. Mean calprotectin, lactoferrin and OHP levels of rats with DSS

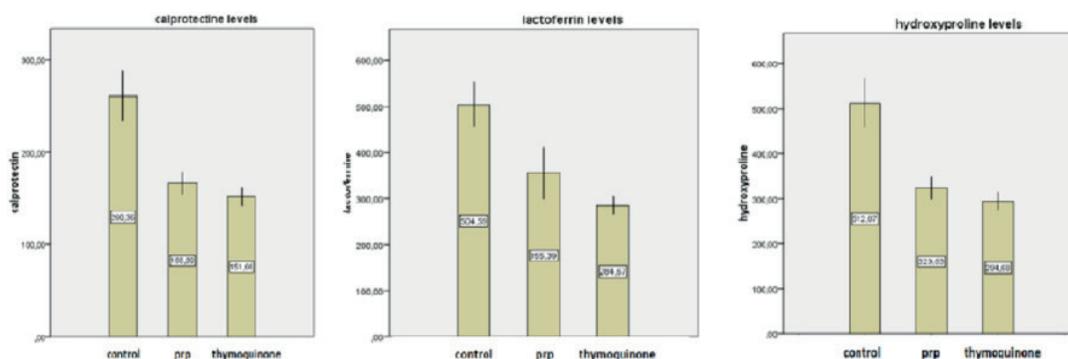


Figure 1. Calprotectin, lactoferrin and hydroxyproline levels of the groups

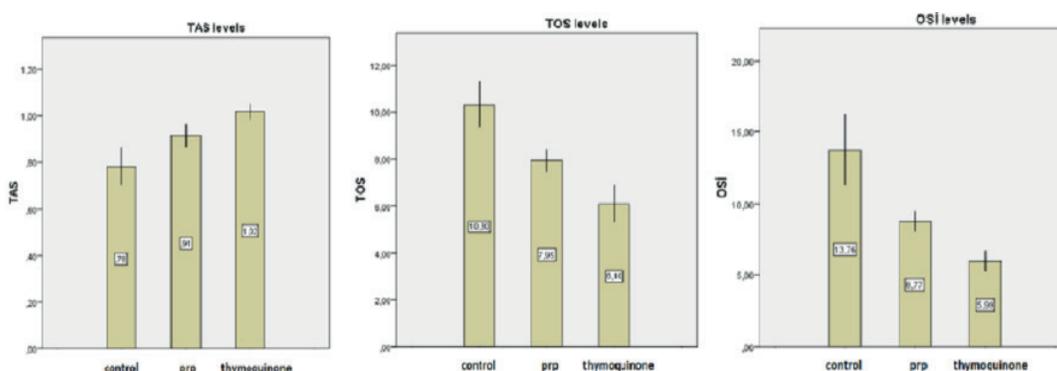


Figure 2. TAS, TOS and OSI levels of the groups

induced colitis were found to be significantly higher than those of PRP and thymoquinone groups ($p < 0.001$ and $p < 0.001$ for each parameter, respectively). There was a significant difference between PRP and TQ ($p < 0.05$) groups in terms of lactoferrin levels. No significant difference was found in the TQ and PRP groups for calprotectin and OHP. Rectal tissue TAS, TOS and OSI levels of groups were detailed in Figure 2, respectively. The lowest TAS levels and the highest TOS and OSI levels were noted in IBD group animals. TQ group animals had a higher level of rectal tissue TAS than PRP group animals ($p < 0.05$). TQ treatment group animals had the lowest rectal tissue TOS and OSI levels. The results of data analysis are presented Table 1.

Table 1. Statistical comparisons of the groups in terms of parameters

Statistical Comparisons	IBD vs. PRP	IBD vs. Thymoquinone	PRP vs. Thymoquinone
Calprotectine levels	$P < 0.001$	$P < 0.001$	$P = 0.093$
Lactoferrin levels	$P < 0.001$	$P < 0.001$	$P = 0.011$
Hydroxyproline levels	$P < 0.001$	$P > 0.001$	$P = 0.093$
TAS levels	$P = 0.014$	$P < 0.001$	$P = 0.008$
TOS levels	$P = 0.001$	$P < 0.001$	$P = 0.002$
OSi levels	$P = 0.001$	$P < 0.001$	$P < 0.001$

DISCUSSION

Today, IBD is accepted as a worldwide disease with increasing frequency especially in the westernized societies of newly industrialized countries. The increase in the frequency of IBD results in note worthy challenges in health care systems that labor over the treatment of this disease with a complex etiology (18).

When compared to other animal models that were developed to aid in comprehending the pathophysiological mechanisms, the dextran sulfate sodium (DSS) induced colitis model is the most commonly employed model due to its simplicity, cost-effectiveness and similarity with clinical features of human IBDs (19). Hence, we used the DSS induced colitis model in our experimental study. Colitis was induced with 3%w/v DSS dissolved in drinking water for a week as described in the literature (20).

In this study, we measured rectal tissue calprotectin and lactoferrin levels as the markers of the presence of colitis and response to the treatment. Colitis group animals had significantly high rectal tissue calprotectin and lactoferrin levels while PRP and TQ group animals had significantly low rectal tissue calprotectin and lactoferrin levels. These results indicated that these molecules could make the treatment of DSS induced colitis easier.

OHP may be employed as a biochemical marker to understand the pathogenesis and treatment of disease (21). Heimesaat et al. stated that the synthetic OHP-containing collagen analogue (Gly-Pro-Hyp) 10 relieved

acute DSS colitis (22). In the present study, the animals that were administered PRP or TQ had lower rectal tissue OHP levels than animals with colitis. This result suggested that inflammation was decreased in animals treated with TQ or PRP.

Chronic intestinal inflammation causes the reactive oxygen species (ROS) to be excessively produced in patients with IBD. Oxidative stress due to imbalance between the overproduction of ROS and antioxidant activity has a crucial role in IBD. Reduced antioxidant levels in the inflamed mucosa due to oxidative stress results in chronic tissue damage. Intestinal tissue lipid peroxidation, disruption of intercellular junctions and leukocyte and neutrophil infiltration causes inflammatory process with cytokines (23-25). We assessed total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) in serum samples of all study groups. The PRP and TQ groups had higher TAS levels than the control group and it was the highest in the TQ group. Thus, the healing effects of PRP and TQ treatments on colitis model have proven with oxidative stress parameters.

Potent antioxidant and anti-inflammatory effects of TQ were reported in many inflammation-based experimental models such as encephalomyelitis, edema peritonitis and arthritis (26,27). Tekeoğlu et al. reported the healing effects of TQ on induced arthritis in rats (28). Maghoub found that TQ had defensive effects on acid induced colitis in rats (29).

In this study, we observed that rectal tissue calprotectin, lactoferrin and OHP levels were beneficial indicators in case of colitis. DSS induced colitis was a serious source of oxidative stress. TAS, TOS and OSI were useful parameters in determining the severity and level of response to treatment.

LIMITATIONS

The limitations of this study were limited sample size and lack of histological parameters. Although it has limitations, this study would give a force to further detailed studies using more advanced research methods to investigate the healing effects of PRP and TQ in the colitis model.

CONCLUSION

Both PRP and TQ had anti-inflammatory and oxidative stress-reducing effects in the DSS induced colitis model. Rectal tissue calprotectin, lactoferrin and hydroxyproline levels and oxidative stress markers such as TAS, TOS and OSI levels were in line with the literature. When contrasted with PRP, the healing effect of TQ was more distinct in the experimental colitis model.

Competing Interests: The authors declare that they have no competing interest.

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Ethical Approval: The study was endorsed by the Kirikkale University Commission of Scientific Research Projects, Kirikkale, Turkey (2014/89).

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