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Original Article

Effects of Saint John's Wort extract on intestinal injury and apoptosis

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Abstract

Aim: Reactive oxygen species play an important role in the pathophysiology of intestinal ischemia/reperfusion (I/R) injury by causing apoptosis. The present study aimed at exploring the possible protective effect of Saint John's Wort (SJW) extract in I/R injury.

Materials and Methods: Twenty four healthy male Wistar rats of 6 to 8 weeks old weighting averagely 200 to 250 g were studied. They were fed on standard rat food and drinking water at room temperature with 12/12 hours periods of day and night. SJW (300 mg/kg, peroral) or saline was given by oral route for 3 days prior to ischemia, 30 minutes before the ischemia, and just before reperfusion. To the rats in the control group, sham operation and application of saline were done. Levels of TNF-α, IL-1β and LDH were measured in the serum samples. In the small bowel tissue, levels of malonaldehyde (MDA) and glutathione (GSH) and activity of myeloperoxidase (MPO) and Na-K ATPase and level of caspase-3/β-actine and Bcl-2/β-actine were measured.

Results: I/R increased serum levels of LDH, TNF-α and IL-1β, small bowel level of MDA and MPO whereas it decreased level of GSH and activity of Na-K ATPase in the small bowel tissue. The level of caspase-3/β-actine increased while the level of Bcl-2/β-actine decreased in the I/R group compared to the controls. With the application of SJW, these values approached the control levels.

Conclusion: These results indicate that SJW recovers small bowel function by reducing oxidation injury during I/R.

Keywords: Apoptosis; intestinal injury; ischemia; reperfusion; Saint John's Wort

INTRODUCTION

Mesenteric ischemia is caused by a group of diseases with an extremely high rate of morbidity and mortality which are difficult to diagnose early and treat in spite of advanced medical science (1). Intestinal ischemia/reperfusion (I/R) injury may be caused by such conditions as repair of thoracoabdominal aortic aneurysm, cardiopulmonary bypass, small bowel transplantation, severe acute pancreatitis, trauma and shock. All these conditions may initiate a process ranging from ischemia with an injury of small bowel mucosa to necrosis (2,3). I/R injury may accelerate tissue necrosis progression and may cause cell injury, syndrome of multiple organ dysfunction and serious metabolic impairments due to generation of reactive oxygen species, bacterial translocation, the release of pro-inflammatory cytokines and inflammation (4,5).

Hypericum perforatum, so-called Saint John's Wort (SJW) is a perennial plant with yellow flowers raised in temperate and subtropical climates that has been used in the patients with skin and wound problems, nervous diseases, muscle pain, mood disorders such as depression or anxiety (6,7). Many studies have demonstrated its anti-inflammatory, anti-oxidant, anti-microbial effects and its positive impacts on wound healing (8-10).

Apoptosis is a physiological process by which dangerous or unnecessary cells originated from cellular stress or injury are eliminated (11). SJW has been shown to suppress intestinal epithelial apoptosis due to chemotherapy (12). The aim of the present study was to determine whether SJW recovered cellular injury from I/R by suppressing intestinal apoptosis.

The present study aimed at exploring the possible protective effect of Saint John's Wort (SJW) extract in I/R injury.

MATERIALS and METHODS

Experimental Animals

The present study was approved by the ethics board of experimental animals of Marmara University and

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conducted in the same center (protocol number: 28.2018. mar). Twenty-four healthy male Wistar rats of 6 to 8 weeks old weighting an averagely of 200 to 250 g were studied. They were fed on standard rat food and drinking water at room temperature with 12/12 hours periods of day and night.

The Groups

The rats were divided into three groups with eight animals as follows:

Group 1 (control group): Rats in Group 1 were given saline solution for three days prior to and 30 minutes before the experiment. SMA was isolated but not clamped. Blood and tissue samples were taken 60 minutes later.

Group 2 (I/R group): Before the I/R procedure, this group of rats was given saline solution for three days prior to and 30 minutes before the procedure. Blood and tissue samples were taken following a standard surgical procedure.

Group 3 (IR-SJW group): Before the I/R procedure, this group of rats was given extract of SJW (300 mg/kg, p.o. [by gavage]) for three days prior to and 30 minutes before the procedure. Blood and tissue samples were taken following a standard surgical procedure.

The Experimental Protocol

Following a period of fasting for 12 hours, laparotomy was made with midline incision under sterile conditions under anesthesia with intraperitoneal ketamine 50 mg/kg (Ketalar, Pfizer, Turkey) and xylazine HCl 10 mg/kg (Rompun, Bayer, Turkey). The small bowel was taken out of the abdominal cavity and the superior mesenteric artery (SMA) and its branches were dissected. SMA was occluded using microvascular clamps at the point where it originates from the aorta and subjected to ischemia for 45 minutes. After the ischemia, the occlusion was terminated and the skin and subcutaneous tissues were closed continuously with 3/0 prolene sutures. After 1 hour of reperfusion, the rats were decapitated and the experiment was terminated by taking blood and small intestinal tissue samples.

Serum Examinations

Serum level of TNF-α was studied with Enzyme Immunoassay (ELISA) method in E1x808 IU Ultra Microplate device using Biosource kit (ELISA, BioSource Europe S. A., Catalogue Number. KRC 3014; Nivelles, Belgium). IL-1β measurement was made with the ELISA method in the E1x808 IU Ultra Microplate device using the BioSource kit (ELISA, BioSourceCatalog No. KRC0011, Nivelles, Belgium). Serum LDH activity was determined spectrophotometrically using an automated analyzer (13).

Tissue Examinations

MPO measurement was made with ELISA method and Hillegas method in the E1x808 IU Ultra Microplate device using MPO ELISA kit (ELISA, Uscn Life Science Inc.; Catalogue Number: SEA601Ra, USA) (14). MDA measurement was made with the ELISA method in the E1x808 IU Ultra Microplate device using MPO ELISA kit (ELISA, Uscn Life Science Inc.; Catalogue Number: E0597Ra, USA). It was done with the method of Ohkawa et al. (15), based on the principle of measuring optical density at 532 nm of the color that MDA made with thiobarbituric acid in the acidic environment. Glutathione was measured with spectrophotometric measurement of the reactive substances from the tissue samples added to the Ellman solutions and 0.3 M Na2HPO4 by modifying the Ellman's method (16). The level of Na-K ATPase was determined according to the method defined by Lowry et al. by determining the amount of tissue Na-K ATPase with SIGMA 366-A kit (17). Level of caspase-3/β-actine and Bcl-2/β-actine were determined with the Bradford method by measuring protein concentrations in the homogenized samples (18).

Histopathological Examinations

The tissue samples were rinsed in tap water for at least 3 hours or overnight after they had been kept in 10% formaldehyde and dehydration was made with increasing alcohol concentrations (for 15 minutes in 70% alcohol, for 15 minutes in 90% alcohol, for 30 minutes in 96% alcohol, for 30 minutes twice in 100% alcohol, and for 30 minutes twice in 100% toluene). Then, they were kept overnight in paraffin at 60°C and embedded in paraffin blocks the day after. Following the blocking procedure, sections of 5-6 mm were made from the tissue samples and they were put on the slides and kept in toluene for 2 hours to remove their paraffin and then reduction to water was made with decreasing concentrations of alcohol (treated for 2 minutes in 100% alcohol, for 2 minutes in 90% alcohol, for 2 minutes in 70% alcohol). Then, they were kept in distilled water and in tap water for 10 minutes for empurpling after being treated with hematoxylin for 15 minutes. After being treated in eosin and additional water for 5 minutes, dehydration was made again with increasing concentrations of alcohol (for 2 minutes in 70% alcohol, for 2 minutes in 96% alcohol, for 10 minutes in 100% alcohol) and then they were rinsed with toluene twice (first bath: 5 minutes, second bath: 10 minutes). The slides were covered with entellan and examined at the level of light microscope (Olympus CX41) (19). The examinations were made in single-blind manner by a histopathologist not knowing the groups. In the small intestinal samples, mucosal injury was assessed histopathologically and intensity of the inflammation, epithelial structure, mitosis, and the Goblet cells were evaluated morphologically.

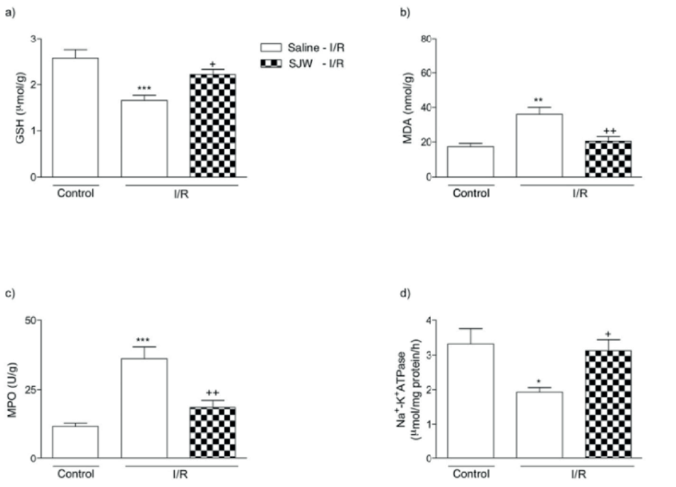
Statistical Analyses

GraphPad Prism 3.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for analyses of data from the present study. The obtained data were expressed as average ± standard deviation. The normal distribution of the variables was assessed with Shapiro-Wilk's test. Homogeneity of the variances of the groups was assessed by Levene's test. Histopathological values were compared using Mann-Whitney's U test, and other parameters were

compared with variance analysis (ANOVA) and Tukey's multiple comparison test. P-value was calculated by Bonferroni correction in the subgroup analysis (post-hoc) of the differences detected in the triple group analyses.

RESULTS

GSH level in the small intestinal tissue was 2.34 ± 0.2 nmol/g in the control group, 0.91 ± 0.1 nmol/g in the IR group and 1.92 ± 0.1 nmol/g in the IR+SJW group. GSH level was found to be lower in the IR group than in the control group and this difference was found to be significant ($p < 0.001$). GSH level was found to be higher in the IR+SJW group than in the controls and the difference was found to be significant ($p < 0.05$). MDA level in the small intestinal tissue was 41.6 ± 5.4 nmol/g in the control group, 82.7 ± 5.8 nmol/g in the IR group, and 51.2 ± 4.1 nmol/g in the IR+SJW group. MDA level was found to be statistically significantly higher in the IR group than in the controls ($p < 0.01$) and to be statistically significantly lower in the IR+SJW group than in the control group ($p < 0.01$). MPO level in the small intestinal tissue was found as 11.7 ± 1.8 U/g in the control group, 41.9 ± 4.8 U/g in the IR group, and 21.7 ± 3.4 U/g in the IR+SJW group. MPO level was statistically significantly higher in the IR group than in the control group ($p < 0.001$) and statistically significantly lower in the IR+SJW group than in the controls ($p < 0.01$). The level of Na-K ATPase in the small intestinal tissue was 2.44 ± 0.32 mmol/mg protein/h in the control group, 1.14 ± 0.20 mmol/mg protein/h in the IR group and 2.38 ± 0.15 mmol/mg protein/h in the IR+SJW group. The level of Na-K ATPase was statistically significantly lower in the IR group than in the control group ($p < 0.05$) and statistically significantly higher in the IR+SJW group than in the control group ($p < 0.05$) (Figure 1). Serum levels of LDH, TNF-α and IL-1β in all groups in the mesenteric IR model in the rats are given in Table 1.



SJW; Saint John's Wort, GSH; Glutathione, MDA; Malondialdehyde, MPO; Myeloperoxidase

Figure 1. Tissue GSH, MDA, MPO and Na-K ATPase values of all groups in mesenteric ischemia/reperfusion (I/R) models in rats

Table 1. Serum TNF-α, IL-1β and LDH values of all groups in mesenteric ischemia/reperfusion (I/R) models in rats. SJW; Saint John's Wort

	Control	IR	IR-SJW
TNF-α (pg/ml)	45.0 ± 2.7	78.7 ± 4.1***	53.7 ± 5.3+++
IL-1β (pg/ml)	313 ± 9	387 ± 10 ***	343 ± 9 +
LDH (U/L)	1275 ± 177	2931 ± 263***	1971 ± 252 +

Mean± Standard error, ***p<0.001 vs control group,+ p<0.05, +++ p<0.001 vs I/R group

In the histopathological examinations epithelial structure, Goblet cells and glandular structures were observed to be normal in the control group (Figure 2a). In the IR group, small intestinal epithelial cells erupted severely and glandular structures were observed to be impaired (Figure 2b). In the IR+SJW group, small intestinal epithelial structure was seen to recover and swollen structure of the Goblet cells was remarkable (Figure 2c). Rate of Bcl-2/β-actine and caspase-3/β-actine activities in all groups in the mesenteric ischemia/reperfusion (IR) model in the rats are given in Table 2.

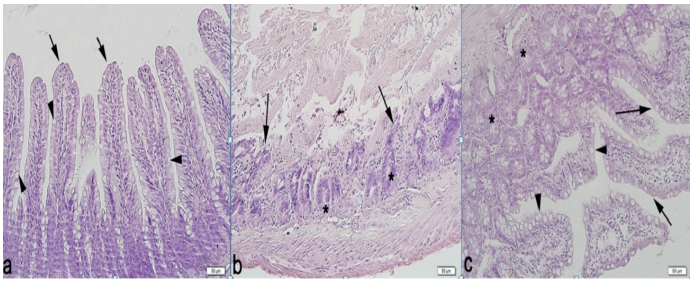


Figure 2. (a)The control group, smooth epithelial structure (arrows) and the Goblet cells (arrow heads); (b) The ischemia group, severely erupted epithelium (arrows) and impaired glandular structures (*); (c) The IR+ SJW group, re-formed smooth epithelial (arrows) and Goblet cells (arrow heads) and glandular structures (*)

Table 2. Tissue Bcl-2/β-actine and caspase-3/β-actine values of all groups in mesenteric ischemia/reperfusion (I/R) models in rats.SJW; Saint John'sWort

	Control	IR	IR-SJW
Bcl-2/β-actine (relative intensity)	0.83 ± 0.01	0.78 ± 0.02**	0.89 ± 0.01+++
Caspase-3/β-actine (relative intensity)	1.24 ± 0.03	1.27 ± 0.01***	1.16 ± 0.02+++

Mean± standarderror, *p<0.05, **p<0.01, ***p<0.001 Comparisons according to the control group, + p<0.05, ++ p<0.01, +++ p<0.001 Comparisons according to the IR group

DISCUSSION

Acute mesenteric ischemia (AMI) is a chronic or emergent vascular pathology of the small or large intestine that causes acute abdomen. It has a high rate of mortality due to difficulties and delays in diagnosis despite medical and surgical treatment (20).

The intestinal ischemic injury occurs due to unmet metabolic needs of the tissue because of vascular circulatory failure and the inability to remove waste metabolites from the tissue. Acute cellular swelling, interstitial edema, cellular dysfunction and at the end cell death occur due to ischemia. Re-start of the blood flow, in other words reperfusion, is required for recovery of the intestinal tissue undergoing ischemia. Although being beneficial, reperfusion of the ischemic organ or tissue may cause injury at the cellular level to progress. Reperfusion of the ischemic tissue may cause more severe injury than ischemia alone causes (21,22). The most important factor responsible for tissue injury due to I/R is reactive oxygen species. They cause membrane injury, DNA destruction, activation of the proteases, lipid and protein peroxidation and cell death resulting in apoptosis and necrosis (23).

In rats, experimental models have been developed in order to investigate I/R injury and the efficacy of various agents in preventing this injury. In these models, SMA was explored at the point where it originated from the aorta and was subjected to occlusion in order to create mesenteric ischemia. There are differences between the studies in the duration of I/R. Intestinal I/R model is a frequently used experimental model. When many studies are reviewed, one can see that conflicting results exist between them.

TNF-α and IL-1β are cytokines released from the macrophages that have important roles in the regulation of the inflammatory reaction and inflammation (24). In the study by Akyuz et al. (25) the inflammatory response was found at a higher level in the rats undergoing I/R. It was noted that reasons for this might be functional results of pro-inflammatory cytokines such TNF-α and IL-1β, weakened intestinal barrier function, impaired intestinal continuity and altered mucus release. In our study, concentrations of pro-inflammatory cytokines of TNF-α and IL-1β were found to be significantly higher in the rats undergoing I/R compared to the IR+SJW and control groups. These high levels were prevented with the addition of SJW compared to the IR group, indicating that activation and infiltration of the neutrophils playing a triggering role in tissue injury was prevented by SJW and thus inflammatory response reduced.

Gluthatione is an anti-oxidant used in measuring oxidative stress. It protects the cells against oxidative injury by reacting with reactive species and peroxides and converting them to harmless products (26). Results of the present study indicated that SJW increased GSH concentration in the small intestinal tissue in the rats and protected the intestine of the rats against oxidative injury. MPO level in the small intestine is an indicator of neutrophil infiltration and acute inflammation. MPO enzyme released from the neutrophils increases injury in the tissue and causes the excessive formation of the reactive species (27). In our study, neutrophil activation and increase in the MPO level initiating the oxidative mechanism in the intestinal injury was prevented by giving SJW.

In addition to beneficial functions in the organism such as playing a role in phagocytosis, the reactive species exert toxic effects on the cellular structures such as lipids when they are present at excessive amounts (28). These harmful species reacting with double-bonds of the polyunsaturated fatty acids lead to the formation of lipid hydroperoxides. MDA is a frequently used indicator of injury because it is known to be a secondary oxidation product of the polyunsaturated fatty acids and its level is known to elevate in many diseases considered to be related to reactive oxygen species injury (29). Our study indicated that SJW prevented elevation of MDA level playing an important role in intestinal injury in I/R and being an important indicator of lipid peroxidation. Na-K ATPase is an important phospholipid-dependent membrane enzyme playing a key role in cellular structure and physiology by providing sodium and potassium gradient that is of importance in the absorption of the food in the basolateral membrane of the enterocytes (30). Reactive oxygen species are released in intestinal injury due to I/R inhibit the activity of membrane Na-K ATPase (25). In the present study, remarkably decreased Na-K ATPase activity in the IR group was prevented in the group given SJW-IR, indicating that SJW is helpful in protecting normal enterocyte structure and physiology.

Apoptosis is well known to have a role in protecting structural continuity during cellular destruction in tissue injury following I/R (31). Oxidative stress manages apoptosis via lipid peroxidation while apoptosis is blocked by the anti-oxidants (32). Bcl-2 is produced as an anti-apoptotic protein through caspase-dependent, capase-independent and anti-oxidant mechanisms. Studies have shown that Bcl-2 protects the cells against ischemia-reperfusion injury (18,33). In our study, the fact that caspase-3 increased and Bcl-2 decreased in the I/R group was an indication of oxidative stress and SJW was observed to reverse it.

Histologically, enterocyte injury and infiltration of the inflammatory cells are observed in the intestinal tissue following I/R (34). In the present study, intestinal mucosa of normal morphology was observed in the tissue specimens of the experimental animals in the control group. In the rats undergoing I/R, severely impaired glandular structures were observed along with cellular eruption which was one of the typical histological findings of ischemia. The epithelium was erupted considerably and the inflammatory cells were found to invade the stroma of the villi. After giving SJW to the rats, the epithelial and Goblet cells were observed to regenerate and increased mitosis was seen. SJW extract reversed intestinal injury and stimulated intestinal recovery by increasing cellular infiltration in mucosal injury following I/R.

CONCLUSION

Over the last 20 years, many experimental studies have been conducted in order to prevent ischemia and reperfusion injury before it begins or to minimize reperfusion injury when ischemia occurs. However, an

agent with clinically proven efficacy hasn't been found yet. We consider that possible effects of SJW to reduce I/R injury should be investigated in future studies with different dose and duration adjustments on the SJW extracts with anti-oxidant and anti-inflammatory features on the experimental animals or humans.

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

Financial Disclosure: There are no financial supports.

Ethical Approval: The ethical consent of this study was obtained (Marmara University Animal Experimental Local Ethics Committee 2018/28.2018.mar).

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