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Gastroprotective Effect Of *Cyperus rotundus* Extract against Gastric Mucosal Injury Induced by Ischemia and Reperfusion in Rats

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Abstract: *Cyperus rotundus* (CR) is widely used as a traditional folk medicine for treating various diseases such as gastrointestinal disorders, inflammatory and infectious diseases in Middle Eastern countries. However, the protective effects of CR extract on gastric mucosa has not been well defined. Thus, the aim of the present study was to investigate the protective effects of CR on gastric mucosal damage induced by ischemia and reperfusion in rats. Ischemia/reperfusion model was designed as of 30 min ischemia followed with 60 min reperfusion by clamping the celiac artery. The CR extracts were given at the doses of 100 or 200 mg kg^{-1} for preventing postischemic gastric mucosal injury. The study was carried out on equal five groups (n = 6) namely as follows: group I (sham, control group), group II (untreated ischemia control group), group III (treated ischemia group) was treated with 200 mg kg^{-1} CR, group IV (treated ischemia group) was treated with 100 mg kg^{-1} CR and group V (treated ischemia/reperfusion group) was treated with 200 mg kg^{-1} CR. Antioxidant enzymes activity such as Malondialdehyde (MDA) and glutathione-peroxidase (GSH-Px) were measured in the gastric tissue. Histopathological sections were examined for ischemic injury. The mean ulcer index of rats treated with 200 and 100 mg kg^{-1} CR were significantly lower ($p<0.05$) than that of control rats. The activities of GSH-Px and MDA were significantly affected ($p<0.05$) by treatment of CR in group III and group IV. These results indicate that both doses of CR extract have gastroprotective effect against acute gastric mucosal lesions induced by ischemia/reperfusion.

Key words: Ischemia/Reperfusion, *Cyperus rotundus*, MDA, GSH-Px, gastroprotection

INTRODUCTION

The stomach is one of the most sensitive tissues against various factors which produce gastric mucosal damage (Wada *et al*., 1995) including systemic events such as thermal stress or application of various irritants that are commonly named gastric mucosal barrier breakers (Brzozowski *et al*., 2000). It has been reported that reactive oxygen (ROS) plays important roles in the pathogenesis of inflammation, diabetes mellitus, apoptosis and ischemia/reperfusion (I/R) (Ueda *et al*., 1989). The tissue damage or death was caused by ischemia itself, but further injuries can occur while oxygen is reintroduced to the tissue injury.

Oxidative stress results from the imbalance between oxidant and antioxidant states, or by the excess of oxidant or depletion of antioxidants in the metabolism events. A considerable mass of recent evidence and the major role is the ischemia and reperfusion. There are many factors that cause (I/R) injury, the increase of ROS production (Schoenberg and Beger, 1993; Kurokawa and Takagi, 1999) is one of the factors. During ischemia, ATP is catabolized to hypoxanthine and is accumulated within the tissue. After reperfusion xanthine oxidase instrumental for formation of the reactive oxygen metabolites to form superoxide (O_2-) and hydrogen peroxide (H_2O_2) (Krenitsky *et al*., 1974).

Gastric mucosal damage has been performed in laboratory animals by clamping the celiac artery generating I/R circumstances (Yoshıkawa *et al*., 1989), which is accompanied by the formation of free radicals and Reactive Oxygen Species (ROS) production (Yoshikawa *et al*., 1991). Free radicals play an important role particularly in injury and ulceration of stomach or

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erosive lesions of the gastrointestinal mucosa (Tanaka *et al*., 1993), due to the damage of many biological systems and the presence of polymorph nuclear leucocytes is indicated as the primary origin of free radicals (Mansour, 2000).

Previous studies have been showed that the herbal origin antioxidants can reduce the gastrointestinal damage by inhibiting free radicals produced by I/R injury. Therefore, treatments with antioxidant and free radical scavengers such as vitamin E, vitamin C and herbal antioxidants were found to decrease the I/R-induced gastric mucosal damage. *Cyperus rotundus* (CR) extract is a folk medicinal herb containing many polyphenolic compounds, flavonoids and phenolic acids, which may act as antioxidative potential. The phytochemical studies have been showed that the major chemical component of CR is essential oil, terpenoids and sesquiterpenes (Ohira *et al*., 1998; Kilani *et al*., 2005a). CR is widely used as a traditional folk medicine for treating various diseases such as gastrointestinal disorders, inflammatory and infectious diseases in Middle Eastern countries (Gupta *et al*., 1971). So, recently studies were subjected to its pharmacological investigations revealed their antioxidant properties (Bhattarai, 1993; Thebtaranonth *et al*., 1995). However, the gastrointestinal mucosal protective activity of CR extract was not well defined. Hence, in this study, a model of ischemia/reperfusion was used to assess the antioxidant effects of *Cyperus rotundus* extract on gastric mucosal injury.

MATERIALS AND METHODS

The research works were done between January-June, 2009.

Animals grouping and experimental design: The study was approved by Ethics Board of Harran University, School of Medicine in accordance with the Helsinki Declaration prior to initiation of the study. Thirty male Wistar albino rats weighing 225±25 g. were purchased from Ankara, Turkey Hıfzıssıhha Institute in this study and ischemia/reperfusion model was designed as of 30 min ischemia followed with 60 min reperfusion by clamping the celiac artery. Rats were kept in Biology Department for two weeks for acclimatization prior to the experiment; groups were housed individually in plastic cages with stainless steel covers in well conditioned rooms (22±2°C) with a 12 h light/dark photoperiod. All rats had free access to water and Rodent chow (Ankara Yem Sanayii) and were kept in individual cages with raised base to prevent coprophagy. Rats were fasted for 18 h prior the experiments but allowed free access to water. The study was carried out on equal five groups (n = 6) namely as follows: group I (sham control group) was subjected to abdomen operation, group II (untreated ischemia control group) was subjected to ischemia of celiac artery, group III (treated group) was treated with 200 mg kg^{-1} *C. rotundus* by orogastric tube and subjected to ischemia, group IV was treated with 100 mg kg^{-1} *C. rotundus* by orogastric tube and subjected to ischemia and group V was treated with 200 mg kg^{-1} *C. rotundus* by orogastric tube and subjected to ischemic period followed by reperfusion.

***Cyperus rotundus* extract preparation:** *Cyperus rotundus* rhizomes were purchased fresh from Sanliurfa herbal market and identified by specialist in taxonomy, voucher specimen was kept in the herbarium of the Medical resources of Biology Department of College of Science for further investigation. The rhizomes were well cleaned to remove all debris, dried and powdered with electrical mill and subsequently macerated in 50% MeOH for 24 h at room temperature, filtered with two layers of fine muslin textile. The extract was obtained by rotary evaporation under negative pressure at 60°C to discharge the methanol then lyophilized with lyophilization apparatus.

Surgery protocol: A protocol for investigation of the protective effects of CR on gastric mucosal damage induced by ischemia and reperfusion in rats was introduced. I/R rats were performed under anesthesia with intramuscular injection of 50 mg kg^{-1} ketamine and 5 mg kg^{-1} xylazine. The bottom wall of each rat was cleaned with antiseptic solution and opened with 3-4 cm midline incision was applied to express the stomach and corresponding vascular supply. Orogastric tube was advanced into the stomach via the mouth for administration the extract. The stomach was exposed; the esophageal and pyloric ends were occluded with bulldog clamps. The celiac artery was declared, clamped and 100 mM HCL (1 mL/100 g) was introduced into the stomach using a syringe fitted with fine insulin needle to maintain acid levels during the ischemia experiment and reperfusion. At one hour of ischemia, the acid was withdrawn with a syringe fitted with fine needle and celiac artery clamp was removed and followed by another one hour to realize reperfusion. At the end of the experiment all rats were sacrificed by dislocation.

Determination of MDA and GSH-Px activities in gastric tissue: The second half of the stomach about (0.5 g) was ice-cooled, homogenized in 5 mL phosphate buffer (pH 7.4) and the homogenate, divided into two portions.

The first portion was used to determine the reduced glutathione-peroxide (GSH-Px) content according to the method of (Paglia and Valentine, 1967) using a spectrofluorometric method with 350 nm excitation and 420 nm emissions. The second half was used for determination of lipid peroxidation level (MDA) content by measuring the thiobarbutiric acid (TBA) reaction according to the method described (Jo and Ahn, 1998) using spectrofluorometer with 520 nm excitation and 550 emissions.

Histopathologic evaluation: Histopathological sections of gastric tissue were examined for ischemic injury as evidenced by inflammation and necrosis of villus-crypt and destruction of the villus architecture. The stomach of each rat was removed; cutted along the greater curvature, rinsed in ice-cooled saline and examined macroscopically for gastric damage, the length and width of the ulcers were measured and the sum of the areas of damage was calculated. Results were expressed as total area of lesions (mm^2). The severity of mucosal ulceration was determined according to (Parks and Granger, 1986). The grade of the lesions was scored as follows: no injury = 0, 1-2 mm = 1, 3-4 mm = 2, 5-6 mm = 3. The stomach then divided into two equal parts, one half was fixed in 10% neutral formalin for 24 h. After routine tissue processing, the stomach was sectioned in 5 μm thick and stained with Hematoxyline Eosin for histopathological evaluation. The second part of the stomach was used for biochemical analyses which were explained below.

Statistical analysis: Values were performed using the SPSS for windows and probability value of 0.05 and less was accepted as statistically significant. All data were expressed as the Mean±SD and analyzed using Analysis of Variance (ANOVA) test. The Kruskal-Wallis, one-way analysis of variance by ranks was used for a simultaneous statistical test of pathologic score for *C. rotundus* + I/R groups. Mann-Whitney test was used for independent samples.

RESULTS AND DISCUSSION

Determination of antioxidants activity in gastric tissue: Treatment with CR in ischemia groups (group III and group IV) were resulted in significant decreased of the tissue MDA levels ($p<0.05$) and increased GSH-Px levels ($p<0.05$) when compared with control untreated ischemia group (Table 1). A significant increase in the MDA activity was occurred after ischemia alone, which was significantly decreased by administration with the extract of *Cyperus rotundus*, but not by group V (treated ischemia/reperfusion group). Exposure of rats to ischemia were resulted in gastric mucosal damage and significantly decreased the levels of GSH-Px and significantly increased the levels of MDA when compared with sham control group (Fig. 1a, b and Table 1). However, significant decreased of the tissue MDA levels and increased GSH-Px levels by treatment with Cyperus rotundus in ischemia groups was were accompanied by decrease in the formation of gastric lesions (Table 1 and Fig. 1c, d). Treatment with CR in ischemia/reperfusion group was showed an elevated MDA content, an effect which was not stopped by CR extract pre-treatment 1 hour after ischemia. On other hand, it was showed the same effect of ischemia action. However, after one hour reperfusion, both substances restored MDA level (Table 1). Regarding GSH-Px content, the effect of CR extract was more pronounced at reperfusion period as compared with ischemia effect.

Table 1: Effects of CR extract on epithelial tissue of stomach according to the groups

Groups	Microvillous injury	Krypt injury	Mucosal injury
Group 1 (sham control)	0	0	No
Group 2 (ischemia control)	3+	2+	Moderate
Group 3 (CR+Ischemia 200 mg kg^{-1} b.wt.)	1+	0	Mild
Group 4 (CR+Ischemia 100 mg kg^{-1} b.wt.)	1+	1+	Mild
Group 5 (CR.+I/R)	2+	1+	Mild

Histopathological properties: Histopathological sections were examined for ischemic injury as evidenced by inflammation and necrosis of villus crypt epithelial cells and destruction of the villous architecture. In the CR + ischemia group, mild microvillus injury characterized by epithelial cells disorgamization was present (Table 2, Fig. 2, 1c, d). These findings were not seen in crypt mucosa cells (Table 2, Fig. 3). However, in *C. rotundus* + I/R group there are extensive disorganization, sloughing, apoptosis and necrosis were present both in microvillus and crypt mucosal epithelium (Table 2 and Fig. 1e). On other hand, the histopathological changes revealed that ischemia was able to induce varying degrees of the histopathological changes in the tissues. Moderate (2+) to severe (3+) injuries were observed in the stomach of the control group, whereas no effect (0) to (1+) injuries were observed in ischemic group treated with 200 and 100 mg kg^{-1} CR extract, respectively (Table 2).

The stomach mucosal barrier protects the stomach against harmful and aggressive factors, gastric mucosal injury has been associated with many factors including ethanol exposure (Whittle, 1993), trauma (Lucas *et al*., 1971), administration of nonsteroidal anti-inflammatory

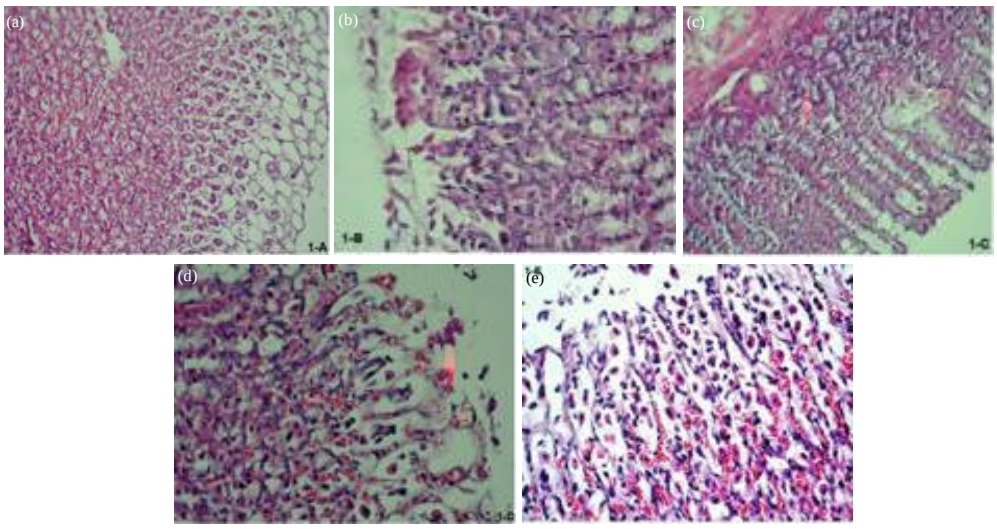

Fig. 1: Photomicrograph examination under light microscope in stomach stained with H and E. (a) Histological examination of gastric mucosa under light microscope in control, group I (orally pretreated with 1 mL normal saline) sham group. Illustrated normal gastric mucosal, (b) control untreated group II subjected to 30 min ischemia and 60 min reperfusion micrograph indicate epithelial exfoliation mucosal hemorrhage and inflammatory cell, (c) micrograph of group III orally pretreated with 200 mg kg^{-1} *C. rotundus* extract and subjected to 30 min ischemia represent clearly medicated gastric injury, (d) micrograph of group orally pretreated with 100 mg kg^{-1} *C. rotundus* extract and subjected to 30 min ischemia represent clearly medicated gastric injury and (e) micrograph of group IV orally pretreated with 200 mg kg^{-1} *C. rotundus* extract and subjected to 60 reperfusion following 30 min ischemia represent abundant neutrophils

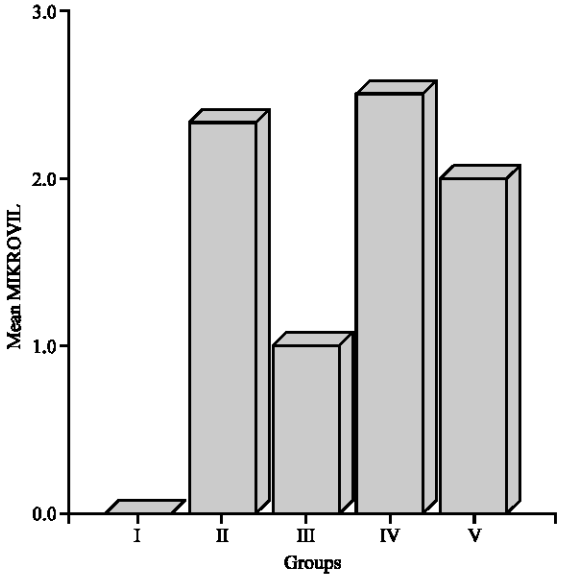

Fig. 2: Effects of CR extract on the villus epithelial tissue of stomach between the groups

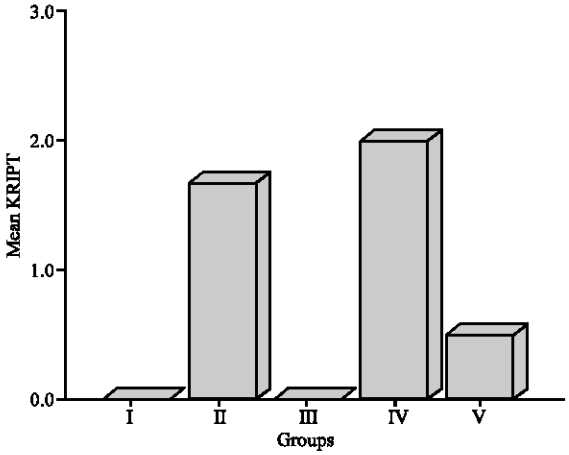

Fig. 3: Effects of CR on the crypt mucosa cells of stomach between the groups

chemicals and association with period of ischemia and reperfusion (Perry *et al*., 1986; Perry and Wadhwa, 1988). When the tissue is subjected to ischemia a serial of biochemical is initiated that finally may lead to necrosis and cellular dysfunction. Moreover, oxygen derived radicals play a major role in the pathogenesis of ischemia/reperfusion injury (Wada *et al*., 1998) by clamping the celiac artery causes an immediately decrease in the gastric blood flow (Wada *et al*., 1995) with the perspective of corrosive lesion one hour after reperfusion

Table 2: Effect of CR 200 and 100 mg kg^{-1} body weight on the mucosal content of GSH-Px and MDA according to all groups

	Parameters	
Groups	GSH-Px	MDA
I (Sham control)	125±0.3	3.1±0.5
II (Ischemia control)	102±0.3	3.5±0.7
III (CR+Ischemia) 200 mg	118±0.2	3.1±0.6
IV (CR+Ischemia) 100 mg	116±0.1	3.1±0.2
V (CR+I/R) 200 mg	114±0.5	3.5±0.6

(Perry *et al*., 1986; Perry and Wadhwa, 1988; Yoshikawa *et al*., 1991; Andrews *et al*., 1994). Although, no single process can be identified as the critical event in ischemia-induced damage, many studies indicate that, depletion of cellular energy accumulation will guide to toxic metabolites may contribute to cell death. However, recent studies have demonstrated that most injuries take place during reperfusion rather than ischemia and the injury can be reduced significantly by pretreatment with free radicals scavengers such as catalase, glutathione peroxidase, allopurinol or melatonin (Zimmerman and Granger, 1994; De La Lastra *et al*., 1997). These investigations implicate oxygen free radicals in the pathogenesis of this irregular event.

Therefore, I/R injury to the stomach causes production of the reactive oxygen species which are known to play an important role in stomach epithelia damage which subjected to increase lipid peroxidation in many organs as in liver, lung and stomach, it was evidenced by significantly increased MDA levels and other free radicals (Zimmerman and Granger, 1994). Recent evidence has suggested that GSH-Px may play a significant role in the maintenance of the mucosal integrity. The ischemia state is associated with increase in tissue oxidative stress due to the increase in the production of free radicals, which might be reflected in the changes in the activities of oxidant and antioxidant enzymes. Moreover, pretreatment with antioxidant scavengers, reported to minimize gastric injury (Matsumoto *et al*., 1993).

The *C. rotundus* is a traditional herbal medicine used widely as anti bacterial, antimalarial, sedative, antispasmodic and relieve diarrhea, (Bhattarai, 1993; Thebtaranonth *et al*., 1995; Zhu *et al*., 1997). Previous studies have been showed that *C. rotundus* and its ingredient compounds inhibit the free radical generation and act as antioxidant and free radical scavengers and it has also been demonstrated that treatment with *C. rotundus* inhibits the generation of superoxide radicals (Oladipupo and Oyedeji, 2009; Ali *et al*., 2008; Kilani *et al*., 2005a, b; Seo *et al*., 2001) and recent evidence suggested that GSH-Px may play a significant role in the elimination of H_2O_2 and lipid peroxidation in the gastric mucosa cell. Thus, inhibition this enzyme may result in the accumulation of the H_2O_2 with subsequent oxidation of lipids. The present study has showed that MDA levels were significantly increased and the GSH-Px levels were decreased with treated both doses of CR in ischemia groups when compared with untreated ischemia control group which confirmed with the histopathological evaluation of gastric tissue (Table 1, 2 and Fig. 1). These findings are in agreement with a recent study by Kilani *et al*. (2005a, b). Flavonoids are the major component in the volatile oil of CR which are able to inhibit the oxidants and to protect the cell membrane by the restoration of Lactate Dehydrogenase (LDH) when compared treatment with high and low dose of *C. rotunds*. Gastric mucosal injury induced by ischemia-reperfusion was mild just after ischemia but severe after reperfusion. Thiobarbituric Acid (TBA)-reactive substances, which are an index of lipid peroxidation (Yagi, 1976), were not increased in the gastric mucosa after ischemia alone but markedly increased after reperfusion. On the other hand, this study assess the effects of oxygen free radical scavengers, both gastric mucosal injury and increase of TBA-reactive substances were inhibited by the treatment of free radical scavengers. *C. rotundus* extract is an effective free radical scavenger showing antioxidant activity against reactive oxygen production and protecting the damage caused by free radicals. Therefore, the extract is useful in diseases in which free radicals are involved as different stomach ulcers, gastrointestinal disorders and anoxia (Zhu *et al*., 2000). In the present study, *C. rotundus* had a marked effect on free radical production and protective action against I/R induced stomach injury. This result is supported with biochemical and histpathological findings which are considerable as there is no previously reported study on the effect of *C. rotundus* on I/R-induced stomach injury. The gastroprotective effect of *C. rotundus* could be attributed to the improvement of stomach ulcer or antioxidant status of the animals (Zhu *et al*., 1997) of the presence of free radical scavenging substances such as flavonoid oil (Kilania *et al*., 2008).

In conclusion, our study indicate that both doses of *Cyperus rotundus* extract have gastroprotective effect against acute gastric mucosal lesions induced by ischemia/reperfusion which may be related to its antioxidant effect.

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