The Role of *Urtica Dioica* (Urticaceae) in the Prevention of Oxidative Stress Caused by Tourniquet Application in Rats

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CETINUS, E., KILINC, M., INANC, F., KURUTAS, E.B. and BUZKAN, N. The Role of *Urtica Dioica* (Urticaceae) in the Prevention of Oxidative Stress Caused by Tourniquet Application in Rats. Tohoku J. Exp. Med., 2005, 205 (3), 215-221 — Tourniquets are used in extremity surgery and provide a relatively bloodless field, thereby minimizing blood loss and helping identify the vital structures. However, they may cause an ischemia-reperfusion injury with potentially harmful local and systemic consequences. Many therapeutic effects such as diuretic, natriuretic, hypotensive, anti-rheumatic, anti-prostatic, and in-vitro antioxidant effects of the *Urtica dioica* (UD) have been determined. In the present study, we aimed to investigate the potential role of UD plant for prevention of oxidative stress in muscle tissues generated by tourniquet application in rats. Wistar rats were used in this study. The UD extract or 1.15% KCl aqueous solution, in which UD leaf samples were homogenized, was given to each group of eight rats once a day for 5 days through an intraesophageal canule. No treatment was applied to untreated group. Tourniquets were applied to the left posterior limb of rats for 1 or 2 h followed by a reperfusion period of 1 h. After the ischemia and reperfusion, the rats were killed with a high dose of anesthetic drug, and malonyldialdehyde (MDA) levels were measured in their tibialis anterior muscles. Basal MDA levels were obtained from tibialis anterior muscles of 8 control rats, which were not exposed to ischemia. MDA levels were lower in the UD-treated rats than those in untreated and KCl-treated rats after either 1 or 2 h of ischemia and 1 h reperfusion. These results indicate that UD has a potential antioxidant effect on ischemic muscle tissues.

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The pneumatic tourniquet is frequently used in orthopedic surgery. This procedure has been universally accepted as an essential instrument for bloodless operating field, because it decreases the amount of blood loss and makes the operation time shorter. However, it causes functional and microscopic changes in the distal neuromuscular tissues with respect to the duration of ischemia...
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KCl. Then, the homogenate was centrifugated at 3,000 g for 20 min. Finally, the supernatant was recovered and stored at 4°C until it is used (Pretsch et al. 1988).

Animals and experimental design

Fifty-six male Wistar rats (Kahramanmaras Sutcu Imam University, School of Medicine, Animal Breeding and Research Laboratory) weighing 170 to 342 g, were used in this study. The rats were kept in standard cages, with one rat in each cage at 21 ± 1°C during the study. The animals were maintained a 12 h light/dark cycle, and fed with standard pellet diet and tap water.

To determine the basal MDA levels of muscle, tibialis anterior muscle specimens were taken from 8 rats which had not been exposed to ischemia (Control group). Remaining 48 rats were randomly divided into three main experimental groups. Each group consisted of 16 animals. These were as follows; (i) the rats which were not given treatment (Untreated group), (ii) the rats which were treated with 1.15% KCl aqueous solution (2.5 ml/100 g body weight/day) (KCl-treated group), (iii) the rats which were treated with UD extract (500 mg/100 g body weight of the UD extract in 2.5 ml of KCl aqueous solution) (UD-treated group) (Table 1).

The UD extract or 1.15% KCl aqueous solution was given to rats once a day through an intraesophageal capsule for 5 days before the experiment.

On the experiment day, before the tourniquet ischemia, rats were anesthetized intraperitoneally with 90 mg/kg body weight of ketamine-HCl (Ketalar, Pfizer Warner Lambert, Turkey) and 3 mg/kg body weight of xylazine (2% Rompun solution, Bayer AG Leverkusen, Germany), and this was repeated throughout the ischemic period if necessary.

Rubber tourniquets (1 × 8 × 100 mm) used by Nylander et al. (1989) were applied to left posterior thighs of rats as proximal as possible for 1 or 2 h followed by a reperfusion period of 1 h. After the reperfusion period, the rats were sacrificed with an over dose of ketamine - xylazine. The tibialis anterior muscles were excised from the legs of the rats. All specimens were washed with 0.9% NaCl to remove hematoma, and then dried out. The tibialis anterior muscles were weighed as 402.3 ± 119.4 mg (min. 190-max. 650 mg), individually placed in plastic bottles and stored at -20°C for pending biochemical analysis.

Biochemical analysis

The tibialis anterior muscle MDA level was mea-
sured according to Ohkawa’s method (Ohkawa et al. 1979). Protein was measured according to the method of Lowry et al. (1951). Results were expressed in nanomoles per milligram protein (nmol / mg prot.).

Statistical analysis

Experimental results concerning this study were expressed as mean ± s.d. (X ± s.d.). Mann-Whitney’s U-test was used for statistical analysis of the data, and \( p < 0.05 \) was considered significant.

RESULTS

The changes in the levels of MDA in the tibialis anterior muscles of the untreated, KCl-treated and UD-treated rats have been summarized together with the values in the control tibialis anterior muscles (Table 1). There were significant differences in the MDA values between tibialis anterior muscle samples of the control group and muscle samples of the UD-treated, KCl-treated, and untreated groups (\( p < 0.001 \)). MDA level of muscle in the UD-treated group was lower than those of the KCl-treated and untreated groups in either 1 or 2 h ischemia (\( p < 0.01 \)).

Fig. 1 shows MDA levels of tibialis anterior muscles in the UD-treated, KCl-treated, and untreated groups after 1 h ischemia-reperfusion and also shows control group. Muscle MDA level was significantly lower in the UD-treated group (1.84 ± 0.08 nmol/mg prot) than those in the KCl-treated group (4.42 ± 1.85 nmol/mg prot) and untreated group (4.47 ± 1.49 nmol/mg prot) \( (p < 0.01) \). The muscle MDA value in control group (0.35 ± 0.26 nmol/mg prot) was significantly lower than those in all the experimental groups \( (p < 0.001) \).

DISCUSSION

The ischemic model used in the present study induces a temporary ischemia of the extremity. Nylander et al. (1989) have shown that this ischemic model causes a total ischemia in the tourniquet applied leg. Following ischemia and reperfusion, ROS are found to be formed and to have a deleterious effect in relation with ischemia-reperfusion injury. Accordingly, ROS are believed to be one of the causes of cell death in where they initiate a chain reaction leading to lipid peroxidation with a consequence of cell membrane damage. Relevantly, cell injury associated

| Table 1. The mean MDA levels of the tibialis anterior muscles (nmol/mg protein). |
|-----------------|----|-----------------------------|
| Group           | n  | MDA level (± S.D.)          |
| Control         | 8  | 0.35 ± 0.26 **              |
| Untreated       |    |                             |
| 1 h I-1 h R     | 8  | 4.47 ± 1.49                 |
| 2 h I-1 h R     | 8  | 4.73 ± 1.60                 |
| KCl-treated     |    |                             |
| 1 h I-1 h R     | 8  | 4.42 ± 1.85                 |
| 2 h I-1 h R     | 8  | 4.39 ± 0.40                 |
| UD-treated      |    |                             |
| 1 h I-1 h R     | 8  | 1.84 ± 0.08 *               |
| 2 h I-1 h R     | 8  | 1.73 ± 0.13 *               |

I, ischemia; R, reperfusion.

*The MDA values in UD-treated group are significantly lower than those in KCl-treated and untreated groups \( (p < 0.01) \).

**The MDA values in the control group are significantly lower than those in the ischemia-reperfusion groups \( (p < 0.001) \).
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with free radical formation occurs either in the situation of overwhelming scavenging systems or in ischemic states depleting protective antioxidant systems (Seyama 1993). Lipid peroxidation, which could be demonstrated by MDA levels, is one of the best known triggers for cytological damage. Higher levels of MDA indicate higher concentrations of free radicals (Sugino et al. 1987; Karakaya et al. 2001). In accordance with the accumulated data informed above, the MDA levels in the untreated group were detected to be increased when compared with those of basal group in the present study. The elevated level of MDA determined in the ischemia-reperfusion group supports the notion that ROS are involved in this pathologic condition.

After the realization that ROS may have a harmful role in ischemia-reperfusion injury, many investigations have revealed a variety of mechanism(s) and/or pharmacological manipulations in preventing such an injury. These include cellular antioxidants like catalase, superoxide dismutase, glutathione peroxidase, and glucose-6-phosphate dehydrogenase, pharmacological antioxidants like allopurinol, alpha-tocopherol, vitamin C, and vitamin A, and physical antioxidants like hypothermia (Irving and Noakes 1985; Ikemoto et al. 1988; Repine 1991; Swanson et al. 1991; Appell et al. 1997; Erdoğan et al. 1999; Molyneux et al. 2002). Bearing in mind that UD could be a suitable candidate as an antioxidant agent, the present study was urged so as to investigate the potential beneficial effect of UD in preventing the increase of MDA levels. Such effect would apparently be associated with a decrease in the amounts of generated ROS since MDA has previously been accepted as an indicator of free radical formation. The previous studies conducted in relation with the remarkable effects of UD were the rationale of the present study to be designed. Thus, Pieroni et al. (2002) studied antioxidant activity of some non-cultivated vegetables using diphenyl-2-picrylhydrazil radical as well as the in vitro inhibition of bovine brain lipid peroxidation and of xanthine oxidase. By this study, it was shown that extract of UD inhibited the lipid peroxidation by more than 50%, and active extract of the UD had a dose dependent activity. Moreover, Gülçin et al. (2004) showed that the water extract of UD had influences on effective reducing power, free radical scavenging, and metal chelating activities. Additionally, the authors reported that the water extract of UD exhibited more effective antioxidant activity than the \( \alpha \)-tocopherol on peroxidation of linoleic acid.

Although mechanisms of antioxidant effect of UD has been studied as in vitro, there is no study in the literature about the antioxidant effect of UD on ischemia-reperfusion damage in muscle tissue depending on tourniquet use. In our study, MDA level in UD-treated group was lower than those in the KCl-treated and untreated groups.
Obviously, there was a significant lowering effect of UD on the MDA levels. Furthermore, the lack of the effect of 1.15% KCl solution on MDA levels confirmed that the effect of UD was original and could not be related to a non-specific action of the vehicle. Indeed, the results obtained in 2 ischemia-reperfusion group were completely similar with those in 1 ischemia-reperfusion group. Despite numerous publications based on experimental studies and empirical clinical data, the absolute limits of tourniquet time have never been firmly established or universally accepted. The “safe” duration of tourniquet ischemia suggested in the literature ranges from 45 min to 4 h, with 2 h being the most widely accepted figure (Green 1993). The lack of difference between two groups (1 h- and 2-h ischemia-reperfusion groups) occurred in the present study is in accordance with the mentioned concept above. Depending on the results of the present study, one would assign a role for UD in terms of preventing ROS induced damage, and thus ischemia-reperfusion injury. Similarly, Karakaya et al. (2001) recently showed that the Urtica sp. had an antioxidant activity. In support of this, Kanter et al. (2003) reported that Nigella sativa L. and UD decreased the lipid peroxidation and liver enzymes, and increased the antioxidant defense activity in the CCl₄-treated rats. Moreover, some authors revealed that a positive relationship was found between total phenols and antioxidant activity in many plant species (Vinson et al. 1998; Gülçin et al. 2002). Phenols have scavenging ability because of their hydroxyl groups (Hatano et al. 1989). Considerably, Gülçin et al. (2004) found that pyrocatechol equivalent of phenols was detected in water extract of UD. Also, Yen et al. (1993) reported that phenolic compounds were associated with antioxidant activity and they stabilized lipid peroxidation. The essential phenolic ingredient in the UD plant is caffeic malic acid which inhibits the proteolitic degradation of I-κB and it is also suggested that anti-inflammatory effect of UD may be ascribed to its inhibitory effect on NF-κB activation (Riehemann et al. 1999). According to the information presented above, the authors of the present study conclude that this antioxidant effect obtained from our results might be explained by the fact that the extract of UD contained the phenolic compounds, and due to phenolic compounds, UD has scavenging ability and stabilizes the lipid peroxidation, which plays an important role in ischemia-reperfusion damage in muscle tissue depending on tourniquet use. Additionally prevention of the NF-κB activation could be responsible for its antioxidant effect.

As a conclusion, the present study has shown that UD pre-treatment diminishes oxidative stress in the muscles generated by applying tourniquet. This finding suggests that UD has a cell protection effect against oxidative stress in rats. It can be proposed that the preoperative use of UD in patients undergoing pneumatic tourniquet operation may reduce oxidative damage in skeletal muscle. Nevertheless, further studies should be carried out for ultrastructural, biochemical, and comparative investigations with UD and other antioxidants in higher animal models for prevention of post-ischemic oxidative stress before clinical applications.

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