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The effects of *Rumex patientia* extract on rat liver and erythrocyte antioxidant enzyme system

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The aqueous extract from the roots of *Rumex patientia* L. (Polygonaceae) (D-1) was investigated for its effects on rat liver and erythrocyte antioxidant enzyme systems and lipid peroxidation. Measurements of the GSH-Px, SOD and CAT activities, and MDA levels of liver and erythrocytes in D-1 administered animals showed that there was an increase in GSH-Px and SOD activities when compared to that of controls. No significant decrease was observed in catalase activity and no changes in malondialdehyde levels were observed.

1. Introduction

Several *Rumex* (Polygonaceae) species are employed in traditional medicine in many parts of Turkey as a laxative, cholagogue [1]. From the chemical point of view, anthraquinones and related compounds have been identified as the active ingredients producing the laxative effect [2]. The roots of *Rumex* species growing in Turkey are very rich in anthraquinones [3]. *Rumex patientia* has been shown to have a fairly high anthraquinone content [4, 5]. It has been reported that *R. patientia* contains anthraquinone, tannin, naphthalene and naphthoquinone derivatives [6–8]. A literature search revealed only limited information on the characterization of tannins and anthraquinone glycosides from *Rumex* species. We previously reported that the aqueous extract of the roots of *R. patientia* exhibited high antiinflammatory, analgesic and antipyretic activities [9–11]. We have isolated several novel naphthalenes, rumexoside; labadoside; orientaloside; patientoside A and B; nepodin-8-*O*-β-D-glucopyranoside; torachrysone-8-*O*-β-D-glucopyranoside [12, 13], a new anthraquinone glycoside, emodin-6-*O*-β-D-glucopyranoside; a new simple halogenated flavan-3-ol, 6-chlorocatechin and seven known compounds, chrysophanol; physcion; emodin; chrysophanol-8-*O*-β-D-glucopyranoside; emodin-8-*O*-β-D-glucopyranoside; catechin and orcinol from the roots of *R. patientia* and elucidated their structures on the basis of spectral analysis. Cytotoxic effects and thin layer chromatographic radical scavenging properties of these compounds have also been investigated [14]. Phenolic substances can produce free radicals. It should be shown that the possible presence of free radicals has no effect on enzyme systems and lipid peroxidation. For this purpose, in this study we examined the effect of varying dosages of the aqueous extract from *Rumex patientia* on rat liver and erythrocyte GSH-Px (glutathione peroxidase), SOD (superoxide dismutase), CAT (catalase) enzyme activities and MDA (malondialdehyde) levels.

2. Investigations, results and discussion

In this study, the effect of varying dosages of the aqueous extract from *Rumex patientia* (D-1) on rat liver and erythrocyte GSH-Px, SOD, CAT enzyme activities and MDA levels were investigated. The roots of *R. patientia* contain anthraquinones, naphthalens and tannins. It is known that endogenous quinone compounds play a vital role in many biological process, while some quinone drugs are used in the treatment of cancer in humans. It has been determined by ESR techniques that many anthraquinone and naphthoquinone compounds produce semiquinone radicals on microsomal incubation. These radicals, reacting with oxygen, produce superoxide anions thus bringing about cellular damage [15]. It is known that antioxidant enzymes such as SOD, GSH-Px and CAT are used to, try to eliminate the superoxide radicals produced in the cell. SOD catalysis of the conversion of superoxide radicals into hydrogen peroxide is inhibited by high hydrogen peroxide concentrations. The excessive amount of superoxide anion in the medium inhibits GSH-Px and CAT [16]. Of the three enzymes mentioned above, CAT is a peroxisomal enzyme and its protection against oxidative stress is less than that afforded by the two other antioxidant enzyme. [17]. It displays an activity in liver, kidneys and erythrocytes at cellular level [18]. GSH-Px has a greater affinity to hydrogen peroxide. As for CAT, it exerts an effect on hydrogen peroxide at high concentration [19].

In our study, the effect of the aqueous extract from *Rumex patientia* on rat antioxidant systems and lipid peroxidation has been investigated. For this purpose, extracts prepared from the plant roots were given to rats at 15 mg/kg and 60 mg/kg doses by gastric lavage for a period of 7 days.

The liver enzyme activities in extract groups and the control group are shown in Table 1 and values of erythrocyte enzyme activities in Table 2. The results obtained are summarized as follows:

When the liver GSH-Px activities are compared to those of controls, 41% activation was observed in group **1** and 53% activation in group **2**. For the erythrocyte GSH-Px activity, there was an increase of 126% in group **1** and

Table 1: Antioxidant parameters of liver in control rats and animals treated with *Rumex patientia* by gastric lavage for a period of 7 days

Parameter	Control	Group 1	Group 2
Glutathione peroxidase (U/mg protein)	1.68 ± 0.3	2.37 ± 0.10	2.57 ± 0.05
Superoxide dismutase (U/mg protein)	336.58 ± 23.20	521.53 ± 57.67	520.47 ± 48.7
Catalase (U/mg Protein)	445 ± 17	343 ± 27	390 ± 43

Data are expressed as mean ± s.e.m. of ten animals in each group

Table 2: Antioxidant parameters of erythrocytes in control rats and animals treated with *Rumex patientia* by gastric lavage for a period of 7 days

Parameter	Control	Group 1	Group 2
Glutathione peroxidase (U/mg Hb)	0.94 ± 0.14	2.13 ± 0.26	3.5 ± 0.5
Superoxide dismutase (U/mg Hb)	28.60 ± 1.4	57.45 ± 3.1	75.7 ± 5.1
Catalase (U/mg Hb)	4.23 ± 1.11	3.52 ± 1.45	3.88 ± 1.86

Data are expressed as mean ± s.e.m. of ten animals in each group

272% in group **2**. These increases were found to be significant when compared to that of the controls ($p < 0.05$). While the liver SOD activity increased 55% in both groups, 101% elevation in erythrocytes was noted in group **1** and 165% in group **2**. This increase was also found to be significant when compared to that of the controls ($p < 0.05$). In both groups, the average decrease was 12% in liver CAT activity and in 6% erythrocyte GSH-Px activity. This decrease was not significant when compared to that of controls ($p > 0.05$). No alteration in MDA levels was observed when compared to those of the controls.

In our investigation, no significant alteration was observed in the liver and erythrocyte CAT activities of rats administered 60 mg/kg D-1. Activation observed in the GSH-Px activity indicates that production of hydrogen peroxide was not at a high level. SOD activity results also support this hypothesis. We can conclude that the superoxide anions produced by semiquinones are at a low level. Furthermore, as no significant change was found in the MDA levels measures in cells, *Rumex patientia* can be considered safe as a drug.

3. Experimental

3.1. Plant material

Plant materials were collected from Niğde-Bor (1050 m) and authenticated. Voucher specimens are deposited in the Herbarium of the Faculty of Pharmacy (HUEF-94102), Hacettepe University, Ankara, Turkey.

3.2. Animals

Adult male Wistar albino rats weighing 150–250 g nourished under normal conditions at the Cumhuriyet University Experimental Animal Laboratory, Sivas were used. In the experimental group, 10 rats were administered 15 mg/ml/day D-1 (group 1) and an other 10 rats were administered 60 mg/ml/day D-1 (group 2) by gastric lavage for a period of 7 days. The control group, consisted of 10 rats not subjected to any application.

3.3. Sample preparation

Roots (5 g) were exhaustively extracted in a soxhlet apparatus with water at 40 °C and the extract was concentrated and then lyophilized to yield a residue (1.2 g, D-1).

3.4. Determination of enzyme activities

The rats were killed by cervical concussion before the livers were perfused and samples of blood were taken from abdominal aorta. The erythrocytes were washed three times with 140 mM NaCl, 40 mM phosphate pH: 7.4 and centrifuged at 1000 g. The precipitate was hemolysed with β-mercaptoethanol in 2.7 mM EDTA, pH: 7.0. This hemolysate was used to determine GSH-Px [20], SOD [21], and CAT [22] activity. Washed erythrocyte suspension was used for MDA measurement. The measurement was made by a literature method [23]. The hemoglobin levels of the samples were measured and the MDA level per g Hb was determined.

The livers were perfused with ice-cold 0.9% saline in situ, removed and weighed, washed and homogenized with 3 vols. of 140 mM NaCl, 40 mM sodium phosphate buffer pH: 7.0 and centrifuged at 18000 g for 20 min at 4 °C. The supernatant obtained after centrifuging for 60 min at 105000 g in a Beckman L5-75B ultracentrifuge was used as the enzyme source [24]. GSH-Px, SOD and CAT activities were determined using the same methods as mentioned above.

All chemicals used were purchased from Sigma Chemical Corp. All numerical data are expressed as the mean ± standard error and the significance between means was assessed by the student t test.

3.5. Acute toxicity

Acute toxicity of D-1 was tested in our previous study. Doses of 500 to 3000 mg/kg of D-1 were administered to rats by the oral route (p.o). None of the rats died [10].

3.6. Statistical methods

All tabulated results were expressed as means ± SEM, and were compared using Student's t test. A p value of less than 0.05 was considered significant.

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