

## Effects of *Rumex patientia* L. extract on some drug-metabolizing enzymes in rat liver

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The effect of aqueous extract from the roots of *Rumex patientia* L. (Polygonaceae) (D-1), a traditional Turkish medicine used as a laxative and cholagogue, on drug-metabolizing enzymes, such as cytochrome P4502E1, NADPH cytochrome *c* reductase, NADH cytochrome *b*<sub>5</sub> reductase and glutathione-S-transferase (GST); and serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were studied in male Wistar albino rat liver. A significant increase was observed in cytochrome P4502E1 and GST activities, but not in NADPH-cytochrome *c* reductase and NADH-cytochrome *b*<sub>5</sub> reductase activities. Serum AST and ALT activities were found within the normal laboratory range values. The results demonstrated that the aqueous extract of *R. patientia* triggers induction of cytochrome P4502E1 in liver and cytosolic GST activity.

**Keywords:** *Rumex patientia*, drug-metabolizing enzymes, cytochrome P4502E1, glutathione-S-transferase, NADPH-cytochrome *c* reductase, NADH-cytochrome *b*<sub>5</sub> reductase

Cytochrome P450 (CYP)-dependent monooxygenase is the primary oxidation enzyme system involved in detoxication and bioactivation of a number of drugs, environmental pollutants and chemical carcinogens. Oxidation catalyzed by monooxygenase system requires a CYP enzyme, NADPH-CYP reductase and phospholipids<sup>1</sup>. The CYP family comprises a group of enzymes with broad substrate specificity, which results in herb-induced drug interactions with selective CYP substrates. Cytosolic GST is an important enzyme for conjugation reactions, particularly glutathione conjugate formation. CYP and GST are responsible for both inductive, as well as inhibitory effects of many exogenous factors including herbal medicine<sup>2-5</sup>.

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In Turkey, genus *Rumex* (Polygonaceae) is represented by 25 species<sup>6</sup>. *R. patientia* is reported to contain anthraquinone, tannin and naphthalene<sup>7-12</sup>. Its dried roots are used in traditional medicine as laxative and cholagogue<sup>13</sup>. The aqueous extract of the roots of *R. patientia* (D-1) is reported to exhibit significant anti-inflammatory, antipyretic and analgesic activities<sup>14,15</sup>. Furthermore, antiulcerogenic effect has also been reported<sup>16</sup>. It is known that endogenous quinone compounds play a vital role in many biological processes, while some quinone drugs are used in the treatment of cancer in humans. Several anthraquinone and naphthoquinone compounds produce semi-quinone radical on microsomal incubation which react with oxygen to produce superoxide anions, thus bringing about cellular damage<sup>17</sup>. Earlier, we reported the effects of *R. patientia* extract on rat liver and erythrocyte antioxidant enzyme system<sup>18</sup>. In this study, we report the *in vivo* effects of *R. patientia* extract on some drug-metabolizing enzymes in rat liver.

### Materials and Methods

Plant material was collected from Nigde-Bor (1050 m) and authenticated. Voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy (HUEF 94102), Hacettepe University, Ankara, Turkey. All chemicals used were from Sigma Chemical Co., USA.

The roots of *R. patientia* (5 g) were exhaustively extracted in a soxhlet apparatus with water and the extract was concentrated at 40°C and lyophilized to yield a residue (1.2 g, D-1). Adult male Wistar albino rats, weighing 150-250 g and nourished under normal conditions at the Cumhuriyet University Experimental Animal Laboratory, Sivas were used. Rats were divided into 3 groups of 10 animals each. The experimental groups were administered with root extract of *R. patientia* @ 15 mg/ml/day (group 1), and 60 mg/ml/day (group 2) after gastric lavage for a period of 7 days. The control group was not subjected to any application<sup>18</sup>.

Animals were killed by cervical dislocation and livers were perfused with ice-cold 0.9% saline, weighed and homogenized in 3 volumes at 140 mM NaCl, 40 mM sodium phosphate pH 7.0, and were centrifuged at 15,000 *g* for 30 min at 4°C. The super-

nant obtained was centrifuged at 105,000 g for 60 min at 4°C. In order to obtain pure microsomal pellet, the pellet was resuspended in fresh buffer and centrifuged again at 105,000 g. The microsomal pellet was resuspended in 0.1 M sodium citrate buffer containing 0.1 M KCl 30 % glycerol (v/v)<sup>19</sup>.

Cytosolic GST activity was determined using 1-chloro-2,4-dinitrobenzene as a substrate in the presence of GSH by monitoring the increase of absorbance at 340 nm and expressed as units/mg protein<sup>20</sup>. Cytochrome P4502E1 (N-nitrosodimethylamine N-demethylation) activity was determined by measuring the formation of formaldehyde using Nash's reagent<sup>21</sup>. NADPH cytochrome *c* reductase was measured at 550 nm using cytochrome *c* as an electron acceptor. Millimolar extinction coefficient was used ( $\epsilon = 0.021 \text{ mM}^{-1} \text{ cm}^{-1}$ ) for calculation<sup>22</sup>. NADH cytochrome *b*<sub>5</sub> reductase activity was determined using potassium ferricyanide as a substrate in the presence of NADH by monitoring the decrease of absorbance at 420 nm. Millimolar extinction coefficient was used ( $\epsilon = 1.02 \text{ mM}^{-1} \text{ cm}^{-1}$ ) for calculation<sup>23</sup>. Microsomal and cytosolic protein concentrations were determined as described by Coomassie brilliant blue method. Bovine serum albumin was used as protein standard<sup>24</sup>. The activities of serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) were determined (Boehringer Mannheim Kit). All numerical data are expressed as the mean  $\pm$  SE and the significance between means was assessed using student's *t* test.

## Results and Discussion

Earlier study on the effects of varying doses of aqueous extract of the roots of *R. patientia* on rat liver and erythrocyte GST-Px, SOD, CAT enzyme activities and malondialdehyde (MDA) levels have

shown that while antioxidant enzyme systems gave different responses, MDA levels showed no changes<sup>18</sup>. In the present study on the effect of aqueous extract of *R. patientia* root on some drug-metabolizing enzymes in rat liver, no significant alteration in NADPH cytochrome *c* reductase and NADH cytochrome *b*<sub>5</sub> reductase activities, was observed compared to control, but for significant increase in the activities of cytochrome P4502E1 and GST (Table 1). The liver cytochrome P4502E1 showed 4% and 14% activity in group 1 and 2, respectively while cytosolic GST activity showed an increase of 15% in group 1 and 139% in group 2, respectively. Serum AST activity displayed no significant change. However, serum ALT activity showed significant decrease compared to control, but the AST and ALT values were within the average normal laboratory range. Thus, it may be possible that there was no liver damage.

Cytochrome P4502E1, a clinically important<sup>25</sup> enzyme metabolizes a wide variety of chemicals, including potential cytotoxic, carcinogenic agents, industrial solvents, toxicological chemical additives and several halogenated anesthetics<sup>26-28</sup>. The GSTs are a family of enzymes that catalyze addition of the tripeptide glutathione to endogenous and xenobiotics substrates, which have electrophilic functional groups. They play an important role in detoxication and metabolism of many xenobiotics and endobiotic compounds<sup>29-31</sup>. Hydroxylation of anthraquinones is reported to be catalyzed by cytochrome P450<sup>32</sup>. It was shown that cytochrome P450-dependent biotransformation of anthraquinones, emodin and chryso-phanol might represent bioactivation pathways for these compounds<sup>33</sup>. Longo *et al*<sup>34</sup> studied the effect of six anthraquinones (AQs) on a variety of drug-metabolizing enzymes in rat liver and found that administration of 9,10-AQ, 1-OH AQ, and 1,4-OH

Table 1—Effects of the *Rumex patientia* extract on some drug-metabolizing enzymes in rat liver and on the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in rat serum  
[Data are expressed as mean  $\pm$  S.E.M. of ten animals in each group]

	Control	Group 1 (15 mg/ml/day)	Group 2 (60 mg/ml/day)
Cytochrome P4502E1 (nmol/mg protein/min)	0.506 $\pm$ 0.019	0.526 $\pm$ 0.013	0.578 $\pm$ 0.012*
Glutathione-S-transfrase (U/mg protein)	0.173 $\pm$ 0.015*	0.199 $\pm$ 0.018*	0.414 $\pm$ 0.065*
NADPH cytochrome <i>c</i> reductase (U/mg Protein)	0.107 $\pm$ 0.036	0.108 $\pm$ 0.017	0.104 $\pm$ 0.021
NADH cytochrome <i>b</i> <sub>5</sub> reductase (U/mg Protein)	8.21 $\pm$ 1.84	8.6 $\pm$ 1.21	7.55 $\pm$ 0.44
ALT (U/L)	70 $\pm$ 1.7	54 $\pm$ 2.1*	52 $\pm$ 2.4*
AST (U/L)	203 $\pm$ 14.6	195 $\pm$ 11.8	213 $\pm$ 11.3

\*Values statistically significant  $P < 0.05$

AQ induce P4501A2 and was also able to increase the activities of phase II enzymes, such as UDP-glucuronosyltransferase and NAD(P)H:quinone acceptor oxidoreductase. In addition, 9,10-AQ also induced cytosolic GST activity<sup>34</sup>. The induction of cytochrome P4502E1 and GST enzyme activities in the present study, is possibly due to anthraquinone constituent of *R. patientia* extract. Anthraquinone compounds are important antitumour agents and are used in anticancer chemotherapy. They exhibit significant activity against several neoplastic diseases such as leukaemia, sarcoma and breast cancer<sup>35-38</sup>. Therefore, the inductive effect of root extract of *R. patientia* on cytochrome P4502E1 and GST activation in rat liver assumes clinical importance.

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