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Structural and Biochemical Alterations in the Rat Liver and Duodenum Following the Administration of *Rumex patientia* L. Extract

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Structural and Biochemical Alterations in the Rat Liver and Duodenum Following the Administration of Rumex patientia L. Extract

Summary : *Although there are some reports on the use of the root extract of Rumex patientia L. (Polygonaceae) to treat constipation, there aren't sufficient studies on their toxic effects. In this study, the effects of the aqueous extract on liver and duodenum of rat were investigated. Biochemical studies were realized using hepatic detoxification (GST; Glutathione-S-Transferase) enzyme. In addition to histopathological researchs on liver and duodenum tissues were performed. The results revealed that the administration of the extract by gastric lavage for a period of 7 days, up to a daily dose of 60 mg/mL, neither caused significant inhibition decrease in the concentration of GST enzyme, nor morbid histological changes.*

Keywords *: Rumex patientia L., duodenum, liver, GST enzyme activity, histopathology*

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Rumex patientia L. Ekstresinin Uygulanmasını Takiben Sıçan Karaciğer ve Duodenumundaki Yapısal ve Biyokimyasal Değişmeler

Özet : *Rumex patientia L. (Polygonaceae) kök ekstresinin kabızlık tedavisinde kullanılışı ile ilgili bazı yayınlar olduğu halde, toksik etkileri üzerinde yeterli çalışma bulunmamaktadır. Bu çalışmada, sıçan karaciğer ve duodenumu üzerine sulu ekstrenin etkileri araştırılmıştır. Biyokimyasal çalışmalar karaciğer detoksifikasyon enzimi (GST) kullanılarak gerçekleştirilmiştir. Buna ilaveten karaciğer ve barsak dokuları üzerinde histopatolojik araştırmalar yapılmıştır. Ekstrenin 7 gün süreyle günlük 60 mg/mL doza kadar gastrik lavajla verilmesi sonucunda ne GST enzim konsantrasyonunda belirgin bir azalma ne de tehlikeli dokusal değişmeler gözlenmiştir.*

Anahtar kelimeler *: Rumex patientia L., duodenum, karaciğer, GST enzim aktivitesi, histopatoloji*

INTRODUCTION

The roots of species of *Rumex* (Polygonaceae) are often employed in traditional medicine in many parts of Turkey as a laxative and cholagogue[1]. From the chemical point of view, anthraquinones and related compounds have been identified as the active ingredients for the laxative effect[2]. The roots of *Rumex* species growing in Turkey are highly rich in anthraquinones[3]. *R. patientia* has shown fairly high amounts of anthraquinones[4].

The aim of the present study was to examine the effect of the varying dosages of *R. patientia* on GST (Glutathione-S-Transferase) enzyme activity in the liver and to investigate several biochemical parameters such as transaminases and cholesterol and histopathological alterations, which are likely to occur in the liver and duodenum.

MATERIALS AND METHODS

Plant material

The roots of *R. patientia* were collected from Niğde-Bor (alt. 1050 m) in September 1994. The plant was authenticated at the Herbarium of the Faculty of

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Pharmacy, Hacettepe University in Ankara (HUEF), where voucher specimens were deposited.

Extraction

The underground parts of the plants were dried in shadow, reduced to powder and was heated in 100 mL water for 30 min at 40°C under reflux. The extract was filtered from Schleicher-Schüll 2040a paper at room temperature and evaporated to dryness in vacuo. From 5 g root, 1.22 g of solid material was obtained, giving a percentage yield of 24.4 %.

Thin Layer Chromatography (TLC)

Thin layer plates : Pre-coated TLC plates, silica gel 60 (Merck 5554)

Solvent system and Development:
Ethylacetate: methanol:water (100: 17: 13)
Chloroform: methanol: water (80: 20: 2)
Cyclohexane: ethyl formiate: dichloromethane: formic acid (35: 30: 30: 5)
Petrol ether: ethyl formiate: formic acid (94: 25: 1)

Detection:
1. The spots were studied directly on the chromatogram in daylight and UV light (Camag).
2. Sprayed with 5 % KOH in methanol (50 % v/v) and heated for 15 min at 100°C.

High Performance Liquid Chromatography Apparatus (HPLC)

The equipment consisted of a Waters 510 solvent delivery system (Waters, Milford, MA, USA) and an autosampler Waters WISP 710 B, Millipore. A Waters Model 481, Lambda-Max, Millipore UV-detector was used. The detector was operated at 430 nm for anthraquinone aglycone. Separation was performed on an 0.8X10 cm, 10µm Radial-Pak, C_{18} column at room temperature. The mobil phase (81.5:18.5:1) consisted of methanol:water:formic acid for aglycone; methanol:water:formic acid (50:50:1) were used for glycoside. The flow rate was 1 mL/min. Each sample was chromatographed three times. The injection volume was 15 µL and the pressure was 1200 psi.

Sample Preparation: 3 mg of fraction was dissolved in exactly 10 mL methanol.

Standard Samples: Aloe-emodin, emodin, chrysophanol, physcion and emodin-8-*O*-glucoside were isolated by us. A solution of 3 mg standard sample in 10 mL methanol was prepared as described in the sample preparation section.

Animals and Experimental Design

In this study, 40 adult male Wistar Albino rats, weighing 150-250 g nourished under normal conditions at the Cumhuriyet University Experimental Animal Laboratory, Sivas have been used.

In the experimental group, 15 rats were administered 15 mg/mL/day *R. patientia* extract and other 15 rats were administered 60 mg/mL/day *R. patientia* extract by gastric lavage for a period of 7 days. As for the control group, it consisted of 10 rats not subjected to any application.

Histopathology

Rat liver and duodenal tissues were obtained by laparotomy under ether anaesthesia. Tissues taken for light microscopical examination were fixed in 10 % formaldehyde. Tissues were processed routinely and cut 4-5 µm and stained with hematoxylin and eosin. Selected sections were also stained with PAS stain.

Biochemistry

Determination of GST (Glutathione-S-Transferase) enzyme activity

The effects of *R. patientia* extract on cytosolic GST enzyme in the liver of rats were investigated. Following the decapitation of the rats the liver tissues were homogenized in 0.15 M KCl and at 1000 rev/min homogenization speed. Homogenate was centrifuged at 18000 g for 20 min at 4°C. The supernatant obtained after the centrifugation for 60 min. at 105000 g in a Beckman L5-75B ultracentrifuge was used as the enzyme source[5].

The enzyme activities were determined in accordance with the method of Habig *et al.*[5] The ex-

periment is based on following up formation of the tioether bond between GSH and 1-chloro 2,4 dinitrobenzene (CDNB) at 340 nm with a spectrophotometer. Coomassie Brillant-Blue method was used for the protein determination[6]. The specific activity was calculated as 1µ-mole S-2.4 dinitrophenyl glutathione (DNPG) formed per mg protein in 1 minute.

Several Biochemical Parameters of Rat Serum

In addition, blood samples were obtained from the same animal's heart to determine some biochemical parameters of the rat serum such as transaminases, lactate dehydrogenase, tryglycerides and cholesterol have been investigated. Biochemical analysis of the control and test groups of rat sera were made by RA-1000 Tecnicon model autoanalyser[7].

Statistical methods

All results are expressed as the mean ± S.E.M. statistical comparisons have been calculated using Student's t test and a probability level of $p = 0.05$ was chosen as the criterion of statistical significance.

RESULTS AND DISCUSSION

Rumex patientia (Polygonaceae) roots are highly rich in anthraquinones and known by its laxative properties in Turkish traditional medicine. The effects of this root extracts on duodenum and liver are examined histopathologically and biochemically.

Histopathological studies

a) Duodenum

The examination of samples obtained from the duodenal part of the small intestine from the rats belonging to both experimental groups had showed villi and simple tubular glands called crypts, which were in normal structural appearence.

The absorptive epithelium of the villi was continuous with that of the glands. In addition, L. propria and T. adventitia were in normal appearance, but the Brunner's glands in T. submucosa were increased significantly (Fig. 1). On the other hand, although it is well known that the goblet cells are less

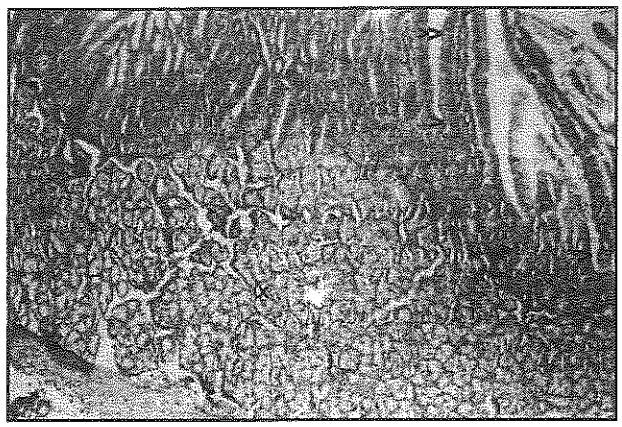

Figure 1: Shows the morphology of duedonum from the *R. patientia* treatment group. Crypts (>) and villous (>>) features seem to be in their normal structure. However, an increase in the amount of Brunner's glands (*) in submucosal layer is evident X40 H+E.

in the duodenum, in the experimental groups, the number of goblet cells have increased significantly (Fig. 2A, 2B). These findings indicated an increase in mucous secretion and a decrease in absorption. Till now, there is no report on the laxative effect of *Rumex* species to be proved histopathologically. In this

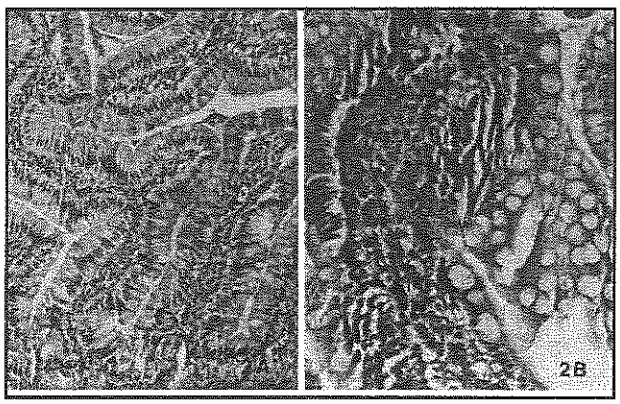

Figure 2A-2B: Crypts and villi belonging to the *R. patientia* treatment group. An increase in the number of the goblet cells (>) among the epithelial cell lining.
2A: X40 H+E
2B: X100 H+E

study, the laxative effect of the aqueous extract of the roots of *R. patientia* has been shown first time histopathologically. In a previous study, the laxative effect of *Rumex patientia* (7 % methanolic extract) has been tried on human subjects; 0.25 g dosing found to be ineffective, while 0.5 g 30-40 % effective, 0.75 g 70 % and 1 g dose 90 % effective[8].

These results and our findings clearly indicated that *R. patientia* possessed laxative effect. Up to date, there were more investigations on other anthraquinone containing plants, such as *Rhamnus* and *Cassia* spec., in the aspect of their laxative activity and anthraquinone content. It is well known that, glucofrangulin A and B are responsible for laxative effect of *Rhamnus* spec. and sennoside A and B are responsible for laxative effect of *Cassia* spec[9]. Additionally, both of the aforesaid plants contain approximately 6 % and 3 % anthraquinones, respectively[10]. In our previous study, it was shown that *Rumex* species growing in Turkey have at the most 2.93 % anthraquinones in *R. gracilescens*[3]. *R. patientia* contained 2.15 % anthraquinone[4]. In our prescreening by HPLC and TLC of *R. patientia*, anthraquinones were identified as emodin, chrysophanol, physcion, aloeemodin and emodin-8-*O*-glucoside. Whereas these compounds are known as worthless substances for laxative activity. Till now, glucofrangulins and sennosides couldn't be found in *R. patientia*. However, pathological studies showed that *R. patientia* possessed laxative effect. These data either point to the presence of other substances, which can potentiate the effect of these anthraquinones on laxative activity of the drug, or to further investigations on the anthraquinone content of *R. patientia*.

Besides, there was not a histopatological alteration in the duodenum. This may show that *R. patientia* extract could be used safely as a laxative.

It may affect the liver function and structure. Therefore, the following section explains the effect of *R. patientia* extract on the rat liver.

b) Liver

Light microscopical examination of the liver morphology in the experimental groups demonstrated a regular organization of hepatocytes and there was no pathological differentiation in the hepatic lobules. In addition, while components of the portal triad, biliary canaliculi between neighbouring hepatocytes, Kuppfer's cells and endothelial cells were in their normal structure, the sinusoids that take place between hepatocytes seemed to be slightly dilated (Fig. 3).

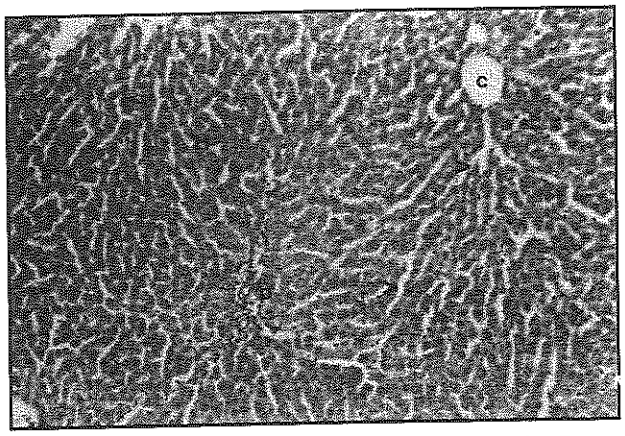

Figure 3: Although the morphology of liver in the *R. patientia* treatment group seemed to be preserved, the sinusoids were slightly dilated (>). Central vein (C), Hepatocytes (H), portal tract (p). X40 H+E

Biochemical studies

a) GST Enzyme Activity

GST constitutes a family of cytosolic isoenzymes and a structurally unrelated microsomal enzyme that is involved in the detoxification of endogenous and exogenous compounds. Therefore, GSTs are important enzymes.

First of all, the activity of GST, which is an important enzyme in liver tissue, was evaluated. In many other tissues including liver, various isoenzymes are present. This is an important part of the mechanism required for the excretion of toxic substances that are uptaken by the cell. The inhibition of this enzyme by a chemical substance or a drug causes an important destruction in the liver[11,12]. In this study, the effects of *R. patientia* extracts were investigated on cytosolic detoxification mechanism and especially in high doses (60 mg) a significant activation has been observed. This data show that the root extracts of *R. patientia*, which has potent cytotoxic activity might be inactivated by this route. Our results confirmed that GSTs are overexpressed in normal and tumor cells following exposure to cytotoxic drugs[13]. In our previous study, it was shown that *R. patientia* has potent cytotoxic activity (LC_{50}=1.30 µg/mL) against brine shrimp[14]. All GST enzyme activity values are shown in Table 1.

Table 1. The effects of *R. patientia* extract on the activity of GST (U/mg/min).

Control (n=10)	15 mg/mL/day *Rumex* applied group (n=15)	60 mg/mL/day *Rumex* applied group (n=15)
0.185	0.204	0.362
0.158	0.225	0.375
0.162	0.192	0.487
0.194	0.176	0.363
0.168	0.196	0.481
0.173±0.015	0.199±0.018	0.414±0.065

b) Several Biochemical Parameters of Rat Serum

The mean ± SD values of the control and the experimental groups for biochemical parameters of rat sera are listed in Table 2.

important indicators of tissue destruction. There was a significant decrease in triglyceride values and partial decrease in cholesterol values in *R. patientia* administired groups (Table 2). The reason for this decrease can be explained as the formation of a nondissolving complex of drug extract with bile salts. Therefore, absorption might be decreased by preventing the cholesterol carrying micelles formation. So the excretion by faeces might be realized.

As a result, it might be stated that the aqueous extracts of *R. patientia* roots are causing some kind of destruction on duodenum and liver and it is a safe plant from this aspect.

Although this was a preliminary investigation of the effect of *R. patientia* on duodenal and liver structure and function, further studies will be performed in order to determine the full identification of the chemical composition of this drug.

Table 2. The cilinical biochemical analysis data in blood given *R. patientia*.

PARAMETER	Control group (n=10)	15 mg/mL/day *Rumex* applied group (n=15)	60 mg/mL/day *Rumex* applied group (n=15)
Glucose (mg/dL)	101±6.1	105±8.2	110±8.0
Triglyceride (mg/dL)	90±9.7	45±6.3	61±6.6
Cholesterol (mg/dL)	52±7.6	50±7.0	46±6.3
Blood ure nitrogen (mg/dl)	23±3.84	19±1.27	20±4.56
Creatinine (mg/dl)	0.4±0.11	0.4±0.10	0.4±0.09
Uric acid (mg/dL)	1.6±0.32	1.5±0.40	1.5±0.38
Total Protein (g/dL)	5.9±0.19	5.6±0.15	5.4±0.16
Albumin (g/dL)	2.2±0.21	2.3±0.27	2.2±0.19
Alkaline phosphatase (U/L)	460±1.31	445±12.1	397±12.2
Alanintransaminase (U/L)	70±1.7	54±2.1	52±2.4
Aspartatetransaminase (U/L)	230±14.6	195±11.8	213±11.3
Lactatedehydrogenase (U/L)	4765±22.4	1780±19.6	2229±20.2
Total Bilirubin (mg/dl)	0.3±0.07	0.4±0.05	0.4±0.05
Direct Bilirubin (mg/dL)	0.1±0.04	0.1±0.04	0.1±0.04
Phosphorous (mg/dL)	7.9±0.44	8.4±0.41	8.7±0.42
Calcium (mg/dL)	9.5±0.45	9.2±0.32	9.4±0.36

Furthermore, the decrease in some parameters as transaminases, lactate dehydrogenases show that there was no adverse effects of *R. patientia*. It is well known that the elevated values of LDH and transaminases are very

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