A BIOCHEMICAL STUDY ON THE PROTECTIVE POTENTIAL OF *Centaurium erythraea* L. IN RATS AT ACUTE ASPIRIN EXPOSURE

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ABSTRACT

The aim of this study was to determine protective effect of *Centaurium erythraea* L. on rats exposed to aspirin (ASA). The protective influence of the 50% aqueous-ethanolic *Centaurium erythraea* L. extract (AECE) (100 mg/kg body weight) was investigated in rats exposed to a single dose of 200 mg/kg body weight ASA administration. Twenty-one Sprague-Dawley albino rats were distributed into three groups equally (n=7) as follows: A (control), B (only ASA-treated) and C (ASA+AECE-treated) group. At the end of 4 h ASA administration to groups B and C, some serum biochemical constituents were assigned and compared between the groups. Results showed that levels of aspartate aminotransferase, alanine aminotransferase, creatinine and potassium increased in group B compared with the control group, but they were decreased significantly with AECE supplementation. While lipase activity decreased only in group B, level of uric acid decreased both in groups B and C compared with control group. In conclusion, the current trial has shown that treatment of AECE with ASA supplied relative protection against acute ASA toxicity through ameliorating some blood parameters.

KEYWORDS: Aspirin toxicity, biochemical constituents, *Centaurium erythraea* L, rats

1. INTRODUCTION

Aspirin, also known as acetylsalicylic acid (ASA), is the most prominent member of non-steroidal anti-inflammatory drugs (NSAID) which can be obtained over the counter in many regions [1]. In recent years, long-term and low-dose ASA has been recommended to reduce the risk of heart attacks; however, gastric bleeding and formation of gastrointestinal ulcers are the most common adverse effects of ASA. In addition, Silagy *et al.* [2] reported renal side effects of ASA.

For a healthier life, some studies have been conducted to determine protection of humans against risk factors by means of chemo-preventive drugs and medicinal plants being used traditionally [3, 4]. Administration of botanical compounds that have preventive features is one of the approaches to avoid the drug-mediated damage. In this respect, we decided to assess the protective effect of aqueous-ethanolic *Centaurium erythraea* L extract (AECE) against high dose ASA administration (200 mg/kg body weight) through evaluating some biochemical constituents. Because monitoring of blood biochemical parameters has essential importance in the physiopathological evaluation of animals [5].

Centaurium erythraea L. (CE) (Family: *Gentianaceae*) is a medicinal plant species that has been used for hepatoprotective, digestive, stomachic, tonic, depurative, blood purifier, hematopoietic, antioxidative, sedative and antipyretic purposes [6-10]. CE was informed to be relatively nontoxic in rodents at acute and subchronic exposure [11]. To the best of our knowledge, the current trial is the first study investigating protective effects of CE on biochemical constituents against ASA administration. Since CE is a potent medical plant, we investigated whether ASA-caused damage and the variables of blood constituents were affected by AECE treatment.

2. MATERIALS AND METHODS

2.1. Plant material and extraction

CE samples were obtained from a herbal store in Sanliurfa, Turkey and identified by Esat Çetin, PhD. Aerial parts of CE (1 kg) were air-dried at room temperature, then powdered coarsely, extracted with 50% aqueous alcohol for 1 day at 40 °C, and filtered (Whatman, grade 1). The CE extract was concentrated under reduced pressure

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and lyophilized (Tel Star Cryodos) to get a dry residue. Dose of CE was determined to be 100 mg/kg body weight according to early studies [12-15].

2.2. Experimental animals

Experiments were realized with 21 Sprague-Dawley male albino rats (230-270 g) kept in plastic cages in a temperature-controlled room (21 °C). Food and water were given to the rats *ad libitum*. The 12-h light/dark cycle was from 7 a.m. to 7 p.m.

2.3. Experimental protocol

Rats were deprived of food for 24 h before the experiment but free access to tap water was allowed. The animals were distributed into three groups, each composed of seven rats: A) Control group received 1 ml saline (0.9 % NaCl) by intragastric administration during 7 days before sacrificing process; B) ASA group received 1 ml saline by intragastric administration during 6 days, but on the 7th day, rats were exposed to saline and also ASA (200 mg/kg b.w.) before sacrificing process; C) ASA+AECE group received 1 ml AECE by intragastric administration during 6 days, but on the 7th day, rats were exposed to AECE (100 mg/kg b.w.) and ASA (200 mg/kg b.w.) combination. All animals received humane care in compliance with the European Community Guidelines on the care and use of laboratory animals.

All solutions were freshly prepared, 30 min before the study. ASA was dissolved in a suspension comprising of 0.025% (w/v) carboxyl methylcellulose suspended in 0.15 N HCl with a pH ranging from 1.3 to 1.5 [16]. AECE was suspended in distilled water, and 1 ml of the solution (25 mg/ml) was given intragastrically via tube feeding.

2.4. Analysis of biochemical parameters

Rats were anesthetized with ketamine (100 mg/kg intraperitoneally (ip)) [17] for measurements of biochemical parameters at 4th h after the ASA administration. Blood samples were taken from animals into tubes by injectors from the heart under light anesthesia. Afterwards, rats were euthanized with inhalation of CO_2. The serum samples were obtained by centrifuging blood samples at 3000 rpm for 15 min at 4 °C. Biochemical parameters including aspartate aminotransferase (AST) (EC 2.6.1.1), alanine aminotransferase (ALT) (EC 2.6.1.2), lipase (EC 3.1.1.3), creatine phosphokinase (CK, CK-MB) (EC 2.7.3.2), alkaline phosphatase (ALP) (EC 3.1.3.1), gamma glutamyl transferase (GGT) (EC 2.3.2.2), amylase (AMY) (EC 3.2.1.1), urea, creatinine, uric acid, total protein, albumin, triglyceride, cholesterol, high density lipoprotein (HDL), glucose, potassium (K^+), chlorine (CL), calcium (Ca), iron, unsaturated iron-binding capacity (UIBC), rheumatoid factor-II (RF-II), C-reactive protein (CRP), antistreptolysin O (ASO), total bilirubin, and direct bilirubin were analyzed by an autoanalyser (Roche, Cobas Integra 400 plus) using the kits.

2.5. Statistical analysis

The statistical analyses were realized using the Minitab 13 for windows package program. All data were presented as means ± standard deviation (SD) (n =7). One-way analysis of variance (ANOVA) was used to determine the differences between means of the experimental groups accepting significance level at $p< 0.05$.

3. RESULTS

The noxious effect of acute high dose ASA administration and protective efficacy of AECE supplementation against this application was monitored by recording the biochemical parameters in the serum specimens obtained from control and treated rats.

3.1. Effect on serum enzymes

As shown in Table 1, activities of AST and ALT increased in ASA group compared to control, but they were decreased significantly with AECE supplementation. Furthermore, lipase activity decreased in ASA group compared to control.

3.2. Effect on serum renal function markers and lipid profile

While ASA treatment led to significant increase of creatinine in comparison with control, its level was decreased markedly in ASA+AECE group. Moreover, level of uric acid decreased both in ASA and ASA+AECE groups compared to control (Table 2).

TABLE 1 - Protective effect of *Centaurium erythraea* L. on serum enzymes of ASA-administered rats (mean ± SD).

Parameters	Control	ASA	ASA+AECE
AST U/L	126.20±5.93	176.60±18.28[a]	137.00±10.65[b]
ALT U/L	54.20±5.81	66.60±8.44[a]	58.00±5.57[b]
ALP U/L	212.80±26.99	191.60±11.84	205.00±16.16
GGT U/L	1.00±0.12	1.06±0.18	1.00±0.14
CK U/L	651.40±38.64	622.00±66.22	624.80±45.57
CK-MB U/L	405.60±74.95	378.20±76.31	393.40±111.18
AMYLASE U/L	1779.0±136.6	1739.8±109.3	1753.0±79.4
LIPASE U/L	4.70±0.35	4.18±0.23[a]	4.24±0.40

Values are given as means ± SD. [a]Control versus treated $p< 0.05$ level. [b]ASA versus treated $p< 0.05$ level.

TABLE 2 - Protective effect of *Centaurium erythraea* L. on serum renal function markers and lipid profile of ASA-administered rats (mean ± SD).

Parameters	Control	ASA	ASA+AECE
Urea mg/dl	57.00±5.05	58.60±8.88	58.80±1.92
Creatinine mg/dl	0.41±0.06	0.48±0.03[a]	0.43±0.02[b]
Uric acid mg/dl	2.18±0.36	1.74±0.38[a]	1.78±0.33[a]
T. protein mg/dl	7.42±0.50	7.30±0.42	7.92±0.61
Albumin g/dl	3.48±0.22	3.46±0.32	3.72±0.25
Triglyceride mg/dl	71.80±17.74	66.40±10.36	68.00±9.22
Cholesterol mg/dl	63.00±8.46	55.20±6.98	62.00±8.25
HDL mg/dl	25.40±7.27	22.20±4.09	26.40±8.65

Values are given as means ± SD. [a]Control versus treated $p< 0.05$ level. [b]ASA versus treated $p< 0.05$ level.

TABLE 3 - Protective effect of *Centaurium erythraea* L. on some biochemical parameters of ASA-administered rats (mean ± SD).

Parameters	Control	ASA	ASA+AECE
Glucose mg/dl	181.80±8.50	176.00±13.55	179.80±7.89
K^+ (mmol/L)	7.98±0.36	8.98±0.42[a]	8.08±0.61[b]
CL (mmol/L)	103.40±1.95	104.20±2.49	105.00±2.24
Ca (mg/dL)	6.52±1.15	6.64±1.09	6.66±0.81
Iron ug/dL	258.20±57.63	237.20±32.17	248.00±25.23
UIBC (ug/dL)	315.00±38.18	354.00±48.53	310.60±61.90
RF-II (IU/mL)	7.26±1.21	6.88±1.05	7.44±0.86
CRP (mg/L)	0.28±0.04	0.26±0.05	0.26±0.05
ASO (IU/mL)	7.80±1.30	7.60±1.14	7.80±1.15
T. bilirubin mg/dl	0.06±0.01	0.06±0.03	0.06±0.01
D. bilirubin mg/dl	0.02±0.01	0.02±0.01	0.02±0.01

Values are given as means ± SD. [a]Control versus treated $p< 0.05$ level. [b]ASA versus treated $p< 0.05$ level.

3.3. Effect on other biochemical parameters

K^+ level was significantly higher in ASA group than control but it was decreased significantly with AECE supplementation (Table 3).

4. DISCUSSION AND CONCLUSION

According to literary data, we evaluated for the first time whether AECE has an ameliorative effect against acute high dose ASA administration through measuring variations in the level of serum enzymes, renal function markers, lipid profile, and some other biochemical constituents.

Enzymes as biomolecules exude from the damaged tissues into the interstitial fluids. Thus, determination of these macromolecules in serum has been found to be crucial in the assignation of related tissue injury. Serum enzymes are used as dependable markers in the definition of organ specific disorders [18]. In the present study, while activities of hepatic marker enzymes AST and ALT increased markedly in ASA group, they were reversed to nearly control in ASA+AECE group. Elevation of AST and ALT might have been resulted from liver injury caused by acute ASA exposure. This result is in accordance with some previous studies [19-22]. However, Demircan *et al.* [23] have not reported high levels of AST and ALT in their studies, in which they investigated the subacute influence of 100 mg/kg b.w. ASA dose (ip) in rats. Reason for this discrepancy may be due to the application of different doses, times and administration routes. Since CE possesses antioxidant [10] and hepatoprotective [6] features, it probably might have recovered AST and ALT activities through preventing leakage of these enzymes from liver cells to interstitial fluid. AECE may have rejuvenated the cell membranes that suffered from ASA in this study. Furthermore, lipase activity declined in ASA group compared to control. Lipase is liable for triglyceride hydrolysis [24]. Activity of lipase might have been inhibited by ASA through acetylating active serine residues of lipase [25], or reducing its extracellular secretion [25].

With regard to serum renal function markers, while ASA application gave rise to significant escalation of creatinine, its level diminished markedly with AECE supplementation. In accordance with our results, high dose of ASA intake led to increase of creatinine, also in some earlier studies [26-28]. Increased creatinine levels in serum may be attributed to its decreased clearance [29-31]. This decline may have been resulted from change of its tubular handling [29]. However, Nogi *et al.* [32] reported that ASA has no effect on the serum creatinine level at a dose of 100 mg/kg b.w. AECE treatment may have given rise to decrease of serum creatinine through elevating its clearance. In current trial, level of uric acid decreased in both ASA and ASA+AECE groups compared to control. The reduction of uric acid levels in ASA-treated groups may be related to its elevated proximal tubular transport [29]. There are conflicting impacts of low and high dose ASA administrations on the level of uric acid [33]. While high dose has a promoting effect [34], low dose has a reducing

influence on uric acid clearance [26]. Based on the above results, it is most likely that acute intragastric ASA treatment (200 mg/kg b.w.) might have caused imbalance on renal functions, and AECE supplementation partly improved this imbalance. In regard to lipid profile, there was no significant difference between groups.

As shown in Table 3, K^+ level was markedly higher in ASA group compared with control, but it dropped significantly with AECE supplementation. Increase in serum K^+ grade was also observed in a study of Mahboob and Haleem [35] where they administered ASA to rabbits and rats. High serum K^+ levels might have been resulted from injury of kidneys [36-38]. Meanwhile, Pamuk and Çakır [26] informed that low dose ASA treatment did not change serum K^+ grade in humans. According to our current results, AECE supplementation may have decreased a high level of K^+ by elevating its urinary excretion [31].

Our data demonstrated that acute high dose ASA application alters several biochemical constituents of circulation system, and AECE could relatively combat these alterations. Thus, we can conclude that AECE may help to amelioration of hepatic and renal functions through reducing adverse effects of ASA.

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