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SUBCHRONIC TOXICITY STUDY OF AQUEOUS INFUSION FROM *COTINUS COGGYGRIA* LEAVES IN WISTAR RATS

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Abstract

The Eurasian smoke tree (*Cotinus coggygria*) is used by the Balkan folk medicine for its antiseptic and antimicrobial properties as well as for treatment of gingival and throat inflammations. Although *C. coggygria* has been applied mainly externally because of the large gallotannins content, there are few reports for internal use of its leaves against gastric ulcer, diarrhoea, nephritis, anthrax, asthma, cardiac and urinal diseases and even diabetes mellitus. The aim of this study is to examine the toxicity of *C. coggygria* leaves aqueous infusion in experimental animals. Male Wistar rats were treated by stomach gavage with different concentrations of herb infusion (1, 2 and 4%) or distilled water at doses of 10 ml/kg b.w. After 30 days of treatment, the animals were sacrificed and the blood and organs were collected for biochemical and histopathological analyses. Results showed that treatment with aqueous infusion from *C. coggygria* did not cause subchronic toxicity on liver and kidney. Histological investigation did not detect pathological deviations in the organs of treated groups compared with control. No significant changes were observed in the serum levels of hepatic enzymes, urea, creatinine, triacylglycerols and total

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thyols. The subchronic administration of *C. coggygria* infusion is non-toxic in the applied concentrations and therefore it can be used to study healing effects as reported by the Balkan traditional medicine.

Key words: *Cotinus coggygria*, histopathology, biochemical markers, toxicology

Introduction. There is a constant search by pharmaceutical industry for new drugs, and the substances produced by plants are the major source for these. Still many of the plants, although reported to have a healing effect, remain unstudied because of their toxicity. Such is *Cotinus coggygria* Scop. (Anacardiaceae) – the Eurasian smoke tree, a medicinal plant species with wide distribution from Southern Europe, the Mediterranean, Moldova and the Caucasus to Central China and the Himalayas [1]. According to some authors [2,3], the whole plant is poisonous due to the large content of gallotannins (above 25%). However, the Balkan folk medicine uses aqueous infusions from leaves to treat gingival and throat inflammations, stomachache, gastric ulcer, diarrhoea, nephritis, anthrax, asthma, cardiac and urinal diseases and even diabetes mellitus, as antiseptic, anti-inflammatory, antimicrobial, anti-haemorrhagic and wound-healing drug [1,3–5]. *C. coggygria* is a common medicinal plant to the Bulgarian folk medicine (well-known as 'smradlika' or 'tetra') for predominantly external use [3]. Studies on the chemical composition of leaves infusion report the presence of gallic acid methyl ester and anthocyanins [6], and galotanins, galic acid, flavonic glycosides, myrcen, alpha-pinene, camphen, linalool, and alpha-terpineol [1,7]. Numerous polyphenol compounds have been isolated, including quercetin, fustin and taxifolin [8]. Antioxidant activity of extracts from the leaves of *C. coggygria* is analyzed in vitro by some Bulgarian and Serbian authors [9–12] and is related to the high polyphenol content. It is noteworthy that the ethanol and aqueous extracts have the highest antioxidant activity and the highest content of polyphenols from several tens of the investigated Bulgarian medicinal plants [9]. The methanol extract is found to be an inhibitor of lipid peroxidation with antioxidant properties similar to these of α-tocopherol [12]. Although the plant seems to be extremely rich in biologically active compounds, it has been somewhat ignored by pharmacological studies because of the reported toxicity.

The aim of the present investigation is to study the plant subchronical toxicity in rat- administered aqueous infusion from *C. coggygria* leaves (AICCL) with relevance to its healing properties.

Material and methods. ***Experimental substances.*** All infusions were obtained from identical and standardized plan material that was purchased from Bilek Ltd., Troyan, Bulgaria. Three concentrations (1, 2 and 4%) of aqueous infusions of *Cotinus coggygria* leaves (AICCL) were prepared one hour before each treatment: 1, 2 and 4 g dried material was scalded in 100 ml boiling distilled water for 10 min. The infusions were filtered through cotton lint following the

traditional preparation of plant infusions in the Bulgarian folk medicine. The low concentration (1%) is commensurable to a traditional Bulgarian recipe for treatment of gastric ulcer [3].

Experimental design. Male albino Wistar rats (2–2.5 months old; 220–250 g) were used for the experiment. The animals were kept under the standard conditions of the animal house with 12 h light-dark cycle (light 7:00–19:00) at a temperature 23–25 °C. They had free access to food and water. All procedures concerning animal treatment and experimentation were in accordance with the EEC Council Directive 86/609, (IL 358, 1, December 12, 1987) and US National Institute of Health Guide for Care and Use of Laboratory Animals (NIH publication No 85–23, 1985, revised 1986), as well as the Guiding Principles in the Care and Use of Animals, approved by the National regulations, adopted by the local Ethical Commission of the Medical University of Varna. The authorization to use rats in experiments was obtained from the Regional Veterinary Agency in Varna (Registration No 13, Protocol of the Ethical Commission No 18/17.10.2010). The cohort comprised four experimental groups containing 10 rats each. All groups were orally treated by direct stomach intubations at a dose of 10 ml/kg b.w. One group was receiving distilled water and served as a control for the other three AICCL-treated groups. The animal treatment lasted for 30 days. The animals were anaesthetized with diethylether four hours after the last treatment.

Sampling. Blood was collected from the sublingual veins in heparinized tubes. It was centrifuged at 2000 rpm for 10 min and serum was obtained for further measurements and stored at 20 °C until analyses: activities of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) and concentrations of Urea, Creatinine, Triacylglycerols (TG), Uric acid (UA), Malondialdehyde (MDA) and Sulphydryl (SH–) groups. After decapitation, the liver, brain and kidneys were removed immediately, gently washed in physiological salt solution and frozen at −18 °C for further analyses. All organs were homogenized in 1:10 w/v 50 mM phosphate buffer (pH 7.4) containing 0.1 mM EDTA, at 4000 rpm for 10 min. The homogenate was centrifuged at 800 rpm for 15 min to discard the sediment. All manipulations were performed at 4–8 °C. All chemicals used for the sampling were of analytical grade and were obtained from Merck (Germany).

Biochemical analyses. Uric acid, MDA and SH-groups were determined in supernatant immediately after thawing of the samples. Plasma activities of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were measured by the enzyme-colorimetric methods of the International Federation of Clinical Chemistry (IFCC) using the standard kits of BioSystems S. A. (Spain). The activity of alkaline phosphatase (ALP) in plasma was measured by an enzyme-colorimetric diethanolamine (DEA) test using the standard kit of BioSystems S. A. Urea concentration in plasma was measured spectrophotometrically by the Blood Urea Nitrogen (BUN) test using the standard kit of BioSystems S. A. The

plasma concentration of Creatinine was measured spectrophotometrically by an alkaline picrate test using the standard kit of BioSystems S. A. Triacylglycerols (TG) in plasma were measured by an enzyme-colorimetric Glycerol 3-phosphate oxidase – 4-aminoantipyrine (GPO-PAP) test with Lipid Clearing Factor (LCF) using the standard kits of HUMAN liquicolor (Germany). Uric acid (UA) levels in plasma and homogenates supernatant were measured by an enzyme-colorimetric 4-aminophenazone (PAP) test with Lipid Clearing Factor (LCF) using the standard kits of HUMAN liquicolour. Membrane lipid peroxidation was monitored by the concentration of malondialdehyde (MDA) measured by its thiobarbituric acid (TBA) reactivity in rat plasma and liver, kidney and brain homogenates using the spectrophotometrical method of Porter [13]. Determination of sulphydryl (SH-) groups was performed spectrophotometrically in plasma and homogenates supernatant using an aromatic disulphide, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) according to the method of Ellman [14].

Histopathological analyses. Pieces of tissues were fixed in 10% neutral buffered formaldehyde solution. Fixed tissues were embedded in paraffin, cut into sections and placed on microscope slides. Staining of the slides with hematoxylin-eosin (H and E) and periodic acid-Schiff (PAS) was used for the histomorphological examination which was performed under light microscopy and documented by an Olympus microphotocamera. The number of apoptotic cells in liver was counted in all samples. All chemicals used for the histopathological examination were obtained from Merck (Germany).

Statistical analyses. Data were analyzed statistically by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison posttest. Each

Table 1

Results from biochemical analyses of blood serum
($^*P < 0.05$ vs control)

Biochemical markers	Control	1% AICCL	2% AICCL	4% AICC
AST [U/L]	120.2 ± 13.21	128.9 ± 15.37	115.5 ± 8.11	129.1 ± 11.42
ALT [U/L]	31.33 ± 2.84	30.12 ± 2.10	34.66 ± 2.53	33.33 ± 3.14
ALP [U/L]	408.0 ± 53.43	416.4 ± 28.45	390.3 ± 48.32	338.6 ± 17.28
Creatinine [µmol/L]	51.40 ± 4.98	55.89 ± 6.38	57.63 ± 6.62	57.34 ± 4.08
Urea [mmol/L]	9.01 ± 0.48	8.21 ± 0.25	8.58 ± 0.51	8.83 ± 0.41
TG [mmol/L]	2.00 ± 0.14	2.15 ± 0.19	2.13 ± 0.19	1.78 ± 0.17
UA [µmol/L]	92.13 ± 6.95	$^*79.39 \pm 5.86$	86.16 ± 7.57	89.69 ± 3.43
MDA [µmol/L]	1.33 ± 0.06	$^*1.21 \pm 0.04$	1.20 ± 0.09	1.22 ± 0.07
SH-groups [µmol/L]	129.2 ± 6.16	125.4 ± 5.58	135.6 ± 4.22	130.6 ± 5.77

two independent groups were compared also by Student's t-test. A value of $P < 0.05$ was considered as statistically significant. Data are expressed as mean±SEM. GraphPad Prism v. 5.00 statistical software was used.

Results. The biochemical measurements did not reveal any change in the liver and kidney of AICCL-treated groups as compared to the control group. No significant variations were observed in serum activities of hepatic enzymes (AST, ALT, ALP), as well as in the serum concentrations of TG, creatinine, urea and SH-groups. Significant decrease in UA and MDA in the group treated by 1% AICCL was detected ($P < 0.05$ vs control, see Table 1).

No significant changes in levels of MDA as a marker of lipid peroxidation and SH-groups as a marker of antioxidative status were observed in organ homogenates from liver and kidney and brain. Increase in UA in groups, treated by 1 and 2% AICCL was registered for liver homogenates ($P < 0.001$, $P < 0.05$ respectively vs control, see Table 2).

Histological investigation did not detect pathological deviations in kidneys and liver of treated groups compared to controls (Figs 1 and 2). Significant decrease ($P < 0.01$) in the number of apoptotic cells was registered in the livers of the group, treated with 1% AICCL (Fig. 3).

Discussion. Serum levels of biochemical markers did not reveal any liver or kidney toxicity in the AICCL-treated group as compared to the control group

T a b l e 2

Results from biochemical analyses of organ homogenates
($^{***}P < 0.001$ vs control; $^{*}P < 0.05$ vs control)

Biochemical markers	Control	1% AICCL	2% AICCL	4% AICC
Liver				
UA [µmol/L]	155.6 ± 15.48	$^{***}180.9 \pm 17.48$	$^{*}171.0 \pm 16.57$	158.2 ± 16.77
MDA [µmol/L]	0.77 ± 0.09	0.72 ± 0.06	0.79 ± 0.06	0.67 ± 0.06
SH-groups [µmol/L]	112.2 ± 5.56	119.9 ± 5.52	114.4 ± 5.14	112.9 ± 4.07
Kidney				
UA [µmol/L]	155.0 ± 12.62	156.0 ± 13.72	157.3 ± 13.41	159.9 ± 12.65
MDA [µmol/L]	2.08 ± 0.13	1.95 ± 0.15	1.96 ± 0.16	1.95 ± 0.11
SH-groups [µmol/L]	121.5 ± 2.38	125.9 ± 2.32	118.9 ± 5.91	122.1 ± 4.94
Brain				
UA [µmol/L]	62.21 ± 2.44	60.95 ± 2.30	59.68 ± 4.17	63.20 ± 4.49
MDA [µmol/L]	1.78 ± 0.21	2.08 ± 0.29	1.93 ± 0.28	1.95 ± 0.22
SH-groups [µmol/L]	90.4 ± 3.90	93.0 ± 3.10	99.66 ± 3.60	93.08 ± 3.12

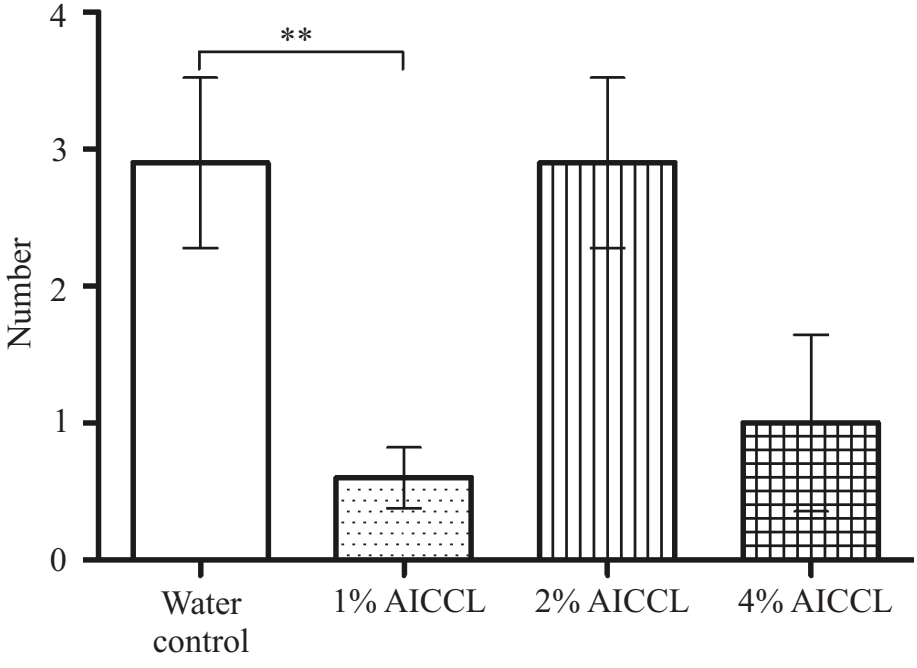

Fig. 3. Count of apoptotic cells in the liver
Legend: Data are presented as mean ± SEM. $^{**}P < 0.01$ vs control

(Table 1). Furthermore, histological investigation did not detect pathological deviations in kidneys and livers of AICCL-treated groups as compared to the control group (Figs 1 and 2). The estimated values of serum urea and creatinine suggest that kidney function is not affected by the AICCL. Liver AST, ALT and ALP enzyme activities remain unchanged indicating preserved liver function. Even more, a significant decrease ($P < 0.01$) in the number of apoptotic cells in the liver was registered in the group treated with 1% AICCL (Fig. 3), indicating healthy liver status. In addition, lower serum UA levels for group 2 exclude possible nuclear breakdown and confirm the lack of toxicity.

Besides the lack of renal or liver toxicity, the AICCW had also some beneficial effect on serum redox status estimated by lower MDA values in group 2. The lower levels of lipid peroxidation might be associated with the reducing properties of the polyphenols ($P < 0.05$ for the 1% AICCL-treated group and a trend for a decrease in the other two groups).

Study of organ homogenates allowed us to assume that the infusion did not impair kidney or brain redox status, as MDA, UA and SH groups did not change significantly upon treatment (Table 2). High liver UA values might be related to

intensified polyphenol metabolism carried out predominantly there. Polyphenols which are reported to induce the expression of many genes from different signalling pathways [15,16] would contribute to the increase of RNAs in the hepatocytes, resulting in activated nucleotide metabolism and the subsequent degradation of nucleotides associated with higher UA levels.

Conclusion. Although *C. coggygria* is indicated as a poisonous plant, the subchronic administration of aqueous infusion from leaves is non-toxic in the applied concentrations. This provides an explanation why Balkan folk medicine reports ulceroprotective and anti-inflammatory properties of orally-administrated infusions from *C. coggygria* leaves.

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