1st International Conference
on Chemo and BioInformatics
ICCBIKG 2021

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BOOK OF PROCEEDINGS

October 26-27th, 2021,
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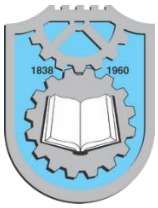

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DNA PROTECTIVE ACTIVITY OF TWO SPECIES OF THE *SCROPHULARIA* GENUS

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Abstract:

Plants from the genus *Scrophularia*, family Scrophulariaceae have numerous biological activities such as antibacterial, antioxidant, antiprotozoal, antitumor, hepatoprotective, and antidiabetic. However, as far as we know, genotoxic and antigenotoxic effects of these two plant species remain unexplored. The present study aimed to evaluate possible *in vivo* protective effects of the methanol extracts of two plant species of the *Scrophularia* genus, *Scrophularia canina* L. and *S. alata* Gilib., against carbon tetrachloride (CCl_4)-induced DNA damage in albino Wistar rat. A significant increase in total comet score has been shown in animals receiving CCl_4 compared with the negative control. Treatment with either *S. alata* or *S. canina* extracts reduced CCl_4 induced DNA damage as indicated by the percentage of reduction in total comet score with a value above 50%.

Key words: *Scrophularia canina*, *Scrophularia alata*, comet assay, *in vivo*

1. Introduction

The genus *Scrophularia*, family Scrophulariaceae, consists of about 300 species commonly present in Asia, Europe, and North America [1]. Plants from the genus *Scrophularia* have been used in traditional medicine in the treatment of various diseases as fever, erythema, eczema, wounds, ulcers, abscesses, fistulas, allergy, rheumatics, and cancer, among other health problems such as mental, nervous, and gastrointestinal disorders [2,3]. Many of these plants have numerous biological activities such as antibacterial, antioxidant [4], insecticidal [5], antitumor, hepatoprotective, and antidiabetic [6], which are related to their richness in secondary metabolites.

The insecticidal activity of aqueous, methanol, hexane and petroleum ether extracts of the aerial part of *S. canina* against the second and fourth-instar larvae and adult females of *Culex pipiens* molestus was investigated [7]. The highest toxicity was exhibited by the petroleum ether and hexane extracts against second- and fourth -instar larvae, respectively.

The total antioxidant capacity and total phenolic and flavonoid levels of methanolic extracts from aerial part of *S. alata* and *S. canina* were evaluated by Šipovac et al. [8]. The higher amount of phenols and flavonoids was found in *S. alata* extract, while the methanol extracts obtained from aerial part of the plant *S. canina* exhibited higher antioxidant activity. According to Guarrera and Lucia [9] *S. canina* is a rich source of iridoid glycosides. This plant has been used in the traditional medicine to treat rhagades on the breast, wounds and haematomas, to bring out abscesses and fistulas [10]. It was used as an anti-

inflammatory, antibiotic and antimalaric agent, while the plant was a generic febrifuge [11]. As far as we know, the antigenotoxic effect of these two plant species remains unexplored. This study was designed to investigate possible protective effect of the methanol extracts of the *S. alata* and *S. canina* against CCl_4-induced DNA damage in albino Wistar rat via the comet assay.

2. Materials and methods

2.1 Plant material and extract preparation

The aerial parts and roots of *S. canina* were collected in August 2011 around Sarajevo, along the road to Pale, Bulozi. The aboveground parts of *S. alata* were collected on the mountain Jahorina during August 2011. Plant materials were chopped into small pieces and air-dried in darkness at room temperature.

Dried aerial parts and roots of *S. canina* and aboveground parts of *S. alata* were separately extracted three times with methanol at room temperature. The extracts were filtered and concentrated under vacuum at 40°C by using a rotary evaporator, and stored in darkness at 4°C until further analysis.

2.2 Animals and study design

Male albino Wistar rats weighing 220 ± 20 g used in this study were obtained from the Animal House of Military Medical Academy, Belgrade, Serbia and acclimatized for three days prior to the experiment. Maintenance was under a 12 h light-dark cycle, with food and water available *ad libitum*. All animal procedures were approved by the University Committee of the Ethics of Animal Experimentation, which acts in accordance with the relevant Serbian guidelines, including the Guidelines for the Care and Use of Laboratory Animals.

Twenty male albino rats were equally divided into four groups consisting of five animals in each group and treated orally for seven days, as follow: the first group, which represents the negative control group, was daily given distilled water for seven days and then intraperitoneally 1 mL/kg body weight olive oil. The second group served as the positive control group, was orally given distilled water for seven days and then intraperitoneally a single dose of CCl_4 (1 mL/kg body weight, 1:1 mixture in olive oil). The animals of the third and fourth groups received orally the methanolic extract of *S. alata* and *S. canina* at 200 mg/kg body weight, respectively. On the last day of the treatment, these animals received intraperitoneally a single dose of CCl_4. Twenty-four hours after CCl_4 injection, all of the animals were anesthetized with ethyl ether, sacrificed and liver samples were collected.

DNA damage in liver samples was measured using the alkaline comet assay under alkaline conditions and dim indirect light according to Singh et al. [12]. Before analysis the images, the slides were stained with 75 μl ethidium bromide (20 μg/ml).

2.3 Data scoring and photomicrographs

Comets were visualized and captured with 400x objective lens of fluorescence microscope Nikon (Ti-Eclipse) attached to CCD camera. Comets without heads and those with almost all the DNA in the tail or with a very wide tail were excluded from the analysis, since they could represent dead cells [13]. The comets were analyzed by a visual scoring method as described by Collins [14]. A total comet score and the percentage reduction (%R) in the comet score in the treatments with extracts showing antigenotoxicity was calculated according to Manoharan and Banerjee [15] and Waters et al. [16].

2.4 Statistical analysis

The data obtained were statistically analyzed using SPSS statistical software package (version 13.0). Hypothesis testing methods included one way analysis of variance (ANOVA). The results were considered to be statistically significant at $p < 0.05$.

3. Results and Discussion

The results of the comet assay in evaluating the *S. alata* and *S. canina* extracts, namely data on the total number of cells with damage and scores for rat treated with 200 mg/kg body weight, besides negative and positive control (1 mL/kg body weight CCl_4) are presented in Table 1.

In rats receiving only CCl_4, marked DNA damage was measured in the liver compared to the control group. Analyzing the comet class distribution shown in Table 1 for the treatments with *S. alata* extract, it is clear that cells with classes 0, 1, and 2 (very low, low and medium damage, respectively) were most prevalent and that cells with classes 3 and 4 were rare. When rats were treated with the *S. canina* extract prior to CCl_4, most of the cells examined on slides were in classes 0, 1 and 2, and very few showed a long DNA migration (class 3).

Table 1. Detection of DNA damage using the comet assay in livers of rat pretreated with *S. canina* and *S. alata* extracts

	Comet class					Total score[a]	% R
	0	1	2	3	4		
I	83.4±0.53	13.3±0.2	3.3±1.01	0.00±0.00	0.00±0.00	19.9±1.42†	
II	6.07±0.81	30.3±0.62	42.4±0.31	18.2±0.12	3.03±0.3	181.82±1.45*	
III	52.7±0.23	18.4±0.32	15.8±0.41	10.5±1.02	2.6±1.1	91.9±0.23*†	55.5
IV	45.3±0.11	34.2±0.43	17.8±0.6	2.7±0.4	0.00±0.00	77.9±0.95*†	64.2

I-Control group; II-CCl_4 1 mL/kg; III-*S. alata* 200 mg/kg+CCl_4; IV- *S. canina* 200 mg/kg+CCl_4.
[a]Values represented mean ± SEM from three independent experiments; n = 5 rats per group
$^{*}p < 0.05$ when compared with the negative control group
$^{\dagger}p < 0.05$ when compared with the CCl_4 group.

A statistically significant reduction in the extent of DNA damage was found in the group of animals treated with 200 mg/kg body weight of the *S. alata* and *S. canina* extracts (55.5 and 64.2%, respectively) to those treated only with CCl_4.

Both extracts at the dose of 200 mg/kg body weight presented protective activity, since the results were statistically different from those obtained from the positive control group. Although, the total score were intermediate between obtained in the untreated control group and the group treated with the CCl_4, statistically, the comet scores were not reduced to the levels of the untreated control.

3. Conclusions

The presented results demonstrated that methanolic extracts of *S. alata* and *S. canina* had antigenotoxic activity. Treatment with extracts prior to treatment with the CCl_4 revealed statistically significant protection attributable to the extracts with percentage reduction in comet score of 55.5 and 64.2%, respectively. These effects can be explained due to the presence of phenols and flavonoids. Further investigation is in progress in order to isolate the mentioned compounds and to determine their biological activities.

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