Anti-genotoxic potential of *Gentiana lutea* extracts against the food sweetener saccharin

S Djukanovic¹, S Cvetkovic¹, D Mitic-Culafic¹, J Knezevic-Vukcevic¹ and B Nikolic¹

¹ Department of Microbiology, Faculty of Biology, University of Belgrade, Studentski Trg 12-16, 11000 Belgrade, Serbia

E-mail: stefana.d@bio.bg.ac.rs

Abstract: Sweeteners are now an integral part of the food industry. Despite its long history of use, saccharin has been in the spotlight for many reasons including toxicity, carcinogenicity and other health issues. Because of this, it is necessary to find natural supplements that could reduce the negative effect of this sweetener. The aim of the study was to investigate the potential anti-genotoxic effect of *Gentiana lutea* plant extracts against saccharin-induced genotoxicity. *G. lutea* is widely used in traditional medicine and in the food and pharmaceutical industries. The anti-genotoxicity of root and leaf 50% aqueous-ethanolic extracts was tested on normal human fetal fibroblasts (MRC-5) and human hepatocarcinoma cells (HepG2) using the comet assay. *G. lutea* extracts significantly reduced the genotoxic effect of saccharin at all tested concentrations. The results obtained in this study indicate the strong protective effect of *G. lutea* against saccharin-induced DNA damage and encourage further studies in order to use this plant as a source of natural food supplements.

1. Introduction

Food additives are used in the food industry to improve food quality, safety, flavor, sweetness and other properties of food [1]. In order to determine the relationship between diet and cancer, it is necessary to determine which food chemicals are potential mutagens. Most chemicals are present in food in relatively low concentrations; however, some of them possess carcinogenic potential even at very low concentrations, and so it is important to reduce or eliminate their harmful effect. It is known that extensive sugar consumption has harmful effects on human health, and moreover, its use by diabetics is limited. To overcome this problem, artificial sweeteners started to appear in the 1800s. One of the most common is saccharin, discovered in 1879 [2]. Since its discovery, saccharin has been the center of many controversies regarding its potential toxic effects. It is the most intensively investigated sweetener for its possible carcinogenic effects.

In order to decrease side effects of artificial food additives, there is a constant need to develop natural products that are able to substitute artificial additives, or to attenuate their harmful potential. Numerous available drugs originating from plant material are widely used in traditional medicine. One of them is manufactured from *Gentiana lutea* or great yellow gentian, a medicinal plant that possesses various biological activities. Gentian was considered successful in the treatment of liver and stomach problems, fever, infected wounds, and is still used in folk medicine to improve appetite and stimulate
digestion. Also, it has anti-inflammatory, antioxidant, hepatoprotective, diuretic, and antidiabetic effects [3, 4]. Gentian root has a long history of use because of its bitterness, and it is the main ingredient of many pharmaceutical products.

Accordingly, the aim of this study was to examine the anti-genotoxic potential of *G. lutea* extracts towards the genotoxic effect of saccharin.

2. Materials and Methods

Plant extracts were prepared as previously described by Nastasijević et al. (2012) [3]. Firstly, to determine non-cytotoxic concentrations of *G. lutea* root (GIR) and leaf (GIL) 50% aqueous-ethanolic extracts and saccharin (SH) towards normal human fetal fibroblasts (MRC-5) and human hepatocarcinoma (HepG2) cell lines, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed. Tested concentrations ranged from 0.062 mg/mL to 2 mg/mL for extracts and 0.312 mg/mL to 10 mg/mL for saccharin. MTT assay was performed as described by Vasilijević et al. (2018) [5], measuring the absorbance at 570nm to determine cell viability. To investigate genotoxic and anti-genotoxic potentials of extracts and sweetener, the alkaline comet assay was applied as previously described by Nikolić et al. (2015) [6]. The DNA damage was quantified by Comet Assay IV software. The highest non-genotoxic concentrations were selected for determination of anti-genotoxic effect of extracts. Both cell lines were exposed to co-treatment of extracts and saccharine for 24 h. For statistical analysis, Statistica 7.0 (StatSoft, Inc.) was used. To determine statistical significance, the Mann-Whitney U test was applied.

3. Results and Discussion

According to MTT results, all tested concentrations of *G. lutea* extracts (up to 2 mg/mL) did not show significant cytotoxicity towards MRC-5 or HepG2 cell lines, and were further used in genotoxocity/anti-genotoxicity studies. Furthermore, because the aim of the study was to examine the anti-genotoxic potential of *G. lutea* extracts, it was necessary to determine non-genotoxic concentrations of extracts and concentrations of saccharin that induced sufficient DNA damage. Using the comet assay (data not shown), concentrations ranging from 0.5 mg/mL to 2 mg/mL for root extract and 0.062 mg/mL to 0.25 mg/mL for leaf extract, and the saccharin concentration of 10 mg/mL, were chosen to test anti-genotoxic potential. Anti-toxigenicity was examined on both cell lines co-treated with genotoxic doses of saccharin (10 mg/mL) and serial concentrations of each plant extract for 24 h.

4-Nitroquinoline N-oxide (4NQO)-positive control (10µM); *** Significant differences between co-treated groups and saccharin; *p<0.05; **p<0.01; ***p<0.001 +++ Significant differences in regard to dimethyl sulfoxide (control solution); +p<0.05; ++p<0.01; +++p<0.001
**Figure 1.** Anti-genotoxic potential of *G. lutea* 50% ethanolic root (a) and leaf (b) extracts against saccharin on MRC-5 cell line. Results are presented as mean values of tail moment ± SD

![Figure 1](image)

**Figure 2.** Anti-genotoxic potential of *G. lutea* 50% ethanolic root (a) and leaf (b) extracts against saccharin on HepG2 cell line

Results of testing the anti-genotoxic potential of *G. lutea* extracts against saccharin on the MRC-5 and HepG2 cell lines are shown in Figure 1 and Figure 2. The results indicated that all tested concentrations significantly reduced the genotoxicity induced by saccharin. Interestingly, the lower concentrations exhibited higher statistically significant anti-genotoxic effect, being in accordance with the dual features of some anti-genotoxic agents, termed *Janus* substances [7]. Bearing in mind that saccharin could induce oxidative stress/damage [8], the protective effect of *G. lutea* extracts could be attributed to their high antioxidative properties [3]. Our findings are in line with the work of Hudecová et al. (2010) [9] who showed that hydrogen peroxide-induced DNA damage could be significantly reduced with plants from Gentiana genus. Moreover, the radio-protective effect of *G. lutea* extracts was observed on peripheral blood mononuclear cells and human cervix carcinoma cells [10].

### 4. Conclusion

The results obtained in this study showed that *G. lutea* ethanolic root and leaf extracts exhibited strong protective effects against DNA damage induced by saccharin. All tested concentrations of *G. lutea* extracts significantly reduce the genotoxicity of this sweetener. Taking into account the controversial nature of saccharin, data obtained in this study are a promising contribution to possibly reducing the genotoxicity of this sweetener. Further studies are needed in order to examine the potential of *G. lutea* extracts to be used as dietary supplements.

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