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Short communication

Non-toxicity and anti-inflammatory effect of extracts from *Echinacea purpurea* and *Onopordum acanthium*

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Introduction

Echinacea purpurea and *Onopordum acanthium* belong to the family Asteraceae (Compositae) are among the most widely used medicinal plant species in traditional medicine (Brunia et al., 2018; Garsiya et al., 2019). The plants of genus *Echinacea* possess healing effect due to their high content of phenolic compounds, alkyl amides and polysaccharides (Brunia et al., 2018). There are four species of the genus *Onopordum* in Bulgaria, the most common being *Onopordum acanthium L.* Its biological activity is associated with the presence of apigenin, quercetin, scutellarin, lignans, coumarins and terpenes and sterols. The high amount of biological active compounds in these medicinal plants determines their antioxidant, anti-inflammatory, antiviral, antibacterial and antitumor activity (Garsiya et al., 2019). It is known that the combined extracts provides an optimal ratio of the components of the individual drugs, which eliminates the possibility of overdose when used individually and allows use for a longer period of time without possible side effects (Tewari et al., 2014).

Serum cytokine levels of IFN-γ, IL-10, TNF-α change with increased stress levels and inflammation in the body (Aucoin et al., 2020; Garsiya et al., 2019). This justifies the aim of the study to determine the non-toxicity and anti-inflammatory action of individual and combined extracts of *Echinacea purpurea* and *Onopordum acanthium*.

Materials and methods

Materials

Materials used in the study included ethanol extracts (60%) of *E. purpurea* and *O. acanthium* and Combination 1 (1:1) and Combination 2 (3:1) *E. purpurea*:*O. acanthium*, respectively. Other materials used were: carrageenan, diclofenac sodium, lipopolysaccharide, cytokines (TNF-α, IFN-γ, IL-10), and experimental animals 142 white male Wistar rats. The experiments were approved by the Committee on Animal Ethics of the Bulgarian Agency for Food Safety permit №299 and decision of the Ethical Committee at MU Plovdiv protocol №3 / established on May the 20th 2021.

Methods

In an acute toxicity test of plant extracts, 54 male Wistar rats were treated orally with individual and combined plant extracts at a single dose of 5 g/kg b.w. and 10 g/kg b.w. The survival of the animals 24 hours after administration of the extracts was recorded.

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Two models of induced inflammation were used to study the anti-inflammatory effect of the extracts and their combinations:

1) With carrageenan. In this model of inflammation, 48 male rats, divided into 6 groups, were treated for 14 days with the tested extracts at a dose of 500 mg/kg b.w. Criteria for anti-inflammatory effect were the changes in the size of the hind right paw of rats, which were reported on the second, third, fourth and 24 th hour after carrageenan treatment.

2) With lipopolysaccharide. In this model, 40 animals were used, divided into 5 groups. They were treated for 14 days with the tested extracts at a dose of 500 mg/kg b.w, after which the animals were decapitated and blood was taken to test the levels of pro- and anti-inflammatory cytokines - TNF-α, IFN-γ, IL-10.

The analyses of cytokines were conducted by ELISA with commercial kits (DiaClone) in strict compliance with the manufacturer's guidelines.

Results and discussion

After the acute toxicity experiment, the results showed 100% survival of the animals, proving the non-toxicity of the individual and combined extracts.

In a model of carrageenan-induced inflammation, Combination 2 was found to provide statistically significant reduction of paw edema at 24 hours. In other groups, a decrease in edema was also observed, but only as a trend, without statistical significance.

The decrease in serum TNF-α concentration in the group treated with Combination 1 was most pronounced compared to the control treated with lipopolysaccharide, followed by the group treated with Combination 2. Regarding the individual extracts, *O. acanthium* decreased the TNF-α level to a greater extent than the *E. purpurea* extract. The most pronounced change in serum IFN-γ levels was observed in animals treated with the Combination 1 compared to the control group, followed by the groups treated with the extracts from *O. acanthium* and *E. purpurea*. Compared to the lipopolysaccharide-treated control group, IL-10 levels increased in all groups of animals treated with the test extracts, most notably in Combination 2, where the difference was statistically significant.

A number of authors reported the anti-inflammatory action of extracts of *E. purpurea*, which may be due to the presence of chicory and kaftaric acids that is characteristic of this species (Brunia et al., 2018; Hussain et al., 2016). The anti-inflammatory effect of *Onopordum* is due to the presence of chlorogenic acid, apigenin and arctigenin, found in its extracts (Garsiya et al., 2019).

In our previous study, we found significant amounts of these substances and thus prepared Combination 1 and Combination 2 to test their effect on serum concentrations of the above cytokines. From the results obtained, it is clear that Combinations 1 and 2 showed a better anti-inflammatory effect compared to the individual extracts of *E. purpurea* and *O. acanthium*, significantly reducing serum levels of TNF-α, IFN-γ and increasing IL-10 levels.

Conclusion

The non-toxicity of the single and combined extracts of *E. purpurea* and *O. acanthium* has been proven. Combined extracts have more apparent effect on serum levels of TNF-α, IFN-γ, IL-10 compared to the extracts alone. This is most likely due to the synergistic action of biological active compounds contained in the combined extracts.

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