STUDY ON ANTI-INFLAMMATORY AND ANALGESIC EFFECTS OF TOTAL EXTRACT OF GERANIUM SANGUINEUM, ASTRAGALUS GLYCYPHYLLOS, ERODIUM CICUTARIUM AND VINCETOXICUM OFFICINALIS

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ABSTRACT

The use of plants and parts of plants for treatment of various diseases dates back to antiquity. Our research team came across such a recipe associated with the use of extract of *Geranium Sanguineum (Geraniaceae)*, *Astragalus Glycyphyllos (Fabaceae)*, *Erodium Cicutarium (Geraniaceae)* and *Vincetoxicum officinalis (Apocynaceae)* for the treatment of certain diseases of the female reproductive system. Based on this information, we prepared a total thick extract of equal amounts of herbal using the method of percolation with 70% ethyl alcohol, removal of the solvent with rotary evaporator. The received extract was tested for acute toxicity. We investigated the anti-inflammatory and analgesic effect of the obtained extract. Anti-inflammatory effect was tested using the method of carageenan-induced paw edema (1), the analgesic effect - using Hot Plate test - the method of Woolfe & McDonald (Ugo Basile) and test "Randall-Selitto" – analgesy-meter (2). The investigated model extract did not show anti-inflammatory effect. Analgesic effect was found only on chronic administration and against thermal irritation.

Keywords: *plant extract, anti-inflammatory effect, analgesic effect*

Introduction

In the last years we witnessed an extraordinary interest in phytopreparations (3). They are obtained after a suitable technological processing of the plant material. Among the biologically active substances, accompanying substances without biological activity are extracted, but in many cases they favor the pharmacological effect. Adverse and toxic effects of phytopreparations are much more limited compared to the synthetic drugs (3). The following biologically active substances were found in Geranium Sanguineum - flavonoids, tannins, phenolic acids. It has a significant antioxidant, hypotensive effect, antispasmodic and cardio-tonic action (4). Bacteriostatic effect on some pathogenic microorganisms was determined experimentally (5, 6, 7). In Astragalus Glycyphyllos were found flavonoids, ascorbic acid, tannins, saponins, organic acids and trace elements (8). They determine its laxative, diuretic, anti-inflammatory, antiperspirant, hepatoprotective, antioxidative and antiviral effect (9, 5, 6, 10). It has been used for painful and irregular menstruation, infertility, uterine diseases and leucorrhoea. *In vitro* antimicrobial activity was found for the flavonoids (7). Erodium Cicutarium contains tannins, flavonoids and vitamin C. It has an antioxidant, astringent and haemostatic action (11). It has been used in the treatment of uterine and other bleeding. Vincetoxicum officinalis have a rich phenolic composition and in the last years was used in homeopathic medicines (12, 13).

Aim

The object of this study was the preparation of total thick extract from equal amounts of the aerial parts of Geranium Sanguineum, Astragalus Glycyphyllos, Erodium Cicutarium and Vincetoxicum officinalis, and study of its anti-inflammatory and analgesic effects.

Materials and methods

The herbal drugs are collected in ecologically clean regions of Bulgaria, at the most appropriate stage of vegetation.

Indomethacin - European Pharmacopoeia (EP) Reference Standar, Fluka; metamizole sodium amp. injectable 500 mg/ml, 2 ml Sopharma PLC; NaCl 0.9%, 10 ml amp. injectable, Sopharma PLC; Λ-carrageenan, Sigma – 1 % solution; methyl cellulose, Sigma - 0.7% methyl cellulose solution; spiritus aethylicus 70% v/v, Chemax pharma; male "Wistar" rats with weight of 180 – 200g.

Obtaining of model extract: the extract was obtained from equal amounts of herbal drugs, using the method of percolation with 70% ethyl alcohol. With thus obtained liquid extract follows removal of the solvent with rotary evaporator – Rotavapor R II, BUCHI, to a thick extract.

Phyto-chemical analysis of the extract was made using HPLC system Varian, RP C18 column and UV detector with variable wavelength. Mobile phase used is A (H2O, pH=3):B (CH3CN) in gradient mode from 90(A):10(B) to 10(A):90(B) and a flow rate of 1 ml/min.

Study of acute toxicity: Experiments were conducted with 10 groups of 6 animals in group, with male "Wistar" rats with a weight of 180-200 g. The animals were placed in standard conditions. Examined doses are 1 mg/kg, 10 mg/kg, 100 mg/kg, 5.0 g/kg and 10.0 g/kg. Extracts were introduces orally by gavage. Rats were observed 24 hours.

The study of ***anti-inflammatory effect*** was conducted by the method of carageenan-induced paw edema.

The animals were divided into groups of eight animals. The compounds are tested in different doses. A control with saline and positive control with indomethacin were used for comparison.

Paw edema was induced by injecting 100 μl of a 1 % solution of Λ-carrageenan in saline into right hind paw of the rat. Hind paw volume was measured prior to carrageenan injection and at 2^{nd}, 3^{rd}, 4^{th} and 24^{th} thereafter with a pletismometer (Ugo Basile, Italy).

Immediately after carrageenan injection the control recieved 0.1 ml/100 g bw saline orally by gavage (p. o.), the animals in the postive control group were treated with 2 mg/kg bw IMC in 0.7% methylcellulose solution (p. o.) and the animals of the experimental groups were treated with the test extract in doses 1 and 2 g/kg bw (p. o.). The difference between the initial paw volume (at time zero) in ml of treated with carrageenan right hind paw (V_0) and the volume at 2^{nd}, 3^{rd}, 4^{th} and 24^{th} after treatment (V_t) was calculated for each experimental animal. Trinus formula was used to calculate the percent paw edema.

To study the ***analgesic activity*** of examined extract rats were divided in four groups (6 per group) as follows: control group treated with saline (p. o.), the positive control – with metamizole sodium (MS) 150 mg / kg bw (p. o.) and two experimental groups with the studied extract in the doses tested (1 and 2 g/kg bw p. o.).

In the first series of experiments the analgesic effect of a single dose from the extract was studied. In the second series of experiments the analgesic effect of repeated doses was studied.

Nociceptive (analgesic) tests.

1. HOT/COLD PLATE (Ugo Basile, Italy). Animals were individually placed on a plate with a constant temperature of 55°C ± 0,5 °C, enclosed in plexiglass cylinder. The latency to the first sign of paw licking or response to avoid the heat was taken as an index of the pain threshold. To avoid tissue damage, the maximum stay on the plate is 30 seconds. Animals are tested before treatment and the 1st, 2nd and 3rd hour after treatment. Analgesic activity criterion is an extension of the normal reaction time in test animals, compared to control treated with saline. MS treated control group is used as a benchmark for the analgesic effect.

2. RANDALL & SELITTO TEST (ANALGESY-METER, Ugo Basile)

Nociceptive threshold was measured with an analgesimeter by applying an increasing pressure to the right hind paw of unrestrained rats. The strength of the pressure at which the animal withdraws testing paw was recorded. The maximal possible pressure is 250 grams (cut-off). The rats were tested before the treatment and on the 1st, 2nd and 3rd hour after the treament.

Statistical analysis

Data were analyzed using the Independent – Samples T test from the software product SPSS 11.0. Mean values ± SEM were calculated. Results were considered significant at $p<0.05$.

Results

The phytochemical analysis shows content of rutin 266.4 ± 14.2 μg/g, quercetin 53.6 ± 4.1 μg/g and pyrogallol 880.8 ± 43.9 μg/g.

Results from acute toxicity – no animals died during the observation. We can conclude that the proposed extract is non-toxic.

In single dose treated animals, the group receiving IMC as a reference substance with anti-inflammatory effect showed a significant reduction of the carrageenan induced edema, when compared with the control group treated with saline. Statistically reliable results were registered at 2, 3 and 4 hours ($p<0,0001$; $p = 0,001$; $p = 0,001$). Experimental groups showed no statistically significant differences compared with the control group. In repeated dose treated animals, IMC demonstrated significant anti-inflammatory activity when compared with the control group. Experimental groups treated with the extract in both doses tested (1 g/kg bw and 2 g/kg bw), showed no statistically significant differences compared to the control group.

Tabl.1 Anti-inflammatory effect of examined extract in carrageenan-induced rat paw edema after single dose treatment.

	Mean ± SEM (%) at 2 hour	Mean ± SEM (%) at 3 hour	Mean ± SEM (%) at 4 hour	Mean ± SEM (%) at 24 hour
Control	65,14 ± 3,09	76,69 ± 6,7	79,05 ± 6,09	51,56 ± 7,14
Indomethacin	21 ± 6,23	29,81 ± 7,44	32,99 ± 7,51	48,15 ± 7,94
EXT 1,0g	72,64 ± 11,35	88,83 ± 9,23	76,91 ± 8,51	57,82 ± 7,6
EXT 2,0g	67,35 ± 8,49	88,11 ± 13	73,22 ± 12,95	51,54 ± 7,82

The analgesy-meter test uses the mechanical pain stimulus. As a reference substance for the analgesic effect was used Metamizole sodium at a dose 150mg/kg. In single dose treated animals, metamizole showed a significant analgesic effect on the 2nd hour ($p = 0.049$). The extract showed no reliable analgesic effect (excluding the dose of 1g/kg bw, 1st hour, $p = 0.031$). In repeated dose treated animals, metamizole has analgesic effect on the 1st, 2nd and 3rd hour, ($p = 0.014$, $p = 0.009$, $p = 0.002$). From the experimental groups, reliable analgesic effect shows only the group treated with extract 1 dose 2 g/kg bw on the 2nd and 3rd hour ($p = 0.037$, $p = 0.022$).

Tabl.2 Analgesic effect of examined extract in analgesimeter test after single dose treatment.

	Mean ± SEM (g) before treatment	Mean ± SEM (g) at 1 hour	Mean ± SEM (g) 2 hour	Mean ± SEM (g) 3 hour
Control	3,67 ± 1,15	8,58 ± 1,54	6,08 ± 1,8	8,92 ± 1,85
Metamizole	1,75 ± 0,38	14,5 ± 2,79	14,08 ± 2,97	16,67 ± 2,9
EXT 1,0g	3,67 ± 0,51	4 ± 0,74	7,83 ± 2,26	12,5 ± 3,45
EXT 2,0g	3 ± 0,62	12,33 ± 3,99	5,67 ± 0,71	7 ± 1,29

Tabl.3 Analgesic effect of examined extract in analgesimeter test after repeated dose treatment.

	Mean ± SEM (g) at 1 hour	Mean ± SEM (g) 2 hour	Mean ± SEM (g) 3 hour
Control	3,85 ± 1,05	3,5 ± 0,98	4,75 ± 1,7
Metamizole	13,17 ± 2,63	15,17 ± 2,9	13,83 ± 1,3
EXT 1,0g	5,75 ± 1,34	8,67 ± 3,64	8 ± 2
EXT 2,0g	8,17 ± 3,54	5,92 ± 1,95	6,67 ± 1,87

The hot plate test uses a thermal pain stimulus. In single dose treated animals, MS showed a significant analgesic effect on the 1st hour ($p = 0.024$) (Tabl.4). In the experimental groups no statistically significant differences with the control.

Tabl.4 Analgesic effect of examined extract in Hot Plate test after single dose treatment.

	Mean ± SEM (s) at 0 hour	Mean ± SEM (s) 1 hour	Mean ± SEM (s) 2 hour	Mean ± SEM (s) 3 hour
Control	3,67 ± 1,15	8,58 ± 1,54	6,08 ± 1,8	8,92 ± 1,85
Metamizole	1,75 ± 0,38	14,5 ± 2,79	14,08 ± 2,97	16,67 ± 2,9
EXT 1,0g	3,67 ± 0,51	4 ± 0,74	7,83 ± 2,26	12,5 ± 3,45
EXT 2,0g	3 ± 0,62	12,33 ± 3,99	5,67 ± 0,71	7 ± 1,29

In repeated dose treated animals, metamizole showed reliable analgesic effect on the 2nd and 3rd hour ($p = 0.012$, $p = 0.007$) (Tabl.5). In the extract treated groups, statistically reliable analgesic effect was observed in the group treated with the extract, dose of 1g/kg bw, on the 1st, 2nd and 3rd hour ($p = 0.024$, $p = 0.029$, $p = 0.021$). At a dose 2g/kg in the first 2 hours, although not statistically reliable, the effect is significantly more pronounced than that of the control. At the 3rd hour was statistically significant ($p = 0.005$).

Tabl.5 Analgesic effect of examined extract in Hot Plate test after repeated dose treatment

	Mean ± SEM (s) at 1 hour	Mean ± SEM (s) 2 hour	Mean ± SEM (s) 3 hour
Control	11,67 ± 0,86	9,88 ± 1,18	8,52 ± 0,79
Metamizole	15,65 ± 1,8	20,8 ± 3,36	21,48 ± 3,71
EXT 1,0g	16,58 ± 1,65	15,43 ± 1,77	13,48 ± 1,64
EXT 2,0g	15,33 ± 2,11	16,62 ± 2,9	20,3 ± 3,26

Conclusion

The extract examined is non-toxic.

The combination of equal parts of Geranium Sanguineum, Astragalus Glycyphyllos, Erodium Cicutarium and Vincetoxicum officinalis showed no anti-inflammatory effect.

The extract showed **analgesic effect only on repeated administration** against thermal irritation, which is mediated from supraspinal mechanisms.

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