THE RESEARCH OF THE REGENERATING ACTIVITY OF GELS BASED ON BIDENS TRIPARTITA HERB EXTRACTS ON MICE AND RATS

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The abundance of atopic dermatitis among children is 5–20% and among adults is 2–10%. One of the advanced solutions is the development of drugs, based on phytosubstances. One of the well-known medical plants for skin treatment is Bidens tripartita (Asteraceae). It is known for its anti-inflammatory and regenerating properties. On the base of SPCPA a technology of Bidens tripartita extract obtained by 60% ethanol extraction and standardized with flavonoids was developed. The aim of this study is to examine the regenerating properties of a hydrophilic gel based on Bidens tripartita extract obtained by 60% ethanol extraction and compare them with properties of gels, based on Bidens tripartita extracts obtained by another extracting agents. Pharmacological studies were performed on 50 white outbred male mice with weigh of 18–20 g, which were kept on a standard diet in vivarium. The mice got a medical discission of 2 cm long with fixed edges. The mice were devided into 5 groups according to the substance applied. The substances were: 1 — a gel, based on Bidens tripartita extract obtained by 60% ethanol extraction; 2 — a gel, based on Bidens tripartita extract obtained by 20% ethanol extraction; 3 — a gel, based on Bidens tripartita extract obtained by water extraction; 4 — a comparator — an ointment “Calendula”; 5 — the control group. These substances were applied on mice’s discissions every day during a week until forming of scars. After that the scars were measured tensile tensiometrically. When comparing the groups of mice upon the mass of the weight at which the scar have broken, it was found out, that the heaviest weight is at the group of the gel, based on Bidens tripartita extract obtained by 60% ethanol extraction. The weight is 425 g and thus the scars of the first group are the toughest. The other groups were compared with the first one. The order of decreasing of scar toughness is: group 3, group 2, group 4, group 5. The ratio of these groups relatively to the first group is –23,5 %, —33,0 %, —34,1 %, —38,9 % respectively. Statistically significant differences comparing with control group were not observed. Therefore, the gel, based on Bidens tripartita extract obtained by 60% ethanol extraction has good regenerative properties and accelerates the process of scar-forming. The regenerative ability is regular and stable.

INFLUENCE OF THE COMBINED PHYTODRUG ON THE NEUROTROPIC ACTIVITY OF MICE

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A dry extract of mixture containing linden leaves, clover herb and St John’s wort herb (3:2:2) enriched with flavonoid fraction was developed in SPCPA. The aim of this research is studying the neurothropic activity of the phytodrug with the extract of mixture containing linden leaves (Tilia cordata folia), clover herb (Trifolium pratense herba) and St John’s wort herb (Hypericum maculatum herba). Pharmacological studies were performed on 50 white outbred male mice with weigh of 18–20 g, which were kept on a standard diet in vivarium. The assessment of neurothropic activity was performed in the open field test. The phytodrug was dosed orally with preliminary solution in water. The range of doses was from 2,2 mg/kg to 220 mg/kg. The comparator agent was a phytodrug “Negrustin” at a dose of 14,6 mg/kg. The mice in a control group received water. After 60 minutes of dosing, searching activity (SA), orientating activity (OA), emotional lability (EL) and agression (AG) were assessed. When comparing a group of mice, who received “Negrustin” at a dose of 14,6 mg/kg with control group, decreasing of SA, OA, EL, AG up to 74 %, 25 %, 25 % and 33,7 % respectively was observed. In groups of mice, who received doses of 0,22 mg/kg, 22 mg/kg, 220 mg/kg, decreasing of OA up to 59 %, 41 %, 74 % respectively, decrease of EL up to 50 %, 25 %, 0 % respectively, decrease of SA up to 30 %, 35,3 %, 13 % respectively and decrease of AG up to 26 %, 16,3 %, 12 % respectively were observed. When dosing the phytodrug at different doses, statistically significant differences comparing with control group were not observed. Maximal sedative effect was observed in group of animals, which received the phytodrug at a dose of 0,22 mg/kg. The effect of the phytodrug was consistent with the effect of comparator agent “Negrustin” at a dose of 14,6 mg/kg. In the com-
plex phytodrug the sedative effect is associated with the presence of biologically active substances. Particularly, the effect was due to the triterpenic saponines, glicose hesperedin and polysaccharides of linden leaves. Flavonoids of St John’s wort herb possess antidepressive activity. Thus, hypericin “softly” inhibits monoamine-oxidase and hyperforin supresses the capture of the serotonin, noradrenalin and dopamin, and also inhibits GABA. So the phytodrug is consistent with the effect of comparator agent.

UNCOMMON APPROACHES FOR NATURAL PRODUCTS ANALYSIS — CAPILLARY ELECTROPHORESIS AND CAPILLARY ELECTROCHROMATOGRAPHY

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Analytical sciences are steadily evolving, with new techniques like capillary electrophoresis (CE) and capillary electrochromatography (CEC) being reported frequently. They often claim to be advantageous in respect to separation efficiency and selectivity, but sometimes fail to convince in terms of practical use. In this presentation the audience is briefly introduced to theory and characteristics of CE and CEC, followed by several respective applications on natural products analysis conducted in our lab. They range from the determination of flavonoids and phenolic acids in Arnica montana (CE-UV) (1), over quinolizidine alkaloids in Lupinus species (CE-MS) (2), to adrenergic amines in Citrus aurantium (3) and naphthoquinones in Eleutherine americana (monolithic CEC) (4). For the latter a comparison to HPLC was attempted. It showed that a novel methacrylate-based monolithic stationary phase enabled the baseline separation of five markers (including eleutherin and isoeleutherin) in less than 11 min (HPLC: 22 min), and permitted their quantitative analysis in herbal material with the same precision (e.g. total naphthoquinones by CEC: 0.41%, by HPLC: 0.45%). Only in respect to sensitivity CEC was less advantageous (limit of detection: 2–8 µg/ml, compared to 0.4–0.8 µg/ml by HPLC), otherwise all validation criteria were met and comparable. This and the above mentioned examples indicate the practical applicability of CE and CEC, and hopefully raise further interest in these “exotic” techniques.


STRUCTURE AND BIOACTIVITY OF 1 MG VATIPAROL BY SLIM TUBE NMR

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Bioactive natural products offer multiple opportunities for the discovery of novel chemical entities with potential pharmaceutical and agrochemical applications. But, it is increasingly difficult to determine the structure and bioactivity of novel natural compounds available only in minor or trace quantities (1). Vatiparol, a resveratrol trimer from Vatica parvifolia with an unprecedented carbon skeleton, showed selective inhibitory effect on the expression of monocyte chemoattractant protein-1 (MCP-1) (2). Of particular importance for the structure determination of this organic compound that was available only in trace amounts is our determination of the relative configuration and accurate conformation of vatiparol with RDC (residual dipolar coupling) enhanced NMR using only one milligram of the sample in a 1.7 mm NMR tube. Based on the NMR determined conformation, the absolute configuration was elucidated with chiroptical methods (Figure).

In conclusion, the presented methodology and strategy will be generally useful for the determination of structure and bioactivity of novel natural compounds with limited availability.