and related enzymes activities. Cell migration was measured by Boyden chamber. S1P and Sphk-1,2 was quantitatively measured both by HPLC-fluorescence detection after OPA derivatization and by LC–MS/MS system. HDAC activity assay kit (Cayman Co) was used. Sphk mRNA level was measured by qRT-PCR using ΔΔCt method. Antibodies for PKC isoform was obtained from Cell Science Co. and detected using western blot. Decursin inhibited sphingosine kinase (Sphk) induced angiogenic processes *in vitro*, including cell proliferation, migration of human umbilical vein endothelial cells (HUVEC). Interestingly, Sphk-1 activity was significantly decreased by 43 % compared to control, while Sphk-II activity was 1.6 fold increased by decursin treatment. The S1P in cells and in cultured media was decreased dose-dependently by decursin, indicating that the reduced synthesis of angiogenic lipid mediator S1P which binding to S1P receptors (S1PRs) is essential to transmit S1P-S1PR axis signaling for angiogenesis. Decursin specifically reduced Rac-1.The increased Sphk-II activity and thus S1P production in nuclear fraction blocked histone deacetylase (HDAC) activity. Indeed, the mRNA expression of Sphk-II was 3-fold increased by decursin. Sphingosine level was also increased in 2-fold. Decursin treatment enhanced the migration of Sphk-1 and PKCα into nuclear membrane. The expression of PKCα and PKCη (eta) were decreased while other PKC isoforms were not changed. Our data suggests that decursin have a potent anti-angiogenic property via S1PR-S1P axis by regulating both SPHK-I and -II activites and thus reducing S1P synthesis and release.

ISOLATION OF GELATIN HYDROLYSATES EFFECTIVE ON BONE FORMATION AND RESORPTION

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Gelatin is a mixture of polypeptides obtained by partial hydrolysis of collagens extracted from connective tissues of animals. The aim of this study was to provide the gelatin hydrolysate (GH) which is effective on bone formation. Porcine skin gelatin was hydrolyzed by manipulating two reaction parameters; enzyme combination and reaction time. Four combinations of enzymes (alcalase+protamex, protamex+flavourzyme, flavourzyme+alcalase and alcalase+protamex+flavourzyme) and 3 reaction times (4, 12, and 24 h) were examined. The resultant 12 GHs were fractionated into 3 ranges of molecular weight (total, >3kDa, and < 3kDa), and 36 various GH were obtained. An *in vitro* study on bone formation was carried out in osteoblast-like MG63 cell proliferation and optimal hydrolysis condition for the maximal bone formation activity was selected. The enzyme combination of protamex+flavourzyme provided the GH with the highest activity. Higher bone formation activity was observed in small molecular weight (< 3kDa) GH at reaction time of 4 hour. The effects of selected GH on bone resorption were determined in bone marrow-derived osteoclasts cells driven by RANKL and M–CSF. GH suppressed the formation of TRAP-positive osteoclasts. Furthermore, RANKL induced TRAP activity was greatly inhibited by GH treatment. The effects on bone formation and resorption were not observed with gelatin treatment. These results suggest that GH isolated in this study is a promising agent for the prevention and treatment of bone loss.

INTESTINAL ABSORPTION AND PRESYSTEMIC ELIMINATION OF VARIOUS CHEMICAL CONSTITUENTS PRESENT IN GBE50 EXTRACT, A STANDARDIZED EXTRACT OF *GINKGO BILOBA* LEAVES

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The nature and level of systemic exposure to the active herbal constituents will profoundly affect their effects at action sites, which is fundamental in understanding their roles in the overall beneficial effects of an herbal medicine. The objective of this study is to gain a full picture of the systemic exposure to various putatively active ginkgo constituents after p. o. administration of GBE50 extract, a standardized extract of Ginkgo biloba

leaves, to rats and understanding of the relevant mechanisms governing the intestinal absorption and presystemic elimination. To define the ginkgo compounds to be studied, literature informatics-guided chemical profiling revealed that GBE50 extract contained 72 ginkgo constituents, including terpene lactones, flavonols, flavones, an isoflavone, biflavones, flavanols, and carboxylic acids, at levels ranging from 0.01 to 55.3 mg/g. Among the ginkgo constituent groups were the terpene lactones and the flavonols that were significantly measurable in plasma after p.o. administration of GBE50 extract to rats. The intestinal absorption of terpene lactones appeared to be dictated by their intermediate membrane permeability, while the influences of MDR-1-and MRP-2-mediated intestinal efflux and the presystemic metabolism and biliary excretion might be relatively limited. Because of their deglycosylation absent in the small intestine and relatively slow presystemic elimination, many intact flavonol glycosides appeared in the rat plasma albeit with a limited extent of absorption. Colonic deglycosylation of the flavonol glycosides occurred and the glucuronides of flavonol aglycones were also measured in the plasma. Although some biflavones also had relatively high abundance in GBE50 extract, these ginkgo constituents were not measured in the rat plasma because of their poor solubility and poor permeability that hindered the intestinal absorption. The levels of the remaining ginkgo constituents in GBE50 extract were too low to be measured in the rat plasma. The current study enabled us to better understand the nature of systemic exposure to ginkgo compounds after p.o. administration of GBE50 extract and to more precisely implement multicomponent PK study of the extract.

BIOPHARMACEUTICAL ASPECTS OF THE DEVELOPMENT OF A PHYTOECDISTEROIDS (PE) OINTMENT COMPOSITION

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PE are analogues of insect steroid hormones occurring in plants. The establishment of reparative activity of PE (1) makes it relevant to develop compositions on their base. PE molecules have six or more hydroxyl groups and hydrophobic core and therefore the influence of the ointment composition nature on the bioavailability of PE was trialed. In these investigations preparation Serpisten (mixture of 20-hydroxyecdisone and 25S-inocosterone obtained from the plant Serratula coronata L.) has been used. For this purpose three types of compositions have been prepared at a concentration of Serpisten 0.02% (w/w): hydrophobic (lanolin and vaseline — C1), hydrophilic (3% sodium *carboxymethyl cellulose* gel — C2), emulsive (aerosil, vaseline oil, water purified, monoglyceride purified — C3). Biopharmaceutical evaluation of prepared formulations has been performed using drug releasing from the compositions by dialysis through a membrane with subsequent determination of the release profile in the UV region at $\lambda = 240$nm. The concentration of PE was determined at 30, 60 and 120 minutes of dialysis. The test revealed that the PE release from the C3 was almost entirely (100.2%), while from C2 76.5% of the total amount of PE was passed to aqueous medium, and from C1 only 37.5%. During the rheological studies it has been established that the shear stress at the C1 was less than 80 Pa*s at C2–300 Pa*s and at C3 156 Pa*s. C3 is the closest to the «Bepanthen» ointment (Bayer, Germany), which has the wound-healing activity (211 Pa*s.). For further PE reparative properties trials, C3 have been chosen, for this test linear aseptic wound model has been used. In five days it was found that, the scar strength in experimental group was 281.6 ± 40.4 gr., in control group 160.8 ± 18.0 gr. and in intact — 123.4 ± 20.0 gr. This data are higher 1.75 times in experimental group, compared with a control group ($p = 0.021$) and 2.28 times in the intact ($p = 0.009$). The result of this work was the development of preparing technology topical emulsive formulation having the high bioavailability.

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