Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 20, issue 4, 2010, pp. 5-9

A PHARMACOTOXICOLOGICAL EVALUATION OF A BETULIN TOPICAL FORMULATION TESTED ON C57BL/6J MOUSE EXPERIMENTAL NEVI AND SKIN LESIONS

Sorina A. CIURLEA[1], Corina TIULEA[1], Erzsébet CSANYI[2], Szilvia BERKO[2], Claudia-Crina TOMA[3], Cristina A. DEHELEAN[1]*, Felicia LOGHIN[4]

[1] University of Medicine and Pharmacy "Victor Babes" Timisoara, Faculty of Pharmacy, Eftimie Murgu Square no.2, 300041, Timisoara, Romania
[2] University of Szeged, Faculty of Pharmacy, Department of Pharmaceutical Technology, Eotvos u.no.6, Szeged, Hungary
[3] "Vasile Goldis" Western University of Arad, Romania
[4] University of Medicine and Pharmacy "Iuliu Hatieganu" Cluj-Napoca, Faculty of Pharmacy, Romania

ABSTRACT. Betulin is an important triterpenic compound obtained from natural sources such as birch bark. It has antiinflammatory and antitumor activity. The birch bark ointment and betulin added in specific topical formulations are very active in actinic keratosis, pathology with damaged keratinocytes correlated from the histological point of view a squamos carcinoma. The aim of present study was to test the biological activity of a topical formulation with 5% betulin on chemical damaged mouse skin. Materials and methods used betulin, a composed cream with the next active agents: 2-propanol, transcutol, isopropyl myristate, labrasol and PEG 4000 and C57BL/6J type mice. The tumoral applied substances were DMBA (7,12-dimethylbenz[α]anthracene) and croton oil. The mexametric measurements were performed using a mexameter MX18 (Courage&Khazaka Electronics, Cologne, Germany). The protocol followed the observations on melanin evolution and haemoglobin status (pigmentation and erythema). Results and discussions indicated that the substances applied as carcinogens determined the apparition of skin pigmentation and lesions as it is mentioned in literature protocol. The cream applied on the skin had adequate rheological properties and a composition that determined a good delivery of active substance. Our investigations confirm the activity on betulin in reducing skin lesions and irritation by significantly decreasing erythema (haemoglobin) indices. The main conclusion of present study is that betulin could be applied as prophylactic and therapeutic compound in skin pathology more important in skin lesions.

Keywords: betulin, topical, skin, melanin, erythema

INTRODUCTION

Betulin is an important triterpenic compound with antiinflammatory and antitumor activity. Birch bark extract that contains important quantities of betulin (over 30%) is used in cosmetic creams (Huyke et al., 2006). Birch bark and betulin act as emulsifiers, propriety that could be helpful in semisolid formulation (Huyke et al., 2006). The birch bark ointment and betulin added in specific topical formulations are very active in actinic keratosis, a pathology with damaged keratinocytes representing from the histological point of view a squamos carcinoma (Huyke et al., 2006, 2009). The topical formulations with birch bark and especially with betulin are water free oleogels or ointments (Huyke et al., 2006, 2009). Betulin is a compound easily detectable and isolated because of its property of sublimation (Guidoin et al., 2003). Betulin is an alcohol related as structure to betulinic acid (alcohol and acid), a lupan skeleton compound that is also a very important antitumor compound effective in melanoma treatment (Dehelean et al., 2010). These triterpenes were classified as low toxicity compounds (Patocka, 2003, Huyke et al., 2009, Dehelean et al., 2010). Is well known that skin is an organ exposed to different environmental factors such as UV-irradiation or chemical compounds with tumoral potential which can induce skin lesions, papiloma or pigmented nevi. All the damages on the skin surface could lead to important pathologies such as skin carcinoma or cutaneous melanoma. The treatment and prevention of this pathologic change is an important aim in research. Betulinic acid in topical applications is effective in dysplastic nevi that have the potential to be transformed in melanoma (Patocka, 2003, Fulda, 2008, Drag et al., 2009). Polyaromatic hydrocarbons such as benzathracens and phorbol esters are well known as tumor initiators and promoters, with important applications in skin pathology experimental models (Dwivedi et al., 2005, Tormo et al., 2006). Melanin evolution and skin pigmentation could be measured even experimentally by non-invasive devices such as Mexameter (Nguyen et al., 2002).

The aim of present study was to test the biological activity of a topical formulation with 5% betulin on chemical damaged mouse skin.

***Correspondence:** Cristina A. Dehelean, University of Medicine and Pharmacy "Victor Babes" Timisoara, Faculty of Pharmacy, Eftimie Murgu Square no.2, 300041, +40723604614, email:cadehelean@umft.ro*
Article received: July 2010, Published: November 2010

MATERIALS AND METHODS

Betulin was purchased from Sigma Aldrich (Taufkirchen, Germany). Components of cream active agents: 2-propanol, transcutol, isopropyl myristate, labrasol and PEG 4000 were kindly offered and prepared by the Faculty of Pharmacy from Szeged, Department of Pharmaceutical Technology. The carcinogens were 7.12-dimethylbenzanthracene (DMBA) as tumor initiator and 12-O-tetradecanoylphorbol-13-acetate (TPA) as tumor promoter. Both substances were prepared as solutions in acetone as in the literature (Dwivedi et al., 2005, Tormo et al., 2006) and the quantity of applied substance was 200 nmol in 100 µl acetone and 5 nmol TPA in 100 µl acetone. The pigmented areas are transient.

Preparation of topical formulation

Only the one part of betulin dissolves in the mixture of liquid components (2-propanol, transcutol, isopropyl myristate and labrasol). After mixing this suspension with the melted PEG 4000 it was stirred until cooling (solidification).

Table 1

Semisolid formulation and its composition for100g formulation (5% betulin)

Active agent	**5 g**
2-propanol	**37.3 g**
Transcutol	**9.3 g**
Isopropyl myristate	**9.3 g**
Labrasol	**0.3 g**
PEG 4000	**38.8 g**

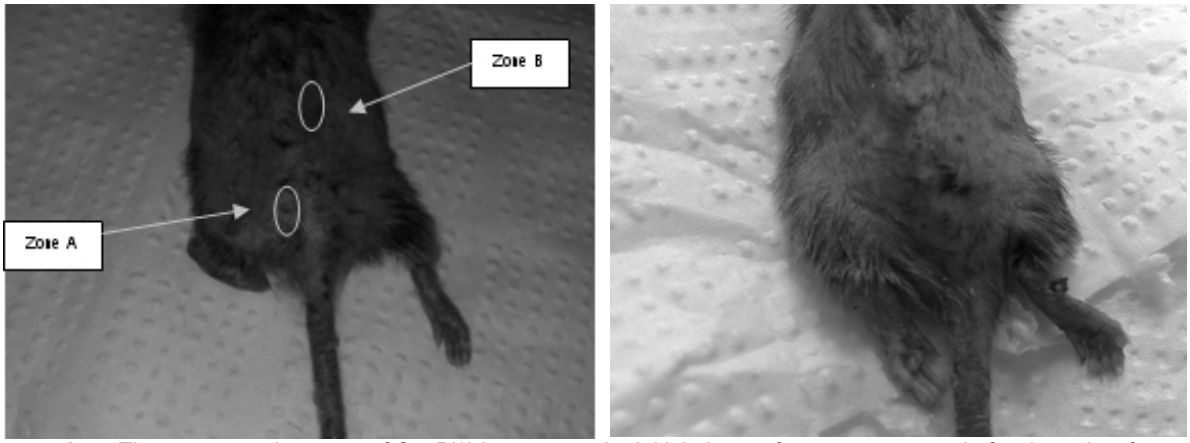

Fig. 1 The macroscopic aspect of C57Bl/6J mouse on the initial phase of measurement and after 3 weeks of treatment – minor damages

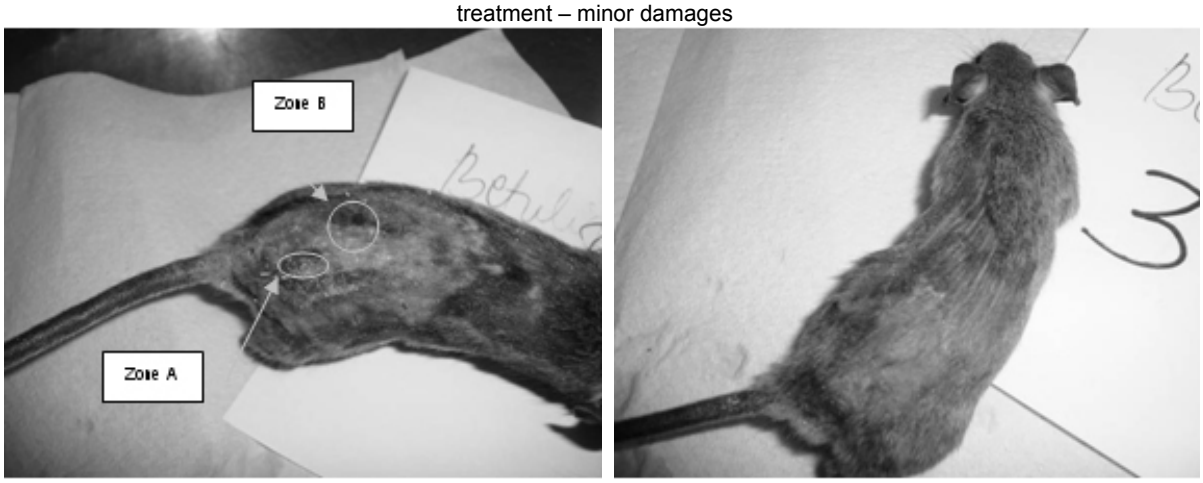

Fig. 2 The macroscopic aspect of C57Bl/6J mouse on initial phase of measurement and after 3 weeks of treatment – important damages

Animals

8 Weeks old C57BL/6J type mice were purchased from Charles River (Germany) and adapted to UMFVBT Animal Breeding Station. Were tested 2 groups: first one after 5 weeks from the initial application of chemical carcinogens and the second 11 weeks after the initial application of chemicals. The tumoral substances were applied, at the beginning for 3 times DMBA in 2 weeks and after this TPA 2 times/week.

The mexametric measurements were performed using a research device from Courage Khazaka, with a

mexameter MX18 (Courage&Khazaka Electronics, Cologne, Germany). The maximum units for Mexameter are 999 (interval 0-999) and the measurement is based on the absorption/reflexion. For the measurements, the mice were anesthetised with xylazine and ketamine. The time of measuring was continuous, for 20s. The protocol followed the observations on melanin evolution and haemoglobin status (pigmentation and erythema). The device was applied on most obvious affected areas and maintain on the skin for 20 seconds. The data were registered by the specific soft from the Mexameter MX18 device and then expressed as units. All data were processed as initial and final measurements values on the same area.

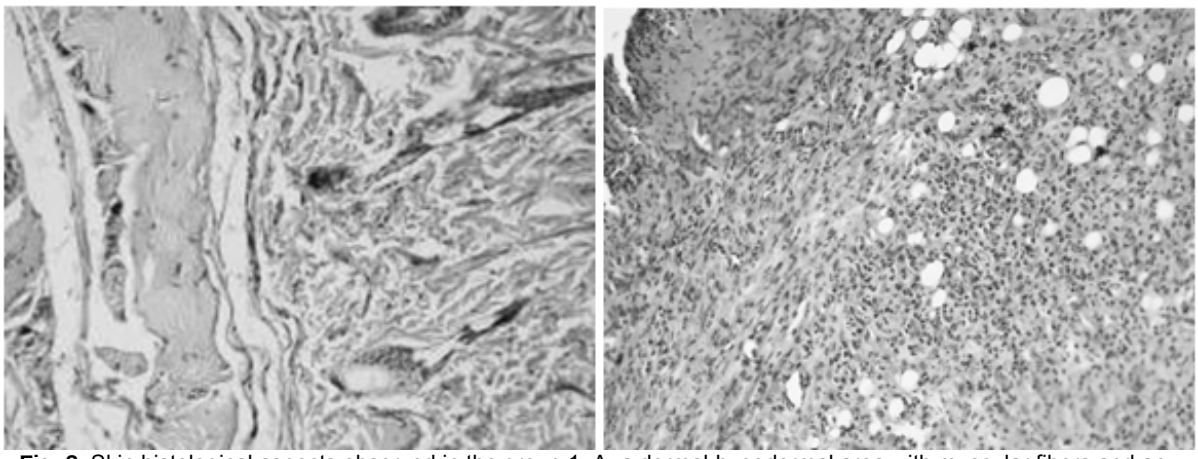

Fig. 3. Skin histological aspects observed in the group 1. A. a dermal-hypodermal area with muscular fibers and an important edema (HEx400) B. skin with a reduced epidermal ulceration and a dermal inflammatory process (HEx200)

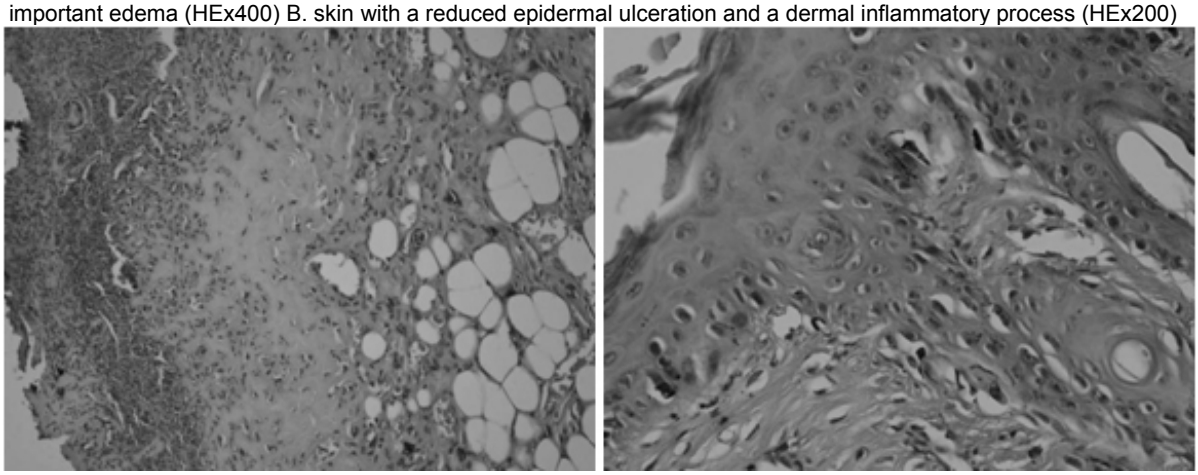

Fig. 4. A dermal-epidermal tissue with an ulcerating epithelium and a necrotic-granulocitary detritus, hemorrhagic suffusions and a lymphocitary and granulocytary infiltrate determined by tumorigenic compounds

RESULTS AND DISCUSSIONS

The substances applied as carcinogens determined the apparition of skin pigmentation and lesions as it is mentioned in literature protocol (Dwivedi et al., 2005). The time of exposure was enough for the apparition of superficial pathology. The age of the animal can influence the time of tumor apparition and the evolution of pathology. The cream applied on the skin had adequate rheological properties and a composition that determined a good delivery of active substance (Figure 5). The modern ingredients offer the possibility to obtain a medium penetration through skin and for this to be indicated in our type of cutaneous damages. The visual observations of skin demonstrated the intervention of active ingredient on skin recovery (Figures 1 and 2). The data presented in other investigations indicated betulin as a compound with an effect on lesions and important skin damages (Patocka, 2003). It should be considered not only a precursor in obtaining an active antitumor compound but a potent antitumor agent (Mullauer et al., 2009). Our investigations confirm the activity on betulin in reducing skin lesions and irritation by significantly decreasing erythema, as appreciated by hemoglobin indices (Figures 6 and 7). This aspect was detected by the Mexameter. Even if there is a reduction in melanin values, these values are not significantly decreased compared to other compounds. All these data contribute to a reevaluation of betulin activity and confirm the last investigations presented in the literature regarding betulin activity which reveal that it is not less important compared to betulinic acid.

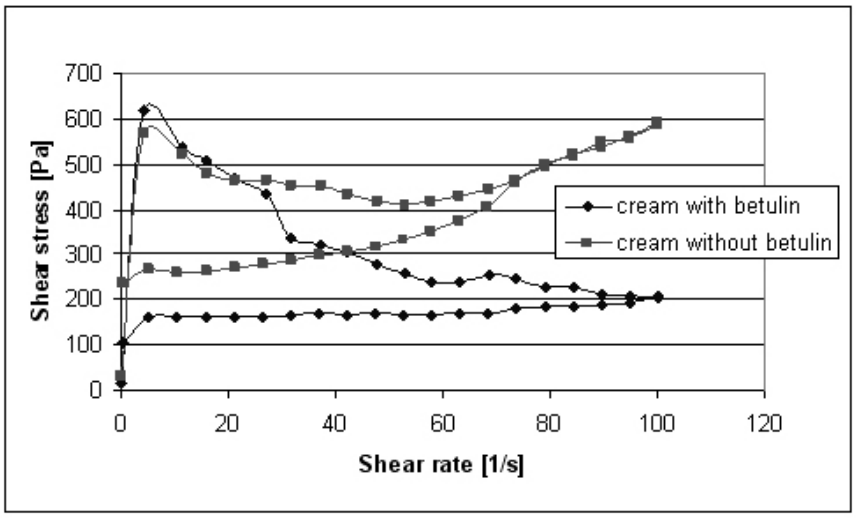

Fig. 5. Flow curves of the tested semisolid formulations, for cream with and without betulin

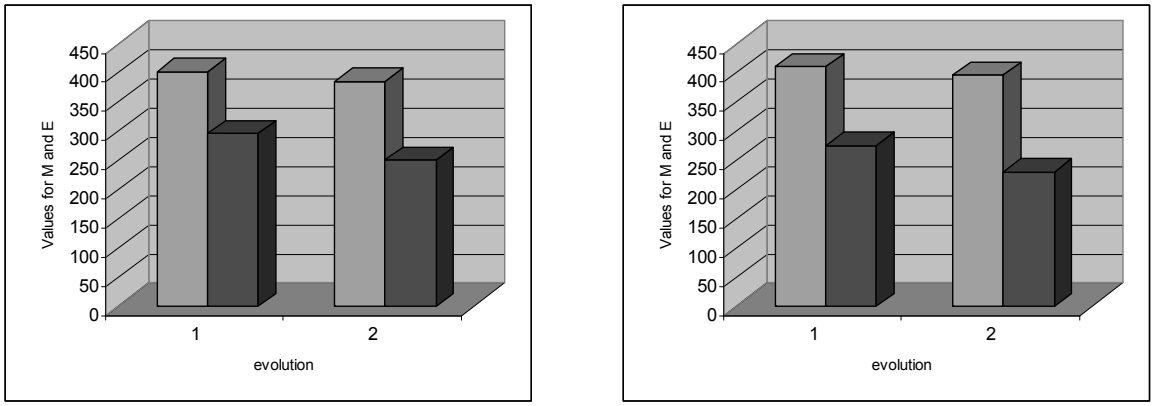

Fig. 6. The evolution of melanin (M, S1) and erythema (E, S2) on first group of mice initially (1) and after 15 days of treatment (2) on the 2 types of zones

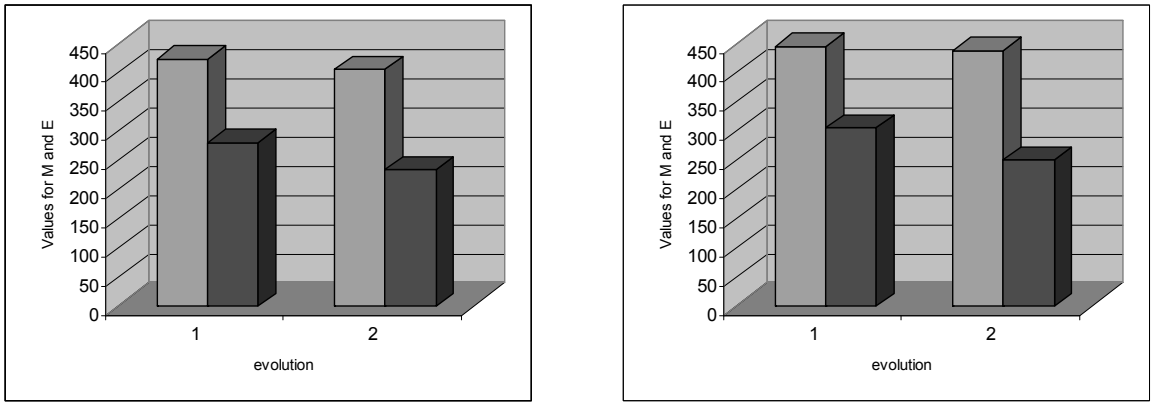

Fig. 7. The evolution of melanin (M, S1) and erythema (E, S2) on second group of mice initial (1) and after 15 days of treatment (2) on the 2 types of zones

CONCLUSIONS

Betulin is an important lupan skeleton compound that could be used in topical applications. The area of its activity could be extended to skin pathology. The importance of its topical effect could be correlated with the activity of reducing skin lesions. This property is very important in pathologies that are accompanied by these damages. Betulin could be applied as prophylactic and therapeutic compound in skin pathology. Early detection of skin pathology presenting damages that could be treated with semisolid formulations can use betulin as active compound. It developed an important role in recovery of skin lesions and a reduce influence on melanin levels.

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 20, issue 4, 2010, pp. 5-9

ACKNOWLEDGEMENTS

This work was supported by CNCSIS-UEFISCSU, project number (contract 212) PNII-IDEI code 1257/2007.

REFERENCES

Dehelean CA, Soica CM, Toma CC, Feflea S, Gruia AT, Kasa P jr., Antitumoral activity of betulin, a compound present in birch tree, in formulations with cyclodextrin, Studia Universitatis Vasile Goldis din Arad, Seria Stiintele Vietii, 20(1), 55-58, 2010;

Drag M, Surowiak P, Drag-Zalesinska M, Dietel M, Lage H, Oleksyszyn J, Comparision of the cytotoxic effects of birch bark extract, betulin and betulinic acid towards human gastric carcinoma and pancreatic carcinoma drug-sensitive and drug-resistant cell lines, Molecules, 14, 1639-1651, 2009;

Dwivedi C, Maydew ER, Hora JJ, Ramaeker DM, Guan X, Chemopreventive effects of various concentrations of α-santolol on skin cancer development in CD-1 mice, European Journal of Cancer Prevention, 14, 473-476, 2005.

Fulda S, Betulinic acid for cancer treatment and prevention, Int.J.Mol.Sci., 9, 1096-1107, 2008;

Guidoin MF, Yang J, Pichette A, Roy C, Betulin isolation from birch bark by vacuum and atmospheric sublimation. A termogravimetric study, Thermochimica Acta, 153-166, 2003;

Huyke C, Laszczyk M, Scheffler A, Ernst R, Schempp CM, Treatment of actinic keratoses with birch bark extract, a pilot study, JDDG, 4, 132-137, 2006;

Huyke C, Reuter J, Rodig M, Kersten A, Laszczyk M, Scheffler A, Nashan D, Schempp C, Treatment of actinic keratoses with anovel betulin-based oleogel. A prospective, randomized, comparative pilot study, JDDG, 7, 128-133, 2009;

Mullauer BF, Kessler JH, Medema JP, Betulin is a potent anti-tumor agent that is enhanced by cholesterol, Plosone, 4(4), e5361, 2009;

Nguyen T, Novak EK, Kermani M, Fluhr J, Peters LL, Swank RT, Wei ML, Melanosome morphologies in murine models of Hermansky-Pudlak syndrome reflect blocks in organelle development, The Journal of Investigative Dermatology, 119(5), 1156-1164, 2002;

Patocka J, Biologically active pentacyclic triterpenes and their current medicine signification, Journal of Applied Biomedicine, 1, 7-12, 2003;

Tormo D, Ferrer A, Gaffal E et al., Rapid growth of invasive metastatic melanoma in carcinogen-treated hepatocyte growth factor/scatter factor-transgenic mice carrying an oncogenic CDK4 mutation, The American Journal of Pathology, 169(2), 665-672, 2006.