

## Full Length Research

# Hystopathological and clinical investigations of five gemmo-derivatives from plants and their biotherapeutical properties

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**Gemmo-therapy, a branch of biotherapy, is a treatment method which is specific by the fact that uses fresh embryonic plant tissue, also known as meristematic tissues. Gemmo-derivatives from branches of *Rosmarinus officinalis* (rosemary), branches of *Vaccinium myrtillus* (blueberry), ament of *Salix Alba* (white willow), shoots of *Ribes nigrum* (black currant) and buds of *Betula Pubescens* (downy birch) were obtained as glycerin macerates. Histologic studies were carried out in kidney and liver, and clinical tests were performed by orally administering a dose of 1 g/kg body weight over 14 days. The interpretation of the variation of animals' weight was performed by t-Student test. The results indicated no negative effects of the studied gemmo-derivatives and no significant changes in motor behavior, body weight, and appearance of treated mice.**

**Key words:** gemmo-therapy, bio-pharmacy, histopathology, meristematic tissues, extracts

## INTRODUCTION

Gemmo-therapy is a biotherapeutical treatment method based on embryonic plant tissue extracts that uses fresh - meristematic tissues (buds, young twigs, bark of young branches, aments, seeds, rootlets, seedlings) (Munro *et al.*, 2015; Blake, 2014; Raiciu and Mihele, 2011; Pitera,

2006). Gemmo-therapy derivatives are used both as a remedy with their own action, and as adjuvant to allopath therapy. They are typically extracted in an optimum mixture of alcohol, water, and glycerol, being also known as glycerol hydro-alcoholic extracts (Pagliarulo *et al.*, 2016;

Pauliuc and Botău, 2013). Glycerol hydro-alcoholic extracts do not present intrinsic or extrinsic toxicity or unwanted side effects, are easily administrated, and can be individually prescribed or in combination with various remedies from the traditional herbal therapy or with other medicinal products.

In this regard, the combination regimens that use multiple gemmo-therapeutic remedies achieve both the intensification by synergism of the similar pharmacodynamic properties, as well the ensuring of a complex activity that address the medical condition of the patient as a whole (Khursheed, 2010; Pitera, 2006). In this way, the doctor can associate different gemmo-derivatives, and can increase or decrease the dose, rate and duration of the administration.

Glycerin macerates are liquid formulations of phyto-complex medicinal herbs, which result from the extraction of raw vegetal materials (gemma, buds, young roots, secondary roots, young branches, seeds, internal bark of roots) with a mixture balanced of alcohol and glycerin (Acharya et al., 2016; British Institute of Homeopathy, 2007; Cavin, 2009). This solution performs complete extraction of plant components without distortion noble and delicate active principles contained in the plant (Andrianne, 2008). All glycerin macerates are indicated by the abbreviation MG and are used in the first decimal dilution (1DH) (Philippe, 2002).

The method of preparation of gemmo-derivatives, whose description is contained in the monograph entitled "Homeopathic" was rigorously defined in 1965 in the French Pharmacopeia and its successive editions (Iancu and Părvu, 2007; Rozenchwajg, 2008; Raiciu et al., 2009).

## MATERIALS AND METHODS

For this study there were used gemmo-derivatives from branches of *Rosmarinus officinalis* (rosemary), branches of *Vaccinium myrtillus* (blueberry), ament of *Salix Alba* (white willow), shoots of *Ribes nigrum* (black currant) and buds of *Betula Pubescens* (downy birch). The chemical composition of GD extracts was previously determined by HPLC analysis (Raiciu et al., 2009).

For the histopathology analyses, the animals were treated with gemmo-derivatives from offshoots of rosemary, buds of blackcurrant, aments of willow, offshoots of bilberry, buds of downy birch, all prepared in 5% aqueous solutions and administrated orally, 10 mL/kg body, for 7 days. It was studied the appearance of different cross-sections of liver and kidney in mice treated with gemmo-derivatives aqueous solution compared to the controls treated with 0.9% NaCl, 10 mL / kg orally for 7 days. Two hours after the last administration, the animals were sacrificed under ethyl ether anesthesia in order to excised liver and kidney samples.

Five groups of white mice consisting of 10 males and

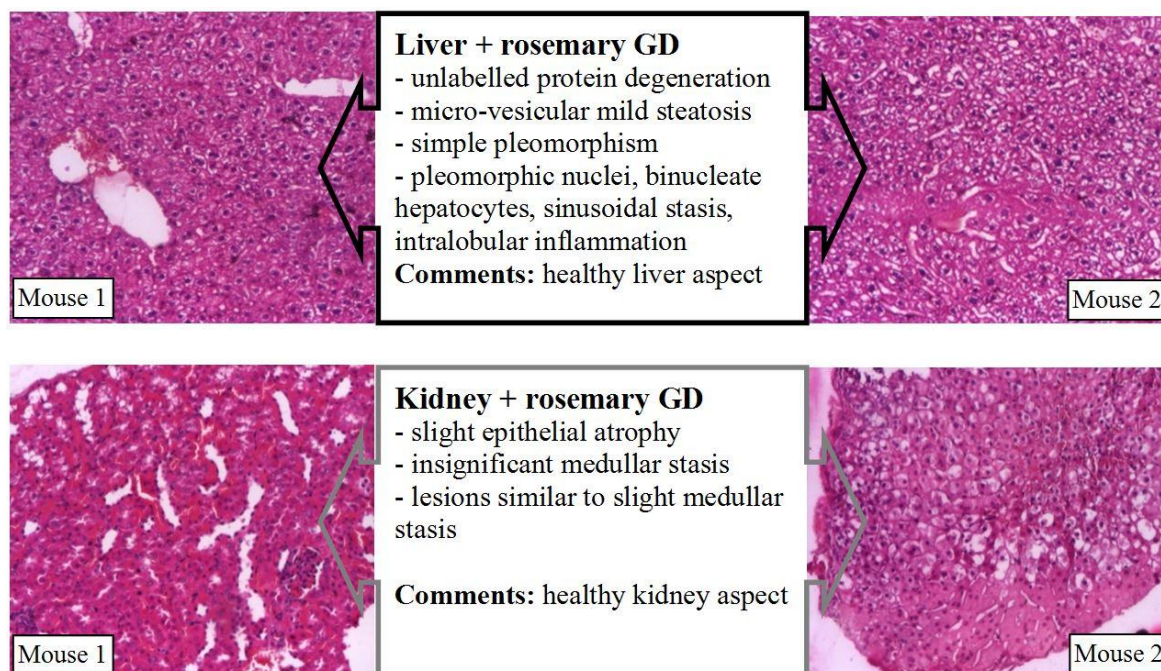
weighing 20-28g were used for clinical tests. The animals were brought from loft and left for two days inside the new habitat. Food was administrated at 8 a.m. and 5 p.m. and the mice received water *ad libitum* in bottles. Administration of the mean dose was performed every day for 14 consecutive days.

The following treatments were administered:

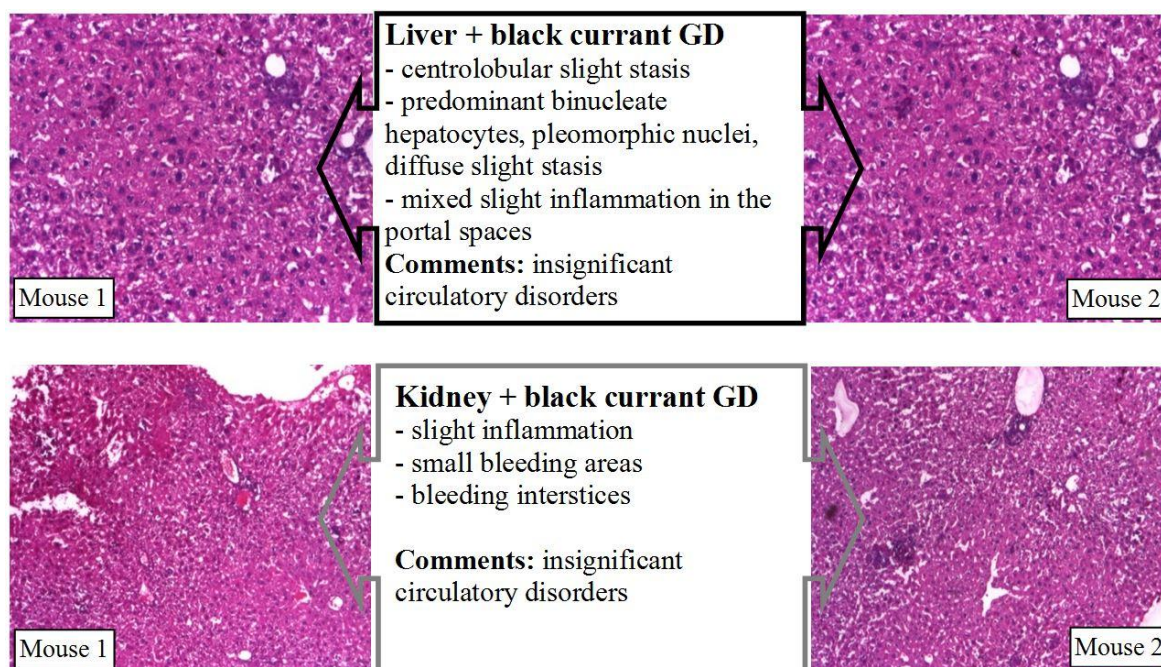
- gemmo-derivatives (GD) from branches of *Rosmarinus officinalis* (rosemary), mean dose of 1 g/kg body weight, aqueous solution of 5% concentration, oral (0.1 mL sol. 5% / 5 g body weight).
- gemmo-derivatives from branches of *Vaccinium myrtillus* (blueberry), mean dose of 1 g/kg body weight, aqueous solution of 5% concentration, oral (0.1 mL sol. 5% / 5 g body weight).
- gemmo-derivatives from buds of *Salix alba* (willow), mean dose of 1 g/kg body weight orally, aqueous solution of 5% concentration, oral (0.1 mL sol. 5% / 5 g body weight).
- gemmo-derivatives from buds of *Ribes nigrum* (black currant), the mean dose of 1 g/kg body weight orally, aqueous solution of 5% concentration, oral (0.1 mL sol. 5% / 5 g body weight).
- gemmo-derivatives from buds of *Betula pubescens* (downy birch), mean dose of 1 g/kg body weight, aqueous solution of 5% concentration, oral (0.1 mL sol. 5% / 5 g body weight).

## RESULTS AND DISCUSSIONS

The histopathologic examination of the 40 selected cases consisted in the following steps. The first step was the collecting and fixation of liver and kidney fragments in 10% neutral formalin, followed by washing with tap water. Next, the fragments were dehydrated by passing through successive hydro-alcoholic baths with increased concentration of alcohol (70%, 80%, 90%, and ethanol absolute). For clarifying, the fragments were passed through three benzene baths to eliminate the alcohol from tissue. The impregnation in melted paraffin was done in a thermostated bath at 55-56°C and was followed by the inclusion of samples in the form of blocks. The sectioning of paraffin blocks was realized with a microtome, the obtained cross-sections being of 4-5 microns thick. The slices were displayed on microscopic glass lamellae previously degreased and treated with Mayer albumin, and after that were dried at 37°C. The paraffin was removed from the lamellae using three benzene baths and the re-hydration was done in three hydro-alcoholic baths with decreasing alcohol concentrations. The sections were stained using the haematoxylin-eosin system. In all cases, nuclei and basophils structures are colored in blue-purple and the cytoplasm in pink-red. The dehydration of the stained

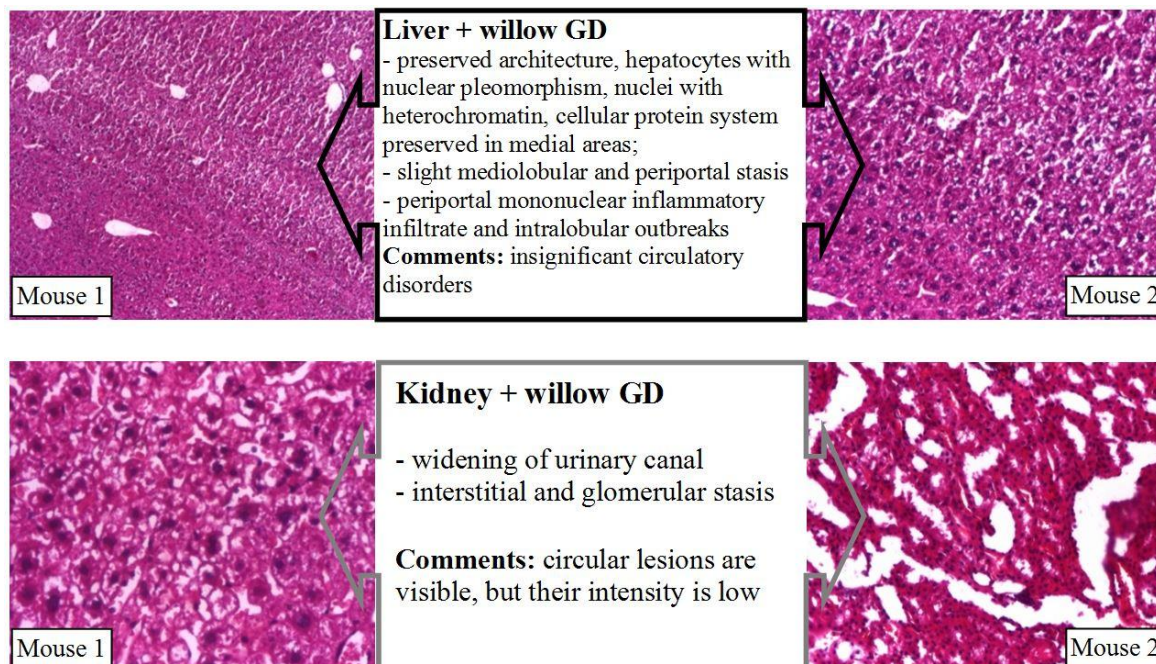


**Figure 1.** Histopathological examination of liver and kidney tissues of two mice treated with 5% rosemary gemmo-derivatives (GD).

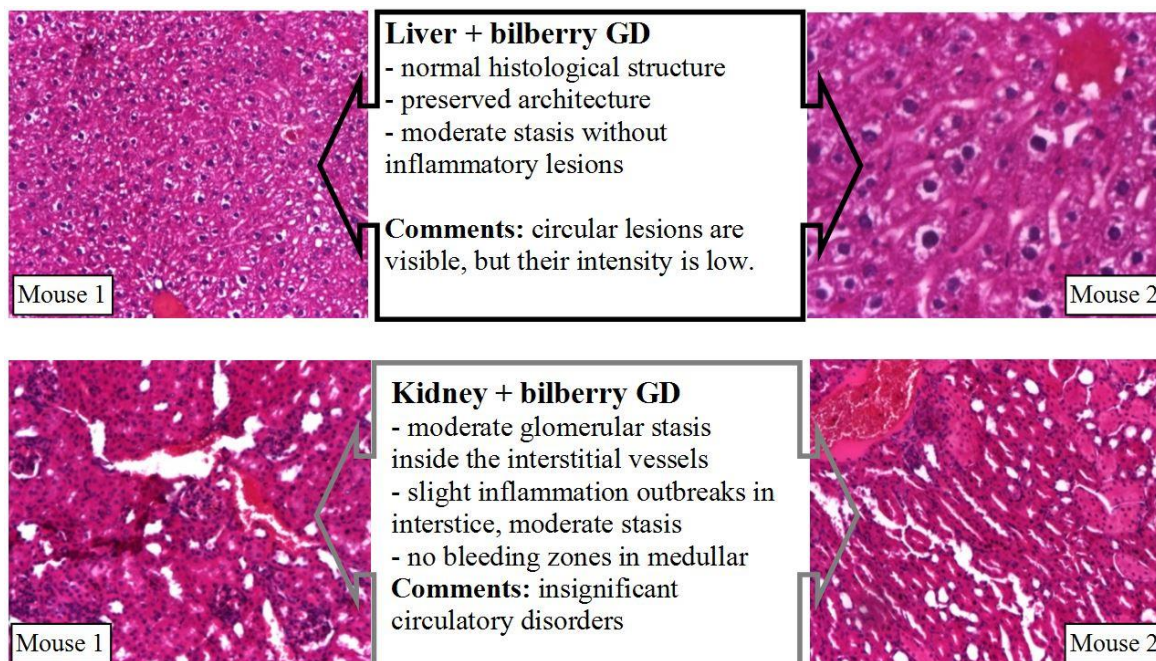


**Figure 2.** Histopathological examination of liver and kidney tissues of two mice treated with 5% black currant gemmo-derivatives (GD).



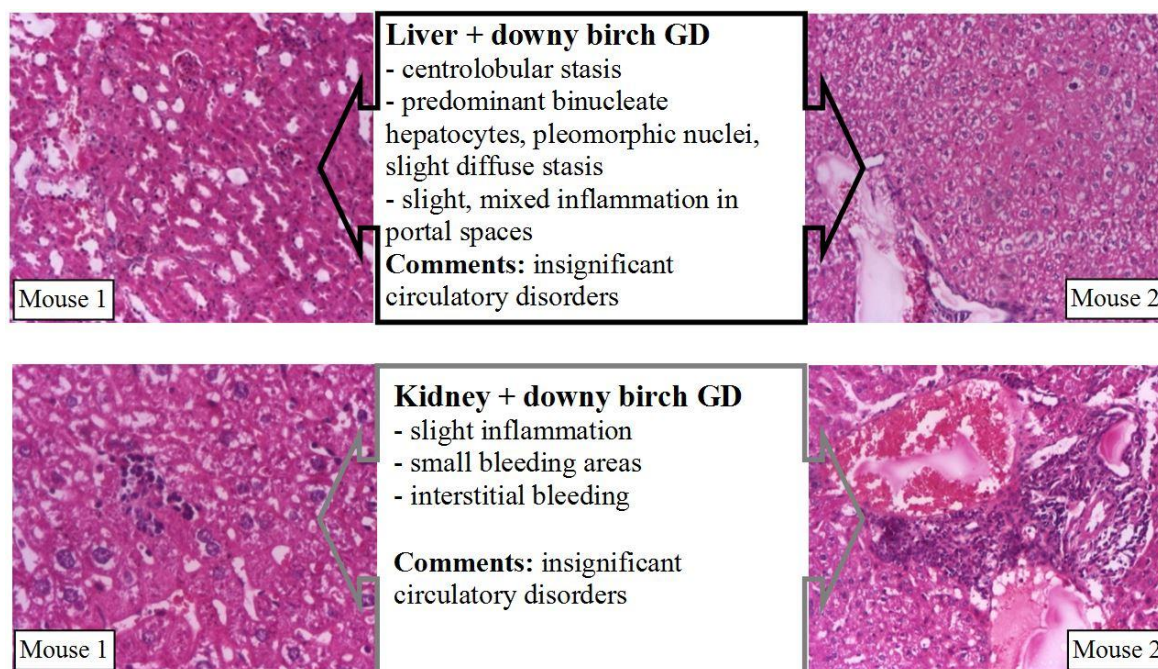


**Figure 3.** Histopathological examination of liver and kidney tissues of two mice treated with 5% willow gemmo-derivatives (GD).

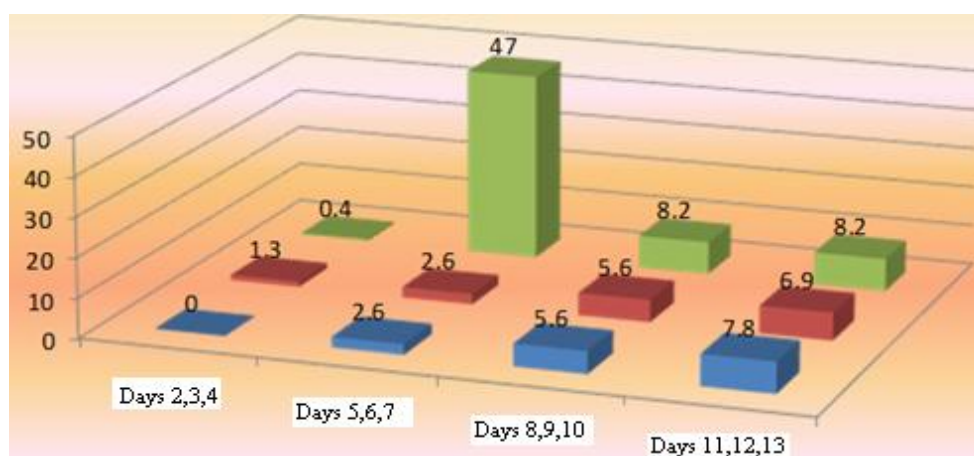


**Figure 4.** Histopathological examination of liver and kidney tissues of two mice treated with 5% bilberry gemmo-derivatives (GD).





**Figure 5.** Histopathological examination of liver and kidney tissues of two mice treated with 5% downy birch gemmo-derivatives (GD).



**Figure 6.** Weight evolution of animals treated with a single dose of GD from *Rosmarinus officinalis* (rosemary), mean dose of 1 g/kg, 5% aqueous solution

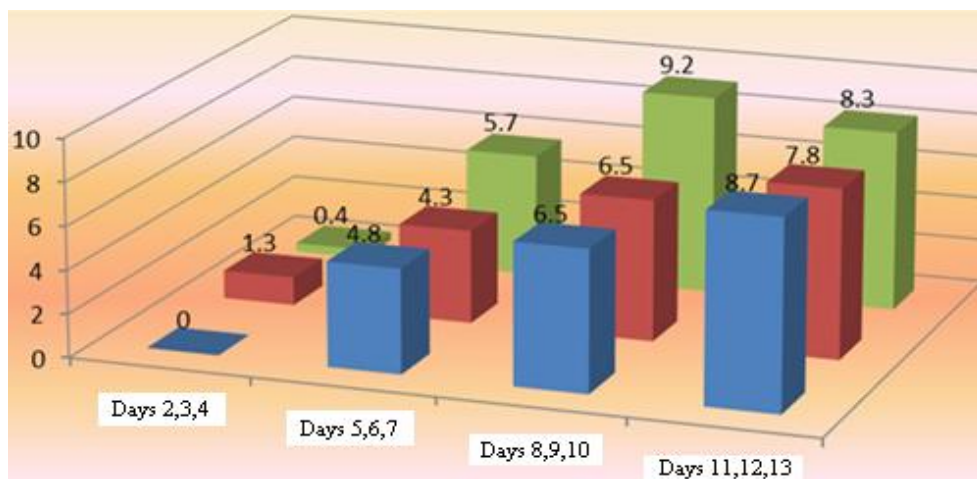
lamellae was performed in three ethylic baths with increasing alcohol concentrations and the clarifying was done in three benzene baths. The samples were fixed in Canada balsam as anhydrous medium and were analyzed using a Nikon microscope. The results are presented in the figures 1-5.

From the figures 1-5 it can be observed that the histological studies performed on the kidney and liver evidenced that the tested gemmo-derivatives have no negative effects. GDs have the potential to regenerate the intoxicated cells (Greaves, 2003; Soescu *et al.*, 2008; Zafar *et al.*, 2014), this being one of the future studies to

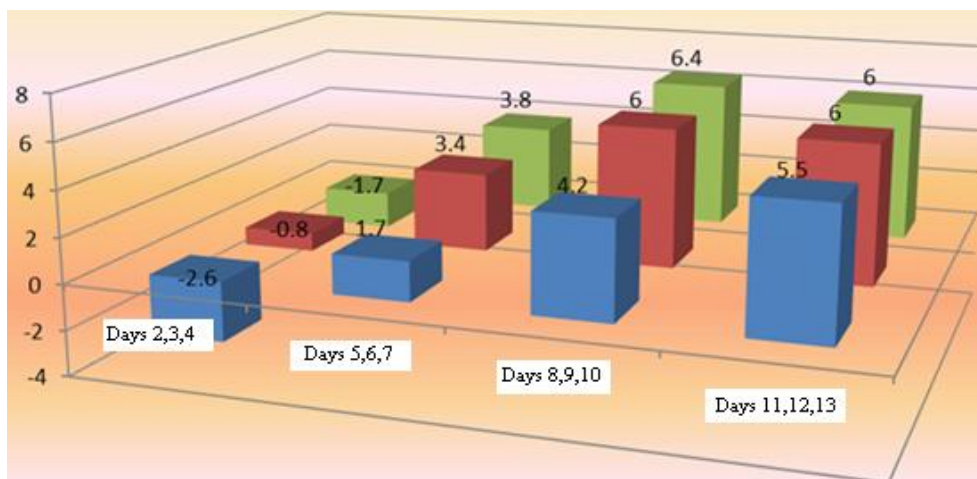
be conducted on the gemmo-derivatives prepared from *Rosmarinus officinalis*, *Vaccinium myrtillus*, *Salix Alba*, *Ribes nigrum* and *Betula Pubescens*.

Further are being presented and discussed the clinical tests on alive animals. The tests were conducted for 14 days and were monitored parameters like: lethality, body weight, motor behavior, aggressiveness, and physical appearance (fur, mucus).

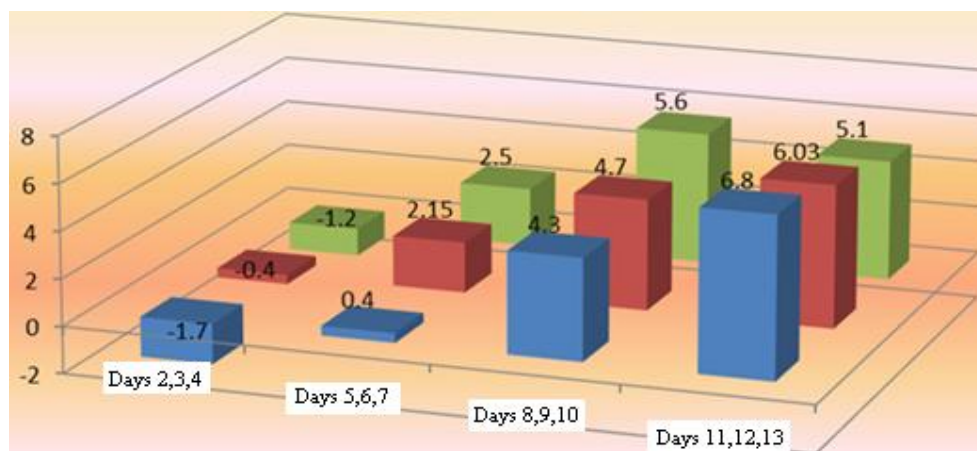
The experimental results on body weight changes are shown in figures 6-10. The interpretation of the variation of animals' weight was performed by t-Student test, the results being shown in table 1.



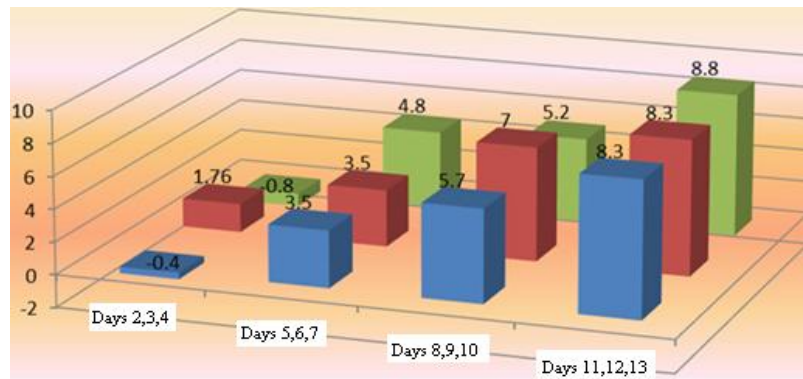
**Figure 7.** Weight evolution of animals treated with a single dose of GD from *Ribes nigrum* (black currant), mean dose of 1 g/kg, 5% aqueous solution



**Figure 8.** Weight evolution of animals treated with a single dose of GD from *Salix alba* (willow), mean dose 1 g/kg, 5% aqueous solution



**Figure 9.** Weight evolution of animals treated with a single dose of GD from *Vaccinium myrtillus* (blueberry), mean dose 1 g/kg, 5% aqueous solution



**Table 1.** Experimental results on changes in body weight of the animals subjected to acute toxicity, statistical significance

The experimental data revealed that the dose of 1 g/kg body weight did not register any lethality cases, or negative effects. The results demonstrate that this type of products has no toxicity or side effects. This incipient study showed also that there were not identified any significant changes in motor behavior, body weight, and appearance of animals.

## CONCLUSIONS

Hystopathological and clinical tests were performed with gemmo-derivatives from branches of *Rosmarinus officinalis* gemmo-derivatives (rosemary), buds of *Ribes nigrum* gemmo-derivatives (black currant), buds of *Salix alba* gemmo-derivatives (willow), branches of *Vaccinium myrtillus* (blueberry), and buds of *Betula pubescens* (downy birch). Five groups of white mice weighing 20-28 g were used for clinical tests. A mean dose of 1 g / kg body weight, 5% aqueous solution was administrated orally for 14 consecutive days. The results showed that for the mice treated with gemmo-derivatives of *Vaccinium myrtillus*, the body weight increased steadily during experiments. All types of gemmo-derivatives registered any negative effects, or other significant changes in animals' health during tests.

## ACKNOWLEDGEMENT

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