EXPERIMENTAL RESEARCHES ON ACUTE TOXICITY OF A BIDENS TRIPARTITA EXTRACT IN MICE - PRELIMINARY INVESTIGATIONS

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EXPERIMENTAL RESEARCHES ON ACUTE TOXICITY OF A BIDENS TRIPARTITA EXTRACT IN MICE - PRELIMINARY INVESTIGATIONS (Abstract): Plants take up an important place in traditional medicine and scientific research confirmed properties about their use as alternative therapy. Bidens tripartita, commonly known as Three-lobe Beggarticks, Three-part Beggarticks, Trifid Bur-marigold, is a flowering plant in the genus Bidens, family Compositae, subfamily Asteroideae. Evaluation of the chemical composition of this plant has revealed the presence of flavonoids, xanthophylls, volatile oil, acetylene and polyacetylene, sterols, aurones, chalcones, caffeine and tannins. Aim: Theoretical data investigation regarding Bidens tripartita plant and experimental researches on acute toxicity of an original extract in mice. Material and methods: The vegetal product of Bidens tripartita used for study was obtained by maceration and extraction in alcohol, and its chemical composition was determined. Acute toxicity of the alcoholic extract of Bidens tripartita was assessed by median lethal dose (LD50) calculation, using a limit dose test of up- and- down procedure at a limit dose of 2000mg/kbw after intraperitoneal administration in mice. Results: In the alcoholic extract of Bidens tripartita different active principles were identified: tannins, anthracene derivatives, triterpenes, coumarins, antocyanosides. The toxicity of plant product was evaluated by different characteristic signs for the mouse which can be retained as toxicity elements of the extract. Using the intraperitoneal route, the animals showed dose-dependent signs of toxicity, ranging from lack of appetite, depression, immobility and respiratory distress to death. Single-dose intraperitoneal LD50 value of the alcoholic Bidens tripartita extract in mice was 4038 mg/kg. No macroscopic changes were seen in the organs of mice that died following extract administration. Histopathological lesions were not found in all examined organs. Conclusions: The obtained LD50 value classifies the study plant extract as slightly toxic according to Hodge and Sterner toxicity scale. We determined the low toxic dose at a rate of 4038 mg of body weight for the alcoholic extract of this medicinal plant. These results suggest that the alcoholic extract of Bidens tripartita is relatively safe toxicologically when administered intraperitoneally, and this product could be used with some degree of safety to continue the investigation for in vivo biocompatibility evaluation. Keywords: BIDENS TRIPARTITA, LETHAL DOSE 50, MICE.

Adaptogens are naturally occurring substances found in rare plants and herbs, which appear to increase body ability to adapt to stress and changing situations (1).
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The exact mechanism through which an adaptogen works is unclear so far, as it seems to be caused by the effects of different chemical compounds. In general, adaptogens are all potent antioxidants and they are reported to improve endurance and produce effects associated with stress reduction, for instance, improved sleep and enhanced physical performances (2).

The main effects of adaptogens are represented by: an increased availability of energy during the day, a reduction of stress feelings, increased endurance, greater mental alertness, and deep and restful sleep (3). Also, adaptogens significantly accelerate the recovery process after illness. They can have individual effects, depending on general health, constitution, specific nutrient deficiencies, and medical conditions present in an individual (4).

Bidens tripartita (B. tripartita), commonly known as Three-lobe Beggarticks, Three-part Beggarticks, Trifid Bur-marigold, is a flowering plant in the genus Bidens, family Compositae, subfamily Asteroideae. It is found along streams and ditches, and flowers during late summer and autumn.

Literature data reported that B. tripartita contains a significant amount of flavonoids, xanthophylls, volatile oil, acetylene and polyacetylene, sterols, aurones, chalcones, caffeine and tannins (5).

In traditional medicine, infusions of B. tripartita are widely used in the treatment of catarrhal rhinitis, angina, acute respiratory infection, and as an anti-inflammatory in colitis and gout (6). It also proved antiseptic, anti-inflammatory, antioxidant, astringent, diuretic, febrifuge, narcotic, sedative and sudorific effects (1,7).

MATERIAL AND METHODS
The study consisted in obtaining an alcoholic extract of Bidens tripartita plant, establishing its composition and determining the lethal dose 50% (LD50) after acute intraperitoneal administration in mice.

Collection and processing of plant material. The plant material for the study was represented by an alcoholic extract of Bidens tripartita L. plant. Plants were collected during flowering stage in July and August 2009, 2010, 2011 from the Ciric area, Iasi district, where it grows naturally. This medicinal plant species was taxonomically identified and authenticated by botanical specialists.

The vegetal product of Bidens tripartita used for the study was obtained by maceration and extraction in alcohol, and its chemical composition was determined.

The plants were washed in water and dried in laboratory using continuous ventilation away from sun light and dust. Then, they were cut into smaller pieces and ground into a coarse powder with a blender. This procedure ensures proper penetration of the extracting solvent into the cell to facilitate the release of the flower’s active ingredients. The vegetable product was kept in closed plastic bag. The powder was dissolved in absolute chloroform (96%) and re-extracted seven (times (repeated maceration). The suspension was filtered by filter paper and dried using a Rota vapour. Then, a jet air was used to remove the solvent smell. After complete dryness, the product was weighed by digital balance, stored in bottles and kept in the laboratory refrigerator for further research (8).

Animals. Swiss white male mice (30-40 g) were used. The animals were housed under standard laboratory conditions (rela-
tive humidity 55-65%, room temperature 23.0±2.0°C and 12 hours light: dark cycle (lights on at 6:00 a.m.). The animals were fed with standard diet and water *ad libitum*, except during the time of experiments. Before the experiment, mice were placed on a raised wire mesh, under a clear plastic box and allowed 2 hours to acclimate to the testing room.

**Acute toxicity investigation.** This study was designed to evaluate the *Bidens tripartita* extract acute toxicity *in vivo*, after intraperitoneal administration in mice.

Median lethal dose (LD50) is the dose, given all at once, which causes the death of half the number of test animals. LD50 is one way to measure the short-term toxic potential of a product (9). Establishing of LD50 in lab animals is very important to estimate the degree of acute toxicity of a tested substance (12).

The acute toxicity of the alcoholic *Bidens tripartita* extract was assessed by LD50 calculation, using a limit dose test (up-and-down procedure) at a limit dose of 2000 mg/kg bw of each vegetal extract after intraperitoneal administration in mice (five animals per group) (OECD-OCDE 425 Guide).

LD50 determination of *Bidens tripartita* extracts was estimated where all doses were expressed in terms of extract weight/animal weight. Preliminary experiments were done to determine the minimal dose that kills all animals (LD100) and the maximal dose that fails to kill any animal. Several doses at equal logarithmic intervals were chosen in between these two doses, each dose being injected intraperitoneally in a group of five animals. The mice were observed for 14 days and symptoms of toxicity and mortality rates in each group were recorded and LD50 was calculated.

The toxicity of plant product was evaluated by different characteristic signs for the mouse which can be retained as toxicity elements of the extract. Using the intraperitoneal route, the animals showed dose-dependent signs of toxicity, ranging from lack of appetite, depression, immobility and respiratory distress to death (11).

The arithmetic method of Karber with the following formula was used:

$$LDL_{50} = LD_{100} - \frac{\Sigma(axb)}{n}$$

*a* = the difference between two successive doses of administered substance

*b* = the average number of dead animals in two successive groups

*n* = the number of animals in a group

DL100 = lethal dose 100% (representing the dose, given all at once, which causes the death all test animals).

Graphic curve was realized with experimental data to have LD01, LD50, LD99. Much more, two ratios: LD99/LD50 and LD50/LD01 were calculated and compared.

The determined LD50 value allowed us to find out the level of toxicity of the plant product on the toxicity scale suggested by WHO/IPCS (2002) representing an adaptation of the Hodge and Sterner scale (1980) (1).

Data were statistically analyzed with SPSS software for Windows version 17.0 and ANOVA one-way method. The results were expressed as mean of LD01, LD50 and LD99 ± S.E.M. (standard error of mean).

Experimental protocols were implemented according to recommendations of the “Grigore T. Popa” University Committee for Research and Ethical Issues. Each animal was used only once, and the duration of the experiments was kept as short as possible. For ethical reasons, all the animals were sacrificed at the end of the ex-
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RESULTS AND DISCUSSION

Phytochemical analysis of the vegetal extract of *Bidens tripartita*, consisting of simple chemical tests, detected the presence of different active principles, especially tannins, anthracene derivatives and triterpenes (tab. I).

<table>
<thead>
<tr>
<th>Extract</th>
<th>Active substances</th>
</tr>
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<tbody>
<tr>
<td>Ethanol</td>
<td>tannins, anthracene derivatives, triterpenes, coumarins, antocyanosides</td>
</tr>
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</table>

The toxicity of *Bidens tripartita* extract was evaluated according to different signs for the mouse, which can be retained as toxicity elements of the vegetal product: decrease in spontaneous behavior normal manifestations and exploration instinct, somnolence, lack of appetite, depression, respiratory distress, and death.

The LD50 value of *Bidens tripartita* extract administered intraperitoneally in a single dose was estimated to be 4038 mg/kbw in mice (fig.1)

DL01 and DL99 of the alcoholic *Bidens tripartita* extract injected intraperitoneally in mice were determined to be 2500 mg/kbw and 4950 mg/kbw, respectively.

The calculation of DL99/DL50 ratio = 1.22 and DL50/DL01 ratio = 1.61 showed comparable values expressing the steadiness of regression line for mortality in relation to dose and validate the biologic significance of the results.

![Fig. 1. Curve of the general acute toxicity of alcoholic extract from Bidens tripartita](image)

**TABLE II**

<table>
<thead>
<tr>
<th>Toxicity rating</th>
<th>Commonly used term</th>
<th>LD50 (rat, oral)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Extremely Toxic</td>
<td>less than 1 mg/kg</td>
</tr>
<tr>
<td>2</td>
<td>Highly Toxic</td>
<td>1-50 mg/kg</td>
</tr>
<tr>
<td>3</td>
<td>Moderately Toxic</td>
<td>50-500 mg/kg</td>
</tr>
<tr>
<td>4</td>
<td>Slightly Toxic</td>
<td>500-5000 mg/kg</td>
</tr>
<tr>
<td>5</td>
<td>Practically Non-toxic</td>
<td>5000-15000 mg/kg</td>
</tr>
</tbody>
</table>
According to Hodge and Sterner toxicity scale, the LD50 value of the alcoholic *Bidens tripartita* extract is in the slightly toxic category (tab. II). For the future researches the retained dose of the alcoholic extract from *Bidens tripartita* for mouse administration is 1/20 LD50, that is around 201.9 mg/kg bw.

**CONCLUSIONS**

These results suggest that the alcoholic extract of *Bidens tripartita* is relatively safe toxicologically when administered intra-peritoneally, and this product could be used with some degree of safety to extend the researches for *in vivo* biocompatibility evaluation.

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