Influence of hyperforin on the morphology of internal organs and biochemical parameters, in experimental model in mice

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Abstract

Background and Aims: Hyperforin (HY) is used to treat depression and skin irritation and has been shown a number of pharmacological activities. The literature does no cite data on changes that may occur in the body after HY intake (ethylene diammonium salt – EDS) in long-term administration. From this point of view, the present work is a key to determining the pharmacotoxicological profile of the HY-EDS, in long-term administration. Materials and Methods: In present research, the influence of toxic doses of HY-EDS was investigated on the biochemical serum parameters and the histopathological changes in internal organs on the experimental mice model. For acute toxicity determination, the HY-EDS was tested in doses of 2000–5000 mg/kg, administered once per day orally. For subacute toxicity, the HY-EDS was tested in three groups of mice, in doses of 50, 75 and 100 mg/kg/day, administered once daily, for 28 consecutive days.

Results and Conclusions: As concern acute toxicity, a lethal effect has not occurred at any of the two tested doses and HY-EDS was classified as Class V toxic: median lethal dose (LD50) >5000 mg/kg, p.o. After 14 days of follow-up in acute toxicity, the experimental results showed a statistically significant increase of aspartate transaminase (AST) and alanine transaminase (ALT), compared to the control group. There were no changes in creatinine and serum glucose compared to the control group. After the administration of repeated doses, it was observed an increase of serum transaminases and alkaline phosphatase. Histological examination revealed that the liver injuries were in an initial stage, making them reversible in case of HY-EDS treatment discontinuation. There was no evidence of kidney damage to any of the doses of HY-EDS.

Keywords: hyperforin toxicity, histopathological changes, motor activity, Hypericum perforatum (St. John’s wort) extract.

Introduction

Hyperforin (HY) is the main active ingredient of the medicinal plant Hypericum perforatum (St. John’s wort), which is commonly use for the treatment of depressions and skin irritations, such as atopic dermatitis (Figure 1). Beside these properties, HY has several pharmacological activities, including antibacterial and antitumor properties [1]. Its antitumor effect in vivo was comparable to that of paclitaxel with absence of any signs of acute toxicity [2]. HY has been shown to be neuroprotective against ischemic damage in the acute phase of ischemic stroke. Previous research has demonstrated that HY up-regulates the vascular endothelial growth factor (VEGF) levels in central nervous system (CNS) tumor cells and induces endothelial proangiogenic behavior [3, 4]. Recent study reported DNA-protective activities of HY [5]. The herb is the main source of HY and hypericin, which are interesting active pharmaceutical ingredients [6]. One recent study reported that HY-loaded gold nanoparticles used in the treatment of experimental autoimmune encephalomyelitis (EAE) in animal model of multiple sclerosis significantly reduced clinical severity of EAE, and decrease the number of inflammatory cell infiltration in the spinal cord [7].

Figure 1 – Hyperforin: chemical structure.

Materials and Methods

Method for determination of acute toxicity

The method of toxicity class [according to Organization...
for Economic Co-operation and Development (OECD) Test Guidelines No. 423] was employed, consisting in use of predefined doses which allow for the substances to be placed within a toxicity class, depending on the presence or absence of lethality (deciding for each dose whether to be tested on other three animals or to switch to testing the following higher or lower dose).

When literature data suggest a broad safety limit for the active substance, the limit test is employed, considering the following:

- administration of a 2000 mg/kg (three animals/group) dose; if no lethality is recorded, the test is repeated with the same dose on another group of three animals;
- lack of lethality in previous determination leads to administration of a 5000 mg/kg (three animals/group) dose.

**Animal stocks**

We used 24 white mice, NMRI (Naval Medical Research Institute) strain, males, 24.19±1.14 g average weight, coming from Animal Facility of “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania. HY-EDS was semi-synthesized in the labs of “Nicolae Testemițanu” State University of Medicine and Pharmacy, Chișinău, Republic of Moldova.

**Experimental groups**

Initially, to a group of six animals was administered a dose of 2000 mg/kg body weight (bw), p.o. (per os), aqueous suspension of 20% HY-EDS. As no lethality was registered, 48 hours after the first administration, another group of six animals was treated with the same dose of HY-EDS as in the previous experiment.

Lack of lethality in previous stages of the research led to administration of a 5000 mg/kg bw dose, p.o., suspension of 50% HY-EDS.

Concomitantly, two control groups were treated with distilled water (in which Tween 80 suspension agent was added), administered in 0.2 mL/10 g bw, p.o., respectively, 0.5 mL/10 g bw, p.o., corresponding to the tested two doses of hyperforin.

After HY-EDS administration, animals were observed for four hours, then daily for 14 days. They were observed for signs of toxicity: pathological changes in the skin and/or mucous membranes; changes in appearance; pathological impairment in organs and systems; changes in behavior (eating, sexual). At the end of 14 days follow-up, the biochemical parameters were determined: liver transaminases, creatinine, serum glucose.

The animals were given free access to food and water in an environment of constant:

- temperature and humidity: 21–24°C and 40–60%, respectively;
- artificial light source: 12 hours light/dark cycle.

**Method for determination of subacute toxicity**

**Animal stocks**

We used a community of 80 white mice, NMRI strain, males, 25.3±1.1268 g average weight, coming from Animal Facility of “Carol Davila” University of Medicine and Pharmacy, Bucharest. The animals were brought from the Animal Facility and left for three days with food and water ad libitum for accommodation. The temperature was maintained constant at 22±1°C and the relative humidity at 45–50%. With one hour before administering the tested substance, the food was removed and water was given ad libitum.

**Doses, route of administration and duration of treatment**

Based on evaluated maximum tolerated dose, HY-EDS selected doses were 50, 75 and 100 mg/kg/day. Aqueous suspension of HY-EDS was administered by gastric intubation (oral, p.o.) per kg/day, once daily, for 28 days.

The suspensions were prepared daily prior to administration. In the suspension formulation, Tween 80 was used as a suspending agent in order to ensure uniformity of dispersion of the administered dose.

**Experimental groups**

After three days of accommodation, the animals were divided into four groups (20 animals/group) treated as following: control group, treated with distilled water, 0.1 mL/10 g bw/day dose, p.o.; HY-EDS group, 50 mg/kg/day dose, p.o.; HY-EDS group, 75 mg/kg/day dose, p.o., HY-EDS group, 100 mg/kg/day dose, p.o.

After administration, the animals were followed-up for 28 days regarding lethality, body weight, feeding behavior, motor behavior, aggressiveness, appearance (furring, mucous).

From the day 9 of treatment, the animals showed clinical signs of agitation, which is likely due to a CNS stimulating effect by HY-EDS. Because of this, we decided to investigate the motor behavior of animals, in all four research groups, at day 15 and day 22 of treatment.

**Determination of motor activity**

Motor activity was determined initially and at days 15 and 22 of the experiment, using the Activity Cage system (Ugo Basile, Italy). This system records motor activity on the vertical axis and the horizontal axis. The device is connected to a computer that inputs the experimental data through Ugo Basile software and export them into Excel documents.

We choose a monitoring interval of five minutes to collect data beyond initial exploration period, when normal animals are investigating any new space in which they are placed.

The animal is introduced in the Activity Cage and recording starts. Each determination shall receive an identification number in order to process later the data sent to the computer. In the five minutes, it takes each individual determination, the animal is protected from the external stimuli that could affect its behavior (i.e., sounds, sudden changes in light intensity, etc.).

After five minutes, the recording automatically stops and the mouse is removed. The total amount of movements on the two axes is obtained by adding the values registered in each of the five minutes of test. Before introducing a new animal into the cage, this must be cleared of manure or other traces left by the previous animal, because the evolution of the next animal should not be influenced by these factors.

**Biological tests**

At the end of the observation period, animals were sacrificed and biological samples were collected (whole
Influence of hyperforin on the morphology of internal organs and biochemical parameters, in experimental model…

blood, serum, organs: liver, kidneys, brain), that were sent to determine the biochemical parameters of interest and histopathological changes. Measurements of transaminases, creatinine, bilirubin and alkaline phosphatase were performed with the automatic analyzer Hitachi 911. Blood counts parameters were determined using the automatic analyzer Pentra 80.

**Histopathological technique**

In order to remove the organs, the mice were euthanized by anesthesia with chloroform extended until exitus. Organs were fixed in 10% formaldehyde solution and prepared by the histological technique of inclusion in paraflin, and stained by Masson’s trichrome method. When the internal organs were harvested, we have performed their macroscopic examination. There were no changes compared to the control group, in terms of color or appearance of internal organs. Cross-sections processing was performed at the Department of Pathology of the University of Agricultural Sciences and Veterinary Medicine, Bucharest.

**Ethics rules**

Acute and subacute toxicity studies for the HY-EDS was conducted in accordance with Directive 2001/83/EC of the European Parliament and of the Council of Europe (November 6, 2001), establishing a Community Code relating to medicinal products for human use. The experiment was conducted in accordance with Directive 86/609/EEC of November 24, 1986 and in accordance with the Order No. 143/April 1, 2002 (Ministry of Agriculture, Food and Forestry), Order No. 400/May 20, 2002 (Ministry of Water and Environmental Protection) and Order No. 84/August 30, 2005 (National Sanitary Veterinary and Food Safety Authority – ANSVSA), on the protection of animals for research purposes.

**Statistical analysis**

The statistical analysis was carried out using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California, USA, www.graphpad.com). Normality was established using the D’Agostino & Pearson test. In order to compare the “n” groups, the tests used were the ANOVA parametric test (compare “n” groups) followed by Dunnett’s post-hoc test (when compared to the baseline response). The value of $p<0.05$ was considered significant.

## Results

### Acute toxicity

The experimental results revealed that the lethal effect has not appeared at any of the doses tested experimentally: 2000 mg/kg, and 5000 mg/kg respectively, administered p.o. The biochemical parameters values are revealed in Figure 2, for the group treated with the dose of 2000 mg/kg and in Figure 3, for the group treated with the dose of 5000 mg/kg.

### Subacute toxicity

The animals that received the HY-EDS in doses of 50, 75 and 100 mg/kg, p.o., showed body weight gain, similar to that observed in the control group (Figure 4).
In this experiment, the research data showed that the HY-EDS, one of the active principles contained in St. John’s wort (SJW) extract, induces an increase in body weight similar to the one registered for control group. The recorded results, that revealed evolution of motor activity on horizontal and on vertical (Figures 5 and 6) have been statistically processed using ANOVA test, followed by Dunnett’s post-hoc test, due to normal distribution of response in collectivity as evaluated by D’Agostino and Pearson test (Table 1).

After 28 days of HY-EDS administration, animals were sacrificed and biological samples were collected, which were sent to determine the following biochemical parameters: AST, ALT, alkaline phosphatase (Table 2), creatinine, bilirubin and blood count (Figure 7).

Table 1 – Evolution of horizontal motor activity (HMA) and vertical motor activity (VMA) for control group and for HY-EDS treated groups

<table>
<thead>
<tr>
<th>Motor activity Parameter</th>
<th>Control Distilled water 0.1 mL/10 g</th>
<th>HY-EDS 50 mg/kg</th>
<th>HY-EDS 75 mg/kg</th>
<th>HY-EDS 100 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M±SD / basal</td>
<td>M±SD / Day 15</td>
<td>M±SD / Day 22</td>
<td>M±SD / Day 22</td>
</tr>
<tr>
<td>D’Agostino and Pearson test</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Effect % vs. basal</td>
<td>-3.7</td>
<td>-0.90</td>
<td>-3.03</td>
<td>-5.24</td>
</tr>
<tr>
<td></td>
<td>M±SD / basal</td>
<td>M±SD / Day 15</td>
<td>M±SD / Day 22</td>
<td>M±SD / Day 22</td>
</tr>
<tr>
<td>D’Agostino and Pearson test</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Effect % vs. basal</td>
<td>26.47</td>
<td>-4.32</td>
<td>-2.41</td>
<td>-5.34</td>
</tr>
</tbody>
</table>

HY-EDS: Hyperforin ethylene diammonium salt; M: Mean; SD: Standard deviation; ND: Normal distribution; ns: Statistically non-significant; *p<0.05.

When examining the cross-sections at the microscope, we observed the following parameters: for brain – neuron integrity, vascular changes, presence/absence of edema, any other inflammatory reaction; for liver – appearance of hepatocyte, changes in the Disse space, circulatory changes, eventual necrosis, other relevant comments; for kidney – aspect of renal glomeruli, proximal convoluted tubule aspect, appearance of renal interstitial space.
Table 2 – Statistically significant alterations of the biochemical parameters: AST, ALT, alkaline phosphatase for the animals in groups treated with HY-EDS comparative with control group

<table>
<thead>
<tr>
<th>Test</th>
<th>Parameter</th>
<th>Control Distilled water 0.1 mL/10 g</th>
<th>HY-EDS 50 mg/kg</th>
<th>HY-EDS 75 mg/kg</th>
<th>HY-EDS 100 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M±SD</td>
<td>27.24±5.339</td>
<td>167.9±47.37</td>
<td>159.1±97.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ANOVA test</td>
<td>0.0002**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td></td>
<td>Dunnett’s post-hoc test / control</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Effect % / control</td>
<td>513.35</td>
<td>484.06</td>
<td>533.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M±SD</td>
<td>33.87±12.79</td>
<td>60.39±26.26</td>
<td>70.12±54.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ANOVA test</td>
<td>0.02411*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td></td>
<td>Dunnett’s post-hoc test / control</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

Table 3 – Mean values for control group and for groups treated with hyperforin: (a) Leukocyte count [×10³/μL]; (b) Thrombocyte count [×10³/μL]; (c) Erythrocyte count [×10⁶/μL]; (d) Hemoglobin [g/dL]. HY-EDS: Hyperforin ethylene diammonium salt.
Figure 7 (continued) – Mean values for control group and for groups treated with hyperforin: (e) Hematocrit [%]; (f) Total bilirubin [mg/dL]; (g) Serum creatinine [mg/dL]. HY-EDS: Hyperforin ethylenediammonium salt.

Table 3 – Histopathological exam results for control group

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Liver</th>
<th>Kidney</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Preserved morphological architecture, discrete vacuolization of hepatocyte cytoplasm in liver perivascular areas, respecting the centrality of the nucleus; granulovascular hepatitis.</td>
<td>Preserved morphological architecture, focal oxyphilic and discrete vacuolization of nephrocytes cytoplasm; normal appearance.</td>
<td>Cerebral hemispheres and other components: preserved morphological architecture; cerebellum – oxyphilic cytoplasm of Purkinje neurons; Purkinje neurons necrosis.</td>
</tr>
<tr>
<td>3.</td>
<td>Normal morphological architecture; sporadic hepatocytes with pyknotic nuclei and oxyphilic cytoplasm; normal appearance.</td>
<td>Normal morphological architecture; normal appearance.</td>
<td>Cerebral hemispheres (small pyknotic neurons, oxyphilic cytoplasm, focal located); focal neuronal necrosis.</td>
</tr>
<tr>
<td>6.</td>
<td>Normal morphological architecture; normal appearance.</td>
<td>Normal morphological architecture; normal appearance.</td>
<td>Cerebral hemispheres (sporadic small pyknotic neurons, oxyphilic cytoplasm); normal appearance.</td>
</tr>
</tbody>
</table>

Observations: Normal appearance – 6/7; Granulovascular hepatitis – 1/7.
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Figure 8 – Histopathology – control group: (a) Liver – normal aspects; (b) Liver – oxyphilic hepatocyte; (c) Liver – granulovacuolar hepatosis; (d) Kidney – normal aspects; (e) Cerebellum – oxyphilic Purkinje neurons. Masson’s trichrome staining: (b and c) ×200; (a, d and e) ×400. HY-EDS: Hyperforin ethylene diammonium salt.

Table 4 – Histopathology results for group treated with HY-EDS of dose 50 mg/kg, p.o.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Liver</th>
<th>Kidney</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Preserved hepatic architecture, hepatocyte with obvious vacuolated cytoplasm and nucleus keeping the central position, on very large areas; granulovacuolar hepatosis.</td>
<td>Normal morphological architecture; normal appearance.</td>
<td>Cerebral hemispheres; normal morphological architecture; isolated presence of glial nodules (phagocytosis of neurons); normal appearance.</td>
<td></td>
</tr>
<tr>
<td>2. Preserved hepatic architecture, hepatocyte with obvious vacuolated cytoplasm, and nucleus keeping the central position, homogeneous; granulovacuolar hepatosis.</td>
<td>Normal morphological architecture; normal appearance.</td>
<td>Normal morphological architecture; normal appearance.</td>
<td></td>
</tr>
<tr>
<td>3. Hepatocyte with obvious vacuolated cytoplasm, and nucleus keeping the central position, central located; granulovacuolar hepatosis.</td>
<td>Not available.</td>
<td>Normal morphological architecture; normal appearance.</td>
<td></td>
</tr>
<tr>
<td>4. Hepatocyte with obvious vacuolated cytoplasm, and nucleus keeping the central position, central located; the presence of cell clusters that discrete dissociated hepatic lobe architecture – possibly islands of extramedullary hematopoiesis – normal in rodents; focal granulovacuolar hepatosis.</td>
<td>Normal morphological architecture; normal appearance.</td>
<td>Normal morphological architecture; normal appearance.</td>
<td></td>
</tr>
<tr>
<td>5. Hepatocyte with vacuolated cytoplasm, and nucleus keeping the central position, central located; focal granulovacuolar hepatosis.</td>
<td>Not available.</td>
<td>Normal morphological architecture; normal appearance.</td>
<td></td>
</tr>
<tr>
<td>6. Can be seen areas with lipid accumulation, which deforms the cells, and moving the nucleus: eccentric nucleus; diffuse granulovacuolar hepatosis; hepatic lipidosis.</td>
<td>Normal morphological architecture; normal appearance.</td>
<td>Cerebral hemispheres; sporadic presence of glial nodules (phagocytosis of neurons); glial nodules; sporadic presence of nonpurulent perivascular sleeves – nonpurulent encephalitis (inflammatory lesion with viral etiology, parasitic).</td>
<td></td>
</tr>
</tbody>
</table>

Figure 9 – Histopathology – group treated with HY-EDS of dose 50 mg/kg: (a) Liver – granulovacuolar hepatosis (animal No. 1); (b) Liver – granulovacuolar hepatosis (animal No. 2); (c) Liver – extramedullary hematopoiesis (animal No. 4); (d) Liver – hepatic lipidosis (animal No. 6); (e) Liver – hepatic lipidosis (animal No. 6); (f) Brain – perivascular sleeves, nonpurulent encephalitis (animal No. 6); (g) Brain – glial nodules (animal No. 6). Masson’s trichrome staining: (a) ×100; (d and g) ×200; (b, c, e and f) ×400. HY-EDS: Hyperforin ethylene diammonium salt.

Table 5 – Histopathology results for group treated with HY-EDS of dose 75 mg/kg, p.o.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Liver</th>
<th>Kidney</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.</td>
<td>Focal hepatic lipidosis; extramedullary hematopoiesis.</td>
<td>Normal morphological architecture; normal appearance.</td>
<td>Cerebral hemispheres; sporadic presence of glial nodules (phagocytosis of neurons); oxyphilic Purkinje neurons from cerebellum.</td>
</tr>
<tr>
<td>5.</td>
<td>Regional hepatic lipidosis on the periphery of hepatic lobe; extramedullary hematopoiesis.</td>
<td>Normal morphological architecture; normal appearance.</td>
<td>Cerebral hemispheres; sporadic presence of glial nodules (phagocytosis of neurons).</td>
</tr>
</tbody>
</table>


Figure 10 – Histopathology – group treated with HY-EDS of dose 75 mg/kg: (a) Liver – normal appearance (animal No. 4); (b) Liver – hepatic lipidosis (animal No. 5). Masson’s trichrome staining: (a and b) ×200. HY-EDS: Hyperforin ethylene diammonium salt.
Table 6 – Histopathology results for group treated with HY-EDS of dose 100 mg/kg, p.o.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Liver</th>
<th>Kidney</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Necrotic hepatocytes in small clusters and marked of phagocytic cells; diffuse granulovacuolar hepatosis.</td>
<td>Normal morphological architecture; normal appearance.</td>
<td>Normal morphological architecture; normal appearance.</td>
</tr>
<tr>
<td>2.</td>
<td>Normal appearance; extramedullary hematopoiesis</td>
<td>Normal morphological architecture; normal appearance.</td>
<td>Normal morphological architecture; normal appearance.</td>
</tr>
<tr>
<td>5.</td>
<td>Focal granulovacuolar dystrophy; discreet lipidosis.</td>
<td>Normal morphological architecture; normal appearance.</td>
<td>Normal morphological architecture; normal appearance.</td>
</tr>
</tbody>
</table>

Observations: Normal appearance – 1/7; Granulovacuolar dystrophy – 4/7; Lipidosis – 1/7; Necrotic hepatocytes – 1/7.

Figure 11 – Histopathology – group treated with HY-EDS of dose 100 mg/kg: (a) Liver – normal appearance (animal No. 6); (b) Liver – necrotic hepatocyte, phagocytosis (animal No. 6); (c) Liver – vacuolar hepatosis (animal No. 6). Masson’s trichrome staining: (a) ×100; (c) ×200; (b) ×400. HY-EDS: Hyperforin ethylene diammonium salt.

Discussion

The aim of our study was to investigate toxicological profile of hyperforin, one of the main derivatives of SJW extract, because there are no data especially about possible changes that occurred in the organs after “well-established use” as drugs or “traditional use” as dietary supplements, for a long time. Therefore, we investigated both acute and subacute toxicity.

Acute toxicity

In 14 days of follow-up after acute toxicity, the experimental results showed a statistically significant increase of AST and ALT, compared to the control group. There were no changes in the creatinine and serum glucose compared to the control group.

According to OECD Test Guidelines No. 423 on determining the acute toxicity of the HY-EDS, administered p.o., in single dose, the HY-EDS was classified as Class V toxic: LD₉₀=5000 mg/kg, p.o. [8] [as shown in Global Harmonized System (GHS) – Environment (ENV) Directorate, Joint Meeting (JM) of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology/Monography – ENV/JM/MONO(2001)6, 2001].

Subacute toxicity

Body weight

Body weight decrease in treated animals is suggestive for treatment toxicity on organism, but modifications in food intake, and accordingly in body weight, may also suggest a depressive event [9].

In our present study, we found that weight gain is statistically significant from day 3 of the study and it is similar to that observed in the control group; therefore, hyperforin did not influence body weight of treated animals. It has been observed an increase in intestinal transit of the animals treated with HY-EDS, from the fourth day of dosing. Nevertheless, there has not been detected weight loss of the animals treated. Research results concur with other literature data showing that for ovariectomized female rat, H. perforatum extract (HPE) does not influence statistically significant the food intake and the amount of adipose tissue (total, mesenteric or visceral) [10]. In Vieira et al. study, no significant differences in body weight reported in rats treated with SJW extract during gestation, suggesting that this mixture of natural compounds was not toxic [9].

Motor activity

Horizontal motor activity for the animals in control group and in groups treated with HY-EDS has decreased compared to initial statistically non-significant at day 15 evaluation, probably because of accommodation with research cage (Figure 5). By contrast, vertical motor activity for the animals treated with HY-EDS was reduced statistically significant at day 22 evaluation, suggesting a depressant effect of the tested substance on CNS (Figure 6). Although Buchholzer et al. [11] reported that locomotor activity in mice treated intraperitoneally
with high-dose hyperforin (10 mg/kg) spontaneously decreased in their study whilst the lower dose of hyperforin (1 mg/kg) was ineffective in this respect. These researchers presented the first data on HY interactions with cholinergic system, and found that HY inhibited choline reuptake in rat synaptosomes, which create premises for the investigation of the use of HY in cognitive disorders. In other study, in mice treated with SJW extract during gestation, there is no interference with motor and behavioral development, as assessed immediately after weaning [9].

CNS depressant effect for total extract of H. perforatum [12, 13] or of hyperforin [14] was also highlighted in other non-clinical experiments by potentiating the depressant action of diazepam and phenobarbital, postulating an interaction with GABA-ergic receptors [13] or a reduction in cerebral GABA (γ-aminobutyric acid) concentration [14]. The results of Uzbay et al. study indicate that caffeine-induced locomotor activity was blocked by pretreatment with SJW extract in mice [15].

Liver damage and SJW-drug interactions

In our study, histopathologically diagnosed liver injuries were dependent on concentration, generally in an initial stage, making them reversible in case of HY-EDS discontinuation. Literature data suggest that in therapeutic concentrations, the extract of H. perforatum has a protective effect on the liver of lab animals [16, 17], although no considerations are made on the effect of different active principles contained in the total plant extract. Nevertheless, clinical data highlights toxic liver effects for several species of Hypericum [18, 19] taken as nutritional supplements, alone or associated with other plant products.

It is well known that SJW is one of the most commonly used herbal extract, and has been described as a strong inducer of cytochrome P450 enzyme [20, 21]. This is a major safety concern, because its interactions with involving liver drugs metabolizing enzymes leading to the alteration of the pharmacokinetics and/or clinical response of a variety of drugs. SJW extract and hyperforin induce the expression of cytochrome P450 enzymes (particularly hepatic and intestinal CYP3A4), induce the expression of P-glycoprotein (P-gp), and activate the pregnane X receptor [22, 23]. The use of St. John’s wort decreases the bioavailabilities and increases the metabolism of a variety of drugs. SJW may interact with oral contraceptives, anticoagulant drugs like warfarin or phenprocoumon, the cardiac medication like digoxin, the asthma drug theophylline, the hypercholesterolemia medication 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, the anti-HIV (human immunodeficiency virus) drug indinavir, and the immunosuppressant drug cyclosporine [24–26]. CYP3A4 induction with SJW extract was associated with HY content, suggesting that drug cyclosporine [24–26]. CYP3A4 induction with SJW extract and hyperforin and HY on CYP1A2 and CYP2D6 expression are still unclear.

Kidney and brain damages

There was no evidence of kidney damage to any of the doses of HY-EDS. Brain injuries are yielded by other etiological factors than those used in the experiment (viruses, protozoa).

Biochemical parameters

Research results revealed that HY-EDS administered in repeated doses (for 28 consecutive days) induced significant changes compared to the control group: increased the levels of serum transaminases and alkaline phosphatase. The effects appears not to be dose-dependent probably because of a limiting process involved in absorption and, consecutively, in bioavailability of hyperforin (Table 2). We have not found information in the literature about the hyperforin action on these biochemical parameters.

The blood count parameters (Figure 7, a–e) did not vary significantly against the control group.

We found no significant changes in bilirubin levels in treated groups compared with control group (except the dose of 100 mg/kg HY-EDS; Figure 7f). Creatinine levels (Figure 7g) in treated groups were similarly with control group, except the group of dose of 100 mg/kg HY-EDS.

Conclusions

Hyperforin is classified in Class V of toxicity, according to literature. Subacute toxicity studies have shown that administration of the HY-EDS induces in all treated groups statistically significant increase in serum transaminases and alkaline phosphatase ($p<0.0001$), compared with controls. Histopathological examination revealed early liver damage that occurs in a dose-dependent manner, reversible upon discontinuation. There was no evidence of renal or brain damage from any of the HY-EDS tested doses.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contribution

Simona Negreş and Corina Scutari equally contributed to the manuscript.

Acknowledgments

The paper was supported by bilateral Project No. 136/2012 “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania / “Nicolae Testemiţanu” State University of Medicine and Pharmacy, Chişinău, Republic of Moldova.

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Received: January 18, 2016
Accepted: August 30, 2016