EFFECTS OF HYPERICUM PERFORATUM AND HYPERICUM MACULATUM EXTRACTS ADMINISTRATION ON SOME MORPHOLOGICAL AND BIOCHEMICAL PARAMETERS IN RAT LIVER INTOXICATED WITH ALCOHOL

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ABSTRACT: The aim of the experiment was to study the effects of two different alcoholic extracts, containing 0.3% and 0.15% Hypericin, of two different species of Hypericum (H. Perforatum L. And H. Maculatum Crantz.) On some biochemical and morphological parameters in rats liver. Extracts were administered for 10 days in white female Wistar rats, weighing 200 ± 20 g, that were previously intoxicated with alcohol (6g/kg body) for 35 days. We found that the most effective curative effect (hepatoprotective and immunostimulating), proved to be those of Hypericum perforatum, with a content of 0.15% hypericin (hyp2 group), as well as for Hypericum maculatum, 0.15% hypericin (hyP1 group). On the other hand, administration of alcoholic extract of Hypericum perforatum containing 0.3%ypericin/kg corp resulted in increases of morphological changes induced by treatment with alcohol.

Key words: hypericum perforatum, hypericum maculatum, alcohol, liver, rats.

INTRODUCTION

Since ancient times, St. John’s Wort has been used to treat many human diseases. Thus, Discorde (Greece) and Pliny (Rome) as well as the father of medicine, Hippocrates, used John’s Wort to treat wounds and hemorrhoids, kidney and lung diseases and digestive system diseases, but also neurological diseases, as eg depression, long before this state to be declared itself as a disease. Currently, St. John’s Wort is mainly used to treat mild depression, but there are studies on its effect on other diseases such as AIDS, various cancers, alcoholism, epilepsy, psoriasis, rheumatoid arthritis, ulcers, nightmares at children, etc. (Fergert et al, 2006)

Herb and flowers contain different polyphenols: flavonoids (rutin, hyperoside, isoquercetin, quercetin, quercitin, 13,118-biapigenin, amentoflavone, astilbin, miquelianin), phenolic acids (chlorogenic acid, 3-O-coumaroylquinic acid), different napthodianthrones (Hypericin, pseudohypericin, protohypericin, protopseudohypericin), phloroglucinols (hyperforin, adhyperforin). It seems that the pharmacological action of biologically active compounds contained in the plant genus Hypericum is given mainly by hypericin and hyperforin, volatile oil and tannins, that determine the effects of antiseptic, astringent and healing properties (Umek et al, 1999; Schwob et al, 2002; Tatsis et al, 2007). Flavonoids determine vasodilating and hypotensive action, and caffeic and chlorogenic acids explain anti-inflammatory and chologogue properties of the plant.

In Romania’s spontaneous flora 12 species of Hypericum are found (Stanescu et al., 2004), two of which are more widely-separated: Hypericum perforatum L. (in hill area), recognized as official plant (Ciulei et al., 1993) and Hypericum maculatum Crantz (in mountain area).

For our study were chosen two hydroalcoholic extracts of Hypericum (H. Perforatum and H. Maculatum) obtained by University of Pharmacy from umph “Iuliu Hatieganu” Cluj. There was used the qualitative analysis of the plant extracts by TLC which evidenced the presence of flavonoids: hyperoside and quercitoside in both species, while rutoside was identified only in H. Perforatum extract. This aspect can be used as a criterion for rapid identification of the species H. Maculatum, by TLC. The two extracts were then used to compare their protective effects on vital organs in rats under alcohol intoxication in order to produce pharmaceutical preparations in phytotherapy used to protect vital organs (liver, kidney, brain) potentially affected by this malady.

Restoration of liver and kidney affected by various xenobiotics, also including the ethilic alcohol, the most common and oldest known drug, under the influence of liver-and kidney-protective products such as medicinal plant extracts, in our case those extracts obtained from two plants: Hypericum perforatum, respectively, Hypericum maculatum, has no much data, thus being an important argument for carrying out this study.

MATERIAL ŞI METHODS

The experiment was conducted on mature white females Wistar rats, weighing 200 ± 20 g, divided into 6 experimental groups of 6 animals each, as follows:

- Control group, C;
- Alc group, treated for 35 days with ethilic alcohol 50° in amount of 6 g/kg., which was administered in drinking troughs, along with drinking water;
- HyP1 group, which was treated for 25 days with alcohol 50° in amount of 6 g/kg. After this period animals were further treated for
10 days with a mixture consisting of tincture of *Hypericum perforatum* containing 0.3% hypericin / kg corp (675 mg dried subst.) In ethyl alcohol 50%, given by intragastric gavage for 10 days;

- **HyP2** group, which was treated for 25 days with alcohol 50% in amount of 6 g/kg. After this period the animals were further treated for 10 days with a mixture consisting of tincture of *Hypericum perforatum* containing 0.15% hypericin/kg corp (340 mg dried subst.) In ethyl alcohol 50%, given by intragastric gavage for 10 days;

- **HyM1** group, was treated for 25 days with alcohol 50% in amount of 6 g/kg. After this period animals were further treated for 10 days with a mixture consisting of tincture of *Hypericum maculatum* containing 0.3% hypericin/kg corp (552.5 mg dried subst.) In ethyl alcohol 50%, given by intragastric gavage for 10 days;

- **HyM2** group, was treated for 25 days with alcohol 50% in amount of 6 g/kg. After this period the animals were further treated for 10 days with a mixture consisting of tincture of *Hypericum maculatum* containing 0.15% hypericin / kg corp (276.24 mg dried subst.) In ethyl alcohol 50%, given by intragastric gavage for 10 days;

Animals were kept under standardized zoohigienical conditions: range of light/dark 12:00; standardized diet, administered as a concentrated food (pelleted feed), balanced protein, carbohydrates, lipids and vitamins, drinking water and food were given „ad libitum”.

The alcoholic extract of *Hypericum perforatum* is a tincture 1:10, containing 0.0442 g flavonoids expressed in rutoside/10 ml tincture (4.421% rutoside) and 0.0044 g total hypericin /10 ml tincture (0.444% total hypericin).

The alcoholic extract of *Hypericum maculatum* is a tincture 1:10, containing de 0.0495 g flavonoids expressed in rutoside/10 ml tincture (4.957% rutoside) and 0.0054 g total hypericin /10 ml tincture (0.543% total hypericin).

Dosage of analyzed substances was not made randomly, in our research being used existing data and models described in the experimental variations in the literature.

On 35th experimental day animals were sacrificed by decapitation after a previous ether anesthesia. The blood and internal organs (brain, liver and kidney) were removed and than processed according to analyzed morphological or functional parameters. Fragments of organs were fixed in Bouin fluid for 24 hours, the parts being processed for inclusion in paraffin. Histological examination of liver and kidney taken from both control and treated animals was performed on hematoxylin-eosin (HE) - stained sections, according to current conventional techniques described by Muresan et al., (1974).

To study the general morphology of the brain and to highlight the neurosecretory cells activity (Nissl granulations), we used the Klaver-Barrera (1953) method. The obtained histological preparations were examined at a photonic microscope Olympus, Japan, the analyzed images being retrieved and processed on a Pentium IV computer.

In our study we kept track of some proteins, lipids and carbohydrates metabolic parameters, as well as indicators of renal function parameters. For all biochemical determinations (GPT and GOT levels, serum creatinine and urea, total protein, PT, triglycerides and cholesterol, blood sugar) blood samples were immediately centrifuged, serum harvested and then frozen in Eppendorf vials. Measurements were made with biochemistry semiautomatic analyzer screen point type, with reagents - STATE - FAX 1904 Plus, Global Medical Instrumentation, Inc. 6511 Bunker Lake Blvd Ramsey Minesota, USA 55303.

Haematological examinations were performed with the Abacus Junior Vet automatically analyzer, Diatron Messtechnik, Budapest, Hungary. This is an automated hematology analyzer designed for counting blood cells.

The biochemical data were statistically processed by means of Student’s „t” test. Aberrant values were eliminated by means of Chauvenet’s criterion. A probability value of p ≤ 0.05 was considered.

**RESULTS**

**Histological study**

**Histological study of liver**

Histological examination of C group liver sections revealed a normal appearance, characteristic for the liver structure. Thus, the body of the liver lobule is composed of cell cords separated by sinusoidal capillaries, biliary ducts and connective support tissue. Remack’s cell cords have a radial disposition, converging towards the centrilobular vein, separated by sinusoidal capillaries. Between hepatic cords and sinusoidal capillaries there is a perisinusoidal space - Diss area, occupied by sinusoids basal membrane, reticulin fibers and connective tissue cells. Sinusoidal capillaries have discontinuous basal membrane and endothelium, being composed of endothelial and Kupffer cells, which are stellate macrophages. Intralobular support tissue is represented by the fasciae of small amount of connective tissue, collagen and reticulin fibers (Figure 1).

Animal treatment with alcohol for 35 days in Alc. group determined morphological changes of liver parenchyma characterized by sinusoids capillary dilatations, binucleate hepatocytes hyperplasia, clear and granular dystrophy of hepatocytes, which in pericentrallobulary areas present aspects of a discrete fat storage suggested by the presence of perfectly...
round clear spaces of different sizes located intra and pericellular. These changes are associated with small infiltrating focals of rotundo-perivascular cell elements. Some centrilobular located necrotic cells are added to these (Figure 2).

Administration of Hypericum perforatum alcoholic extract (0.3% hypericin/kg body) determined an emphasises of morphological changes induced by treatment with alcohol in hyp1 group. Most hepatocytes are intensely ballooning and dystrophic. There are pointed out aspects of fatty dystrophy and apoptosis phenomena, suggested by the appearance of cells with pyknotic nuclei, hyperchromatic, located perinuclear clear spaces, cytoplasms being restricted to the cell periphery. Some ballooning hepatocytes are joined, separating areas of hepatic parenchyma (Figure 3).

Administration of Hypericum perforatum alcoholic extract (0.15% hypericin/kg body) determined a significant reduction of the liver degenerative aspects in HyP2 group. Thus, the histopathological changes reported in the group treated with alcohol and high dose of Hypericin are attenuated, the liver morphology being closer to normal. However, there are aspects of a clear and discrete granular dystrophies, small focals of liver parenchyma presenting ballooning hepatocytes with eccentrically located nuclei. Hepatoprotective effect is also manifested by the absence of hepatic parenchymal congestion, significant decreases of dystrophic and apoptotic cells and the disappearance of fatty charge from hepatic parenchymal structure, so that the general appearance of the liver structure is closer to that of the control group (Figure 4).
Morphological changes in the liver structure of HyM1 group are somewhat reduced as intensity compared with those described in HyP1 group. They are characterized by clear and granular generalized dystrophy aspects of hepatocytes, discrete fatty charge, an increased number of binucleate hepatocytes, presence of rare infiltrates with blood elements located perivascular as well as capillary sinusoids congestion, especially under the liver capsule (Figure 5). The morphological changes in hym2 group hepatocytes are significantly lower compared to those described in previously group, the overall picture of the liver structure being closer to the control group (Figure 6).

**Histological study of kidney**

Histological sections of this organ in C group, highlight the normal appearance of kidney morphology, where at the renal cortex level, it can be observed the Malpighi corpuscles presence, of the proximal and distal convoluted tubules and peritubules blood vessels. The Malpighi renal corpuscles consists of a cup-shaped epithelial component - Bowman capsule, renal glomeruli being composed of a bundle of capillaries. Renal glomeruli is highlighted on preparations by a mass of nuclei surrounded by an vacuum optical ring. Proximal convoluted tubules and the greater part of the renal cortex are delimited by a cubic epithelium with large cells with centrally located spherical nuclei. Cells delimit a narrow lumen, the cell limits being unclear because of many lateral interdigitally sides. Distal convoluted tubules are delimited by a cubic epithelium having a greater lumen than proximal convoluted tubules (Figure 7).

Subchronic treatment of animals with ethilic alcohol (Alc group) resulted in marked dilation of renal tubular lumen. Urinifery tubuli present aspects of vacuolar tubules dystrophy, respectively granulo-vacuolar degeneration that most affected nephron components being those located in cortical area. Thus, the proximal tube and it’s distal portion represent the most affected segment, the lesions being present in a variable number of nephrons. Some urinifery tubuli present epithelial desquamation, without affecting the integrity of tubular basal membrane, which favors the regeneration and restoration process. A part of the proximal and distal convoluted tubule have increased in volume epithelial cells. In the glomeruli there were small lesions, characterized by a discrete thickening of glomerular capillary ansaes associated with dilatation of the interglomerulary interstices. The vasodilator effect of alcohol determined the distention (congestion) of blood vessel lumen (Figure 8).

Administration of higher doses (0.3%) of hypericin associated with ethilic alcohol, both in the *H. perforatum* extract and of the *H. maculatum* case (lots HyP1 and HyM1) resulted in induction of somewhat marked changes of kidney morphology, compared with the Alc group (Figures 9 and 11). As a result of lower doses of hypericin (0.15%) administration, in both HyP2 and HyM2 groups, there was a significant reduction of tubular degenerative aspects this phenomenon suggesting a protective effect of hypericin low concentrations in maintaining of the structural kidney integrity (Figures 10 and 12).

![Fig. 5 – Clear hepatocytes diystrophy, infiltrates with perivascular blood elements in HyM1 group, (x 40).](image1)

![Fig. 6 – Aspect of liver parenchim structure close to normal, in HyM2, (x 40).](image2)
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**Fig. 7** – Normal aspects of kidney structure in C group (x 40).

**Fig. 8** – Tubules dilatation and epithelial desquamation in urinifery tubuli lumen in Alc group, (x 40).

**Fig. 9** – Emphasized dilation of urinifery tubuli lumen in HyP1 group (x 40).

**Fig. 10** – Aspect close to normal of kidney morphology in HyP2 group, (x 40).

**Fig. 11** – Urinifery tubules lumen dilatation associated with epithelial desquamation in HyM1 group, (x 40).

**Fig. 12** – Aspect close to normal of kidney morphology in HyM2 group, (x 40).
Results of GPT and GOT serum transaminases determination

Alcohol intoxication caused changes in GPT and GOT serum transaminases activity. Thus in the Alc group GPT value increased with 17.26% compared with controls, this value being statistically insignificant. GOT value increases with 8.37%, also being statistically insignificant compared with C group.

Administration of the plant alcohol extract containing 0.3% Hypericin in HyP1 group determined a faster growth, but without statistical significance, of GPT activity (29.67%) compared with C group. GOT activity increases slightly, insignificant (6.15%) than the control group. In the HyP2 group treated with low dose (0.15%) of hypericin, GPT mean value is very close to that of C group (0.15%), but value of GOT (+12.07), slightly increases being statistically insignificant versus the C group. In HyM1 group who received higher concentration of hypericin (0.3%) in alcoholic extract has been registered more intense increases of GPT activity (29.92%), which is not statistically significant, but the value of GOT (9.51%) very slightly increases compared with C group. Administration of Hypericum maculatum plant alcoholic extract with a low concentration of hypericin (0.15%) in HyM2 group resulted in a decrease with 15.21% (statistically insignificant) of GPT activity, while GOT value is extremely close to the C group (0.032%).

The values of GPT and GOT activity in the 6 experimental groups are presented in Table 1.

Table 1

| GOT and GPT serum aminotransferase activity (mu / ml serum) in the six experimental groups |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|
| C                              | Alc            | HyP1 (0.3%)    | HyP2 (0.15%)   | HyM1 (0.3%)    | HyM2 (0.15%)   |
| GPT (u/L)                      |                |                |                |                |                |
| x ± ES                         | 51.57 ± 4.19   | 60.47 ± 1.78   | 66.87 ± 6.33   | 51.65 ± 3.72   | 67.00 ± 8.7    | 43.72 ± 4.25   |
| n                              | 6              | 6              | 6              | 6              | 6              | 6              |
| p                              | -              | NS             | NS             | NS             | NS             | NS             |
| D%                             | -              | +17.26         | +29.67         | +0.15          | +29.92         | -15.21         |

| GOT (u/L)                      |                |                |                |                |                |
| x ± ES                         | 290.54±15.03   | 314.9±37.68    | 308.45±41.14   | 325.65±20.48   | 262.92±45.64   | 290.47±27.16   |
| n                              | 6              | 6              | 6              | 6              | 6              | 6              |
| p                              | -              | NS             | NS             | NS             | NS             | NS             |
| D%                             | -              | +8.37          | +6.15          | +12.07         | +9.51          | +0.032         |

DISCUSSION

The contents of our research was based on an experiment concerning the study of the effects of plant extracts obtained from Hypericum perforatum and Hypericum maculatum on hematological, morphological and functional liver parameters, kidney and brain, under subchronic intoxication with a xenobiotic agent (ethilic alcohol) in white rats.

According to literature data, extracts provided from those two plants, which due to their biologically active principles content (currently classified as chemopreventors) is expected to have hepatoprotective actions as well as modulation of brain functions action. In this context, the complex investigation of renal and liver injuries by various xenobiotics, and of natural recovery or under the influence of pharmaceutical products with no toxicity or low toxicity, represents a potential pathway to deepen the two components of living - damage and recovery - and has a great fundamental and applied importance.

Our results, both on liver, kidney, brain, and the hematological parameters, following the alcohol intoxication, and those relating to protection of liver, kidney and brain, exerted by the two extracts obtained with an original experimental model, is a chain link of the contributions made by many researchers to clarify the fundamental biological processes: damage, destruction and recovery of cells and tissues. Administration of alcohol produced morphological and functional liver damages, seen by some authors (Chanda and Mehendi, 1996; Rusu, 1997), a phenomenon of “chemical hepatectomia”, which is based on biochemical processes of biotransformation of xenobiotics, with the emergence of highly reactive free radicals which attack the extracellular, intracellular, mitochondrial membranes, etc. These free radicals act as “chemical scalpels” sectioning the liver parenchyma.

The liver histological examinations revealed liver injuries consecutive to subchronic alcohol treatment, generally characterized by hepatocytes ballooning degeneration of various degrees of intensity, which suggests a moderate lipid liver charge phenomenon. Hepatic changes induced by subchronic intoxication with alcohol is of variable intensity, ranging from dilatation of sinusoid capillaries, ballooning of both Kupffer endothelial cells and hepatocytes, easy fatty charge and of hepatocellular injuries until small necrotic foci on the liver parenchyma limited areas.

The appearence of cell necrosis areas, disseminated in the form of small islands in the liver parenchyma, are viewed by hydropic cytoplasm vacuolation, often containing homogeneous hyaline material. Is also noticed more or less pronounced injuries of sinusoids.
cells endothelial capillary. Centrilobular lesions appear first and gradually extend to the periphery of the lobules, concomitantly evolving with intertrabecular stasis issues. Our results are confirmed by a set of data from the literature (Kovalovics et al., 2000; Rusu et al., 1994a, b; Rusu et al., 2005; Uray et al., 1986). According with Timar’s (1983) data, the increase in volume of hepatocytes is a consequence of the mitochondria ballooning and of increases of cell and undercell membranes permeability.

Animals treatment with alcoholic extracts containing different hypericin concentrations (0.15% and 0.3%) from two plant species: Hypericum perforatum and Hypericum maculatum resulted in a depending on dose reactivity of the liver intoxicated with alcohol. Thus, administration for 10 days of higher concentration (0.3%) of hypericin contained in the vegetable extracts of H. perforatum and H. maculatum did not induce a recovery process of the animals liver intoxicated with alcohol, contrary, the liver morphology changes are increased in intensity compared with that of animals treated only with alcohol, as confirmed by the general liver morphostructural appearance. In the H. maculatum treated group it is emphasized a restriction of the liver parenchyma areas affected by that alcohol administration, of the number and volume of ballooning hepatocytes and centrilobular necrotic foci.

Morphopathological changes of the liver, more pronounced in the two groups receiving higher concentrations of hypericin, may be due to this compound which induced a potentiating effect of the alcohol toxic action, as confirmed and also correlated with the results of GPT biochemical activity - hepatocytolysis enzyme marker. Data from the literature regarding the potential toxicity of Hypericum are very sporadic. It is known that many biologically active substances derived from plant extracts may have toxic effects in overdose or prolonged administration. (Gregoretti et al., 2004)

Administration of hypericin lower concentrations (0.15%) in both experimental groups (HyP2 and HyM2), has caused obvious remission of hepatotoxic effects induced by alcohol treatments, the liver morphology being closer to normal. The liver structural recovery is increased slightly, insignificantly in both experimental groups compared with control group. GOT values are very close to those of C group.

The assembly of histological and GPT and GOT activity obtained results in case of animals treatment with lower hypericin concentrations, may be according to experimental data obtained by Kumar et al. in 2006, who observed that hypericin and hiperforina - other constituent of the Hypericum perforatum plant extract, have proved effective in the treatment of alcoholism. Lower level of liver morphological and functional changes in HyP2 and HyM2 experimental groups demonstrate that administration of lower hypericin concentrations of Hypericum perforatum and Hypericum maculatum results in renal protection against the alcohol toxicity, kidney histological images being closer to those of the control group.
recover potential particularly on alcohol-damaged liver functioning.

In conclusion, the lower level of liver and kidney morphological changes occurred mostly in the groups treated with lower doses of Hypericin, suggests a hepatarecovery potential of *Hypericum perforatum* and *Hypericum maculatum* plant extracts, versus the alcohol hepatotoxic effect. Our results demonstrate the protective potential, respectively, the stimulation of the liver and kidney structural regeneration, following administration of the bioactive plant extract.

**CONCLUSIONS**

Our experiment emphasized the following results:

- In the alcohol intoxicated group liver, we remarked moderate histological lesions consisting of ballooning cells that are connected and separates areas of liver parenchyma. To these, is added clear and fatty dystrophy and small necrotic foci. Serum transaminases, especially GPT increases with an average values.
- Alcohol intoxication induces reduced kidney damage, too.
- Administration of alcoholic extracts of *Hypericum perforatum* plant *Hypericum maculatum* at a concentration of 0.3% hypericin determined an emphasis of the adverse effects induced by ethanol, particularly in the liver, while lower doses (0.15% of hypericin) had positive effects, notably manifested by a tendency to return to normal histological and biochemical studied parameters, as a result of active processes of recovery.
- Histological changes consist in hepatocytolisis reduction, but with persistence of some infiltrations of rotundo-cell elements, often arranged pericentrolobular as well as dystrophic aspects. All these changes are smaller compared with the group intoxicated with alcohol.
- From the biochemical point of view we remarked a decrease of the transaminases GPT and GOT serum level.
- At kidney level we noticed reduced changes compared with the liver, the elective organ affected in alcohol intoxication.
- Of the four variants of *Hypericum* extracts, from our point of view, the most efficient curative effect (hepatoprotective), we recommend the *Hypericum perforatum*, of which hypericin content was 0.15% (group HyP2), as well as for *Hypericum maculatum*, 0.15% hypericin (HyM2).
- The protective effect of vegetable extracts of *Hypericum perforatum* and *Hypericum maculatum*, enable us to recommend them especially in clinical adjuvant therapy of liver diseases.

**REFERENCES**


Effects of Hypericum perforatum and Hypericum maculatum extracts administration on some morphological and biochemical parameters in rat liver intoxicated with alcohol


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