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CITOTOXIC EFFECTS OF THREE SPECIES OF *EPILOBIUM (ONAGRACEAE)* HERBAL EXTRACTS IN RATS

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ABSTRACT. Experiments were performed on albino male Wistar rats that were treated with three Herbal extracts of *Epilobium hirsutum, Epilobium angustifolium* and *Epilobium parviflorum (Onagraceae),* with a rich content in flavonoids. We investigated the possible cytotoxic effects of the hydro-alcoholic extracts on some metabolic – endocrine – immunologic organs (brain, hypophysis, adrenals, liver, kidney, thymus and spleen) with biochemical, histological and histoenzymological methods. There were no cytotoxic effect. *Epilobium hirsutum*, which has the best content in flavonoids, has had the most favorable effects

Keywords: herbal extracts, vital organs, epilobium, rats

INTRODUCTION

Phytotherapy has certain advantages that can't be denied (Weiss et al., 2000; Stănescu et al., 2004; Tămaş et al., 2005; Rusu et al., 2007; Brown et al., 2007). In spontaneous flora of Romania there are 16 species of the genus *Epilobium* (Fam. *Onagraceae*). Our attention was directed to 3 species used in popular medicine of central Europe, namely: *Epilobium parviflorum*, *Epilobium angustifolium* and *Epilobium hirsutum*. Their composition is complex, having a rich content in flavonoids (kemferol, quercetol, miricetol etc.), polyphenols, free sterols, gallic acid derivatives, ellagitannines. Principles are active during the peak flowering (Hierman et al., 1991). Chemical composition, pharmacological actions and phytotherapeutical recommendations are largely similar in those three species with certain characteristics: antiinflammatory effects of *E. Parviflorum* are even 10 times higher than indometacine (Arredondo et al., 2004) and with antioxidant effects. *Epilobium angustifolium* contains flavonoids, phenolic compounds and ellagitannins (Ducrez et al., 1997) as enotein A and B (Okuda et al., 1989) which have antiviral and anticancerigene effects, inhibiting DNA synthesis (Vitalone et al., 2001, 2003). *E. Hirsutum* is less known and used even if it inhibits Influenza virus (Ivancheva et al., 1992) and also it has a strong antitumoral action (Voznova et al., 1991). In relation to those three species, traditional phytotherapeutic recommendations are: benign prostate hypertrophy, epididimite, prostate, ginecomasty, urinary tractus infections, antidiarrohea, skin diseases, etc., being considered as local cicatriceant and disinfectant (Willoherb, 2002) etc.

These spontaneous species, in particular *E. Hirsutum,* are less studied in terms of pharmacological and especially toxicological aspects. First we determined the total flavonoids content (g% in rutozide) of material with which we worked (in Herba-stem, leaves, flowers): *Epilobium hirsutum* - 5.8%; *Epilobium angustifolium* - 3.4%; *Epilobium parviflorum* - 1.6%.

Our results showed that the species which is less used, *Epilobium hirsutum*, have the highest content in flavonoids - which could produce more intense phytotherapeutical effects. Because there are few data concerning the *Epilobium* extracts toxicity, we started evaluating the citotoxicity of bioactivity extracts on some matabolic-endocrine-immune organs.

MATERIALS AND METHODS

Experimental model

Experiments were performed on male albino Wistar rats, two month old, weighing 140±15 g. Animals were housed under the following laboratory conditions: lights on 06.00-18.00 h, 60% relative humidity; 200±20°C room temperature and access to commercial food pellets, and the tap water *ad libitum*. The animals were divided into the following experimental groups each consisting of 7-8 rats, as follows: control group – C; *Epilobium hirsutum* group - E1; *Epilobium angustifolium* group - E2; *Epilobium parviflorum* group - E3.

The ***duration of treatment*** was for 10 days. Treatment was performed daily by intragastric gavage with an adapted rubber stomach pump.

Dose: was of 1.5 ml Herbal extract for each rat, daily.

Sacrifice: animals were killed by decapitation after a pre-anesthesia, according to the EU rules of ethics. Blood was collected and fragments were removed from the following organs: brain, hypothalamus, hypophysis, adrenals, liver, kidney, spleen and thymus.

Biochemical determinations

- transaminase serum levels (GOT and GPT) (Reittman and Frankel, 1957);
- the lipoperoxidation rate in brain, liver and kidney (Wilbur et al., 1949);
- determination of adrenal cholesterol concentration (Zack et al., 1954).

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Histological determinations

Fragments of organs were fixed in Bouin liquid fixative and prepared for histology.

- Haematoxilin-eosin staining for histological structure of liver, kidney, spleen, thymus and adrenals (Muresan et al., 1976)
- Gabe Dawson-staining for neurosecretion in the hypothalamus (brain) (Muresan et al., 1976)
- Hurduc staining for hypophysis (Muresan et al., 1976).

Histoenzymological determination

Fragments of organs (liver, kidneys, adrenals, timus and spleen) were frozen in nitrogen liquid at -1960C. Then were cuts to a criotom mark Shandon AS London, in sections with a thickness of 7 µ. In these sections was performed the determination of some enzymes activity with usual methods (Muresan et al., 1976; Van Norden and Junges, 1995): lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), cytochrome c oxidase (cyox), Mg2+ dependent adenosine triphosphatase, lipids (Sudan black staining) in liver and kidney and steroid dehydrogenase (stdh) and glucose-6-phosphate dehydrogenase (G6PDH) in adrenals only.

Epilobium herbal extracts obtaining

All plants were harvested in the summer period (June and July 2008) from Alba, Cluj, Salaj, Mures area. The plants were in flower and were harvested only Herb (stems, leafs, flowers) and identified by Professor N. Tămaţ, Phd, from the "Iuliu Haţieganu" University of Medicine and Pharmacy, School of Pharmacy, Cluj-Napoca. The voucher specimen was deposited in the Herbarium of Institute of Biological Research, Cluj-Napoca, Romania. The fluid extracts (Herbal extracts) were obtained from dried, powdered material by repercolation with 700 ethanol.

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 to indicate a significant difference (Snedecor et al., 1978; Weber, 1980).

RESULTS AND DISCUSSIONS

The species of Epilobium genus have a fairly complex composition that includes a large proportion of flavonoids (kaemferol, quercetol, miricetol, etc.), polyphenols, sterols, tannins, gallic acid derivatives, etc. (Steenkamp et al., 2006). Flavonoids have antioxidant properties of cleaning free radicals (Arredondo et al., 2004). Between the 16 species that vegetate in Romania 2 are most known in folk medicine: *Epilobium angustifolium* (with 3.4% flavonoids) which are utilised as antiinflammatory, antidiarrohea, antibacterial and of benign prostate hypertrophy treatment and *Epilobium parviflorum* (with 1.6% flavonoides) (Ducrey et al., 1997; Vitalone et al., 2001, 2003; Willoherb 2002; Arredondo et al., 2004). The 3rd species, *Epilobium hirsutum*, although having 5.8% of flavonoides, is less used; it appears that has antiinflammatory (Hierman et al., 1991), antitumoral (Vaynova et al., 1991) and antiviral action (Ivancheva et al., 1991). Though they are used in popular phytotherapy systematic research on the chemical composition and hidroalcoholic extracts citotoxicity of some species of the Epilobium genus lacks.

Therefore, we proposed to study the extracts of 3 species: *Epilobium parviflorum*, *E. Angustifolium* - best known in local etnopharmacology and *E. Hirsutum* (less known) at the level of some metabolic-endocrine-immunological organs. We previously determined the flavonoids content and have been taken into account this parameter. We used a complex methodology, biochemical, histological, histochemical and histoenzymological that allow evidencing the changes also including cytotoxic changes, induced by the extracts of those 3 plant species at the level of important or vital organs. We have considered inclusive *Epilobium hirsutum* extract which is less studied.

An important parameter is hepatocytolysis, observed by determining serum transaminase activity, especially GPT. The GPT and GOT obtained results showed no major change with pathological significance. (Table 1). Hypothalamic-hypophyso-adrenal axis reactivity is involved in the stress state. In our experiment case the neurosecretion analysis of hypothalamus as well as hypophyses cell types doesen't show notable differences between C and E1, E2, E3 groups. At adrenal level, the central organ in stress reaction, there aren't significant changes between the control group and those 3 treated groups as concern the cholesterol concentration. Also, the stdh activity and G6POH enzymes "marker" for adrenals activity is not altered. That means that administration of Epilobium extracts not induced a stress state.

We also determined the lipoperoxidation process which is an oxidative process being a result of cells exposure to xenobiotics and free radicals (Rusu et al., 2005; Rusu et al., 2007). Our results show a decrease in the lipoperoxidation phenomenon, especially in the experimental groups E1 and E2, particularly in the brain and liver, which proves that the active principles contained in the 3 species of the genus Epilobium, especially flavonoids, have antioxidant activity cleaning of free radicals, which confirm and complete some of the literature data (Arredondo et al., 2004, Steenkamp et al., 2006). *Epilobium hirsutum*, which has a richer content in flavonoids, although is much less utilised, has greater antioxidant effects (Table 1).

Table 1

Serum transaminases (GPT and GOT) activity, cholesterol level in adrenals and lipoperoxidation rate in brain, liver and kidney in experimental groups

Group / Probe	*C*	*E1*	*E2*	*E3*
SERUM (µg.pyr/ml)				
GPT x ± ES	86,64 ± 15,31 (6)	88,128 ± 3,14	97,8 ± 5,61	73,44 ± 5,72
(n)	-	(5)	(6)	(5)
P	-	<0,05	<0,001	<0,001
D%		**+1,71**	**+12,88**	**-15,23**
GOT x ± ES	50,09 ± 2,06	49,454 ± 1,64	54,44 ± 4,76	45,03 ± 1,84
(n)	(6)	(5)	(6)	(5)
P	-	>0,5	>0,1	<0,001
D%	-	-1,27	+8,68	**-10,10**
ADRENALS (mg%)				
Colest. X ± ES	1588 ± 59.87	1695 ± 50.68	1394 ± 61.25	1499 ± 37,5
(n)	(5)	(6)	(6)	(6)
P	-	>0,5	=0,05	>0,5
D%	-	+6,73	**-12,21**	-5,60
BRAIN (nmol/MDA/hr)				
X ± ES	3,12 ± 0,19	1,42 ± 0,05	2,50 ± 0,01	2,57 ± 0,03
(n)	(6)	(6)	(6)	(6)
P	-	**-54,32**	**-19,87**	**-17,6**
D%	-	<0,001	<0,02	<0,05
LIVER (nmol/MDA/hr)				
X ± ES	1,05 ± 0,05	0,67 ± 0,03	0,70 ± 0,05	0,80 ± 0,04
(n)	(6)	(6)	(6)	(6)
P	-	**-36,20**	**-33,05**	**-23,60**
D%	-	<0,001	<0,01	<0,01
KIDNEY (nmol/MDA/hr)				
X ± ES	1,05 ± 0,06	0,88 ± 0,05	0,84 ± 0,04	0,88 ± 0,04
(n)	(6)	(6)	(6)	(6)
P	-	**-15,87**	-19,84	**-15,87**
D%	-	<0,001	<0,02	<0,05

Legend: C = control group; E1 = Epilobium hirsutum group; E2 = Epilobium angustifolium group, E3 = Epilobium parviflorum group. Are given: percentage difference vs. The control group (±D %); mean values homogeneity (x) ± SE, ± D% was considered to be statistically significant for p = 0.05

Histological structure of the liver and kidneys (evidenced by haematoxilin-eosine staining) doesn't change. In the liver and kidneys - vital metabolic organs - we noted a moderate increase of enzymatic activity of some oxido-reducing enzymes (LDH, SDH and cyox) (Table 2) under the influence of plant extracts, especially in E1 and E2 groups. Thymus and spleen limfoide organs do not undergo changes in E1, E2 and E3 groups, so immunological capacity is not affected. The bioactivity of Epilobium extracts doesen't produce pathological changes in the studied metabolic-endocrine-immune organs.

Contrary, we observed a moderate stimulation of oxidoreduction enzyme activity as well as the existence of some antioxidant qualities. Both, bioactivity and shown changes are correlated with increased contents in flavonoids, tannins, polyphenols, etc. Especially in E1 group and also in E2 group. This may be one of the main mechanisms of these extracts action. Is a novelty that *Epilobium hirsutum*, less used in popular phytotherapy has an increased concentration in flavonoids, compared with *Epilobium parviflorum* and *Epilobium angustifolium.* Therefore, *Epilobium hirsutum* species deserves more attention in future.

CONCLUSIONS

The hidroalcoholic extracts bioactivity of *Epilobium hirsutum*, *Epilobium angustifolium* and *Epilobium parviflorum* showed no cytotoxicity in rat at the brain, hypothalamic-hypophyso-adrenal axis, liver, kidneys, spleen and thymus levels.

Hidroalcoholic extracts of the genus Epilobium not produce a state of stress.

Hidroalcoholic extracts induce a moderate increase of the oxidoreduction enzymes (LDH, SDH, cyox) activity in liver and kidney in *Epilobium. Hirsutum* and *Epilobium angustifolium* groups.

The Epilobium extracts produce decreases of lipoperoxidation activity in the brain, liver and kidney, showing certain antioxidant qualities.

Extracts of *Epilobium hirsutum* and *Epilobium angustifolium*, which have the highest concentration of flavonoids, have proved to be most reactive.

The extract of *Epilobium hirsutum* has great phytotherapeutical perspectives having a rich content in flavonoids.

Table 2

Histoenzymological and histochemical results

Enzyme / Groups	LDH	SDH	Cyox	ATP-ase	Stdh	G6PDH	Lipids
LIVER							
C	+++	+++	+++	++	-	-	0
E1	$+++^{+}$	$+++^{+}$	$+++^{+}$	++	-	-	0
E2	$+++^{+}$	$+++^{+}$	$+++^{+}$	++	-	-	0
E3	+++	+++	+++	++	-	-	0
KIDNEY							
C	++	++	++	+	-	-	-
E1	$++^{+}$	$++^{+}$	$++^{+}$	+	-	-	-
E2	$++^{+}$	$++^{+}$	$++^{+}$	+	-	-	-
E3	++	++	++	+	-	-	-
ADRENALS							
C	-	-	-	-	++	++	-
E1	-	-	-	-	++	++	-
E2	-	-	-	-	++	++	-
E3	-	-	-	-	++	++	-

Legend: C = control group; E1 = E. Hirsutum group; E2 = E. Angustifolium group, E3 = E. Parviflorum group. Intensity of enzymes activity: 0 = no activity; + = slow activity; ++ = moderate activity; +++ = intense activity; ++++ = very intense activity.

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