SOME HISTOENZYMEOLOGICAL (HISTOCHEMICAL) AND BIOCHEMICAL CHANGES INDUCED BY PEUCEDANUM HERBAL EXTRACTS UPON THE LIVER AND KIDNEY IN RATS

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Summary
The experiments were performed on adult albino female Wistar rats. We tested the action of 4 species of Peucedanum herbal extracts, for 10 days, on the liver and kidneys. We used histoenzimological determinations (LDH, SDH, CyOx and ATP-ase activity) and biochemical (serum transaminase activity) methods. Herbal extracts of Peucedanum sp. not produce toxic effects in liver and kidney, but produced a moderate increase of LDH, SDH and CyOx activities, both in liver and kidneys, especially in the group treated with Peucedanum officinale extract which has the highest content in flavonoids and coumarines.

Key words: herbal extracts, Peucedanum sp., liver, kidney, rats.

Introduction
Plants are able to synthesize and store a series of primary and secondary substances (Tiina, 2001) used by man in phytotherapy (Ciulei et al., 1993; Weiss, Fintelmann, 2000; Stănescu et al., 2004; Rusu et al., 2007).

The most frequent chemical compounds from plants are the very large category of polyphenols. They are very widespread in plants and probably may be carbohydrates that are more abundantly (Ciulei et al., 1993). Polyphenols are different not only in molecular weight but also in structure.

Polyphenols are under some aspects truly traps for free radicals. They must take into account not only as xenobiotics and free radicals (Rusu et al., 2005) that act but also as a complex structure of the polyphenols that are used. In the polyphenols category are also included the flavonoid compounds and coumarins and phurano coumarins.

The Genus Peucedanum contain many polyphenols that contribute together with other vegetal compounds to their utilization in phytotherapy, having the following effects: anti-inflammatory, vasoprotective, antiseptic, antimitotic (peucedamine), vasodilator, hepatoprotective, antioxidative, K and P vitamins effects (Tiina, 2001; Hierman, Schantl, 1998; Tamaș, 1999; Barnard et al., 2002).

The herbal alcoholic extracts of Peucedanum sp. contain especially flavonoids, but also coumarins, phenolic compounds, volatile oils, fatty oil, resins, etc. The most important compounds are flavonoids and coumarins extracted from herbs.

The flavonoids and phenolic compounds content depending on Peucedanum species used. Thus, for flavonoids were emphasized routine and quercetine, and for phenolic compounds were emphasized chlorogenic acid and caffeic acid. The content was expressed in µg/ml, as is presented in Table 1.

Although phytotherapy has generally favorable effects on some diseases, it is not completely free of unwanted side effects. So, it is favorable in the case of organs, but may harm in other cases. Therefore, it is necessary to investigate possible toxic effects on some vital organs.
Table 1. The main active substances content of some species of *Peucedanum*

<table>
<thead>
<tr>
<th>Species</th>
<th>Flavonoids from Herba (µg/ml)</th>
<th>Phenolic compounds from Herba (µg/ml)</th>
<th>Coumarines from Herba (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Peucedanum officinale</em></td>
<td>111.7</td>
<td>489.5</td>
<td>230</td>
</tr>
<tr>
<td>2. <em>Peucedanum cervaria</em></td>
<td>52.8</td>
<td>243.6</td>
<td>67.50</td>
</tr>
<tr>
<td>3. <em>Peucedanum palustre</em></td>
<td>31.7</td>
<td>52.6</td>
<td>56.30</td>
</tr>
<tr>
<td>4. <em>Peucedanum oreoselinum</em></td>
<td>25.1</td>
<td>0</td>
<td>50.90</td>
</tr>
</tbody>
</table>

The aim of our research was the study of biochemical and histoenzymological (histochemical) modifications induced by *Peucedanum* extracts on two important organs: the liver and kidneys.

**Material and Methods**

Experiments were performed on albino female Wistar rats, weighing 126 ± 10g. The animals were maintained under the following laboratory conditions: lights on 06.00-18.00; 60% relative humidity; 22°C ± 2°C room temperature, access to commercial food pellets, and tap water *ad libitum*.

Animals were divided into the following experimental groups, consisting of 7-8 rats each: C (control) and 4 experimental groups notated with P, as follows:
- P1 = *Peucedanum officinale*
- P2 = *Peucedanum palustre*
- P3 = *Peucedanum cervaria*
- P4 = *Peucedanum oreoselinum*

Obtain vegetable extracts of *Peucedanum* sp; it was used herba (stems with leaves and flowers). Extracts were obtained by the maceration technique in ethylic alcohol 50%.

Dosage and way of administration: from a mother solution obtained from 7 ml alcoholic (50%) vegetal extract from each plant + 93 ml distilled water, was taken 1.5 ml from each extract and was given to each rat daily, by intragastric gavages, during 10 days.

Sacrification: The rats were killed by decapitation after a previous anesthesia with ether and were collected blood, liver and kidney.

Biochemical determination:
- The serum transaminase activity (GPT and GOT) (Reitmann, Frankel, 1957).
- Histological determination:
  - Liver and kidney fragments were fixed in Bouin liquid and prepared for histology (hematoxylin-eosine staining) for study of liver and kidney histological structure (Muresan et al., 1974).
  - Histoenzymological determination:
    - Liver and kidney fragments were frozen in liquid nitrogen at -196°C. After that, were sectioned in a Shandon cryothome, into thin sections of 7 µ. On these section were determined some enzymes with usual methods (Muresan et al., 1974; Van Noorden C, Jonges, 1995).
    - Lactate-dehydrogenase (LDH),
    - Succinate-dehidrogenase (SDH),
    - Cytochromeoxidase (cyox),
    - Mg-dependent adenosine triphosphatase (ATP-ase).

**Results**

Biochemical, histological and histoenzymological parameters:

The level of GPT and GOT serum transaminases (Fig. 1).
- GPT level: comparative with C group decrease significantly with 55.82% in P2 group, with 60.64% in P3 group and with 53.56% in P4 group.
- GOT level: comparative with C group decrease in experimentally group as follows: with 9.19% in P1 group, with 11.34% in P2 group, with 17.13% in P3 group and with 16.54% in P4 group, but all these values doesn’t have a pathological signification.
Figure 1. The level of GPT and GOT serum transaminases in rats treated with different alcoholic extracts of *Peucedanum sp.* Are given: percentage difference vs. the control group (±D %); it was considered to be statistically significant for p = 0.05.

**Histological parameters**

**Hematoxylin-eosine staining:**

Liver: in C group one may observe cordons of hepatocytes with cytoplasm stained in red and nuclei in blue-black, as well as in portal and centrolobular spaces. There are no changes between experimental groups and control group.

Kidney: in C group one may observe urinary tubules and Malpighi glomeruli. There are no changes between groups.

**Histoenzymological parameters** (Table 2)

<table>
<thead>
<tr>
<th>Enzimatic activity</th>
<th>LDH</th>
<th>SDH</th>
<th>CyOx</th>
<th>ATP-ase</th>
<th>Sudan Black for lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LIVER</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>P1</td>
<td>+++ (+)</td>
<td>+++ (+)</td>
<td>+++ (+)</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>P2</td>
<td>+++ (+)</td>
<td>+++ (+)</td>
<td>+++ (+)</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>P3</td>
<td>+++ (+)</td>
<td>+++ (+)</td>
<td>+++ (+)</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>P4</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td><strong>KIDNEY</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>P1</td>
<td>++ (+)</td>
<td>++ (+)</td>
<td>++</td>
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<td>0</td>
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<td>P2</td>
<td>++</td>
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<td>P3</td>
<td>++</td>
<td>++</td>
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<td>+</td>
<td>0</td>
</tr>
<tr>
<td>P4</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

Legend: 0 – no activity; + - slow activity; ++ - moderate activity; +++ - intense activity

*Liver*
- LDH activity: in C group show a very intense blue color, with a zonal distribution, more intense in periportal area or acinous zone I. In P1, P2, P3 groups but especially in P1 group it can remark a moderate increases of LDH activity.
- SDH activity: in C group it appears like an intense blue color with a zonal distribution, being more intense in periportal area. Comparative with C group, SDH activity is moderate increased especially in P1 group, also in P2 and P3 group.
- ATP-ase activity: in C group appear like a brown color having a pericanalicular distribution, in blood vessel walls, as well as in hepatocyte cytoplasm. There are no differences between C group and experimental groups.
- CyOx activity: in C group appear like a blue-green color having a zonal distribution. The enzymatic activity is a little bit increase especially in P1 group, and also in P2 and P3 group, too. (Fig. 2a, b)

**Figure 2a.** CyOx, Liver, C group, 6x. Heterogeneous distribution of the enzymatic activity predominantly in the acinar I area (periportal).

- Kidney

- LDH activity: is intense expressed at the urinary tubules and less expressed at the Malpighi glomerulus’s in C group. The enzymatic activity easily increases especially in P1 group.
- SDH activity: in C group is positive in renal tubules, but it is not emphasized at the Malpighi glomeruli. The enzymatic activity is moderate increase especially in P1 group.
- ATP-ase activity: is intense especially at the Malpighi glomerulus’s level, being less intense in renal tubules. There are no differences between groups.
- CyOx activity: in C group, enzymatic activity is reduced at the Malpighi glomerulus’s level, but is intense in urinary tubules. Enzymatic activity is moderately increased in P1 group. (Fig. 3 a,b)

**Figure 2b.** CyOx, Liver, P1 group, 6x. Show the same distribution of CyOx activity but is moderately high.

**Figure 3a.** CyOx, Kidney, C group, 6x. Enzymatic activity is normal distributed both in Malpighi corpusculi and in urinary tubules.
Discussion

Our researches utilized a complex, interdisciplinary, biochemical, histological and histoenzymological adequate methodology, which is necessary to analyze for phytotherapy some vital organs (liver and kidney).

In the frame of our analyses, determination of serum transaminases level has a great importance. Thus, the GPT level decreases with a significantly high value in P2, P3 and P4 group, without any pathological significance. The GOT values are not significant. Thus, in our experiment we may certainly affirm that administration of those four different species extracts of Genus *Peucedanum*, have not toxic effects upon the liver, and doesn’t produce hepatocitolsy.

Our researches included two vital metabolic organs, liver and kidney, that present reactivity under vegetal extracts action with a great importance. Histoenzymological determinations allow us to estimate “in situ” some enzymatic activities not of some enzyme, like it is believed (Van Noorden, Jonges, 1995). The great advantage of histoenzymology consists in description of enzymatic activity distribution at the tissue level. Thus, as concern the liver we could describe an oxide reduction activity (LDH, SDH, CyOx) more intense in periportal areas or acinous zone I. Enzymatic distribution respect the acinar structure of the liver, described by Rappaport (1958) and make evident on this basis, the functional heterogeneity of the liver (Katz, Jungermann, 1993).

At the kidney level is a different distribution between urinary tubules and Malpighi glomerulus’s. Both in liver and in kidney it remark an easier or a moderate increases of some oxide reduction activity enzymes under herbal extracts influences, especially in P1 group and less in P2 and P3 group.

Conclusion

Administration of alcholic extracts obtained from four *Peucedanum* species doesn’t produce pathological modifications at the level of analyzed organs (liver and kidney) in experimental rats. Contrary, it can remark a moderate stimulation of some enzymes activity and the possibility of some antioxidant qualities presence. The presented modifications are in direct correlation with flavonoids and coumarins content. Thus, on the first place is *Peucedanum officinale* (that have the greatest content in flavones and coumarins) *Peucedanum palustre* and *Peucedanum cervaria*. On the “last place” remain *Peucedanum oreoselinum*.

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References


