Effect of hydro-alcoholic extract of Heracleum persicum during pregnancy on liver enzymes (AST-ALT-ALP) and biochemical factors (Albumin and protein) of infant male rats

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Abstract

Introduction:
Liver is one of the vital organs in the body with a key role in regulating many physiological mechanisms. The present study was conducted to examine the effect of the hydroalcoholic extract of Heraclum persicum administered to the mother rats during pregnancy on liver enzymes and blood biochemical factors in their infant male rats.

Materials and Methods:
The present experimental study was conducted on 40 infant male Wistar rats divided into 5 groups of 8, whose mothers were then divided into 5 groups of 8 during their pregnancy, including a negative control group, a sham control group and three experimental groups. The negative control group did not receive any treatments while the sham control group received only distilled water. Experimental groups 1, 2 and 3 received, respectively, 100, 200 or 400 mg/kg oral doses of Heracleum persicum on a daily basis for a period of 21 days (the pregnancy term). At the end of the pregnancy and when the male infant rats were 25 days old, the infant rats’ blood samples were taken and their liver enzymes (alanine transaminase [ALT], aspartate aminotransferase [AST] and alkaline phosphatase [ALP]) and biochemical factors (Albumin and total serum protein) were measured. The data obtained were analyzed using the ANOVA and Tukey's post-hoc test. P<0.05 was taken as the level of statistical significance.

Results:
The AST and ALT levels had increased significantly in experimental groups 2 and 3 compared to in the negative and sham control groups (P<0.05). The Albumin level had also increased significantly in experimental group 3 compared to in the negative and sham control groups (P<0.05). There was also a significant difference between ALP and serum protein levels in the experimental groups and the negative and sham control groups.

Conclusion:
The hydroalcoholic extract of Heracleum persicum may have changed the liver function and increased ALT and AST as a result of its furanocoumarin and trans-anethole content. The extract’s alkaloid and flavonoid content also increase serum Albumin levels.

Keywords: Heracleum Persicum, AST, ALT, Albumin, Rat

Introduction
Liver is the largest internal organ of the body and is involved in many essential functions of the body, such as the removal of red blood cells in coordination with the
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spleen, production of bile, regulation of glucose through glycogen storage, and control of blood homeostasis through secreting blood clotting factors and serum proteins, including albumin (1). Given that the liver is responsible for biochemical, synthetic, and secretory functions, its enzymes are used as biochemical markers in diagnosis of hepatic disorders (2, 3).

Golpar, scientifically known as heracleum persicum, is a plant in the family Apiaceae, of the species parsley, and native to Iran (4). Golpar is a 2-year, 3-year, or perennial plant with six different species. It has a hollow, thick, straight cylindrically shaped, grooved, and a bit fluffy stem that can be up to 150 cm high. It has a thick root and fully cutoff leaves. It grows near rivers and is considered an ornamental plant with very harmful and invasive species (5, 6). Golpar is used to treat epilepsy in Iran. Its fruit and leaves are used as antiseptics, carminatives, digestives, and pain relievers (7). Golpar oil has a wide range of antibacterial and antifungal properties (8). Golpar is recommended as a contraceptive for women (9). Predecessors have mentioned many properties for golpar. It increases the gastric secretions and excretes toxins of the body. It is a strong antiseptic and microbicde. It increases women’s breast milk and the body’s secretion of sweat and is diuretic. According to the traditional medicine, the overuse of golpar during pregnancy may result in abortion (10). This study examined the effect of different doses of golpar extract during pregnancy on liver enzymes, including alanine transferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and some biochemical factors of blood, such as total protein and male newborns’ albumin, in order to determine the side effects of golpar misuse in infants.

Materials and Methods

This study was performed on 10 adult male Wistar rats and 25 adult female Wistar rats. The rats were provided by the animal breeding center of the Islamic Azad University, Kazeroon Branch. The female rats weighed approximately 180 \pm 10 g and aged approximately 70 days, and male rats weighed approximately 220 \pm 20 g. The rats were housed with 12:12 dark-light cycle at 22-24 °C, and male rats received only potable water. Once the initial weight of the rats was recorded, every five female rats were kept in one cage (10). Then, two male rats were added to each of those cages for six days in order to impregnate the female rats (11).

To make hydroalcoholic extract of golpar, an amount of golpar flower was dried in the shadow and then powdered with an electric mill. The powder was soaked in 96% ethanol/distilled water (1:1) for 72 hours, and the container was shaken several times during that period. The solution was filtered, and the resultant hydroalcoholic extract was centrifuged at 4500 rpm for 8 minutes in order to remove its suspended particles. The obtained solution was placed in an oven at 40 °C in order to achieve a concentrated liquid. Eventually, certain amounts of the extract were dissolved in distilled water to obtain different concentrations (12).

Grouping the rats

In this experimental study, 40 Wistar rats were divided into five groups of eight rats as follows:

The control group: It included the male rats whose mother was kept without any pharmacological therapy during pregnancy.

The sham group: It included male rats whose mother received 2 ml of distilled water as a solvent daily during pregnancy (10).

The case groups 1, 2, and 3: These groups included male rats whose mother received respectively 100 mg/kg, 200 mg/kg, and 400 mg/kg of the hydroalcoholic extract of golpar per day orally for 21 days during pregnancy using a feeder (4).

The menstrual cycle of the female rats was made concurrent before pregnancy. To do
so, 1 cc of 10 mg estradiol valerate vaccine was mixed with 2 ml of olive oil in a 5 cc syringe. About 2 ml of the syringe was injected intramuscularly to each female rat. About 48 hours later, 1 cc of 25 mg progesterone vaccine was mixed with 2 ml of olive oil in a 5cc syringe, and 2 ml of the syringe was injected to each female rat. Six hours later, a smear test was performed from rats’ vagina. To perform the smear test, an amount of physiological serum was injected into the vagina using a sterilized Pasteur pipette, and the cells washed out of the uterine wall were collected using the same pipette. Smears were prepared from the collected material and then dried. The smears were fixed in alcohol and stained with Geimsa stain (England, BBDE) diluted at 1/20 ratio for 15 minutes. Then, the samples were gently rinsed with distilled water and examined in order to confirm their menstrual concurrence using an optical microscope (13,14). The newborn male rats were kept with their mother in separate cages and were fed with breast milk. The newborns comprised 40 rats with approximate weight of 100 g and age of 25 days. The floor of the cages was replaced cautiously every three days without injuring the newborns. The newborns were cared for 25 days. At the end of the 25th day, all male newborns were weighed using a digital scale with 0.001 precision and then anesthetized with ether, and their heart’s blood was drawn. The blood tubes were centrifuged at 5000 rpm for 15 minutes, and their serum was removed and kept at -20 °C up to the enzymatic measurement. Serum samples were delivered to the laboratory in order to measure the activity of liver enzymes (ALP, AST, & ALT) and biochemical blood parameters (albumin & total protein) using kits that were specific to measurement of liver enzymes and manufactured by Pars Azmun Co., Iran. The one-way ANOVA was used to compare the data in different groups, and then Tukey’s test was used to determine the difference between groups. The figures were drawn using the Excel® software. The statistical significance level was determined as P < 0.05. The data were presented as Mean ± SEM.

Results

The effect of hydroalcoholic extract of golpar on the level of liver enzymes

According to the results, the level of AST in the moderate and maximum case group at higher doses of the extract was respectively 49.55 ± 86 and 622.55 ± 22.622, which showed a significant increase against that in the control and sham group (371 ± 27.10 & 430.55 ± 13.64, respectively) (Table 1) (P < 0.05). The level of ALT in the moderate and maximum case group at higher doses of the extract was respectively 120.55 ± 8.89 and 142.33 ± 7.61, which showed a significant increase against that in the control and sham group (90.77 ± 7.49 & 92.88 ± 4.54, respectively) (Table 1) (P<0.05).

There was no significant difference between case groups in terms of the level of ALP (1164.44 ± 67.77, 1301.00 ± 96.40, & 1320.91 ± 59.98, respectively) against that in the control and sham groups (1138.22 ± 44.05 & 1169.00 ± 48.37, respectively) (Table 1).

The effect of hydroalcoholic extract of golpar on the level of albumin and total protein

A significant increase was observed in the level of albumin in the maximum case group against that in the control and sham groups (P < 0.05) (Table 1).

The level of total protein in the minimum, moderate, and maximum case group was respectively 3.22 ± 0.10, 3.71 ± 0.18, and 3.63 ± 0.24, which was not significantly different from that in the control and sham groups (2.83 ± 0.50 & 2.94 ± 0.40, respectively) (Table 1).
Table 1: The serum level of liver enzymes (ALP, AST, & ALT) and biochemical parameters of the blood (albumin & total protein) in the studied groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (u/l) Mean±SEM</th>
<th>ALT (u/l) Mean±SEM</th>
<th>ALP (u/l) Mean±SEM</th>
<th>Albumin (mg/dl) Mean±SEM</th>
<th>Protein (mg/dl) Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>371 ± 27.1</td>
<td>90.77 ± 7.49</td>
<td>1138.22 ± 44.05</td>
<td>2.83 ± 0.50</td>
<td>4.51 ± 0.10</td>
</tr>
<tr>
<td>Sham (receiving 2 ml of distilled water)</td>
<td>430.55 ± 13.64</td>
<td>92.88 ± 4.54</td>
<td>1169.00 ± 48.37</td>
<td>2.72 ± 0.40</td>
<td>4.52 ± 0.70</td>
</tr>
<tr>
<td>Case group 1 (receiving 100 mg/kg of golpar extract)</td>
<td>490.11 ± 57.06</td>
<td>100.55 ± 10.40</td>
<td>1164.44 ± 67.77</td>
<td>3.14 ± 0.10</td>
<td>4.58 ± 0.80</td>
</tr>
<tr>
<td>Case group 2 (receiving 200 mg/kg of golpar extract)</td>
<td>622.55 ± 86*</td>
<td>120.55 ± 8.89*</td>
<td>1301.00 ± 96.40</td>
<td>3.21 ± 0.18</td>
<td>4.56 ± 0.15</td>
</tr>
<tr>
<td>Case group 3 (receiving 400 mg/kg of golpar extract)</td>
<td>622.55 ± 22.4*</td>
<td>142.33 ± 7.61*</td>
<td>1320.91 ± 59.98</td>
<td>3.92 ± 0.16*</td>
<td>4.57 ± 0.12</td>
</tr>
</tbody>
</table>

Asterisk (*) shows a significant difference between case groups and control and sham groups. The values have been presented as mean ± standard error of the mean (Mean ± SEM).

**Discussions**

This study was performed to examine the effect of the hydroalcoholic extract of golpar on liver enzymes and biochemical factors of the blood during pregnancy in male newborn rats. The results showed that the level of AST and ALT enzymes significantly increased in the case groups receiving the moderate and maximum dose of the extract against that in the control and sham group. The level of albumin also significantly increased in the case group receiving maximum dose of the extract against that in the control and sham group. The case groups did not significantly differ from the control and sham group in terms of the level of ALP and total protein.

Enzymes, as biological catalysts, perform all enzymatic reactions of the body cells. The quantitative and qualitative changes in the level of these enzymes reflect the health or sickness (2). The high level of liver enzymes in the serum indicates the damages to hepatic cells (extensive hepatic necrosis) and even liver death (2,15). ALT is a hepatocellular enzyme that enters and accumulates in the blood following cellular damages. The increased ALT causes acute hepatic failure. AST is in the cytoplasm and mitochondria of the cells in heart, muscles, and liver tissues and will be increased in the serum if those tissues are damaged (2,16). ALP is a hydrolase with a half-life of 7-10 days, and its production is coded by various genes. So far, 16 isoenzymes of ALP have been identified. Maximum plasma level of ALP occurs following the effect of the gene on chromosome 1 and is called tissue-nonspecific isoenzyme that originates from the kidneys, the liver, and bones (17).

One of the most important components of golpar extract is coumarin and its derivatives (18). Coumarin is a class of organic compounds with biological activities. Clinical evidence has shown the toxicity of furanocoumarin in some food materials and plants (19). Furanocoumarin was administered orally to the rats for 28 days in order to examine its toxicity on the kidney and the liver. The results showed that the level of ALT and AST increased in rats receiving isopsoralen (a derivative of coumarin) (20). Moreover, the furanocoumarin in the grapefruit extract is a very strong inhibitor of microsomal cytochrome P450 and increases the cell toxicity (20). Furanocoumarin is an inhibitor of cytochrome P4503A and other cytochrome isoenzymes that play the main role in metabolism of many narcotics and drugs (21). The cytochrome P450 in the liver tissue is concentrated mostly in centrilocubular liver cells. The inhibition of
cytochrome P450 occurs through bonding to this enzyme, ceasing NAPD activity, and reducing the cytochrome results in an increase in the lipid peroxidation, production of reactive oxygen species, and the secondary damage to cell membranes and mitochondria (21, 22). Hepatic microsomal enzymes interfere with many drugs and change their pharmacology (20, 22). It has been proved that coumarin as an inhibitor of CYP2B inhibits the metabolism of drugs and steroids. Moreover, coumarin derivatives inhibit the activity of CYP1A1 and CYP1A2 in the liver (21). Furanocoumarins are also phototoxic and can produce psoralen complex between base pairs of the DNA through a non-covalent interaction and form cyclobutane monoadduct in the pyrimidine base that causes mutations in cells (18, 19). Furthermore, furanocoumarin may produce free or complex oxygen under the influence of UV ray. The oxygen attacks the fat in the cell membrane and results in a decrease in fluidity of cell membranes and leakage of liver enzymes to the blood. Oxygen binds to proteins and DNA and produces lipid peroxidation and consequently reduces glutathione and produces free radicals. Furthermore, furanocoumarin damages the lysosomes as they are formed in the cell (19, 20). Studies have shown ethyl acetate as another component of golpar (23). Ethyl acetate increases the serum ALT and AST mildly in rabbits (23). Other studies have shown that golpar extract increases the nitric oxide (NO) through macrophages. The NO inhibits the DNA repair proteins and thus inhibits the cells’ ability to repair the DNA. The DNA damages and mutations in the liver lead to an increase in the serum AST and ALT (4, 24, 25). Studies have shown that golpar extract contains a substance called anethole (7). A partial increase in proliferative lesions of the liver and a low prevalence of neoplasm of the liver were observed in rats receiving anethole; and the neoplasm of the liver increased with an increase in dose of anethole. The results showed that the used dose of trans-anethole is not carcinogenic for humans but may increase liver tumors at higher doses and increase ALT and AST (26, 27). In this study, the case groups did not significantly differ from the control and sham groups in terms of the level of ALP. Studies have shown that isopsoralen, a derivative of coumarin, increases the ALP (19). Moreover, studies have shown that ethyl acetate increases the ALP in rabbits. Given that golpar contains ethyl acetate (7), the increased dose of golpar extract during pregnancy probably makes a significant difference regarding the conformity of the results of this study with those of other studies. In this study, the level of albumin increased at the maximum dose of the extract. The serum albumin is a spherical protein encoded by ALP gene in humans. The half-life of albumin in the body is 20 days and is destroyed and replaced by 4% per day (28). The main function of albumin is the adjustment of colloid osmotic pressure of blood. The level of albumin depends on the production, destruction, nutritional status, and oncotic pressure of the plasma, cytokines, and alkaloids (7). Golpar also contains quercetin that is a major flavonoid in humans’ diet and has a broad range of biological properties (7). Quercetin is anti-inflammatory and anticancer (29) and significantly reduces the oxidative stress through affecting the P53 gene. The treatment with quercetin considerably increases the relative mRNA, glutathione peroxidase, activity of the liver Gpx, and concentration of the liver glutathione (29). The activity and increase in glutathione deactivate the reaction of lipid peroxidation (30, 31). Studies have shown that alkaloids make the plasma membrane of the organs permeable and cause the normal function, normal secretion, synthesis, and excretion in the organs, which result in an increase in serum albumin (32). The significant increase in biosynthesis of albumin shows the sustainability of the plasma membrane.
Therefore, golpar increases the serum albumin through changing the membrane permeability probably due to its flavonoids, quercetin, and alkaloids (7).

Conclusion
Golpar increases the liver AST and ALT due to its compounds, including ethyl acetate, trans-anethole, and coumarin and its derivatives. Furthermore, it increases the serum albumin through changing the permeability of plasma membrane of the organs due to its flavonoids and alkaloids.

Conflicts of Interests
The authors of this study do not have any conflicts of interests regarding the authorship and publication of this study.

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