Investigating Effects of Alcohol Aqueous Extract of Harmala Seed on the Plasma Levels of Pituitary - Thyroid Hormones Axis and Liver Enzymes and Body Weight in Adult Male Rats

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ABSTRACT
Introduction and Objectives: Harmala plant has activations of anti-bacterial, anti-fungal and anti-tumor, and inhibit monoamine oxidase enzyme, stimulate nervous system and modulate activation of Endocrine. The aim of this study was to investigate the effect of harmala seed extract on the levels of hormones of pituitary-thyroid and liver enzymes.

Materials and methods: This research is an experimental study that was done on 50 adult male Wistar rats. In this study, animals were divided into 5 groups. The control group did not receive any treatments, the sham group for 14 consecutive days received orally 1 ml of distilled water and experimental groups for 14 consecutive days received respectively 90 mg/kg, 180 mg/kg and 270 mg/kg harmala seed extract that was given orally and on the fifteenth day, stimulating hormones concentration of thyroid, thyroxine and triiodothyronine and liver enzymes were measured and the results analyzed using SPSS software and ANOVA test.

Results: Harmala seed extract at the minimum and maximum doses significantly reduced the rate of thyroid stimulating hormone, and in medium and maximum doses significantly reduced the rate of thyroxine and triiodothyronine, but body weight and liver enzymes did not show significant changes (P<0.05). Conclusion: The results indicate that the harmala seed extract can be used in the treatment of hyperthyroidism.

INTRODUCTION
Maintaining homeostasis of the body is the key point in human health that is controlled by several factors, especially pituitary-thyroid hormone axis. Underactive and overactive thyroid with disruption in body health imposes a high cost for patient, and in many cases there is no possible treatment [1]. From the past to now, in medical and treatment of diseases medicinal plants have generally been used and peganum is one of the medicinal plants with the scientific name of Peganum harmala, from the family of zigu filaseh. Peganunharmala is a perennial grass that grows to a height of approximately 30 to 40 cm. This plant grows in semi-arid and arid lands and has general distribution in Europe, Turkey, Iran, Afghanistan, Pakistan, India, Iraq, Syria, Jordan, Lebanon, Palestine, Arabia and North Africa. It can be found in Iran in areas where there is little salt. This plant is also found in America and Australia. Harmala seeds contain compounds such as Alkaloids like Harmaline, Harmaine, Harmalole, Harman, Vasicine and Vazizinone [1]. Harmala traditionally produce effects such as sleepiness, sweating, anti-parasite, binding rule, the embryo abortion, anti-cancer, anti-bacterial, nervous system stimulants and inhibitors of the enzyme monoamine oxidase [2,3]. Harmala alkaloid composition of the seeds of the neurotransmitters dopamine and serotonin stimulates a dose-dependent manner [4,5]. The most important factor controlling the synthesis and secretion of thyroid hormones is applied by regulating hypothalamus-pituitary hormonal axis. Thyroid-stimulating hormone, which is released from hypothalamus has an impact on
anterior pituitary and causes secretion of thyroid stimulating hormone [6,7]. Thyroid hormones T4 (thyroxine) and T3 (triiodothyronine) also affect the secretion of thyroid stimulating hormones that are secreted by adnoid pituitary [8,9]. Thyroid hormones stimulate the transcription of numerous genes, intensify production of large amounts of enzymes, structural proteins and carrier and increase the basal metabolic [10,11]. Because of the many uses of this plant in traditional medicine and the effect of this plant in endocrine researches, other axes have been considered but a study of the effect of harmala seed on performance of pituitary-thyroid hormone axis associated with changes in liver enzymes and body weight has not been done, so the aim of the present study is to investigate the influence of hydro-alcoholic seed extract on plasma levels of hormones pituitary-thyroid axis.

MATERIALS AND METHODS

In this study 50 adult male Wistar rats weighing approximately 260-320 gr were used. The samples were divided into five groups of 10 that include control and sham groups and 3 experimental groups. Each of the groups were in separate cages and were given one week to adapt to the laboratory environment. During the prescribed period, all animals were given equal and unrestricted food and water at a constant temperature (25 ±2 °C) and natural light (12 hours light, 12 hours dark). In the entire project all ethics in relation to working with laboratory animals have been considered. In the spring season the required amount of harmala seeds was collected from Nurabad-Mamasani in Iran. Then a perfect sample of the above-mentioned plant in order to identify plant species in the herbarium of the Research Center of the Jahad university of Kohkiluyeh and Boyer Ahmad province in Iran, Herbarium No. 4633 inside the country was recorded and coded. 500 gr of powdered seeds were soaked in 96% alcohol and after 48 hours exposure on shaker machine, at room temperature and laboratory animals have been considered. In the spring season the required amount of harmala seeds was collected from Nurabad-Mamasani in Iran. Then a perfect sample of the above-mentioned plant in order to identify plant species in the herbarium of the Research Center of the Jahad university of Kohkiluyeh and Boyer Ahmad province in Iran, Herbarium No. 4633 inside the country was recorded and coded. 500 gr of powdered seeds were soaked in 96% alcohol and after 48 hours exposure on shaker machine, at room temperature and away from heat and light the mixture was smoothed. The filtered solution was passed through Whatman filter paper (number 1) for better refining. The resulting solution was used as raw material for the extraction stage and was concentrated in a rotary machine in vacuum condition. The resulting material was placed in an oven for 5 days at 40 to 45°C. The final material weight of the above process was 94 gr. The animals were randomly grouped. Animals in the control group did not receive any special treatment. The sham group received daily 1 cc distilled water for 14 days orally and three experimental groups, 1-3 respectively received 90 mg/kg, 180 mg/kg and 270 mg/kg of harmala seed extract orally. On day 15, after weighing, the animals were anesthetized by ether and blood samples were taken directly from the heart. To obtain plasma, blood samples were taken into tubes containing EDTA anticoagulant and then the required centrifuged blood plasma samples were kept frozen in the freezer at -20°C. Plasma samples for measuring stimulating hormones of thyroid, thyroxine and triiodothyronine were assessed by radioimmunoassay method(12). Liver enzymes were measured by spectrophotometric method. Data were analyzed by SPSS software and ANOVA test.

Results:
The results indicate that plasma levels of thyroid stimulating hormone in the receiving groups of minimal doses (90 mg /kg) and maximum (270 mg/ kg) compared to control and sham groups had a significant decrease (P≤0.05) while in the receiving dose of (180 mg/kg) groups there was no significant difference (Table 1). Also, after measuring plasma levels of thyroxine and triiodothyronine hormones it was determined that the rate of the above hormones, just in the groups receiving the intermediate (180 mg/kg) and maximum (270 mg/kg) doses compared with control and sham groups showed significant decrease (P ≤0.05) (Table 1). These findings suggest that increasing the rate of receiving extract has decreased the concentration of thyroxine hormone. Scheffe test between experimental groups receiving medium and maximum dose, and control and sham groups showed significant difference, but in other cases there was no significant difference (P≤0.05). Also, by increasing the concentration of harmala extract the amount of triiodothyronine hormone in the rats under study has decreased. Scheffe test between experimental groups receiving the intermediate and maximum dose, and control and sham groups has shown significant decrease (P≤005), but between experimental groups 1-3 there was no significant difference. Body weight (Table 2) and liver enzymes have not shown significant difference in P≤0.05 levels in any of the groups with control group.

Table 1: Comparison of changes in plasma of pituitary-thyroid hormone axis in examined groups based on the mean and standard deviation

<table>
<thead>
<tr>
<th>Variable groups</th>
<th>Thyroid stimulating hormone (DI)</th>
<th>Thyroxine hormone (DI)</th>
<th>Triiodothyronine hormone (DI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.16±0.09</td>
<td>3.02±1.1</td>
<td>1.03±0.25</td>
</tr>
<tr>
<td>Sham</td>
<td>0.17±0.09</td>
<td>3.38±0.79</td>
<td>0.83±0.15</td>
</tr>
<tr>
<td>Experimental 1  (mg/kg)</td>
<td>0.13±0.04</td>
<td>2.56±0.69</td>
<td>0.71±0.08</td>
</tr>
<tr>
<td>Experimental 2  (180 mg/kg)</td>
<td>0.18±0.05</td>
<td>2.06±0.47</td>
<td>0.63±0.11</td>
</tr>
<tr>
<td>Experimental 3  (270 mg/kg)</td>
<td>0.12±0.04</td>
<td>1.78±0.64</td>
<td>0.62±0.11</td>
</tr>
</tbody>
</table>

The asterisk sign *indicates significant difference with control and sham groups.
Table 2: Changes in rats weight to the harmala extract received on the first, tenth and fifteenth days based on mean and standard deviation

<table>
<thead>
<tr>
<th>Weight of fifteenth day</th>
<th>Weight of tenth day</th>
<th>Weight of first day</th>
<th>Concentration group</th>
</tr>
</thead>
<tbody>
<tr>
<td>315±47.46</td>
<td>300±49.752</td>
<td>294.4±47.879</td>
<td>Control</td>
</tr>
<tr>
<td>303.1±31.405</td>
<td>297.5±27.837</td>
<td>295.3±29.154</td>
<td>Sham</td>
</tr>
<tr>
<td>298.8±44.374</td>
<td>264.8±41.483</td>
<td>278.96±44.187</td>
<td>Experimental 1 (180 mg/kg)</td>
</tr>
<tr>
<td>284.7±36.174</td>
<td>282.2±32.117</td>
<td>273.2±31.371</td>
<td>Experimental 2 (180 mg/kg)</td>
</tr>
<tr>
<td>284.8±37.424</td>
<td>279.1±29.414</td>
<td>269.5±27.89</td>
<td>Experimental 3 (180 mg/kg)</td>
</tr>
</tbody>
</table>

Table 3: Changes in liver enzymes of the groups of receiving harmala extract.

<table>
<thead>
<tr>
<th>AST (mean±SD)</th>
<th>ALT (mean±SD)</th>
<th>Concentration group</th>
</tr>
</thead>
<tbody>
<tr>
<td>185.2±65.072</td>
<td>89±18.024</td>
<td>control</td>
</tr>
<tr>
<td>154.1±38.526</td>
<td>83.7±7.746</td>
<td>sham</td>
</tr>
<tr>
<td>153.4±27.399</td>
<td>85.50±11.266</td>
<td>Experimental 1</td>
</tr>
<tr>
<td>159.6±26.496</td>
<td>96.6±33.635</td>
<td>Experimental 2</td>
</tr>
<tr>
<td>202.9±69.589</td>
<td>93.6±14.206</td>
<td>Experimental 3</td>
</tr>
</tbody>
</table>

Discussion and conclusion:
Harmala seed, having alkaloid compounds such as harmala, harmaline, harmalole, and steroidal compounds of lygan and Saponins have many medicinal effects including regulation of endocrine functions [1]. Thyroid disorders, if not treated properly impose irreparable damage to patients and in medicine a variety of methods are used to treat thyroid disorders. The aim of this study was to investigate the effect of hydroalcoholic extract of harmala seeds on plasma levels of the pituitary–thyroid hormone axis. The results showed harmala seed extract has a lowering effect on hormone axis function of pituitary-thyroid. Secretion of thyroid stimulating hormone is stimulated by thyrotropin-releasing hormone of Para ventricular hypothalamus core. Several neurotransmitter structures control secreting neurons of thyrotropin-releasing hormone in hypothalamus. One of these structures of neurotransmitter, is neuropeptide Y [13]. Since the fibers in harmala seed extract through the increase of leptin secretion have an inhibitory effect on activation of secreting neurons of neuropeptide Y, by reducing the activity of neuropeptide-secreting neurons that have a stimulating effect on secretion of the thyrotropin-releasing hormone, the rate of this neuro-hormone and so secretion of thyroid stimulating hormone would decrease [14,15]. Regarding the stimulatory role of thyroid stimulating hormone on secretion of thyroid hormones of thyroxine and triiodothyronine, plasma levels of thyroid hormones would decrease. Several studies have shown that harmala is one of the alkaloids in the harmala seed extract that has inhibitory properties for Monoamine oxidase enzyme and with regard to the inhibition of this enzyme, catecholamine neurotransmitters levels (dopamine, norepinephrine, epinephrine) and Indole amine (serotonin) will increase. Further, since serotonin is known as an inhibitory neurotransmitter in the secretion of thyrotropin-releasing hormone, harmala seed extract, by increasing the rate of serotonin causes a decrease of secretion of thyrotropin-releasing hormone and subsequently thyroid stimulating hormone levels in plasma, so thyroxine and triiodothyronine would decrease [16]. Other studies have shown that dopamine, in the level of hypothalamus with decreased secretion of thyrotropin-releasing hormone, and in the level of pituitary directly prevent secretion of thyroid stimulating hormone, and causes decreased plasma levels of thyroid stimulating hormone, either free or bound to plasma proteins. Harmala seed extract, also with inhibition of the enzyme monoamine oxidase, causes increase in the rate of dopamine that can decrease plasma levels of pituitary-thyroid hormones axis [17]. Dopamine through receptors can prevent secretion of thyroid stimulating hormone from the anterior pituitary. Also, dopamine by stimulating somatostatin secretion can reduce thyroid stimulating hormone secretion, and so plasma levels of hormones thyroxine and triiodothyronine would decrease [18]. Based on the investigation, steroid compounds cause a decrease of thyroid hormones transporter proteins in serum [19]. Thus, despite the steroids in harmala seed extract decrease of thyroxine and triiodothyronine hormones through decrease of thyroid hormones transporter proteins is justified (20). According to the results in Table 2 it was determined that harmala does not apply significant changes to body weight. Based on research conducted by Sebastian et al it was determined that the combinations in harmala seed extract cause an increase in leptin in adult mammals and finally, by inhibiting the secretion of NPY causes reduction of food intake, which is consistent with this research study [15]. According to the results in Table 3, plasma concentration of enzymes ALT and AST in the control and experimental and sham groups did not show significant changes. Regarding the fact that in the hypothyroidism conditions through the damages that are incurred on the liver tissue, plasma levels of liver enzymes such as ALT and AST would increase and Harmala seed extract reduces plasma levels of thyroid hormones, so the lack of significant changes in plasma levels of these enzymes is justified [21]. According to the results of this study the effect of harmala seed extract on pituitary-thyroid axis is examined, it is recommended that the combined effect of harmala extract on this axis also be examined, as the findings indicate that the inhibitory effect of compounds in harmala seed extract effect the secretion of thyroid hormone, so its use in overactive thyroid is recommended.
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REFERENCES


