THE EFFECTS OF ACUTE OR CHRONIC IMMOBILIZATION STRESS AND ALOE VERA EXTRACT ON SERUM LEVELS OF TSH, T3 OR T4 IN MALE RATS

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ABSTRACT
Thyroid gland function is influenced by various types of stress and plant extracts. The aim of this study was to investigate the role of immobilization stress and Aloe vera extract on serum levels of TSH, T3 and T4 in male rats. Male Wistar rats were divided into control, normal saline receiving, Aloe vera extract (300mg/kg/body weight) receiving, acutely immobilized (8h/day for 8 days), chronically immobilized (2h/day for 21 days) and under acutely or chronically immobilized Aloe vera extract receiving groups of 10 each. Following serum collection, hormone levels were measured by electrochemiluminescence method. Data were compared statistically between groups using ANOVA. Serum level of TSH was not significantly altered in experimental groups compared to control rats. However, serum T3 and T4 levels were significantly increased in acutely immobilized rats compared with control rats (P<0.001, P<0.01 respectively); however, there was no significant difference in serum T3 and T4 levels between chronically immobilized rats and control animals. Serum T3 and T4 levels were significantly decreased in extract receiving and chronically immobilized extract receiving rats compared to control animals (P<0.05, P<0.001, P<0.05, respectively). Serum T3 level was significantly decreased in acutely immobilized extract receiving rats compared to control animals (P<0.001). Serum T3 and T4 levels were significantly lower in chronically immobilized extract receiving rats compared to chronically immobilized animals (P<0.01). Our findings indicate that acute immobilization stress has excitatory effects on thyroid function to enhance serum T3 and T4 level, however, Aloe vera extract administration can withstand against.

Keywords: Immobilization, Aloe vera, TSH, T3, T4, Rat

INTRODUCTION
Immobilization stress can influence many physiological aspects of organism. It is followed by alterations in normal body functions including hypothalamus-pituitary-endocrine axis. The effects of immobilization stress on thyroid function have been reported in past and recent studies (Armario et al., 1993; Bartanusz et al., 1993; Maccari and Morley-Fletcher, 2007). The thyroid gland is the most important endocrine gland that controls various functions in our body (Kar et al., 2002). Thyroid hormones, triiodothyronine (T3) and thyroxine (T4), have a major impact on many physiological functions (Kioukia et al., 2000; Watt and Panksepp, 2009). Thyroid hormones influence every organ, tissue and cell in the body; therefore, abnormal thyroid hormone levels bring about physiological abnormalities (Kar et al., 2002). Despite the prevalence of thyroid disorders worldwide, there are few studies on the effects of stress on thyroid gland function (Miyata and Ose, 2012) and there are also very few studies on the effects of stressors on the pathways of thyroid hormones metabolism and secretion and the changes occurring in target organs (Turakulov et al., 1994).

Although there are various plant extracts affecting on thyroid hormone levels in laboratory animals (Vogler and Ernst, 1999; Ajabnoor, 1990; Chithra et al., 1998), there are few reports on the application of plant extracts in thyroid disorders treatment (Kar et al., 2002). Aloe vera is one of the plants studied concerning its effects on thyroid gland function. Aloe Vera is a member of the Lily family that mainly
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grows in arid climates. Studies on Aloe Vera and Lily family plants show that the Aloe vera extract has various physiological functions including heart function improvement (Pigatto and Guzzi, 2005), joint pain relief, antidiabetic effect (Health, 1985), controlling of blood cholesterol, energy production, strengthening of immune system (Pittman, 1992), treatment of skin ulcers and wounds, asthma and digestive disorders (Aguilera et al., 1995; Pettersson et al., 1990). There are also studies indicating the effects of Aloe vera extract on thyroid gland functions (Kar et al., 2002; Moghaddam, 1993).

Since the various types of stresses, in particular immobilization stress can influence thyroid gland function and since Aloe vera extract has major impact on thyroid gland function, the present study was undertaken to determine antagonistic or synergistic effects of Aloe vera extract and immobilization stress on thyroid gland function in male rats.

MATERIALS AND METHODS

Animals

Adult albino (Wistar) rats weighting 200 -250g were purchased and raised in our colony from an original stock of Pasteur institute (Tehran, Iran). The temperature was at 20-25°C and animals kept under a schedule of 12h light: 12h darkness (light on at: 08: 00 a.m.) with free access to water and standard laboratory chow. Animals were kept under consistent conditions during during all the experimental period (Turakulov et al., 1992). This study was performed according to ethical guidelines relating to working with laboratory animals and all applicable institutional and/or national guidelines for the care and use of animals were followed. All rats were behaviourally suited to the research environment. There was not any observable evidence of diseases in animals during the period of experiment.

Extract Preparation

Aloe vera leaves were purchased from Hamedan Bu Ali Sind Medicinal Herb Garden. Aqueous extract of Aloe vera leaves was prepared according to previous studies with slight modifications (Aguilera et al., 1995). Briefly, fresh Aloe vera leaves weighing between 500-600 g with approximate length 60-80 cm were collected and the thick epidermis was removed. Fleshy mucilaginous pulp (parenchymatous tissues) was selectively scraped out, homogenized and centrifuged at 6000 g for 15 min to remove the fibers. The supernatant was lyophilized. A 20% extract solution was prepared by suspending the extract in sterilized distilled water and stored at 4°C.

Immobilizing Method

In the present study, an immobilization system that allows rats free intake of feed and water while restraining their movement was established. Animals assigned to stress groups underwent immobilization using restraining device. Rats were immobilized but not compressed, pinched, or screaming/screeching. Animals were unable to turn or barrel-roll. Studies show that repeated immobilization stress causes structural remodeling in areas of the brain responsible for emotional memories and regulation of the stress response (Miller and McEWEN, 2006).

Protocol of Study

Animals were divided into control, normal saline receiving, orally aqueous Aloe vera extract (300 mg/kg) receiving, acutely immobilized (8h/day for 8 days), chronically immobilized (2h/day for 3 weeks), acutely immobilized normal saline receiving, chronically immobilized normal saline receiving, acutely immobilized Aloe vera extract (300 mg/kg) receiving, and chronically immobilized Aloe vera extract (300 mg/kg) receiving groups of 10 rats in each. In all Aloe vera extract receiving groups, Aloe vera leaf extract administered orally by gavage.

Hormonal Assay

Blood samples were collected using cardiac puncture technique after anesthetizing animals by ether. Heparinized blood was centrifuged for 5 min at 3500 rpm. Following serum collection, T3, T4 and TSH levels were determined by electrochemiluminescence method using commercially available kits (Immunotech A Beckman Coulter/ Ref.2121).
Statistical Analysis
All results are presented as mean±SD. The significance of differences between groups was determined by analysis of variance (ANOVA) using SPSS 18. Games-Howel test was used for post-hoc comparisons. Differences were considered significant when p < 0.05.

RESULTS AND DISCUSSION

Results
Statistical analysis suggests that serum level of TSH was not significantly changed in experimental groups compared to control rats Serum T3 and T4 levels were significantly increased in acutely immobilized rats compared with control rats (P<0.001 and P<0.01, respectively); however, there was no significant difference in serum T3 and T4 levels between chronically immobilized rats and control animals.

Serum T3 and T4 levels were significantly decreased in extract receiving and chronically immobilized extract receiving rats compared to control animals (P<0.05, P<0.01 and P<0.05, respectively). Serum T3 level was significantly lower in chronically immobilized extract receiving rats compared to chronically immobilized extract receiving rats compared to chronically immobilized animals (P<0.01) (table 1).

<table>
<thead>
<tr>
<th>Animals</th>
<th>TSH UU/ml±SD</th>
<th>T3 UU/ml±SD</th>
<th>T4 UU/ml±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.098±0.01</td>
<td>73.2±0.58</td>
<td>3.65±0.20</td>
</tr>
<tr>
<td>AI</td>
<td>0.095±0.01</td>
<td>82.92±0.58</td>
<td>5.28±0.20</td>
</tr>
<tr>
<td>CI</td>
<td>0.089±0.03</td>
<td>73.26±0.40</td>
<td>3.67±0.13</td>
</tr>
<tr>
<td>Av</td>
<td>0.001±0.02</td>
<td>71.00±0.70</td>
<td>2.84±0.01</td>
</tr>
<tr>
<td>AI+Av</td>
<td>0.099±0.01</td>
<td>68.07±0.60</td>
<td>3.83±0.278</td>
</tr>
<tr>
<td>CI+Av</td>
<td>0.095±0.03</td>
<td>70.58±0.70</td>
<td>2.67±0.01</td>
</tr>
</tbody>
</table>

AI, CI, AV, AI+AV and CI+AV indicate acutely immobilized, chronically immobilized, Aloe vera extract receiving, acutely immobilized Aloe vera extract receiving and chronically immobilized Aloe vera extract receiving rats, respectively. Data represent the mean±SD of 10 rats. *, ** and *** indicate P<0.05, P<0.01 and P<0.001, respectively versus control.

Discussion
Our findings indicated that short term immobilization results in increased serum T3 and T4 levels, however, does not significantly influence serum TSH levels. In line with this finding, there are reports indicating that immobilization, as a stress condition, has a capability to alter serum level of various hormones (Moolchandani et al., 2008). It has also been shown that hypothalamic-pituitary axis is influenced by exerting of various types of stress (Aguilera et al., 1995; Pettersson et al., 1990). Stress of various types can enhance excitatory amino acids levels in brain (Moghaddam, 1993) leading to functionally activated endocrine glands including thyroid glands. Activation of the pituitary-thyroid axis was also found in experiments with rhesus monkeys enduring acute immobilization stress (Mason and Mougey, 1972). Enhanced serum levels of thyroid hormones have also been demonstrated in acutely immobilized animals (Helmreich and Tylee, 2011). There is also a report indicating that serum level of TSH is not significantly changed in animals enduring acute immobilization stress (Parra et al., 1980).

Most types of stress can influence variety of aminergic neurons in brain by which activate peptidergic pathways in hypothalamus to induce the secretion of TRH, which is followed by enhanced serum levels.
of T3 and T4 (Kioukia et al., 2000). It seems that the physiological effect of acute immobilization stress on nervous system is mediated by alpha-adrenergic system. In this respect, studies show that prazosin, an alpha 1-adrenergic receptor blocker, can neutralize the effects of immobilization stress (Takita et al., 1995). The other mechanism associated with increased serum T3 and T4 levels in acutely immobilized rats may come from enhanced serum thyroxine binding globulin (TBG) level (Cizza et al., 1995).

In our study long term immobilization did not significantly influence TSH, T3 and T4 level. In accordance with this finding there are studies indicating nonsignificant change in serum levels of TSH, T3 and T4 in chronically immobilized animals (Turakulov et al., 1994). However, in contrast to this finding, some reports have shown a decrease in serum level of thyroid hormones in chronically immobilized animals (Langer et al., 1983). Chronic immobilization also has capability to reduce TRH and serum TSH levels in mice (Cizza et al., 1996). Nonsignificant change in serum levels of TSH, T3 and T4 which occurred in our study may come from biologically adaptation mechanisms occurring in long term stresses (Turakulov et al., 1994).

On the other hand, our finding indicated that serum T3 and T4 level was significantly decreased in Aloe vera extract receiving rats. Similar decrease in serum T3 and T4 level was also occurred in several experiments with Aloe vera (Moghaddam, 1993). There are also other plants in Aloe family or other families that can influence serum levels of thyroid hormones (Panda and Kar, 1998). Because of simultaneous decrease in serum T3 and T4 level, it is conceivable that Aloe vera extract administration could not influence extrathyroid enzymatic conversion of T4 to T3.

In our study, serum T3 and T4 levels were also significantly increased in acutely immobilized rats but were not increased in acutely immobilized extract receiving animals, indicating the potentially protecting role of Aloe vera extract against the effects of acute immobilization stress on thyroid function. In addition to this finding, our study indicated that the serum T3 and T4 levels were lower in chronically immobilized extract receiving rats compared to chronically immobilized animals demonstrating the synergistic effects of extract Aloe vera extract administration and chronic immobilization stress on decreasing of serum T3 and T4 levels.

Conclusion

In conclusion, our findings indicate that acute immobilization stress has excitatory effects on thyroid function to enhance serum T3 and T4 level, however, Aloe vera extract administration can withstand against. On this ground, protective effect of Aloe vera on thyroid hyperactivity is conceivable.

Compliance and Ethics

Conflict of Interest: The authors declare that they have no conflict of interest.

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