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EXPERIMENTAL STUDY

Lawsonia inermis – An alternative treatment for hyperthyroidism?

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Abstract: *Aim:* The goal of our study was to determine the effects of *Lawsonia inermis (L. inermis)* in mice, in which hyperthyroidism had been caused by thyroid stimulant hormone (TSH).
Material and method: The first phase of the study aimed to detect the effects of *L. inermis* on the amount of ionized hydrogen (pH) in cells. For this aim, the effect of *L. inermis* on pH levels in the liver tissues of mice, in whom Escherichia coli (E. coli) had caused peritonitis, was examined. In the second phase of the study, the effect of *L. inermis* on the serum T4 levels in the 24th and 48th hour in mice, whose thyroid cells showed an increased activity by TSH was measured.
Results: In the first phase, in mice, in whom E.coli had caused peritonitis, the pH in the liver tissue of the group that had been given *L. inermis* was found to be significantly alkaline ($p<0.05$). In the second phase, in mice, in whom TSH had caused hyperthyroidism, it was noted that serum total T4 levels were significantly lower than in the group that had been given *L. inermis* in the 48th hour ($p<0.05$).
Conclusion: In our study, we detected that *L. inermis* significantly decreased serum total T4 levels in the 48th hour in mice in whom TSH had caused hyperthyroidism. These results suggest that *L. inermis* can be used as an alternative treatment for the Graves' disease *(Tab. 2, Fig. 1, Ref. 34)*. Text in PDF *www.elis.sk.*
Key words: Lawsonia inermis, hyperthyroidism.

L. inermis (henna leaf) is a popular natural dye to colour hands and hair in eastern cultures. *L. inermis* contains 2 OH 1.4 naphtoqinone's that has oxidant effects (1–3). It is also known for its benefits for medical treatment. In many studies, the antimicrobial, antitumoral and tuberculostatic effects of *L. inermis* were investigated (4–8). Graves' Disease is an autoimmune disease with hyperthyroidism. In this disease, thyroid stimulant immunoglobulin (TSI) attaches itself to thyroid stimulant hormone (TSH) and creates the thyroid hormone synthesis that is much more powerful than TSH. A need arises for more hydrogen ions (H+) for thyroid hormone synthesis in thyroid cells exposed to the disease in this way. The objective of this study was to determine whether *L. inermis*'s oxidant effects would decrease the thyroid hormone synthesis by decreasing the hydrogen ions in the hyperactive thyroid cells. According to our knowledge, this is the first study in English literature up to date.

Methods

H+ is important both for myeloperoxidase (MPO) and for thyroidperoxidase (TPO) enzymes. Oxidant molecules created by the neutrophils against microbial agents play a critical role in the host defense mechanism. The active phagocytes secrete the enzyme MPO and produce hydrogen peroxide. In the formation of thyroid enzymes, the enzyme TPO causes a reaction resembling that of the enzyme MPO.

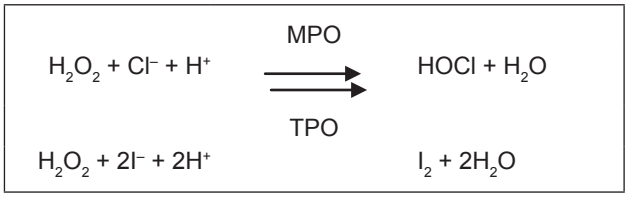

Fig. 1.The pathways of MPO and TPO enzymes.

Both MPO and TPO show the substrate and product similarities during the reactions (Fig. 1). TPO allows the formation of iodine (I) using H2O2 with iodide (I^-). Iodine is given to tyrosine residues in the form of iodine and thyroid hormone synthesis starts. For this reason, the ion concentration is very important in thyroid hormone synthesis. A need arises for more H+ for thyroid hormone synthesis in thyroid cells exposed to Grave's disease.

It is known that the best indicator of H+ concentration is pH measurement. The thyroid glands in mice are so small, it was thought that the pH measurement in this tissue would not be trustworthy, so the study was planned in two phases.

In the first phase of the study, in order to better understand the effects of *L. inermis* on TPO and H+ concentrate in the hyperactive thyroid cells, we wanted to make use of the MPO enzyme, which is very similar in terms of substrate, product, and type of function to TPO. Yao et al showed that in the case of infection, the enzyme MPO became active in the liver in the 24th hour (9, 10). We created peritonitis in mice by administering E. Coli intraperitonially to examine the effect of *L. inermis* on pH levels in the liver tissue

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in the 24th hour. In the second phase of the study, we investigated the effect of *L. inermis* on the serum total T4 hormone level in the 24th and 48th hours in mice, in which hyperthyroidism had been caused by administering TSH intraperitoneally.

Subjects

Sixty female Swiss albino mice (25–30 g) were used in the first phase and 60 mice were used in the second phase. In order to ensure a natural environment before the experiment, the mice were kept in seclusion for 5 days at the same room temperature with 12 hours each of light and darkness every day. Throughout the entire duration of the observation and experiment, all the mice received the same food and water. Animal ethics approval for the entire project was obtained from the Medical Experimental Research Center of the University of Cukurova Animal Ethics Committee.

Creation of L. inermis solution

The *L. inermis* solution (2 %) was made by mixing the freeze-dried form of this plant (henna powder) with distilled water. The solution was heated to boiling using a magnetic stirrer, and was allowed to cool to room temperature and the sediment was removed by straining (8).

Formation of E. coli peritonitis in first phase

The E. coli culture, which had been formed in a Luria Bertani medium at 37 °C, was washed twice with sterile saline before injection. 108 CFU of E. coli was injected intraperitoneally inside of 200 µl of sterile saline. Following the inoculation of E. coli, its viability was checked with an emergency blood agar plate (11–15).

Preparation of liver tissue samples and measurement of pH

Mice were euthanized by cervical dislocation in the 24th hour following E. coli inoculation. A midline laparotomy was performed and approximately 1g of tissue from the right liver lobe was placed in a tube containing 1.5 ml 0.02 M EDTA and homogenized in a container of ice (Ultra Turrax T25). The pH of the homogenized liver tissue was assessed with a pH meter (InoLab) after centrifuging at 4 ºC.

Work Plan

In the first phase, 60 mice were divided to 3 groups (n=20). In the 1st group, E.Coli was inoculatated intraperitoneally and 400 mg/kg/day of *L. inermis* orally by gavage was given in the 1st, 11th, and 22nd hours. In the 2nd group, E.Coli was inoculatated intraperitoneally and SF was given orally by gavage in the 1st, 11th and 22nd hours. In the 3rd group (control group), after SF administration intraperitoneally, SF was given orally by gavage in the 1st, 11th and 22nd hours. The pH was measured in 24th hour in liver for all groups.

In the second phase, 60 mice was divided to 6 groups (n=10). In the 1st group, TSH was given intraperitoneally and SF was given orally by gavage in the 1st, 11th and 22nd hours. In the 2nd group, TSH was given intraperitoneally and 400 mg/kg/day of *L. inermis* orally by gavage in the 1st, 11th and 22nd hours. The T4 levels of both these groups were measured in the 24th hour. In the 3rd group, TSH was given intraperitoneally and SF was given orally by gavage in the 1st, 11th and 22nd hours for two days. In the 4th group, TSH was given intraperitoneally and 400 mg/kg/day of *L. inermis* was given orally by gavage in the 1st, 11th and 22nd hours for two days. In the 5th group after SF administration intraperitoneally, SF was given orally by gavage in the 1st, 11th and 22nd hours for two days. In the 6th group after SF administration intraperitoneally, 400 mg/kg/day of *L. inermis* was given orally by gavage in the 1st, 11th and 22nd hours for two days. The T4 levels of groups 3, 4, 5, 6 were measured in the 48th hour.

The creation of hyperthyroidism

We caused hyperthyroidism in mice by administering TSH intraperitoneally using the method Chiu et al had described (16–18). To the groups 1 and 2, a single dose of 0.3 units (in 150 µl of sterile saline per dose), to the groups 3 and 4, two doses of bovine TSH 24 hours apart were administered intraperitoneally. The groups 5 and 6, which were not given TSH, were administered 150 µl of sterile saline in order to produce the same stress. The bovine TSH was purchased from Sigma (Germany).

Serum total T4 level measurement

After TSH application, following a chest wall incision under anesthesia **(**Xlazine 10 mg/kg + Ketamine 200 mg/kg), 1 cc of blood was obtained from the right atrium of each mice at 24 hours in the groups 1 and 2 and at 48 hours in the groups 3,4,5 and 6. Serum samples were obtained by centrifuging blood samples for 10 minutes at 5000 rpm. Serum total T4 hormone levels were detected by microelisa at 450 nm. The T4 kit was purchased from Alpha Diagnostics (adjusted test for mouse-serum T4 level measurement, Cat. No. 1100, USA). Results were determined as ug/dl by the standard curve, which was calculated from the samples.

Statistical analysis

Statistical analyses were performed using the statistical package SPSS v 12.0. For each continuous variable, normality was checked. The Student t-test was used for normally distributed data (pH) and the Mann–Whitney U test was used for non-normally distributed data (T4). p value <0.05 was considered as statistically significant.

Results

Phase 1 results

The liver tissue pH level of the mice in the group 1 were statistically significantly higher than in the group 2 ($p<0.05$). The pH levels of all groups in the Phase I study are shown in Table 1.

Phase 2 results

In the Phase 2, the mean serum total T4 level in the 24th hour in the group 1 was 7.91 ug/dL, while the T4 level in the group 2 was found to be 7.67 ug/dL ($p>0.05$). In the group 3, the mean serum total T4 level in the 48th hour was found to be 6.39 ug/dL, while it was found to be 5.00 ug/dL in the group 4($p<0.05$). Group

Tab. 1. pH values of liver tissue samples.

	Mean pH± SD	Median pH (Min–Max)
Group-1 Inoculated E.Coli and L.inermis	6.58±0.10*	6.59 (6.45–6.77)
Group-2 Inoculated E.Coli and water	6.53±0.11	6.53 (6.29–6.71)
Group-3 Control	6.52±0.08	6.52 (6.38–6.62)

Tab. 2. Serum total T4 levels (ug/dL) in mice with hyperthyroidism after getting L. Inermis or placebo.

Groups	24 hr T4 level	48 hr T4 level	p value
1 IP TSH + Water	7.91±1.93		=0.570
2 IP TSH + L. inermis	7.67±1.67		
3 IP TSH + Water		6.39±1.04	=0.041
4 IP TSH + L. inermis		5.00±1.59	
5 IP Saline + Water		4.03±0.85	=0.820
6 IP Saline + L. inermis		3.92±0.49	

IP – intraperitoneal

5 had a mean T4 level of 4.03 ug/dL, while the group 6 had T4 level of 3.92 ug/dL (p>0.05) (Tab. 2).

Discussion

Diseases associated with hyperthyroidism can occur with different mechanisms and its treatment is performed with different methods that are conservative and/or surgical. Hyperthyroidism model created in our study is an adaptation of the model performed by Chiu et al on TSH receptors (18). In the model of this study, the TSH administered intraperitoneally increases the production of the T4 hormone by connecting to the TSH receptors in the thyroid follicle cells.

It was reported by various researchers that *L. inermis* created serious hemolysis with the oxidant properties in children who suffer deficiency of the enzyme G6PD in the order of usages. G6PD is one of the basic sources that provide H+ to the cell with the help of NADPH+H+. It was shown that a minor oxidant effect related to *L. inermis* in a child who is G6PD deficient could lead to a serious illness.

In our study, we measured the effect of *L. inermis* on serum T4 levels in mice, in whom hyperthyroidism had been caused. This might be related to the oxidant effect of L. inermis. For this aim we wanted to measure pH in mice thyroid gland. However, the pH measurement might not be healthy due to the small size of the mice thyroid gland. So we benefited from the liver MPO enzyme, which resembles in both structure and function the thyroid TPO enzyme used in making thyroid hormones. Yao et al showed an increased MPO enzyme activation in liver tissue in the 24th hour in mice that had peritonitis (9, 10). In our study, in mice in whom peritonitis had been induced, the pH values in the liver tissues of mice in the group that had been given *L. inermis* shifted significantly to the alkaline side (p<0.05).

In the group of mice with peritonitis, which had not been given *L. inermis*, the pH was not different from that of normal mice in whom peritonitis had not been induced. These results showed that H+ levels had decreased with the oxidant effect of the *L. İnermis*. Austin et al showed that the pH (19) difference in the tissue is related to the changes in the intracellular pH. In this experimental study, we showed that oxidant effect of the *L. inermis* increased pH by decreasing the H ions in hypermetabolic cells.

The use of H+ increases in the hyperthyroid tissue that the TPO enzyme activated. In the second phase of our study, in the mice in which hyperthyroidism had been induced with the TSH stimulation, a significant decrease was noted in the serum T4 level in the 48th hour by *L.inermis*. These data, when they were evaluated with the first phase of our study, suggested that the oxidant effect of *L. inermis* decreased the production of T4 in the thyroid cells stimulated with TSH by connecting the hydrogen ions in.

In previous studies, it was shown that intracellular H_2O_2 increased as a response to TSH (20–23) and the increase in intracellular level of H_2O_2 (24–27) induce apoptosis through oxidative stress in the cell. The reduction of H_2O_2 in the cell under stimulation of TSH due to the H+ deficiency of both its use and its destruction, the increasing H_2O_2 concentration's killing of the diseased cell via apoptosis may be another mechanism related to the *L. inermis*'s oxidant effect.

There is still no definite information about substrate input and formation of the product during activation of TPO (28–33) in thyroid hormone synthesis. One of the intermediate molecules in the thyroid hormone synthesis is the hypoiodous acid (HOI) (34), which shows an effect like the hypochlorous acid (HOCl). Iodine, an electronegative ion found in the 7A group of the periodic table, and which is missing an electron in its outer orbital, tries to become stable by taking on an electron. We can say that with this feature it will stabilize itself in the form of HOI when it cannot create iodine from iodide in thyroid hormone synthesis. By this way, T4 synthesis might be decreased.

In Grave's disease, TSI stimulate the increase in the H_2O_2 level and oxidative stress much more than TSH. With *L. inermis* decreases the reduction of H_2O_2 and by this way the increase of H_2O_2 may cause the cell to start apoptosis. We think that the cells under TSI stimulation might be apoptosized by *L. inermis.*

Conclusion

L. inermis may be effective as an alternative treatment of hyperthrodisim and may be a therapeutic approach in Grave's disease by oxidant effects. Further studies are needed at the molecular level.

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