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Original Research Article

Effect of Ecklonia kurome extract on thyroid hormone disorder in rats

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Abstract

Purpose: *To investigate the effect of Ecklonia kurome extract (EKE) on thyroid hormone concentrations in male mice.*
Methods: *Mice were administered EKE by gastric intubation for 15 days. PTU or L-T4 was set as positive control. Twenty-four hours after the last administration, all animals were sacrificed by cervical dislocation. Blood was collected and serum samples assayed for T3 and T4. Furthermore, the liver was removed for biochemical analysis.*
Results: *T3 and T4 serum levels in mice decreased after the administration of EKE. The relative potency of EKE was calculated in terms of percent increase or decrease in thyroid hormones. Compared with the control value, the decrease in T3 concentration by a high dose of EKE was approximately 64.32 %. Compared with the control group, hepatic LPO decreased ($p < 0.01$) while superoxide dismutase (SOD, $p < 0.01$) and catalase (CAT, $p < 0.01$) activities were significantly increased by the high dose of EKE, thus indicating its anti-peroxidative role.*
Conclusion: *This suggests that EKE may be useful for the treatment of hyperthyroidism, but further studies are required to ascertain this.*

Keywords: *Ecklonia kurome, Thyroid hormone, Anti-peroxidative, Hyperthyroidism*

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INTRODUCTION

Two thyroid hormones (thyroxine [T4] and triiodothyronine [T3]) are involved in the regulation of a myriad of body functions including lipid and carbohydrate metabolism, oxygen consumption, nerve conduction and reproduction [1]. Alterations in their normal levels lead to physiological/clinical abnormalities such as hypothyroidism and hyperthyroidism.

Despite the fact that day-by-day herbal drugs are gaining much importance due to its affordable and safe nature, scientific investigations towards the mitigation of thyroid disorders *via* plant extracts are meager [2-4]. Therefore, in our endeavor to determine a plant extract that can regulate the levels of both thyroid hormones, we have investigated the potential activities of *Ecklonia kurome* on thyroid functions. Traditional Chinese medicine has many advantages in treating metabolic disease. *Ecklonia kurome* is a common herb usually used for treating thyroid disease.

In this study, the effect of EKE on the efficacy of thyroid function was investigated. Hepatic lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT) activities were also studied.

EXPERIMENTAL

Plant material and extraction

Ecklonia kurome samples were collected from Qingdao City, Shandong Province, China in May 2016. Taxonomic identification of the plant was performed by Professor Liang Lu of Henan University of Traditional Chinese Medicine in China. A voucher specimen (no. EKE 20160503) was deposited in the herbarium of the College of Pharmacy, Henan University of Traditional Chinese Medicine, China for future reference.

The whole *Ecklonia kurome* plant was dried in a drying oven at 100 °C for 12 hours. The *Ecklonia kurome* aqueous extract was obtained by steeping the dried *Ecklonia kurome* in water at 60 °C three times, each for one hour before first drying in an oven and freeze-drying the last extract. One gram of powder was obtained from approximately 1.6 g of dried sample; that is, a yield of 62.5 %.

Animals and groups

Colony-bred healthy Swiss albino male mice (30 ± 2 g) were purchased from the Experimental Animal Center of Henan Province (Certificate no. SYXK 2006-02), maintained at 27 ± 1°C with a light/dark schedule (14 hours light/10 hours dark), and provided with food and water *ad libitum*. These mice were randomly assigned to six groups: normal control group, propylthiouracil (PTU, 3 mg/kg) group, L-tyroxine (L-T4, 0.5 mg/kg) group, and various concentrations of *Ecklonia kurome* extract (EKE; 80, 160 and 320 mg/kg) group.

The animals were administered EKE, PTU, or L-T4 by gastric intubation. All the treatments were given twice daily and continued for 15 days. The experiment was approved by the Animal Care and Use Committee of Henan University of Science and Technology (Approval ref. no. 20101005) and was carried out in compliance with Directive 2010/63/EU on the handling of animals used for scientific purposes [5].

Sample preparation

On the last day of the experiment, mice were sacrificed by cervical dislocation. Blood was collected and serum samples were stored at -80 °C for one day until assayed for T3 and T4. The liver was quickly removed and washed thoroughly with phosphate-buffered saline (PBS, pH 7.4) for biochemical index analysis.

Biochemical assays

LPO was estimated by TBA reaction with malondialdehyde (MDA), a product formed due to the peroxidation of membrane lipids, according to the method performed by Ohkawa *et al* [6], and as modified by Jamall and Smith [7]. LPO was expressed as nM of MDA formed as per h/mg of protein. Hepatic SOD activity was assayed according to the method reported by Marklund and Marklund [8]. Enzyme activity was expressed as units/mg of protein, and one unit of enzyme is defined as the activity that inhibits the autoxidation of pyrogallol by 50 %. CAT activity was estimated following the method reported by Aebi [9].

Determination of thyroid hormones

Serum concentrations of total T3 and T4 were estimated by radioimmunoassay, as per routine protocol. Lower limits of sensitivity for T3 and T4 were 0.07 and 0.12 ng/mL, respectively. Inter-assay variation was <5 % for both hormones.

Statistical analysis

All the data were analyzed using Statistical Package SPSS 16.0 (SPSS Inc., Illinois, Chicago, USA) and were expressed as mean ± standard error of the mean (SEM). These data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test. A *p*-value < 0.05 was considered statistically significant.

RESULTS

Effect of EKE on serum concentrations of T3 and T4

Compared with the control value, serum T3 and T4 concentrations in mice significantly decreased when treated by middle and high doses of EKE ($p < 0.05$), while serum T3 and T4 concentrations of mice significantly decreased when treated by middle and high doses of EKE ($p < 0.01$). Thyroid hormone concentrations also decreased in PTU and increased in T4 treated groups (p </0.01, for all groups).

When relative potencies of different doses of EKE with respect to control values were calculated, the maximum inhibition in T3 concentration was observed by a high dose of EKE (64.32 %), followed by a middle dose of EKE (32.43 %); while T4 was inhibited by 32.67 % by a high dose of EKE. Middle doses of EKE increased T4 concentrations by 15.41 % (Table 1).

Table 1: Effect of *Ecklonia kurome* extract on serum concentrations of T3 and T4

Group	Dose (mg/kg)	T3 concentration (nmol/l)	T4 concentration (nmol/l)	Change in T3 concentration (%)	Change in T4 concentration (%)
Control	-	1.85 ± 0.14	42.12 ± 2.5	-	-
EKE-L	80	1.63 ± 0.21	40.27 ± 3.3	-11.89	-4.39
EKE-M	160	1.21 ± 0.15*	35.63 ± 1.5*	-32.43	-15.41
EKE-H	320	0.66 ± 0.12**	28.36 ± 1.1**	-64.32	-32.67
PTU	3	0.61 ± 0.07**	16.34 ± 0.89**	-67.03	-61.21
T_4	0.5	2.67 ± 0.15**	104.59 ± 7.38	+44.32	+148.31

EKE-L: low dose of EKE; EKE-M: middle dose of EKE; EKE-H: high dose of EKE. $^{**}P < 0.01$, $^{*}p < 0.05$, compared to the respective controls

Table 2: Effect of *Ecklonia kurome* extract on hepatic LPO, CAT and SOD activities in male mice

Group	Dose (mg/kg)	LPO (nM /mg protein)	SOD (unit/mg protein)	CAT (mmol /mg protein)
Control	-	0.93 ± 0.07	4.88 ± 0.18	39.26 ± 2.35
EKE-L	80	0.84 ± 0.05	5.12 ± 0.23	41.25 ± 2.12
EKE-M	160	0.71 ± 0.06*	6.36 ± 0.21*	45.18 ± 1.95*
EKE-H	320	0.52 ± 0.04**	8.34 ± 0.17**	57.24 ± 1.84**
PTU	3	0.97 ± 0.09	4.92 ± 0.19	25.33 ± 3.62**
T_4	0.5	2.37 ± 0.19**	4.53 ± 0.17	34.12 ± 3.27

EKE-L: low dose of EKE; EKE-M: middle dose of EKE; EKE-H: high dose of EKE. $^{**}P < 0.01$, $^{*}p < 0.05$, compared to the respective control values

Effect of EKE on hepatic LPO, CAT and SOD activities in male mice

The hepatic LPO level of mice decreased when treated by high (*P*</0.01) and middle (*P*</0.05) doses of EKE. On the other hand, SOD and CAT activities were significantly enhanced in high dose EKE (*P*<0.01) and middle dose EKE (*P*<0.05, for both) treated animals. In PTU treated animals, CAT activity was inhibited (*P*<0.01, Table 2).

DISCUSSION

These results reveal that serum levels of both T3and T4 were inhibited by EKE. When the relative potency of EKE was calculated in terms of percent increase or decrease in thyroid hormones, as compared to the control value, the decrease in T3 concentration through high doses of EKE was approximately 64.32 %. This indicates its possible use in the regulation of hyperthyroidism.

The predominant thyroidal hormone in circulation is T4, while T3 is mostly generated from T4 in peripheral tissues by outer ring mono-deiodination [10,11]. Since only T4, and not T3, was enhanced by EKE, it appears that EKE might be stimulating the synthesis and/or release of T4 directly at the glandular level, and not through the peripheral conversion of T4 to T3. The former one appeared to have a better potency, as it could inhibit T3 by approximately 64.32 %; which is comparable to that of a standard drug, PTU. Although EKE decreased both T3 and T4 concentrations, the inhibition was only approximately 64 and 32 %, respectively. This indicate that EKE may not be very effective in the reduction of thyroid hormone concentrations. However, EKE may be a better choice for *thyroid disease*, as it does not exhibit any hepatotoxic effect, and is rather hepatoprotective in nature.

It was reported that LPO occurs in biological membranes with potential injurious consequences [12,13]. Anti-oxidative enzymes (SOD and CAT) usually help maintain cellular integrity by protecting it against the deleterious effects of lipid peroxides [14]. Interestingly no hepatotoxic effects were observed in EKE. The decrease in hepatic LPO with a concomitant increase in the activities of antioxidative enzymes (SOD and CAT) by EKE suggest its antiperoxidative nature.

CONCLUSION

EKE may augment thyroid function, and can potentially be developed as a therapeutic agent for the treatment of hyperthyroidism in the future.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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