MECHANISTIC ROLE OF VARUNA (CRATAEVA NURVALA) EXTRACT ON THYROID GLAND AND ITS HISTOLOGY THROUGH IODOTHYRONINE DEIODINASES

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Received: 11 May 2018, Revised and Accepted: 20 June 2018

ABSTRACT

Objective: Crataeva nurvala (CN) is used for its therapeutic effects, but its effect on the thyroid gland in euthyroid conditions and mechanism behind its thyrotropic activity in hypothyroidism is still not explored. This study screened the pharmacological effect of the ethanolic extract of the bark of CN on thyroid hormones, free and total thyroxine (FT4 and T4), triiodothyronine (T3), thyroid-stimulating hormone (TSH) levels, and thyroid histology in normal Swiss albino female mice.

Methods: Eighteen animals of 28–33 g were segregated into three groups: Group I treated with vehicle (NOR+VEH), Group II administered CN 400 mg/kg (NOR+CN 400), and Group III given CN 600 mg/kg (NOR+CN 600) for 15 days, per os (p.o.). The variation in the T4, FT4, T3, and TSH levels recorded using ELISA, 24 h after the last dose, and T4/T3 ratio thus calculated along with the histopathological studies of the thyroid gland.

Results: The findings were presented as mean ± standard error of the mean, using one-way ANOVA, followed by Dunnett’s post-tests to compare all columns with the control. NOR+CN 600 has shown thyroid protective effect through retaining euthyroid profile, normal T4/T3 ratio, and near-normal histology. However, NOR+CN 400 had shown the significant decline in T4/T3 ratio and pathological changes in thyroid histology, in comparison with the control and NOR+VEH group.

Conclusion: The higher dose of CN was found to sustain the euthyroid levels through retention of iodothyronine deiodinases activity, facilitating the peripheral conversion of T4 to T3, and in retaining normal histoarchitecture of the thyroid gland in contrary to a lower dose.

Keywords: Varuna, Thyroxine, Triiodothyronine, Iodothyronine deiodinase, Euthyroid.

INTRODUCTION

Crataeva nurvala (CN) commonly known as Varuna is reported to possess various pharmacological activities such as analgesic, antiarthritic, antiinflammatory, antimycotic, antioxidant, antiurolithiatic, anticancer, antidiabetic, antidiarrheal, antifertility, anti-hemolytic, anti-snake venom, antianthelmintic, antiarthritic, antibacterial, anticancer, antidiabetic, antiinflammatory, antimycotic, antioxidant, antiurolithiatic, cardioprotective, hepatoprotective, neuroprotective, and antimalarial activities. It is also found to be effective in treating urinary tract infections as evident from various in vivo–in vitro studies in disease conditions [1-6].

Furthermore, Varuna is a part of various polyherbal Ayurvedic, Siddha, and commercially manufactured formulations, used for certain pharmacological actions such as Asmarihara kasaya (antihypertriglycerideremia and hepatoprotective), Pashanabhedadi Ghrita (anti-inflammatory, antiinflammatory, anti-inflammatory), Himplasia (Himalaya Herbal Healthcare, Bengaluru) used for Benign Prostatic Hyperplasia, and Neeri (Amil Pharmaceuticals India Ltd., New Delhi) as nephroprotective [7-11].

In a recent study, the bark extract of CN (CN 600 mg/kg) had shown to possess significant thyroid stimulant activity when compared with the standard therapy i.e. levothyroxine in propylthiouracil (PTU) induced hypothyroidism. It showed significant reduction in cholesterol levels and improved thyroid hormone levels, proving its beneficial role in the treatment of hypothyroidism and associated hypercholesterolemia. However, the lower dose (CN 400 mg/kg), despite raising T4 levels, in an erratic manner, raised thyroid stimulating hormone (TSH) also, for which mechanism was not clear [12]. Moreover, its effect in the euthyroid state, when used for other ailments in the form of polyherbal preparations or certain extracts, is still unexplored. For estimating the mechanism, an additional diagnosis like T4 is also required, which was not estimated in previous studies, needed to be estimated.

Hence, this study was framed to evaluate the per se effect of CN on thyroid hormone levels, thyroid histology, and its mechanism through estimating thyroid hormones, i.e. T4 (total and free) and T3 levels, T4/T3 ratio, and histopathological studies in the normal healthy female mice.

METHODS

Animals
Swiss Albino healthy female mice, having age around 3–5 months and body weight 28–33 g were purchased from “Panacea Biotec Ltd., LaRú (140501), India.” Animals were kept in cages made of polypropylene, under specified temperature conditions such as 25±2°C and relative humidity 30–70% with the maintenance of 12-h night and 12-h day cycle. Animals were nourished with standard pellets of food purchased from “Shree Jagdambey Feed Industries” situated in Moga (Punjab), and potable water was supplied on a free basis. The prior approval for the conduct of the study was taken from the Institutional Animal Ethics Committee (IAEC) under Protocol no.: IAEC-CTIPS/2015/VII/0042 (PCL-M) as per the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPSEA), New Delhi.

Procurement of plant material: CN
The CN bark (3.5 kg) was purchased from Herbal Health Research Consortium Pvt., Ltd. (HHRC), Amritsar, from Lot No. VRN-024 along with Certificate of Analysis (COA) whose A. R. No. was 06/2015/
Fig 3. 4. 5. 6a), whereas the thyroid gland T .S. of (NOR+CN 400) (**p<0.01) and significantly NOR+CN 600 (**p<0.01) as compared to NOR+VEH (Fig. 3).

T₃/T₄ ratio
The calculated T₃/T₄ ratio was found to be significantly less in NOR+CN 400 (**p<0.01), with respect to the normal control, whereas this ratio has been found to be near normalcy in case of NOR+CN 600 (Fig. 4).

Effect on TSH
Administration of CN for 15 days in normal healthy female mice had not significantly altered the TSH levels in any of the test group with respect to normal control, thus depicting the subclinical changes at T₃ levels (Fig. 5).

Histology of thyroid gland
The transverse section (TS) of thyroid gland of the normal group (NOR+VEH) showed the appearance of normal structural features such as follicular cells embedded in cuboidal epithelium (F), colloid appearance in follicles with slight variation in size (co), parafollicular cells or C-cells clustered in between the follicles (pf), and fenestrated capillaries (co) with visible appearance of interlobular connective tissue (Ich) (Fig. 6a), whereas the thyroid gland in NOR+CN 600 appeared to have reduced follicular size (fr) with undistinguished columnar epithelium (ue), the presence of C-cells (pf), few capillaries (co), and large vacuole spaces (vo) (Fig. 6b). The thyroid gland TS of group NOR+CN 600 depicted the bunch of follicles of variable size (fr) with cuboidal epithelium and abundant follicular and cluster of C-cells (pf) with the presence of colloid in different intensity (co) and blood capillaries (co) (Fig. 6c).

DISCUSSION
Thyroxine (T₄) is the principle prohormone secreted from the thyroid gland. T₃ thus produced is metabolically converted into its biologically active form T₂ through the process of outer-ring monodeiodination by thiorodoxin fold-containing selenoenzymes, known as iodothyronine deiodinases in cytoplasm and nucleus of target/extrathyroidal tissues mainly liver, kidneys, etc. T₃ is secreted in small amount by the thyroid gland (13%) and the majority is formed in peripheral tissues through Type I 5'idothyronine monodeiodinase (5'DI) by peripheral monodeiodination to carry out pro-metabolic, pro-enzymatic, and lipolytic effects [19,20]. Suppression in levels of both T₃ and T₂ is seen in conditions of hyperthyroidism or due to the effect of certain goitrogens like bamboo shoots as a food entity or bark of Ficus racemosa Linn. [21,22]. Any change in 5'DI, i.e., inhibition is reflected by a decrease in T₃ concentration and T₄/T₃ ratio, despite the increase in T₄ concentration [23].
In this study, administration of the ethanolic extract of CN to healthy mice for 15 days significantly increased the levels of $T_4$ (**$p<0.01$) and FT$_4$ (*$p<0.05$) in NOR+CN 400, with a significant reduction in the T$_3$ levels (**$p<0.001$) (Figs. 1-3). Whereas, in NOR+CN 600, i.e. at the higher dose, the insignificant change was observed in thyroxine levels with significant (**$p<0.01$) decrease in T$_3$ levels as compared to normal control i.e. NOR+VEH (Figs. 1-3). However, no significant change was observed in TSH levels (Fig. 4). Hence, this extract might have some inhibitory effect on 5’DI, thus affecting the peripheral deiodination, but this 5’DI inhibition is more marked with the lower dose as compared to the higher dose as T$_3$/T$_4$ ratio in NOR+CN 400 has significantly (**$p<0.01$) decreased as compared with NOR+CN 600 that retained the levels compared to normal group.

These results are in line with the study by Panda and Kar, 1999, done on root extract of *Withania somnifera*, given at the dose 1.4 g/kg to female mice for about 20 days along with the additional parameter observed, i.e., hepatic glucose-6-phosphatase [24]. Tahiliani and Kar 1999 reported similar findings in their study on *Moringa oleifera* leaf extract, which showed approximately 30% reduction in T$_3$ despite marked increased in T$_4$ levels with antiperoxidative effects and is suggested to be used in hyperthyroidism conditions [25].
The thyroid gland comprises of acini or follicles that are spherical bodies that selectively absorb iodine in the form of iodide ions, I\(^{-}\), from the blood circulation for the production of the thyroid hormones, and also for its adequate storage in thyroglobulin (Tg). 25% of the body’s I\(^{-}\) ions are in the thyroid gland. Follicles contain a region called the follicular lumen, containing colloid comprising of a protein, Tg that serves as the reservoir of materials for the thyroid hormone production and, to a lesser extent, also acts as a reservoir for already synthesized thyroid hormones. The follicles are lined by multiple cuboidal cells, whose size varies depending on age and locality [31,32]. The size of the follicles and the follicular lumen is found to be reduced in NOR+CN 400 group, thus indicating the depletion of colloid accompanied by vacuole formation, whereas normal follicular size with no vacuolization was seen in NOR+CN 600 (Fig. 6a and b). “C cells” or parafollicular cells which secrete calcitonin were found in abundance, scattered within the follicular cells and in the spaces between the spherical follicles in NOR+VEH. As the acini size has decreased in NOR+CN 400, the acinar epithelium appears more cuboidal as compared to NOR+VEH and NOR+CN 600, in which with the increased size of follicles and the epithelial lining becomes flatter or low cuboidal.

As per the findings of this study, CN 400 mg/kg was found to be beneficial to be used in hyperthyroidism, as evident from raised T\(_3\) and reduced T\(_4\)/T\(_3\) ratio, whereas CN 600 mg/kg was able to maintain euthyroid state in per se or hypothyroid mice, compared to the normal group. For future studies, CN extract must be studied extensively for its effect on peripheral organs also such as determination of a additional parameter for thyroid function and glucose-6-phosphatase (G-6-Pase) activity in liver tissues as carbohydrate metabolism is also influenced by thyroid hormones and moreover the anti-peroxidative effects in relation to thyroid disorders [33]. However, before human therapy, further investigations are required such as the direct measurement of 5'DI using specific radioimmunoassay for more confirmation.

CONCLUSION

The ethanolic extract of CN is thyrotropic, stimulatory at glandular level but possesses the 5'DIs inhibitory activity in a dose-specific manner. Lower dose, i.e. CN 400 mg/kg is suitable to be used in hyperthyroidism, whereas higher dose, i.e. CN 600 mg/kg is found to be effective in hypothyroidism, in maintaining euthyroid levels and in retaining normal histoarchitecture of the thyroid gland as evident from preclinical studies.
ACKNOWLEDGMENTS

This project was a part of M. Pharmacy (Pharmacology) research protocol. The support and guidance of all teaching, non-teaching, and technical staff of the Department of Pharmacology, CT Institute of Pharmaceutical Sciences, School of Pharmaceutical Sciences, Lovely Professional University, for providing the basic facilities, resources, and in the completion of the study and communication of research outcomes is highly appreciable.

AUTHOR’S CONTRIBUTIONS

All the authors have significantly contributed to the concept, design, definition of intellectual content, literature research, the conduct of the study, manuscript editing, preparation, and review.

CONFLICTS OF INTEREST STATEMENT

The authors mentioned in this paper do not have any personal or financial relationship with any other person or organization that can influence the content of the paper.

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