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

ARSHVIR KAUR1, SANTOSH KUMAR VERMA1,2
1Department of Pharmacology, CT Institute of Pharmaceutical Sciences, Shahpur, Jalandhar, Punjab, India. 2Department of Pharmacology, Faculty of Pharmaceutical Sciences, Motherhood University, Roorkee, Haridwar, Uttarakhand, India. Email: archie.dhwal@gmail.com

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 (FT3 and FT4), 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 T3, FT3, T4, and TSH levels was recorded using ELISA, 24 h after the last dose, and T3/T4 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 T3/T4 ratio, and near-normal histology. However, NOR+CN 400 had shown the significant decline in T3/T4 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, antibacterial, anticancer, antidiabetic, anti diarrheal, antifertility, anti-hemolytic, anti-snake venom, anti-inflammatory, antimitotic, antioxidant, antilithiatic, cardioprotective, hepatoprotective, nephroprotective, neuroprotective, and antioxidant, antiurolithiatic, cardioprotective, anthelmintic, antiarthritic, antibacterial, anticancer, antidiabetic, antipyretic (anti-inflammatory) and hepatoprotective. 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 kasya (anti-hypertriglyceridaemia and hepatoprotective), Pashanabhedadi Ghrita (antinephrolithiatic and antioxidant), Vedikara silasathu parpam and Nerunjil kudineer (anti-inflammatory), Himplasia (Himalaya Herbal Healthcare, Bengaluru) used for Benign Prostatic Hyperplasia, and Neeri (Amin 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 stimulating 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 T3 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 T3 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. T3 (total and free) and T4 levels, T3/T4 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., Lahru (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 (CPSECA), 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/
Preparation of extract
The CN bark extract was prepared through triple maceration strategy [13], 3.5 kg of CN bark was dried in shade and rendered free of dust. The size reduction was achieved, first by manual crushing, thereafter undergoing an electrical grinding (sieve size #16). Ethanol (1 kg in 3 L) was used as a solvent to macerate the coarse powder at room temperature with periodic shaking. The straining was done by layer muslin fabric in 2 folds, and the marc pressing was done to extract the solvent. The individual filtrates were obtained, combined, and filtered through Whatman No. 1 paper. This procedure was rehearsed every 3rd day to achieve triple maceration. All the filtrates were stored in light-resistant bottles. The recovery of the solvent was done under vacuum at 37°C and allowed to concentrate to get dark semisolid mass. The yield obtained was 1.37%.

Chosen amount of extract, i.e. 400 mg/kg and 600 mg/kg was used, based on available literature, scientific evidence for its neurological, hepatic and renal safety, therapeutic efficacy, and thyrotropic activity in PTU-induced hypothyroidism to evaluate its mechanism [12]. For administration, suspension using Gum Acacia [1%] and preserved at 2-8°C in light-resistant bottle [14-17].

Preliminary phytochemical screening
The qualitative phytochemical estimation of CN ethanolic extract was conducted using tests such as Keller-Killiani test, sodium hydroxide test, lead acetate test, Salkowski test, Lieberman’s test, silver nitrate test, and frothing test. Every reagent taken was of analytical grade.

Experimental procedure
Mice of 28-33 g were segregated into three groups, i.e. Group I, vehicle treated, and Groups II and III, administrated with CN 400 mg/kg BW and CN 600 mg/kg BW for 15 days, orally. The variation in the T3, FT3, T3, and TSH was analyzed on the 15th day. Dosage administration was done every day between 9.00 am and 10.00 am to avoid any circadian alteration.

Serum preparation
The blood samples were ensured to be collected after 24 h of the last dose administered, through retro-orbital puncture method. The blood specimen was permitted to get coagulate, and centrifugation for 20 min was carried out thereafter to obtain serum. The serum, thus, obtained was preserved at −2°C−−8°C until examined for biochemical examinations.

Biochemical parameter estimation in serum
Serum T3, FT3, TSH, and TSH were estimated by ELISA as per the instructions given in protocol by ERBA Lachema s.r.o., Czech Republic, and Calbiotech Inc., CA, at end of the study.

Histological studies
The histology of thyroid gland was performed using hematoxylin and eosin staining method [18]. The thyroid glands were removed, rinsed, and immersed in formaldehyde solution. The section was first deparaffinized, and slide was flame over the burner and repeatedly agitated in xylene for 3–5 min. The section is first hydrated with water and then in decreasing concentration of alcohol, i.e. 100%, 90%, 80%, and 70% for 30–60 s, then washed under tap water, rinsed with distilled water and drained properly before staining with hematoxylin and eosin solution. The cell nuclei will be visible in blue color, erythrocytes in red color, and muscle and other connective tissue with cytoplasm in shades of pink.

Statistical analysis
All the findings are presented as mean ± standard error of the mean (n=6), for serum biochemical parameter estimation, using one-way RM ANOVA followed by Dunnett’s post-test to compare all columns with the control group.

RESULTS
Preliminary phytochemical screening
The CN was screened for phytochemical estimation using various qualitative tests and ensured the presence of various phytochemicals such as glycosides, saponins, alkaloids, flavonoids, and terpenoids.

Effect of CN on thyroid hormones
Thyroxine (T4, FT4) and Triiodothyronine (T3)
Administration of the CN to healthy mice for 15 days, significantly reduced T4 (**p<0.01) and FT4 (**p<0.05) levels in NOR+CN 400, whereas no deviation from normal levels, on the significant basis, was observed in NOR+CN 600 when compared to normal control, i.e., NOR+VEH (Figs. 1 and 2).

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), colloidal 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 (it) (Fig. 6a), whereas the thyroid gland in NOR+CN 600 appeared to have reduced follicular size (fr) with undistinguished columnar epithelium (ce), 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 T4, 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. T4 is secreted in small amount by the thyroid gland (1.3%) and the majority is formed in peripheral tissues through Type 1 iodothyronine monodeiodinase (S-DI) by peripheral monodeiodination to carry out pro-metabolic, pro-enzymatic, and lipolytic effects [19,20]. Suppression in levels of both T3 and T4 is seen in conditions of hypothyroidism or due to the effect of certain goitrogens like bamboo shoots as a food entity or bark of Cinnamomum zeylanicum Linn. [21,22]. Any change in S-DI, i.e., inhibition is reflected by a decrease in T4 concentration and T3/T4 ratio, despite the increase in T3 concentration [23].
Fig. 1: Effect of *Crataeva nurvala* on total thyroxine levels (the values are presented as mean ± standard error of the mean (n=6), for serum biochemical parameter estimation, using one-way RM ANOVA followed by Dunnett’s post-test to compare all columns with the control group, **p<0.01**)

Fig. 2: Effect of *Crataeva nurvala* on free thyroxine levels (the values are presented as mean ± standard error of the mean (n=6), for serum biochemical parameter estimation, using one-way RM ANOVA followed by Dunnett’s post-test to compare all columns with the control group, *p<0.05*)

Fig. 3: Effect of *Crataeva nurvala* on triiodothyronine levels (the values are presented as mean ± standard error of the mean (n=6), for serum biochemical parameter estimation, using one-way RM ANOVA followed by Dunnett’s post-test to compare all columns with the control group, **p<0.01** and ***p<0.001**)

Fig. 4: Effect of *Crataeva nurvala* on T₃/T₄ ratio (the values are presented as mean ± standard error of the mean (n=6), for serum biochemical parameter estimation, using one-way RM ANOVA followed by Dunnett’s post-test to compare all columns with the control group, **p<0.01**)
et al. 2015 demonstrated that the administration of l-thyroxine or availability of thyroid hormones is characterized by the presence of large size follicles, availability of more colloid, reduction in resorptive vacuoles with flattened cuboidal epithelium as it is evident from the images of NOR+CN 400 mg/kg, thus inhibiting the formation of T₃ and rising the TSH.

The thyroid gland comprises of acini or follicles that are spherical bodies that selectively absorb iodine in the form of iodide ions, from the blood circulation for the production of the thyroid hormones, and also for its adequate storage in thyroglobulin (Tg), which make it suitable for use in hyperthyroidism, while being higher dose remaining safe to be used for other ailments with respect to thyroid function.

Finding from previous studies using the ethanolic extract of CN in PTU-induced hypothyroidism revealed that CN 400 mg/kg has thyrotropic action as it significantly raised T₄ levels (***p<0.001) with a concomitant decrease in TSH (p<0.05) and associated hypercholesterolemia (**p<0.01). However, the lower dose proved to be less effective in correcting the disorder and seen to increase TSH. This may be attributed to more marked inhibition of 5'DIs by CN 400 mg/kg, thus inhibiting the formation of T₃ and rising the TSH.

Structurally, the thyroid gland in rodents is similar to that of humans, except the difference in size of the follicles that are comparatively small and are surrounded by the cuboidal epithelium [27,28]. Petrova et al. 2014 demonstrated that the administration of l-thyroxine or availability of thyroid hormones is characterized by the presence of large size follicles, availability of more colloid, reduction in resorptive vacuoles with flattened follicular epithelium as it is evident from the images of normal (NOR+VEH), and the group administered with CN 600 mg/kg, where no or less vacuolization was present and follicular size was large with flattened cuboidal epithelium, colloidal appearance in follicles, slightly variable in size, parafollicular cells, or C-cells in between the follicles, fenestrated capillaries with visible appearance of interlobular connective tissue [29]. Ali Rajab et al. 2015 demonstrated that the supernormal levels of thyroid hormones or conditions resembling hyperthyroidism distort the morphology of the gland characterized by distortion of lumen of follicles, reduction in thyrocyte height, follicular remodeling (fusion), and thyrocyte death due to lack of trophic effect and cytoprotection offered by TSH [30]. NOR+CN 400 (Fig. 6b) depicted the hypotrophic follicles i.e., reduced in size and undistinguished epithelium, with presence of C-cells, capillaries, and vacuoles as compared to NOR+VEH group (Fig. 6a).

NOR+CN 600 (Fig. 6c) depict the bunch of follicles with fused epithelium and abundant follicular and a cluster of C-cells with the presence of colloid in different intensity. However, blood capillaries and C-cells are visible with a number of follicles.

The thyroid gland comprises of acini or follicles that are spherical bodies that selectively absorb iodine in the form of iodide ions, from the blood circulation for the production of the thyroid hormones, and also for its adequate storage in thyroglobulin (Tg), which make it suitable for use in hyperthyroidism, while being higher dose remaining safe to be used for other ailments with respect to thyroid function.

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 T₄ and reduced T₃ and T₃/T₄ 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 TSH 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.

REFERENCES

1. Bopana N, Saxena S. Crataeva nurvala: A valuable medicinal plant. J Herbs Spices Med Plants 2008;14 I Supp 2:107-27.
2. Khattar V, Val A. Utilities of Crataeva nurvala. Int J Pharm Pharm Sci 2012;4:21-6.
3. Kamath R, Shetty D, Bhat P, Shabaraya AR, Hegde K. Evaluation of antibacterial and anthelmintic activity of root extract of Crataeva nurvala. Pharmacologyonline 2011;1:671-2.
4. Chidambaram K, Albert J, Karpagam K. Antipyretic activity of Crataeva magna bark on tab-vaccine induced pyrexia. Int J Pharm Sci Res 2011;2:856-9.
5. Shelkea TT, Bhaskarb VH, Adkara PP, Jhaa U, Oswala RJ. Nephroprotective activity of ethanolic extract of stem barks of Crataeva nurrula Buch Ham. Int J Pharm Pharm Sci 2011;2:2712-7.
6. Pattanaik SO, Si SC, Nayak SS. Evaluation of free radical scavenging activity, wound healing activity and estimation of phenolic, flavonoid and proanthocyanidin contents of the plant “Crataeva magna”. Asian J Pharm Clin Res 2012;5:168-71.
7. Bulbul L, Choudhuri MS. Effect of Asmarihara kasaya curma (ASM)-an ayurvedic formulation on lipid profile after chronic administration. Int J Basic Med Sci Pharm 2012;2:83-7.
8. Gupta SK, Baghel MS, Bhuyan C, Ravishankar B, Ashok BK, Patil PD. Evaluation of anti-urlothiatic activity of pashanabhedadi ghrita against heavy metal induced nephrotoxicity in rats. Indian J Pharm Educ Res 2012;46:371-82.
9. Akila B, Manickavasakam K. Anti-inflammatory and antinociceptive activity of two Siddha formulations in combination. Int J Pharm Sci Res 2013;4:856-61.
10. Chakraborty S, Karamakar D, Kolhapare SA. Evaluation of the efficacy and safety of Himplasia in BPH: A randomised, double-blind, placebo-controlled, phase III clinical trial. Mod Med 2004;12:39-48.
11. Barwal A, Kumar S, Verma SK, Goyal PK, Sharma I, Sharma S, et al. Evaluation of herbal formulation neeri (NS-RF) for protective effect against heavy metal induced nephrotoxicity in rats. Indo Am J Pharm Sci 2015;5:2790-8.
12. Kaur A, Khurana N, Verma SK. Potential thyrotropic and antihypercholesteronemic activity exhibited by ethanolic extract of Crataeva nurrula bark. J Appl Pharm Sci 2017;7:69-73.
13. Hussain M, Bakhsh H, Aziz A, Majeed A, Khan IA, Muejeeb A, et al. Comparative in vitro study of antimicrobial activities of flower and whole plant of Jasminum officinale against some human pathogenic microbes. J Pharm Altern Med 2013;2:33-43.
14. Sikarwar MS, Patil MB. Antihyperlipidemic activity of Crataeva nurrula stem barks extracts. Indian J Pharm Educ Res 2012;46:378-82.
15. Bhattacharjee A, Shashidhara SC, Saha S. Neuroprotective activity of Crataeva nurrula Buch-Ham stem bark against scopomalone - Induced cognitive impairment via antioxidative activities in rats. Am J Ethnomed 2014;1:371-83.
16. Patel A, Rath S, Pradhan D, Mahanty A, Gupta BK, Bala NN. Hepatoprotective activity of leaves of Crataeva magna (Lour.) DC. In different types of hepatotoxic rat models. Indo Am J Pharm Res 2014;4:125-31.
17. Shelkea TT, Bhaskarb VH, Adkara PP, Jhaa U, Oswala RJ. Nephroprotective activity of ethanolic extract of stem barks of Crataeva nurrula Buch. Ham. Int J Pharm Pharm Sci Res 2011;2:2712-7.
18. Sayaki H, Azure B. Counterstain in the immunohistochemical evaluation of heavily pigmented nevomelanocytic lesions. Appl Immunohistochem 1995;5:268-71.
19. Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocr Rev 2002;23:38-89.
20. Greenspan FS. The thyroid gland. In: Greenspan FS, Baxter JD, editors. Basic and Clinical Endocrinology. Englewood Cliffs, New Jersey: Prentice Hall International Inc.; 1994. p. 160-226.
21. Sarkar D, Chakraborty A, Bhattacharya C, Singh LH, Chandra AK. Exploration of goitrogenic/antithyroidal potentiality of bamboo-shoots in relation to thiourea. Int J Pharm Pharm Sci 2017;9;7-12.
22. Azharuddin M, Atif M, Ahmed MI, Bakhtarya SA, Ibrahim M. Evaluation of anti-thyroid activity of Ficus racemosa Linn bark in male rats. Int J Pharm Pharm Sci Res 2015;7:118-22.
23. Visser TJ, Vander dose-toibe I, Hennemann G. Conversion of thyroxine into triiodothyronine by rat liver homogenate. Biochem J 1978;150:489-93.
24. Panda S, Kar A. Withania somnifera and Bashania purpurea in the regulation of circulating thyroid hormone concentrations in female mice. J Ethnopharmacol 1999;67:233-9.
25. Tahiliani P, Kar A. Role of Moringa oleifera leaf extract in the regulation of thyroid hormone status in adult male and female rats. Pharmacol Res 2000;41:319-23.
26. Panda S, Kar A. Evaluation of the antithyroid, antioxidative and antihyperglycemic activity of scopoletin from Aegle marmelos leaves in hyperthyroid rats. Phytother Res 2006;20:1103-5.
27. Choksi NY, Jahnke GD, Hilaire CS, Shelby M. Role of thyroid hormones in human and laboratory animal reproduction health. Birth Defects Res (Part B) 2003;68:479-91.
28. U.S. EPA. Assessment of Thyroid Follicular Cell Tumors. Washington, DC: EPA 1998.
29. Petrova I, Mitevska E, Gerasimovska Z, Milenkova L, Kostovska N. Histological structure of the thyroid gland in apolipoprotein E deficient mice. J Morpho Sci 2015;3:268-71.
30. Rajab NM, Ukropina M, Cakic-Milosevic, M. Histological and molecular characterization of heavily pigmented nevomelanocytic lesion. Appl Immunohistochem 2014;2014;4:125-31.
31. Petrova I, Mitevska E, Gerasimovska Z, Milenkova L, Kostovska N. Histological structure of the thyroid gland in apolipoprotein E deficient mice. J Morpho Sci 2015;3:268-71.
32. Rajab NM, Ukropina M, Cakic-Milosevic, M. Histological and molecular characterization of heavily pigmented nevomelanocytic lesion. Appl Immunohistochem 2014;2014;4:125-31.