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Effects of *Commiphora mukul*, *Withania somnifera* and Thyroxine on Thyroid Profile in Murine Model of Hypothyroidism

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ABSTRACT

Introduction: Thyroid disorders, especially hypothyroidism are on the rise these days due to unhealthy lifestyle and nutritional deficiencies which calls for research in this domain. Monotherapy with thyroxine is considered the main line of treatment up till now but besides its beneficial role in normalizing thyroid hormone levels, it has serious cardiac effects which leads to the search of safer natural remedies. *Commiphora mukul* and *Withanias omnifera* are considered two such plants with beneficial role in correcting T3 and T4 levels respectively. However keeping in mind the diagnostic importance of TSH, the effects of these phyto extracts on TSH levels needs to be established. **Aims & Objectives:** So the present study is designed to explore the effects of Ethanolic extract of *Commiphora mukul* (CMEE) and *Withania somnifera* (WSEE) on T3, T4 and TSH levels keeping thyroxine as a gold standard in murine model of hypothyroidism. **Place and duration of study:** This study was carried out in the animal house of PGMI, Lahore. Total duration of study was 38 days. **Material & Methods:** 50 female rats were divided into 5 groups. G-1 the control group. G-2 received methimazole 60mg/kg/day for 21 days in order to induce hypothyroidism and left untreated for self recovery & G3-5 were induced with methimazole in the same way. After induction all the groups were treated for 15 days, G-3: CMEE 0.2g/kg/day orally, G4: WSEE 1.4g/kg/day orally and G-5 received the gold standard Thyroxin 0.6µg/100gm BW S/C. Thyroid function tests were performed in all groups on Day 1, repeated on Day 22 after methimazole administration and on Day 38 following completion of treatment. **Results:** The results showed that both CMEE and WSEE corrected T3 and T4 levels to significant extent. CMEE had more effect on T3 levels whereas WSEE corrected T4 levels more. TSH levels were also reverted to normal showing the potential of both these extracts to correct TSH besides T3, T4 levels. **Conclusion:** This proves that these extracts may play a significant role in correcting thyroid profile (T3, T4 & TSH) if used clinically for the treatment of hypothyroidism. However, clinical research is highly recommended in this domain.

Key words: *Commiphora mukul, Withania somnifera*, hypothyroidism, thyroxine.

INTRODUCTION

Hypothyroidism, a common endocrine disorder; most commonly presents with generalized slowing of all the bodily systems causing weight gain, tiredness, decreased memory, bradycardia, skin and voice changes etc.[1] Iodine deficiency as noted to be the commonest cause of hypothyroidism is prevalent in mountainous areas, like northern Asia, central Africa, and central South America. WHO estimates that about 2 billion people are at a risk of developing hypothyroidism and consequently cretinism due to iodine deficiency.[2] Other important causes of hypothyroidism include autoimmune disease (Hashimoto's thyroiditis), postpartum thyroiditis or post thyroidectomy and drug induced hypothyroidism.[3] Antithyroid drugs like methimazole are the most common cause of drug induced hypothyroidism. They are also used to induce hypothyroidism in study population as their superiority for murine model is already established in literature.[4] Hormone replacement therapy with thyroxine is considered the mainstay of treatment of hypothyroidism with adequate monitoring of response. The main concern in this therapy is cardiac adverse effects especially in elderly due to which it is initiated in low doses and increased

gradually under supervision.[5] Therefore, considering the importance of this emerging endocrine disorder; exploring safer alternatives from the vast field of herbal medicine is the dire need of hour. *Commiphora mukul* and *Withania somnifera* are two traditional medicinal plants used for centuries for various ailments. They have a beneficial role in regulating thyroid hormone levels (T3, T4) as seen in literature making them novel treatment modalities for hypothyroidism.[6,7] Murine propylthiouracil (PTU) induced hypothyroidism studies have shown the beneficial role of *Commiphora mukul* in treatment of hypothyroidism.[8] It mainly increases T3 levels with less effect on T4 levels. A proposed mechanism in boosting the T3 levels is that it enhances the action of enzyme 5 deiodinase which catalyzes peripheral conversion of T4 to T3.[6] The thyroid enhancing action of *Commiphora mukul* is thought to be mainly caused by *Z*-guggulsterone .[9] A human case study also showed its beneficial role in hypothyroidism.[10]

Euthyroid rat studies have shown that *Withania somnifera* root extract has direct stimulatory effect on thyroid activity reflected *as* increased circulating levels of T4 with minimal effect on T3 levels.[11] The ethanolic extract of roots of *Withania somnifera* contains active constituent withanolides, which are considered to be responsible for its extraordinary therapeutic potential.[12] These results clearly show that *Withania somnifera* have thyrotropic effect and may prove to be useful botanical in the treatment of hypothyroidism.[11,7] The target of successful treatment in hypothyroidism is a euthyroid state, which is determined by a normal circulating thyroid stimulating hormone (TSH) levels.[5,13] The effects of these extracts on serum T3 and T4 levels are previously investigated and proven. However no study is conducted to show the beneficial role of these herbs in decreasing TSH levels to normal. Therefore our present study was conducted to show not only the effect of these plants on T3, T4 levels but also on TSH levels which has diagnostic importance in hypothyroidism. Thyroxine being the drug of choice in hypothyroidism is taken as a gold standard for comparison.

MATERIAL AND METHODS

Animals: 50 healthy female Sprague Dawley albino rats aged 6-7 weeks weighing 150-170 gm were purchased from University of Health Sciences, Lahore (Fig-5.1). Similar ambient room temperature and light and dark cycles were maintained for all the groups to ensure uniformity. Throughout the study duration i.e 38 days, rats were given free access to standard laboratory diet[14] two times a day and full access to water *ad libitum* was also ensured at the animal house of PGMI, Lahore

Plants: *Commiphora mukul* and *Withania somnifera* were purchased from a renowned herbs shop in Lahore and verified by PCSIR Laboratories, Lahore. Ethanolic extracts of both the herbs were prepared by method previously illustrated in literature.[15,16]

Medicines: Neomercazole (Ray Pharma Pvt Ltd) and Thyronorm(Abbott Pharmaceuticals)

Biochemical Kits: Thyroid function tests were performed by using T3, T4 and TSH ELISA kits provided by Calbiotech.

Preparation of Drugs:

MMI was dissolved in distilled water. It was administered in dose 60mg/kg/day for 21 days to induce hypothyroidism.[17] Thyroxine was dissolved in normal saline (0.9%). 1 tablet of 25µg was dissolved in 5ml of normal saline. Then it was filtered and the required dose of thyroxine according to 0.6µg/100gm body weight was given subcutaneously by insulin syringe.[18]

Grouping of animals: 50 animals were divided into 5 groups with 10 animals in each group. One week was given to rats for acclimatization to the new environment before starting the experiment. Ethical approval was obtained through Ethical Review Committee and instructions given by them were followed to minimize animal suffering. Thyroid function tests were performed at Day 0, Day 22 after inducing hypothyroisim and Day 38 post treatment. Following is the description of groups:

Group 1: The Control Group The animals in this group were maintained on standard laboratory diet only for 38 days.

Group 2: All animals in this group were given methimazole once daily orally in a dose of 60mg/kg/day for 21 days. This high dose of MMI was used to induce hypothyroidism based on an earlier report.[17] In the remaining groups each rat was administered the same dose of MMI daily for 21 days to induce hypothyroidism after which following drugs or plant extracts were given for the next 15 days .

Group 3: *Commiphora mukul* 0.2g/kg/day orally by gastric gavage.

Group 4: *Withania Somnifera* 1.4g/kg/day by gastric gavage.

Group 5: The animals in this group were given only Thyroxine 0.6µg/100gm BW S/C.

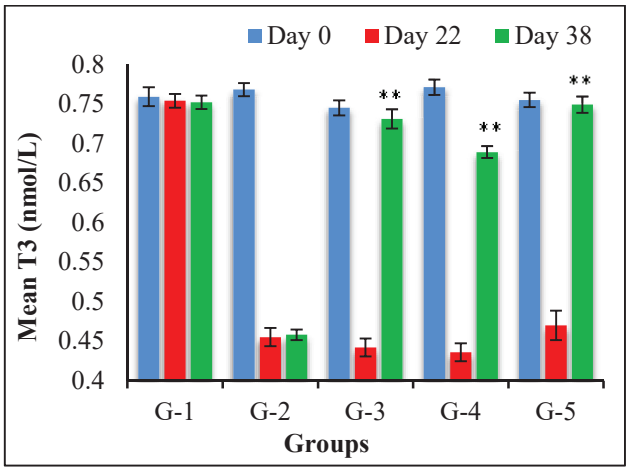
Statistical analysis:

The data was entered and analyzed by using SPSS version 20.0. Paired wise comparison of T3, T4, TSH, T3:T4 at Day 0, Day 22 and Day 38 in

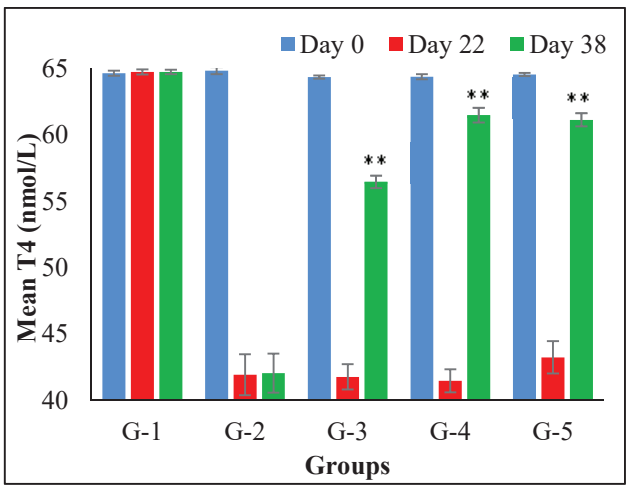
different study groups was done using Tukeys test. Repeated measure ANOVA was used for comparison among groups of all the quantitative variables at different days. A p-value of < 0.05 is considered statistically significant for all the measured parameters.

RESULTS

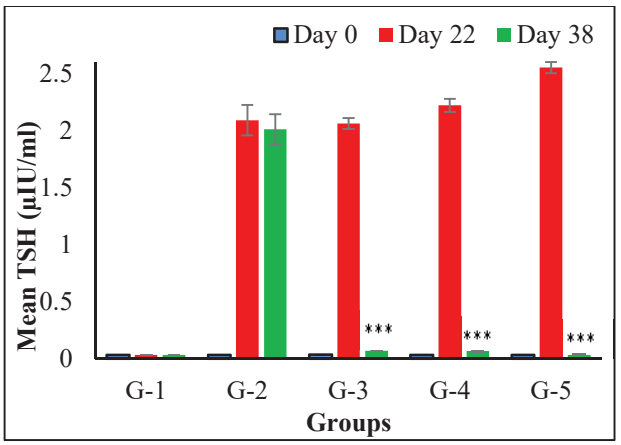
Thyroid function tests were performed at baseline i.e Day 0, then on day 22 after induction and finally on Day 38 to see the effects of treatment. Detailed result on respective days are given below in Graphs.
Group 1 (CONTROL)
Group 2 (MMI only)
Group 3 (MMI & CMEE 0.2g/kg/day)
Group 4 (MMI & WSEE 1.4g/kg/day)
Group 5 (MMI & Thyroxine 0.6µg/100gm)



Graph-1: Comparison of T3 at Day 0, 22& 38 in different study groups (**p-value<0.01)



Graph-2: Comparison of T4 at Day 0, 22 & 38 in different study groups (**p-value<0.01)



Graph-7: Comparison of TSH at Day 0, 22 & 38 in different study groups (***p-value<0.001)

DISCUSSION

Hypothyroidism is a common thyroid endocrine disorder found not only in underdeveloped but also in developed countries like UK having prevalence as high as 9% for females and 1% for males with mean age at diagnosis around 60 years.[19] The increasing incidence of this endocrine disorder forces us to search for safer medicinal plant that can employed for its treatment as an alternative to thyroxine. Previous research reveals both *Commiphora mukul* and *Withania somnifera* to have a miraculous role in regulating thyroid hormone levels T3 & T4 respective.[8,11] However their impact on TSH levels which has diagnostic importance in hypothyroidism and comparison of their affectivity with thyroxine in regulating thyroid hormone levels in murine methimazole induced hypothyroididsm still needs to be determined. So our present study is novel in this regard.

Thyroid Function tests:

According to literature the normal value of adult rats Total T4 is 65.6nmol/L, Total T3 is 0.8nmol/L[20] and TSH is 0.037 µIU/ml.[21] In our study baseline thyroid function tests i.e at Day 0 showed that all the groups had T3, T4 and TSH levels within the normal range having a p-value >0.05 which is statistically insignificant. At Day 22 after methimazole (MMI) administration group 2 -5 showed significantly low T3, T4 levels whereas TSH levels were increased. This showed that biochemical derangements had begun with methimazole (MMI), which works by multiple mechanisms like inhibiting thyroid peroxidase catalyzed reactions, iodination of tyrosine residues in thyroglobulin and coupling of iodotyrosine residues to form T3 and T4 all of which lead to decreased thyroid hormone levels and increased TSH levels by negative feedback mechanism.[22] In

addition to this TSH levels are also increased probably by phosphodiesterase 8B modulation. It decreases cAMP activity in gland, leading to decreased stimulation of thyroid by TSH which causes TSH levels to increase so that adequate levels of thyroid hormones are produced.[23]

Thyroid function was compared in all groups with group 1 (healthy control), group 2 (disease control) and group 5 (Thyroxine0.6µg/100gm BW) which served as gold standard. At Day 38, healthy control group and disease control group i.e group 1 and 2 respectively showed no significant difference in their T3, T4 and TSH levels from Day 22. However thyroxine treated group had statistically insignificant difference in T3, T4 and TSH levels from healthy control group which proves its therapeutic ability to normalize thyroid profile. After 15 days of treatment with individual doses of ethanolic extract of *Commiphora mukul* (CMEE) and Ethanolic extract of *Withania somnifera* (WSEE) on Day 38, when the groups were tested for serum T3 levels then p-value came out to be <0.001 which is statistically significant.

Among the treatment groups, group 4 (WSEE 1.4g/kg/day), showed 50%, improvement in serum T3 levels whereas thyroxine treated group showed 63% increase in serum T3 level as compared to disease control group. This shows that WSEE has a potential to correct T3 levels but it is 13% less than gold standard thyroxine which is also statistically significant. The ability of WSEE to correct T3 levels though less can be explained on the basis of its ability to reduce the oxidative stress imposed by high dose of methimazole.[17,11] However animals which received CMEE 0.2g/kg/day showed significantly better results than WSEE. It showed 61% increase in T3 level when compared with disease control group which is only 2% less than the thyroxine treated group and this difference is statistically insignificant. This remarkable increase in T3 value after receiving CMEE was in accordance with a previous study showing the presence of guggulsterone in extract which stimulates enzyme 5 deiodinase leading to increased peripheral conversion of T4 to T3 and consequently greater T3 levels.[6,8] Genetic studies have shown that nearly 67% of the thyroid hormone levels are genetically determined employing an important role of genes in it. Important genes identified so far in this respect are iodothyronine deiodinase 1 and 2.[23] A plausible explanation for CMEE extract activity could be binding with genetic response elements of iodothyronine deiodinase 1 and 2, thus enhancing the activity of this crucial enzyme leading to enhanced T3 levels. In addition, CMEE & WSEE could have worked by their commendable antioxidant property to reduce the oxidative stress imposed by methimazole (MMI) and helped to improve thyroid function. Oxidative stress was caused by methimazole at high doses by altering, reduced & oxidized glutathione concentration (GSH-GSSG) and by decreasing the activity of enzyme catalase, hence increasing reactive oxygen species (ROS).[17] CMEE & WSEE have the potential to increase the activity of antioxidant enzymes like catalase, superoxide dismutase and lipid peroxidase, thus works as antioxidant.[6,11]

Similarly following 15 days of treatment with extracts and thyroxine when we evaluated T4 levels, significant differences were found with p value <0.001. Group 3 (CMEE 0.2g/kg/day) showed 34%, correction of T4 levels while the group which received Thyroxine 0.6µg/100gm BW showed 45% increase in serum T4 levels. Improvement in T4 levels in CMEE treated group is 11% less than the thyroxine treated group which is also statistically significant. CMEE showed less potential to correct T4 levels, it has more effect on T3 levels than T4 level as discussed previously. The role of CMEE in improving T4 levels can be explained based on its antioxidant property which leads to an increase in the activity of superoxide dismutase (SOD) and catalase (CAT).[24] However WSEE treated group showed better results with 46% improvement as compared to disease control. Surprisingly, this effect was even 1% better than the Thyroxine treated group proving its immense potential of increasing T4 levels. This effect of WSEE on T4 levels can be explained by research which showed significant increase in serum T4 levels with little effect on serum T3 levels after administration of WS root extract containing withanolides, showing direct stimulatory effect at the glandular level.[11,7] It clearly demonstrated that WS has thyrotropic effects. In addition WSEE also has antioxidant properties which decrease lipid peroxidation by blocking lipid peroxidase and also increase activity of superoxide dismutase thus helping it to correct the oxidative stress imposed by MMI as discussed previously and therefore improved thyroid functioning.[11,18] At the genetic level, plausibly they may enhance the expression of thyroid-specific genes. These are required in production of thyroglobulin, its lysosomal breakdown and ultimately release of T3 & mainly T4.[25]

When we evaluated TSH levels after 15 days of treatment at Day 38, significant differences were found with p-value <0.0001 which is also statistically significant. Group 3 (CMEE 0.2g/kg/day) and group 4 (WSEE 1.4g/kg/day),

showed similar decline in TSH level around 95% which was nearly equal to normalization of TSH levels achieved with gold standard thyroxine treatment (98%) as compared to disease control. The decrease in TSH level towards normalization can be explained on the basis of enhancement in T3 and T4 levels which led to negative feedback on TSH production. Genes regulating the TSH concentration include phosphodiesterase 8B, F-actin-capping protein beta subunit and the gene for TSH receptor. F-actin-capping protein beta subunit and the gene for TSH receptor work to decrease the TSH levels. Genes for receptor work by altering sensitivity of the receptors for TSH.[23] These extracts might have played a modulatory role in the activity of these genes by binding to effector or promoter regions. Further genetic studies should be carried out to determine these effects. No previous data could be found to describe the effect of these extracts individually and in comparison, with thyroxine on TSH levels. The above discussion on thyroid profile clearly elucidates that both the plant extracts have the potential to regulate T3, T4 levels. In addition, both extracts also have a phenomenal impact in declining TSH levels to normal.

CONCLUSION

The results of present study clearly elucidates that both extracts are beneficial not only in increasing T3, T4 levels but also in declining TSH levels to normal making them novel medicinal plants in the treatment of hypothyroidism. With this research the door opens to further evaluate the potential of these extracts when used in different combinations.

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