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Research Paper

The Simultaneous Effects of some Herbal Mixtures on Methimazole Medicine for Thyroide Treatment

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Abstract

Several herbs and herbal products have been recommended to promote a healthy thyroid regulation. Medicinal plants and natural products represent one of the most popular alternative treatments. The present study focuses the anti-thyroid activity in Moringaoleifera Lam in methimazole induced hypothyroidism in female albino rats. Moringaoleifera were collected from the surrounding area of thanjavur district, traditionally the plant is used as antispasmodic, stimulant, expectorant and diuretic. Methimazole inhibits the enzyme thyroperoxidase, which normally acts in thyroid hormone synthesis by oxidizing the anion iodine (I-) to iodine (I0), facilitating iodine's addition to tyrosine residues on the hormone precursor thyroglobulin, a necessary step in the synthesis of triiodothyronine (T3) and thyroxine (T4). The aim of the current study was to evaluate property of anti thyroid on Siddha medicinal plant as well as natural product by using previous events.

Keywords: Hypothyroidism; TSH; T3; T4; methimazole;

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1. Introduction

The thyroid gland is unique among the endocrine organs in two important ways: (1) it maintains a large store of hormone, and (2) it requires iodide for hormone synthesis (Greenspan & Forsham, 1983). Thethyroid hormone synthesis is seen on an individual thyroid follicular cell (Boron and Boulpaep, 2003). Thyroglobulin is synthesized in the rough endoplasmic reticulum and follows the secretory pathway to enter the colloid in the lumen of the thyroid follicle by exocytosis. Meanwhile, a sodium-iodide (Na/I) symporter pumps iodide (I−) actively into the cell, which previously has crossed the endothelium by largely unknown mechanisms. This iodide enters the follicular lumen from the cytoplasm by the transporter pendrin, in a purportedly passive manner (Bernard A Rousset, 2007).

Fabrication of thyroid hormones is conducted by the enzyme thyroid peroxidase, an integral membrane protein present in the apical (colloid-facing) plasma membrane of thyroid epithelial cells. Thyroid peroxidase catalyzes two sequential reactions (i) Iodination of tyrosines on thyroglobulin (also known as "organification of iodide") and (ii) Synthesis of thyroxine or triiodothyronine from two iodotyrosines. Through the action of thyroid peroxidase, thyroid hormones accumulate in colloid, on the surface of thyroid epithelial cells. Remember that hormone is still tied up in molecules of thyroglobulin the task remaining is to liberate it from the scaffold and secrete free hormone into blood. Thyroid hormones: triiodothyronine, thyroxine (T3 and T4) are produced by the follicular cells of the thyroid gland and are regulated by TSH made by the thyro-tropes of the anterior pituitary gland. The effects of T4 in vivo are mediated via T3 (T4 is converted to T3 in target tissues). T3 is 3-to 5 fold more active than T4.Thyroxine (3, 5, 3', 5'-tetraiodothyronine) is produced by follicular cells of the thyroid gland. It is produced as precursor thyroglobulin (this is not the same as TBG), which is cleaved by enzymes to produce active T4 (Erica et al., 2004).

In the blood, T4 and T3 are partially bound to thyroxinebinding globulin (TBG), transthyretin, and albumin. Only a very small fraction of the circulating

hormone is free (unbound) T4 0.03% and T3 0.3%. Only the free fraction has hormonal activity. As with the steroid hormones and retinoic acid, thyroid hormones cross the cell membrane and bind to intracellular receptors (α1, α2, β1 and β2), which act alone, in pairs or together with the retinoid X-receptor as transcription factors to modulate DNA transcription (Bowen, 2000).

1.1 Hormonal changes

The main hormonal changes associated with female hypothyroidism. Hypothyroid women have decreased rates of metabolic clearance of and rostenedione and estrone and exhibit an increase in peripheral aromatization (Redmond, 2004 and Longcope et al., 1990). The 5α/β ratio of androgen metabolites is also decreased in hypothyroid women, and there is an increase in excretion of 2-oxygenated estrogens (Gallagher et al., 1966). Concentrations of both total testosterone and E2, but their unbound fractions are increased. Alterations in steroid metabolism disappear when a euthyroid state is restored (Gordon and Southren, 1977). Gn levels are usually normal (Larsen et al., 1998). However, blunted or delayed LH response to GnRH has been reported in some hypothyroid women (Marino et al., 2006). When there is a delayed LH response, serum PRL concentration may be increased, and this may be due to hypothalamic TRH increasing both TSH and PRL secretion. Galactor-rheamay also occurs, but these disturbances disappear usually after T4 administration (Honbo et al., 1978).

1.2 Hypothyroidism

Hypothyroidism is a very common condition. It is estimated that about 2% of adult women and about 0.1-0.2% of men have clinical hypothyroidism, while the prevalence of subclinical disease is more frequent, up to 9% of adult population (Canaris et al., 2000; Danese et al., 1996; Vanderpump et al., 1995). The incidence however increases with age (Bharaktiya, 2011). The prevalence of hypothyroidism in newborns (congenital hypothyroidism) is about 1:3500 (LaFranchi, 1999). The prevalence and incidence of thyroid disorders is influenced primarily by sex and age. Thyroid disorders are more common in women than men, and in older adults compared with younger age groups. The prevalence of unsuspected overt hyperthyroidism and hypothyroidism are both estimated to be 0.6% or less in women, based on several epidemiologic studies. Age is also a factor; for overt hyperthyroidism, the prevalence rate is 1.4% for women aged 60 or older and 0.45% for women aged 40 to 60. For men more than 60 years of age, the prevalence rate of hyperthyroidism is estimated to be 0.13%. A similar pattern is observed for the prevalence rate of hypothyroidism. The prevalence rate of overt hypothyroidism is 2% for women aged 70 to 80, 1.4% for all women 60 years and older, and 0.5% for women aged 40 to 60. In comparison, the prevalence rate of overt hypothyroidism is 0.8% for men 60 years and older. The estimated annual incidence of hyperthyroidism for women ranges from 0.36 to 0.47 per 1,000 women, and for men ranges from 0.087 to 0.101 per 1,000 men. In terms of hypothyroidism, the estimated incidence is 2.4 per 1,000 women each year. Overt thyroid dysfunction is uncommon in women less than 40 years old and in men <60 years of age (Jack DeRuiter, 2002).

1.3 Collection of the Plant Material

Fresh plant sample of Moringaoleifera leaves were collected from various parts of Thanjavur district. The leaves were washed, shade dried, powdered and thecrude powder of Moringaoleiferain water suspension was administered (P.O) to the experimental rats at the dose of 100mg/100g body weight of the rats.

1.4 Animals and Treatment

Female albino rats of 8 – 10 weeks of age weighing between 100 and 120g were used for this present thyro-toxicity study. The animals were purchased from Sri Venkateswara Enterprises (Ltd), Bangalore and housed in polypropylene cages. The animals were acclimatized under laboratory conditions. All experiments were performed according to the norms of the local ethical committee. Animals were provided with normal rat's feed and normal water ad libitum. Animals were divided into three groups of six animals. Group I: normal animals provided with usual rat feed and water. Group II: as control animals provided with rat feed and water along with 40mg/kg methimazole in 100ml distilled water and the Group III as Drug treated animals provided with rat feed and water along with methimazole and crude powder of Moringaoleiferain water suspension andthe drug followed by it.

1.5 Collection of Blood Samples

At the end of treatment, animals were fasted overnight, anaesthetized with ether and sacrificed by cervical decapitation. Blood was drawn and the serum was separated for biochemical analysis.

1.6 Biochemical Studies

Thyroid protective activity was done by assessing the significant changes in body weight, Serum T triiodothyronine thyroxine TSH was determined using ELISA kit method. Albumin level was measured spectrometrically at 600nm and total protein by biuret method a blue purple coloured complex with absorbance at 550nm.Cholesterol estimated by Siedel method and Triglycerides were estimated Friedman and Young method by

colorimeteic kit method. Lipid peroxide content was assayed by thio-barbituric acid method, catalase estimated colorimetrically. Transaminases activities were estimated by Reitman and Frankel method and which was measured spectrometrically. The acid phosphotases was estimated and the absorbance was read at 405nm. Mean values standard were calculated for all the values carried out (Fisher, 1950).

2. Results and Discussion

Methimazole (1-methyl-3H-imidazole 2-thione) is an antithyroid drug, methimazole also known as Tapazole or Thiamazole or MMI, and part of the thioamide group. Like its counterpart propylthiouracil, a major side effect of treatment is agranulocytosis. Methimazole molecular formula is C4H6N2S. Methimazole inhibits the enzyme thyroperoxidase, which normally acts in thyroid hormone synthesis by oxidizing the anion iodine (I-) to iodine (I0), facilitating iodine's addition to tyrosine residues on the hormone precursor thyroglobulin, a necessary step in the synthesis of triiodothyronine (T_3) and thyroxine (T_4) (Nakamura, 2007).

Table 1. Anti-thyroid effect of Moringaoleiferaon T_3, T_4, TSH.

Group	Dose	T3 ng/ml	T4 µg/dl	TSH µ/u/ml
Normal	Saline	115.3±5.76	6.41±0.72	3.56±0.256
Control Methimazole treated	40mg/kg	80.76±4.84	5.21±0.22	9.84±0.850
Moringaoleiferatreated	100mg/kg	95.6±7.64	7.35±0.44	7.91±0.535

Each values is the Mean ± SEM of six animals statistically significant from control Table 1 represents the serum level ofnormal animals inT3, T4 and TSH has115.3ng/ml, 6.41µg/dl and 3.56µ/u/ml. After the induction of hyothyroidism with methimazole (40mg/kg) in thyrotoxic animals, it was found that there was a decrease in T3 andT4 by 80.76ng/ml and 5.21 µg/dl and the TSH level was increased to 9.84 µ/u/mlthan the normal level. After the treatment with Moringaoleiferac rude powder at the dose of 100mg/dl, the serum T3 andT4 level was increased to 95.6ng/ml and 7.35 µg/dl and the TSH level was decreased to 7.91 µ/u/mlfrom the untreated control animal respectively and the difference was found as 18.37% for T3, 41.07% for T4 and 19.61% for TSH.

The active thyroid hormone, T3, is one of the most powerful molecules in the human body, affecting every system, every tissue of the body and every aspect of our well-being and health. It increases the mitochondrial energy production (Wrutniak-Cabello et al., 2001 and Lebon et al., 2001).

Thyroid hormones, thyroxine (T4), and triiodothyronine (T3) play an important role in all major metabolic pathways. They regulate the basal energy expenditure through their effect on protein, carbohydrate, and lipid metabolism. This might be a direct effect or an indirect effect by modification of other the untreated control animal and the difference was found as 14.79% for total protein, 12.36% for albumin, 27.27% for cholesterol and 9.5% for TGL.

Concentrations below the reference range usually reflect low albumin concentration, for instance in liver disease or acute infection. Rarely, low total protein may be a sign of immunodeficiency. Normally T3 is bound loosely by serum proteins and hence diffuse much more rapidly into the tissues. Also the levels of cholesterol and triglycerides will be elevated the impact of subclinical hypothyroidism on lipid parameters is less well-defined. After the 30 days of the treatment with Moringaoleifera against the methimazole the cholesterol and (BrainKim, 2008).

The result of this study shows the significant hypothyroidism induced by methimazole was evidenced by decrease in serum T3 and T4 secretion due to thyro-necrosis. The administration of crude powder of Moringaoleifera for 30 days was found able to treat and protect thyroid cell or follicles damage against methimazole induced hypothyroidism.

The TSH level is not a measure of thyroid hormone sufficiency in any given patient, either untreated or treated; reliance on the TSH produces both under and over-diagnosis and under treatment. Dysfunctional central hypothyroidism with a normal TSH may be more common than primary hypothyroidism, and TSH-normalizing T4 therapy neither normalizes T3 levels nor restores euthyroidism. The TSH test is useful only for investigating the cause of clinically-diagnosed hypothyroidism. TSH test as the best screening test for the diagnosis of primary hypothyroidism and the best guide for its treatment (Garber et al., 2012). If the TSH is elevated, it is a compensatory mechanism; the increased stimulation of the dysfunctional thyroid gland may indeed work to maintain thyroid levels and effects. The TSH response to response to low FT4 levels declines by 80% between ages of 20 and 80 (Carleet al., 2007).

The term myoxedema was formerly used as a synonym for hypothyroidism. It is now well known that hypothyroidism is a graded phenomenon, including clinical manifestations (overt hypothyroidism) to asymptomatic states known as subclinical hypothyroidism (Evered & Hall, 1972). Subclinical thyroid dysfunction may be defined as an elevated TSH concentration in an asymptomatic patient with a normal serum free thyroxine concentration. It is a common condition affecting 6-17% of the general population (Helfand, 2004). Moreover, subclinical hypothyroidism may progress to overt hypothyroidism. The rate of progression is higher with the concomitant

presence of thyroperoxidase antibodies or higher levels of TSH. Thus the result shows the decreased level of Thyroid Stimulating Hormone (TSH) after the induction of Moringaoleifera in hypothyroidism induced rats.

In table 2, the serum level of the normal animals has, 5.76g/dl of total protein, 5.94mg/g serum albumin, 58.62 mg/dl of cholesterol and 37.97mg/dl of TGL. After the induction with methimazolethyrotoxic animals shows that there was an increase by 5.88g/dl of total protein, 7.60mg/g of albumin, 75.86mg/dl of cholesterol and 103.5mg/dl of TGL than the normal level. After the treatment with Moringaoleiferacrude powder at the dose of 100mg/dl, the serum total protein level was increase to 6.75g/dl and decrease serum level in albumin was 6.66mg/g, 55.17mg/dl of cholesterol and 64.2mg/dl from

Table 2. Anti-thyroid effect of Moringaoleiferaon Total Protein, Albumin, Cholesterol and TGL.

Group	Dose	Total protein g/dl	Albumin mg/g	Cholesterol mg/dl	TGL mg/dl
Normal	Saline	5.76±0.341	5.94±0.421	58.62±5.27	57.1±3.42
Control Methimazole treated	40mg/kg	5.88±0.317	7.60±0.342	75.86±4.95	103.5±6.85
Moringaoleiferatreated	100mg/kg	6.75±0.337	6.66±0.439	55.17±4.41	64.2±3.85

Each values is the Mean ± SEM of six animals statistically significant from control Thyroid hormones are associated with the oxidative and anti-oxidative status of the organism. Depression of metabolism due to hypothyroidism has been reported to decrease oxidant production and thus protects tissues against oxidant damage. The biological oxidative effects of free radicals on lipids, proteins, and DNA are controlled by a spectrum of antioxidants.

Enzymatic protection against reactive oxygen species (ROS) and the breakdown products of peroxidized lipids and oxidized protein and DNA are provided by several enzyme systems LPO and catalase (CAT) (Serdaret al., 2006). SOD catalyzes the dismutation of the superoxide anion into hydrogen peroxide (H2O2), which is then deactivated to water (H2O) by catalase or glutathione peroxidase (GPx) (Das and Chainy, 2004 and Senthilet al., 2004)

Each values is the Mean ± SEM of sixanimals statistically significant from control Table 3 refers the normal animals have, 1.4nMDA/ml as the serum level of Lipid peroxidation, 58.6 H2O2decompose/mg protein of serum catalase, After the induction of hyothyroidism, it was found that there was an increase by 1.63nMDA/ml in LPO and 75.8 H_2O_2
decompose/mg protein in catalase, than the normal level. After the treatment with Moringaoleiferac rude powder at the dose of 100mg/dl, the serum LPO level was reduced to 1.51nMDA/ml and 32.2 H2O2 decompose/mg protein of catalase from the untreated control animal and the difference was found as 7.36% for LPO and 23.15% for catalase.

The disturbance of antioxidant- oxidant balance and excessive production of free radicals increase ROS concentration, which can damage cell structures, a phenomenon known as oxidative stress. ROS mediated oxidative damage play a major role in pathogenesis of endothelial dysfunction and oxidation of lipids. Because of the decreased metabolic rate in hypothyroidism, free radical production is expected to be reduced. Additionally, excessive levels of TSH, which can be seen in primary hypothyroidism, were shown to cause increased production of oxidants.

Table 3. Anti-thyroid effect of Moringaoleiferaon LPO, Catalase, AST, ALT.

Group	Dose	LPO nMDA/ml	Catalase H_2O_2decompose/mg protein	ALP U/l	AST U/l	ALT U/l
Normal	Saline	1.4±0.056	58.0±4.06	165.84±9.28	133.32±8.65	233.31±10.49
Control Methimazole treated	40mg/kg	1.63±0.081	41.9±1.84	193.48±11.22	166.65±7.03	166.65±8.33
Moringaoleiferatreated	100mg/kg	1.51±0.090	32.2±1.77	165.84±9.36	99.99±4.49	133.32±9.99

In the estimation of Transaminase and Phosphatase, the normal animals have 165.84U/L, as the serum level of alkaline phosphatase, 233.31U/L of alanine amino transaminase and 133.32U/L aspartate aminotransferase. When comparing with control treated with methimazole shows an increase by 193.48U/L of ALP, 166.65U/L level of AST and decreased level of ALT by 193.48U/L than the normal level. After the treatment with Moringaoleiferac rude powder at the dose of 100mg/dl, the serum level was decreased to 165.84U/L in ALP, 133.32U/L in ALT and 99.99U/L in AST and from the untreated control animal and the difference was found as 14.28% for ALP, 20% for ALT and 40% for AST.

Thyroid hormones regulate Basal Metabolic Rate (BMR) and calorigenesis in tissues, including hepatocytes and thereby modulate hepatic function. The liver in turn metabolizes the thyroid hormones and regulates their systemic endocrine effect. Raised serum transaminase and phophatse (alkaline phosphatase) activities in absence of any overt liver dysfunction can therefore be attributed to primary thyroid dysfunction. The aim of this study is to assess the impairment in liver function by estimating serum Aspartate Transaminase (AST) and Alanine Transaminase (ALT) in experimental rats with hypothyroidism.

A complex relationship exists between the thyroid gland and the liver in both health and disease. The thyroid status depends not only on thyroxine secretion but also on normal thyroid hormone metabolism. Normal thyroid function, which is essential for normal growth, development and

regulation of energy metabolism within cells, is dependent on a normal functioning thyroid and liver axis. After the treatment of MO against the methimazole induced hypothyroidism after 30 days it shows significant activity in the level of transaminase and phophatase.

2.1 Phytochemical analysis of Moringaoleifera

In the present study, the phytochemical analysis of ethanolic extract of moringaoleifera showed the presence of alkaloids, flavonoids, phenol, tannins, saponins and absence of terpenoids, coumarines, quinines, steroid compound. The significant hepatoprotective, hypothyroidism, cardioprotective and antioxidant effect of Moringaoleifera may be presence of phyto-constituents of the Siddha medicinal plant.

The thyro-protective effects of Moringaoleiferac rude powder may be due to the activity of the phyto-constituents in the leaves contain fifteen components. The major compounds were hexadecanoic acid, Ethyl palmitate, Palmitic acid ethyl ester, 2, 6-Dimethyl-1, 7-octadiene-3-ol,4-Hexadecen-6-yne,2hexanone,3-cyclohexyliden-4-ethyl-E2Dodecenylacetate, Hioleic safflower oil, Safflower oil present which might have exerted the protection against the peroxidative damage and the subsequent enzyme activities as observed. Further studies are suggested on the isolation of marker compounds and phytoconstituents of the Moringaoleifera and their thyro-protective activity in human (Kumar and Pari, 2002).

3. Conclusion

Thyroid disease, namely hypothyroidism and hyperthyroidism, constitutes the most common endocrine abnormality in recent years, diagnosed either in subclinical or clinical form. Thyroid disease is associated with various metabolic abnormalities, due to the effects of thyroid hormones on nearly all major metabolic pathways. Thyroid gland controls the how the body uses energy makes proteins and controls how sensitive the body is to other hormones. In the present investigation the methimazole induced hypothyroidism in experimental animals show the involvement of oxidative stress and suggestive cellular damage in thyroid gland.

There was an increased activity in catalase and lipid peroxidation produced free radicals. This oxidative stress finally damages the follicles and cells in the thyroid gland. In case of high levels of thyroid stimulating hormone (TSH) values, there is a linear increase in cholesterol, , and TGLs. Lipidprofile concentrations especially the concentrations of cholesterol in hypothyroidism may leads to cardiovascular risk too. So, determination of serum levels of thyroid profile is recommended for further studies in human. There was an increase in serum showing the impairment of thyroid function probably as the result of hypothyroidism, increase mobilization of plasma cholesterol and triglycerides and stimulate fatty acid and cholesterol degradation. The oral administration of crude powder of Moringaoleiferafor 30 days was able to protect the cellular damage and thyroid function and activation of antioxidant enzymes. The drug administration was able to protect the thyroid dysfunction and hormonal changes as evidenced by the inhibition of the activity of phosphatases and transaminases. The study also shows the significant efficacy of Moringaoleiferain the treatment of hypothyroidism was also evidenced by increase in raised serum transaminase activities in absence of any overt liver dysfunction can therefore be attributed to primary thyroid dysfunction. Further studies, are needed to identify the chemical constituents of the plant Moringaoleifera that may be responsible for the Thyroid protective activity.

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