IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
June 2024 Vol.:30, Issue:6

Anti Thyroid Activity of *Morinda tinctoria* Roxb. Leaves against Thyroxine Induced Hyperthyroidism in Albino Wistar Rats

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Submitted: 25 May 2024
Accepted: 31 May 2024
Published: 30 June 2024

Keywords: *Morinda tinctoria* Roxb.; Propylthiouracil; Hyperthyroidism; Hormone analysis; Graves' Disease; Toxic Nodular Goitre.

ABSTRACT

Objective: The objective of this study was to evaluate the Antithyroid activity of *Morinda tinctoria* Roxb. Leaves against Thyroxine induced Hyperthyroidism in Albino Wistar rats. **Methods:** *Morinda tinctoria* Roxb. (Family: Rubiaceae), is traditionally used for inflammation, wound healing and ulcer. The preliminary phytochemical analysis performed in the Ethanolic Extract of *Morinda tinctoria* Roxb. leaves (EEMT), revealed the presence of polyphenols & flavonoids. The *in vitro* & *in vivo* Anti thyroid activity of EEMT was evaluated by Thyroid Peroxidase inhibition assay and Thyroxine induced Hyperthyroidism model in Male Albino wistar rats. Hyperthyroidism was induced in experimental rats by administering Thyroxine (0.6 mg/kg p.o.) for 14 days. Hyperthyroid rats were treated with 200 mg/kg & 400 mg/kg of EEMT and Propylthiouracil (10mg/kg) for 21 days. **Results:** EEMT showed an IC_{50} value of 183.34 µg/ml in the *in vitro* Thyroid peroxidase inhibition assay. In the *in vivo* Thyroxine induced Hyperthyroidism model, the thyroxine treated group demonstrated increased levels of Triiodothyronine (T_3) & L-Thyroxine (T_4) and decreased levels of TSH. Simultaneous administration of EEMT lowered the levels of T_3 & T_4 and increased the levels of TSH in comparison with the standard drug Propylthiouracil.

1 INTRODUCTION

Hyperthyroidism is a common Thyroid disorder, associated with excess thyroid hormone production. Hyperthyroidism can be overt with low or suppressed Thyroid Stimulating Hormone (TSH) levels and elevated Triiodothyronine (T_3) levels and/or elevated Thyroxine (T_4) levels or Subclinical with low or suppressed TSH and normal T_3 & T_4 levels. Common manifestations include unintentional weight loss, palpitations, tremors, heat intolerance, dyspnoea on exertion, increased anxiety, irritability, fatigue, muscle weakness, increased frequency of bowel movements, hair loss, loss of libido and oligomenorrhea or amenorrhea in women.(1) Hyperthyroidism is mostly caused by Graves' Hyperthyroidism (GD) or toxic nodular goitre. Acute granulomatous thyroiditis, medications such as amiodarone, tyrosine kinase inhibitors, and immune checkpoint inhibitors can also result in hyperthyroidism.

Antithyroid medications are currently the preferred treatment for Graves' hyperthyroidism. Toxic nodular goitre is mostly treated with radioiodine (^{131}I) or thyroidectomy.(2) Untreated hyperthyroidism can adversely affect health, leading to increased risks for abnormal heart rhythms, heart failure, osteoporosis, adverse pregnancy outcomes, metabolic abnormalities and increased mortality risk.(3) Although Synthetic Thyroid regulating compounds are available, their prolonged use may have some side effects. There is a need for safe, alternative medicine for the regulation of thyroid problems. Botanicals are very often considered as safe and economic therapeutics. (4)

Morinda tinctoria Roxb. that belongs to the family Rubiaceae is an important folklore medicine. The major components identified in the plant include octanoic acid, potassium, vitamin C, terpenoids, scopoletin, flavones, glycosides, linoleic acid, anthraquinones, morindone, rubiadin, and alizarin. (5) This plant exhibited potent antidiabetic, antimicrobial, hepatoprotective, anti-inflammatory, free radical scavenging & cytoprotective activity, antidiabetic and antihyperlipidemic activities. (6)

The components of the plant such as Flavonoids and Phenols may help in the amelioration of Hyperthyroidism. As per Literature review, the Anti Thyroid activity of *Morinda tinctoria* Roxb. Leaves was not evaluated. Thus, this study was aimed to evaluate the Anti Thyroid Activity of *Morinda tinctoria* Roxb. Leaves against Thyroxine Induced Hyperthyroidism in Albino Wistar Rats.

2. MATERIALS AND METHODS

2.1 PROCUREMENT OF PLANT AND EXTRACTION PROCESS

The leaves of *Morinda tinctoria* Roxb. were collected from Madhavaram, Chennai and were authenticated by Dr. S. Sankaranarayanan, HOD, Medical Botany and Pharmacognosy, Govt. Siddha Medical College, Chennai – 600106. The Ethanolic Extract of *Morinda tinctoria* Roxb. leaves (EEMT) was prepared by Soxhlet extraction with ethanol as the solvent. [7] The percentage yield of Ethanolic Extract of *Morinda tinctoria* Roxb. (EEMT) was calculated using the following formula.

$$\% \, Yield = \left(\frac{Weight \, of \, the \, Dry \, Extract}{Weight \, of \, the \, Dry \, Plant}\right) 100$$

2.2 PHYTOCHEMICAL ANALYSIS [8]

Methodology for Chemical analysis

Test for Carbohydrate

a) Molisch's Test: To the 0.5ml of sample, few drops of alcoholic alpha naphthol and 0.2ml of concentrated sulfuric acid were added slowly through the sides of the test tube. A purple to violet colour ring at the junction indicates the presence of Carbohydrate.

b) Benedict' Test: To 1ml of sample, few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) were added and boiled on water bath. The formation of a reddish-brown precipitate indicates the presence of Reducing Sugars.

c) Fehling's Test: To 1ml of sample, Fehling's solution A and B were added and heated for few minutes. The presence of Carbohydrates is indicated by the appearance of brick red precipitate.

Test for Proteins and Amino acids

a) Millon's Test: The sample was treated with 2ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid). Appearance of a white precipitate which turns red upon gentle heating indicates the presence of Proteins.

b) Biuret Test: The sample was treated with 1ml of 10% sodium hydroxide, 1ml of 1% copper sulphate solution. Formation of a Violet colour is an indication of the presence of Proteins.

c) Xanthoprotein Test: The sample was treated with 2ml of con. nitric acid. Appearance of Orange colour indicates the presence of Proteins.

Test for Alkaloids

a) Mayer's Test: To 1ml of sample, Mayer's reagent [Potassium mercuric iodide solution] was added. Formation of Cream colour precipitate indicates the presence of Alkaloids.

b) Dragendroff's Test: To 1ml of sample, Dragendroff's reagent [Potassium bismuth iodide solution] was added. Formation of a Reddish-brown precipitate is an indication of the presence of Alkaloids.

c) Hager's Test: To 1ml of sample, Hager's reagent [Saturated solution of Picric acid] was added. Appearance of a yellow colour precipitate is an indication of the presence of Alkaloids.

Test for Glycosides

Legal's Test: To 1ml of sample, few drops of pyridine and alkaline sodium nitroprusside solution were added. Appearance of blood red colour indicates the presence of Glycosides.

Test for Cardiac Glycosides

Keller Killani Test: The Test substance was added with 0.4ml of glacial acetic acid and a little amount of ferric chloride. The mixture was transferred to a small test tube and then 0.5ml of con. sulphuric acid was added. Appearance of blue colour in the acetic layer indicates the presence of Cardiac Glycosides.

Test for Phenolic compounds

Ferric chloride Test: The sample was treated with 1ml of water and boiled for few minutes then it was filtered. The filtrate was treated with ferric chloride solution. Appearance of bluish black colour indicates the presence of Phenolic compounds.

Test for Flavonoids

Alkaline reagent Test: The sample was treated with 1ml of sodium hydroxide. Appearance of yellow colour indicates the presence of Flavonoids.

Test for Saponin

Foam froth Test: The sample was treated with 10ml of water and boiled for few mins, then it was filtered. The filterate was shaken well and noted for the stable froth. A 1 cm layer of foam is an indication of the presence of Saponins.

Test for Tannins

a) Gelatin Test: The sample was treated with 2ml of 1% Gelatin and 10% sodium chloride. Appearance of a white precipitate is an indication of the presence of Tannins.

b) Lead acetate Test: To 2ml of sample, few drops of lead acetate solution were added. Appearance of a white precipitate indicates presence of tannins.

Test for Sterols

Libermann Burchard Test: The sample is treated with 2ml of chloroform, small amount of acetic anhydride and 1ml of con. Sulphuric acid. The colour changes from red to bluish green indicate the presence of Sterols.

Test for Terpenoids

Noller's Test: Two or three granules of tin metal were dissolved in 2ml thionyl chloride solution and added to 1ml of the extract and warmed. The formation of a pink colour is an indication of the presence of Terpenoids.

Test for Fats and Fixed oils

Stain Test: A small quantity of sample was pressed between two filter papers. A stain on the filter paper is an indication of the presence of fixed oils.

2.3 *IN-VITRO* ANTI-THYROID ACTIVITY

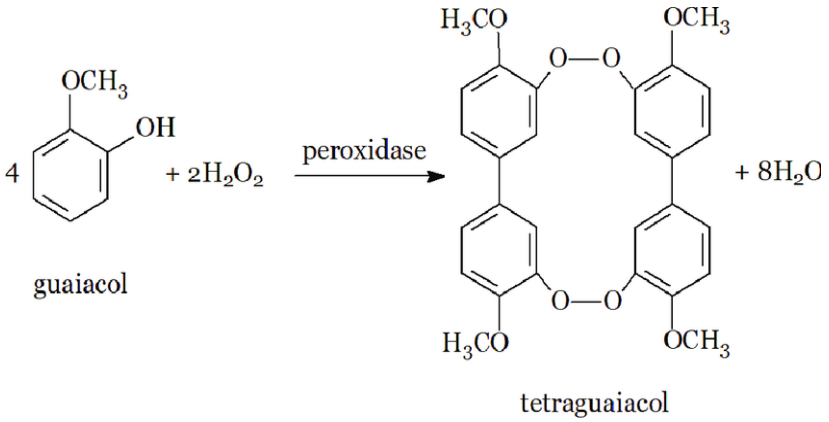
The *In Vitro* Anti Thyroid activity of Ethanolic Extract of *Morinda tinctoria* Roxb. (EEMT) leaves was studied using Thyroid Peroxidase Inhibition Assay.

THYROID PEROXIDASE INHIBITION ASSAY: [9]

The Thyroid Peroxidase Inhibition Assay is used to determine the *In Vitro* Anti Thyroid activity as it has been demonstrated that Thyroid Peroxidase (TPO) inhibitors are potential therapeutic agents in hyperthyroidism.

PRINCIPLE

Thyroid Peroxidase, also called Thyroperoxidase, is an enzyme secreted in the thyroid colloid. It oxidizes iodide ions to form iodine atoms for addition onto tyrosine residues on thyroglobulin for the production of Thyroxine or Triiodothyronine. The peroxidases are capable of reducing hydrogen peroxide. They can oxidize aromatic electron donors such as guaiacol and pyrogallol at the expense of H_2O_2. This assay is based on the theory that in the presence of Hydrogen peroxide, peroxidases oxidize the substrate Guaiacol and catalyze it with the formation of Tetra guaiacol, a coloured compound that can be quantified by its absorbance at 470nm.



guaiacol

tetraguaiacol

PROCEDURE

- The assay was conducted by adding 50 µl of buffer, 40 µl of pure substance solution, 50 µL of guaiacol, 20 µL of TPO enzyme and 50 µL H_2O_2 in a cuvette.
- The cuvette was then placed into the spectrophotometer in 96 – well plates and the reaction was started by the addition of 100 µL H_2O_2.
- Extracts were used in place of buffer for the assay of samples.
- Absorbance readings were recorded at a wavelength of 470 nm, every minute for a total of 3 min in 37˚, as a unit of TPO activity is defined as the change of absorbation per minute.
- Thyroperoxidase inhibitory activity was calculated as follows:

$$\%\ Inhibition = \left\{1 - \frac{\frac{\Delta A}{\text{mintest}}}{\Delta\text{Aminblank}}\right\} \times 100$$

Where:

ΔA/min test - the linear change in absorbance per minute of the test substance.

ΔA min blank - the linear absorbance change per minute of the blank.

2.4 *IN VIVO* ANTI THYROID ACTIVITY

ETHICAL APPROVAL:

The present study was conducted after obtaining approval from the Institutional Animal Ethics Committee, Madras Medical College, Chennai-03 and this protocol met the requirements of national guidelines of CPCSEA/IAEC approval no.1917/GO/ReBi/S/16/CPCSEA/20.09.2021 for the experimental protocol no. 08/AEL/IAEC/MMC dated 26.12.2023.

EXPERIMENTAL ANIMALS:

Male Albino Wistar rats (150-200g) were used for this study and they were procured from Animal experimental laboratory, Madras Medical College, Chennai-03, India. The procured Wistar Albino rats were quarantined for one week to minimize the chances of pathogen introduction to the established animals and to develop psychological, physiological & nutritional stabilization before their use. The animals were housed in a well-ventilated animal house which was maintained at a constant temperature and relative humidity of 55-60%. The animals were kept in polypropylene cages and paddy husk was utilized as bedding material. The bed material was changed twice a week. The animals were maintained on standard pellets and purified water. The animals were provided food and water ad libitum except during fasting. Each animal in the cage was marked on the tail with picric acid for identification.

MODEL USED: THYROXINE INDUCED HYPERTHYROIDISM MODEL [10]

Thyroxine was chosen to be administered for inducing Hyperthyroidism in Wistar Rats. Thyroxine at a dose of 0.6 mg/kg was given orally for 14 days to the experimental rats of all groups except the Control group.

PROCUREMENT OF DRUGS

The standard drug Propylthiouracil (10 mg/kg) and the inducing drug L-Thyroxine (0.6 mg/kg) manufactured by Macleods Pharmaceutical Pvt. Ltd and Glaxo SmithKline Pharmaceuticals Ltd, were procured from a retail pharmacy.

PREPARATION OF DRUGS

- Ethanolic extract of *Morinda tinctoria* Roxb. was dissolved in distilled water.
- Propylthiouracil (PTU) tablets were weighed, powdered and dissolved in distilled water.
- Thyroxine tablets were weighed, powdered and dissolved in distilled water.

EXPERIMENTAL DESIGN

30 Male Albino Wistar rats were weighed and divided into 5 groups each containing 6 animals.

Group I: Served as Control and received Normal diet and water for 35 days

Group II: Served as Negative control and received Thyroxine (0.6 mg/kg) p.o. for 14 days.

Group III: Served as Positive control and received Propylthiouracil (10 mg/kg) p.o. for 21 days.

Group IV: Served as Treatment control and received Low dose of EEMT (200 mg/kg) p.o. for 21 days.

Group V: Served as Treatment control and received High dose of EEMT (400 mg/kg) p.o. for 21 days.

Groups II, III, IV and V were administered with Thyroxine 0.6 mg/kg p.o. for the period of 14 days to induce Hyperthyroidism. After 14 days, Group III rats were administered with Propylthiouracil at the dose of 10 mg/kg and Groups IV & V received 200 mg/kg and 400 mg/kg of the Ethanolic Extract of *Morinda tinctoria* Roxb. (EEMT) Leaves respectively for 21 days. Group II received distilled water for the next 21 days. The Doses of the EEMT were selected based on the previous Acute Toxicity [5] results of the Ethanolic Extract of *Morinda tinctoria* Roxb. Leaves. 200 mg/kg, the 1/10th dose of the standard 2000 mg/kg and 400 mg/kg, the 1/5th dose of the standard 2000 mg/kg of the Ethanolic Extract of *Morinda tinctoria* Roxb. Leaves used for the Acute Toxicity testing as per the OECD Guideline No. 423 were selected.

2.5 EVALUATION OF ANTI-THYROID ACTIVITY

After 35 days of treatment, the blood was collected from the retro-orbital plexus puncture of all groups of overnight fasted rats using micro capillary. The serum was separated by

centrifuging at 2000 rpm for 15 mins which was used for the estimation of thyroid hormones (TSH, T_3, and T_4). The animal was then sacrificed by euthanasia. The thyroid gland was immediately dissected out, washed in ice cold saline to remove the blood and stored in 10% formalin for histopathological studies.

EVALUATION PARAMETERS

2.5.1 EFFECT ON BODY WEIGHT

Rats were weighed every week throughout the experimental period. Weight gain was calculated after the last week of the experiment.

2.5.2 ESTIMATION OF SERUM THYROID HORMONES

Serum separated by centrifugation was stored at $-70^{\circ}C$ before analysis. Serum levels of T_3, T_4 and TSH were analyzed by colorimetric competitive enzyme immunoassay using individual ELISA kit.

2.6 HISTOPATHOLOGICAL STUDIES

The Thyroid glands dissected out from the animals were rinsed in ice cold 0.9% saline, fixed in 10% formalin embedded in paraffin and cut into 5µm thick section using a microtome. Sections were mounted on glass slides. The sections were stained with Haematoxylin – Eosin, examined under a microscope using 100 x magnifications and photographed under a light microscope equipped for photography.

2.7 STATISTICAL ANALYSIS

The results were expressed as mean ± SEM. The data were statistically analyzed by means of one-way ANOVA followed by Dunnett's multiple comparison test using Graph pad prism software version 10.2.2. One way ANOVA was used to analyse the statistical difference between the variables. P values such as $P<0.05$, $P<0.01$, $P<0.001$ were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 EXTRACTION

The Percentage Yield of the Ethanolic Extract of the *Morinda tinctoria* Roxb. leaves obtained through Soxhlet Extraction is found to be **20 % w/w**.

3.2 PRELIMINARY PHYTOCHEMICAL ANALYSIS

The Phytochemicals present in the EEMT is shown in Table -1.

Table - 1 PHYTOCHEMICAL TESTS AND RESULTS

S. NO	PHYTOCHEMICAL	RESULT
1.	Carbohydrates	+
2.	Proteins and Amino acids	+
3.	Alkaloids	+
4.	Glycosides	+
5.	Cardiac Glycosides	+
6.	Phenolic compounds	+
7.	Flavonoids	+
8.	Saponins	-
9.	Tannins	+
10.	Sterols	+
11.	Terpenoids	+
12.	Fats and Fixed oils	+

The Phytochemical screening of EEMT revealed the presence of Alkaloids, Glycosides, Flavonoids, Phenols, Tannins, Terpenoids and Carbohydrates (Table - 1). As the extract possessed the most phytochemical constituents, it was selected for the evaluation of Anti-Thyroid activity.

3.3 *IN VITRO* ANTI THYROID ACTIVITY

The *In vitro* Anti Thyroid activity was carried out using **Thyroid Peroxidase Inhibition Assay**. The Mean Absorbance, percentage inhibition of TPO, determined using the TPO

Assay and the IC_{50} value obtained for the different concentrations of EEMT and PTU are represented in the Table - 2 and Table - 3.

TABLE - 2 Effect of EEMT in Thyroid Peroxidase Inhibition Assay

S.No	Concentration of the Sample (µg/ml)	Mean Absorbance	% Inhibition of TPO	IC_{50} (µg/ml)
1.	Control	2.935	-	183.34
2.	25	2.316	21.09	
3.	50	2.134	27.29	
4.	100	2.056	29.94	
5.	200	1.644	43.98	
6.	300	0.447	84.77	
7.	400	0.113	96.14	

Table - 3 Effect of PTU in Thyroid Peroxidase Inhibition Assay

S.No	Concentration of the Sample (µg/ml)	Mean Absorbance	% Inhibition of TPO	IC_{50} (µg/ml)
1.	Control	2.948	-	154
2.	25	2.21	25.03	
3.	50	1.56	47.08	
4.	100	1.414	52.03	
5.	200	1.215	58.78	
6.	300	0.788	73.27	
7.	400	0.183	93.79	

From the Tables 2 and 3, the IC_{50} values of EEMT and PTU are found to be 183.34 µg/ml and 154 µg/ml. This comparison reveals that the Ethanolic Extract of *Morinda tinctoria* Roxb. is found to have significant Anti Thyroid activity.

3.4 *IN VIVO* ANTI – THYROID ACTIVITY

EVALUATION PARAMETERS

3.4.1 EFFECT ON BODY WEIGHT

Rats were weighed every week for the whole period of the experiment. Body Weight changes were calculated after the last week of the experiment.

Table - 4 Body weight changes of Rats during the Experimental period.

GROUPS		BODY WEIGHT (kg)				
		INDUCTION PERIOD		TREATMENT PERIOD		
		Day 0	Day 14	Day 21	Day 28	Day 35
I	Control	157.2±0.47	172.6±0.55***	176.3±0.49	185.3±0.61	206.6±0.55***
II	Negative control	175.6±0.55	150.6±0.55***	135.3±0.61	131±0.85	110.6±0.91***
III	Positive control	170±0.36	149.5±0.61***	147.5±0.76	152.6±0.91	166.5±0.61**
IV	Low dose EEMT	167.6±0.55	142.6±0.92***	145.5±0.62	154.5±0.76	160.3±0.61***
V	High dose EEMT	174.5±0.76	149.6±0.61***	157.8±0.7	163.5±0.62	172.1±0.47*

All the values are expressed as Mean ± SEM.

***p<0.001, **p<0.01 and *p<0.05 compared to Day 0

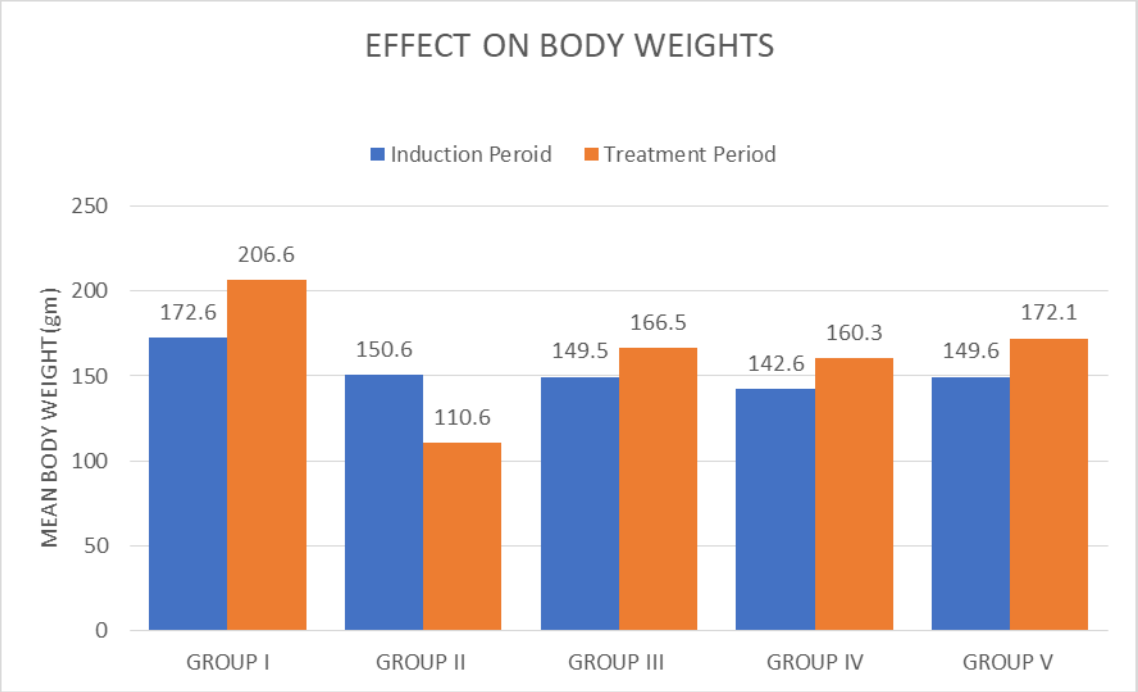


Fig - 1 Body weight Changes of Rats during the Induction and Treatment periods

It is seen from Table – 4 and Fig - 1, that the animals in Control group fed with Normal Diet from Day 1 to Day 35 showed a gradual increase in Body weight. After the Induction period, there was a significant decrease ($p<0.001$) in Body weight of the animals in Groups II, III, IV and V. After the Treatment Period, there was a significant increase in Body weight of the animals in Groups III, IV and V. The decrease in Body weight in Group II continued to low significantly from Day 21 to Day 35.

3.4.2 EFFECT ON THYROID HORMONE PROFILE

3.4.2.1 ESTIMATION OF SERUM T_3 LEVELS

Table - 5 Estimation of Serum T_3 Levels

Groups	Treatment	T_3 (ng/dL)
I	Control	113.6±0.494
II	Negative control	225.16±0.833###
III	Positive control	126±0.683***
IV	Low Dose EEMT	165±0.730***
V	High dose EEMT	135.6±0.494***

All values are expressed as Mean± SEM (n= 6).

###p<0.001 compared with Group I

***p<0.001 compared with Group II

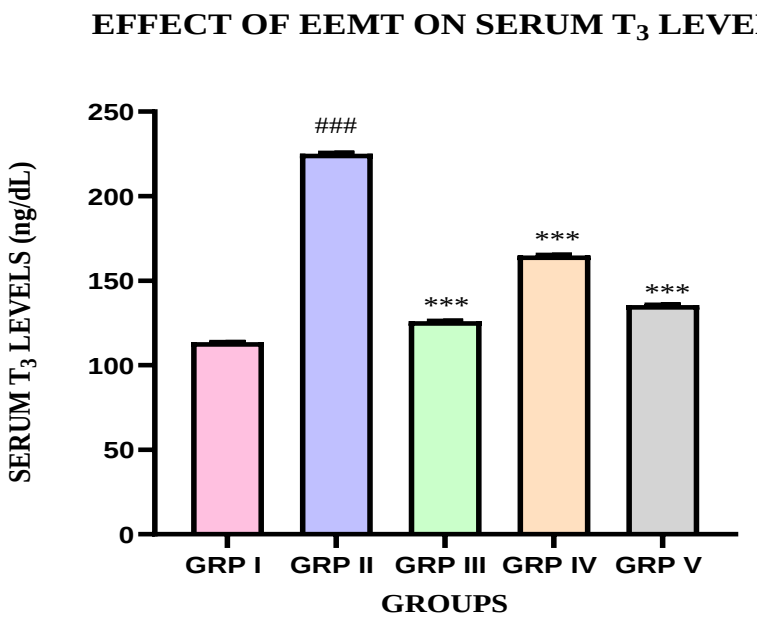


Fig - 2

From Table - 5 and Fig - 2, it was found that there was a significant ($p<0.001$) increase in the Serum T_3 levels in Group II when compared with Group I rats. This is an indication of development of Hyperthyroidism. Treatment with Propylthiouracil (Group III), EEMT at the dose of 200mg/kg (Group IV) and 400mg/kg (Group V) showed significant ($p<0.001$) decrease in the Serum T_3 level when compared with Group II.

3.4.2.2 ESTIMATION OF SERUM T_4 LEVELS

Table - 6 Estimation of Serum T_4 Levels

Groups	Treatment	T_4 (µg/dL)
I	Control	4.84±0.037
II	Negative control	11.83±0.024###
III	Positive control	6.34±0.051***
IV	Low Dose EEMT	9.53±0.055***
V	High dose EEMT	7.61±0.027***

All values are expressed as Mean± SEM (n= 6).

###p<0.001 compared with Group I

***p<0.001 compared with Group II

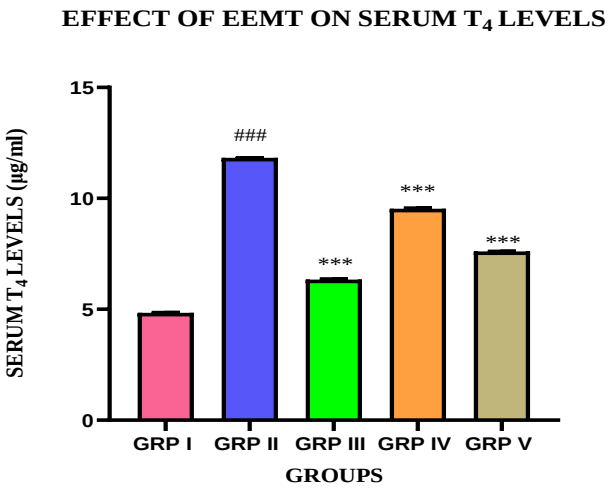


Fig - 3

From the Table - 6 and Fig - 3, it is found that there was a significant ($p<0.001$) increase in the Serum T_4 levels in Group II when compared with Group I rats. This is due to the development of Hyperthyroidism. Treatment with Propylthiouracil (Group III), EEMT at the dose of 200mg/kg (Group IV) and 400mg/kg (Group V) showed significant ($p<0.001$) decrease in the Serum T_4 level when compared with Group II.

3.4.2.3 ESTIMATION OF SERUM TSH LEVELS

Table - 7 Estimation of Serum TSH Levels

Groups	Treatment	TSH (µIU/ml)
I	Control	0.081±0.001
II	Negative control	0.05±0.0007###
III	Positive control	0.066±0.0007***
IV	Low Dose EEMT	0.055±0.0004**
V	High dose EEMT	0.061±0.0004***

All values are expressed as Mean± SEM (n= 6).

###p<0.001 compared with Group I

***p<0.001 and **p<0.01 compared with Group II

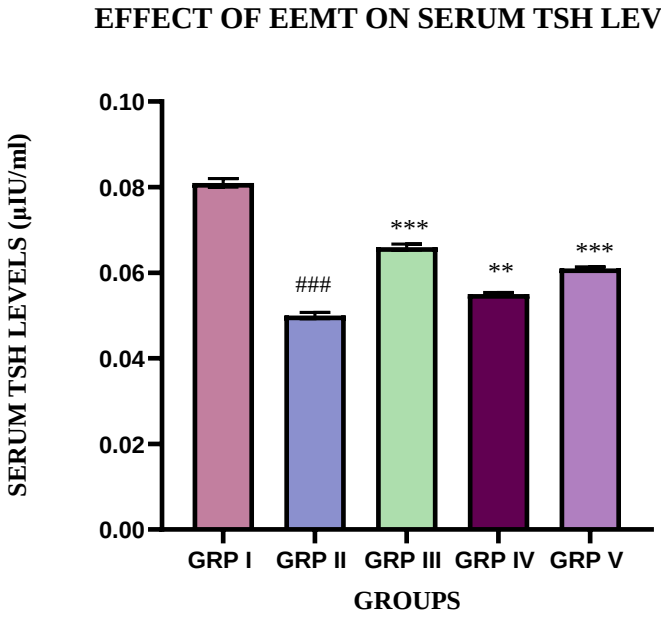


Fig - 4

From the Table - 7 and Fig - 4, it is found that there was a significant ($p<0.001$) decrease in the Serum TSH levels in Group II when compared with Group I rats. This is an indication of development of Hyperthyroidism. Treatment with Propylthiouracil (Group III) and EEMT at the dose of 400mg/kg (Group V) showed significant ($p<0.001$) increase in the Serum TSH levels when compared with Group II. EEMT at 200mg/kg (Group IV) also showed a significant ($p<0.01$) increase in the Serum TSH levels when compared with Group II.

3.5 HISTOPATHOLOGY OF THYROID GLANDS

GROUP I - Histopathological studies revealed that the thyroid gland sections from Control rats (Fig - 5) showed normal thyroid follicles with cuboidal lining epithelium (E), filled with colloid (C) and Inactive follicles (F) with cuboidal lining epithelium. The Interstitium (I) appears normal.

GROUP II - Thyroid gland sections from rats, which served as Negative control (Fig - 6), showed predominantly active thyroid follicles with flattened lining epithelium, filled with minimal colloid. Interstitium showed edema and mild inflammatory infiltration.

GROUP III - Thyroid gland sections from rats, which served as Positive control (Fig - 7), showed normal thyroid follicles with greater thickness of the cuboidal lining epithelium, filled with maximal colloid and inactive follicles with cuboidal lining epithelium.

GROUP IV - Thyroid gland sections from rats, which received Low dose of EEMT (Fig - 8), showed thyroid follicles with mild increase in the thickness of the cuboidal lining epithelium, filled with moderate colloid and inactive follicles with cuboidal lining epithelium.

GROUP V - Thyroid gland sections from rats, which received High dose of EEMT (Fig - 9) showed thyroid follicles with moderate increase in the thickness of the cuboidal lining epithelium, filled with significant colloid and inactive follicles with cuboidal lining epithelium.

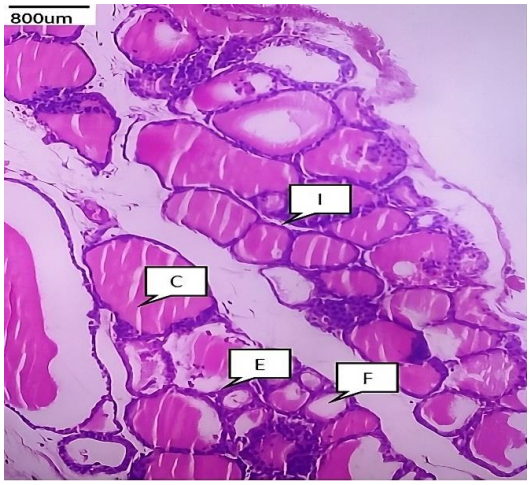


Figure - 5 GROUP I

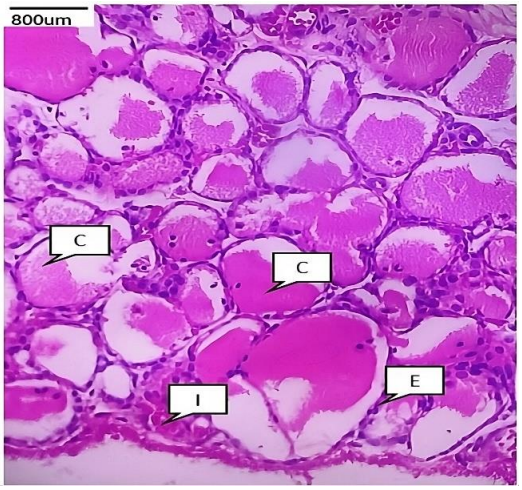


Figure - 6 GROUP II

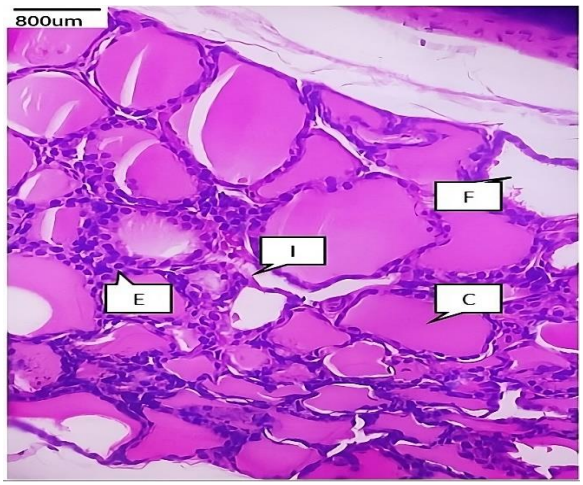


Figure - 7 GROUP III

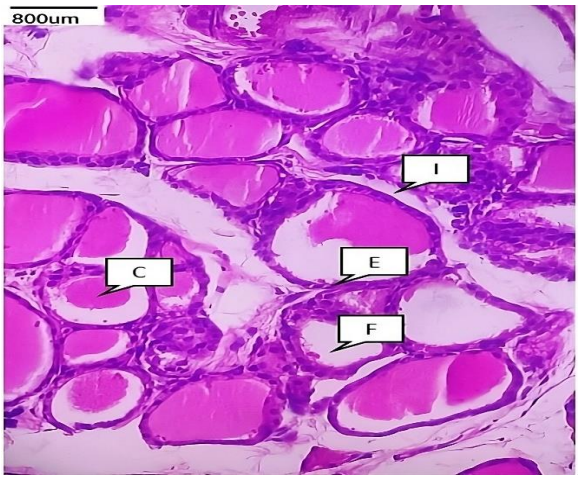


Figure - 8 GROUP IV

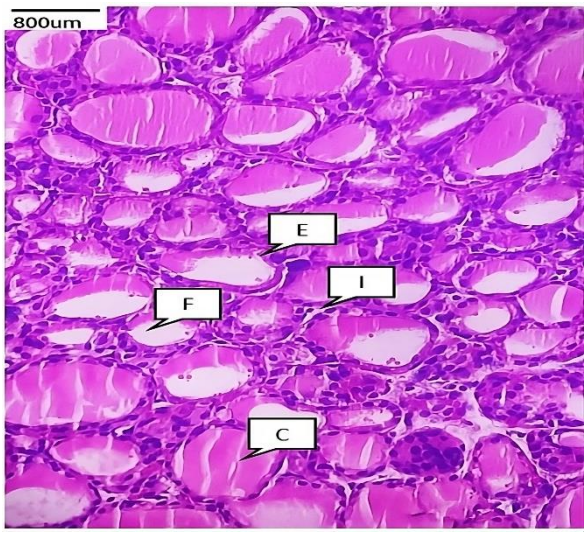


Figure - 9 GROUP V

E - Cuboidal lining epithelium

C - Filled with colloid

F - Inactive follicles

I - Interstitium

CONCLUSION

Based on the traditional use, secondary metabolites and their biological activities, the leaves of *Morinda tinctoria* Roxb. were chosen for the study. The Preliminary phytochemical screening of the Ethanolic Extract of *Morinda tinctoria* Roxb. (EEMT) reported the presence of Alkaloids, Phenols, Terpenoids, Flavonoids, Glycosides and Tannins. EEMT showed better Thyroid Peroxidase inhibitory activity in the *in vitro* Thyroid Peroxidase Inhibition Assay. Further, the *in-vivo* study was conducted with 5 groups of Male Albino wistar rats, 6 in each group using Thyroxine induced Hyperthyroidism model. Hyperthyroidism was induced in all animals using Thyroxine except Normal control rats. Standard and test groups were treated with Propylthiouracil and EEMT (low and high dose respectively) as per experimental design.

Various parameters were evaluated such as Body weight changes, Thyroid hormone profile and Histopathology of Thyroid glands. The results were analyzed using one-way ANOVA and P – values were calculated to find the statistical significance of the results in comparison with

the Standard drug, Propylthiouracil. Results showed that treatment with EEMT has been effective against Thyroxine induced Hyperthyroidism, by increasing Body weight, decreasing T_3 & T_4 levels and increasing TSH levels significantly. This was further confirmed by the histopathological examination of Thyroid glands.

From this study, it is concluded that the Ethanolic Extract of *Morinda tinctoria* Roxb. leaves (EEMT) has shown profound Anti-Thyroid effect against Thyroxine induced Hyperthyroidism which is similar to that exhibited by standard Propylthiouracil on both *in-vitro* and *in-vivo* evaluation. The study also provides strong evidence for the use of *Morinda tinctoria* Roxb. leaves in treating Hyperthyroidism. The activity may be due to the presence of Polyphenolic compounds and Flavonoids present in the Ethanolic Extract of *Morinda tinctoria* Roxb. leaves.

For future perspective, it can be further confirmed by molecular studies of the compounds responsible for the Anti-Thyroid activity to explore the exact mechanism of action by which the plant possesses the activity.

Acknowledgements: I would like to express my heartfelt gratitude to God for guiding and supporting me throughout the completion of my project. I am immensely grateful to my parents, Mr. Subramani and Mrs. Shanthi for their unwavering love, support and belief in me. I owe my deep gratitude to my project guide Dr. M. Sakthi Abirami, M. Pharm., Ph. D., Assistant Professor, Department of Pharmacology, College of Pharmacy, Madras Medical College, Chennai -03, for the watchful and in-depth guidance provided by her throughout my project. I would like to express my heartfelt gratitude to my Friends, Seniors and Juniors for their invaluable support throughout my project.

Conflict of interest statement: The Authors declared no conflict of interest.

REFERENCES:

1. Mathew P, Rawla P. Hyperthyroidism PubMed. Treasure Island (FL): StatPearls Publishing; 2020. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537053/
2. Wiersinga WM, Poppe KG, Effraimidis G. Hyperthyroidism: aetiology, pathogenesis, diagnosis, management, complications, and prognosis. The Lancet Diabetes & Endocrinology. 2023 Feb;11(4).
3. Medicine BUS of. Recognizing clinical signs of hyperthyroidism leads to appropriate treatments, reduces adverse impact on health. medicalxpress.com. Available from: https://medicalxpress.com/news/2023-10-clinical-hyperthyroidism-treatments-adverse-impact.html
4. Dixit Y, Panda S, Kar A. *Lagenaria siceraria* peel extract in the regulation of hyperthyroidism, hyperglycemia and lipid peroxidation in mice. Int J Biomed Pharm Sci. 2008;2(2):79-83.

5. Sivaraman D, Muralidharan P. Cytoprotective effect of *Morinda tinctoria* Roxb. against surgical and chemical factor induced gastric and duodenal ulcers in rats. Ulcers. 2011;2011.
6. Raju SK, Sekar P, Kumar S, Sundhararajan N, Nagalingam Y. Pharmacoinformatics and Antiangiogenic Activity of *Morinda tinctoria* Phytochemicals by Targeting Vascular Endothelial Growth Factor Receptor-2.
7. Sivakumar TH, Sivamaruthi BS, Priya KL, Kesika PE, Chaiyasut CH. Evaluation of bioactivities of Morinda tinctoria leaves extract for pharmacological applications. Evaluation. 2018 Feb 1;11(2):100-5.
8. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. India: Nirali Prakashan; 2008.
9. Habza-Kowalska E, Kaczor AA, Żuk J, Matosiuk D, Gawlik-Dziki U. Thyroid peroxidase activity is inhibited by phenolic compounds—impact of interaction. Molecules. 2019 Jul 30;24(15):2766.
10. Santhi T. Evaluation of Anti Thyroid Activity of *Asparagus Racemosus* Root Extract against Thyroxine Induced Hyperthyroidism in Rats. J of Pharmacol & Clin Res. 2019; 7(5): 555721.

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