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Anti-Thyroid Activity Of Myrica Salicifolia (Bayberry) Methanol Root Extract In Levothyroxine-Induced Hyperthyroidism In Male Wistar Albino Rats

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Abstract
Hyperthyroidism Is Defined As The Excess Production And Release Of Thyroid Hormones By The Thyroid Gland Resulting In Inappropriately High Serum Levels. The Most Common Causes Include Diffuse Toxic Goiter, Toxic Multinodular Goiter And Toxic Adenoma.Traditional Medicine Strategy By WHO Aims To Support Member States In Developing Proactive Policies And Implementing Action Plans That Will Strengthen The Role Traditional Medicine Plays In Keeping Populations Healthy. Myrica Salicifolia Is A Shrub Of 1 Meter In Height .It Belongs To The Family Myricaceae Found In The Subtropical Regions Of The World. The Aim Of This Study Was To Determine Antithyroid Activity Of Myrica Salicifolia (Bayberry) Methanol Root Extract (MSRE) In Levothyroxine-Induced Hyperthyroidism In Male Wistar Albino Rats. The Study Adopted A Controlled Experimental Study Design And The Study Site Was The SAFARI Animal House Of JKUAT. Forty Five Male Wistar Albino Rats, Acquired From The SAFARI Animal House Were Randomly Divided Into Five Groups. The First Group Served As The Normal Control And Received Distilled Water Only. At The Onset Of The Experiment, Rats In 2^{nd},3^{rd}, 4^{th} And 5^{th} Groups Were Treated With Levothyroxine In Order To Induce Hyperthyroidism. Three Rats From Group 2 Were Sacrificed On Day 14 And Blood Serum Processed For Immediate Confirmation Of Induction Of Hyperthyroidism Through Chemiluminescence Assay Test For TSH And T3. Subsequently, From Day 15 Of Experiment GP3 Rats Were Treated With Propylthiouracil (Standard Drug), GP4 Treated With Low Dose Of MSRE (200mg/Kg) And GP5 Treated With High Dose MSRE (400mg/Kg) For A Period Of 21 Days Post-Induction. During The Course Of The Experiment The Rats Were Closely Observed Daily For Any Behavioral And Clinical Changes And Body Weights Recorded Weekly. Serial Sacrifice Of Three Animals Per Group Was Done On Days 21 And 28 And Experiment Terminated On Day 35. Prior To Euthanization, Using CO_2 Gas, The Rats Were Fasted For 12 Hours. Fresh Blood Samples Were Obtained Through Intracardiac Puncture For Determining TSH And T3 Levels And The Thyroid Gland Harvested For Microscopic Examination. Prior To The Animal Experiment, Phytochemical Screening Of The Myrica Salicifolia Root Extract Was Done And It Was Found To Contain Alkaloids, Flavonoids, Sterols, Phenolics And Tannins. Acute Oral Toxicity Tests Of The Extract On LT4 Induced Hyperthyroid Rats Were Also Carried Out At Different Doses Of Extract, From 10mg/Kg To 5000mg/Kg Body Weight. No Mortality Nor Significant Behavioral Changes Were Recorded Except For Some Overcrowding And Reduced Activity For Rats Administered With 5000mg/Kg Body Weight Of The Root Extract. From The Current Study The LD50 Of M. Salicifolia Root Extract Was Found To Be > 5000 Mg/Kg Body Weight. Data Analysis For Hormone Levels Was Undertaken Using Statistical Package For Social Sciences (SPSS) -Version 21.0). Median (Interquartile Range-IQR) And Kruskal Wallis Test Were Employed In The Analysis And P-Value < 0.05 Was Considered Statistically Significant. Levothyroxine Administration Altered Thyroid Function By Significantly Decreasing Serum Levels Of TSH (P=0.0162) And Significantly Increasing T3 (P= 0.0081) Serum Levels In Group 2 Rats Sacrificed At Day 14, Confirming Successful Induction Of Hyperthyroidism. Treatment With The Standard Drug, PTU, Reversed The Trend By Significantly Increasing Serum TSH Levels On Days 21 (P=0.0022), Day 28 (P=0.0055) And Day 35 (P=0.0175) And Decreasing T3 Serum Levels Significantly On Days 28 (P=0.0066) And 35 (P=0.0016) Post-Treatment. Following Treatment With Low Dose (200mg/Kg Body Weight) MSRE (G4), There Was No Significant Change In Hormone Levels (P>0.05). Treatment With High Dose MSRE (G5) Led To Significant Increase Of T3 Serum Levels On Day 28 (P=0.0235) And Day 35 (P=0.0398) Accompanied By Non-Significant Decrease Of TSH. Comparing Serum Levels Of TSH And T3 Between Groups Treated With PTU And High Dose MSRE Did Not Show Any Significant Difference Over The Treatment Period. Histological Analysis Of Thyroid Gland In Normal Control Rats (G1) Showed Normal Cuboidal Epithelial Cells And Follicles Full Of Colloidal Material. Histopathology Of Thyroid Gland Showed Marked Changes In The

Follicular Cells Of The Treated Male Wistar Albino Rats, As Compared To The Positive Control And Normal Control Groups. Thyroid Follicles In Hyperthyroid But Untreated Rats (G2) Were Atrophied And Contained Scanty Colloid Material. Histological Examination Of Thyroid Glands From Formerly Hyperthyroid Rats Treated With PTU (G3) And MSRE Showed Normal Cuboidal Epithelium And Follicles Containing Colloid. From The Study Findings, It Can Be Inferred That The Rats Treated With The MSRE Concentrate(400mg/Kg) Shows A Great Effect As Like That Of The Standard Medication Hence, Upon All Findings And Assumption It Can Be Said That The Methanol Extract Of Myrica Salicifolia Root Extract Can Possibly Overcome Hyperthyroidism In Albino Rats .

***Key Words**:Thyroid Hormones, Hyperthyroidism, Levothyroxine, Myrica Salicifolia*

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I. INTRODUCTION

Background information

Hyperthyroidism occurs due to an inappropriately high synthesis and secretion of thyroid hormone (TH) by the thyroid (Bahn et al, 2011). TH increases tissue thermogenesis and the basal metabolic rate, and reduces serum cholesterol levels and systemic vascular resistance. The complications of untreated hyperthyroidism include weight loss, osteoporosis, fragility fractures, atrial fibrillation, embolic events, and cardiovascular dysfunction (Bartalena et al., 2013). The prevalence of hyperthyroidism is 1.2–1.6, 0.5–0.6 overt and 0.7–1.0% subclinical (Ross et al., 2016). The most frequent causes are Graves' disease (GD) and toxic nodular goiter. GD is the most prevalent cause of hyperthyroidism in iodine-replete geographical areas, with 20–30 annual cases per 100,000 individuals (Smith et al., 2016). GD occurs more often in women and has a population prevalence of 1–1.5%. Approximately 3% of women and 0.5% of men develop GD during their lifetime (Nystrom et al., 2013). The prevalence of hyperthyroidism in the United States is approximately 1.2% (0.5% overt and 0.7% subclinical) (Zimmerman et al., 2015). In Africa the epidemiology of thyroid dysfunction has proved more challenging to monitor due to a lack of comprehensive population-based studies (Okosieme et al., 2016). Existing studies are largely sourced from hospital-based cohorts that exclude large segments of the rural population and are unlikely to be representative of the general population (Ogbera et al, 2011). Recent hospital-based studies from Ghana show that contrary to earlier reports, Graves' disease is not uncommon, comprising 54% of all cases of thyroid dysfunction (Sarfo et al., 2017). While this may be due to improvements in iodine nutrition, subsequent, subsequent studies in the aftermath of iodisation in Ghana have shown marked increases in the incidence of both Graves' disease and nodular disease suggesting a role for improved diagnosis (Sarfo et al., 2017). It is instructive to note that thyrotoxicosis is a notable cause of cardiac morbidity in this part of the word. In a report from Togo, cardiac complications were documented in 46.6% of patients with thyrotoxicosis. (Ogbera et al, 2011). At present, more than 80% of the world's population relies on ethnopharmacologic healing modalities and plants for their primary health care and wellness(Pan et al.,2018) Due to cultural acceptability, physical accessibility, and economic affordability as compared with modern medicine, traditional medicines are used widely in Ethiopia (Asnake et al., 2015) and it is estimated that about 90% of the population is dependent on traditional medicine, essentially plants Nureye et al ., 2018) Myrica salicifolia A Rich (Myricaceae) is a shrub of 1 m in height and is found in several central and east Africa countries such as Burundi, Ethiopia, Kenya, Malawi, Rwanda, Tanzania, Uganda, and Zaire (Bordolo et al., 2014). In kenya M. salicifolia is known by the number of vernacular names in Kenya, Olkitoloswa (Maasai), Mukikia, Muthogoya (Kikuyu) Kibogen (Marakwet) and Kabuneto (Kipsigis) (Korir et al., 2015). In the Marakwet community in Kenya, its powder is taken to treat hyperthyroidism (Korir et al., 2015). The study indicates that local people along with local herbalist use M salicifolia A Rich (Myricaceae) root and bark extract with tea for aliment of different disease such as chest congestion, pneumonia, diarrhea, nervous disorders, diabetes, hypertension, and respiratory diseases (Maara et al.,2014). To verify the traditional uses of M salicifolia, various in vitro and in vivo studies have been conducted. The studies showed that this plant has most of the claimed activities, including antibacterial activities against Gram negative bacterial strains namely Pseudomonas aeruginosa, Klebsiella oxytoca, Proteus mirabilis, Klebsiella pneumoniae, Salmonella kisarawe, Salmonella typhi, and Escherichia coli. (Kilonzo et al., 2016). The plant is a cough suppressant, and possesses wound healing and aphrodisiac activities (Kariuki et al 2014) Studies have shown that the root extract of M salicifolia A Rich (Myricaceae) is a non hypnotic central nervous system depressant with muscle reluctant, analgesic, hypothermic, and antipyretic properties Kariuki et al.,2014). Its widely used herbal leaf extract proved to be a central nervous system depressant effect (Eshete et al.,2016). Similar study reveals that the root of M salicifolia A Rich (Myricaceae) is used as slow acting medicine in stomach problems and headache (symptoms associated with malaria), the barks chewed for toothache problem whereas powdered young leaves are used to treat skin infections (Gakio et al.,2004).The root part of M salicifolia has been used in the treatment of malaria

orally as claimed in Ethiopia and Uganda (Seifu et al.,2020) So, this study is designed to Antithyroid activity of Myrica salicifolia (bayberry) methanol root extract in levothyroxine-induced hyperthyroidism in male Rats.

Study Objective

To determine the antithyroid activity of *Myrica salicifolia* (bayberry) methanol root extract in levothyroxine-induced hyperthyroidism in male wistar albino rats.

Specific Objectives

1. To determine the phytochemical compounds in *Myrica salicifolia* methanol root extract.
2. To determine the acute toxicity profile of myrica salicifolia in male wistar albino rats
3. To determine the serum levels of T3 in levothyroxine-induced hyperthyroidism in male wistar albino rats before and after treatment with *Myrica salicifolia* methanol root extract.
4. To determine the serum levels of TSH levothyroxine-induced hyperthyroidism in male wistar albino rats before and after treatment with *Myrica salicifolia* methanol root extract?
5. To determine the thyroid gland histological changes in levothyroxine-induced hyperthyroidism in male wistar albino rats before and after treatment with *Myrica salicifolia* methanol root extract?

Study site

The study was carried out at the small animal facility for research and innovation (SAFARI), of the Jomo Kenyatta University of Agriculture and Technology (JKUAT). SAFARI animal house is located next to the College of Health Sciences complex. The rats were kept in appropriate animal cages and taken care of by trained animal technicians. All procedures were conducted in the SAFARI house procedure room.

Blood specimens were analyzed in the Biochemistry laboratory at Thika Level 5 Hospital, Kiambu County while tissue specimens were processed in the histology laboratory at JKUAT.

Study design

Controlled experimental, laboratory-based study.

Study population

Wistar albino rats of the species of *Rattus Norveginicus* from a pure breed were purchased from SAFARI animal house at JKUAT and housed at the same place in standard cages that measured 330 cm cubed and the floor was 60cm2 for the rats. Each cage accommodated six rats. The wistar albino rats (*Rattus norveginicus*) have long ears, short tail compared to their body size and their head is characterized to be wide (Pritchett & corning 2016). The wistar albino rats (*Rattus norveginicus*) were preferred because of the; share 90% of the genome with human being, they have a relatively high survival rate, big body size as compared to mice but small and easy to take care of them, they have a short gestational span (4 weeks) hence easier to find the study subjects, they are also resilient in withstanding most of the study medicine, male are always larger as compared to females approximately 450-650grams and 350-450 grams respectively (Bailey *et al*., 2014).

Sample size calculation.

Sample size was arrived at using the resource equation method (Charan and Biswas (2013).
E = Total number of animals - Total number of groups (The value of E should lie within 10 to 20 for optimum sample size)
Number of groups: **5**
Total number of animals: **15**
E value is: **10**
Each group had 3 animals and 20 percent was added to cater for non- response and mortality, totaling to 18 rats.

Selection of Laboratory Animal

Rats are the most commonly used animals in the study of thyroid disorders. Animals such as sheep, cats, dogs, rabbits and guinea pigs are also used.

In the present study rats have been used because the thyroid hormone production and metabolism of rats resembles that of humans which is believed to contribute to hyperthyroidism studies (Chakrabarti et al., 2007).

Animal acquisition and feeding

Rats were purchased from SAFARI animal house at JKUAT and housed at the same place in standard cages that measured 330 cm cubed and the floor was 60cm2 for the rats. Each cage accommodated six rats. The

rats had free access to rat pellets sourced from Unga Feeds Limited, Kenya and water ad libitum. They were handled humanely and the rules and regulations of SAFARI animal house were adhered to at all times.

Collection of plants and preparation of extracts

Five kilograms of medicinal plant part (root) of *M. salicifolia* was collected from Timboroa forest in Elgeyo Marakwet, Kenya, about 15 km from Eldoret town in October 2020, by the researcher with the help of a plant taxonomist from JKUAT. Authentication was achieved by comparison with Herbarium specimens by taxonomists and a voucher specimen was deposited at the JKUAT herbarium (voucher number of YK001). The roots were chopped and air dried under shade in ambient temperature for three weeks. The dried roots were ground into coarse powder using an electric grinder made at the Mechanical Engineering Department in JKUAT. Organic extraction was done using petroleum ether, dichloromethane (DCM), ethyl acetate and methanol by cold maceration, whereas aqueous extraction was done using hot maceration. Organic extraction was done by adding 500 mL of petroleum ether, methanol, DCM and ethyl acetate to each quantity of 50 g of the *Myrica salicifolia* root powder for 72 hours by cold maceration then the extract was concentrated by use of a rotary evaporator (BUCHI Vac® V-500) at 45°C and stored at 4°C (Kisangao et al., 2007). In aqueous extraction, 50 g of the powder was added to 500 mL of distilled water in a 1 L flask then boiled for 15 minutes. The boiled mixture was then filtered using Whitman No. 1 filter paper and the extract were freeze-dried using a freeze dryer (BUCHI Lyovapor™ L-300). The lyophilized sample was kept at 4°C (Menino et al., 2019).

Materials

- Animals: male wistar rats (200gms -250gms)
- Drugs
- L-Thyroxine (T4) (Sigma, USA
- Propylthiouracil (Macleods Pharmaceuticals Ltd)
- Methanol extract of myrica salicifolia

Experimental design.

Forty-two male albino rats were weighed and randomly assigned into 5 experimental groups. Each group had 3 animals. Group 1 consisted of normal controls that received distilled water and rat pellets only for the whole course of experiment without intervention. Group 2 was the negative control, out of which 3 rats were sacrificed on day fourteen to confirm hyperthyroidism and histopathological changes of the thyroid gland following induction with levothyroxine. The 3rd group is positive control which was treated with propylthiouracil, the standard drug while 4th, and 5thgroups and their respectful subgroups were hyperthyroid and treated with low dose MSRE and high dose MSRE respectively. The reason for having subgroups is because sub group a, b and c were sacrificed on 21st, 28th and 35thday respectively of the experiment. Grouping and treatment is as follows.

Group 1- normal control: animals were not induced with hyperthyroidism and were not treated. The group only received distilled water.

Group 2-negative control: 12 Wister rats received levothyroxine (600ug/kg/ml) of which 3 animals were then sacrificed on day 14 to confirm hyperthyroidism and histopathological changes in the thyroid gland. The remaining 9 hyperthyroid animals did not receive treatment for 21 days and were sacrificed as follows:

Sub group II-3 animals sacrificed on 21st day of experiment

Sub group II b -3 animals sacrificed on 28th day of experiment

Sub group II c- 3 animals sacrificed on 35th day of experiment

Group 3 -9 hyperthyroid animals were treated with the standard drug (PTU 10mg/kg) for 21 days and sacrificed as follows:

Sub group III-3 animals were sacrificed on 21st day of experiment

Sub group III b -3 animals were sacrificed on 28th day of experiment

Sub group III c- 3 animals were sacrificed on 35th day of experiment

Group 4 -9 hyperthyroid animals were treated with (MSRE 200mg/kg) for 21 days and sacrificed as follows:

Sub group IV -3 animals were sacrificed on 21st day of experiment

Sub group IV b -3 animals were sacrificed on 28th day of experiment

Sub group IV c- 3 animals were sacrificed on 35th day of experiment

Group 5 -9 hyperthyroid animals were treated with (MSRE 400mg/kg) for 21 days and sacrificed as follows:

Sub group V -4 animals were sacrificed on 21st day of experiment

Sub group V b -4 animals were sacrificed on 28th day of experiment

Sub group V c- 4 animals were sacrificed on 35th day of experiment

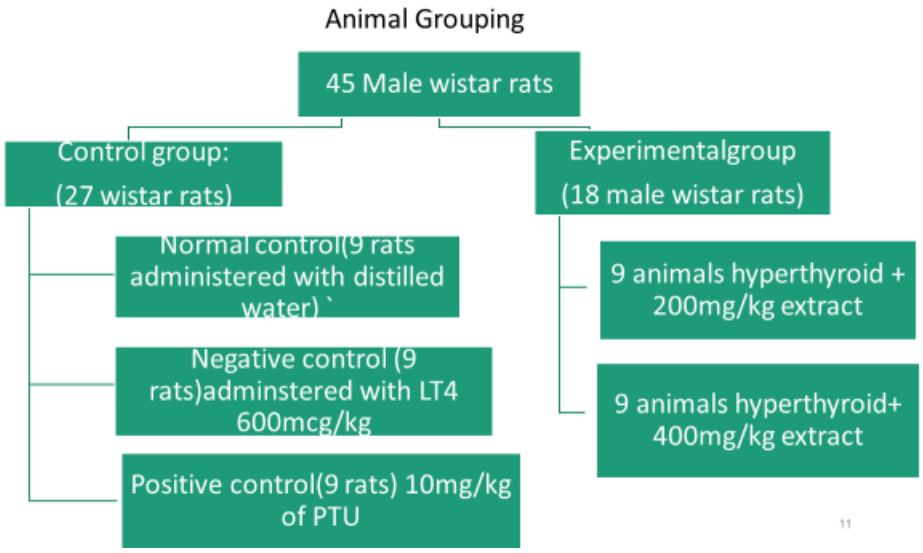


Figure 1: Animal Grouping

Base line measurements before induction of hyperthyroidism
Using electronic weighing machine, the animals were weighed and their body weights recorded.

Preparation of levothyroxine solution
The required quantity (600 ug/kg) was dissolved in 5ml of water and given by gavage

Experimental induction of hyperthyroidism
Levothyroxine at a dose of 600 ug/kg body weight administered by gavage daily for 14 days induces hyperthyroidism in rats (Chakrabarti et al., 2007).

Confirmation of hyperthyroidism
Induction of hyperthyroidism was confirmed by analyzing the serum TSH, and T3 levels on day fourteen after induction using Chemiluminscence assay method

Principle of the Assay for TSH.

The CLIA kit test (Human) utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the intact TSH molecule. Rat monoclonal anti-TSH antibody is used for solid phase (microtiter wells) immobilization and a goat anti-TSH antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the TSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 60 minutes incubation at room temperature, the wells are washed 5 times by wash solution to remove unbound anti-TSH conjugate. A solution of chemiluminescent substrate is then added and read relative light units (RLU) in a Luminometers. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of TSH in the sample. By reference to a series of TSH standards assayed in the same way, the concentration of TSH in the unknown sample is quantified.

TSH Assay procedure

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. It's recommended that all samples and standards be assayed in duplicate.

1. Add Sample: Add 100µL of Standard, Blank, or Sample per well. The blank well is added with Reference Standard & Sample Diluent. Solutions are added to the bottom of micro CLIA plate well, avoid inside wall touching and foaming as possible. Mix it gently. Cover the plate with sealer provided. Incubate for 90 minutes at 37℃.
2. Biotinylated Detection Ab: Remove the liquid of each well, don't wash. Immediately add 100µL of Biotinylated Detection Ab working solution to each well. Cover with the Plate sealer. Gently tap the plate to ensure thorough mixing. Incubate for 1 hour at 37°C.
3. Wash: Aspirate each well and wash, repeating the process three times. Wash by filling each well with Wash Buffer (approximately 350µL) (a squirt bottle, multi-channel pipette, manifold dispenser or automated washer are needed). Complete removal of liquid at each step is essential. After the last wash,

remove remained Wash Buffer by aspirating or decanting. Invert the plate and pat it against thick clean absorbent paper.

4. HRP Conjugate: Add 100μL of HRP Conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 minutes at 37°C.
5. Wash: Repeat the wash process for five times as conducted in step 3.
6. Substrate: Add 100μL of Substrate Mixture Solution to each well. Cover with a new Plate sealer. Incubate for not more than 5 minutes at 37°C. Protect the plate from light.
7. RLU Value Measurement: Determine the RLU value of each well at once after the substrate reaction time. You should open the Chemiluminescence immunoassay analyzer ahead, preheat the instrument, and set the testing parameters.
8. After experiment, put all the unused reagents back into the refrigerator according to the specified storage temperature respectively until their expiry.

T3 Assay procedure summary

1. Prepare all reagents, samples and standards;
2. Add 50μL standard or sample to each well.
   And then add 50μL prepared Detection Reagent A immediately.
   Shake and mix. Incubate 1 hour at 37°C;
3. Aspirate and wash 3 times;
4. Add 100μL prepared Detection Reagent B. Incubate 30 minutes at 37°C;
5. Aspirate and wash 5 times;
6. Add 90μL Substrate Solution. Incubate 10-20 minutes at 37°C;
7. Add 50μL Stop Solution. Read at 450 nm immediately.

3Determination of *Myrica salicifolia* methanol extract and PTU dosages

The dosages of MSRE extracts used in this study were selected based on the previous reports in which 100mk/kg of MSRE showed enough in vivo pharmacological effects in rats (B. Lee et al., 2009). And PTU 10mg/kg was selected based on previous in vivo efficacy test on levothyroxine induced hyperthyroidism in rodents (S. Panda et al., 2007).

Administration of *Myrica salicifolia* methanol root extract

For a period of 21 days of experimentation, the extract was administered by oral gavage to respective groups between 11:00 and 12:00h of the day to avoid circadian interference.

PTU administration

PTU was administered by oral gavage, in a dosage of 10 mg/kg prepared as (2mg/5mls), dissolved in distilled water.

Body weight measurements

All the animals were weighed and their body weights recorded on day 0, 7 and 14 of experiment,

Estimation of Biochemical Parameters

Blood samples were collected at the SAFARI animal house at JKUAT lab on 21st , 28th and 35th day after treatment by cardiac puncture after anaesthetizing the rats with carbon dioxide narcosis and sacrificing them. 6mls of blood was collected from each rat. The samples were carefully introduced into plain vacutainers free from anticoagulant, properly labelled and transported immediately under room temperature to JKUAT Laboratory for Immunology laboratory where hormonal assay was done. The blood samples were allowed to clot, retract and then centrifuged for 5minutes at a speed of 3000 revolutions per minute. The serum was then aliquoted and, refrigerated at -20°C waiting assaying of the hormones.

Humane sacrifice of the animals and collecting specimens

The procedure of anaesthetizing the animal and collecting the specimens

The rat was put into the bell jar containing cotton wool or gauze and cover via a plastic tubing coated to a regulator attached to the gas cylinder. 70% concentrated carbon dioxide or more was introduced into the bell jar for 1 minute. After 3 to 5 minutes the anaesthetized rats were removed from the bell jar and were mounted onto the board using mounting pins with dorsal side of the board. Using a pair of scissors and forceps the animal was cut through the ventral medial side from the symphysis pubis to the sternal angle of the thoracic cage. Approximately 1 ml of serum is required per duplicate determination. 4-5 ml of blood were collected into an appropriately labelled tube and allowed to clot. Centrifuge was carefully done and the serum layer was

removed. Serum was stored at -20 for analysis to be done at a later date. All human specimens were considered as possible bio hazardous materials and taken appropriate precautions when handling. The remaining blood in the heart was cleared using physiological saline (200 mls of 0. 85%) after sufficiently clearing, the saline drip is removed and the desired fixator was introduced and the firmness of the tail was checked as an indicator of effective fixation. Finally, the thyroid gland was excised and immersed in a clearly labelled container with fresh fixative to continue fixation for 12 hours.

Induction of hyperthyroidism

After acclimatization, hyperthyroidism was achieved by daily oral administration of L-Thyroxine (T4) (Sigma, USA) at a dose of 600µg/kg for 14 consecutive days according to the previous established method. (Chakrabarti et al., 2007). Levothyroxine acts like the endogenous thyroid hormone thyroxine (T4, a tetra-iodinated tyrosine derivative). It is a synthetic form of the thyroid hormone thyroxine, which is normally secreted by the follicular cells of the thyroid gland. L-thyroxine is commonly used to produce hyperthyroidism in experimental animals due to its ability to be converted into T3, the active form in the liver and kidney by the enzyme 5'-deiodinase.

Preparation of Drugs:

- Methanol extract of *Myrica salicifolia* root extract was dissolved in sterile water.
- Propylthiouracil tablets were weighed, powdered and titrated with saline. The dosages of MSRE extracts used in this study were selected based on the previous reports in which 100mk/kg of MSRE showed enough in vivo pharmacological effects in rats (B. Lee et al., 2009). propylthiouracil 10mg/kg was selected based on previous in vivo efficacy test on levothyroxine induced hyperthyroidism in rats (S. Panda et al., 2007).

Phytochemical Screening

The solution of Petroleum ether, chloroform, ethylacetate, methanol and ethanolic extract was prepared using distilled water and subjected to preliminary phytochemical screening. Test for common phytochemicals were carried out by standard methods described in practical pharmacognosy by Kokate, Khandelwal and Trease and Evans (Pan et al 2018) Phytochemical screening was done by observing precipitate formation and color change (Getahun 2004).

Saponins test (foam test)

1 ml of the extract was put in a test-tube then 50mls of tap water was added. The mixture was shaken vigorously for 15 minutes. Formation of honeycombs foam that persists for 15 minutes after shaking was subjected to a confirmatory test, which involved dissolving 1 ml of the extract in anhydride tetrachloride to which 5 ml of concentrated sulphuric acid was added on the mixture. A blue, green or red color accompanied with a pink ring was indicative of saponins

Alkaloids (mayers reagent)

1ml of extract was tested with Mayer's reagent prepared by dissolving 35gm of mercury chloride in distilled water and a solution of 5 gm. potassium iodide in 10mls of water. the mixture then was diluted to 100mls. The appearance of opalescence or yellow color indicted presence of alkaloids.

Flavonoids (alkaline reagent test)

5 mL of hydrochloric acid solution and magnesium turnings was added to myrica salicifolia root extract. Appearance of a pink or magenta red was indicative of the presence of flavonoids (Getahun 2004).

Sterols and steroids (Salkowaski method)

Salkowaski method was used. To 1 mL of extract in a test tube, 0.5 mL acetic anhydride and 0.5 ml chloroform were added. Concentrated sulfuric acid was slowly added along the sides of the test tube. A red coloration was an indication of the presence of sterols and a green color was indicative of presence of steroids (Nureyev et al., 2018).

Tannins test (Braymer's test)

1ml of extract was dissolved in water in which 1 %gelatin and 10% sodium chloride and salt solution (10%Nacl) were added. Allow tannins were indicated by presence of a blackish blue color while catechol tannins were indicted by a greenish back coloration.

Acute toxicity test of *M. salicifolia* extract

The acute toxicity was determined by use of modified Lorke's method (Lorke 1984).

Fifteen male wistar albino rats weighing approximately 200 -250 grams were obtained from SAFARI animal Biomedical department in Jomo Kenyatta University of Agriculture and Technology (JKUAT). They were housed in standard rat cages and exposed to 12hour light/dark cycles under humid tropical conditions. Litter papers in the cage was changed on alternate days. Each cage was labelled with a cage card showing experiment number, date of starting the experiment, dosage level, and age, number of animals, species and sex of the animal. The rats were allowed unrestricted access to standard feed Rodent pellets obtained from UNGA Mills and water ad libitum throughout the experimental period. The rats were handled in accordance with the guidelines for the care and use of laboratory animals.

The acute toxicity study was conducted in two phases. The animals were fasted overnight prior administration of the methanol root extract of *Myrica salicifolia*. Phase one; four groups each with 3 rats, group 1, 2 & 3 animals were administered with single oral dose of 10, 100 & 1000 mg/kg of the root extract in 5% dimethyl sulfoxide (DMSO), respectively. Group 4; was the control group with three rats, which were given 5% DMSO in distilled water (5 ml/kg body weight).

Phase II; had three animals, and each received a single oral dose of 1600, 2900 and 5000 mg/kg of the bark extract in 5% DMSO respectively. The root extract was administered orally using sterile gavage needles. All the animals were monitored closely for signs of toxicity which are mortality, changes in gross appearance of the skin and fur, mucous membrane of the eye, respiratory distress, somatomotor activity, behaviour, and special attention was given to observation of tremors, salivation, diarrhoea, coma and convulsions, changes during the first 48 hours post dosing. The observation schedule was as follows; immediately, ½ an hour, 1 hour, 4 hours, 24 and 48 hours, then monitoring for signs of toxicity continued daily for 14 days. The body weight was monitored as follows; day 0 (initial weight), day 7 and day14 (terminal weight)

Then the LD50 was calculated by the formula;

$LD50 = \sqrt{D0 * D100}$

D0 = Highest dose that gave no mortality

D100 = Lowest dose that produce mortality

Histological studies

Histological studies were carried out in JKUAT histology laboratory. The first group to be sacrificed was the negative control group to confirm hyperthyroidism and the histological changes in the thyroid gland. The 2nd 3rd and 4th sacrifice was done on days 21st, 28 and 35th in all subsequent groups of the experiments descried in the experimental design.

Material used for staining thyroid sectioned for histology

-DPX mount
-specimen bottles
- Paraffin wax
-slide holders
-microtome
-dropper
-toluidine solution
-formaldehyde 40% concentration
-Rotary microtome
-the specimen (the kidney)
-distilled water
-glass staining square jars
-zenkers solution (acetic acid 5mls and distilled water)
-xylene
-wood blocs
-isopropyl alcohol
-beakers
-egg albumin
-dropper
-cedar wood oil
-heater and water bath container

Procedure for processing the thyroid specimens for the light microscopy

i. The thyroid glands were fixed in formaldehyde solution for a period of 24 hours.
ii. The thyroid gland tissues were dehydrated in ascending concentration of alcohol that is 50%>60%>70%>80%>90%>95>100% each for one hour.

iii. Were cleared with xylene.
iv. Then infiltrated with paraplast wax for about 12hours at 56%.
v. The infiltrated tissue was embedded on paraffin wax on a wood blocks.
vi. 4 micrometer thick sections were cut using leitz sledge rotary microtome.
vii. The cut section was floated on water at 37^0 to spread the tissue.
viii. The section was stacked onto glass slides firmly by micro- dropper.
ix. The slides were dried in an oven at about 37% for 24 hours.
x. The slides were stained with hematoxylin and eosin (H&E).

Procedure to be followed in taking photomicrograph
i. The slides were mounted on the stage of the microscope.
ii. The focus was adjusted until the image to be photographed to be in focus.
iii. The field magnified appropriately.
iv. The photographs of the regions were taken at best under the focus.
v. The photograph were transferred to the computer, stored and uploaded using adobe fireworks application.

Photography
Materials needed
i. Memory
ii. Histological glass slide
iii. Digital camera

Procedure followed in taking photomicrographs
i. The prepared tissues were mounted on the stage of the microscope.
ii. Thereafter the images were focused and adjusted to be photographed.
iii. The images were viewed under the focus of the microscope and the photographs were taken.
iv. The photographs were transferred into the memory cards.
v. The images were uploaded using the adobe application.

Statistical analysis

Data entry was done using Microsoft excel and later exported to SPSS V.21 for analysis. Normality test was performed using the Shapiro wilks test with Ho being C A B D 35 that data follows the normal distribution. Since the data failed the normality test (skewed), it was summarized using median interquatile range (IQR) and variance among the groups and across time was tested using the non-parametric alternative to Anova (Kruskal Wallis test). Trend analysis was done to establish significant changes in estimates with increase in time. Significance was set at $p<0.05$(95% confidence interval). The results were presented in terms of column graphs and tables.

Ethical consideration

Ethical approval was sought from the Animal Ethics Committee for Care and Use of laboratory animals of Jomo Kenyatta University of Agriculture and Technology. Animal experimentation was carried out in a Level 2 Biosafety laboratory. All activities during the studies conformed to accepted principles for laboratory animal use and care (EU directive of 1986: 86/609/EEC). All the technical team observed institutional biosafety guidelines for protection of personnel and laboratory. Animals were handled in a humane manner and were monitored twice daily for any ill health related to experimental interventions. Sacrificing of the animals was after euthanizing using an overdose of concentrated carbon dioxide. Reduction: The resource equation method was employed to reach at the desired scientific sample size that led to reliable, valid and a robust research. Refinement: paper shredding for enrichment, proper and standard housing was provided. A stand-by veterinary doctor was available to identify and take care of sick study animals. For humane end point, all animals were to be moved to fresh air chamber immediately they developed body weakness. Animals were euthanized on day 35 of the experiment. All animals having laboured breathing, acute weight loss of more than 20% of baseline body weight and inability to ambulate were to be removed from the experimental group and euthanized.

II. RESULTS

Acute oral toxicity of *Myrica salicifolia*
Mortality rate and behavioral observations

The *Myrica salicifolia* methanol root extract was administered with the aim of establishing a standard dose that can cause toxicity and marked distress to the animals. There was no mortality observed within the first 48 hours and during the entire 14 days of observation in all groups administered with methanol root extract of

Myrica salicifolia. General behaviour of each animal was observed for the first 30 minutes,1 hour, 4 hours, 24 hours, 48 hours and then daily for entire 14 days. The following general parameters were normal for the entire period of observation; fur appearance, alertness, muscle tone on hind limbs, pain, feeding, activity, respiratory rate apart from overcrowding and inactivity that was observed at a dose of 5000mg/kg.

Table 1: mean body weight +/- SEM for *Myrica salicifolia* treated groups and the control group

M.salicifolia (mg/kg)	**Fasting day**	**Day 0**	**Day 1**	**Day 7**	**Day 14**
Control group	177.80±2.747	171.90±1.751	178.67±0.998	184.78±1.358	188.95±2.193
1600	183.74±1.529	168.21±3.292	182.51±1.150	188.63±3.549	194.97±3.703
2900	182.00±1.8179	9.11±1.67	177.09±1.58	176.21±2.11	178.00±1.20
5000	176.95±4.084	165.02±5.269	173.63±4.713	180.27±4.159	183.34±4.709
p-value	0.275	0.639	0.393	0.365	0.270

Table 1: Acute toxicity of M.salicifolia root extract as per behavioral observation and mortality rate

Experiment	**Doses (Mg)**	**Observation hours Immedi**	**½ hour**	**1 hour**	**4 hours**	**24 hrs**	**48 hrs**	**Mortality**	**Mortality rate (%)**
Phase I (M.E in distilled H20)	10 100	Normal activity	Normal activity	Normal activity	Normal Activity	Normal activity	Normal activity	0/3 0/3	0 0
		Normal activity	Normal activity	Normal activity	Normal Activity	Normal activity	Normal activity		
	1000	Normal activity	Normal activity	Normal activity	Normal Activity	Normal activity	Normal activity	0/3	0
Control [Distilled water]	2mls	Normal activity	Normal activity	Normal activity	Normal Activity	Normal activity	Normal activity	0/3	0
Phase II (M.E in distilled H20)	1600 2900	Normal activity Normal activity	Normal activity Normal activity	Normal activity Normal activity	Normal Activity Normal activity	Normal activity Normal activity	Normal activity Normal activity	0/1 0/1	0 0
	5000	Over crowding	Over crowding	Over crowding	Over Crowding	Normal activity	Normal activity	0/1	0

Table 2: Median (IQR) statistics for FT3 and TSH levels by day of sacrifice and treatment groups.

Variable	**Day**	**Normal**	**Negative**	**Standard**	**Low Dose**	**High Dose**
FT3	21	2.7 (0.3)	4.3 (0.15)	2.98 (0.25)	2.55 (0.1)	2.81 (0.19)
	28	2.7 (0.3)	4.3 (0.15)	2.1 (0.11)	2.55 (0.1)	2.12 (0.21)
	35	2.8 (0.2)	3.3 (0.35)	1.94 (0.03)	2.24 (0.14)	2.2 (0.18)
TSH	21	3.2(0.96)	0.7 (0.15)	2.42 (0.03)	2.2 (0.12)	2.42 (0.03)
	28	3.1(0.95)	0.9 (0.5)	2.46 (0.02)	2.4 (0.06)	2.41 (0.12)
	35	3.4 (0.98)	0.42 (0.05)	2.45 (0.06)	2.35 (0.05)	2.46 (0.02)

As shown in the table 3 above, there is a variation in FT3 hormone levels between the days of sacrifice – within each treatment, and, also between the treatments. The negative control group (rats which received distilled water after being induced by hyperthyroidism) has a median of 4.3 for rats sacrificed on day 21 and 28 then median reduced to 3.3 for the rats sacrificed on day 35. Data observed on day 35 for the negative control had a wider spread of FT3 levels compared to day 21 and 28. There is a general decrease in the observed median FT3 levels from day 21 to day 35. Negative group expresses higher FT3 levels compared to the rest of groups. Less variance is observed in the low dose between values observed in days 21,28 and 35 compared to the standard dose and the high dose of the MSRE. The observed median FT3 value (2.7) observed on the normal group (group not induced with hyperthyroidism and received neither of the treatment) is higher than the medians observed on the standard group, low and high dose, however, it is lower than the negative group.

The negative group TSH observed median levels are generally low compared to the other treatment groups. There is an overall increasing trend in the TSH levels from day 21 to day 35. Also, the negative group levels have a higher spread than the rest of the groups except the normal group, which has a wider spread compared to the rest for day 35. Although the observed medians for the standard, low and high dose groups are comparable, the standard groups had higher observed medians compared to the other investigational extract subgroups for the three time points. For day 35, the observed median TSH value in the normal group (3.4) was higher than the observed values in the other treatment groups as shown in the column graphs below.

The FT3 hormone levels explicit higher between and within treatment variation compared to the TSH hormone levels as shown figures 1 and 2 respectively. Among the active treatments, TSH shows very minimal variance between treatments, with day 21 showing lower levels compared to later time points.

Figure 4: Column graph showing the distribution of the observed FT3 values by treatment groups and colored by timepoints.

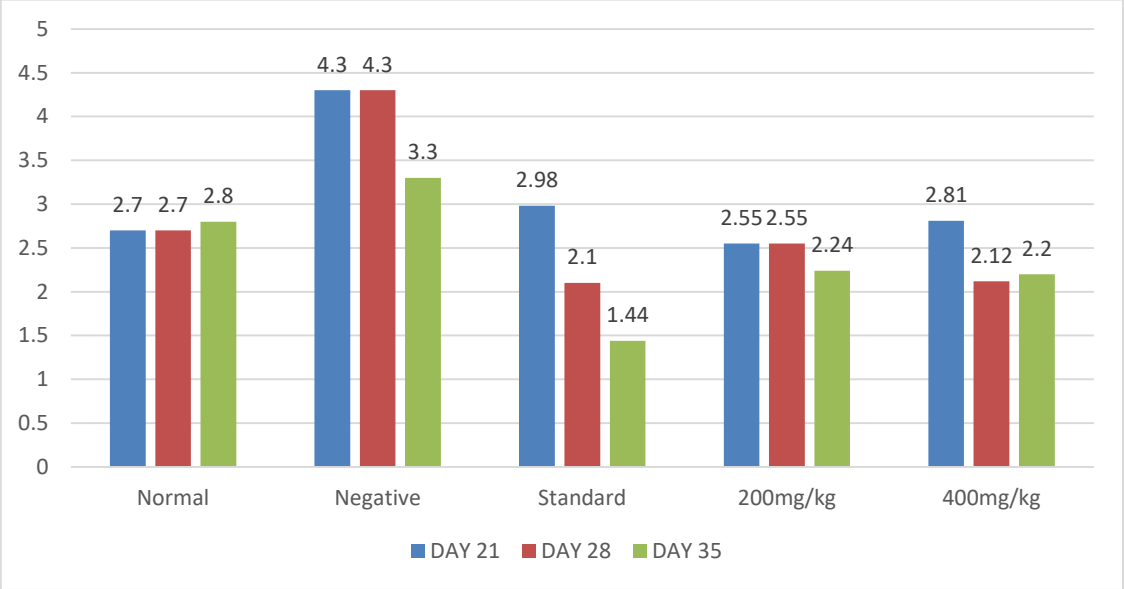
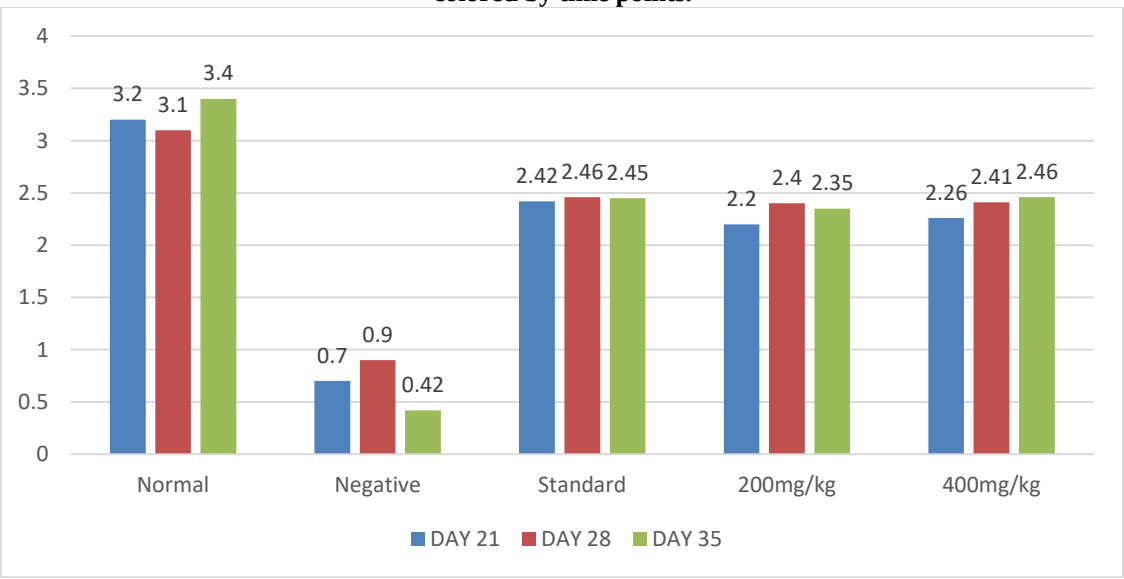


Figure 5; Column graph showing the distribution of the observed TSH values by treatment groups and colored by time points.



Between treatment groups differences

Table 6: Kruskal Wallis test – test for differences among groups (Normal, Negative, Standard, Low Dose, and High Dose).

Variable	Day	Chi-square value	P value
FT3	21	7.3333	0.062
	28	8.8974	**0.0307**
	35	12.3804	**0.0147**
TSH	21	9.6185	**0.0221**
	28	7.9123	**0.0479**
	35	11.746	**0.0193**

Kruskal-Wallis H test was performed on the observed FT3 and TSH levels to determine any statistically significant differences between the treatments by time points. By comparing the FT3 levels among the treatment groups, neither of any treatment group pairs would express any statistically significant differences at 5% level of significance at day 21. However, the Kruskal-Wallis H test results were significant for days 28 and 35 – indicating that the observed FT3 levels were significantly different in at least two treatment groups. For the TSH, all the three time points shown significant results. Therefore, to determine the specific treatments that has these significant differences, Dunn's post-hoc tests were performed, and the results are shown in table 4.5 below.

After the post-hoc tests, it was observed that the significant differences in FT3 levels observed in day 28 and 35 were between high dose and Negative, and Standard and negative treatment groups for both time points. Also, the Normal and Standard groups had significantly different FT3 levels at day 35.

The observed differences in TSH levels among treatment groups were between Negative and Standard groups across time points. In addition, Low-dose and Normal, and Negative and Normal groups showed significantly different TSH levels at 5% level of significance.

Table 7:Dunn's post-hoc tests for differences in ft3 and TSH levels between treatment groups – Z-statistic (unadjusted p-values) are presented.

Variable	Comparison (Treatments)	Day-21	Day-28	Day-35
ft3	High Dose - Low Dose		-1.1323 (0.2575)	-0.1371 (0.891)
	High Dose - Negative		**-2.2646 (0.0235)**	**-2.0558 (0.0398)**
	Low Dose - Negative		-1.1323 (0.2575)	-1.9187 (0.055)
	High Dose - Standard		0.4529 (0.6506)	1.0964 (0.2729)
	Low Dose - Standard		1.5852 (0.1129)	1.2335 (0.2174)
	Negative – Standard		**2.7175 (0.0066)**	**3.1522 (0.0016)**
	High Dose - Normal			-1.4162 (0.1567)
	Low Dose – Normal			-1.2792 (0.2008)
	Negative – Normal			0.6396 (0.5224)
	Normal – Standard			**2.5126 (0.012)**
TSH	High Dose - Low Dose	0.4545 (0.6495)	0.1701 (0.8649)	0.1827 (0.855)
	High Dose - Negative	1.7612 (0.0782)	1.7581 (0.0787)	1.4162 (0.1567)
	Low Dose - Negative	1.3067 (0.1913)	1.588 (0.1123)	1.2335 (0.2174)
	High Dose - Standard	-1.3067 (0.1913)	-1.0208 (0.3073)	-0.9594 (0.3374)
	Low Dose - Standard	-1.7612 (0.0782)	-1.191 (0.2337)	-1.1421 (0.2534)
	Negative – Standard	**-3.0679 (0.0022)**	**-2.7789 (0.0055)**	**-2.3756 (0.0175)**
	High Dose - Normal			-1.7817 (0.0748)
	Low Dose – Normal			**-1.9644 (0.0495)**
	Negative – Normal			**-3.1979 (0.0014)**
	Normal – Standard			0.8223 (0.4109)

Within Treatment group Differences

Table 8: Kruskal-Wallis H test for differences in FT3 and TSH levels by comparing days 21, 28 and 35 within each treatment.

Variable	Treatment group	Chi-square value	P value
FT3	Negative	5.7931	0.0552
	Standard	7.2	**0.0273**
	Low Dose	5.5385	0.0627
	High Dose	5.4222	0.0665
TSH	Negative	4.1356	0.1265
	Standard	0.8136	0.6658
	Low Dose	3.322	0.1899
	High Dose	2.4	0.3012

Further analysis was conducted to determine any within treatment group differences by time points. At 5% level of significance, it was observed that on the observed FT3 levels within the Standard treatment group differed among days 21, 28 and 35 as shown in table 4 above. Further, a post-hoc analysis was conducted to identify with time points if there was significant difference. As shown in table 4.8 below, it was observed that the difference in FT3 levels within the standard drug differed significantly between days 21 and 35 (p value **0.0073**).

Table 9: Dunn's Post-hoc test of difference in FT3 levels between days 21, 28 and 35 within the Standard treatment group.

Variable	Comparison (Days)	Chi-square value	P value
FT3 (Standard drug)	21 – 28	1.3416	0.1797
	21 – 35	2.6833	**0.0073**
	28 – 35	1.3416	0.1797

HISTOLOGY OF THE THYROID GLAND

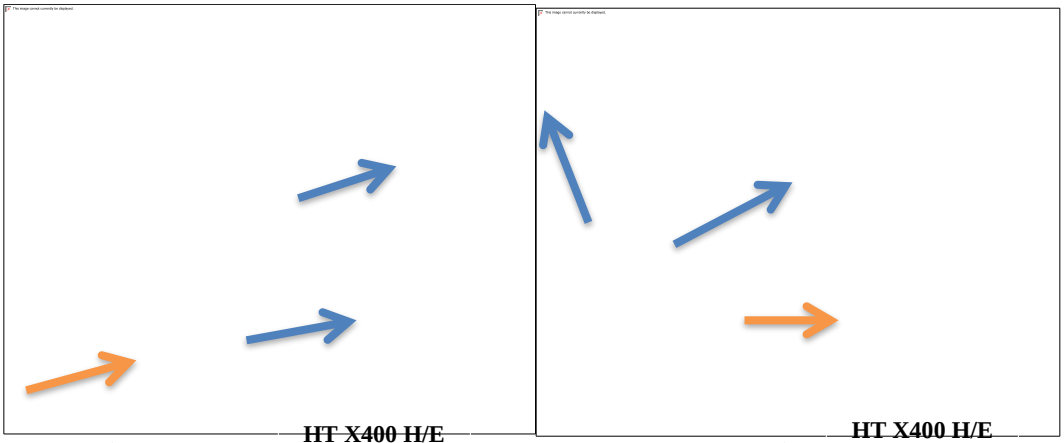


Figure 2 : Group 1 **Figure 3: Group 2**

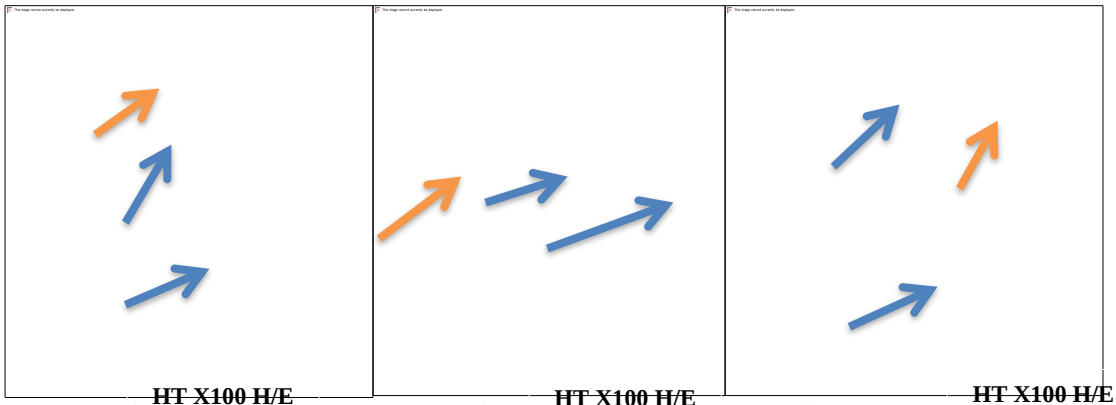


Figure 4: Group 3 **Figure 5: Group 5** **Figure 6: Group 4**

Figure 1::Thyroid gland section of GP1 rats (normal control) showing follicles lined by cuboidal epithelial cells filled with abundant colloid. showed normal histological structure of thyroid gland and there are no any pathological alterations

Figure 2:Thyroid gland section of GP2 rats (hyperthyroid control) showing follicles lined by cuboidal epithelial cells filled with scanty

Thyroid gland section of GP2 rats (hyperthyroid control) showing follicles lined by cuboidal epithelial cells filled with moderate colloid. This demonstrates thyroid follicles of variable sizes. Thyroid follicles were lined by cubical follicular cells with rounded basophilic nuclei. Also, many follicle lumen were empty from colloid. In these groups, multiple follicular cells exhibited pale nuclei and vacuolated cytoplasm (which nearly obliterated their cavities). Atrophied of some thyroid gland follicles and minute blood capillaries were also recorded in these sections.

Figure 4: **Thyroid gland section of GP3 rats (standard control at a dose of 10mg/kg) there was an increased number of thyroid follicles compared to the previous groups.**

This demonstrated thyroid follicles showed uniformly distributed variable size with little number of follicles with single layered flattened epithelium filled with abundant colloid. The lumen of follicles contained uniformly distributed colloid with peripheral vacillations. In these thyroid glands, the hydrophobic degeneration changes were seen in some follicles. The congested blood capillaries were still observed in these sections

Figure 5:**Thyroid gland section of GP4 rats (MSRE extract at a dose of 200mg/kg) showing follicles lined by cuboidal epithelial cells filled with moderate**

Thyroid gland section of GP4 rats (MSRE extract at a dose of 200mg/kg) showing follicles lined by cuboidal epithelial cells filled with moderate colloid demonstrates that some of follicular cells lining thyroid follicles slumped inside the lumen but some of them were partially filled with colloid. The colloid of the follicular spaces exhibited peripheral vacillations. Also, the cytoplasm follicular cells revealed, clear vacuoles with pyknotic or karyolitic nuclei. In additional, the degenerated lining of cells in some of the follicles were detected in these sections. Congested blood capillaries were extended between thyroid follicles and packed with red cells before being detected.

Figure 6: Thyroid gland section of GP5 (MSRE extract at a dose of 400mg/kg/rat) rats showing follicles lined by cuboidal epithelial cells filled with abundant

Thyroid gland section of GP5 (MSRE extract at a dose of 400mg/kg/rat) rats showing follicles lined by cuboidal epithelial cells filled with abundant colloid. there were variable sizes of thyroid follicles. The lumen of the follicles contained basophilic colloid with peripheral vacillations. Also, scanty colloid was observed in most of remaining thyroid follicles. In these glands, the escape of blood from capillaries was recorded. In order to avoid this, we found that there was an increase in lining cells number of follicles compared to the previous group

III. DISCUSSION, CONCLUSION AND RECOMMENDATION

Methanol extract phytochemicals of *Myrica salicifolia* root extract

The safe use of the extracts tested and of active substances they contain, explain probably their common uses by traditional healers in the treatment of numerous human and animal diseases (DMP, 2004; CAPES, 2006). Alkaloids, flavonoids, sterols, phenolics and tannins were observed in the root extracts of *M. salicifolia*. The therapeutic properties of these large chemical groups have been reported by various authors (Delaveau, 1998; Cowan, 1999).

Its methanol extract was found to contain alkaloid, tannins, saponins flavonoid, sterols and phenolics. Additionally, some tannins were able to inhibit HIV replication selectively beside their use as diuretics. It was reported saponins are used as mild detergents and in intracellular histochemistry staining to allow antibody access intracellular proteins (Thamara et al.,2012). Medicinally, saponins are used in hypercholesterolemia, hyperglycemia, antioxidant, anti-cancer, anti-inflammatory, central nervous system activities and weight loss (Poornima et al.,2009).Saponins generally help humans to fight fungal infections, combat microbes and viruses and knock out some tumor cells, particularly lung and blood cancers(wadwood et al.,2013) Alkaloids have been reported from *Morella salicifolia* and their medicinal values indicated these compounds serve as natural antibiotics, which help the body to fight infections and microbial invasion .(Hanson et al., 2013 The presence of alkaloids in the stem bark *of M. salicifolia* might be one of the reasons for its traditional medicinal values. While phenolic compound and flavonoids are known to possess biological activity such as antibacterial activity, antioxidant, anti-inflammatory, etc. (Sudip et al 2000). Therefore, the phytochemical screening results reveal that the presence of these phytochemical constituents supports the use of *Myrica salicifolia* plant in folk medications.

Acute toxicity

Upon administration of *M. salicifolia* root extract to the LT4 induced hyperthyroid at different doses, no mortality was recorded across all doses though overcrowding and reduced activity was noted for the rats administered with 5000mg/kg body weight of the MSRE.

Conversely; this behavior was not observed in the other dose categories. From the current study the LD50 of *M. salicifolia* root extract was found to be > 5000 mg/kg body weight.

It was also observed that the plant extract caused a slight but insignificant decrease in the body weight of the test animals; this may be due to a decrease in appetite, which may be secondary to a feeling of fullness after administration of the extract. It may also be due to the effect of the plant on the body fat metabolism. This, however, remained to be rationalized.

The effect of MSRE on serum triiodothyronine (T3) and serum thyroid stimulating hormone (TSH)

From the data of the blood samples collected on the 14th day of the study, the T3 and TSH of the normal control rats was found to be 2.7ng/dl, and 3.4 mIU/ml respectively.this results falls within the normal range of FT3 and TSH. On the other hand, rats in all other groups (II, III, IV, V) were treated with the LT4 and showed an increase in the levels of FT3 and decreased serum values of TSH, confirming successful induction of hyperthyroidism in those groups. The current study showed that there was a significant increase in serum FT3 levels as well as a significant decrease in serum TSH levels in rats treated with levothyroxine, when compared to the control group ($P < 0.01$). This indicates that LT4 was convenient for induction of hyperthyroidism.LT4 stimulates thyroid activity and exerts its primary effect on the synthesis of the thyroid hormones thyroxine and triiodothyronine by blocking oxidative iodination within the thyroid gland itself (muller et al., 2014).in addition levothyroxine triggers the metabolism of thyroid hormones outside of the thyroid gland by interfering with the peripheral deiodination of T4. (Brent et al., 2014). The decrease in TSH secretion by anterior pituitary gland extends a negative feedback effect on the thyroid gland secretion of T3 and T4. As shown in the table 1 above, there is a variation in ft3 hormone levels between the days of sacrifice – within each treatment, and, also between the treatments. There is a general decrease in the observed median FT3 levels from day 21 to day 35. Less variance is observed in the low dose between values observed in days 21,28 and 35 compared to the standard dose and the high dose of the investigation extract.

The negative group TSH observed median levels are generally low compared to the other treatment groups. There is an overall increasing trend in the TSH levels from day 21 to day 35. Also, the negative group TSH levels have a higher spread than the rest of the groups except the normal group, which has a wider spread compared to the rest for day 35. Although the observed medians for the standard, low and high dose groups are comparable, the standard groups had higher observed medians compared to the other investigational extract subgroups for the three time points.

The FT3 hormone levels are higher between and within treatment variation compared to the TSH hormone levels as shown figures 1 and 2 respectively. Among the active treatments, TSH shows very minimal variance between treatments, with day 21 showing lower levels compared to later time points. Kruskal-Wallis H test was performed on the observed ft3 and TSH levels to determine any statistically significant differences between the treatments by time points. By comparing the ft3 levels among the treatment groups, neither of any treatment group pairs would explicit any statistically significant differences at 5% level of significance at day 21. However, the Kruskal-Wallis H test results were significant for days 28 and 35 – indicating that the observed ft3 levels were significantly different in least two treatment groups. For the TSH, all the three time points shown significant results. Therefore, to determine the specific treatments that has these significant differences, Dunn's post-hoc tests were performed, which observed that the significant differences in ft3 levels observed in day 28 and 35 were between high dose and Negative, and Standard and negative treatment groups for both time points. Also, the Normal and Standard groups had significantly different ft3 levels at day 35. The observed differences in TSH levels among treatment groups were between Negative and Standard groups across time points. In addition, Low-dose and Normal, and Negative and Normal groups showed significantly different TSH levels at 5% level of significance. The group treated with 400mg/kg of methanol extract of *M. salicifolia* showed better result than the other concentration 200mg/kg and was found to be effective as the standard drug propylthiouracil.

Obtained data of MSRE showed that flavonoids may influence on thyroid function through reduction of thyroid peroxidase activity. These effects may be due to flavonoids of MSRE constituents, such as dillapiole, costunolide and caffeic acid, on the thyroid function, which is more pronounced when iodine is deficient (Divi et al., 1996). Another pathway for decreasing HT in our model may due to tannins included in MSRE, such as gallic acid and epigallocatechin gallate, which act as a chelating agent through bonding with inorganic iodide (Ali nagi 1999). Subsequently, the correction of iodine deficiency leads to normalization of THs. Indeed, THs were positively correlated with flavonoid ingestion owing to the biosynthesis of T4 confined to thyroid gland. The majority of THs are T3 (80%), and synthesized in the liver (out of the thyroid). Each of the pathways has illustrated that polyphenolic compounds may affect the thyroid gland but no other organs (Ali nagi 1999). Certainly, MSRE is an active treatment which may ameliorate thyroxin over secretion via tannins, caffeic acid and garlic acid, and can act as a chelating agent of iodine liberated in the bloodstream (Peleg et al., 1998). The synergetic effects of quercetin and ferulic acid may ameliorate the side effects of thyroid over secretion due to total antioxidant capacity and their antioxidant properties. Recent studies have reported that quercetin acts as a tranquilizer agent in hyperactive rats (Okamura et al., 1990). On the whole, herbal extract is a new direction for exchange of chemical drugs due to its side effects and unknown interactions. Indeed, MSRE containing mixture of flavonoids and tannins have stabilized the most common side effect of hyperthyroidism- via stimulating different pathways whether internal or external cell processes. The internal pathway may be due to succinate dehydrogenase inhibitor and Na/k — ATPase inhibitor, according to epigallocatechin gallate content (Moreira et

al., 2005). External pathway may be due to iodine trapping, suppression of chemotactic factors, and suppression of reductive markers, which due to the phenolic component contribute to the synergetic action (Moreira et al., 2005). In the present study, alterations in thyroid function after LT4 exposure were confirmed by the histological examination of the thyroid follicles by H&E. Our hyperthyroidism model- induced by LT4- showed histological changes which may be due to TSH suppression. It is well-known that low levels of TSH affect thyroid gland function and structure. On the other hand, normal levels have yielded simulative effects on the follicles, which are modulated by the action of a variety of molecules, such as peptides and/or neuropeptides, derived from para follicular cells and other growth factors. The hyperthyroidism model demonstrated that follicles have irregular shape and many cubical follicular cells are lined with basophilic nuclei accompanied by empty luminal colloids. Also, multiple follicular cells exhibited pale nuclei, vacuolated cytoplasm and nearly obliterated their activities. Takamatsu et al. illustrated irregular and atrophied follicles shaped with LT4, which induced hyperthyroidism modulated condensed nuclei walls and follicle loss of thyroid gland (Takamatsu et al., 1992.) Histopathological analysis of thyroid gland was conducted and light photomicrographs were taken (Figure 5), which showed marked changes in the follicular cells of the treated animals as compared to the positive control and normal control groups. The follicular cells in normal control animals were observed to be cuboidal and epithelium full of colloidal material. On the other hand, LT induced animals show follicular atrophy, scanty colloid material and epithelial hyperplasia. MSRE administered group reversed the follicular atrophy and increased colloid material. Almost similar histological changes were observed for PTU treated groups. Results of histopathology are in correlation with previous literature. This clearly suggests the PTU like activity of MSRE. Mechanism responsible for anti-thyroid activity of the extract can be suggested as iodine complexation, inhibition of thyroid peroxidase, protease, 5 α- deiodinase enzymes as flavonoids exhibit antithyroid activity through the above mechanisms.

Effect of *M. salicifolia* methanol root extract on the histology of the thyroid gland

In the present study, alterations in thyroid function after LT4 exposure were confirmed by the histological examination of the thyroid follicles by H&E. Our hyperthyroidism model- induced by LT4- showed histological changes which may be due to TSH suppression. It is well-known that low levels of TSH affect thyroid gland function and structure. On the other hand, normal levels have yielded simulative effects on the follicles, which are modulated by the action of a variety of molecules, such as peptides and/or neuropeptides, derived from para follicular cells and other growth factors. The hyperthyroidism model demonstrated that follicles have irregular shape and many cubical follicular cells are lined with basophilic nuclei accompanied by empty luminal colloids. Also, multiple follicular cells exhibited pale nuclei, vacuolated cytoplasm and nearly obliterated their activities. Takamatsu et al. illustrated irregular and atrophied follicles shaped with LT4, which induced hyperthyroidism modulated condensed nuclei walls and follicle loss of thyroid gland 31.

MSRE treated rats showed increase of columnar follicular cells size, vacuolated cells and deeply stained nuclei. The findings of MSRE may be owing to the accumulation of fluid and glands over stimulation. Therefore, the increase in TSH threshold in a MSRE -treated hyperthyroid model could be the causative factor for rebuilding cells and follicles, and could regenerate thyroid hypertrophy and increase cell size and functional capacity for normal status 32.

According to the reference drug for treating the hyperthyroidism model, PTU showed the variable size of follicles filled with abundant colloid. These disruptions may be due to the hydrophobic degeneration changes and minute blood capillaries extended between them.

Conclusion

The present investigation was conducted to study the effect of the potential antithyroid activity of methanol extract of *Myrica salicifolia* on L-thyroxine induced hyperthyroidism in male wistar rats. From the findings it can be inferred that the animals treated with the plant concentrate(400mg/kg) shows a great effect as like that of the standard medication and the histopathological studies of the thyroid gland of data also proves the same. Hence, upon all findings and assumption it can be said that the methanol extract of Myrica *salicifolia* root extract can possibly overcome hyperthyroidism in albino rats.

Recommendations

1. Though use of M. salicifolia as a medicinal plant has proved to be safe for use and therefore further pre-clinical and clinical studies need to be carried out to validate its safety furthermore its safety on chronic use.
2. Further scientific validation is encouraged and more specific investigation should be done on M. salicifolia extract for a clear understanding on its mode of action.
3. The study recommends that *M. salicifolia* extract should be used in high doses since low doses have not proved to be effective.

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