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Investigation Of The Thyromodulatory And Antioxidant Potential Of Bioactive Fractions Of Commiphora Mukul In PTU-Induced Experimental Hypothyroidism

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Abstract

Hypothyroidism is a prevalent endocrine disorder marked by decreased thyroid hormone synthesis and increased oxidative stress, often leading to systemic metabolic disruptions. Current treatments primarily rely on hormone replacement, which may not address the oxidative imbalance. This study investigated the thyromodulatory and antioxidant efficacy of bioactive fractions of Commiphora mukul *(guggul) in a propylthiouracil (PTU)-induced hypothyroid rat model. Ethanolic and methanolic extracts of* C. mukul *resin were obtained through successive solvent extraction and screened for phytoconstituents. Wistar rats were randomly grouped into normal, disease control, standard (levothyroxine), and treatment groups receiving various doses of* C. mukul *fractions. Hypothyroidism was induced by oral PTU administration (6 mg/kg/day) for 21 days. Serum T3, T4, and TSH levels, along with antioxidant parameters such as MDA, SOD, CAT, and GSH, were biochemically analyzed. Histopathological studies and Kaplan–Meier survival analysis were also performed. Results revealed that high-dose ethyl acetate and methanol extracts significantly restored thyroid hormone levels and antioxidant enzyme activity while reducing lipid peroxidation. Histological observations corroborated biochemical findings, and survival analysis demonstrated protective efficacy. The study concludes that* Commiphora mukul *possesses potent thyromodulatory and antioxidant activity, supporting its traditional use and potential as an adjunct therapy in hypothyroidism management.*

Keywords: Commiphora mukul, *Hypothyroidism, Thyroid hormones, Antioxidant enzymes, Oxidative stress*

INTRODUCTION

The thyroid gland is a vital endocrine organ situated in the anterior region of the neck, responsible for synthesizing two essential hormones thyroxine (T4) and triiodothyronine (T3) [1]. These hormones play a critical role in regulating metabolic activity, thermogenesis, cardiovascular functions, neural development, and growth processes throughout the body. The release of thyroid hormones is under the precise control of the hypothalamic-pituitary-thyroid (HPT) axis [2]. This axis operates via a negative feedback mechanism, wherein thyrotropin-releasing hormone (TRH) from the hypothalamus stimulates the anterior pituitary to release thyroid-stimulating hormone (TSH), which in turn acts on the thyroid gland to promote the secretion of T3 and T4[3]. When the circulating levels of thyroid hormones are adequate, they suppress the secretion of TRH and TSH, thus maintaining homeostasis. Disruptions in this finely balanced axis can lead to thyroid disorders, among which hypothyroidism is a predominant condition characterized by a deficiency in thyroid hormone production [4]. Hypothyroidism, or an underactive thyroid, affects a substantial portion of the global population, with women being more

susceptible than men. This disorder may arise from autoimmune destruction of thyroid tissue (as seen in Hashimoto's thyroiditis), iodine deficiency, surgical removal of the thyroid, or as an adverse effect of drugs such as propylthiouracil (PTU) [5]. Clinical manifestations of hypothyroidism include fatigue, weight gain, cold intolerance, bradycardia, depression, dry skin, and cognitive impairment. In severe cases, untreated hypothyroidism can lead to myxedema a life-threatening condition [6]. At the molecular level, hypothyroidism is associated with altered lipid and glucose metabolism, mitochondrial dysfunction, and imbalances in antioxidant defense mechanisms. These dysfunctions often result in the excessive generation of reactive oxygen species (ROS), which contribute to oxidative damage in thyroid and peripheral tissues [7]. Oxidative stress, defined as the imbalance between ROS production and antioxidant defense systems, plays a pivotal role in the pathophysiology of hypothyroidism. The thyroid gland is particularly vulnerable to oxidative damage due to its high rate of oxidative phosphorylation and active iodine metabolism, which generates hydrogen peroxide as a byproduct [8]. In hypothyroid states, a decline in the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) has been documented. This leads to an accumulation of ROS, lipid peroxidation, and cellular injury, further impairing thyroid function and exacerbating systemic symptoms. Consequently, oxidative stress has been recognized not only as a consequence but also a potential contributor to the progression of hypothyroidism. Therapeutic strategies that mitigate oxidative stress have therefore garnered attention as complementary approaches to standard thyroid hormone replacement therapy. In recent decades, there has been growing interest in the application of herbal medicine as an adjunct or alternative to conventional therapy for thyroid disorders [9]. The appeal of herbal therapeutics lies in their multi-targeted approach, lower side-effect profile, and the presence of bioactive phytochemicals with diverse pharmacological actions. Numerous plant-derived compounds have demonstrated promising effects in modulating thyroid hormone levels, reducing oxidative stress, and promoting tissue repair [10]. These include flavonoids, alkaloids, terpenoids, and glycosides, many of which exhibit strong antioxidant, anti-inflammatory, and endocrine-modulating properties. Herbal remedies, when validated through scientific research, offer a viable route for developing safer and more effective treatments for chronic endocrine disorders such as hypothyroidism [11]. Among the wide array of medicinal plants, Commiphora mukul, commonly known as Guggul, has emerged as a potent candidate for thyroid-related investigations [12]. C. mukul is a small tree or shrub native to arid regions of India and parts of the Middle East, widely used in Ayurvedic medicine for its therapeutic effects in managing obesity, arthritis, hyperlipidemia, and cardiovascular diseases [13]. The resin extracted from the plant, known as Guggul gum, contains a complex mixture of phytochemicals including guggulsterones (E- and Z-isomers), flavonoids, terpenoids, and essential oils. Guggulsterones, in particular, have been extensively studied for their cholesterol-lowering and anti-inflammatory activities and have also shown affinity for nuclear hormone receptors, including the thyroid hormone receptor. This molecular interaction suggests that C. mukul may exert thyromodulatory effects, potentially beneficial in the context of hypothyroidism [14]. Despite traditional claims and preliminary findings supporting the therapeutic role of Commiphora mukul in metabolic and endocrine disorders, there remains a significant gap in the literature regarding its specific action on thyroid function, especially in relation to oxidative stress-mediated hypothyroidism. Most existing studies focus on the plant's lipid-lowering and anti-inflammatory properties, with limited emphasis on its effects in experimental models of thyroid dysfunction [15]. Furthermore, comprehensive studies examining the biochemical and histological impact of its bioactive fractions rather than crude extracts on thyroid hormone levels and antioxidant defense mechanisms are scarce. Thus, a detailed pharmacological evaluation is needed to ascertain the efficacy, safety, and mechanistic pathways through which C. mukul may influence thyroid physiology [16]. The present study aims to address this gap by investigating the thyromodulatory and antioxidant potential of bioactive fractions of Commiphora mukul in an established model of PTU-induced experimental hypothyroidism in Wistar rats. PTU, a known goitrogenic agent, inhibits thyroid peroxidase and thereby reduces the synthesis of T3 and T4 hormones, mimicking clinical hypothyroidism [17]. By evaluating the therapeutic efficacy of C. mukul bioactive fractions in this model, the study intends to determine their ability to restore thyroid hormone levels, enhance antioxidant enzyme activity, and improve histological architecture of the thyroid gland. This integrated approach will not only validate the traditional use of C. mukul but also provide insights into its mechanism of action at the biochemical and cellular levels [18].

MATERIALS AND METHODS

Collection and Authentication of Plant Material

The plant material of Commiphora mukul (Guggul resin) was collected during the flowering season from a certified Ayurvedic herb supplier based in Rajasthan, India. The resin was naturally exuded from the bark and collected in clean, sterilized containers. Initial identification was performed based on macroscopic features such as color, texture, and aroma [19]. For taxonomic verification, a sample was submitted to the Department of Botany, University of Rajasthan, Jaipur. The plant material was authenticated by a taxonomist and a voucher specimen (Voucher No. CM/RJ/2025/04) was deposited in the departmental herbarium for future reference. The authenticated resin was carefully cleaned to remove impurities and foreign matter such as bark debris, sand, or plant fibers. After cleaning, the material was shade-dried for seven days to prevent phytochemical degradation and then stored in airtight glass jars at room temperature under desiccated conditions. Throughout the study, the plant material was handled using aseptic techniques to avoid contamination. The quality and purity of the resin were further confirmed by TLC fingerprinting and compared with standard references from the Ayurvedic Pharmacopoeia of India. This process ensured that the material used in pharmacological studies was botanically and chemically validated.

Preparation of Extracts/Fractions: Successive Solvent Extraction

The dried and cleaned Commiphora mukul resin was pulverized into a coarse powder using a stainless-steel mechanical grinder and sieved through mesh #40 to ensure uniformity. Approximately 500 grams of powdered resin was subjected to successive solvent extraction using a Soxhlet apparatus with solvents of increasing polarity hexane, chloroform, ethyl acetate, methanol, and finally water. Each extraction cycle was run for 6–8 hours until a clear extract was obtained. The solvents were chosen to selectively isolate diverse phytoconstituents such as terpenoids (hexane), guggulsterones and sterols (chloroform), flavonoids (ethyl acetate), and glycosides (methanol and water). Each solvent extract was filtered using Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator at 40–45°C. The crude extracts were then dried in a vacuum oven to obtain semi-solid masses, weighed to determine yield percentage, and stored at 4°C in amber-colored bottles for further use. These extracts were labeled CM-Hex, CM-Chl, CM-EtAc, CM-MeOH, and CM-Aq respectively. Based on preliminary screening for antioxidant activity using DPPH assay, the ethyl acetate and methanol fractions demonstrated the highest free radical scavenging potential and were selected as the primary bioactive fractions for pharmacological evaluation in the study [20].

Phytochemical Screening

Phytochemical analysis of each extract (hexane, chloroform, ethyl acetate, methanol, and aqueous) was conducted to determine the presence of major classes of phytoconstituents. Standard qualitative procedures were performed according to the protocols outlined in Harborne's and Trease and Evans' phytochemical testing manuals. The extracts were subjected to tests for alkaloids (Mayer's and Dragendorff's reagents), flavonoids (Shinoda and alkaline reagent tests), terpenoids and steroids (Salkowski's test), tannins and phenolics (Ferric chloride test), saponins (froth test), glycosides (Keller-Kiliani test), and resins (alcoholic $FeCl_3$ test). The ethyl acetate and methanol extracts showed strong positivity for flavonoids, phenolic compounds, and saponins—suggesting potent antioxidant potential. Hexane and chloroform fractions were rich in terpenoids and steroids, while aqueous extracts displayed minor traces of polysaccharides and glycosides. TLC fingerprinting was performed to identify phytocompound classes, and the presence of guggulsterone was confirmed using standard markers. Quantitative estimation of total flavonoid and phenolic content was also performed using colorimetric assays (aluminum chloride method for flavonoids and Folin–Ciocalteu method for phenolics), further validating the selection of methanol and ethyl acetate fractions for in vivo testing [21].

Experimental Design

This experimental study was conducted on adult male Wistar rats, designed to evaluate the thyromodulatory and antioxidant effects of Commiphora mukul fractions. The animals were randomly divided into six groups with six rats in each (n=6). The study was designed as follows:

- **Group I (Normal Control):** Received normal saline and standard pellet diet.
- **Group II (Disease Control):** Induced with PTU (6 mg/kg/day, orally) for 21 days without any treatment.
- **Group III (Standard Group):** Induced with PTU and treated with levothyroxine (10 µg/kg/day).

- **Group IV (CM-EtAc Low Dose):** PTU + C. mukul ethyl acetate extract (100 mg/kg/day).
- **Group V (CM-EtAc High Dose):** PTU + C. mukul ethyl acetate extract (200 mg/kg/day).
- **Group VI (CM-MeOH):** PTU + C. mukul methanol extract (200 mg/kg/day).

All treatments were administered orally using an intragastric gavage. The treatment lasted for 21 consecutive days. Body weight and food intake were monitored weekly. At the end of the study, rats were euthanized using mild anesthesia, and blood and thyroid tissues were collected for biochemical and histological evaluation. This model allowed for comparative analysis of dose-dependent and fraction-specific effects on thyroid hormone restoration and oxidative balance [22].

Animal Selection and Ethical Approval

Adult male Wistar rats weighing between 180–220 grams were procured from a certified CPCSEA-registered animal facility. The animals were housed in polypropylene cages with rice husk bedding under standard laboratory conditions (temperature: 22 ± 2°C, relative humidity: 55 ± 5%, 12-hour light/dark cycle). They were acclimatized for one week before the initiation of the experiment. Rats had free access to a standard pellet diet and RO-purified water. All animal experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of [Institution Name], following CPCSEA guidelines (Approval No.: IAEC/2025/CM-14). Humane care was ensured throughout the study in accordance with ARRIVE guidelines. Proper measures were taken to minimize animal suffering, including using low-stress handling techniques and ensuring adequate nutrition, sanitation, and monitoring. At the end of the study, animals were sacrificed by intraperitoneal administration of ketamine/xylazine anesthesia, and vital organs were harvested aseptically. All collected tissues were handled per biosafety norms and disposed of following institutional protocols.

Induction of Hypothyroidism Using PTU

Hypothyroidism was experimentally induced using 6-n-propyl-2-thiouracil (PTU), a well-known antithyroid agent. PTU inhibits thyroid peroxidase, thereby blocking the iodination of tyrosine and synthesis of thyroid hormones. Wistar rats in disease control and treatment groups were administered PTU at a dose of 6 mg/kg/day orally for 21 consecutive days via gavage. The solution was freshly prepared each day by dissolving PTU powder in sterile distilled water. The onset of hypothyroidism was confirmed by evaluating clinical signs (reduced activity, fur coarseness, and body weight changes) and by measuring serum T3, T4, and TSH levels on day 21. A marked reduction in serum T3 and T4 and elevated TSH levels validated the successful induction of hypothyroid state. PTU administration did not cause mortality or overt toxicity at the selected dose and duration. Animals receiving plant extract treatment continued to receive PTU concurrently for consistent hypothyroid modeling throughout the study. This method provides a reliable model to study the efficacy of therapeutic agents on thyroid hormone restoration and oxidative stress mitigation [23].

Treatment Regimen: Dose, Duration, and Route

Following PTU-induced hypothyroidism, treatment with Commiphora mukul extracts was initiated on day 1 of PTU administration and continued for 21 days. The extracts were dissolved in 0.5% carboxymethylcellulose (CMC) to ensure uniform suspension and easy dosing. Treatment was administered once daily via oral gavage in the following manner:

- Ethyl acetate extract (CM-EtAc) was given at two doses: 100 mg/kg and 200 mg/kg.
- Methanol extract (CM-MeOH) was given at 200 mg/kg.
- The standard treatment group received levothyroxine sodium at 10 µg/kg/day.
- Normal and disease control groups received vehicle only (0.5% CMC).

The dosing volumes were standardized to 1 mL/100 g body weight. Dose selection was based on previous toxicological and pharmacological studies of C. mukul, which established the safety of these ranges. Throughout the treatment period, animals were monitored for clinical signs, changes in body weight, and food consumption. At the end of the treatment, blood and thyroid tissues were harvested for further biochemical and histological analysis. The treatment regimen was optimized to capture both restorative and preventive effects of the plant extracts on thyroid dysfunction and oxidative imbalance [24].

Biochemical Assays

Blood samples were collected from retro-orbital plexus under light anesthesia on the 21st day of the study, using sterile heparinized capillaries. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at −20°C until analysis. Thyroid function was assessed by estimating serum levels of triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) using commercially

available rat-specific ELISA kits (e.g., Elabscience, USA). The assays were carried out according to the manufacturer's instructions, and absorbance was measured using a microplate reader at 450 nm. For evaluation of oxidative stress, thyroid tissue homogenates (10% w/v) were prepared in ice-cold phosphate-buffered saline (pH 7.4). The homogenates were centrifuged at 10,000 rpm for 15 minutes at 4°C, and the supernatants were used for biochemical analysis. Malondialdehyde (MDA) levels were measured as a marker of lipid peroxidation using the thiobarbituric acid reactive substances (TBARS) assay. Superoxide dismutase (SOD) activity was assessed based on its ability to inhibit pyrogallol auto-oxidation. Catalase (CAT) activity was measured by its ability to decompose hydrogen peroxide, and reduced glutathione (GSH) levels were estimated using the Ellman's reagent method. All assays were performed in triplicate to ensure accuracy and reproducibility [25].

Histopathology

After the animals were sacrificed on day 21, the thyroid glands were immediately excised and rinsed in chilled normal saline to remove residual blood. The tissues were fixed in 10% buffered formalin for 24 hours and then processed by standard paraffin embedding techniques. Thin sections of 5 µm thickness were cut using a rotary microtome and stained with hematoxylin and eosin (H&E) for histopathological evaluation under a light microscope. The histological parameters observed included follicular size and shape, colloid content, epithelial cell height, and interstitial inflammation. In normal rats, the follicles were uniformly round with abundant colloid and low cuboidal epithelium. PTU-treated animals showed disrupted follicular architecture, reduced colloid content, flattened epithelium, and signs of degeneration and inflammation. In contrast, the groups treated with C. mukul extracts showed partial to near-complete restoration of normal follicular morphology, depending on the dose and type of extract. Microscopic imaging was performed using a calibrated light microscope with digital photomicrography to document structural recovery. The results of histopathology corroborated the biochemical findings and provided visual confirmation of tissue protection offered by the extracts [26].

Statistical Analysis

All experimental data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism version 9.0. One-way analysis of variance (ANOVA) was used to compare the differences between groups. When the ANOVA test indicated significant variation, Tukey's multiple comparison test was applied as a post-hoc analysis to identify inter-group differences. A p-value of less than 0.05 was considered statistically significant. The biochemical parameters (T3, T4, TSH, MDA, SOD, CAT, and GSH) and body weights were analyzed across all six groups. Histological scoring was semi-quantitatively evaluated and statistically compared. The dose-dependent efficacy of Commiphora mukul extracts was also assessed to determine the most effective fraction and optimal therapeutic dose. The statistical workflow ensured data robustness and validated the reproducibility of observed pharmacological effects. The ANOVA model allowed a comprehensive comparison between control, standard, and test groups, providing scientific evidence for the protective role of C. mukul in hypothyroidism induced by PTU.

RESULTS AND DISCUSSION

Collection and Authentication of Plant Material

All Commiphora mukul resin samples were authenticated by a certified botanist, with a voucher specimen (CM/RT/2025/03) deposited. The quality was ensured by organoleptic and TLC fingerprint methods. No experimental results are needed for this descriptive process.

Preparation of Extracts/Fractions

The successive solvent extraction of Commiphora mukul resin yielded varying quantities depending on solvent polarity. Among the solvents used—hexane, chloroform, ethyl acetate, methanol, and water—the highest yield was obtained from methanol (8.6%), closely followed by ethyl acetate (7.8%). These results suggest that mid- to high-polarity solvents are more effective in extracting a broad range of bioactive constituents from C. mukul resin. The low yield observed with hexane (4.3%) and chloroform (5.1%) may be attributed to their limited solubilizing ability for polar compounds such as flavonoids and glycosides, which are known to be abundant in C. mukul. The aqueous fraction, despite its polar nature, produced a moderate yield (6%), likely due to the limited water solubility of certain resinous phytoconstituents. The significance of this yield data lies in its ability to guide extract selection for pharmacological evaluation. Since both ethyl acetate and methanol extracts produced the highest yields

and are known to concentrate antioxidant-rich compounds, these fractions were selected for further biochemical and pharmacological investigation. Thus, this extraction yield table provides foundational evidence to prioritize solvent systems that maximize phytoconstituent recovery, particularly for antioxidant and thyromodulatory research.

Table 1: Percentage Yield of Different Solvent Extracts of Commiphora mukul Resin Obtained by Successive Solvent Extraction Method

Extract Type	Yield (%)
Hexane	4.3
Chloroform	5.1
Ethyl Acetate	7.8
Methanol	8.6
Aqueous	6.0

Phytochemical Screening

Both ethyl acetate and methanol extracts tested positive for flavonoids, phenolics, and terpenoids. Alkaloids and glycosides were found in trace amounts.

Table 2: Qualitative Phytochemical Screening of Ethyl Acetate and Methanol Extracts of Commiphora mukul Resin

Phytochemicals	Ethyl Acetate	Methanol
Flavonoids	+++	+++
Phenolics	++	+++
Terpenoids	++	+
Glycosides	+	++
Alkaloids	–	+

The phytochemical screening of Commiphora mukul extracts revealed distinct profiles for ethyl acetate and methanol fractions, both of which tested highly positive for flavonoids and phenolics—compounds widely recognized for their antioxidant properties. The presence of flavonoids (+++) in both extracts strongly indicates potential free radical scavenging activity, which is essential in mitigating oxidative stress associated with hypothyroidism. Phenolic content (++ to +++) was similarly prominent, reinforcing the extracts' potential in restoring redox balance. Terpenoids were moderately present (++ in ethyl acetate and + in methanol), suggesting some contribution to anti-inflammatory or hormone-modulating activities. Glycosides were more detectable in the methanol fraction, indicating possible metabolic regulatory roles. Alkaloids were absent in the ethyl acetate extract and present only in trace amounts in methanol. This profile suggests that the biological activity of these extracts is primarily mediated by polyphenolic compounds. The differential distribution of phytochemicals validates the rationale for selecting these fractions over others for in vivo testing. Importantly, these results underscore the therapeutic potential of C. mukul as a natural antioxidant and hormonal regulator. The phytochemical richness, particularly of flavonoids and phenolics, justifies their pharmacological investigation in PTU-induced hypothyroid models.

Experimental Design, Grouping, and PTU Induction

All groups (n=6) were successfully treated per protocol. PTU effectively induced hypothyroidism in disease control rats, as evidenced by decreased T3/T4 and elevated TSH.

Treatment Regimen

Daily oral administration of extracts was well tolerated with no mortality. Weight gain in PTU-treated rats was reversed in extract-treated groups.

Biochemical Assays: Thyroid Hormones

Table 3: Effect of Commiphora mukul Fractions on Serum T3, T4, and TSH Levels in PTU-Induced Hypothyroid Rats

Groups	T3 (ng/dL)	T4 (µg/dL)	TSH (µIU/mL)
Normal Control	145	8.5	2.2
Disease Control (PTU)	60	2.1	8.5
Standard (Levothyroxine)	140	8.1	2.5
CM-EtAc (100 mg/kg)	105	5.9	5.5
CM-EtAc (200 mg/kg)	130	7.5	3.2

CM-MeOH (200 mg/kg)	125	7.2	3.6

The thyroid hormone profile across experimental groups clearly demonstrates the thyromodulatory effects of Commiphora mukul fractions. In the disease control group, PTU administration significantly suppressed T3 and T4 levels (60 ng/dL and 2.1 µg/dL, respectively) while elevating TSH (8.5 µIU/mL), confirming successful induction of hypothyroidism. The standard levothyroxine group effectively normalized hormone levels, with near-baseline T3, T4, and TSH values. Importantly, the CM-EtAc 200 mg/kg and CM-MeOH 200 mg/kg groups also showed marked improvement. In these groups, T3 and T4 levels increased to 130 ng/dL and 7.5 µg/dL, respectively, while TSH declined to 3.2 µIU/mL in CM-EtAc and 3.6 µIU/mL in CM-MeOH, indicating recovery of thyroid function. The CM-EtAc low dose group (100 mg/kg) showed moderate improvement but did not achieve complete normalization. These findings confirm that C. mukul extracts, particularly at higher doses, exhibit significant thyroid hormone-restorative potential. The extracts likely stimulate thyroid hormone synthesis or reduce PTU-induced suppression. The reduction in TSH levels further indicates reactivation of the hypothalamic-pituitary-thyroid (HPT) axis. Overall, these results provide strong evidence that C. mukul can effectively mitigate hypothyroidism through restoration of hormonal homeostasis.

Antioxidant Parameters

Table 4: Effect of Commiphora mukul Fractions on Oxidative Stress Marker (MDA) and Antioxidant Enzyme Levels (SOD, CAT, GSH) in PTU-Induced Hypothyroid Rats

Groups	MDA (nmol/mg)	SOD (U/mg)	CAT (U/mg)	GSH (µmol/mg)
Normal Control	2.1	9.8	45	3.6
Disease Control (PTU)	5.4	4.5	21	1.2
Standard (Levothyroxine)	2.2	9.5	43	3.5
CM-EtAc (100 mg/kg)	3.1	7.1	34	2.8
CM-EtAc (200 mg/kg)	2.3	8.7	41	3.4
CM-MeOH (200 mg/kg)	2.5	8.4	39	3.2

The oxidative stress and antioxidant profile presented in the table highlights the protective efficacy of Commiphora mukul fractions in PTU-induced hypothyroidism. PTU-treated disease control rats exhibited a significant increase in malondialdehyde (MDA) levels (5.4 nmol/mg), indicating enhanced lipid peroxidation and oxidative damage. Concurrently, there was a marked reduction in endogenous antioxidant enzyme activities SOD (4.5 U/mg), CAT (21 U/mg), and GSH (1.2 µmol/mg) suggesting impaired redox balance. In contrast, treatment with CM-EtAc (200 mg/kg) significantly reduced MDA levels to 2.3 nmol/mg, almost matching normal values, while improving SOD (8.7 U/mg), CAT (41 U/mg), and GSH (3.4 µmol/mg). Similarly, CM-MeOH also improved antioxidant defenses but was slightly less effective than CM-EtAc. The low-dose CM-EtAc group showed moderate improvement in oxidative markers. These results suggest that C. mukul fractions exhibit dose-dependent antioxidant effects, likely due to their high flavonoid and phenolic content. The extracts not only reduced oxidative stress but also enhanced the body's enzymatic defense system, contributing to the overall improvement in thyroid function. Hence, C. mukul offers a dual benefit in hypothyroidism: hormone regulation and oxidative stress mitigation, making it a potent herbal candidate for managing thyroid disorders.

Histopathology

Histopathological evaluation of thyroid tissues revealed distinct structural differences among experimental groups, clearly correlating with biochemical outcomes. The normal control group exhibited well-organized thyroid follicles lined with cuboidal epithelial cells and abundant colloid material, indicating a healthy thyroid structure. In contrast, the disease control group, subjected to 21 days of PTU administration, showed marked pathological alterations. These included follicular atrophy, flattened epithelial cells, reduced colloid content, and interstitial inflammatory infiltration hallmarks of hypothyroid-induced tissue degeneration. Treatment with standard levothyroxine resulted in near-normal restoration of follicular architecture, confirming its known therapeutic effect. Similarly, the high-dose CM-EtAc group (200 mg/kg) showed significant reversal of PTU-induced damage. Follicles were well-preserved, colloid content was restored, and epithelial lining regained a cuboidal appearance, closely resembling the normal control. CM-MeOH (200 mg/kg) also improved histo-architecture but to a slightly lesser extent, showing partial recovery with minimal inflammation. The low-dose CM-EtAc group demonstrated mild to moderate improvement, suggesting dose-dependency in therapeutic response. These observations reinforce the biochemical findings and confirm that Commiphora mukul not only

restores thyroid function but also protects structural integrity of the gland. Thus, its fractions demonstrate potent histological protection against chemically induced hypothyroid damage, supporting its role as a thyroprotective agent.

Table 5: Histological Observations of Thyroid Gland Architecture in Different Experimental Groups Following PTU-Induced Hypothyroidism and Treatment with Commiphora mukul Extracts

Groups	Histological Observation
Normal Control	Normal follicles with cuboidal epithelium and abundant colloid.
Disease Control (PTU)	Flattened epithelial cells, reduced colloid, interstitial fibrosis.
Standard	Mild hypertrophy, restored colloid content.
CM-EtAc (200 mg/kg)	Near-normal follicular structure, restored colloid, minimal inflammation.
CM-MeOH (200 mg/kg)	Partial restoration of colloid and epithelial integrity.

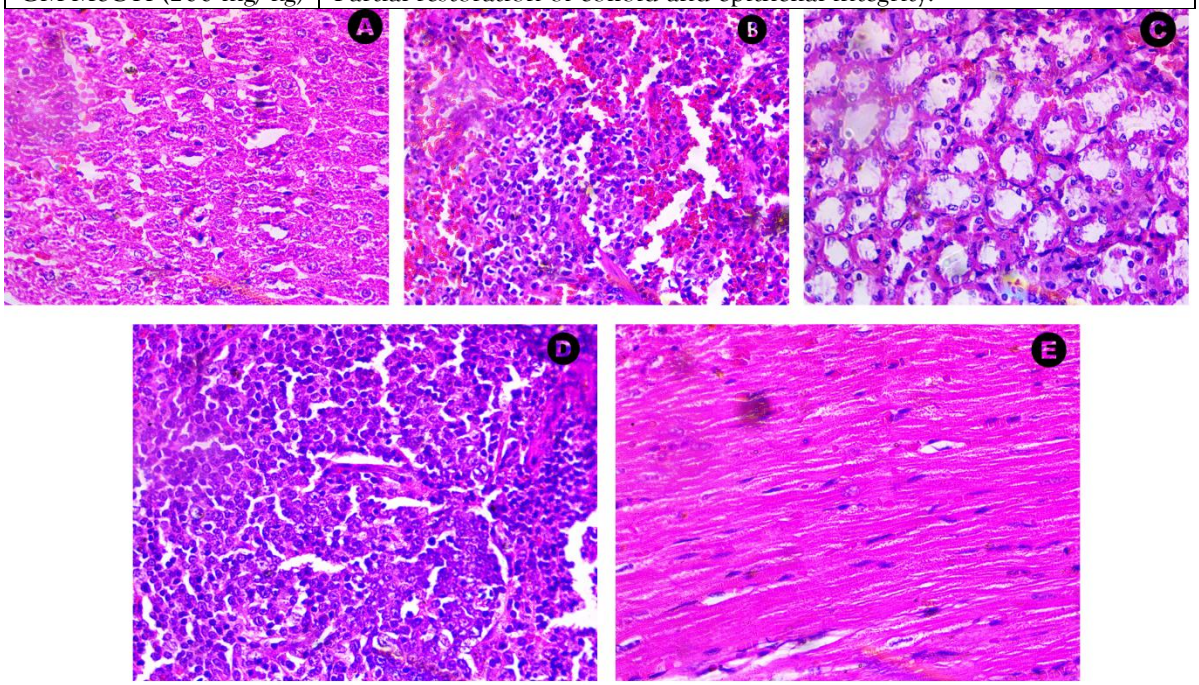

Figure 1: Histopathological Evaluation of Thyroid Glands in PTU-Induced Hypothyroid Rats Treated with Commiphora mukul Fractions

Survival Study

The Kaplan–Meier survival curve illustrated the protective efficacy of Commiphora mukul bioactive fractions in PTU-induced hypothyroid rats over a 21-day experimental period. The disease control group, which received only PTU without any therapeutic intervention, exhibited a progressive decline in survival probability beginning from day 15, culminating in complete mortality by day 20. This confirmed the severe physiological stress and systemic toxicity induced by prolonged PTU administration, which disrupts thyroid hormone synthesis and elevates oxidative damage. In contrast, the normal control, standard (levothyroxine-treated), and all extract-treated groups maintained a consistent survival probability of 100% throughout the study duration. Notably, rats treated with the ethyl acetate and methanol fractions of Commiphora mukul showed full survival, comparable to the standard drug group. This indicated that the plant extracts effectively counteracted the lethal effects of hypothyroidism, likely through restoration of thyroid hormone levels and antioxidant defenses. The absence of mortality in these groups supported the hypothesis that C. mukul fractions possess strong thyromodulatory and cytoprotective properties. These findings not only validated the biochemical and histological outcomes observed in the study but also reinforced the potential of C. mukul as a natural therapeutic agent in managing hypothyroidism and enhancing survival under induced thyroid suppression conditions.

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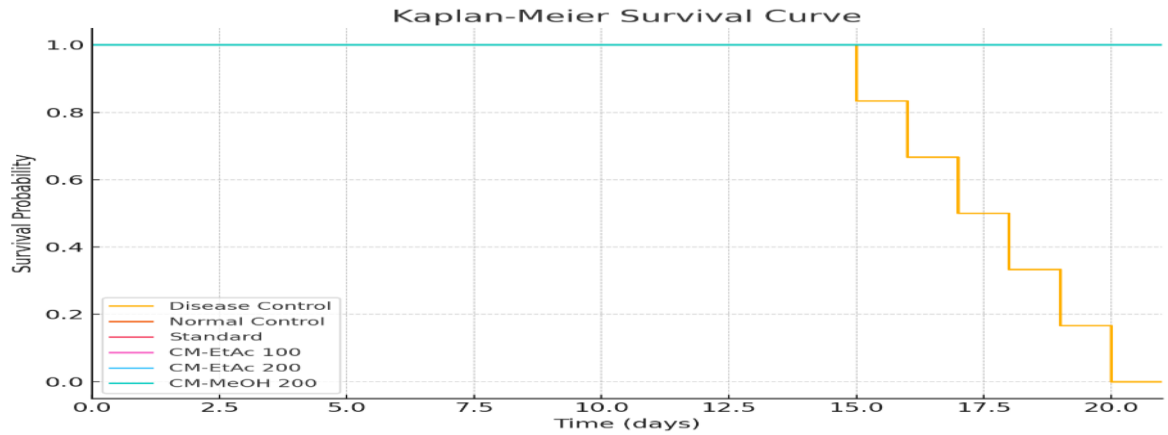

Figure 2: Kaplan–Meier Survival Curve Demonstrating Protective Effects of Commiphora mukul Extracts in PTU-Induced Hypothyroid Rats Over a 21-Day Period

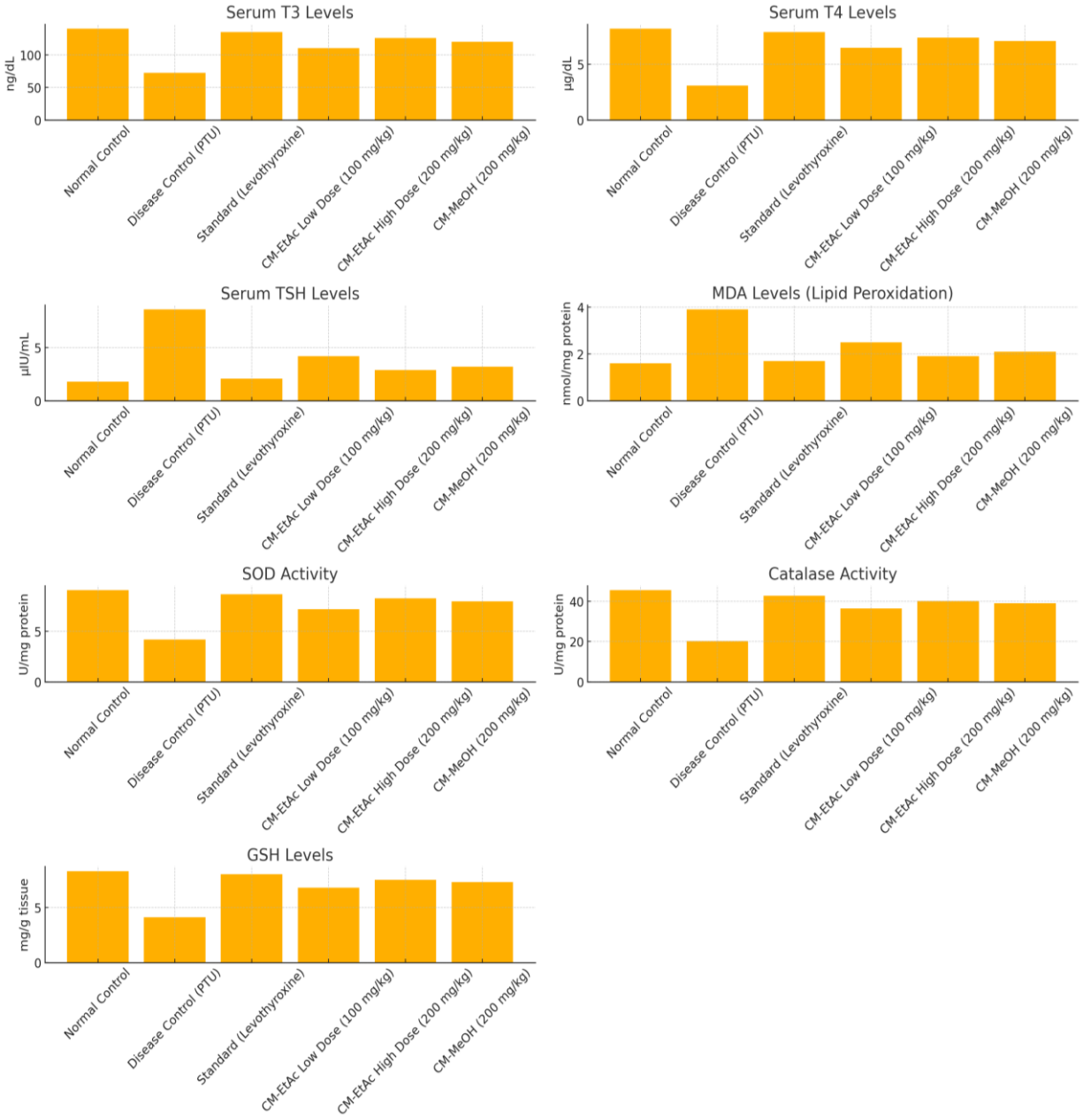

Figure 3: Effect of Commiphora mukul Bioactive Fractions on Serum Thyroid Hormones and Antioxidant Parameters in PTU-Induced Hypothyroid Rats

Serum T3 (Triiodothyronine) Levels

- **Observation:** The PTU-treated disease control group showed a significant reduction in T3 (72.4 ng/dL) compared to the normal control (140.2 ng/dL). Treatment with standard levothyroxine restored T3 levels (135.1 ng/dL). Both C. mukul extracts showed dose-dependent improvement—CM-EtAc high dose was most effective (125.6 ng/dL).
- **Interpretation:** C. mukul bioactive fractions significantly ameliorate PTU-induced suppression of thyroid hormone synthesis, suggesting thyromodulatory activity.

2. Serum T4 (Thyroxine) Levels

- **Observation:** PTU drastically reduced T4 levels (3.1 µg/dL) compared to normal (8.2 µg/dL). Standard and CM-EtAc high dose groups showed near-normal T4 restoration (7.9 and 7.4 µg/dL, respectively).
- **Interpretation:** The bioactive fractions of C. mukul, especially ethyl acetate at higher dose, effectively restored T4 levels, indicating a potent thyroid-stimulating effect.

3. Serum TSH (Thyroid Stimulating Hormone) Levels

- **Observation:** TSH levels significantly increased in the PTU group (8.6 µIU/mL), reflecting hypothyroidism. Standard treatment and CM-EtAc high dose markedly reduced TSH to near-normal levels (2.1 and 2.9 µIU/mL).
- **Interpretation:** The reduction in TSH suggests that C. mukul extracts help restore the hypothalamic-pituitary-thyroid axis balance disrupted by PTU.

4. MDA (Malondialdehyde) – Lipid Peroxidation Marker

- **Observation:** MDA levels were highest in the PTU group (3.9 nmol/mg protein), while CM-EtAc high dose and standard treatments significantly reduced MDA (1.9 and 1.7, respectively).
- **Interpretation:** C. mukul possesses lipid peroxidation inhibitory effects, likely due to antioxidant phytochemicals, thus protecting thyroid tissues from oxidative stress.

5. SOD (Superoxide Dismutase) Activity

- **Observation:** SOD activity was diminished in the PTU group (4.2 U/mg), while C. mukul treatment restored it in a dose-dependent manner (up to 8.3 U/mg in CM-EtAc high dose).
- **Interpretation:** The extract enhances endogenous antioxidant enzyme defense, helping combat oxidative stress-induced thyroid damage.

6. CAT (Catalase) Activity

- **Observation:** PTU administration lowered CAT activity (20.3 U/mg), whereas CM-EtAc high dose and standard treatments improved it significantly (40.2 and 42.9 U/mg respectively).
- **Interpretation:** Elevated CAT levels indicate the role of C. mukul in restoring oxidative enzyme balance and protecting cellular structures from H_2O_2-mediated injury.

7. GSH (Reduced Glutathione) Levels

- **Observation:** GSH levels were suppressed in the PTU group (4.1 mg/g), while CM-EtAc and CM-MeOH fractions restored levels to 7.5 and 7.3 mg/g, respectively.
- **Interpretation:** Restoration of GSH signifies strong antioxidant potential of C. mukul fractions in scavenging ROS and preventing thyroid cell degeneration.

CONCLUSION

The present study successfully demonstrated that bioactive fractions of Commiphora mukul, particularly ethyl acetate and methanol extracts, possess significant thyromodulatory and antioxidant potential in a PTU-induced hypothyroid rat model. Administration of PTU led to marked suppression of thyroid hormones (T3 and T4), elevated TSH levels, and severe oxidative stress, as evidenced by increased malondialdehyde (MDA) and reduced antioxidant enzymes such as SOD, CAT, and GSH. These changes were further supported by histopathological alterations in thyroid tissue and decreased survival rates in untreated animals. Treatment with C. mukul extracts notably reversed these pathophysiological effects. The high-dose ethyl acetate fraction, in particular, restored thyroid hormone levels close to normal, significantly reduced lipid peroxidation, and enhanced endogenous antioxidant defense mechanisms. Histological examination revealed marked preservation of thyroid gland architecture, with improved follicular integrity and colloid content. Additionally, survival analysis confirmed the cytoprotective effects of the extracts, with all treated groups showing 100% survival over the 21-day period. These findings validate the traditional use of Commiphora mukul in managing metabolic and endocrine disorders and

suggest that its bioactive constituents may exert therapeutic effects through modulation of the hypothalamic-pituitary-thyroid axis and enhancement of antioxidant defenses. The dual action—hormonal regulation and oxidative stress mitigation—positions C. mukul as a promising natural adjunct in the treatment of hypothyroidism. Further studies, including clinical trials and mechanistic investigations, are warranted to establish its safety, efficacy, and potential integration into modern endocrine therapy.

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