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Anti-hypothyroid potential of *Bauhinia variegata* and *Commiphora mukul* extracts in albino wistar rats: A histopathological investigation

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Abstract

The present strategies used for the management of hypothyroid conditions are associated with various side effects. To overcome this, ethanolic extracts of *Bauhinia variegata* and *Commiphora mukul* gum resin were evaluated for their effect in the regulation of hypothyroidism in Albino wistar rat model. The study initiated with the preparation of ethanolic extract of non-woody aerial parts of *Bauhinia variegata* and *Commiphora mukul* gum resin. The phytochemical screening was followed via acute oral toxicity studies. To evaluate the effect of extracts, Methimazole-induced hypothyroid Albino wistar rats were used followed via histopathological examination. Animals were treated with individual and combined dose (100, 200 and 400 mg/kg) of plants extracts. As per the acute toxicity studies, 400mg/kg body weight was chosen as the maximum dose for the animal studies. Histopathology studies showed that a combined extract dose of *Bauhinia variegata* and *Commiphora mukul* gum resin showed more beneficial effects than the individual plant extract doses.

Keywords: *Bauhinia variegata*, *Commiphora mukul*, hypothyroidism, ethanol extract, phytochemical, gum resin

Introduction

The hormones delivered by thyroid gland regulates protein synthesis, use of oxygen, basal metabolic rate, metabolic rate at cellular rate and overall growth and development [1]. The thyroid hormones have their direct or indirect vital impact on various body functioning, including cardiovascular, respiratory, reproductive, metabolic and neurological [2]. The concentration of the pituitary glycoprotein hormone, thyroid stimulating hormone transcendently direct the activity of the thyroid gland [3]. Two thyroid hormones, thyroxine (T4) and tri-iodothyronine (T3) are involved in the regulation of major of body functioning. Measurement of the thyroid hormones T3 and T4 in the blood plasma are viewed as a substantive assessment of thyroid capacity.

Hypothyroidism, specifically, is the most common of thyroid disorders in India, affecting one in ten adults. It has been estimated that about 42 million people in India suffer from thyroid diseases [4]. The fall in the levels of thyroid hormones leads to hypothyroidism. To date, the hormone replacement therapy is the most common strategy in the management of thyroid dysfunction. However, the therapy has not remained untouched from the possible side effects which includes, cardiovascular changes, left ventricular hypertrophy, reduced bone density, skin rash, itching, and mild leucopenia. In a rare condition, the therapy may also cause aplastic anemia, thrombocytopenia and lupus erythematosus like syndrome [5]. Therefore, there is an unmet need of an alternative approach for a safe and effective management of hypothyroidism.

From past few years, there has been a tremendous increasing in using natural products in the management of various diseases including hypothyroidism. To date, various plants, such as Gotu Kola (*Centella asiatica*), Ashwagandha (*Withania somnifera*) and Bladder wrack (*Fucus vesiculosus*) have been investigated for the management of thyroid diseases [6, 7]. In the present investigation, areal parts of *Bauhinia variagata* Linn. and *Commiphora mukul* gum resin has been investigated for their effects in the management of hypothyroidism. *Bauhinia variagata* Linn (family Leguminosae) is commonly known as Kachnar (Hindi), Rakta Kanchan (Sanskrit), mountain ebony, orchid-tree and camel's foot [8]. It was native to Southeast Asia and has been investigated to various pharmacological effects including, anti-inflammatory, anti-carcinogenic, anti-mutagenic, anti-microbial, anti-hyperlipidemic, hepatoprotective, insulin release enhancer, nephroprotective, proteinase inhibitor and wound healing activity [9]. *Commiphora mukul* (family Burseraceae) commonly known as guggul and found in India, Bangladesh, and Pakistan [10].

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It has been investigated for various pharmacological activities, such as arthritis, anti-hyper lipidemic, cardio protective, anti-diabetic, anti-helminthic, antioxidant and anti-neoplastic [11].

The present study was designed to explore the effect of *Bauhinia variagata* Linn. (non-woody arrial parts) and *Commiphora mukul* gum resin on thyroid gland of *Albino wistar* rats. The hypothyroidism in *Albino wistar* rats was induced via methimazole. The ethanol extract of the plants were evaluated for their phytochemical constituents and acute oral toxicity. The *Albino wistar* rats with methimazole-induced hypothyroidism were used as an animal model. Histopathological studies on thyroid gland were used to screen the anti-hypothyroid potential of the plant extracts.

Materials and Methods

The plants for the study was collected from the natural habitat of Assam. The non woody aerial part of the *Bauhinia variegata* including leaves, twigs and flowers has been collected from North Jalukbari area, Kamrup (metro) District, Assam, India. *Commiphora mukul* (Guggulu) gum resin was purchased from Assam Ayurvedic Bhandar, Tokobari road, Guwahati, Assam, India. *Bauhinia variegata* Linn. was authenticated via Department of Botany, Gauhati University, Guwahati, Assam, India (Herb/Bot/GU/2018/90/Acc. No.18477). Methimazole (Tapazole) was purchased from Loknath Medical Store, Pandu Port Road, Guwahati 12. All other chemicals were of analytical grades and obtained from Pharmacognosy laboratory, Institute of Pharmacy Gauhati Medical College, Guwahati 14.

Preparation of *Bauhinia variegata* extract

The non-woody arial parts of *Bauhinia variegata* Linn. (Leaves, twigs and flowers) were collected, dried in a shade under $28\pm 2^{\circ}\text{C}$ (for six to seven days and then crushed into coarse powdery substance by using electric grinder. A weighed quantity of the powder (1200g) was passed through sieve number 40. The filtered powder was subjected to hot solvent extraction in a soxhlet apparatus using ethanol, at a temperature range of 55°C to 65°C . Before and after every extraction the marc was completely dried and weighed. The filtrate was evaporated to dryness at 40°C under reduced pressure in a rotary vacuum evaporator.

Preparation of *Commiphora mukul* gum resin extract

The collected plant sample (resin) was washed thoroughly with tap water, dried at room temperature away from sun light, cut into small pieces, and then powdered. Ethanolic extract was prepared by hot solvent extraction in a soxhlet apparatus using ethanol, at a temperature range of 55°C to 65°C . The extract was filtered, concentrated under reduced pressure and finally dried in a vacuum desiccators.

Phytochemical screening

The prepared extracts were screened for various phytochemical constituents, such as alkaloid, carbohydrate, phytosterol, fixed oil and fats, phenols, tannins, flavonoids, gums and mucilage, proteins and saponins. The presence of phytochemical constituents were detected via the methods as described in the literature [12, 13].

Experimental Animals

Albino wister rats (150-200 g) were procured from animal house of Girijananda Chowdhury Institute of Pharmaceutical Science (GIPS), Hatkhowaapara, Azara, Guwahati, Assam,

India. Animals were kept in different cages in Animal house, Department of Pharmacology, Guwahati Medical College, Guwahati, Assam, India. All rats were acclimatized to the laboratory condition for a period of one week prior to dosing. The rats were housed in air conditioned room at $24 \pm 2^{\circ}\text{C}$ on a 12/12 h light-dark cycle. The animals were fed on diet of fish, bread, corn and water *ad libitum*. All the animal experimental protocols were approved (MC/32/2012/9) by Institutional Animal Ethical Committee CPSEA Regd no 351 3/1/2001, Gauhati Medical College and Hospital, Guwahati, Assam, India.

Acute oral toxicity study

The acute toxicity studies were performed in accordance with the OECD (Organization for Economic Co-operation and Development) guideline no 425(up and down procedure)[14]. The rats were divided into 3 groups (4 rats each). Group 1 served as the control, and the other two groups were treated with ethanolic extract of *Bauhinia variegata* Linn and *Commiphora mukul* gum resin. The control group was given purified water, while the treatment groups received extract s at 2000 mg/Kg body weight suspended in purified water through oral gavage. The administered volume was adjusted to 8 ml/Kg for every rat. The vehicle and the extract were administered only once (on day 0) at the start of the experiment. The rats were monitored for 14 days for mortality, convulsions, salivations, sleep and coma.

Evaluation of anti-hypothyroid activity

The ethanolic extracts of *Bauhinia variegata* and *Commiphora mukul* gum resin were evaluated for their potential in regulating hypothyroid condition. Methimazole was used to induce hypothyroidism in the *Albino wister* rats. Different doses of the plants extracts (100, 200 and 400 mg/kg of body weight) were administered. In addition, the plant extracts from *Bauhinia variegata* and *Commiphora mukul* gum resin were also co-administered to evaluate their combination effect. The combined extracts were also evaluated at different doses of each extract, such as 100, 200 and 400 mg/kg of body weight.

Experimental design

Dose of Methimazole (oral) -60mg /kg /day.

Doses of *Bauhinia variegata* extract, X1= 100mg/kg, X2=200 mg/kg and X3=400mg /kg body weight.

Doses of *Commiphora mukul* gum resin extract, Y1=100mg/kg, Y2=200mg/kg and Y3=400mg /kg body weight).

- Group 1:** Control group. (Euthyroid)
- Group 2:** Received Methimazole 60mg / kg body weight orally with water.
- Group 3:** Received Methimazole and levothyroxine standard drug
- Group 4A:** Received Methimazole and dose (X1) of *Bauhinia variegata*.
- Group 4B:** Received Methimazole and dose(X2) of *Bauhinia variegata*.
- Group 4C:** Received Methimazole and dose(X3) of *Bauhinia variegata*.
- Group 5A:** Received Methimazole and dose (Y1) of *Commiphora mukul*.
- Group 5B:** Received Methimazole and dose (Y2) of *Commiphora mukul*.
- Group 5C:** Received Methimazole and dose (Y3) of

Commiphora mukul.

- Group 6A:** Received Methimazole and dose (X1) of *Bauhinia variegata* combined with Dose (Y1) of *Commiphora mukul*.
- Group 6B:** Received Methimazole and dose (X2) of *Bauhinia variegata* combined with dose (Y2) of *Commiphora mukul*.
- Group 6C:** Received Methimazole and dose (X3) of *Bauhinia variegata* combined with dose (Y3) of *Commiphora mukul*.

Histopathology study of thyroid gland

Animals were sacrificed by cervical dislocation and the

thyroid gland was isolated from each animal. Isolated thyroid glands were cleaned off extraneous tissue and kept in freshly prepared formalin (10% v/v) for histopathological analysis. The effect of plant extracts on the thyroid was evaluated through comparing histopathology report of each group.

Results and Discussion

Results

Phytochemical screening

The prepared extracts of *Bauhinia variegata* Linn. (Non-woody arial parts) and *Commiphora mukul* gum resin were evaluated for the presence of different phytoconstituents. The results of phytochemical screening were mentioned in table-1.

Table 1: Preliminary phytochemical studies

Constituents	Ethanollic extract of <i>Bauhinia variegata</i> Linn	Ethanollic extract of <i>Commiphora mukul</i> gum resin
Alkaloid	+	-
Carbohydrate	+	+
Phytosterol	-	+
Fixed oil and fats	-	-
Phenols	+	+
Tannins	+	+
Flavonoids	+	+
Gum and mucilage	+	+
Proteins	+	+
Saponins	+	+

Acute oral toxicity study

The acute toxicity studies were performed in accordance with the OECD (Organization for Economic Co-operation and Development) guidelines. As per the results, no abnormality has been observed up to a period of 4-6 hrs or mortality up to a period of 24 hrs at the maximum tolerated dose level of 2000 mg/kg body weight. From the results, 400mg/kg body weight was chosen as the maximum dose for further experimentation.

Histopathological investigation

The ethanollic extracts of the plants were evaluated for their effects in *Albino wistar* rats having methimazole-induced hypothyroidism. As per the results, Group-1 (Control, euthyroid) showed numerous follicles with round nuclei surrounded by a clear cytoplasm (Fig.1A).

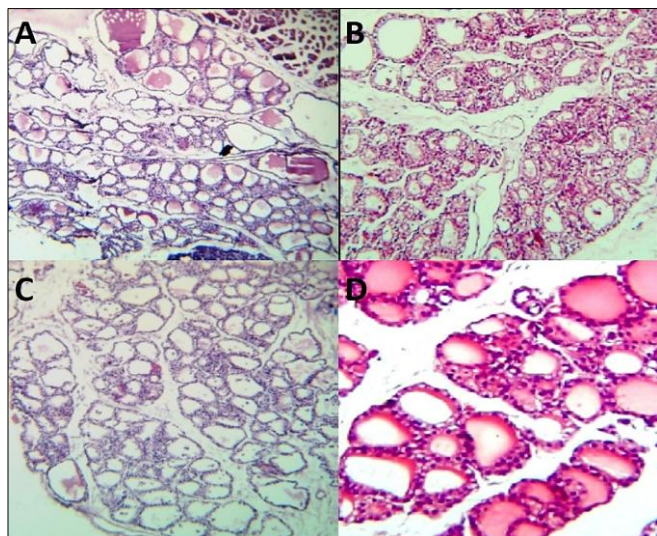


Fig 1: Histopathology of thyroid glands. (A) Group-1 (control group, Euthyroid), (B) Group-2 (Methimazole 60mg / kg treated), (C) Group-3 (Methimazole + levothyroxine treated) and (D) Group-4A (Methimazole and dose (X1) of *Bauhinia variegata*).

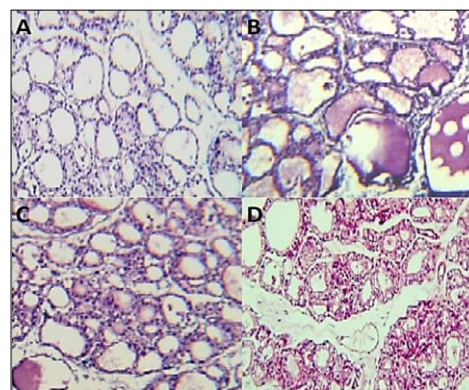


Fig 2: Histopathology of thyroid glands.(A) Group 4 B (Methimazole and dose(X2) of *Bauhinia variegata*), (B) Group 4 C (Methimazole and dose(X3) of *Bauhinia variegata*), (C) Group 5 A (Methimazole and dose (Y1) of *Commiphora mukul*) and (D) Group 5 B (Methimazole and dose (Y2) of *Commiphora mukul*).

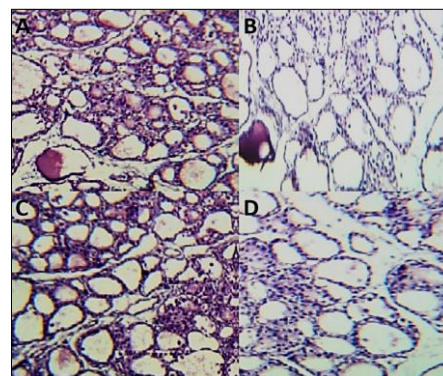


Fig 3: Histopathology of thyroid glands. (A) Group 5C (Methimazole and dose (Y3) of *Commiphora mukul* extract), (B) Group 6A (Methimazole and dose (X1) of *Bauhinia variegata* combined with Dose (Y1) of *Commiphora mukul*), (C) Group 6B (Methimazole and dose (X2) of *Bauhinia variegata* combined with Dose (Y2) of *Commiphora mukul*) and (D) Group 6C (Methimazole and dose (X3) of *Bauhinia variegata* combined with Dose (Y4) of *Commiphora mukul*).

Histopathology studies of Group-2 rats showed that the thyroid gland is small, shrunken lobules and comprising of micro follicles. These micro follicles were partially filled or completely devoid of colloid and lined by cuboidal epithelium. The follicles also lacked absorptive vesicles. These features suggested markedly suppressed functional status of the thyroid gland (Fig.1B). Group-3 showed that about 30-40 % of the follicles were colloidal in nature (Fig.1C). The follicular epithelial cells appeared normal. Follicles were filled with homogenous colloids. Group 4A showed follicles lined by follicular epithelial cells which appeared to be normal (Fig.1D). In Group 4B, the thyroid follicles showed 30-40% of luminal colloids. There was no papillary in folding of the epithelium (Fig.2A). Histopathology studies of Group-4C showed normal sized follicles as well as a few macro follicles (Fig.2B). In Group-5A, the follicular epithelial cells appeared normal (Fig.2C). Histopathology studies of Group 5B showed follicles with thick colloid show a flattened cuboidal lining epithelium. The follicular epithelial cells appeared normal (Fig.2D). Histopathology studies of Group 5C showed that the thyroid follicles were filled with homogenous colloid. The glands showed cuboidal lining epithelium of the follicles (Fig.3A). In Group 6A, the thyroid follicles were lined by low cuboidal epithelial cells filled with colloid. All the follicles were almost equal and regular in size (Fig.3B). Group 6B showed numerous follicles some of which contain colloidal material (40-50%) (Fig.3C). In Group 6C, the follicular epithelial cells appeared normal. Follicles were filled with homogenous colloids (Fig.3D).

Discussion

The present therapeutic strategies of the management of the hypothyroidism were associated with various side effects. To overcome this, the present investigation deals with the evaluation of beneficial effects of *Bauhinia variegata* Linn. and *Commiphora mukul* gum resin in the management of hypothyroidism. In the present study, non-woody areal parts of *Bauhinia variegata* Linn. and *Commiphora mukul* gum resin were collected and authenticated. For the extract preparation, hot solvent extraction method was used. Ethanol was used as an extracting solvent for the extraction of *Bauhinia variegata* Linn. and *Commiphora mukul* gum resin. The phytochemical screening revealed that the ethanolic extract of *Bauhinia variegata* Linn has all the phytoconstituents, such as alkaloid, carbohydrate, phenols, tannins, flavonoids and, gum and mucilage, proteins and saponins. However, phytosterol and, fixed oil and fats were absent in the extract (Table-1). The phytochemical screening of *Commiphora mukul* gum resin showed the presence of Carbohydrate, phytosterol, Phenols, tannins, flavonoids, gum and mucilage, proteins and saponins. However, alkaloids, fixed oil and mucilage were absent in the extract (Table-1).

The acute toxicity studies of the prepared extracts were performed in accordance with the OECD (Organization for Economic Co-operation and Development) guidelines. At the maximum tolerated dose level of 2000 mg/kg body weight, no abnormalities has been observed. A dose of 400mg/kg body weight was selected as the maximum dose. *Albino wistar* rats with methimazole-induced hypothyroidism were used as an animal model. Different doses of *Bauhinia variegata* extracts (X1= 100mg/kg, X2=200mg/kg and X3=400mg /kg body weight) and *Commiphora mukul* gum resin (Y1=100mg/kg, Y2=200mg/kg and Y3=400mg /kg body weight) were used in the study. At the end of the animal studies, histopathological examination of the thyroid glands of different group animals

was conducted. The thyroid glands of methimazole-induced *Albino wistar* rats showed characteristic histopathological features of hypothyroidism. The lobules were appeared small and shrank with presence of micro follicles. These micro follicles were partially filled or completely devoid of colloid and lined by cuboidal epithelium. The absorptive vesicles were absent in the follicles (Fig.1B). The levothyroxine treatment in methimazole-induced Albino wistar rats showed colloidal follicles. The epithelial cells were appeared normal filled with homogenous colloids (Fig.1C). The histopathological examination of thyroid glands (Group 4A, 4B and 4C) treated with methimazole and different doses *Bauhinia variegata* ethanolic extract showed normal follicular epithelial cells with no papillary unfolding of the epithelium (Fig. 1D and Fig. 2(A and B)). With increase in the dose of *Bauhinia variegata* ethanolic extract (100 to 400 mg/kg), thyroid follicle shows luminal colloids (30-40%) with few macro follicles. The histopathological examination of thyroid glands (Group 5A, 5B and 5C) treated with methimazole and different doses *Commiphora mukul* gum resin ethanolic extract showed normal follicular epithelial cells were appeared normal. With increase in the dose of *Commiphora mukul* gum resin ethanolic extract (100 to 400 mg/kg), thick colloid with flattened cuboidal epithelium lining was appeared. In addition, cuboidal epithelium lining of the follicles were also appeared.

A combined doses of ethanolic extract of *Bauhinia variegata* and *Commiphora mukul* gum resin showed significant effect on the methimazole-induced hypothyroid glands. At low dose combination (100 mg/kg of *Bauhinia variegata* and *Commiphora mukul* gum resin), the thyroid follicles were surrounded by low cuboidal epithelial cells filled with colloid. The follicles were almost equal and regular in size. At median dose combination (200 mg/kg of *Bauhinia variegata* and *Commiphora mukul* gum resin), numerous follicles were filled with colloidal material (40-50%). At high dose combination (400 mg/kg of *Bauhinia variegata* and *Commiphora mukul* gum resin), the follicular epithelial cells appeared normal filled with homogenous colloids. The histopathological examination showed that the combination of the different ethanolic extracts showed better results than the individual extract treatment groups.

Conclusion

The present findings inferred that the combination of *Bauhinia variegata* and *Commiphora mukul* gum resin ethanolic extract could be a potential strategy for the treatment of hypothyroid condition. In addition, disease management using natural products will be helpful in overcoming the possible side effects associated with present treatment strategies. The results are encouraging, but further molecular studies are needed to clarify the exact mechanism behind the anti-thyroid activity.

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Conflict of interest: None

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