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Research Article

Effect of Ethanolic Extract of *Bauhinia variegata* and *Commiphora mukul* in Regulating Thyroid Stimulating Hormone in Hypothyroidism Induced Albino Wistar Rats

Singha Mahua Bhaumik^{1*}, Sarma Tikendrajit², Lahakar Mangala³

¹ Lecturer, Institute of Pharmacy, Guwahati Medical College, Guwahati, Assam, India.

² Associate Professor, Department of Sharir Rachna, Government Ayurvedic College, Guwahati, Assam, India.

³ Professor and Head of Department, Department of Pharmacology, Guwahati Medical College, Guwahati, Assam, India.

ABSTRACT

The present clinical interventions used for the management of the hypothyroidism are still associated with various side effects. In order to avoid this, the aim of the present investigation is to evaluate the effect of *Bauhinia variegata* and *Commiphora mukul* in regulating serum thyroid stimulating hormone levels in rats. Ethanolic extract of *Bauhinia variegata* (non-woody aerial parts) and *Commiphora mukul* (gum resin) was prepared via hot solvent extraction in a Soxhlet apparatus. Preliminary phytochemical screening showed that the ethanolic extract of *Bauhinia variegata* linn has all the phytoconstituents except phytosterol, fixed oil and fats. The *Commiphora mukul* gum resin extract showed the presence of all phytoconstituents except alkaloids, fixed oil and mucilage. The oral toxicity studies showed no abnormalities at the maximum tolerated dose level of 2000 mg/kg body weight. Methimazole was used to induce hypothyroidism in the rats. The animals were treated with different individual and combined dosages (100, 200 and 400 of two plant extracts. Serum TSH level analysis showed that a combination of high dose (400mg /kg body weight) of *Bauhinia variegata* and *Commiphora mukul* extract has a significant effect in lowering serum TSH levels raised due to hypothyroidism. To conclude, a combined extract of plants could be a potential therapeutic alternative for the management of hypothyroidism.

Keywords: *Bauhinia variegata* Linn., *Commiphora mukul*, Hypothyroidism, Thyroid stimulating hormones, Methimazole.

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*Address for Correspondence:

Mahua Bhaumik Singha, Lecturer, Institute of Pharmacy, Guwahati Medical College, Guwahati, Assam, India.

INTRODUCTION

The thyroid hormones have their impact on the different body working, including cardiovascular, respiratory, regenerative and neurological.¹ These hormones control various events, such as oxygen utilization, basal metabolic rate, cellular metabolism and protein synthesis.² Thyroxine (T₄) and tri-iodothyronine (T₃) are the two thyroid hormones that moderate various body functioning.³ Hypothyroidism is a medical condition where there is a fall in the levels of thyroid hormones. In India, it has been estimated that about 42 million people are suffered from thyroid diseases.⁴ The present clinical intervention for the management of hypothyroidism includes hormone replacement therapy (HRT). However, the HRT has also been associated with various side effects, such as alteration in cardiovascular functioning, low osteo-density, itching and leucopenia. In addition, aplastic anemia, thrombocytopenia and lupus erythematosus has also been reported in rare

cases.⁵ considering the side effects of the HRT, there is a need of an alternative approach that can treat hypothyroidism in a safe and effective manner.

It has been estimated that about 80% of the world population uses plant products for the management of their primary health issues.⁶ In the previous studies, various plants have been investigated for the management of thyroid diseases, such as Gotu Kola (*Centella asiatica*), Ashwagandha (*Withania somnifera*) and Bladder wrack (*Fucus vesiculosus*).^{7,8} In the present investigation, *Bauhinia variegata* Linn. (Non woody aerial part) and *Commiphora mukul* gum resin has been investigated for their potential in regulating thyroid stimulating hormone (TSH). *Bauhinia vareagata* Linn (Kachnar (Hindi), Rakta Kanchan (Sanskrit), mountain ebony, orchid-tree and camel's foot) belongs to the family Leguminosae.⁹ To date, *Bauhinia variegata* has been investigated for various activities, such as anti-carcinogenic, anti-microbial, anti-inflammatory, anti-hyperlipidemic,

nephroprotective, proteinase inhibitor and insulin release enhancer.¹⁰ *Commiphora mukul* (Guggul) belongs to the family Burseraceae.¹¹ To date, *Commiphora mukul* has been investigated for various activities, such as anti-hyperlipidemic, anti-diabetic, anti-helminthic, anti-arthritis, antioxidant and anti-neoplastic.¹²

In our previous studies, we have investigated the anti-hypothyroid effect of ethanolic extracts of *Bauhinia variagata* Linn. And *Commiphora mukul* gum resin through the histopathological studies. The results have shown promising results regarding the potential anti-hypothyroid effect of ethanolic extracts of *Bauhinia variagata* Linn. and *Commiphora mukul* gum resin.¹³ In the present investigation, *Bauhinia variagata* Linn. (Non-woody aerial parts) and *Commiphora mukul* gum resin has been evaluated for their potential in regulating elevated TSH level in *Albino wistar* rats due to hypothyroidism. The drug methimazole was used to induce hypothyroidism in *Albino wistar* rats. The ethanolic extracts of the plants were subjected to phytochemical screening and acute oral toxicity. Finally, the animals were treated with individual and combined plant extracts, and the level of TSH in animals was evaluated.

MATERIALS AND METHODS

Bauhinia variagata (non-woody aerial part) was collected from North Jalukbari area, Kamrup district, Assam, India. *Commiphora mukul* gum resin was purchased from Assam Ayurvedic Bhandar, Guwahati, Assam, India. The plant, *Bauhinia variagates* Linn. was authenticated via Department of Botany, Guwahati University, Guwahati, Assam, India (Herb/Bot/GU/2018/90/ Acc. No.18477). Methimazole (Tapazole) was purchased from Loknath Medical store, Pandu Port Road, Guwahati, Assam, India. All other chemicals were of analytical grades and obtained from S. D. Fine Chemicals, Mumbai, India.

Bauhinia variagata and *Commiphora mukul* gum resin extract

The leaves, twigs and flowers of *Bauhinia variagata* Linn. were dried in a shade for six to seven days. The dried leaves, twigs and flowers were crushed into coarse powdery substance followed by screening through sieve number 40. The screened powder was extracted using a Soxhlet apparatus and ethanol as an extraction solvent. The extraction was performed under a temperature range of 55°C to 65°C. Finally, the solvent was removed from the filtrate under reduced pressure in a rotary vacuum evaporator at 40°C.

For the preparation of *Commiphora mukul* gum resin extract, the gum resin was properly washed, dried, reduced in size and powdered. The powdered gum resin was subjected to hot solvent extraction using a Soxhlet apparatus and ethanol as an extraction solvent. The extraction was carried out at a temperature range of 55°C to 65°C. The extract was filtered, concentrated under reduced pressure and finally dried in a vacuum desiccator.

Phytochemical screening

The prepared extracts of *Bauhinia variagata* and *Commiphora mukul* gum resin were evaluated for the presence of alkaloid, carbohydrate, phytosterol, fixed oil and fats, phenols, tannins, flavonoids, gums and mucilage, proteins and saponins. The presence of phytoconstituents was investigated via the methods mentioned in the literature.^{14,15}

Experimental Animals

For in-vivo studies, *Albino wistar* rats (150-200 g) were acquired from the animal house of Girijananda Chowdhury Institute of Pharmaceutical Science (GIPS), Hatkhowaapara, Azara, Guwahati, Assam, India. For the animal studies, the animals were stored in the animal house, Department of Pharmacology, Guwahati Medical College, Guwahati, Assam, India. All rats were acclimatized to the lab condition for a week preceding dosing. The rats were kept in an air-conditioned room at $24 \pm 2^\circ\text{C}$ on a 12/12 h light-dark cycle. The animals were fed with fish, bread, corn and water ad libitum. The protocol for the animal study was approved (MC/32/2012/9) from Institutional Animal Ethical Committee (IAEC), Guwahati Medical College and Hospital, Guwahati, Assam, India.

Acute oral toxicity study

The OECD (Organization for Economic Co-operation and Development) guideline no 425 (up and down procedure) was followed to perform acute toxicity studies.¹⁶ Rats were divided into three groups, Group 1 served as the control, and the other two groups (Group-II and III) were treated with ethanolic extract of *Bauhinia variagata* Linn and *Commiphora mukul* gum resin. Group-I was fed with purified water and Group-II and III were administered with 2000 mg/Kg body weight extract suspended in purified water through oral gavage. Following the administration of the vehicle and the extracts (only once, day 0), the rats were monitored for mortality, convulsions, salivations, sleep and coma for about 14 days.

Effect on thyroid stimulating hormone

Bauhinia variagates and *Commiphora mukul* gum resin extracts were evaluated for their potential in regulating thyroid stimulating hormones (TSH). Methimazole was used to induce hypothyroidism in the *Albino Wister* rats. In order to evaluate the effect on the TSH, different doses of the individual and combined extracts (X1, Y1= 100, X2, Y2= 200 and X3, Y3= 400 mg/kg of body weight) were administered (Table-1).

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

Dose of T4 injection-2 nmol /g through subcutaneous route

Doses of *Bauhinia variagata* 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).

Table 1: Animal groups and treatment used to evaluate the effect of extracts on TSH.

Group	Treatment
Group 1	Control group (Euthyroid)
Group 2	Methimazole 60mg / kg body weight orally with water
Group 3	Methimazole and levothyroxine standard drug
Group 4A	Methimazole and dose (X1) of <i>Bauhinia variegata</i>
Group 4B	Methimazole and dose(X2) of <i>Bauhinia variegata</i>
Group 4C	Methimazole and dose(X3) of <i>Bauhinia variegata</i>
Group 5A	Methimazole and dose (Y1) of <i>Commiphora mukul</i>
Group 5B	Methimazole and dose (Y2) of <i>Commiphora mukul</i>
Group 5C	Methimazole and dose (Y3) of <i>Commiphora mukul</i>
Group 6A	Methimazole and dose (X1) of <i>Bauhinia variegata</i> combined with Dose (Y1) of <i>Commiphora mukul</i>
Group 6B	Methimazole and dose (X2) of <i>Bauhinia variegata</i> combined with dose (Y2) of <i>Commiphora mukul</i>
Group 6C	Methimazole and dose (X3) of <i>Bauhinia variegata</i> combined with dose (Y3) of <i>Commiphora mukul</i>

Serum analysis

In the test blood sample was collected on 4, 6, 8 and 10 weeks following the treatment by retro-orbital puncture under anaesthetic conditions (thiopental sodium 40 mg/kg) and serum was separated¹⁷. The serum TSH was estimated by ELISA, catalog no 55- TSHRT Size 96 wells Ver. 1a-07/120704- ALPCO.

RESULTS AND DISCUSSION

Acute oral toxicity

The acute toxicity study was performed as per the OECD (Organization for Economic Co-operation and Development) guidelines no 425. No abnormalities have been reported at the maximum tolerated dose level of 2000 mg/kg body

weight. In the present investigation, a dose of 400mg/kg body weight was selected as the maximum dose. The investigation was performed at three different dosage range, 100, 200 and 400mg /kg body weight.

Phytochemical screening

The extracts of *Bauhinia variegata* Linn. and *Commiphora mukul* gum resin were analysed for the presence of various phytoconstituents. The ethanolic extract of *Bauhinia variegata* Linn has all the phytoconstituents except phytosterol, fixed oil and fats (Table-1). The *Commiphora mukul* gum resin extract showed the presence of all phytoconstituents except alkaloids, fixed oil and mucilage (Table-1).

Table 1: Preliminary phytochemical studies

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

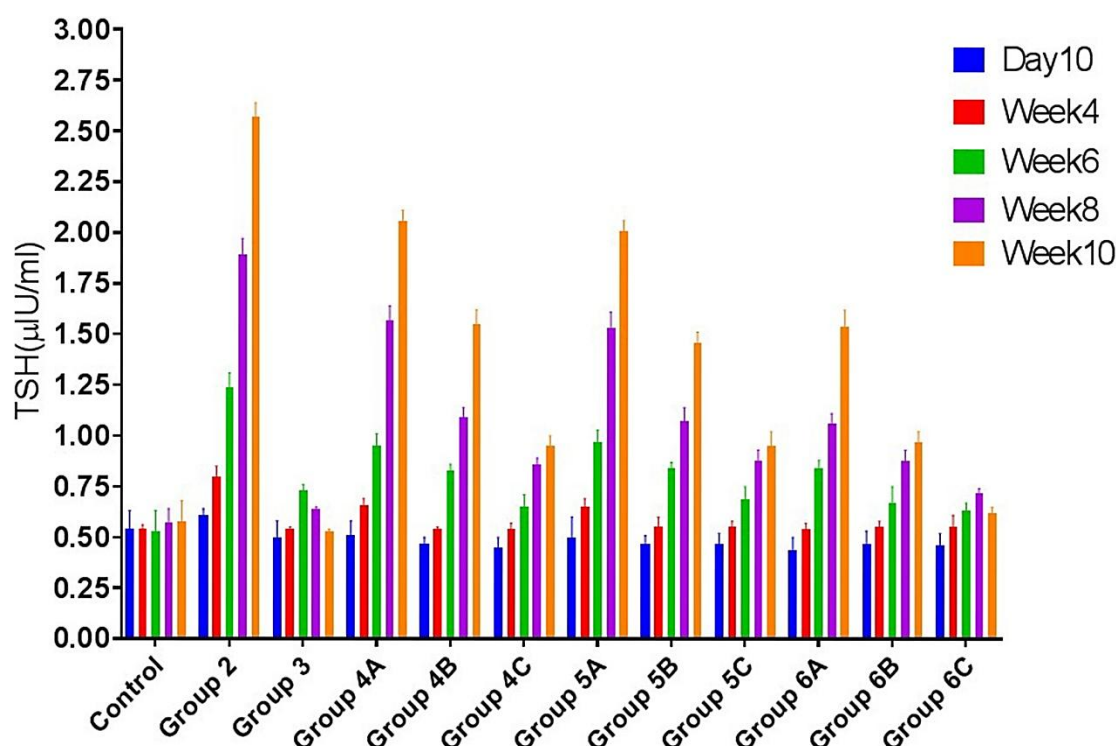


Figure 1: Effect of plant extracts on serum TSH levels after week 4, 6, 8 and 10 [Mean±SE, n=6].

Effect on TSH

Different doses of extracts (individual and in combination) were tested. At the end of 6 week, group 4C, 5C, 6B and 6C showed a reduction in the serum TSH level compared to the group-3 (Figure-1). However, group 4A, 5A and 6A showed an increase in the serum TSH level compared to the control group-3 (Figure-1). In addition, control group-3 treated with levothyroxine (T₄) has also showed a slight increase in the serum level of TSH (Figure-1). This could be due to a decrease in effectiveness of the T₄ with time. The methimazole induced group 2 showed a further rise in TSH level (Figure-1).

At the end of 8 weeks, group 4C, 5C and 6B showed a reduction in the serum TSH level compared to the group 3 (Figure-1). Group-6C (Methimazole and dose (X3) of *Bauhinia variegata* combined with dose (Y3) of *Commiphora*

mukul) showed a significant reduction in serum TSH level compared to group 3, 4C, 5C and 6B (Figure-1). The methimazole induced group 2 showed a further rise in TSH level. In addition, group 4A and 5A showed a further increase in serum TSH level (Figure-1).

At the end of 10 weeks, group 4C, 5C and 6B maintained the low serum TSH level compared to the group 3 (Figure-1). However, group 4A, 5A and 6A showed an increase in the serum TSH level compared to the control group (Figure-1). The methimazole induced group 2 showed a further rise in TSH level. Group-6C (Methimazole and dose (X3) of *Bauhinia variegata* combined with dose (Y3) of *Commiphora mukul*) showed a significant reduction in serum TSH level compared to group 3, 4C, 5C and 6B (Figure-1). The quantified alteration in serum TSH level following plant extract treatment at day 10, week 4, 6, 8 and 10 is also shown in table-1.

Table 1: Effect of plant extracts on serum TSH levels after day 10, week 4, 6, 8 and 10 [Mean±SE, n=6].

Treatment Group	TSH (µIU/ml)				
	Day10	weeks 4	weeks 6	weeks 8	weeks 10
	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Control	0.54±0.09	0.54±0.02	0.53±0.10	0.57±0.07	0.58±0.10
Group 2	0.61±0.03	0.80±0.05	1.24±0.07	1.89±0.08	2.57±0.07
Group 3	0.50±0.08	0.54±0.01	0.73±0.03	0.64±0.01	0.53±0.01
Group 4A	0.51±0.07	0.66±0.03	0.95±0.06	1.57±0.07	2.06±0.05
Group 4B	0.47±0.03	0.54±0.01	0.83±0.03	1.09±0.05	1.55±0.07
Group 4C	0.45±0.05	0.54±0.03	0.65±0.06	0.86±0.03	0.95±0.05
Group 5A	0.50±0.10	0.65±0.04	0.97±0.06	1.53±0.08	2.01±0.05
Group 5B	0.47±0.04	0.55±0.05	0.84±0.03	1.07±0.07	1.46±0.05
Group 5C	0.47±0.05	0.55±0.03	0.69±0.06	0.88±0.05	0.95±0.07
Group 6A	0.44±0.06	0.54±0.03	0.84±0.04	1.06±0.05	1.54±0.08
Group 6B	0.47±0.06	0.55±0.03	0.67±0.08	0.88±0.05	0.97±0.05
Group 6C	0.46±0.06	0.55±0.06	0.63±0.04	0.72±0.02	0.62±0.03

Results showed that group 4C treated with Methimazole and dose (X3) of *Bauhinia variegata*, group 5C treated with Methimazole and dose (Y3) of *Commiphora mukul* and group 6C treated with Methimazole and dose (X3) of *Bauhinia variegata* combined with dose (Y3) of *Commiphora mukul* showed significant decline in the serum TSH levels (Figure-1, Table-2). A combination of high dose (400mg /kg body weight) of *Bauhinia variegata* and *Commiphora mukul* gum resin showed significant effect compared to control and other treated groups (Figure-1, Table-2).

CONCLUSION

In the present investigation, the effect of ethanolic extract of *Bauhinia variagata* and *Commiphora mukul* on serum TSH level was evaluated in Albino rats. Animal studies have shown that, compared to other treatment groups, a combination of high dose (400mg /kg body weight) of *Bauhinia variegata* and *Commiphora mukul* showed a significant effect on serum TSH levels. The study showed that the combined extract dose of *Bauhinia variagata* and *Commiphora mukul* significantly reduced the serum TSH levels through a synergistic effect. This can be a potential therapeutic alternative for the management of hypothyroidism. However, further investigation is required to establish these plant extract as a safe and effective natural product.

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CONFLICT OF INTEREST

None

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