

Research Article

Role of Ethanolic Extract of Bauhinia Purpurea Leaves on Amelioration of Hyperthyroidism in L-Thyroxin Induced Female Albino Wistar Rats.

Revathy Nai R^{1*}, Joseph L², Rahees T³

^{1,2,3} University College of Pharmacy MG University, Kottayam, Kerala, India.

*Corresponding author: Revathy R Nai, University College of Pharmacy MG University, Kottayam, Kerala, India, Tel: 00971524678422; E-mail: revathy6nair@gmail.com

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Abstract

Ethanolic extract of Bauhinia purpurea (EEBP) leaves was evaluated for its possible ameliorative effect in the regulation of hyperthyroidism in albino wistar rat model. Serum triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) concentrations were considered as the end parameters of thyroid function in the study. Histopathological study of thyroid gland was also performed. (Levothyroxine) LT4 administration (0.5 mg/kg/d for 12 days) as an inducing agent increased the levels of serum T3, T4 with a concomitant decline in TSH levels. However, simultaneous administration of the EEBP (100 mg/kg, 150 mg/kg 12-30th day) to the LT4 induced hyperthyroid animals reversed all these effects indicating their potential in the regulation of hyperthyroidism. Propylthiouracil (PTU) (10mg/kg, 12-30th day) was administered as standard drug. PTU is selected as the standard drug in the light of many antithyroidic studies done [18]. When relative efficacy was compared with that of (PTU), a standard antithyroidic drug, experimental B.purpurea extract appeared to be comparable by showing a positive effect towards the amelioration of hyperthyroidism in albino wistar rat model. Furthermore histopathological analysis confirmed the result obtained.

Key words: antioxidant; antithyroidic activity; Bauhinia purpurea; flavonoids; hyperthyroidism; propylthiouracil.

Introduction

Thyroid hormone is essential for normal development, especially of the central nervous system (CNS). Thyroid disorders are often described as 'the great masqueraders'. This is because they can have such a varied and variable presentation. Thyroid hormones regulate almost all functional aspects of the body, including metabolic, respiratory, cardiovascular, nervous and reproductive functions, either directly or indirectly [1]. Alterations in the level of these hormones lead not only to altered basal metabolic rate but also to many health problems. People with these conditions may present with something completely unrelated, but on further investigation the underlying cause is decreased thyroid activity. It is therefore prudent for health professionals to always be alert on

thyroid disorders, and actively investigate the possibilities.

Triiodothyronine (T3) and thyroxine (T4) hormones are known as cell metabolism regulators, being associated with different biological processes in all vertebrates [2,3]. Thyroid dysfunctions are considered as some of the most important endocrinopathic disorders both in human and in veterinary medicine [4]. Hyperthyroidism is the most common endocrine disease in women [5]. Prevalence of hyperthyroidism in women is between 0.5 and 2%, and is ten times more common in women than in men. Approximately half the cases of thyroid disease involve hyperthyroidism and the other half involves hypothyroidism.

Recently there is an increased demand for using plants in therapy "back to nature" instead of using synthetic drugs which may have adverse effects that may be more dangerous than the disease itself. Different herbs show thyroid modulating activity; prothyroidic as well as antithyroidic.

Saxena et al 2012 studied Thyroidic regulatory activity of *Ficus carica* leaf extract in rat models and revealed the presence of tyrosine in the leaf extract which may be suggestive of thyroidal activity of *Ficus carica* leaf extract depending on the well established mechanism of T₃, T₄ formation in the body. The authors recommend further research work to fully explore its mechanism of its effect of T₃, T₄ formation in the body [6]. Similarly, Azharuddin et al, 2014 studied antithyroidic potential of *Ficus racemosa* Linn bark in Albino rats and concluded that *Ficus racemosa* significantly by virtue of the presence of tyrosine, which may be useful for further molecular studies to determine the exact mechanism for its antithyroidic activity [7]. Nagarathna and Deepak Kumar Jha, 2013, reviewed property of antithyroidic on herbal plants as well as natural products by using previous events and concluded that flavonoids have profound effects on thyroid hormones and on the hypothalamus–pituitary axis. These results suggest that alternative thyroid treatments place more importance on improving lifestyles and nutritional diet, providing spiritual support along with natural thyroid medication [8]. Krishnaveni 2014 evaluated *Bauhinia purpurea* leaf extract for its antioxidant activity and proved to be a good antioxidant and needs further characterization to confirm its diversified therapeutic applications [9].

In an attempt to further establish the pharmacological properties of *Bauhinia purpurea* (Fabaceae), Yahya et al 2013 investigated the hepatoprotective potential of methanol extract of *B. purpurea* leaves (MEBP using the paracetamol-(PCM-) induced liver toxicity in rats. And they concluded that, MEBP exerts potential hepatoprotective activity that could be partly attributed to its antioxidant activity and high phenol content and thus warrants further investigation [10].

Megha Chaudhari 2013 studied the antioxidant capacity of *Bauhinia purpurea* stem bark extracts obtained by sequential extraction with various polarities of solvents, by hot continuous percolation method. From the tested *Bauhinia purpurea* crude extracts showed potent different level of in vitro antioxidant activity. Results indicate that *Bauhinia purpurea* may be a potential source of natural antioxidant.[11] Neelima et al 2012 had done the general phyto chemical screening of the aerial parts of *Bauhinia purpurea* (Fabaceae) revealed the presence of flavonoids, carbohydrates, glycosides, tannins and terpenoids. Also they studied the nephro protective activity of the plant and concluded that the ethanol extract of leaves and unripe pods of *B. purpurea* possessed potent nephro protective activity [12].

The people use this plant in several ways for the treatment of skin diseases (leucoderma and leprosy), wounds, ulcers, cough, dysentery, snakebite, tumors, flatulence, indigestion, piles and also lots of other ailments [13,14].

Medicinal plants are the richest bio resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Considerable contributions have been made on medicinal plants by many workers.

Compliance is a major problem with synthetic drugs, hence it was thought worthwhile to explore the available herbal option using Ethanolic extract of *Bauhinia purpurea* leaves. Despite the fact that plant-based drugs are gradually becoming the choice of the patients for their safe and economic nature, on herbal regulation of hyperthyroidism scientific investigations are meager [15,16]. The rationale behind this study is *B. purpurea* leaves possess different flavonoids such as quercetin in [17] and so may be antithyroidic in nature [18]. *Bauhinia purpurea* bark was found to be prothyroidic in nature [19] the same time its leaves possess flavonoids and is antithyroidic in nature. From the light of this finding *Bauhinia purpurea* leaf extract was tested for antithyroidic activity.

Materials and Methods:

Plant Material

The leaves of *Bauhinia purpurea* was collected from MG University campus, Kottayam, Kerala and authenticated by Prof. Dr. Jomy Augustine, HOD Botany, St .Thomas college of Palai, Kottayam. The plant part was collected in the months of January to March. (Voucher no 2258)

Drugs and Chemicals

L-thyroxine (LT₄), Propylthiouracil (PTU), Carboxymethyl cellulose, Potassium phosphate buffer, Methionine, riboflavin, Nitro blue tetrazolium (NBT), Potassium ferricyanide, Trichloro acetic acid (TCA), Thio barbituric acid (TBA), Hydrogen peroxide.

Animals

Female albino Wistar rats weighing 180-220g, maintained under standard husbandry conditions (temp 23 ± 2° C, relative humidity 55 ± 10% and 12 hr light dark cycle) were used for the screening which was obtained from the animal house of the University College of Pharmacy, Cheruvandoor. Animals were fed with standard laboratory food and ad libitum during the study period. The experiments were performed after getting the approval for experimental protocol from the institutional animal ethics committee, of University College of Pharmacy, Cheruvandoor, Kottayam, India 2013 under the IAEC no: 011 /MPH/UCP/CVR/13.

Experimental Details

Preparation of Ethanol Extract

Plant materials were dried in shade for 2 weeks and coarsely powdered. About 280 g of coarsely ground, dried leaves of

Bauhinia purpurea Linn was placed in a porous bag placed in the middle chamber of the Soxhlet apparatus. Hot soxhlet extraction was carried out using ethanol as solvent. After extraction, ethanol was distilled out and extract was concentrated to obtain a sticky mass.

Phytochemical Screening

The chemical tests were performed in the preliminary phytochemical screening to identify various secondary metabolites such as alkaloids, glycosides, saponins, phenol compounds, tannins, flavonoids using standard methods (Table 1) [20].

- Test for alkaloids: A small portion of extracts were stirred separately with few drops of dilute hydrochloric acid and filtered, tested carefully with various alkaloidal reagents.
 - a) Mayer's reagent - cream precipitate
 - b) Dragendroff's reagent- orange brown precipitate
 - c) Hager's reagent- yellow precipitate
 - d) Wagner's reagent-reddish brown precipitate
- Test for carbohydrates and Glycosides
 - a) Molisch's test -violet ring shows presence of carbohydrate

- b) Fehling's test - reddish brown precipitate
 - Test for glycosides
 - a) Legal's test -pink colour obtained
 - b) Borntrager's test- no colour change in ammoniacal layer was observed.
 - Test for saponins: formation of 1cm layer of foam.
 - Test for phenol compounds and tannins: The extract was treated with
 - a) Dil ferric chloride solution (5%) -violet colour formation
 - b) 1% sodium gelatin containing 10% sodium chloride -white precipitate
 - c) 10% lead acetate solution- white precipitate
 - Test for flavonoids: The extract was treated with
 - a) With aq sodium hydroxide solution -yellow colour
 - b) With conc. Sulphuric acid- orange colour
 - c) Shinoda test - extract with magnesium turnings followed by drop wise conc. hydrochloric acid shows appearance of magenta colour.

S. No	Groups	Drug Given	Period Of Treatment (Days)	Dose
1	Normal control	Vehicle	30	---
	(Group I)			
2	Positive Control	Inducing Agent (L T4)	30	0.5 mg/kg
	(Group II)			
3	Treatment Group Low Dose	LT4	0-30	0.5 mg/kg,
	(Group III)	EEBP	13- 30	100mg/kg
4	Treatment Group High Dose	LT4	0-30	0.5 mg/kg,
	(Group IV)	EEBP	13-30	150mg/kg
5	Standard	LT4	0-30	0.5 mg/kg,
	(Group V)	standard drug (PTU)	13-30	10mg/kg

Table 1: Treatment Protocol for in vivo studies

Serum Analysis

In the test blood was collected on 12th and 30th day by retro-orbital puncture under anesthetic conditions (thiopental sodium 40 mg/kg) and serum was separated. Serum concentrations of T3, T4 and TSH were analyzed using Chemiluminiscent micro particle immune assay in Abbott architect; about diagnostic kit (Me division laboratory, Kottayam).

Thyroid Gland Histopathology

At the end of studies, all animals were sacrificed by cervical dislocation. Thoracic and neck cavity was cut open to isolate thyroid gland from each animal. Isolated glands were cleaned off extraneous tissue, kept in freshly prepared 10% formalin for histopathological analysis.

Liver Homogenate Analysis (Exvivo)

After removing thyroid gland, liver was also isolated from each animal. Isolated liver was homogenized in 10% (w/v) ice-cold phosphate buffer (0.1 M, pH 7.4) and the homogenate was centrifuged at 15,000 x g for 30 min. Then it was analyzed for Lipid Peroxidation (LPO), Superoxide Dismutase (SOD), Catalase (CAT) estimations [21].

Statistical Analysis

Data were processed with graph pad prism version5 software. The results were expressed as mean \pm SD /SEM standard deviation/standard error of the mean). Comparisons of variables were performed using one way analysis of variance (ANOVA) non-parametric test followed by post hoc Turkey's multiple comparison test P value at < 0.05 was considered as statistically significant.

Results

Percentage Yield

The percentage yield of Ethanolic extract of Bauhinia purpurea (EEBP) leaves was approximately 14.4% w/w.

Phytochemical Screening

Preliminary Phytochemical screening revealed the presence of flavonoids, Phenolic compounds, tannins and glycosides.

In Vivo Assay

Serum Analysis

From the results, it was observed that on 12th day all the four groups except normal control LT4 induced group showed elevated T3, T4 value and diminished TSH value. On EEBP administration after 30th day the elevated T3, T4 level and diminished TSH reversed to that of normal in a dose dependent manner and the high dose EEBP shows a comparable activity to that of standard PTU used (Figure 1-3, 4, 5),(Table 2,3).

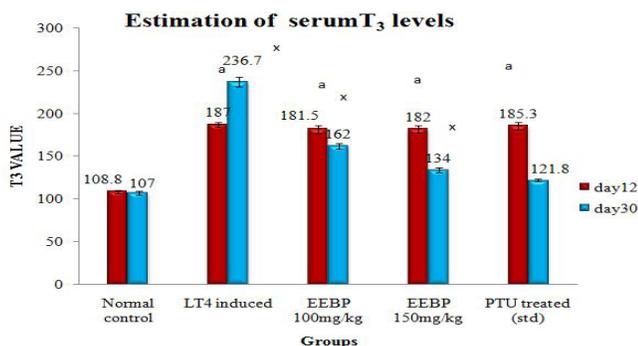


Figure1: Effect of EEBP on serum T3 level.

Values expressed as Mean \pm SEM, n=6 in each group. One way ANOVA followed by Turkey's multiple comparison Test. x P value < 0.001 , y P value < 0.01 , z P value < 0.05 as compared to the values of LT4 induced animals; a p < 0.001 , bp < 0.01 , Cp < 0.05 as compared to the standard PTU value in 30th day and x P value < 0.001 , y P value < 0.01 , z P value < 0.05 as compared to the values of normal control animals in 12th day.

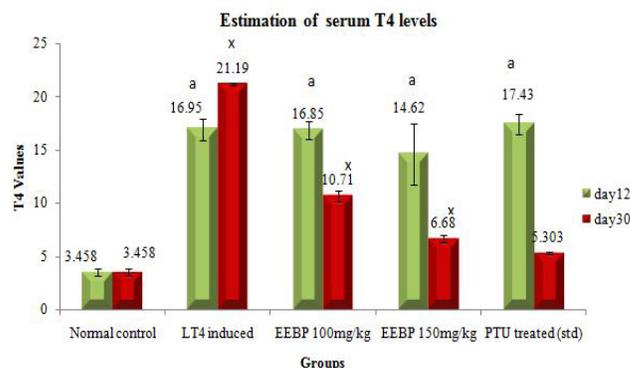


Figure 2: Effect of EEBP on serum T4 level.

Values expressed as Mean \pm SEM, n=6 in each group. One way ANOVA followed by Turkey's multiple comparison Test. x P value < 0.001 , y P value < 0.01 , z P value < 0.05 as compared to the values of LT4 induced animals; a p < 0.001 , bp < 0.01 , Cp < 0.05 as compared to the standard PTU value in 30th day and x P value < 0.001 , y P value < 0.01 , z P value < 0.05 as compared to the values of normal control animals in 12th day.

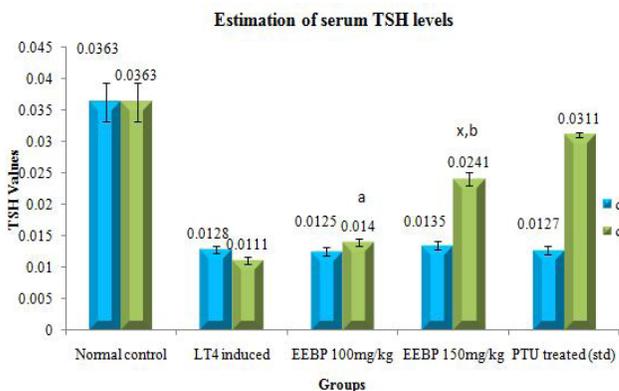


Figure 3: Effect of EEBP on serum TSH level.

Values expressed as Mean \pm SEM, n=6 in each group. One way ANOVA followed by Turkey's multiple comparison Test. x P value < 0.001 , y P value < 0.01 , z P value < 0.05 as compared to the values of LT4 induced animals; a p < 0.001 , b p < 0.01 , Cp < 0.05 as compared to the standard PTU value in 30th day and x P value < 0.001 , y P value < 0.01 , z P value < 0.05 as compared to the values of normal control animals in 12th day.

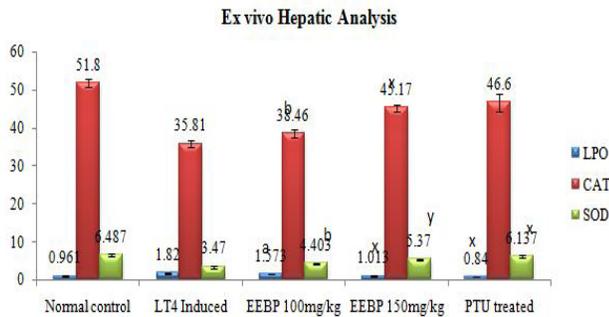


Figure 4: Effect of EEBP on ex vivo hepatic analysis.

Values expressed as Mean ± SEM, n=6 in each group. One way ANOVA followed by Turkey's multiple comparison Test. x P value < 0.001, y P value < 0.01, z P value < 0.05 as compared to the values of LT4 induced animals; a p < 0.001, b p < 0.01, Cp < 0.05 as compared to the standard PTU value

Groups	T ₃ value(n g/dl)	T ₄ values (µg/dl)	TSH values (m IU/ml)
Normal control	108.8 ± 2	3.458 ± 0.3	0.0363 ± 0.003
LT4 induced	187 ± 2.898x	16.95 ± 1.01x	0.0128 ± 0.0006x
EEBP 100mg/kg	181.5 ± 4.877x	16.85 ± 0.83x	0.0125 ± 0.0007x
EEBP 150mg/kg	182 ± 4.155x	14.62 ± 2.84x	0.0135 ± 0.0006x
PTU Treated	185.3 ± 4.128x	17.43 ± 0.93x	0.0127 ± 0.0007x

Table 2: Thyroid function test values (TFT) on 12th day (induction of hyperthyroidism).

Values expressed as Mean ± SEM, n=6 in each group. One way ANOVA followed by Turkey's multiple comparison Test. x P value < 0.001, y P value < 0.01, z P value < 0.05 as compared to the values of normal control animals.

Groups	LPO (n M MDA formed/h/mg protein)	CAT (µM of H2O2 decomposed/min/mg protein)	SOD (units/mg protein)
Normal control	0.961 ± 0.11	51.8 ± 1.13	6.487 ± 0.39
LT4 Induced	1.82 ± 0.083	35.81 ± 0.83	3.47 ± 0.31
EEBP 100mg/kg	1.573 ± 0.12a	38.46 ± 0.99b	4.403 ± 0.21b
EEBP 150mg/kg	1.013 ± 0.10x	45.17 ± 0.87x	5.37 ± 0.26y
PTU treated	0.84 ± 0.12x	46.6 ± 2.35x	6.137 ± 0.33x

Table 4: Effect of EEBP on LPO, CAT and SOD values.

Values expressed as Mean ± SEM, n=6 in each group. One way ANOVA followed by Turkey's multiple comparison Test. x P value < 0.001, y P value < 0.01, z P value < 0.05 as compared to the values of LT4 induced animals; a p < 0.001, bp < 0.01, Cp < 0.05 as compared to the standard PTU value.

Thyroid Gland Histopathology

Histopathological analysis of thyroid gland follicles was conducted. From the results obtained it was found that LT4 induced group shows atrophy of thyroid follicles with scanty colloid material. And on EEBP administration atrophy of thyroid follicles reversed to a greater extent.

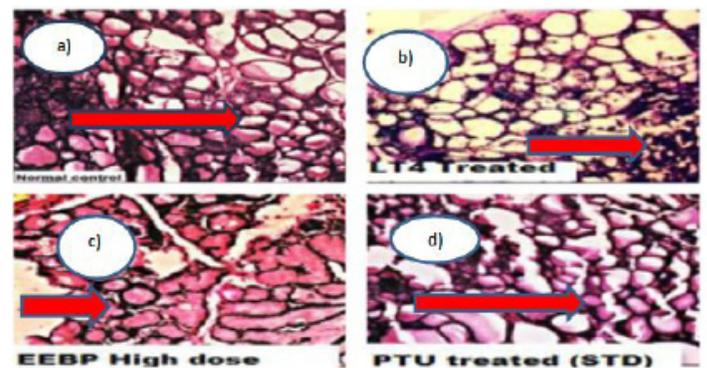


Figure 5: Light photomicrographs of thyroid gland follicles from treated albino wistar rats (Magnification 10X, stain used: Eosin).

- a) Normal control: showing thyroid follicles with colloid material.
- b) LT4induced: showing atrophy of thyroid follicles with scanty colloid material.
- c) EEBP Treated: showing atrophy of thyroid follicles reversed to a greater extent.
- d) PTU Treated: showing atrophy of thyroid follicles reversed to that of normal.

Discussion

Herbal medicines are gaining growing interest because of their cost-effective, eco-friendly attributes and true relief from disease condition. The plant Bauhinia purpurea of family cesalpinaeceae is claimed to be used for thyroid dysfunctions in folklore medicine. In the present study leaf extract of Bauhinia purpurea was evaluated for the amelioration of thyroid dysfunction. The preliminary phytochemical screening of the ethanolic extract of Bauhinia purpurea leaves (EEBP) indicated the presences of flavonoids, alkaloids, tannins, phenolics, glycosides, steroids and terpenoids and the most prominent one was found to be flavanoids. Flavonoids are large class of benzo-pyrone derivatives, ubiquitous in plants exhibit antioxidant activity. Flavonoids inhibit many enzymes including thyroid peroxidase (TPO), 5α-deiodinase, the key

enzymes of thyroid hormone synthesis etc [22, 23].

Divi and Doerge (1995) in their study Inhibition of Thyroid Peroxidase by Dietary Flavonoids found that almost all of the flavonoids except flavanone and flavone inhibited tyrosine iodination by thyroid peroxidase (TPO), but with markedly different potencies [24]. Consumption of flavonoids by experimental animals reduces both iodide ion uptake and iodide ion incorporation into thyroid hormones [25]. In vitro, several flavonoids reduce iodide ion uptake as well as inhibited TPO dependent iodination [26]. These data are consistent with the antithyroidic effects of flavonoids observed in humans and experimental animals [22, 27].

As leaves are rich in flavonoid content it may exhibit high amount of antioxidant property as well as antithyroidic property. In hyperthyroidism thyroid hormones T3, T4 levels get elevated at same time TSH level decreases and this condition was achieved on administration of LT4 for 12 consecutive days. Results of hyperthyroidic induction from the current study correlate with previous literatures [18].

In this study, following LT4 administration, an increase in LPO was observed as reported earlier by Panda and Kar. However, it was significantly decreased in the LT4 treated group that received EEBP extract, suggesting that the plant extract may reduce the hepatotoxic effects in hyperthyroid animals. On the other hand, antioxidant enzymes, SOD and CAT were enhanced to near normal levels in this group. Decrease in LPO and an increase in enzyme activities in the liver do indicate not only the safe nature of the plant extract but also its antiperoxidative value [28].

Induction of hyperthyroidism was confirmed from 12th day results of thyroid function test. And from the 30th day results of *in vivo*, it was evident that administration of LT4 (positive control group) increased the serum T3 and T4 concentrations as well as hepatic LPO, and decreased TSH levels, hepatic SOD and CAT activities. However, when 100 mg/kg (low dose) and 150 mg/kg (high dose) of EEBP was administered along with an equivalent amount of LT4, it reversed the altered effects as evidenced by a marked decrease in the concentration of both the thyroid hormones (T3, T4) and hepatic LPO activity with a concomitant increase in TSH levels, hepatic SOD and CAT activities, indicating the antithyroidic as well as anti peroxidative nature of the extract. This may be due to the high amount of flavonoids present in the leaves of *Bauhinia purpurea*.

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 untreated (control) animals were observed to be cuboidal and epithelium full of colloidal material. On the other hand, LT4 induced animals (positive control) show follicular atrophy, scanty colloid material and epithelial hyperplasia. EEBP 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 [29]. This clearly suggests the PTU like activity of EEBP. Mechanism responsible for antithyroidic activity of the extract can be suggested as iodine complexation, inhibition of thyroid peroxidase, protease, 5 α - deiodinase enzymes as flavonoids exhibit antithyroidic activity through the above mechanisms.

Conclusion

Preliminary phytochemical screening revealed the presence of flavonoids, phenol compounds, alkaloids, tannins, glycosides, terpenoids and steroids. From the results of serum analysis it can be concluded that EEBP shows elevated T3, T4 and diminished TSH level in a dose dependent manner when compared to PTU. From the results obtained in *ex vivo* liver homogenate analysis, it was found that EEBP administered group show decreased LPO levels with an elevation in CAT and SOD levels in a dose dependent fashion compared to the standard drug PTU.

The studies confirmed that the ethanolic extract of *Bauhinia purpurea* leaves possess significant anti thyroidic and anti peroxidative activity. The results are encouraging to pursue further studies to propose the underlying pharmacological mechanism and also to isolate and characterize probable bioactive molecule responsible.

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