e-ISSN:2322-0139
p-ISSN:2322-0120

Research & Reviews: Journal of Pharmacology and Toxicological Studies

Role of Ethanolic Extract of *Bauhinia purpurea* Leaves on Amelioration of Hyperthyroidism in LT4 Induced Female *Albino wistar* Rats

Revathy R Nair*, Litty Joseph and Rahees T

University College of Pharmacy, MG University, Kottayam, Kerala, India

Research Article

Received date: 09/07/2015
Accepted date: 25/09/2015
Published date: 01/10/2015

*For Correspondence

Revathy R Nair, University College of Pharmacy, MG University, Kottayam, Kerala, India, Tel: 919446429739

E-mail: revathy6nair@gmail.com

Keywords: *Bauhinia purpurea;* Hyperthyroidism; Flavonoids; Antithyroidic activity; Propylthiouracil; Antioxidant

ABSTRACT

Ethanolic extract of *Bauhinia purpurea* leaves was evaluated for its possible ameliorative effect in the regulation of hyperthyroidism in *Albino wistar* rat model. Serum triiodothyronine (T_3), thyroxine (T_4) 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.

LT_4 administration (0.5 mg/kg/d for 12 days) as an inducing agent increased the levels of serum T_3, T_4 with a concomitant decline in TSH levels. However, simultaneous administration of the EEBP (100 mg/kg, 150 mg/kg 12-30th day) to the LT_4 induced hyperthyroid animals reversed all these effects indicating their potential in the regulation of hyperthyroidism. PTU (10 mg/kg, 12-30th day) was administered as standard drug. PTU is selected as the standard drug in the light of many anti-thyroidic studies done. When relative efficacy was compared with that of propyl thiouracil (PTU), a standard antithyroidic drug, experimental *B. purpurea* extract appeared to be comparable. Furthermore histopathological analysis confirmed the result obtained.

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 [1,2].

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 (T_3) and thyroxin (T_4) hormones are known as cell metabolism regulators, being associated with different biological processes in all vertebrates [3,4]. Thyroid dysfunctions are considered as some of the most important endocrinopathic disorders both in human and in veterinary medicine [5]. Hyperthyroidism is the most common endocrine disease in women [6]. 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. 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 T_3, T_4 formation in the body. The authors recommend further research work to fully explore its mechanism of its effect of T_3, T_4 formation in the body [7].

Similarly, Azharuddin et al. studied antithyroid 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 antithyroid activity [8].

Nagarathna and Deepak Kumar Jha reviewed property of antithyroid on herbal plants as well as natural products by using previous events and concluded that isoflavonoids 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 [9].

Krishnaveni 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 [10].

In an attempt to further establish the pharmacological properties of *Bauhinia purpurea* (Fabaceae), Yahya et al 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 phenolic content and thus warrants further investigation [11].

Megha Chaudhari 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 [12].

Neelima et al. had done the general phytochemical screening of the aerial parts of *Bauhinia purpurea* (Fabaceae) revealed the presence of flavonoids, carbohydrates, glycosides, tannins and terpenoids. Also they studied the nephroprotective activity of the plant and concluded that the ethanol extract of leaves and unripe pods of *B. purpurea* possessed potent nephroprotective activity [13].

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 [14,15].

Medicinal plants are the richest bioresource 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 [16,17].

The rationale behind this study is *B. purpurea* leaves possess different flavonoids such as quercetin [18] and so may be antithyoidic in nature [1]. *Bauhinia purpurea* bark was found to be prothyroidic in nature [19] the same time its leaves possess flavonoids and is anti-thyroidic in nature. From the light of this finding *Bauhina purpurea* leaf extract was tested for anti-thyroidic activity.

METHODOLOGY

Study centre

The present study was carried out from January 2013 to October 2013 at Postgraduate and Research Laboratory, Department of Pharmacology, University College of Pharmacy, Mahatma Gandhi University, Cheruvandoor, Ettumanoor, Kottayam, Kerala, India.

MATERIALS AND METHOD

Plant material

The leaves of *Bauhinia purpurea* was collected from M.G. 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 (LT_4), Propyl thiouracil (PTU),Carboxy methyl cellulose, Potassium phosphate buffer, Methionine, riboflavin, Nitro blue tetrazolium (NBT), Potassium ferricyanide, Trichloro acetic acid (TCA), Thiobarbituric acid (TBA), Hydrogen peroxide.

Animals

Female *albino Wistar* rats weighing 180-220 *g,* 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, Kottaym, 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 constituents of ethanolic extract of *Bauhiniapurpurea* leaves (EEBP) were identified by qualitative phytochemical analysis (Table 1) [20].

S. No.	GROUPS	DRUG GIVEN	PERIOD OF TREATMENT (DAYS)	DOSE
1	Normal control (Group I)	Vehicle	30	—
2	Positive Control (Group II)	Inducing Agent (LT_4)	30	0.5 mg/kg
3	Treatment Group Low Dose (Group III)	LT_4 EEBP	0-30 13- 30	0.5 mg/kg, 100mg/kg
4	Treatment Group High Dose (Group IV)	LT_4 EEBP	0-30 13-30	0.5 mg/kg, 150mg/kg
5	Standard (Group V)	LT_4 standard drug (PTU)	0-30 13-30	0.5 mg/kg, 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 T_3, T_4 and TSH were analyzed using Chemilumniscen tmicroparticle immune assay in Abott architect; Abott diagnostic kit (Medivision 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 *(ex-vivo)*

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 LPO, SOD, CAT estimations [21].

Statistical analysis

Data were processed with graph pad prism version 5 software. The results were expressed as mean ± SD/SEM. Comparisons of variables were performed using one way ANOVA non-parametric test followed by post hoc Tukey'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

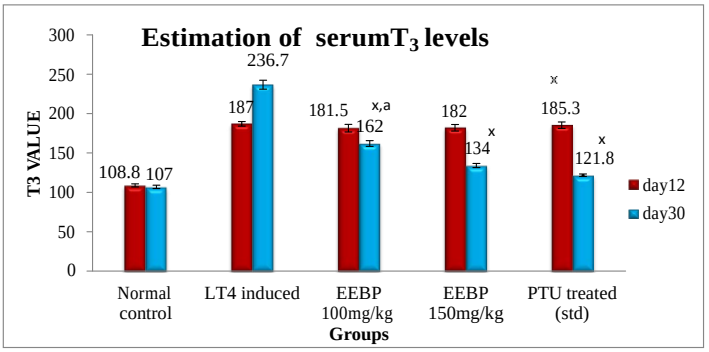
Preliminary phytochemical screening revealed the presence of flavonoids, phenolic compounds, alkaloids, tannins, glycosides, terpenoids and steroids.

In-vivo assay

Serum analysis: From the results, it was observed that on 12th day all the four groups except normal control LT_4 induced

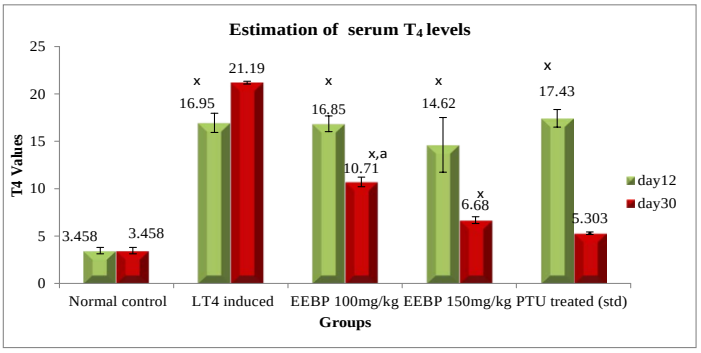
e-ISSN:2322-0139
p-ISSN:2322-0120

group showed elevated T_3, T_4 value and diminished TSH value. On EEBP administration after 30th day the elevated T_3, T_4 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) (Table 2 and 3).



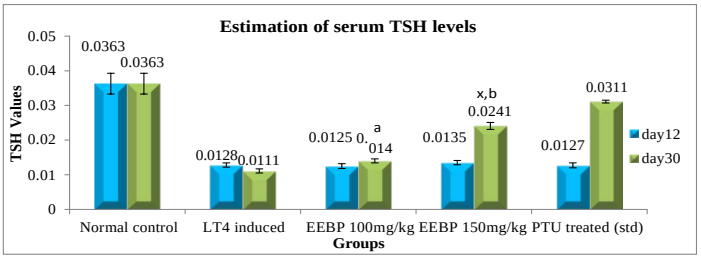
Values expressed as Mean ± SEM, n=6 in each group. One way ANOVA followed by Tukey's multiple comparison test. ^{x}P value<0.001, ^{y}P value<0.01, ^{z}P value<0.05 as compared to the values of LT_4 induced animals; $^{a}p<0.001$, $^{b}p<0.01$, $^{c}p<0.05$ as compared to the standard PTU value in 30th 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 1. Effect of EEBP on serum T_3 level.



Values expressed as Mean ± SEM, n=6 in each group. One way ANOVA followed by Tukey'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 LT_4 induced animals; $^{a}p<0.001$, $^{b}p<0.01$, $^{c}p<0.05$ 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 T_4 level.



Values expressed as Mean ± SEM, n=6 in each group. One way ANOVA followed by Tukey'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 LT_4 induced animals; $^{a}p<0.001$, $^{b}p<0.01$, $^{c}p<0.05$ 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.

Ex-vivo liver homogenate analysis

From the results, it was observed that except normal control LT_4 induced group showed elevated LPO value and diminished CAT, SOD values. EEBP treated groups reversed the elevated LPO levels and diminished CAT, SOD levels to that of normal in a dose dependent manner and the high dose EEBP shows a comparable activity to that of standard PTU group (Figure 4) (Table 4).

e-ISSN:2322-0139
p-ISSN:2322-0120

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

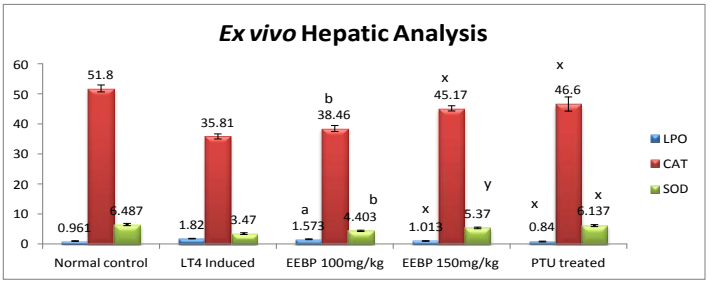
GROUPS	T_3 value(ng/dl)	T_4 values (µg/dl)	TSH values (mIU/ml)
Normal control	108.8 ± 2	3.458 ± 0.3	0.0363 ± 0.003
LT_4 induced	187 ± 2.898^{x}	16.95 ± 1.01^{x}	0.0128 ± 0.0006^{x}
EEBP 100 mg/kg	181.5 ± 4.877^{x}	16.85 ± 0.83^{x}	0.0125 ± 0.0007^{x}
EEBP 150 mg/kg	182 ± 4.155^{x}	14.62 ± 2.84^{x}	0.0135 ± 0.0006^{x}
PTU Treated	185.3 ± 4.128^{x}	17.43 ± 0.93^{x}	0.0127 ± 0.0007^{x}

Values expressed as Mean ± SEM, n=6 in each group. One way ANOVA followed by Tukey'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.

Table 3. Effect of EEBP on Thyroid function test values (TFT) on 30th day.

GROUPS	T_3 value(ng/dl)	T_4 values (µg/dl)	TSH values (mIU/ml)
Normal control	107 ± 2	3.458 ± 0.3	0.0363 ± 0.003
LT_4 Induced	236.7 ± 5.7	21.19 ± 0.14	0.0111 ± 0.0006
EEBP 100 mg/kg	162 ± 3.61^{x,a}	10.71 ± 0.51^{x,a}	0.014 ± 0.0006^{a}
EEBP 150 mg/kg	134 ± 2.67^{x}	6.68 ± 0.36^{x}	0.0241 ± 0.001^{x,b}
PTU treated	121.8 ± 1.45^{x}	5.303 ± 0.13^{x}	0.0311 ± 0.0004^{x}

Values expressed as Mean ± SEM, n=6 in each group. One way ANOVA followed by Tukey'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.



Values expressed as Mean ± SEM, n=6 in each group. One way ANOVA followed by Tukey'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 LT_4induced animals; ^{a}p<0.001, ^{b}p<0.01, ^{c}p<0.05 as compared to the standard PTU value .

Figure 4. Effect of EEBP on *Ex-vivo* hepatic analysis.

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

GROUPS	LPO (nM MDA formed/h/mg protein)	CAT (µM of H_2O_2 decomposed/min/ mg protein)	SOD (units/mg protein)
Normal control	0.961 ± 0.11	51.8 ± 1.13	6.487 ± 0.39
LT_4 Induced	1.82 ± 0.083	35.81 ± 0.83	3.47 ± 0.31
EEBP 100 mg/kg	1.573 ± 0.12^{a}	38.46 ± 0.99^{b}	4.403 ± 0.21^{b}
EEBP 150 mg/kg	1.013 ± 0.10^{x}	45.17 ± 0.87^{x}	5.37 ± 0.26^{y}
PTU treated	0.84 ± 0.12^{x}	46.6 ± 2.35^{x}	6.137 ± 0.33^{x}

Values expressed as Mean ± SEM, n=6 in each group. One way ANOVA followed by Tukey'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 LT_4 induced animals; ^{a}p<0.001, ^{b}p<0.01, ^{c}p<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 LT_4 induced group shows atrophy of thyroid follicles with scanty colloid material.

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 cesalpinaecea 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 in their study Inhibition of Thyroid Peroxidase by Dietary Flavonoids found that almost all of the flavonoids

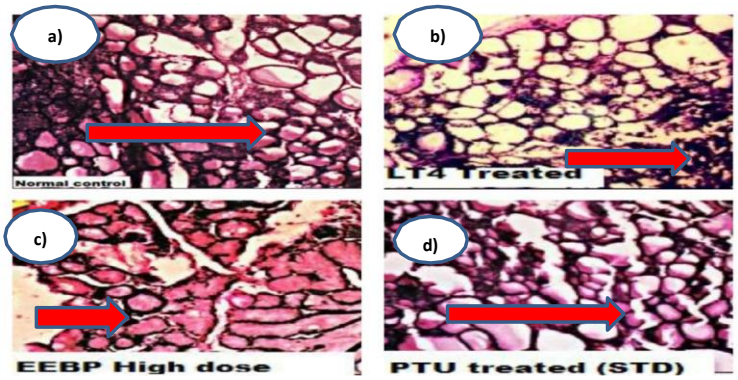
except flavanone and flavone inhibited tyrosine iodination by thyroid peroxidase (TPO), but with markedly different potencies [24]. Consumption of flavonoids by experimental animals reduces both iodide ion uptake and iodide ion incorporation into thyroid hormones [25]. In vitro, several flavonoids reduce iodide ion uptake as well as inhibited TPO dependent iodination [26]. These data are consistent with the antithyroid 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 antithyroid property. In hyperthyroidism thyroid hormones T_3, T_4 levels get elevates at same time TSH level decreases and this condition was achieved on administration of LT_4 for 12 consecutive days. Results of hyperthyroidic induction from the current study correlate with previous literatures [1].

In this study, following LT_4 administration, an increase in LPO was observed as reported earlier by Panda and Kar. However, it was significantly decreased in the LT_4 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 anti-peroxidative 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 LT_4 (positive control group) increased the serum T_3 and T_4 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 LT_4, it reversed the altered effects as evidenced by a marked decrease in the concentration of both the thyroid hormones (T_3, T_4) 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, LT_4 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 anti-thyroid activity of the extract can be suggested as iodine complexation, inhibition of thyroid peroxidase, protease, 5 α- deiodinaseenzymes as flavonoids exhibit antithyroid activity through the above mechanisms.



Values expressed as Mean ± SEM, n=6 in each group. One way ANOVA followed by Tukey'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 LT_4 induced animals; ^{a}p<0.001, ^{b}p<0.01, ^{c}p<0.05 as compared to the standard PTU value .

Figure 4: Effect of EEBP on *Ex-vivo* hepatic analysis.

CONCLUSION

Preliminary phytochemical screening revealed the presence of flavonoids, phenolic compounds, alkaloids, tannins, glycosides, terpenoids and steroids. From the results of serum analysis it can be concluded that EEBP shows elevated T_3, T_4 and diminished TSH level in a dose dependent manner when compared to PTU.

From the results obtained in exvivo 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.

e-ISSN:2322-0139
p-ISSN:2322-0120

REFERENCES

1. Panda S and Kar A. Annonasquamosa seed extract in the regulation of hyperthyroidism and lipid-peroxidation in mice: Possible involvement of quercetin. Phytomedicine. (2007); 14: 799–805.
2. Ganong WF. The thyroid gland. In: Review of Medical Physiology, Appleton and Lange, East Norwalk (CT). (1995); 90–305.
3. Nunes MT. Hormôniostiroideanos: Mecanismodeação e importânciabiológica. Arq Bras Endocrinol Metabol. (2003); 47: 639-643.
4. Shi YB et al. Complex regulation of thyroid hormone action: Multiple opportunities for pharmacological intervention. Pharmacol Therap. (2002); 94: 235-251.
5. Rijnberk A et al. Endocrine diseases in dogs and cats: similarities and differences with endocrine diseases in humans. Growth Horm IGF Res. (2003); 13: S158-S164.
6. Goldman MB. Thyroid diseases and breast cancer. Epidemil Rev. (1990); 12: 16-28.
7. Saxena V et al. *Ficus carica* leaf extract in regulation of thyroidism using elisa technique. Asian J Pharma Clin Res. (2012); 5: 44-48.
8. Mohammed Azharuddin et al. Evaluation of anti-thyroid activity of *Ficus racemosa* Linn bark in male rats. Int J Pharm Pharm Sci. (2014); 7:118-122.
9. Nagarathna PKM and Deepak Kumar Jha. Study on Antithyroid Property of Some Herbal Plants. Int J Pharm Sci Rev Res. (2013); 23: 203-211.
10. Marimuthu Krishnaveni. Antioxidant Potential Of *Bauhinia Purpurea* (L) Leaf. Int J Pharm Sci. (2014); 6: 558-560.
11. Yahya F et al. Hepatoprotective Activity of Methanolic Extract of *Bauhinia purpurea* Leaves against Paracetamol-Induced Hepatic Damage in Rats. Evid Based Complementary and Alternat Med. (2013).
12. Megha Chaudhari. In Vitro Screening of *Bauhinia Purpurea* Stem Bark with Reference Anti-Oxidant Activity. IJRHS. (2013); 1.
13. Neelima N and Sudhakar M. Phytochemical investigation and evaluation of Nephroprotective activity of aerial parts of *Bauhinia purpurea*. Int J of Res in Pharmacology and Pharmacotherapeutics. (2012); 1: 97-102.
14. Gupta D et al. In vitro Antidiabetic activity of stem bark of *Bauhinia purpurea* Linn. Scholars Research Library Der Pharmacia Lettre. (2012); 4: 614-619.
15. Sharma S and Kumar A. Tribal uses of medicinal plants of rajashthan:Kachnar. International Journal of Life science and pharma Research. (2012); 2: 2250-0480.
16. Kar A and Panda S. Ayurvedic therapies for thyroid dysfunctions in Mishra LC Scientific Basis for Ayurvedic Therapies. CRC Press, Boca Raton, FL, London. (2004).
17. Tahiliani P and Kar A. The combined effects of Trigonella and Allium extracts in the regulation of hyperthyroidism in rats. Phytomedicine. (2003); 10: 665–668.
18. Kumar T and Chandrashekar KS. *Bauhinia purpurea* Linn.: A Review of its Ethnobotany, Phytochemical and Pharmacological Profile. Research Journal of Medicinal Plant. (2011); 5: 420-431.
19. Panda S and Kar A. *Withania somnifera* and *Bauhinia purpurea* in the regulation of circulating thyroid hormone concentrations in female mice. Journal of ethnopharmacology. (1999); 67: 233–239.
20. Divakar MC. Plant drug evaluation: A guide. CD remedies. (2002).
21. Naskar S et al. In Vitro and In Vivo Antioxidant Potential of Hydromethanolic Extract of *Phoenix dactylifera* Fruits. J Sci Res. (2010); 2: 144-157.
22. Gaitan E et al. Millet and the thyroid In Gaitan E: Environmental Goitrogenesis. CRC Press, Boca Raton, FL. (1989); 195-204.
23. Cody V et al. Structure-activity relationships of flavonoids as inhibitors of iodothyronine deiodinase. In Gaitan, E: Environmental Goitrogenesis. CRC Press, Boca Raton, FL. (1989); 57-69.
24. Divi RL and Doerge DR. Inhibition of thyroid peroxidase by dietary flavonoids. Chem Res Toxicol. (1996); 9: 16–23.
25. Moudgal NR et al. Studies on goitrogenic agents in food. III. Goitrogenic action of some glycosides isolated from edible nuts. J Nutr. (1958); 66: 291-300.
26. Cooksey RC et al. Intrathyroidal effects of flavonoids. Am Chem Soc Div Environ Chem. (1986);26:75.
27. Klopfenstein C F et al. Goitrogenic effects of pearl millet diets. Nutr Rep Int. (1983); 27: 1039-1047.
28. Panda S and Kar A. Fruit extract of *Emblica officinalis* ameliorates hyperthyroidism and hepaticlipid peroxidation in mice. Pharmazie. (2003); 58: 753-755.
29. Sung-Dae Cho et al. Pre-validation study for OECD enhanced test guideline 407 protocol by gavage for 4 weeks using propylthiouracil and tamoxifen. Toxicology Letters. (2003); 144: 195-204.