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Protective effect of a polyherbal bioactive fraction in propylthiouracil-induced thyroid toxicity in ratsby modulation of the hypothalamic–pituitary–thyroid and hypothalamic–pituitary–adrenal axes

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ABSTRACT

Hypothyroidism is the most frequent consequence of the interaction of a large variety of drugs, environmental pollutants and industrial chemicals with the thyroid gland. It is associated with diminished endocrine function which may lead to hyperlipidemia, diabetes, Alzheimer's disease, weight gain, and other metabolic disorders. The present study evaluates the pro-thyroid activity of a bioactive fraction from a polyherbal teabag in rats with hypothyroidism induced by propylthiouracil. The teabag was formulated to stimulate synthesis and/or release of T4 and affectthe conversion of T4 to T3. Phytoconstituents of the polyherbal teabag are potent antioxidants that may be responsible for the pro-thyroid activity. The tea-extract (1000 mg) was found to contain 1076 µg of gallic acid and 1131 µg of rutin from HPTLC analysis. Rats received propylthiouracil (8 mg/kg) for the first 15days followed by the polyherbal tea-extract (500, 1000 and 1500 mg/kg), the standard drug levothyroxine (0.1 mg/kg), aerobic exercise, and a combination of tea-extract (1000 mg/kg) and aerobic exercise daily along with propylthiouracil for the next 30 days. Finally, rats received their respective treatments alone without propylthiouracil for 15 more days. Lipid profile and levels of glucose, insulin, T3, T4, TSH, cortisol, homocysteine, creatinine, uric acid, malondialdehyde, glucose-6 phosphatase, and endogenous antioxidants were determined. All treatments attenuated significantly the propylthiouracil-elevated TSH, homocysteine, creatinine, uric acid, glucose-6-phosphatase, insulin, and malondialdehyde levels, and restored favorably the propylthiouracil-altered lipid profile, T3, T4, and endogenous antioxidant levels. The polyherbal tea-extract (1000 and 1500 mg/kg) treatment and thecombination treatment of tea-extract (1000 mg/kg) with aerobic exercise displayed significant restoration of the suboptimalthyroid function. This may be due to a favorablemodulation ofthe hypothalamic-pituitary–thyroid and the hypothalamic–pituitary–adrenal axes.

1. Introduction

Thyroid dysfunction is one of the most common endocrine abnormalities and about 1–2% of the adult population is known to suffer fromit [1]. The thyroid gland is the body's "metabolic thermostat," which manufactures, stores and releases the hormones thyroxine (T4), triiodothyronine (T3) and calcitonin. The activity of the thyroid gland is directly associated with all metabolic processes and suboptimal thyroid function often results in a multi-complex of endocrine disorders including diabetes, hypertension, and cardiovascular problems. Hypothyroidism has fundamental effects on cognition, lipid profile, kidney function, cardiovascular system, liver and reproductive system which

Abbreviations: AC, Abdominal circumference; CAT, Catalase;GA- Gallic acid; GPx, Glutathione peroxidase; GR, Glutathione reductase; GSH, Reduced glutathione; HPTLC, High-performance thin layer chromatography; HOMA, IR- Homeostatic model of insulin resistance; HDL, High-density lipoprotein; Hcy, Homocysteine; IR, Insulin resistance; LPO, Lipid peroxidation; LDL, Low-density lipoprotein; MDA, Malondialedhyde; NIS, Sodium-iodide symporter; PTU, Propylthiouracil; Ru, Rutin; SOD, Superoxide dismutase; T500, Tea-extract 500 mg/kg; T1000, Tea-extract 1000 mg/kg; T1500, Tea-extract 1500 mg/kg; AE, Aerobic exercise; TAE, T1000 mg/kg + Aerobic exercise; T3, Triiodothyronine; T4, thyroxine; TSH, Thyroid stimulating hormone; TC, total cholesterol; TG, Triglycerides; VLDL, Very low-density lipoprotein

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may ultimately lead to hyperlipidemia, hyperinsulinemia, diabetes, Alzheimer's disease, cancer, weight gain and other metabolic disorders [2–4].

Environmental pollutants like the pesticides DDT, amitrole, and ethylenethiourea, industrial chemicals such as poly-halogenated hydrocarbons, polychlorinated biphenols, and phthalates, and perchlorate in rocket fuel among others have been shown to be associated with reduced thyroid hormone levels or impaired thyroid hormone action and may also confer an increased risk of autoimmune thyroid disease [5].

Hypothyroidism frequently results due to the interaction of a large variety of drugs with the thyroid gland, the main mechanisms involved being inhibition of the synthesis and/or release of thyroid hormones. The anti-cancer drugs sunitinib and sorafenib,anticonvulsants, sulfonamides, and the antiarrhythmic drug amiodaroneare known to produce profound hypothyroidism as a toxic effect [6–8]. The patients who develop hypothyroidism are put on long term synthetic thyroid drug therapy and need to be treated carefully for the development of hyperthyroidism. Thus,safe and effective thyroid therapy is needed in drug and chemical-induced hypothyroidism.

A better understanding and proof of the biological activity of plant constituents haveseen herbal medicine staging a comeback. Studies report a root extract of *Withania somnifera* at an oral dose of 1.4 g/kg to albino mice for 20 days to stimulateT3 and T4 secretion without any untoward effect [9]. *Bacopa monniere* extract administrationat 200 mg/kg to rats demonstrated anelevationin T4 levels by 41 % [10]. The *Achyranthes aspera* leaf extract, when given to rats at a dose of 200 mg/kg for 7 days, was shown to cause a significant increase in T3 and T4 levels [11]. *Bauhinia purpurea*'s water-soluble fraction of total alcoholic extract at a dose of 2 g/kg when fed to hypothyroidic rats for 20 days enhanced thyroid function [9]. Forskolin, the major phytoconstituent of *Coleus forskohlii i*ncreases the production of thyroid hormones and stimulates their release [12].

Herbal medicine may have multiple active phytoconstituents acting in a synergistic manner to cure a disease by different mechanisms, so they might provide a complete therapy against a disease. Thus, in the present study,a polyherbal teabag was formulated, which contains root of *Withania somnifera* and *Coleus forskohlii*, leaf of *Bacopa monniere* and *Achyranthes aspera* and bark of *Bauhinia purpurea* with the purpose of stimulatingsynthesis and/or release of T4 at the glandular level and affecting conversion of T4 to T3. These herbs which have no known toxicity are proven to possess potent antioxidant activity which may be beneficial in their pro-thyroid effect. Also, they are rich in trace elements such as copper, iron, zinc, and selenium which offer thyroprotective effect at the molecular level [13,14].

Keeping in view the above considerations, this study formulated a polyherbal bioactive teabag and evaluated itspro-thyroid effects in hypothyroidism induced by propylthiouracil (PTU).

2. Materials and methods

2.1. Plant material

Leaves of *Bacopa monniere* and *Achyranthes aspera*, the bark of *Bauhinia purpurea,* and roots of *Withania somnifera* and *Coleus forskohlii* were collected from Pune and Kolhapur region of Maharashtra, India and authenticated at the Blatter Herbarium, St. Xaviers College, Mumbai and Agharkar Research Institute, Pune with the voucher specimens deposited for further reference (AT-128 for Bacopa; AT-129 for Bauhinia; AT-130 for Coleus and AT-131for Achyranthes) at the Agharkar Institute. The *Withania somnifera* plant was authenticated at the Blatter Herbarium after matching with the existing specimen (Accession no. 01,706)

Table 1
Composition of the polyherbal teabag.

Sr.No	Name of Ingredient	Quantity
1.	*Withania somnifera*	0.7g
2.	*Bacopa monniere*	0.1g
3.	*Achyranthes aspera*	0.1g
4.	*Bauhinia purpurea*	0.0013g
5.	*Coleus forskohlii*	0.1g
Total		1.0013g

2.2. Drugs and chemicals

PTU and levothyroxine were obtained as gift samples from Panchsheel Organics Ltd. and MacLeod's Pharmaceuticals Ltd. (Mumbai, India) respectively. Epinephrine, 5,5-dithiobis (2-nitrobenzoic acid), trichloro acetic acid, thiobarbituric acid, reduced glutathione, oxidized glutathione, and nicotinamide adenine dinucleotide phosphate were purchased from Sigma Chemical Co., St Louis, MO, USA. All other chemicals were obtained from authentic sources and were of analytical grade.

2.3. Formulation of teabag

All plant material was dried under shade and powdered mechanically. The dried powderswere blended together in a specific composition to make a tea blend. The composition of the tea blend is given in Table 1. Particle size of the tea blend was determined by microscopy and the average particle size of the tea blend was found to be 0.425 mm. The tea blend (1000 mg) was filled in ateabag to be extracted in hot water prior to administration.

2.4. Quantification of total antioxidants, total flavonoids and phenolic compounds in tea extract using HPTLC

High-Performance Thin Layer Chromatography (HPTLC) of the tea-extract was carried out on the CAMAG HPTLC System for the determination of natural antioxidants, flavonoids, and phenolic compounds. HPTLC plates (HPTLC silica gel 60 F254; 20 × 10 cm) were activated at 110 °C for 30 min. A stock solution of the tea-extract was applied as a band of 8 mm length on the activated HPTLC plates using a Linomat 5 applicator (CAMAG, Switzerland). The extract wasapplied in duplicate at 3 different concentrations (2, 4 & 8 µl). To determine the antioxidants, the plate was developed with 20 mL of a solvent system comprising dichloromethane: toluene: ethyl acetate: methanol: formic acid (4:1:4:0.5:0.5) in the twin trough chromatographic chamber and examined in fluorescent light at 425 nm in the CAMAG visualizer when derivatized with 0.1 Mm DPPH in methanol.

Similarly, the plates were prepared for the determination of flavonoids. The tea-extract was applied in duplicate at 3 different concentrations (2, 5 &10 µl). The plates were developed with 20 mL of a flavonoid specific solvent system comprising ethyl acetate: formic acid: glacial acetic acid: water (10: 0.5: 0.5: 1) in the twin trough chromatographic chamber and examined in white light, fluorescent light (366 nm) and UV remission (254 nm) in the CAMAG visualizer after derivatizing it with2-aminoetheyl diphenylborinate.

For the determination of phenolic acids, the tea-extract was applied in duplicate at 3 different concentrations (2, 5 &10 µl). The plates were developed with 20 mL of a phenolic acid-specific solvent system comprising cyclohexane: ethyl acetate: formic acid (4:6:1) in the twin trough chromatographic chamber and examined in white light (540 nm) in the CAMAG visualizer afterderivatizing it with ASR.

2.5. Quantification of gallic acid and rutin in tea-extract using HPTLC

Analysis of the tea-extract was carried out using CAMAG HPTLC

system (Switzerland) with theCAMAG Linomat V sample applicator and CAMAG TLC scanner III operated using win CATS software (V 1.44 CAMAG). Chromatographic separation of gallic acid (GA) and rutin (Ru) from the tea-extract was carried out on silica gel aluminium plates (silica gel 60 F254; 20 × 10 cm, Merck, Darmstadt, Germany) using a mobile phase comprising toluene: ethyl acetate: ethanol: formic acid (5:2:2.5:0.5). The detection was carried out at 305 nm to quantify gallic acid and rutin. The amount of GA and Ru in the tea-extract was calculated from the calibration curves.

2.6. Experimental animals

Sprague Dawley female rats (150–200 g) were acquired from Glenmark Pharmaceuticals Ltd., India. They were housed in clean polypropylene cages under standard conditions of humidity (50 ± 5%), temperature (25 ± 2 °C), and light (12 h light/12 h dark cycle) and fed with a standard diet (Amrut laboratory animal feed, Pranav Agro Industries, India) and drinking water *ad libitum*. All animals were handled with humane care. Experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee (Animal House Registration no. 25/PO/ReBi/S/99/CPCSEA) and conform to the Indian National Science Academy Guidelines for the Use and Care of Experimental Animals in Research.

2.7. Acute toxicity study

The acute toxicity (LD_{50}) of the tea-extract was evaluated by the oral route as per OECD guideline no. 423.

2.8. Dose fixation of PTU

A pilot study was carried out to fix the dose of PTU to induce hypothyroidism by administering 6, 8 and 10 mg/kg of PTU intra-peritoneally to 3 groups of rats ($N = 6$) for two different durations-15 days and 30days. Doses of PTU were chosen to bracket doses reported in earlier studies. Shifts in levels of serum T3, T4 and thyroid stimulating hormone (TSH) were determined as per standard protocols.

2.9. Preparation of test solutions

PTU solution was prepared by dissolving PTU in 0.01 N sodium hydroxideand making up the volume was made up with water for injection, the pH being maintained at 6.8–7.5 by addition of o-phosphoric acid. Levothyroxine was dissolved in distilled waterand the aqueous solution was used immediately for administration. The tea bag was extracted in hot water (90 °C) for 2 min, the mixture (tea) was cooled and administered torats.

2.10. Experimental procedure

Rats after acclimatization for 7 days in the animal quarters were randomly divided into 9 groups with 6 rats in each group and treated in the following way [15]:

Group I (Normal Control): Rats received drinking water (1 mL/kg,p.o) daily for 45 days

Group II (Toxicant): Rats were administered PTU (8 mg/kg, i.p) for the first 30days and left untreated till the 45th day of the study

Group III (T500):Rats were administered PTU (8 mg/kg, i.p) for the first 30days and the tea-extract (500 mg/kg, p.o) daily from the 15thto the 45th day of the study

Group IV (T1000): Rats were administered PTU (8 mg/kg, i.p) for the first 30 days and the tea-extract (1000 mg/kg, p.o) daily from the 15thto the 45thday of the study

Group V (T1500 mg/kg): Rats were administered PTU (8 mg/kg, i.p) for the first 30days and the tea-extract (1500 mg/kg, p.o) daily from the 15th to the 45thday of the study

Group VI (AE): Rats were administered PTU (8 mg/kg, i.p) for the first 30days and aerobic exercise (running on activity wheel) for 10 min daily from the 15th to the 45thday of the study

Group VII (TAE): Rats were administered PTU (8 mg/kg, i.p) for the first 30days and subsequently administered tea extract (1000 mg/kg, po) and given aerobic exercise (running on activity wheel) for 10 min daily from the 15th to the 45thday of the study

Group VIII(Reference): Rats were administered PTU (8 mg/kg, i.p) for the first 30days and levothyroxine (0.1 mg/kg, p.o) daily from the 15thto the 45th day of the study

All the treatments were given between 9.00 and 10.00 h of the day to avoid circadian variation. Waist (abdominal) circumference, body weight, and rectal temperature were monitored each day. At the end of the treatment period, all animals were fasted for 6 h, blood was collected from the retro-orbital plexus and a drop was used for glucose measurement, the remaining being allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30 °C for 15 min andused for the estimation of T3, T4, TSH, homocysteine (Hcy), total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL),creatinine, cortisol, uric acid, and total protein content. Fasting serum insulinconcentration was also measured using the insulin Radioimmunoassay (RIA) kit. The homeostasis modelassessment of insulin resistance (HOMA-IR) was measured as an indicator of insulin resistance. The obtained values of both fasting blood glucose (FBG) and fasting insulin (FI) levels were then used to calculate insulin resistance as described by Matthews et al. [16]

HOMA-IR = serum glucose (mmol/L) X serum insulin (mIU/L) / 22.5

Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL)were calculated as per Friedevald's equation as follow:

VLDL = Total serum triglycerides/5

LDL = Total serum cholesterol – VLDL – HDL

Animals were sacrificed by cervical dislocation and the thyroid and livers of all animals were dissected immediately and washed with ice-cold saline. The livers were used to prepare a 10 % (w/v) homogenate in phosphate buffer (50 mM, pH 7.4). An aliquot of the liver homogenate was used for estimation of lipidperoxidation (LPO) and the remaining was centrifuged at 7000 × g for 10 min at 4 °C and thesupernatant was used for the assay of glucose-6-phosphatase, reduced glutathione (GSH), superoxide dismutase (SOD),catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR). The thyroid gland was fixed in 10 %buffered formalin and used for histological studies

2.11. Estimation of thyroid hormones, homocysteine,and cortisol

T3, T4, TSH, Hcy, and cortisol levels were determined in serum using ELISA kits supplied by Erba (Mumbai, India).

2.12. Determination of metabolic markers, protein, uric acid and creatinine

TC, TG, HDL, total protein, albumin, globulin, uric acid and creatininelevels were measured in serum using biochemical kits supplied by Erba (Mumbai, India).

2.13. Blood glucose measurement

Blood glucose of all animals was measured using an automated digital glucometer (ACCU − CHECK active, Roche) on the 1st, 15th, 30th and 45thday of the study.

2.14. Insulin assay

Insulin assay was carried out in serum using a radioimmunoassay (RIA) kit supplied by Board of Radiation and Isotope Technology, Mumbai, India.

2.15. Estimation of glucose-6-phosphatase

Glucose 6-phosphatase in liver homogenate was quantified by using ELISA kits supplied by Erba (Mumbai, India).

2.16. Assay of LPO, GSH and antioxidant enzymes

The quantitative estimation of lipid peroxidation (LPO) was performed using the method of Buegeet al. [17]. GSH level wasestimated by the method of Ellman [18]. SOD was assayed by the method of Sun et al. CAT was estimated by the method of Claiborne [19]. GPx estimation was carried out using the method of Rotruck et al. [20]. GR activity was determined by using the method of Mohandas et al. [21]

2.17. Histopathological studies

Thyroid tissue stored in 10 % buffered formalin was embedded in paraffin, sections cut at 5 μm, and stained with haematoxylin and eosin. These sections were examined under a light microscope for histoarchitectural changes.

2.18. Statistical analysis

The results are expressed as mean ± SEM from 6 animals in each group. Results were statistically analyzedusing one-way ANOVA followed by the Tukey- Kramer multiple comparison test; $p < 0.05$ was considered significant. GraphPad InStat version 4.00 of Graph Pad Software Inc, San Diego, USA, was the software used for statistical analysis.

3. Results

3.1. Quantification of total antioxidants, flavonoids and phenolic compounds in tea-extract by HPTLC

HPTLC analysis for total antioxidants showed the presence of 5 antioxidants in the tea-extract (Fig. 1). Rf values of the separated antioxidants were observed to be in the range of 0.081 to 0.815 with two major compounds at Rf 0.353 and 0.690 being present in amounts of 58.76 and 22.98 % respectively (Table 2). Similarly,HPTLC analysis of total flavonoids showed a clear separation of 10 flavonoids from the tea-extract (Fig. 2a and b). Rf values of the separated flavonoids were observed to be in the range of 0.019 to 0.919 (Table 3). HPTLC analysis

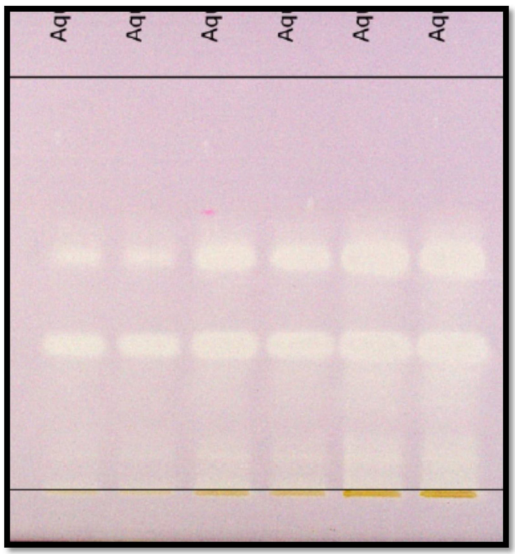

Fig. 1. HPTLC finger printing of tea-extract for total antioxidants at 425 nm.

Table 2
Peak areas and Rf values of antioxidants from tea-extract.

Peak	Start Rf	Max Rf	End Rf	Area (%)
1	0.065	0.081	0.106	10.51
2	0.148	0.156	0.165	8.12
3	0.265	0.282	0.291	2.54
4	0.318	0.353	0.398	58.76
5	0.665	0.690	0.711	22.98
6	0.790	0.815	0.816	4.52

for phenolic compounds showed 7 phenolic acids from the tea-extract (Fig. 3a and b). Rf values of the separated phenolic acids were observed to be in the range of 0.045 to 0.924 (Table 4).

3.2. Quantification of GA and Ru by HPTLC

The tea-extract showed the presence of rutin and gallic acid at Rf 0.166 and 0.65 respectively (Fig. 4). From the calibration curves, the tea-extract (1000 mg) was found to contain 1076 µg of GA and 1131 µg of Ru (Table 5).

3.3. Dose fixation of PTU

PTU administration at all 3 doses (6, 8 & 10 mg/kg) and 2time points (15th and 30th days) significantly attenuated the serum T3 and T4 levels and significantly elevated the serum TSH levels when compared with Normal Control levels (Tables 6a & 6b). However, PTU intoxication for 30 days showed better toxicity than 15 days. The PTU dose of 8 mg/kg showed optimal toxicity with better secondary toxicities like weight gain, hypothermia, and hypoglycemia. Hence, 8 mg/kg was selected as the toxicant dose of PTU in the study.

3.4. Acute toxicity study

The LD_{50} value of the tea-extract by the oral route was found to be greater than 2 g/kg b.w. as no mortality was observed until that dose.There was no significant change in body weight, behavior, renal function test, liver function test, lipid profile and other hematological evaluation. The tea-extract was thus considered safe for consumption.

3.5. Effect on anthropometrical parameters(body weight, BMI, abdominal circumference and body temperature)

All animal groups which started with no significant variation in body weight, BMI and abdominal circumference (AC) among them, on the 15th day showed significant ($p < 0.001$)increment in body weight, BMI and AC when compared with the Normal Control group (Fig. 5a, b &c) due to PTU administration. On the 30thand 45thdays, all treatment groups recorded lower weights, BMI and AC than their previous ones. Also, the body weight, BMI and AC of treatment groups was significantly ($p < 0.001$) lower than the Toxicant group indicative of the beneficial effect of the treatments in PTU-induced obesity.

After 15 days of PTU administration, all treatment groups showed a significant ($p < 0.001$) reduction in rectal temperature when compared with the Normal Control rats (Fig. 5d) due to a hypothyroid condition. On the 30th day, T500, T100, TAE, and levothyroxine treatments showed significant restoration ($p < 0.001$ for TAE, levothyroxine and $p < 0.05$ for T1500 and T1000) of the PTU-decreased rectal temperature. At the end of the study, all treatments significantly ($p < 0.001$) restored the PTU-decreased body temperatureto normal by virtue of their pro-thyroid effect. However, no significant elevation in rectal temperature was observed in AE when compared with the PTU group of animals.

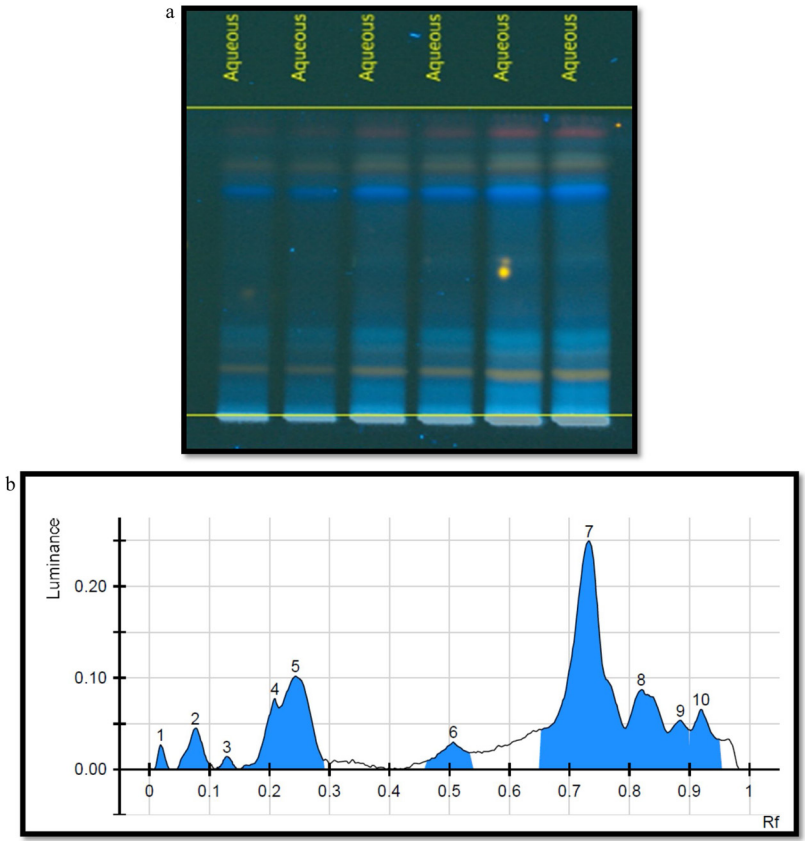

Fig. 2. a HPTLC finger printing of tea-extract for total flavonoids at 366 nm. b HPTLC chromatogram of tea-etract for total flavonoids at 366 nm.

Table 3
Peak areas and Rf values of flavonoids from tea-extract.

Peak	Start Rf	Max Rf	End Rf	Area (%)
1	0.005	0.019	0.033	0.95
2	0.046	0.077	0.102	3.59
3	0.112	0.130	0.146	0.70
4	0.157	0.209	0.216	5.41
5	0.216	0.244	0.291	14.57
6	0.464	0.507	0.534	4.01
7	0.653	0.733	0.792	45.57
8	0.794	0.820	0.864	13.53
9	0.864	0.885	0.900	4.79
10	0.901	0.919	0.954	6.88

3.6. Effect on T3, T4 and TSH

The effect of PTU on serum T3, T4 and TSH is shown in Fig. 6a, b and c respectively. Administration of PTU for 30 days to rats caused a significant ($p < 0.001$) reduction in serum T3 and T4 levels and a significant increase ($p < 0.001$) in serum TSH levels when compared with Normal Control animals. On the 1stday of the experiment, all groups started out with similar basal values. On the 15thday, all PTU-treated groups showed significant attenuation ($p < 0.001$) of T3 and T4 levels and a significant increase ($p < 0.001$) in serum TSH levels when compared with the Normal Control group due to the hypothyroid effect of PTU. At the end of the study on the 45th day, all treatments significantly ($p < 0.001$) restored the PTU-depleted serum T3 and T4 levels and attenuated ($p < 0.001$) the PTU-elevated TSH levels displaying a potent pro-thyroid effect.

3.7. Effect on lipid profile

The PTU-treated group of rats exhibited significant ($p < 0.001$) increase in the levels of TC, TG, VLDL and LDL, and reduction in the levels of HDL when compared with Normal Control rats (Fig. 7). The PTU-elevated TC, TG, VLDL and LDL levels were attenuated significantly and the PTU-depleted HDL levels were restored significantly by all treatmentsdue to an anti-hyperlipidemic effect secondary to their main pro-thyroid effect.

3.8. Effect on hcy

Rats that displayed similar basal levels at the start, after 15 days of PTU administrationshowed a significant elevation ($p < 0.001$) in Hcy levels when compared with the Normal control values (Fig. 8) due to PTU administration. At the end of the treatment period, on the 45th day, all treatments had significantly attenuated ($p < 0.001$) the PTU-elevated Hcy levels indicating their ability to attenuate hyperhomocysteinemia by means of their primary thyroid effect.

3.9. Effect on protein

PTU treatment depleted significantly ($p < 0.001$) the serum albumin, globulin and total protein of rats (Fig. 9). All treatment groups except AE significantly restored ($p < 0.001$) the PTU-attenuated albumin, globulin and total protein levels indicating a restorative effect of the treatments.

3.10. Effect on blood glucose levels

The normal blood glucose levels of all treatment groups recorded on the 1st day of the study were significantly ($p < 0.001$) lowered by 2 weeks of PTU administration due to development of hypothyroidism in the animals (Fig. 10). On the 30th and 45th days, a significant ($p < 0.001$) elevation of the PTU-depleted glucose levels was exhibited by all treatment groups except the AE groupdue to a thyroid boosting

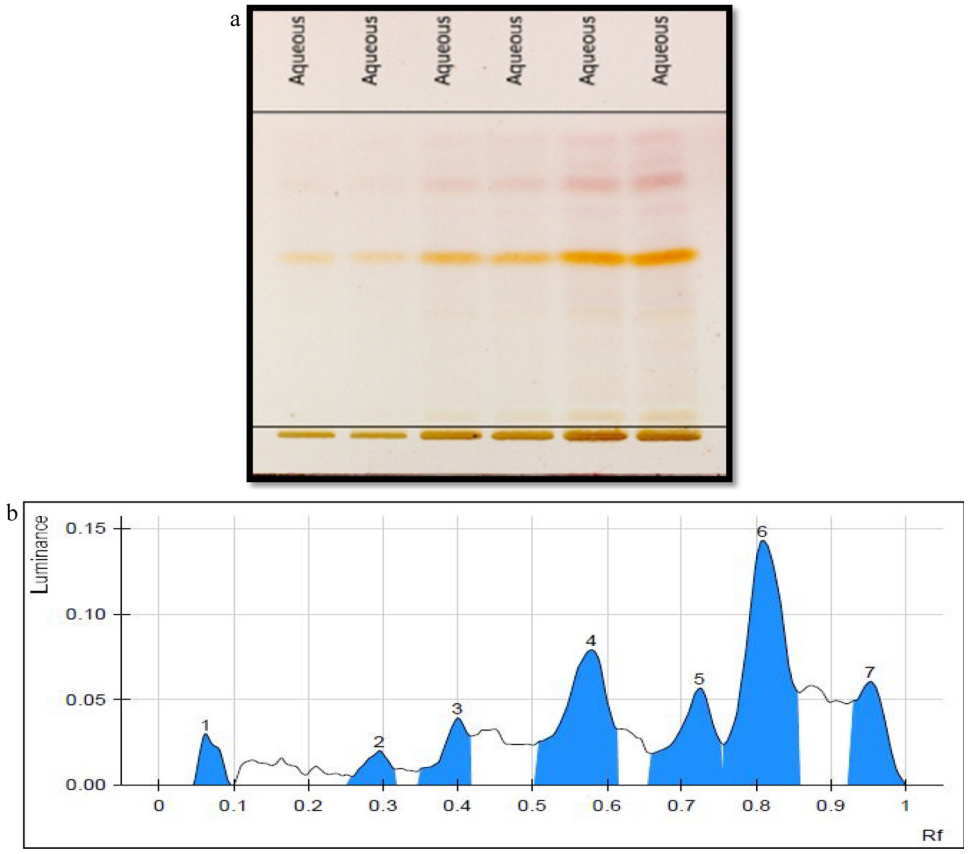

Fig. 3. a HPTLC finger printing of tea-extract for total phenolic compounds at 540 nm. b HPTLC chromatogram of TEA-EXTRACT for total phenolic compounds at 540 nm.

Table 4
Peak areas and Rf values of phenolic acids from tea-extract.

Peak	Start Rf	Max Rf	End Rf	Area (%)
1	0.045	0.063	0.097	3.64
2	0.258	0.295	0.318	3.36
3	0.348	0.400	0.418	6.65
4	0.505	0.579	0.615	23.05
5	0.660	0.724	0.755	13.77
6	0.756	0.808	0.858	37.21
7	0.924	0.953	1.000	12.32

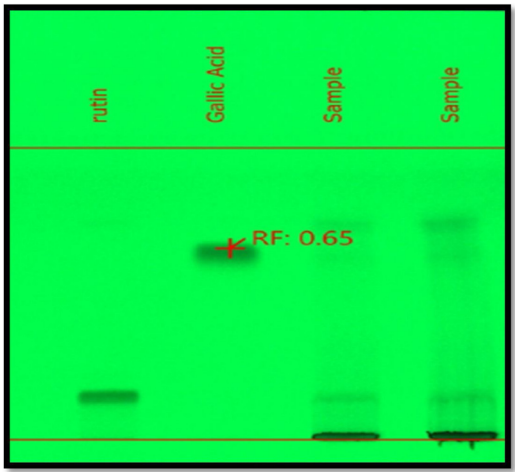

Fig. 4. HPTLC plate with standard gallic acid, rutin and tea-extract at 305 nm.

effect of the treatments which alleviated the PTU-induced hypoglycemia.

3.11. Effect on insulin, cortisol, IR and glucose 6-phosphatase

The effect of all treatments in this study on insulin, cortisol, IRand glucose 6-phosphatase is depicted in Table 7. Insulin and cortisol levels and HOMA-IR were noted to be significantly elevated ($p < 0.001$) in PTU-administered rats when compared with the Normal Control rats due to a disturbance of the hypothalamic–pituitary–adrenal axis. All treatments significantly ($p < 0.001$) attenuated the PTU-elevated insulin and cortisol levels, and HOMA-IR, except for AE due to their favorable effect on the hypothalamic–pituitary–thyroid and the hypothalamic–pituitary–adrenal axes.

PTU administration induced a significant ($p < 0.001$) depletion of the normal glucose 6-phosphatase activity. All treatments significantly ($p < 0.001$) restored the depleted enzyme activity, except for AE, thus indicating their ability to restore T4 which regulates this enzyme.

3.12. Effect on uric acid and creatinine levels

PTU administered rats exhibited a significant ($p < 0.001$) increase in serum uric acid and creatinine levels when compared with the Normal Control rats (Fig. 11) due to the untoward effect of PTU on the kidney. T1000, T1500, TAE and levothyroxine treatments significantly attenuated ($p < 0.001$) the PTU-elevated serum uric acid levels, whereas all treatment significantly attenuated ($p < 0.001$) the PTU-elevated creatinine levels, indicating their restorative effect on PTU-affected renal function.

Table 5
Quantification of gallic acid and rutin in tea-extract using HPTLC.

Sample	Quantity applied (μg)	Start Rf	Max Rf	End Rf	Peak area	% content
Standard gallic acid	5	0.539	0.654	0.739	0.0423	100
Tea-extract	200	0.585	0.658	0.716	0.0022	5.20
Standard rutin	5	0.094	0.168	0.224	0.0241	100
Tea-extract	200	0.095	0.166	0.221	0.0061	25.41

Table 6a
Effect of PTU administration for 15 days on T3, T4 and TSH.

GROUPS	T3	T4	TSH
Normal Control	66.59 ± 0.42	4.03 ± 0.11	1.33 ± 0.12
6mg/kg of PTU	53.09 ± 0.45**	3.18 ± 0.07**	4.71 ± 0.20**
8 mg/kg of PTU	37.77 ± 1.83***	2.63 ± 0.16***	11.65 ± 0.55***
10 mg/kg of PTU	38.50 ± 2.75***	2.68 ± 0.15***	12.95 ± 0.59***

Table 6b
Effect of PTU administration for 30 days on T3, T4 and TSH.

GROUPS	T3	T4	TSH
Normal Control	66.59 ± 0.42	4.18 ± 0.05	1.41 ± 0.18
6mg/kg of PTU	43.09 ± 0.45***	2.68 ± 0.15***	5.22 ± 0.35***
8 mg/kg of PTU	10.78 ± 0.17***	1.56 ± 0.17***	26.01 ± 1.29***
10 mg/kg of PTU	10.50 ± 2.75***	1.88 ± 0.15***	31.95 ± 1.59***

Note: All values are mean ± SEM; N = 6 in each group; One-way ANOVA followed by Tukey-Kramer multiple comparison test is applied for statistical analysis; $^{**}p < 0.01$&$^{***}p < 0.001$ when PTU groups compared with Normal Control.

3.13. Effect on LPO and antioxidant enzymes in liver

The effects of tea extract on LPO, GSH, SOD, CAT, GPx and GR in the liver homogenate are summarized in Table 8. malondialdehyde (MDA), the lipid peroxidation marker was significantly elevated ($p < 0.001$) in PTU-administered rats when compared with Normal Control rats. Administration of all treatments attenuated significantly ($p < 0.001$) the PTU-elevated levels of MDAexcept for AE. TAE treatment was comparable to Normal Control in preventing the formation of MDA.

Significant ($p < 0.001$) depletion in GSH levels, and SOD, CAT, GPx and GR activities was observed upon PTU administration to rats. All treatment groups could significantly restore the depleted levels of these antioxidants except AE which failed to restore GPx and GR activity significantly.

3.14. Histopathological studies

Histopathological studies on the thyroid gland of the Normal Control group of rats revealed a normal histoarchitecture with uniform thyroid follicles lined by cuboidal cells containing a moderate amount of colloid in the lumen. No evidence of fibrosis or inflammation was noted (Fig. 12a). The thyroid follicles of the PTU administered rats were closely packed and displayed abundant granular cytoplasm with reduced lumen size and complete absence of colloid. The features were similar to the degenerate cellular changes seen in Hurthle/Askanazy cells (Fig. 12b). Thyroid follicles of T500 were of normal shape and size and showed mild crowding, overlapping and stratification of cuboidal lining of epithelial cells. Colloid formation was moderate (Fig.12c). The thyroid tissue of T1000 treatment was comparable to Normal Control exceptfor a mild reduction in the size of follicles. No evidence of degenerative changes/ fibrosis or inflammation was seen and the colloid deposition was moderate (Fig. 12d). Thyroid tissue of T1500 showed a moderate reduction in follicular size and marked crowding, overlapping and stratification of epithelial cells. Colloid was the least when compared with T500 and T1000 groups (Fig. 12e). The thyroid slice of AE revealed features comparable to those of T500 but follicular cells were lined by more vesicular nuclei with powdery chromatin (Fig.12f). TAE showed maximum preservation of thyroid histology which was comparable with the Normal thyroid in every way (Fig. 12g). Levothyroxine

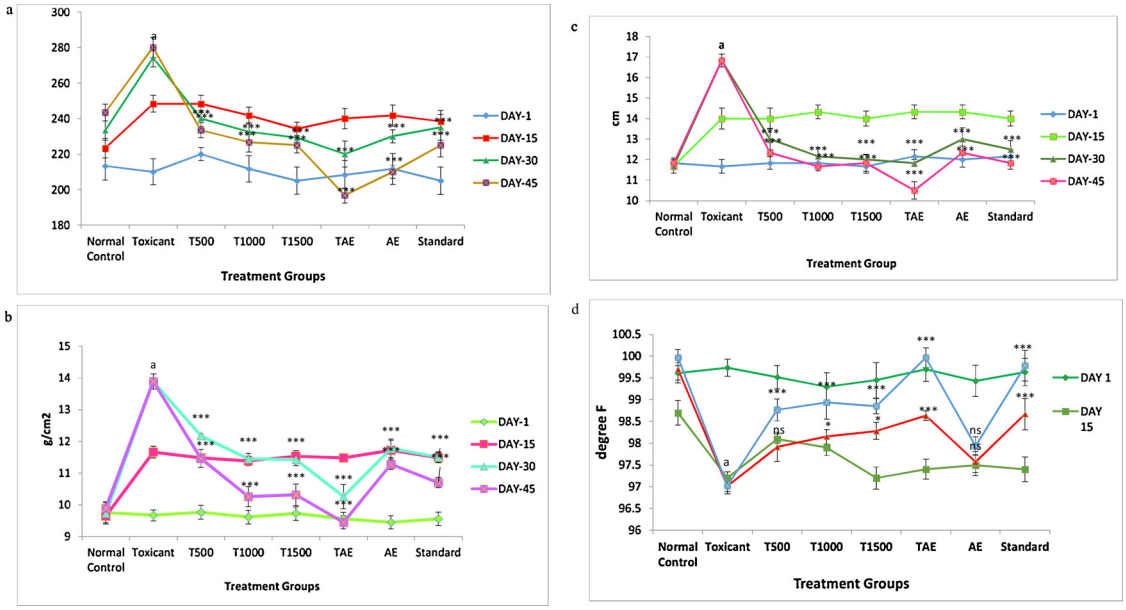

Fig. 5. a Effect of tea-extract, TAE, AE and levothyroxine on body weight. b Effect of tea-extract, TAE, AE and levothyroxine on BMI.c Effect of tea-extract, TAE, AE and levothyroxine on abdominal circumference. d Effect of tea-extract, TAE, AE and levothyroxine on rectal temperature.

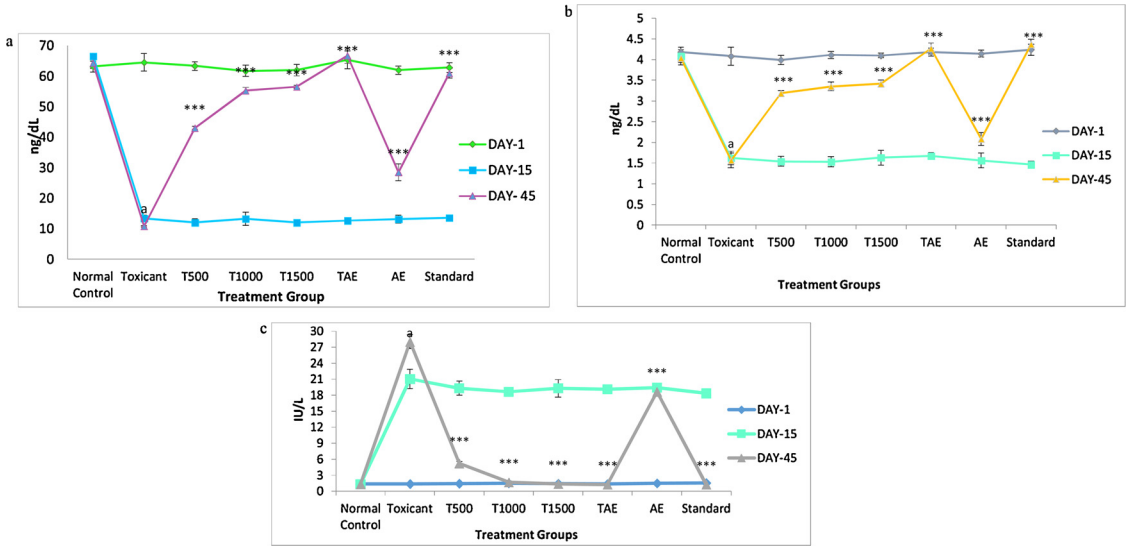

Fig. 6. a Effect of tea-extract, TAE, AE and levothyroxine on T3. b Effect of tea-extract, TAE, AE and levothyroxine on T4. c Effect of tea-extract, TAE, AE and levothyroxine on TSH.

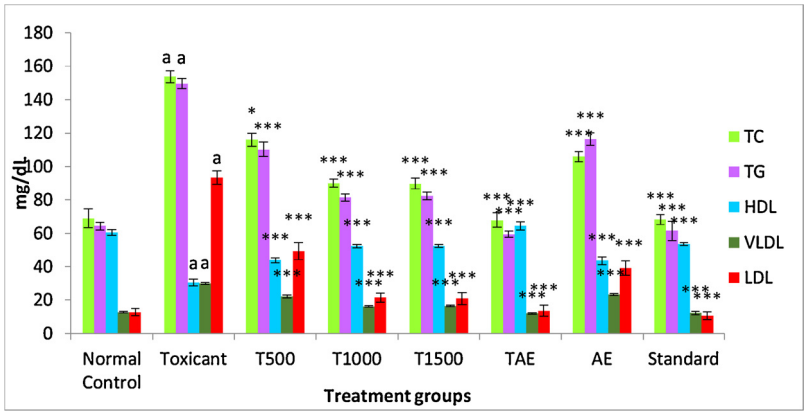

Fig. 7. Effect of tea-extract, TAE, AE and levothyroxine on serum lipid profile.

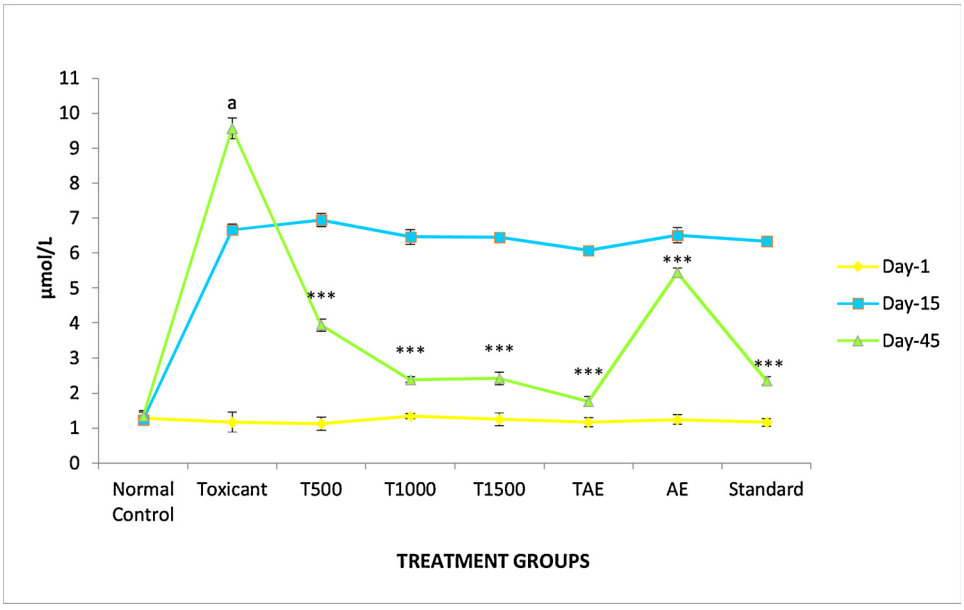

Fig. 8. Effect of tea-extract, TAE, AE and levothyroxine on serum Hcy levels.

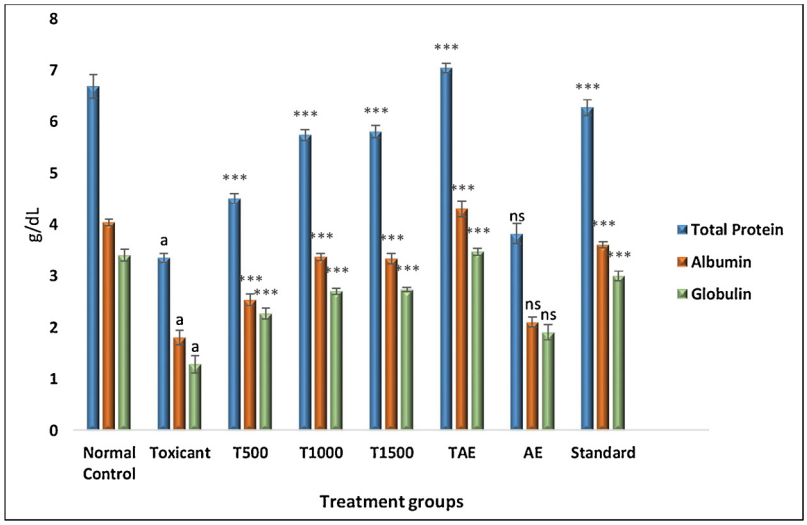

Fig. 9. Effect of tea-extract, TAE, AE and levothyroxine on serum protein levels.

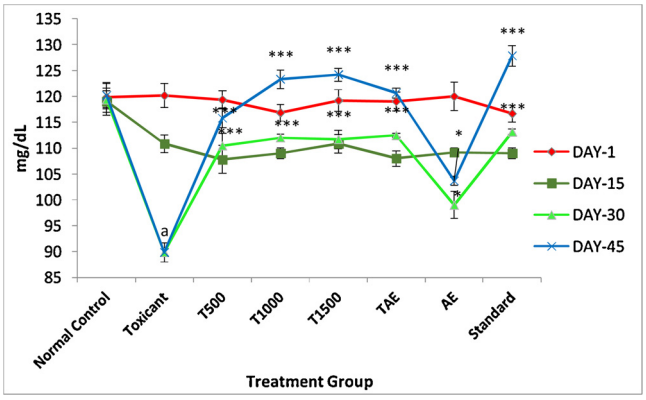

Fig. 10. Effect of tea-extract, TAE, AE and levothyroxine on BGL.

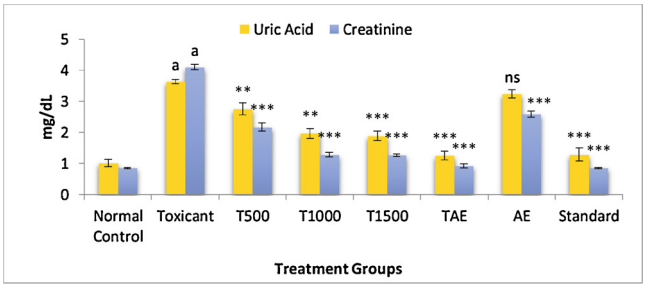

Fig. 11. Effect of tea-extract, TAE, AE and levothyroxine on uric acid. and creatinine levels.

treatment's thyroid showed features almost similar to the Normal group's thyroid. Mild reduction in the size of follicles and moderate colloid was noted (Fig. 12h).

4. Discussion

PTU is a thiouracil-derived drug which is clinically used to treat hyperthyroidism. It acts by two distinct mechanisms to produce hypothyroidism [22]. It inhibits the enzyme thyroperoxidase, which catalyzes the incorporation of iodine into tyrosine residues to form T3 and T4. PTU also inhibits the enzyme tetraiodothyronine 5′deiodinase, which converts T4 to the active form T3. The resulting lowering of serumT3 and T4 levels leads to an increase in serum TSH *via* a feedback system involving the pituitary gland. Studies have also shown that PTU-induced perinatal hypothyroidism leads to hyperactive behavioral phenotypes and altered monoaminergic state in the brain of rodents [23]. The thyroid toxicity produced by PTU is similar to that produced by most environmental toxicants and drugs hence, our study used PTU for inducing hypothyroidism in rats.

Drug therapy for hypothyroidism includes daily use of the synthetic thyroid hormone levothyroxine or triiodothyronine (T3) as an add-on treatment for select individuals.The oral medication restores adequate hormone levels, reversing the signs and symptoms of hypothyroidism.The reference standard used in our study was levothyroxine which is a synthetic form of T4 that is converted to its active metabolite L-triiodothyronine *in situ*. Levothyroxine restores thyroid function adequately, reversing the signs and symptoms of

Table 7
Effect of tea-extract, TAE, AE and levothyroxine on insulin, cortisol, insulin resistance and glucose 6-phosphatase.

GROUPS	INSULIN (mIU/L)	HOMA-IR (m/L)	CORTISOL (mcg/dL)	Glucose-6-phosphatase (U/mg protein)
Normal control	10.19 ± 0.49	3.02 ± 0.04	7.60 ± 0.14	0.74 ± 0.04
Toxicant Control	21.18 ± 1.06[a]	4.88 ± 0.08[a]	13.60 ± 0.31[a]	0.28 ± 0.02a
T500	15.04 ± 0.30***	3.95 ± 0.06***	9.37 ± 0.26***	0.58 ± 0.03***
T100	13.04 ± 0.99***	3.96 ± 0.10***	8.58 ± 0.20***	0.76 ± 0.02***
T1500	12.94 ± 1.04***	3.87 ± 0.17***	8.46 ± 0.17***	0.78 ± 0.02***
TAE	9.45 ± 0.46***	2.97 ± 0.03***	7.82 ± 0.13***	0.88 ± 0.02***
AE	19.98 ± 0.86	4.81 ± 0.07	12.19 ± 0.37*	0.31 ± 0.02
Standard	11.68 ± 0.78***	3.96 ± 0.12***	7.96 ± 0.12***	0.86 ± 0.01***

All values are means ± SEM; N = 6 in each group; One-way ANOVA followed by Tukey-Kramer multiple comparison test is applied for statistical analysis;[a]$p <$ 0.001 when PTU group is compared with Normal Control; *$p < 0.05$&***$p <$ 0.001 when experimental groups are compared with PTU group.

Table 8
Effect of tea-extract, TAE, AE and levothyroxine on LPO and antioxidant enzymes in liver.

GROUPS	LPO (nmol MDA/ min / mg protein)	GSH (μmol/ mg protein)	SOD (U/ mg protein)	CAT (U/ mg protein)	GPx (U/mg protein)	GR (U/ mg protein)
Normal control	18.15 ± 0.95	3.36 ± 0.11	32.22 ± 0.44	25.35 ± 0.89	12.14 ± 0.51	257.82 ± 1.19
Toxicant	46.18 ± 0.51[a]	0.44 ± 0.12[a]	10.77 ± 0.89[a]	8.64 ± 0.47[a]	7.51 ± 0.17[a]	119.40 ± 7.56[a]
T500	29.36 ± 0.75***	2.26 ± 0.14***	20.39 ± 0.84***	17.74 ± 0.57***	9.39 ± 0.27***	221.02 ± 2.01***
T1000	26.27 ± 0.65***	3.21 ± 0.14***	25.46 ± 1.17***	22.33 ± 0.51***	11.18 ± 0.52***	251.11 ± 3.90***
T1500	25.15 ± 0.74***	2.69 ± 0.85***	28.26 ± 0.56***	22.89 ± 0.55***	11.13 ± 0.40***	224.05 ± 6.09***
TAE	14.36 ± 0.71***	3.92 ± 0.14***	35.40 ± 0.90***	28.22 ± 0.74***	13.42 ± 0.35***	254.13 ± 2.70***
AE	41.00 ± 4.82	1.29 ± 0.10***	16.38 ± 0.83***	13.33 ± 0.61***	7.14 ± 0.13*	139.64 ± 1.55
Standard	23.27 ± 0.65***	2.88 ± 15***	28.34 ± 0.64***	23.20 ± 0.64***	11.01 ± 0.50***	201.28 ± 5.54***

All values are means ± SEM; N = 6 in each group; One-way ANOVA followed by Tukey-Kramer multiple comparison test is applied for statistical analysis;[a]$p < 0.001$ when PTU group is compared with Normal Control; *$p < 0.05$ &***$p < 0.001$ when experimental groups are compared with PTU group.

hypothyroidism and its associated disorders. However, treatment with levothyroxine or any other synthetic thyroid drug has to be monitored carefully to prevent the occurrence of hyperthyroidism.

Aerobic exercise increases oxygen consumption, number, size, and density of mitochondria and oxidative enzymes, thus increasing the rate of aerobic fat catabolism leading to energy expenditure and fat burn. Exercise also contributes to an increase in the metabolic rate by improving cardiovascular capacity and suppressing pro-inflammatory cytokine production. It is known to increase the release of nitric oxide and accelerate the consumption of free fatty acids and enhance insulin sensitivity [24]. It has been reported that aerobic exercise, at both low and high intensity, stimulates an increase in lipoprotein lipase, improving the lipoprotein profile, and enhancing the enzymatic processes involved in lipid metabolism [25,26]. Hence, our study included a group with AE alone and in combination with the tea-extract to see its beneficial effects in hypothyroidism.

It has been known that phenolic acids and flavonoids stimulate the activity of type 2 iodothyroninedeiodinase (D2), an intracellular enzyme that activates thyroid hormones. The half-life for D2 is also selectively and significantly increased by these phytoconstituents [27]. Rutin, a flavonoid from the teabag has been proven to aid in the uptake of iodide by the thyroid due to an elevation in the activity of the sodium-iodide symporter (NIS) [28]. Thyroid iodide uptake through NIS is an essential step for thyroid hormones biosynthesis. Also, thyroperoxidase, the key enzyme in thyroid hormone biosynthesis may be boosted by rutin. GA, a phenolic acid from the tea bag is thyroprotective and prevents damage to the thyroid by attenuation of thyroid inducible nitric oxide synthaseexpression, anti-inflammatory cytokines, cyclooxygenase-2, LPO markers and nitric oxide levels, and upregulation of endogenous antioxidant enzymes [29].

T3 is the major thyroid hormone responsible for the metabolic effects of the thyroid gland including calorigenesis, oxygen consumption and maintenance of basal metabolic rate. Hence, a lowT3 level in our study, leads to a low basal metabolic rate which manifested as weight gain, obesity, increased BMI and increased AC.It is also possible that a disturbance in the regulation of the hypothalamic-pituitary-thyroid axisaltered the activity of the anorexigenic hormone leptin leading to central obesity. All our treatments by way of their thyroid boosting and metabolism-enhancing effect could reverse these untoward effects of PTU administration.

Hypothermia has been defined as a body temperature below 95 °F (35 °C). Hypothyroid hypothermia results due to low heat production consequent to depressed thyroid function. Hypothermia may lead to shivering, mental confusion, and ultimately cardiac failure. Low-grade hypothermia was observed in PTU intoxicated animals, which was reversed successfully by the treatment groups indicating their role in improving the hypothyroid condition.

In the present study, the tea-extract could increase the PTU-depleted T4 and T3 levels. The tea-extract may have stimulated hormone production in two ways, *viz.*, by increasing the activity of thyroperoxidase and thereby increasing the secretion of T4 and to a lesser extent T3 or by increasing the conversion of T4 to T3 in the peripheral tissue to increaseT3. Most circulatory T3 is generated in hepatic tissue by the enzyme type-1 5′-iodothyroninemonodeiodinse (5′-DI). This enzyme is inhibited by PTU intoxication. Thus, the increase in serum T3 could also be due to the decrease in hepatic LPO and increased activity of 5′-DI. Elevated serum TSH level in hypothyroidism is due to the feedback mechanism at HPT axis. Our formulation could successfully attenuate TSH levels due to the restoration of T3 and T4 hormones.TAE exhibited an additional benefit because physical exercise has been reported to stimulate the peripheral deiodination of T4 to T3 and increased uptake of T4 in the liver during exercise [30].

Thyroid hormones are known to be key factors in regulating the synthesis, metabolism, and mobilization of lipids [31,32]. T3 up-regulates the LDL receptor by controlling its gene activation and expression. Thyroid hormones control HDL metabolism by increasing the activity of cholesteryl ester transfer protein which collects triglycerides from VLDL or LDL and exchanges them for cholesteryl esters from HDL and *vice versa*. Thyroid hormones stimulate the lipoprotein lipase, which catabolizes the TG-rich lipoproteins, and the hepatic lipase, which converts IDL to LDL and in turn LDL to small dense LDL. The small dense LDL is sent back to the liver to be stored or broken down to create ATP or to the peripheral cells to synthesize various hormones. Another effect of T3 is the up-regulation of apolipoprotein AV, which plays a major role in TG regulation. In PTU-induced hypothyroidism, an increase in serum TC, TG, LDL and VLDL levels and a decrease in HDL levels was noted probably due to a failure of the thyroid to synthesize adequate T3 and T4 to perform their normal functions of lipid synthesis and regulation. The tea-extracts by their thyroid hormone boosting effects could successfully normalize the PTU-skewed lipid profile. Physical exercise is considered an important non-drug intervention for treating dyslipidemias, and hence AE and TAE demonstrated a significant reduction in PTU-induceddyslipidemia.

High homocysteine levels are found to be invariably associated with an imminent or current cardiovascular disease. A hypothyroid state decreases hepatic levels of enzymes involved in the re-methylation pathway of homocysteine to methionine, thus building up homocysteine in blood [33]. Also, a low level of thyroid hormones probably reduces glomerular filtration rate leading to increased homocysteine levels in the blood. Homocysteine levels were thus found significantly

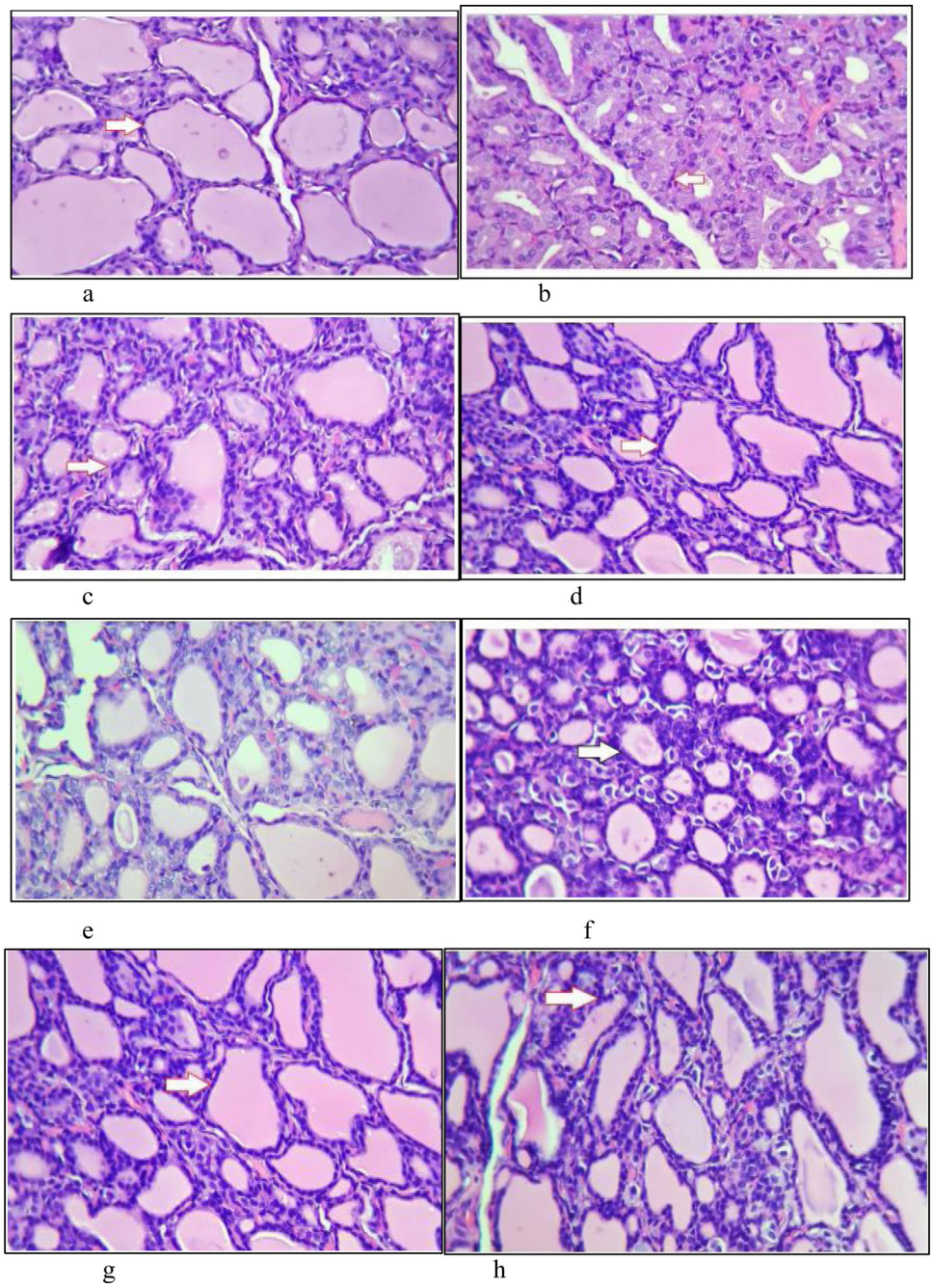

Fig. 12. a Normal Control:H&E 40X Normal thyroid follicles lined by cuboidal cells. b Toxicant (PTU): H&E 40X Loss of colloid. Cells have become columnar with abundant granular cytoplasm and small lumen (Oncocytic/ Degenerative).c T500:H&E 40X Appear almost normal with abundant colloid and flattened to cuboidal epithelium. d T1000: H&E 40X Mild reduction in the size of follicles. Colloid is moderate. e T1500: H&E 40X Mild reduction in follicular cells, marked crowding, Overlapping and stratification. Less colloid observed. f AE: H&E 40X Follicular cells variable (compared to normal)lined by more vesicular nuclei with powdery chromatin. g TAE: H&E 40X Follicles are almost normal in shape and size, and the nuclei shows less crowding and overlapping. Colloid is moderate. h Standard (Levothyroxine): H&E 40X Mild reduction in the size of follicles. Colloid is moderate.

elevated in the PTU group when compared with the Normal Control group of animals. Attenuation of serum homocysteine by the tea-extract may be due to an amelioration of thyroid function by its phytoconstituents.

The thyroid gland is essential for the metabolism and normal function of the liver. The liver, in turn,is responsible for the synthesis of a large number of proteins in the body including the plasma proteins albumin and globulin. Thus, in PTU-induced hypothyroidism in rats, inadequate thyroid hormone synthesis adversely affects liver function and there is a subnormal production of proteins by the liver. PTU-induced hypothyroidism in rats featured a reduction in serum total protein, albumin, and globulin. All the treatment groups significantly restored the PTU-depleted serum total protein, albumin and globulin levels may be due to their thyroprotective and adaptogenic effects. Exercise is known to stimulate protein synthesis and hence the AE and TAE groups too restored the PTU-depleted proteins.

Thyroid hormones stimulate hepatic gluconeogenesis and glycogenolysis and increase expression of genes coding for the glucose transporter type-4 and phosphoglycerate kinase, thus, acting synergistically with insulin to facilitate glucose disposal and utilization in peripheral tissues [34]. They play a significant role in the maintenance of glucose homeostasis. Gluconeogenesis is reduced in hypothyroidism, both in skeletalmuscle and in adipose tissue. Glycogenolysis is also impaired in hypothyroidism. These biochemicaldefects might lead to hypoglycemia in hypothyroidism. In the present study glucose, 6-phosphatase activity was found to be reduced in the PTU-administered rats. Glucose 6 phosphatase is an enzyme that hydrolyzes glucose 6-phosphate to free glucose which is then exported from the cell *via* glucose transporter membrane proteins. Exogenous T4 is known to regulate glucose 6-phosphatase activity, hence reduction in thyroid hormones reduces the activity of glucose 6-phosphatase which is also responsible for hypoglycemia in PTU-administered rats.

In this study, significant hyperinsulinemia and increased insulin resistance wereobserved in the PTU intoxicated animals when compared with the Normal Control animals. In hypothyroidism, there is reduced renal clearance of insulin from blood, because of which hyperinsulinemia occurs [35]. Alleviation of hyperinsulinemia, reduction in insulin resistance and a near reversal of hypoglycemia by the TAE, levothyroxine, T500, T1000 and T1500 treatments was noted. This was probably due to the restoration of the altered thyroid function by the tea-extract's phytoconstituents.

Hypothyroidism causes elevation of cortisol by reducing its peripheral disposal and by blunting its feedback on the hypothalamic-pituitary-adrenal axis [36]. In our experiment, PTU intoxication in rats caused an increase in serum cortisol levels. Another reason for the elevation of cortisol is the metabolic stress which is generated by an underperforming thyroid. This metabolic stress may titillate the hypothalamic-pituitary-adrenal axis to bring about a sustained release of cortisol. Also, TSH levels are positively correlated with cortisol. Hence, an elevation in TSH levels as seen in PTU intoxication would elicit increased cortisol levels. Treatment with tea-extracts, levothyroxine and TAE attenuated the PTU-elevated cortisol levels and thus our study demonstrated a protective effect of tea-extract on impaired glucose parameters by PTU.

The effects of hypothyroidism on the kidney are deleterious. The renal blood flow (RBF) is reduced in hypothyroidism by decreased cardiac output (negative inotropic and chronotropic effect), increased peripheral vascular resistance and intra-renal vasoconstriction [37–39]. Also, due to hypothyroidism, the glomerular histology is altered leading to a thickening of the basement membrane with a progressive expansion of mesangial matrix, which may occlude glomerular capillaries to reduce RBF and GFR by about 40 % [40]. Levels ofcreatinine and uric acid were seen to be elevated in PTU administered animals indicating renal abnormalities associated with hypothyroidism. The PTU elevated serum creatinine and uric acid levels were significantly attenuated by T1000, T1500, TAE and levothyroxine treatments, indicating a restoration of kidney function due to an improved thyroid function.There is an untoward alteration in the oxidative balance of the body in hypothyroidism and hyperthyrotropinemia. Hyperlipidemia and hyperinsulinemia which are present in hypothyroidism may contribute toenhancing oxidative stress in the hypothyroid state, which can impair and decrease the antioxidant capacity of the liver making it more vulnerable to reactive oxygen species-induced damage [41]. Therefore, PTU-injected hypothyroidic rats showed an increased production of reactive oxygen species which caused increased LPO and a marked decline in the GSH and antioxidant enzyme levels. All treatment groups displayed their ability to prevent MDA formation and restore the endogenous antioxidants in PTU-induced hypothyroidism due to their potent antioxidant activity. Our treatment groups restored the PTU-depleted thyroid hormones, which are known to reduce tissue LPO and increase the levels of natural antioxidants. The potent flavonoids of the tea-extracts are responsible for this activity. AE showed a significant restoration of only GSH, CAT and SOD activities depleted by PTU. The reason behind the failure of amelioration of the depleted GR and GPx levels in this group may be due to an acute exercise-induced oxidative stress.

The polyherbal teabag's phytoconstituents, thus, may act through multiple mechanisms to boost the activity of the thyroid.

5. Conclusion

In conclusion, the polyherbal tea-extract (T1000 and T1500 mg/kg) and TAE modulated the hypothalamic–pituitary–thyroid and the hypothalamic–pituitary–adrenal axes to produce a beneficial effect in hypothyroidism and associated disorders. There was no significant difference between T1000 and T1500 treatments possibly due to a ceiling effect of the extract at 1000 mg/kg.This work indicates the beneficial role of exercise in combination with herbs in treating hypothyroidism and its associated disorders. In increasing incidences of hypothyroidism induced by environmental, industrial and pharmaceutical chemicals, our tea-bag may prove useful as a safe alternative to synthetic thyroid drugs which are associated with ample toxic effects.

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CRediT authorship contribution statement

Sneha Singh: Conceptualization, Methodology, Data curation, Writing - original draft. **Vandana Panda:** Conceptualization, Supervision, Writing - review & editing. **Sudhamani S.:** Formal analysis. **Payal Dande:** Formal analysis.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxrep.2020.06.002.

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