INTRODUCTION

Hypothyroidism is one of the most common endocrine disorder [1]. It is defined as a condition when the level of the thyroid hormones decreases below the normal need of the body [2]. The hypothyroidism is a complex hormonal dysfunction rather than a single hormonal defect [3]. Thyroid hormone is a powerful modulator of cardiac function [4]. Hypothyroidism causes the disturbance in body weight and appetite [5]. Even during pregnancy time, a significant change in thyroid hormone metabolism is observed [6]. Synthetic levothyroxine is recommended for hypothyroidism, actually, this drug elevates the $T_3$ and $T_4$ level in blood serum so that thyroid gland activity can be controlled [7]. Whereas, 6-n-propyl-2-thio-uracil (PTU) is prescribed for hyperthyroidism because it can decrease the amount of both thyroid hormones secreted by the thyroid gland [8]. Long duration usage of the synthetic drug can cure the hypothyroidism, but they cause side effects. L-thyroxin can damage the vital organs such as the liver and kidney, and it also causes the reduction in body weight and an increase in the relative body weight of the kidney [9]. Thus, there is a need to develop a drug from plants origin which can work for the hypothyroid treatment. Ayurveda suggests that some plant products cure hypothyroidism and some investigation on the effects of plant products on hypothyroidism is also found. Several bioactive compound has been derived from various plants, having anti-thyroidal activity and low toxicity as compare to synthetic drugs, e.g., Bamboo shoot [10]. These plant products can control the basal metabolic rate of the body and several cardiovascular diseases like atherosclerosis [11]. These extracts have antioxidants and these can affect the metabolism of the body too [12]. Withania somnifera can affect on antioxidants of animal, and it can increase the serum $T_3$ and $T_4$ significantly. Its roots have a compound name withanolides which can cure many diseases [13,14]. Roots of the W. somnifera are used in Ayurveda to cure hypothyroidism. This study is designed to evaluate the effect of W. somnifera leaf extract on hypothyroidism. Therefore, we have performed an experiment to examine the effect of W. somnifera leaf extract on PTU induced hypothyroid rats. This study is aimed to finding out whether W. somnifera leaves can increase Serum $T_3$, $T_4$ level and affect the thyroid gland activity positively.

METHODS

Extraction of plant material

W. somnifera plant’s leaves were collected from Jodhpur, Rajasthan state, India, and scientific identification was done in the Department of Botany, Jai Narai Vyas University, Jodhpur. These leaves were extracted with 70% ethanol for 24–36 h by Soxhlet extraction method. Then, ethanol was separated under reduced pressure to obtain a dark brown crude residue which was dissolved in distilled water and orally administrated to the animals.

Model animals

Wistar rats (150–250 g) were purchased from Certified Institute. Protocols for animal care, maintenance and experiments were followed given by Animal Ethical Committee (IAEC, Reg no: 1646/G0/Re/12/CPCSEA). For this experiment, 20 female animals were housed in polypropylene cages containing corn-cob bedding and maintained at approximately 25–28°C on a 12-h light/dark cycle. For the animal adaptability, all rats had been fed for 1 week before the experiment. Rats were randomly divided into four groups.

Experimentation

Induction of hypothyroidism

Hypothyroidism was induced in euthyroid rats by administration of PTU at the dose of 10mg/kg in drinking water as well as orally for 30 days.

Dose regime of Eltroxin

Eltroxin was used as a standard drug to cure hypothyroid that was orally administrated at the dose of 0.5 µg/100 g body weight dissolved in 50 mL of distilled water.
Preparation of plant drug
The 70% ethanolic extract (500 mg/kg.bwt) was prepared then given to the experimentally induced hypothyroid rats.

Experimental design
The experimental period comprised 60 days and the rats were divided into the following groups (n=5).
- Group I: Intact control: Rats receiving normal slurred rat pallets with saline water.
- Group II: Hypothyroid control: Rats were orally injected PTU for hypothyroidism induction for 60 days.
- Group III: *W. somnifera* leaf extract’s treatment group: PTU treated rats received *W. somnifera* leaf extracts (70% ethanolic extracts) for next 30 days on the dose of 500 mg/kg body weight.
- Group IV: Eltroxin treatment group: PTU treated rats received Eltroxin at the dose of 0.5 µg/100 g body weight for the next 30 days.

Serum biochemistry
At the end of the experiment, all rats were sacrificed under prolonged anesthesia and blood was collected through the direct cardiac puncture. Serum was separated and stored at −20°C until analyzed.

Assessment of hormone assays of T₄ and T₃
Total circulating T₄ and T₃ in serum were quantitatively determined by enzyme-linked immunosorbent assay following the protocols provided in the CALBIOTECH kits as routinely followed in our laboratory.

Assessment of liver function test (LFT), renal function test (RFT), and other parameters
For the RFT determination in blood serum urea, creatinine, and uric acid was estimated by the kit method. Whereas serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), bilirubin direct, bilirubin indirect, and bilirubin total were measured for the analysis of LFT. Other parameters such as blood sugar, cholesterol, alkaline phosphate, and protein were also evaluated by the kit method. All of these tests were analyzed using Biochem Analyzer RX-50 and commercial diagnostic kits (Siemens Healthcare Diagnostics, USA).

Statistical analysis
The mean and standard deviation was calculated in terms of mean ±SEM. One-way analysis of variance was used for parametric analysis of all groups and calculating the statistical differences between the means of the various groups.

Hematology
Blood was collected by direct cardiac puncture at the end of the experimental period. Collected blood was stored in EDTA vials at 20°C. Hematological assessments of hemoglobin, TRBC, HCT, MCV, MCH, MCHC, RDW, TLC, PLT, PCT, MPV, and PDW were examined through standard methods [15].

RESULTS
Hormone assay
Animals treated by PTU demonstrated significant reduction (p≤0.001=c) in serum level of T₄ and slightly significant reduction (p≤0.05=a) in T₃ level as compared to control groups whereas *W. somnifera* leaf extracts treated group’s blood serum have demonstrated no significant changes in T₄ and T₃ level as compared to control groups. Moreover, when this Group III was compared to Group II, then it was observed that T₄ was significantly increase (p≤0.001=g) and T₃ had significant changes (p≤0.01=f). Eltroxin treatment demonstrated the highly significant changes in T₄ level as compared to the control group and no significant changes as compared to PTU group. Actually, *W. somnifera* and Eltroxin, both groups demonstrated the no significant changes in T₃ level as compare to whemmas significant changes (p≤0.01=f) were observed when compared to PTU treated rats. In both groups, T₄ exhibited significance (p≤0.01=f) (Figs. 1 and 2).

Morphological studies

Body weight and thyroid weight
No significant changes were observed in body weight in hypothyroid and all treatment groups. No significant changes were observed in relative body weight of liver, pancreas, and heart, although slight reduction in kidney weight was observed in the PTU treatment group as compared to control.

Relative body weight of pancreas, heart, kidney, and liver of Groups III and IV exhibited slight differences, or no changes as compared to control and PTU treated groups. However, the statistically significant difference was observed in the thyroid weight between Group II (PTU treated Group) and Group I (control group). Whereas, slightly significance or significant changes in relative body weight of thyroid was observed in the next two groups (Groups III and IV) as compared to control and PTU treated groups (Table 1 and Fig. 3).

RFT
PTU causes some toxicity in kidney which can be observed in RFT parameters where significant, slightly significant, and highly significant changes were evaluated in urea, creatinine, and uric acid values as compared to control group. Whereas *W. somnifera* leaf extract treatment (Group III) and Eltroxin (Group IV) treatment groups had slightly or no significantly changes in these parameters as compared to control (Table 2).

LFT
LFT was also affected by PTU in which bilirubin total and bilirubin direct were slightly increased whereas SGOT, SGPT, and bilirubin direct were significantly increased as compared to control group. Whereas *W. somnifera* leaf extract and Eltroxin treatment groups also exhibited some changes as compared to the control group (Table 3).

Other parameters
PTU causes some significant changes in blood, sugar, cholesterol, alkaline phosphate, albumin, and globulin parameters when compared to control group. There is significantly higher blood sugar and...
DISCUSSION

The thyroid gland is a small but most important gland in the mammalian body; it plays a pivotal role in overall body function and homeostasis. Thyroid gland secret 93% of the thyroxine (T₄) which is a metabolically inactive hormone and 7% tri-iodothyronine (T₃) although later on almost all thyroxin is converted in tri-iodothyronine [16]. Thyroid hormone exerts a broad range of effects on growth, metabolism, and development. The medical manifestations of thyroid hormone excess or deficiency are remarkable examples of the innumerable actions of the hormone. The primary secretions of the thyroid are thyroxine (T₄) which are relatively inactive and are converted to the active hormone, the hormone. The primary secretions of the thyroid are thyroxine (T₄) and tri-iodothyronine (T₃) which are relatively inactive and are converted to the active hormone, tri-iodothyronine (T₃), by the enzyme tyroxine 5′-deiodinase [17]. In the present study, PTU was used to induce hypothyroidism in rats. PTU blocks the oxidative iodination in the thyroid gland so that thyroid hormone-like thyroxine (T₄) and tri-iodothyronine (T₃) level decrease in serum [18], in the present investigation, we have reported similar findings. Synthetic drug Eltroxin which is commonly used at

Table 1: Comparative study of body weight and relative body weight of various organs in intact control, PTU treatment, W. somnifera leaf extract treatment and Eltroxin treatment groups

| Group name                        | Body weight | Relative Body Weight |       |       |       |       |
|-----------------------------------|-------------|----------------------|----------------|----------------|----------------|----------------|
|                                   |             |                      | Initial       | Final          | Thyroid         | Pancreas        | Heart           | Kidney          | Liver           | g/100 body weight |
| Group I - Intact control          | 123.33±13.33| 115.11±12.88         | 19.93±1.87    | 316.02±25.4    | 620.25±60       | 990±90.16       | 4.31±0.41       |
| Group II - PTU treated            | 196.66±13.03| 176.66±13.38         | 73.87±6.42    | 106.36±10.95   | 555±45.7e       | 890±86.5e       | 4.53±0.34e      |
| Group III - W. somnifera leaf     | 216.66±16.66| 146.66±13.66         | 12.31±1.21b,e| 258.54±25.43   | 426.43±3.954a   | 829.58±52.63f   | 3.606±0.366e    |
| Group IV - Eltroxin               | 125±13      | 116±12.88            | 16.32±1.65a,e| 362.43±21.23   | 570.13±1.82b,h  | 966.65±94.42e   | 3.87±0.34a      |

Values expressed as mean±SEM (n=5). Groups II-IV compared with Group I, where ‘p≤0.05’, ‘p≤0.01’, ‘p≤0.001’, and ‘non significant. Groups III and IV are compared with Group II, where ‘p≤0.05’, ‘p≤0.01’, ‘p≤0.001’, and ‘non significant. SEM: Standard error of the mean. PTU: 6-propyl-2-thiouracil, W. somnifera: Withania somnifera

Table 2: Comparative analysis of various parameters of RFT in all groups

| Group name                        | Urea (mg/dL) | Creatinine (mg/dL) | Uric acid (mg/dL) |
|-----------------------------------|--------------|--------------------|-------------------|
| Group I (control)                 | 36.33±3.63   | 0.96±0.08          | 4.16±0.48         |
| Group II (PTU treatment)          | 47.36±3.27   | 1.06±0.11          | 15.36±1.38        |
| Group III (W. somnifera leaf extracts treatment) | 35.66±3.61a,b | 0.8±0.08h          | 4.63±0.24c        |
| Group IV (Eltroxin treatment)     | 36.56±3.56c   | 0.92±0.06h         | 3.35±0.46k        |

Values expressed as mean±SEM (n=5). Groups II-IV compared with Group I, where ‘p≤0.05’, ‘p≤0.01’, ‘p≤0.001’, and ‘non significant. Groups III and IV are compared with Group II, where ‘p≤0.05’, ‘p≤0.01’, ‘p≤0.001’, and ‘non significant. SEM: Standard error of the mean. W. somnifera: Withania somnifera, RFT: Renal function test

Table 3: Comparative study of various parameters of LFT in all groups

| Group name                        | Bilirubin total (mg/dL) | Bilirubin direct (mg/dL) | Bilirubin indirect (U/L) | SGOT (U/L) | SGPT (U/L) |
|-----------------------------------|-------------------------|--------------------------|--------------------------|------------|------------|
| Group I (control)                 | 0.49±0.037              | 0.13±0.012               | 0.32±0.02                | 37.66±3.26 | 35.1±3.58  |
| Group II (PTU treatment)          | 0.70±0.05b              | 0.20±0.018               | 0.27±0.02c               | 148.3±10.15c| 312.3±29.18c|
| Group III (W. somnifera leaf extract treatment) | 0.86±0.077c       | 0.51±0.020c              | 0.29±0.021c              | 35.1±3.58 | 312.3±29.18c|
| Group IV (Eltroxin treatment)     | 0.59±0.049d             | 0.12±0.011d,e           | 0.37±0.024d,e           | 134.55±11.88e| 78.5±5.66e |

Values expressed as mean±SEM (n=5). Groups II-IV compared with Group I, where ‘p≤0.05’, ‘p≤0.01’, ‘p≤0.001’ and ‘non significant. Groups III and IV are compared with Group II, where ‘p≤0.05’, ‘p≤0.01’, ‘p≤0.001’ and ‘non significant. SEM: Standard error of the mean. SGOT: Serum glutamic pyruvic transaminase, LFT: Liver function test, W. somnifera: Withania somnifera

Table 4: Comparative study of other parameters in blood serum of the rats in all groups

| Group name                        | Blood sugar (mg/dL) | Cholesterol (mg/dL) | Alkaline phosphate (U/L) | Protein (g/dL) | Albumin (mg/dL) | Globulin (mg/dL) |
|-----------------------------------|---------------------|--------------------|--------------------------|---------------|----------------|-----------------|
| Group I (control)                 | 79.5±5.71           | 75.7±6.7           | 250.7±25.28              | 6.7±0.46      | 2.8±0.2        | 3.9±0.34        |
| Group II (PTU treated)            | 121.26±11.28c       | 151.3±14.6c        | 121.75±13.7c             | 5.28±0.43d    | 2.19±0.21a     | 2.7±1.05c       |
| Group III - W. somnifera leaf     | 84.03±7.96e         | 75.76±4.06e        | 182.91±18.06e             | 5.38±0.441a   | 3.8±0.36c      | 3.56±0.244e     |
| Group IV - Eltroxin               | 91.12±8.24h         | 72.86±4.42e        | 349.6±12.41b              | 7.36±0.56A    | 3.08±0.366                 | 4.2±0.34A        |

Values expressed as mean±SEM (n=5). Group II-IV compared with Group I, where ‘p≤0.05’, ‘p≤0.01’, ‘p≤0.001’ and ‘non significant. Groups III and IV are compared with Group II, where ‘p≤0.05’, ‘p≤0.01’, ‘p≤0.001’ and ‘non significant. SEM: Standard error of the mean. W. somnifera: Withania somnifera
the different dosage to control hypothyroidism in human being shows a significant increase of T₄ and T₃ level. In the present investigation, Eltroxin was used as a standard drug which causes significant improvement in T₄ and T₃ level in hypothyroid rats. Similar findings have been reported by plant-like W. somnifera and Bauhinia purpurea in female mice [19]. This is mainly due to changing the inactive hormone to active hormone [20,21]. The green medicine (W. somnifera’s leaf extract) can also increase the level of T₄ and T₃ in the experimental group without any side effects. This is mainly due to beneficial effects of plant extract on the thyroid gland. The improvement of T₄ and T₃ suggests that plant extract possesses T₄ and T₃ like compound, which mimics like Eltroxin [22,23]. Some plants show thyroid stimulating actions [24] and suggested that green medicines positively effect on serum T₄ and T₃. Besides this, they increase the hepatic glucose-phosphate activity and hepatic-lipid-peroxidase activity [25]. In the present investigation, increase of glucose was reported in blood and abnormal function of kidney and liver by PTU treatment. This is mainly due to the adverse effect of PTU on the vital organs. In the present investigation when the plant extract was given to PTU induced hypothyroid rats, all these parameters come to the normal range which shows a beneficial effect of W. somnifera leaf extract in controlling hypothyroid without showing any toxicity on liver and kidney. These findings reported by a different scientist on plant product which shows low toxicity of green medicine [26]. The current study indicates that the plant extracts are having no toxicity which is reflecting in hematology. All parameters of hematology in treatment groups were within the normal range. The current study recommends that W. somnifera extract can cure hypothyroidism without causing any adversity on the vital organ as well as on hematology.

Table 5: Comparison of various hematological parameters in all groups

| Group name                  | Group I (control) | Group II (PTU treatment) | Group III (W. somnifera leaf extract treatment) | Group IV (Eltroxin treatment) |
|-----------------------------|-------------------|--------------------------|-----------------------------------------------|-------------------------------|
| Hemoglobin (g%)             | 12.6±0.63         | 13.5±1.26                | 11.46±0.5                                     | 13.13±0.7                    |
| TRBC (ml/mm³)               | 7.08±0.51         | 4.25±0.33                | 4.26±0.32                                     | 6.18±0.52                    |
| HCT (%)                     | 37.06±5.32        | 23.6±6.23                | 38.46±1.89                                    | 23.6±6.2                     |
| MCH (pg)                    | 52.53±5.29        | 54.5±6.98                | 88.63±6.6                                     | 54.56±4.63                   |
| MCHC (g/dL)                 | 17.7±1.18         | 16.6±1.05                | 23.93±0.8                                     | 17.7±1.18                    |
| RDW (%)                     | 33.8±3.04         | 36.46±3.58               | 26.6±1.96                                     | 33.6±1.02                    |
| TLC (th/mm³)                | 16.16±1.16        | 16.16±1.16               | 13.66±0.63                                    | 14.8±0.78                    |
| Neutrophil                  | 10.66±1.76        | 16.6±1.84                | 38.66±3.45                                    | 8.63                          |
| Lymphocyte                  | 76.1±2.43         | 65.4±7.22                | 66.5±5.5                                      | 8±0.5                         |
| Monocyte                    | 6.9±0.39          | 10.66±1.66               | 4.5±0.34                                      | 11.3±1.17                    |
| Eosinophil                  | 4.6±0.305         | 3.3±0.38                 | 8±0.5                                         | 5±0.5                         |
| PLT                          | 4.35±0.32         | 2.31±0.28                | 3.1±0.32                                      | 3.5±0.30                     |
| PCT                          | lac/cumm          | lac/cumm                 | lac/cumm                                      | lac/cumm                     |
| MPV                          | 6.8±0.65          | 7.73±0.58                | 6.7±0.5                                       | 6.43±0.48                    |
| PDW                          | 9.5±0.5           | 9.76±0.55                | 8.13±0.84                                     | 7.5±0.6                      |

Fig. 3: Thyroid weight of rats in various experimental groups. Values expressed as mean±SEM (n=5). Groups II-IV compared with Group I, where*p≤0.05, **p≤0.01, ***p≤0.001, and non significant. Groups III and IV are compared with Group II, where *p≤0.05, **p≤0.01, ***p≤0.001, and non significant. SEM - Standard error of the mean

CONCLUSION

The present finding reveals that W. somnifera leaf extract can control the hypothyroidism. It normalize the T₄ and T₃ level in blood serum. Moreover, it does not show any toxicity in any of the LFT and RFT parameters and hematology of the rat’s blood.

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AUTHOR CONTRIBUTION

The complete research work was suggested and designed by Ashok Purohit. Authors drafted and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interests.

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