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Evaluation of Anti Thyroid Activity of *Asaparagus Racemosus* Root Extract against Thyroxine Induced Hyperthyroidism in Rats

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Abstract

Objective: The present study was formulated in order to evaluate the Antithyroid potential of *Asaparagus racemosus* root in wester rats.

Methods: *Asaparagus racemosus* (Family: *Asparagaceae*), is used in the mythical system for anti inflamatory, diabetes and urinary disorders and anti oxident. The genus of *asaparagus* exhibited the presence of tyrosine which is responsible for the formation of T3 and T4 hormones. Hyperthyroidism was induced in experimental rats by administering thyroxine (600µ/kg/ml) orally for 14 days. Hyper thyroid male wester rats weighing 150-300gm were treated with oral doses of 100mg/kg of *asaparagus racemosus* aqueous extract for a period of 21 days. Propyl thiouracil for 21 days. Served as the standard.

Results: In this study, morphological assessment demonstrated that thyroxine treated gathering demonstrates increased levels of Triiodo-Lthyronine and L-thyroxine. Simultaneous administration of aqueous extracts of *Asaparagus racemosus* root lowered the increased levels. The decrease in the levels of T3 and T4 by the extracts was compared with the reference drug propyl thio uracil

Keywords: Thyroxine T3, T4; TSH hormone levels; Hyperthyroidism, *Asaparagus Racemosus* Aqueous extract

Introduction

Herbal medicine has as of late pulled in much consideration as option prescription valuable for treatment and anticipation of way of life related disorder [1]. Thyroid hormonal disorders are associated with the imbalance of T3 and T4 hormones secreted by the thyroid gland directly into the blood, the severity of thyroid hormonal imbalance leads to some of the common diseases like diabetes and hypertension and disturb the BMR (basal metabolic rate) of the body. Approximately half the cases of thyroid disease involve hyperthyroidism and the other half involves hypothyroidism. Even though day-by-day herbal drugs are gaining much importance for their affordable and safe nature, scientific investigations towards the mitigation of thyroid disorders by the plant extracts are meager. In almost all these reports, only one thyroid hormone (T3 or T4) was altered by the plant extract. Therefore, in our endeavor to find out a plant extract that can regulate the levels of both the thyroid hormones. *Asparagus racemosus* Willd. (root) (family-*Asparagaceae*) also known by the name "Shatavari" means "who possesses a hundred husbands or acceptable to many". It is considered both a general tonic and a female reproductive tonic [2] *Asparagus racemosus (AR)* may be translated as "100 spouses", implying its ability to increase fertility and vitality. In Ayurveda, this amazing herb is known as the "Queen of herbs", because it promotes love and devotion. is the main Ayurvedic rejuvenative tonic for the female, (Figures 1&2) as is Withania for the male. Throughout India, Tropical and subtropical parts including Andamans and ascending in the Himalayas up to an altitude of 1500m [3].

Materials and Method

Experimental Animals

Wistar albino male rats (150-300g) were maintained for 7 days in the animal house of Chalapathi Institute of Pharmaceutical Sciences, Guntur under standard temperature conditions (24° ± 1°C) with 60% relative humidity and illuminated 12/24 h [4]. The animals were allowed to acclimatize to laboratory conditions 48 h before the start of the experiment. 6 rats / group were used in all sets of experiments. The animals were allowed free access to drinking water and rat feed [5] (Figures 3&4) All the protocols

were approved by Institutional Animal Ethical Committee (IAEC) and conducted according to Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) registered no: at Department of Pharmacology, Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur [6].

Plant Material

The roots of *Asaparagus racemosus* was authenticated by Dr. P. Satyanarayana Raju (Plant taxonomist) of Department of Botany and Microbiology at Acharya Nagarjuna University, Guntur. *Asparagus racemosus* roots were collected from chalapathi institute of pharmaceutical sciences, lam, Guntur.

Preparation of Extracts

Asaparagus racemosus roots were shade dried at room temperature for 15 days, roots were powdered in a mechanical grinder [7]. These were subjected to extraction by Soxhlet extraction method using aqueous as a solvent. Excess solvent was removed by solvent evaporation to obtain the dry weight of the plant extracts. At the time of oral administration, the concentration of extract selected were 100mg/kg and 200mg/kg body weights [8].

Preliminary Phytochemical Screening

Aqueous extracts of *Asparagus racemosus* were subjected to different chemical tests for identification of phytochemical constituents. Phytochemical tests were carried out by standard methods such as proteins, amino acids, tannins, phenolics, saponins, flavonoids, triterpenoids, steroids, fixed oil [9] (Figures 4&5).

Chemicals

Thyroxine (T4), Propylthiouracil (PTU) was procured from Sigma Chemical Co, USA.

Acute Toxicity Study

The healthy male wester rats starved for 3-4 h were subjected to acute toxicity studies were performed in accordance with the OECD (Organization for Economic Co-operation and Development) guidelines no. 425 (Up and Down Procedure). No death was observed till the end of the study. The test samples were found safe up to the dose of 2000mg/kg and from the results 500mg/kg was chosen as the maximum dose for further experimentation [10].

Experimental Design

Five groups of animals, five in each received the following treatment schedule

Anti-Thyroid Activity

a. Group-I: Normal diet (control)

b. Group-II: Hyperthyroid induced animals (thyroxine-600µg/kg/ml) for 14 days.

c. Group-III: Hyperthyroid induced animals treated with standard drug (PTU propyl thio uracil 10mg/kg) for 21 days (Figures 5&6).

d. Group-IV: Hyperthyroid induced animals treated with *A. racemosus* root extract (100mg/kg) for 21 days.

e. Group-V: Hyperthyroid induced animals treated with *A. racemosus* root extract (200mg/kg) for 21 days.

Twenty-five wester male adult rats which have assigned into five groups each having five animals were selected for the study. Serum from the experimental rats were analyzed for thyroid hormone level (ELISA METHOD) and lipid profile (KIT METHOD) before and during the experiment. Blood was collected by retro-orbital puncture under light diethyl ether anesthesia. Serum was separated by centrifugation at 2000rpm for 15 min in normal centrifuge and used for the analysis. Hyperthyroidism was induced in experimental rats by administrating Thyroxine (600µg/kg/ml) orally for fourteen days and induction of hyperthyroidism was confirmed by analyzing the serum thyroid hormone level and with liver complication of hyperthyroidism [6].

Evaluation parameter

Body and Organ Weights

Body weights of each rat were measured from each day of starting experiment to end of experiment. The rats are to sacrifice with appropriated intervals using automatic electronic balance. At sacrifice, the weight of liver, left thyroid gland, was measured at g levels (absolute weights), and to reduce the differences from individual body weights, the relative weight (% of g or mg/g body weight) was calculated as [(absolute organ weight (g or mg)/body weight at sacrifice (g)) ×100]).(20). [5]

Measurement of Food and Water Intake

All animals were observed daily for clinical signs for 5 weeks from the first injection day. The body weight and food consumption of each rat were measured at the initiation of treatment and once a week during the treatment period. The amounts of food and water intake were averaged every week during the treatment period [6].

Estimation of Total Cholesterol, HDL, LDL and VLDL

Lipid irregularities may ascribe to the disabled thyroid capacity. Before the accessibility of serum thyroid hormone estimations, serum cholesterol level was utilized to help in the judgment of the thyroid issue. Total cholesterol, HDL, LDL, VLDL and triglycerides were discovered. Following twenty-one days of treatment with standard medication and *asaparagus racemosus* root concentrate of two dose separate fixations (100mg/kg, 200mg/kg) total cholesterol, HDL and LDL levels expanded while VLDL and triglycerides diminished essentially and arrived at normal extent [1].

Estimation of Hormone Levels in Blood Serum

Blood samples for hormonal determination were collected from the sacrificed animal by cardiac punch, then the collected samples and centrifuged at 2000 rpm for 15 min. following this

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the serum was separated and placed in micro centrifuge tubes and stored in ice cold condition until analysis. Then the serum concentration of triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) were determined [11].

Statistical Analysis

All results were expressed as mean ± standard error of mean (S.E.M.). Data was analyzed using one-way ANOVA followed by Dunnett's-test. $P < 0.05$ was considered as statistically significant.

Results

Table 1: Phytochemical constituents, += Presence; =Absence.

Phytochemicals	Aqueous extract
saponins	+
Carbohydrates	+
Flavonoids	+
phenolic compound	+
Steroids	-
Protein	-
fixed oil and fatty acid	-
Triterpenoids	-
Tannins	+
Alkaloids	+
Glycosides	+
Phytosterols	-

The phytochemical screening of root extracts of *asaparagus racemosus* revealed the presence of Alkaloids, Glycosides, flavonoids, saponins, tannins and carbohydrates (Table 1). As the aqueous extract possessed the most phytochemical constituents, it was selected for evaluation of anti-thyroid activity

Effects on the Body Weights

Significant ($P<0.01$) decreases of body weights and food and water intake were detected. However, these decreases of body weights were significantly ($P<0.01$ or $P<0.05$) inhibited by treatment of PTU 10mg/kg, AR extracts 100mg/kg and 200mg/kg from 21 days (Table 2).

Table 2: Group-I (control), Group II (thyroxin induced), Group III (induced + PTU), Group IV (induced +AR extract 100mg/kg), Group V (induced+ AR extract 200mg/kg).

Animal groups	Days	Body weight (gm/kg)	Food intake (gm)	Water intake (ml)
Group I	35	210±4.62	13.74±3.53	10.5±3.6
Group II	14 induced	150±2.79 to 125±3.63	11.1±2.76 to 9.3±1.06	8.6±2.2 to 7.9±2.4
	21 induced	125±3.63 to 104±4.29	9.3±1.06 to 6.2±2.10	7.9±2.4 to 6.5±1.5
Group III	14 induced	170±7.25 to 130±5.45	12.65±2.04 to 8.95±1.95	9.5±4.7 to 7.5±2.78
	21 treatment	130±4.26 to 160±3.06	8.95±1.90 to 11.48±0.78	7.5±2.78 to 9.2±1.5
Group IV	14 induced	260±7.25 to 225±3.35	21.51±4.33 to 15.78±2.41	15.5±2.3 to 11.4±1.72
	21 treatment	225±3.3 to 243±2.18	15.78±2.41 to 17.97 ±3.19	11.4±1.72 to 12.65±1.09
Group V	14 induced	270±8.32 to 230±2.45	21.89±3.26 to 14.06±2.78	16.78±1.8 to 11.8±2.44
	21 treatment	230±2.45 to 258±1.11	14.06±2.78 to 18.03±1.14	11.8±2.44 to 13.6±1.25

Effect on Lipid Profile (Total Cholesterol, HDL, LDL, VLDL And Triglycerides)

Lipid abnormalities may attribute to the impaired thyroid function. Prior to the availability of serum thyroid hormone measurements serum cholesterol level was used to assist in the diagnosis of thyroid disorder. Table 3 shows the lipid profile in different experiment groups of rats. total cholesterol, HDL, LDL, VLDL and triglycerides were found to be 124, 40.12, 82.90, 9.21 and 31.86mg/dl respectively in normal rats. The total cholesterol, HDL, and LDL got decreased to 80, 20.16, and 62.68mg/dl whereas VLDL and triglycerides was increased to 14.34, and 43.31mg/dl in hyperthyroid rats. After twenty-one days of treatment with standard drug and *Asaparagus Racemosus* root extract of two different concentration (100, 200mg/kg) total cholesterol, HDL, and LDL levels increased while VLDL and triglycerides decreased significantly at 5%level and reached normal rang.

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Table 3: Effect on lipid profile.

Animal groups	Total cholesterol	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Triglycerides
group I	124±4.30	40.12±0.26	82.90±1.03	9.21±0.11	31.86±1.06
group II	80±1.41	20.16±1.31	62.68±0.12	14.34±0.43	43.31±0.95
group III	120±1.5	38.64±0.26	79.28±0.641	10.72±0.62	34.12±0.9
group IV	97±3.68	26.75±0.42	52.27±0.45	12.83±0.92	40.8±0.6
group V	110±2.27	32.73±0.34	69.72±0.63	10.01±0.54	36.51±1.7

Effect on T3, T4 And Thyroid Stimulating Hormone (TSH) Levels

Table 4: Effect on T3, T4 and TSH levels.

Animal groups	T3(ng/ml)	T4(ng/ml)	TSH(µg/ml)
Group I	1.02±o.26	22.62±0.75	1.82±0.97
Group II	2.63±0.71	73.15±0.31	0.71±0.39
Group III	0.91±0.32	31.07±0.92	1.43±0.53
Group IV	0.69±0.14	45.74±0.15	0.91±0.45
Group V	0.89±0.63	35.26±0.54	1.26±0.81

T3, T4 and TSH level of the control animals were found to be 1.02ng/ml, 22.62ng/ml, and 1.82µg/ml respectively. The T3 and T4 values increased to 2.63ng/ml and 73.15ng/ml, while TSH level decreased to 0.71µg/ml significantly in hyperthyroid induced rats. T3 and T4 were decreased to 0.91 ng/ml and 31.07ng/ml, whereas TSH was increased to 1.43µg/ml for those rats treated with standard drug. Rats treated with *Asaparagus Racemosus* root extract in two different concentration (100mg/kg, 200mg/kg) showed decrease in T3 and T4 levels and increase in TSH levels (Table 4) group treated with 200mg/kg of *Asaparagus Racemosus* root extract showed better result than the 100mg/kg concentration and was found to be effective as the standard drug (PTU).

Effects On the Organ Weights

Table 5: Effect on organ weight.

Animal groups	Thyroid gland weight(mg/g)	Liver weight (g/kg)
Group I	3.41±0.26	3.98±0.12
Group II	2.65±0.18	2.91±0.61
Group III	3.34±0.75	3.72±0.14
Group IV	2.85±0.49	3.13±0.56
Group V	3.21±0.73	3.61±0.27

The weight of the thyroid gland was significantly lower in the experimental group than in the control group. Relative weights of thyroid gland and liver of control rats significantly (P<0.01) decreased as compared with thyroxine induced rats. However, these decreases of organ weights were significantly (P<0.01) increased by treatment of PTU and all two different dosages of *AR* extracts as compared with thyroxine induced, respectively (Table 5).

Histopathology

Histopathological studies were embraced to study the tissue section of the thyroid gland and liver of different experimental groups of rats.

The decreases in sinusoidal space due to hepatocyte hyperplasia in liver in control rats. In histomorpho metrical analysis, significant (P<0.01) decreases of the mean thicknesses of cross thyroid glands and follicular lining epithelium, were detected in hyperthyroid induce with significant (P<0.01) increases of hepatocyte numbers as compared with control rats. These hyperthyroid treatments related histopathological changes of thyroid gland, liver was dramatically inhibited by treatment of all two different dosages of *AR* extracts or PTU 10mg/kg.

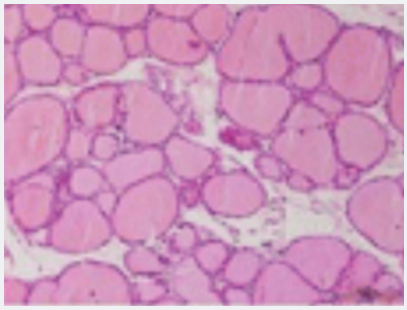

Figure 1: (Group 1) Thyroid gland tissue Group I (control).

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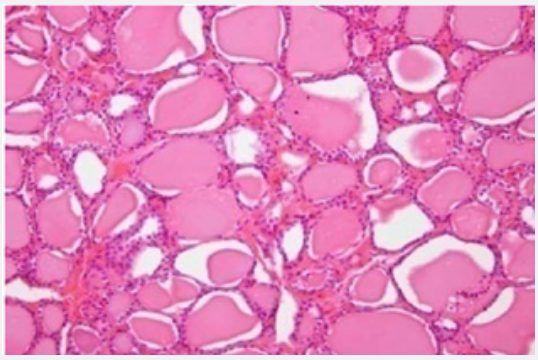

Figure 2: (Group 2) Thyroxine induced.

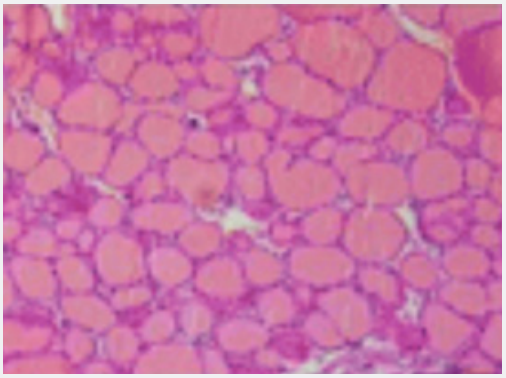

Figure 3: (Group 3) Induced +PTU.

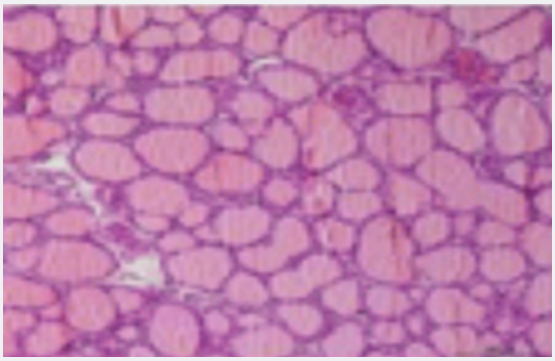

Figure 4: (Group 4) Induced +AR extract100mg/kg.

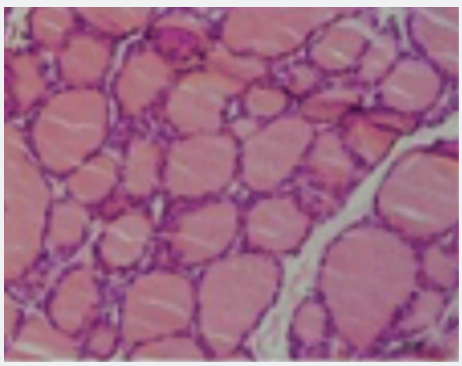

Figure 5: (Group 5) Induced +AR extract 200mg/kg.

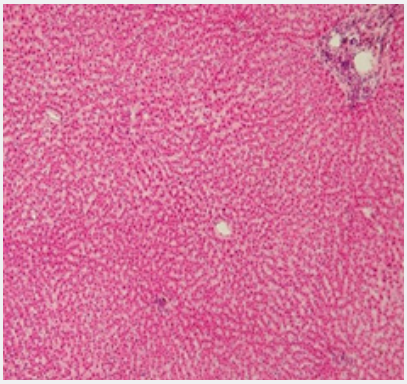

Figure 6: (Group I) liver tissue Group I (control.

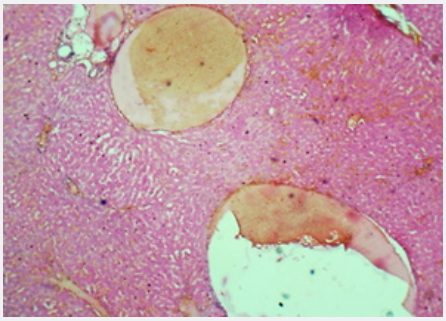

Figure 7: (Group II) Thyroxine induced.

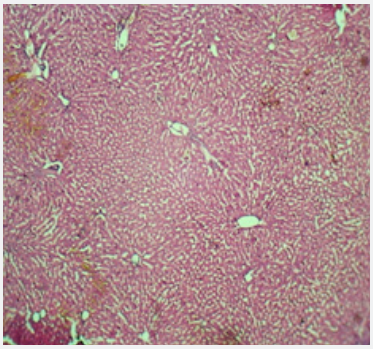

Figure 8: (Group III) Induced+PTU.

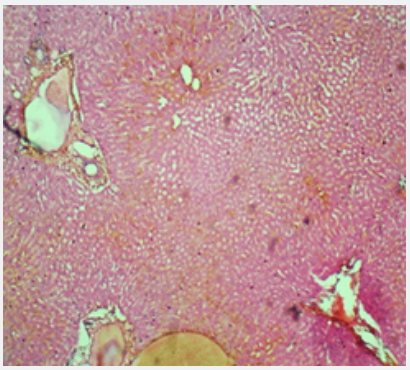

Figure 9: (Group IV) Induced+ AR extract 100mg/kg.

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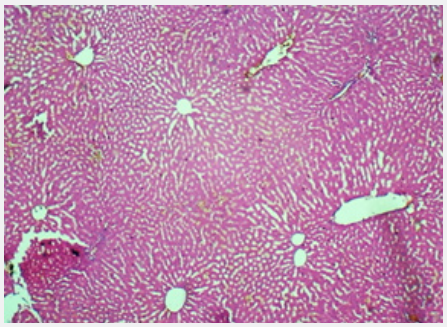

Figure 10: (Group V) Induced + AR extract 200mg/kg.

Conclusion

The present findings inferred that the gathering treated with the most noteworthy convergence of plant concentrate indicated great come about as that of the standard medication and was underpinned concentration of *Asparagus Racemosus* root can possibly overcome hyperthyroidism in albino rats. The treatment of aqueous concentrate of root of *Asparagus Racemosus* have indicated noteworthy changes in thyroid hormone level and lipid profile level in diverse exploratory gatherings of rats. The measurement of *Asparagus Racemosus* extract 100 mg/kg is found to be intense and strong towards the opposition to thyroid action, when contrasted and control. Our preliminary results are encouraging, but further molecular studies are needed to clarify the exact mechanism behind the anti-thyroid activity of *Asparagus Racemosus* root.

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