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"ANTI-THYROID ACTIVITY OF BERRY POWDER OF *Hippophae rhamnoides* AGAINST THYROXINE INDUCED HYPOTHYROIDISM"

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ABSTRACT:

The present research work is to evaluate the Anti-thyroid potential of *Hippophae rhamnoides* [sea buckthorn] in Albino Wister rats. **Methods:** *Hippophae rhamnoides* were used to treat infections, slows down the aging process, improves sight and also used to treat BP (Blood Pressure) heart and liver diseases, common cold, gout, high cholesterol, ulcers and to reduce weight. The Anti-Thyroid activity of sea buckthorn was studied by inducing hyperthyroidism in albino Wister rats by administering Thyroxine orally for 14 days. After 14 days, collect the blood samples from the eye and check the levels of T_3, $T_4$and TSH. From 15th day, administer the sea buckthorn berry powder and Methimazole(standard)for 6 days (21st day) **Results:** In present study, it was found that thyroxin increases the levels of T_3, T_4 and TSH i.e., Hyperthyroidism. By administrating Sea buckthorn berries powder, lowers the increased levels of T_3, T_4 and TSH when compared to standard drug (Methimazole).

Keywords: Thyroid, Sea Buckthorn, Elisa test

Introduction:

Medicinal plants: Medicinal plants have been used for medicinal practices since prehistoric times. Medicinal plants synthesis hundreds of chemical compounds which are used for their functions and also for human use. The Phyto chemical contents in the medicinal plants are having pharmacological actions, many plants having medicinal potential remain unassessed by rigorous scientific researches to define efficacy and safety.[1] Medicinal plants are widely used in non-industrialized societies, which are cheaper than modern medicines. The annual global export values are thousand types of Plants with suspected medicinal properties was estimated to be US$2.2 billion in 2012(2). In 2017, the Potential global market for botanical extract and medicines was estimated at several hundred billion Dollars. (1) In many countries, there is little regulation for traditional medicine, but The WHO coordinates a network to encourage safe and rational usage. Medicinal plants face both general threats, such as climatic change and habitat destruction the specific threat of over-collection to Meet market demand. (1)

Thyroid:

The Endocrine system is the collection of glands that produces Hormones which regulates
Metabolism, Growth and Development. The thyroid gland is one of the largest endocrine glands. The thyroid gland produces two related hormones, Thyroxine(T_4) and Triiodothyronine (T3). Acting through nuclear receptors, these hormones play a critical role in cell differentiation during development and helps to maintain thermogenic and metabolic homeostasis in the adult. The thyroid gland is a Butterfly-shaped organ and is composed of two cone-like lobes or wings, lobes, Dexter (Right lobe) and lobes sinister (left lobe). The two lobes are connected by an isthmus located inferior of the cricoid cartilage. The organ is situated on the anterior side of the neck, y against and around the larynx and trachea, reaching posterior to the oesophagus and carotid sheath. It starts cranially at the oblique line on the thyroid cartilage and extend inferiorly to approximately the 5thor 6th tracheal ring. It is highly vascular gland that weighs about 12g to 20g and is surrounded by a fibrous capsule.
The lobes are roughly cone shaped, and consists of 5cm length and 3cm width.
4 **parathyroid glands**: Producing parathyroid hormone are located in posterior region of each lobe of thyroid [3'⁴] thyroid gland function is known to be involved in the regulation of basal metabolism and thermogenesis, playing an important role in glucose metabolism, food intake and fat oxidation [5,6] According to a projection from various studies on thyroid disease, it has been estimated that about 42 million people in India suffer from thyroid diseases.[7] The results of the National Health and Nutrition Examination Survey NHANES) showed higher levels of thyroid stimulating hormone (TSH) .Concentration and more prevalent positivity of anti-thyroid antibodies in women which increased with age.[8] The prevalence and incidence of thyroid disorders depend on geographic areas, increasing age, ethnicity, and most importantly the amount of iodine intake of the population.[9].. The types of diseases which are associated with the abnormal secretion of thyroid
Hormones are;

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•**Hyperthyroidism**: a condition where there is an increase in production of thyroid hormones due to excessive thyroid function.**[10]** Hyperthyroidism is also known as thyrotoxicosis. It is characterized by hyper metabolism and elevated serum levels of free thyroid hormones. There is enlargement of the thyroid gland and exophthalmos (bulging eyes). The prevalence of hyperthyroidism in community-based studies has been estimated at 2 percent for women and 0.2 percent for men. As many as 15 percent of cases of hyperthyroidism occur in patients older than 60 years.[11] Symptoms are related to the thyroid hormone's stimulation of catabolic enzymopathy activity and catabolism, and enhancement of sensitivity to catecholamines. Older patients often present with a paucity of classic signs and symptoms, which can make the diagnosis more difficult.[12] Thyroid storm is a rare presentation of hyperthyroidism that may occur after a stressful illness in a patient with untreated or undertreated hyperthyroidism and is characterized by delirium, severe tachycardia, fever, vomiting, diarrhoea, and dehydration.[13] Treatment depends on the cause and severity of the disease, the patient's age, goitre size, comorbid conditions and treatment preferences. Treatment includes pharmacotherapy, radioactive iodine and surgical treatment [11]

Hypothyroidism: a condition where there is a decrease in production of thyroid hormones due to impaired thyroid function.**[14]** Hypothyroidism is one of the most common forms of thyroid dysfunction.[15]. It is defined as failure of the thyroid gland to produce sufficient thyroid hormone to meet the metabolic demands of the body [8]. It may be congenital or acquired, primary or secondary, chronic or transient [16]. It refers to a state that results in a deficiency of thyroid hormones, including hypothalamic or pituitary disease and generalized tissue resistant to thyroid hormone, and disorders that affects the thyroid gland directly [8] The signs and symptoms of hypothyroidism are nonspecific and may be confused with those of other clinical conditions, especially in postpartum women and the elderly [17] Infants and children may present more often with lethargy and failure to thrive. Women who have hypothyroidism may present with menstrual irregularities and infertility. In older patients, cognitive decline may be the sole manifestation.[12] Patients with severe hypothyroidism generally present with a group of signs and symptoms that may include lethargy, weight gain, hair loss, dry skin, forgetfulness, constipation and depression. [17]

Hormones	**Normal Range**	**Interpretation**
Thyroxin[T_4]	64-142mmol/L	>142mmol/L indicates hyperthyroidism and <64 mmol/L indicates hypothyroidism.
Tri iodo thyronine[T_3]	1.46-2.92mmol/L	>2.92mmol/L indicates hyperthyroidism and <1.46 indicates Hypothyroidism
Thyroid stimulating hormone[TSH]	0.4-4.8mIU/L	<0.4 mIU/L indicates hyperthyroidism and >0.4 mIU/L indicates hypothyroidism

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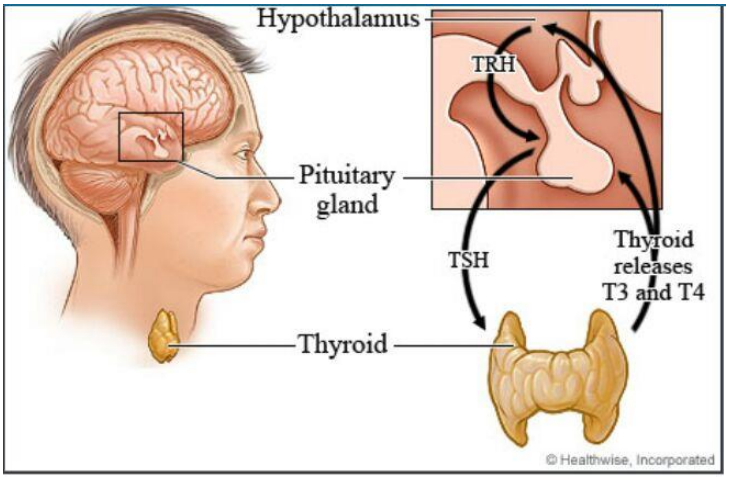
PRODUCTION OF THYROID HORMONE:

The thyroid gland uses iodine from food to make two thyroid hormones: triiodothyronine (T3) and thyroxin(T4). It also stores these two thyroid hormones and releases when they are needed. The hypothalamus and the pituitary gland which are located in the brain, helps to control the thyroid gland. The hypothalamus releases thyrotropin-releasing hormone (TRH), which stimulates pituitary gland to release thyroid-stimulating hormone (TSH). The hypothalamus and pituitary are working normally when,

- Thyroid hormone levels are low, so they secrete more TRH and TSH, which stimulates thyroid to make more hormones.
- Thyroid hormone levels are too high, so they secrete less TRH and TSH, which reduces hormone production by the thyroid [18]

Sea buckthorn:

Sea-buckthorn is a thorny, dioeciously shrub in the oleaster family (elgeagnaceae) growing up to 7mts High [19,22,23]. It is also known as sand thorn, sallow thorn or sea berry [24]. This plant grows in Europe, Asia, Siberia, China, Tibet



[22,25,26]. H.goniocarpa, H. gyantsenais, H. litangensis, H. neurocarpa, H.rhamnoides, H.salicifolia, H.tibetana are the most common species in the hippophae family. Sea-buckthorn can withstand drought and frost [19]. Late April and early May are the flowering seasons for sea-buckthorn. Its leaves are long, lance late and covered with silvery hairs. Small green and brown Flowers are produced in large numbers. After the flowering period, the flowers turn into tasty and Nutritious berries which are yellow or orange in colour, ripen in September. Smooth, small stone Which has a long groove and covers an oily seed can be found inside the berry [19,23-28]. Fruits are bitter and sour in taste and have an odour as that of pineapple [19,20,21]. Berries are rich source of vit-C, vit-E, malic acid and citric acid[19,29,30]. It also contains vit-A i.e. alpha and beta carotene (60mg) and a mixture of other carotinoids (180mg). Moreover, it contains tocopherols i.e. vit-E (110mg to 160mg), folic acid (0.79mg) and vit-B complex group i.e. B1 0.035mg, B2 0.056mg [20,21,29-33]. The nutritious ingredients of sea-buckthorn berry are tested for their application in medicine i.e. in treatment of inflammation, cancer and treatment after chemotherapy [34-41]. Bark and leaves of sea-buckthorn are used to treat diarrhoea and dermatological condition whereas berry oil applied topically softens the skin [42]. In India, China and Tibet, the sea-buckthorn fruits were added to the medicine as their ingredients have a beneficial effect on functions of alimentary, respiratory and

Circulatory systems.

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MATERIALS AND METHODS:

Collection of the see buck throne powder:

Sea buckthorn berries powder was purchased from Mystique Hills , Tamilnadu ,India in the month of March.

Animals:

The animals on which this study ,conducted was Wister albino rats of either sex weighing 200-300 g .The animals were acclimatized for one week in ani

mal house in metal cages at room temperature ,relative humidity of 40-70 % is maintained in the animal house and should be perfectly ventilated as well as air conditioned. All the animals were fed with commercial standard diet for rats (ad.libitium pellets, hyd) and water .The entire procedure of the experiment was conducted according to the ethical norms of animal ethical committee approved by(1629/PO/a/12/CPCSEA).

Chemicals

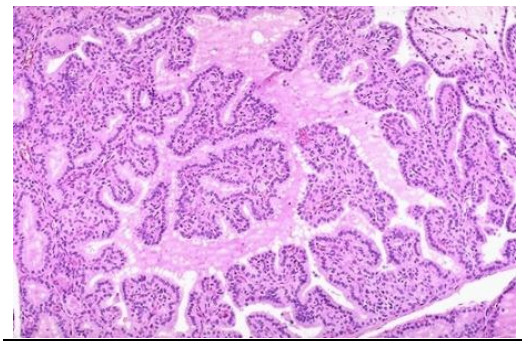
All the chemicals were Analytical grade. Thyroxine was purchased from Manipal Super speciality Hospital, Tadepalli .Methimazole was purchased from Mangalagiri local market of Himalaya Herbal Healthcare company.

Histopathology:

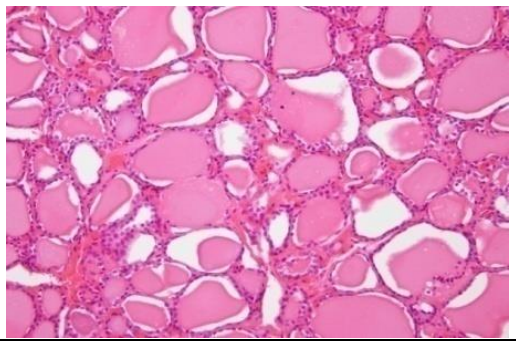
Histopathological studies were embraced to study the tissue section of the thyroid gland of different experimental groups of rats.

Thyroid gland of normal rats (Group-I) showed numerous follicles some of which contain colloid (fig. 1). A colloid varies from thick to thin with occasional scalloping. The follicular cells have round nuclei surrounded by a clear cytoplasm. Thyroid gland of rats induced with Thyroxine showed follicles lined by follicular epithelial cells. Thyroid follicle shows 65-75% of luminal colloids. In about 15% of follicles, the lumen is completely filled with colloid. There is no papillary infolding of the epithelium (Group-II). Thyroid section of Methimazole treated rats showed round nuclei surrounded by a clear cytoplasm (Group-V). Section of thyroid gland of hyperthyroid rats treated with *sea buckthorn* berries extracts (Group-IV, III, and VI) showed follicle lined by follicular epithelial cells which appeared normal. Many of follicular colloids showed scalloping. There is no papillary infolding of the epithelial cells.

Histopathology of thyroid glands



A



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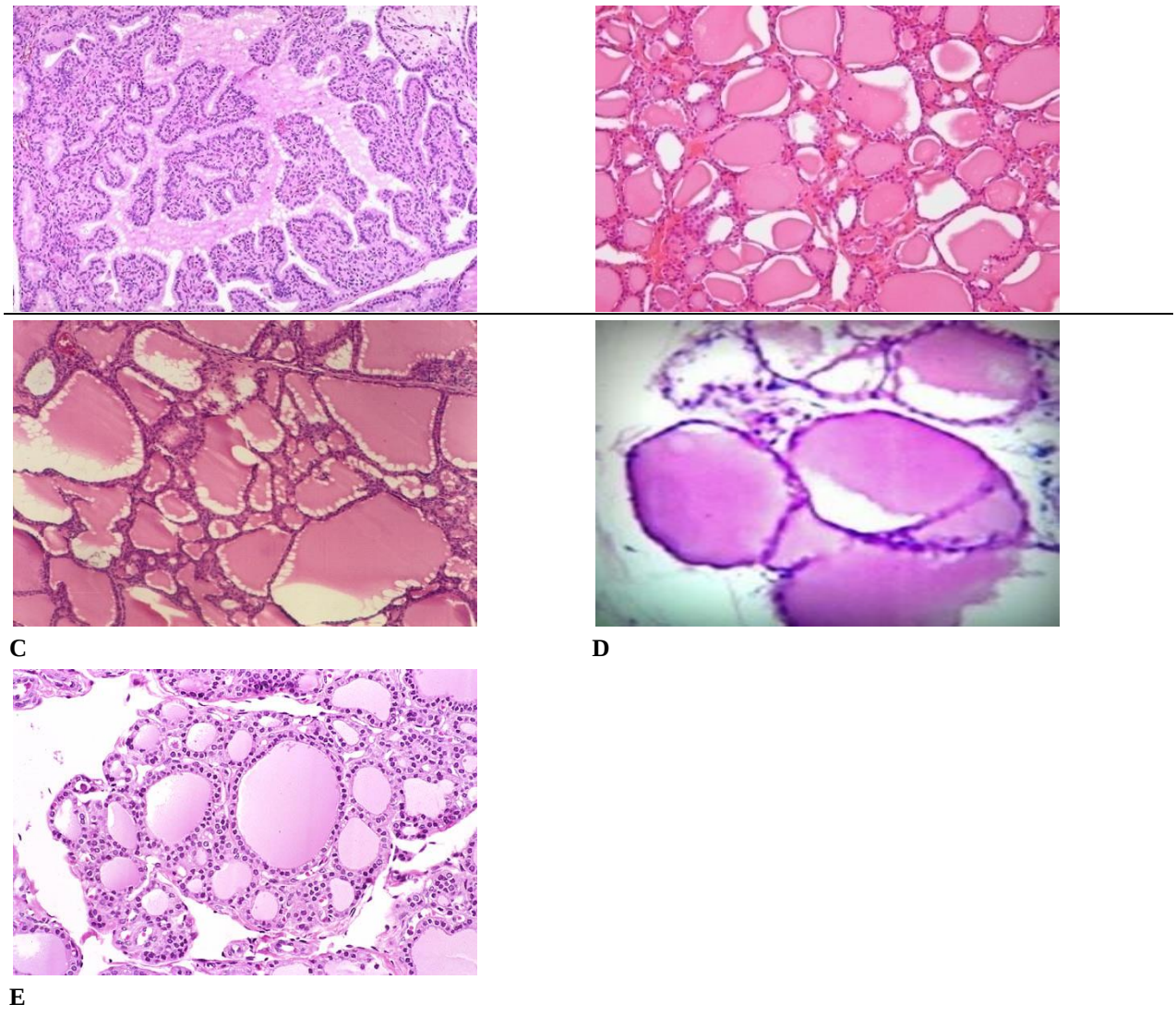

Fig. 1: Histopathology of thyroid glands in different groups a) Normal Control rat b) Hyperthyroid induced rat c) Hyperthyroid induced rat treated with sea buckthorn extract(low dose).d) Hyperthyroid induced rat treated with sea buckthorn extract(high dose). e) Hyperthyroid induced rat treated with standard drug methimazole f) hyperthyroid induced rat treated with sea buckthorn extract.

Experimental design:

Firstly the albino Wister rats of either sex were divided into 6 groups with 3 animals in each group .The grouping of rats is as shown in the following table-

S.No	GROUP s	Treatment	Route of administration
1	I	Control	oral
2	II	Thyroxin	oral
3	III	Low Dose	oral
4	IV	High dose	oral
5	V	Methimazole	oral
6	VI	Sea buckthorn berry powder.	oral

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Group I: Normal diet (control)Group II: Hyperthyroid induced animals (Thyroxine) for 14 days. Group III: Hyperthyroid induced animals treated with sea buckthorn berry powder (low dose). Group IV: Hyperthyroid induced animals treated with sea buckthorn berry powder (high dose). Group V: Hyperthyroid induced animals treated with standard drug (Methimazol).Group VI: Hyperthyroid induced animals treated with sea buckthorn berry powder. Thirty-six adult rats which have assigned into six groups each having six animals were selected for the study. Blood was collected by retro-orbital puncture under light diethyl ether aesthesia. 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 orally for fourteen days and induction of hyperthyroidism was confirmed by analysing the serum thyroid hormone level.

Methods for determination of thyroid hormones:

Five ml of venous blood sample were collected from all the Rats and controls. Serum was stored at –20°C for thyroid hormones assay [43]

ELISA test for the quantitative determination of TSH:

Serum levels of TSH were measured by a classical sandwich ELISA technique, using the ELISA Kit according to the manufacturer's instructions (Stat fax-2600 micro plate washer USA) and (ELISA) Awareness micro plate readers USA) capable of measuring absorbance at 450 nm was used to measure the colour intensity developed in each well. All assays were done in duplicate. The detection of assay range was 0.3 - 12 micro-IU/ml, and 0.15 micro-IU/ml Sensitivity. Serum TSH concentrations were measured using a commercial kit by the Human® chemical company. Wiesbaden-Germany Max-plank-Ring-21 - D-65205, used a highly specific monoclonal Anti-TSH antibody coated on the surface of the micro titer wells. In each well; specimen or calibrators(50µl) and enzyme conjugate (100µl) (peroxidise–labelled anti-TSH, PH 6.25±0.1) were mixed (incubated 60 minutes at 20C°, washed 5 times) to form the sandwich complex which bound to the surface of the wells by the interaction with the immobilized antibody. After washing with the wash solution (pH 7.2±0.2, 10 mmol/l Tris-Buffer, 8 g/l Nacl) a 100µl substrate reagent (1.2 mmol/l TMB, <6.0 mmol/l Hydrogen peroxide) a 100µlwas added and incubated 15 minutes at 20C° and
finally, a 100µl of the stop solution (0.5 mol/l Sulphuric acid) a 100µl was added, turned the colour into yellow. The intensity of colour was proportional to the TSH concentration in the sample.

ELISA test for the quantitative determination of total Thyroxine (T4):

Serum T4 concentrations were measured using a competitive enzyme immunoassay (commercial kits by the Human® chemical company. Max-plank-Ring-21-D-65205 Wiesbaden- Germany), based on the principle of competitive binding between T4 in a test specimen and T4-peroxidase conjugate for limited number of binding site on the anti-T4 coated well. Thus the amount of T4-peroxidase
conjugate bound to the well is inversely proportional to the concentration of T4 in the specimen. In each well; 50µl specimen or calibrators (human, for thyroxin at concentration of 0, 2, 5, 10, 15, and 25 ug/dl) and 100µl working conjugate solution (T4-HRP-Conjugate, pH7.5±0.1 (diluted 1+10 with Phosphate buffer pH 7.42±0.1)) were mixed and incubated 60 minutes at20C°, washed 5 times). After washing with the working wash solution (diluted to1000 ml with fresh, deionised water) unbound. enzyme conjugate was removed. A 100µl working substrate solution (mixed equal volumes of (TMB 4 mmol/l, sodium acetate buffer 0.05mol/l (pH3.5±0.1)) and (Ureahydrogen peroxide10 mmol/l, sodium acetate buffer 0.05mol/l (pH 4.5±0.1)) was addand incubated for 15 minutes at 20C°, finally 50µl stop solution (0.5 mol/l Sulphuric acid) was added. The absorbance was measured at 4nmwithin 10 minutes using ELISA micro plate readers
.

ELISA test for the quantitative determination of Total Triiodothyronine (T3):

Serum T4 concentrations were measured using a competitive enzyme immunoassay (commercial kitsby the Human® chemical company. Max-plank-Ring-21- D-65205 Wiesbaden- Germany), it depends on the principle of competitive binding between T3 (test specimen) and T3-peroxidase conjugate limited number of binding site which are present on anti-T3 coated well. Thus the amount of T3-
peroxidase conjugate bound to the well is inversely proportional to the concentration of T3 in the specimen. In each well; 50µl specimen or calibrators are made into concentration of
0,2,5,10,15,25,µg/dl of human thyroxin and 100µl working conjugate solution(T3-HRP-Conjugate, pH 7.5±0.1 (diluted 1+10 with Phosphate buffer pH 7.42±0.1)) were mixed and incubated 60 minutes at 20°C, washed 5 times. After washing with the working wash solution (diluted to 1000 ml with fresh, deionised water) unbound enzyme conjugate was removed. A 100µl working substrate solution. (mixed equal volumes of (TMB 4 mmol/l, sodium acetate buffer 0.05mol/l (pH 3.5±0.1)) and was added and incubated for 15 minutes at 20°C, finally 50µl stop solution (0.5 mol/l

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Sulphuric acid) was added. The absorbance was measured at 450 nm within 10 minutes by using ELISA micro plate readers.

RESULT:

Effect on T_3, T_4 and thyroid stimulating hormone (TSH) level:

Groups	Treatment	T_3	T_4	TSH
Group I (Positive control)	2% gum acacia	1.53±0.064	8.33±0.261	5.87±0.059
Group II (Negative control)	Thyroxine (1mg/kg b.w)	5.49±0.095	16.5±0.145	0.65±0.047
Group III (Test low dose)	Berry powder of sea-buckthorn (200mg/kg b.w)	3.29±0.075	14.5±0.025	2.58±0.0325
Group IV (Test high dose)	Berry powder of sea-buckthorn (400mg/kg b.w)	2.54±0.042	12.35±0.193	4.67±0.065
Group V(standard)	Methimazole (1mg/ml kg b.w)	1.23±0.073	9.23±0.026	2.99±0.060
Group VI (Only plant powder)	Berry powder of sea-buckthorn (4mg/kg b.w)	1.90±0.096	8.34±0.077	3.84±0.0175

All the values are expressed as Mean±SEM(N=3); compared to normal group ($p<0.001$), significance values are $p<0.001$, $p<0.01$, $p<0.05$ (negative control group by one way ANOVA followed by dunnett multiple comparison test)

Preliminary phyto chemical screening:

The berry powder of sea buckthorn confirmed the presence of chemical constituents like carotenoids, tocopherols, sterols ,flavanoids ,lipids, ascorbic acid and tannins.

Discussion:

In the present study, the medicinal plant *Hippophae rhamnoides* was selected. Hyperthyroidism was induced by thyroxine.T3,T4, TSH levels were evaluated for determining anti- thyroid activity of the plant. Histopathological examination was conducted in order to determine the effectiveness of methimazole and sea buck thorn berry powder.

Thyroid fortifies the rate of digestion system and high temperature generation has been known over a century. Hyperthyroidism impelled rats demonstrated a decline in TSH level and increment in T3, T4 levels which may be incharge of diminishing in body weight. Hyperthyroid rats have expanded oxygen utilization then the typical rats bringing about expanded T3 and T4 levels and diminished TSH level.

From the study, it was discovered the hyper thyroid actuated rats when treated with the standard medication methimazole and sea buckthorn berry powder i.e,200mg/kg ,400mg/kg for 21 days indicated typical thyroid hormone level. The gathering treated with the most astounding amassing of sea buckthorn berry powder demonstrated great come about as that the standard medication. This shows that the sea buckthorn berry powder can possibly cure hyperthyroidism in test rats.

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Histopathology of thyroid organ of hyper thyroid instigated and treated rats were consider and contrast with control rats. The thyroid organ of control rats has lesser measure of colloid in follicular epithelial cells however demonstrated an increment in hyperthyroid instigated rats which got to be typical in the wake of getting treatment with sea buckthorn berry powder.

Conclusion:

The Anti- thyroid activity is evident from the decreased T_3 and T_4 levels. It can be said that *Sea buckthorn berries* significantly by virtue of the presence of thyroxin, which may be useful for further molecular studies to determine the exact mechanism for its Anti-thyroid activity.

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