Can *Nigella Sativa* Oil (NSO) Reverse Hypothyroid Status Induced by PTU in Rat? Biochemical and Histological Studies

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Abstract: ***Background****: Nigella sativa* Linn. or Black cumin was tested anti-oxidant effect including its anti thyroid effect. **Material**: Adult male Wister rats (200g) were divided into: control and experimental. Hypothyroidism was induced by propylthiouracil. Oil of *Nigella Sativa* was administrated to animal models of hypothyroidism in daily doses of 400 mg/ kg / BW via gastric intubation for 4 weeks. Body weight gain, food intake, % food conversion efficiency, water intake, blood thyroid hormones were determined. Histological study of the thyroid gland was carried out .Data were expressed as mean ± SEM and were analyzed by one-way analysis of variance (ANOVA) and *t*-tests. **Results:** Improvement in body weight, food and water intake in treated hypothyroid rats were observed. *Nigella* sativa increased serum triiodothyronine thyroxin and decreased TSH. No change in sodium, potassium, calcium, chloride, magnesium, for all treated hypothyroid rats. Histological examination of the treated hypothyroid rats showed improvement in the follicular cell height and colloid content. **Conclusion***: Nigella sativa* oil has antioxidant effect that could ameliorate PTU induced oxidative stress and damage of thyroid follicles so could be considered to have a significant therapeutic role in hypothyroid disease. Studying the effect of *Nigella sativ*a components on cells of thyroid could be tested in the future to identify which of them is involved in treatment.
[Amel Ahmed Khalawi, Ali Ahmed Al-Robai, Sameer Mohamed Khoja and Soad shaker Ali. **Can *Nigella Sativa* Oil (NSO) Reverse Hypothyroid Status Induced by PTU in Rat? Biochemical and Histological Studies]** Life Science Journal 2013;10(2):802-811]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 113

Key word: hypothyroidism - Nigella sativa oil – thyroid function tests- histology.

1. Introduction

Thyroid hormone level are controlled by feedback regulation to the pituitary and hypothalamus (**Ito, 2010)**, which bears primary responsibility for integrating thyroid function with body needs. When the blood level of thyroid hormone falls the hypothalamus senses the change and secretes thyroid–releasing hormone (TRH). This hormone stimulates the anterior pituitary to secret thyroid stimulating hormone (TSH) **(Sukkar *et al*., 2000).** TSH in turn causes the thyroid gland to increase secretion of thyroid hormone which raises concentration of the hormone in the blood stream **(Cecie *et al*., 2007).**

Thyroid diseases are more common in females than in males. These diseases are either due to thyroid gland overactivity resulting in hyperthyroidism or underactivity of resulting in hypothyroidism **(Fabrizio, 2003 and James *et al*., 2007).** Hypothyroidism is clinically linked with decreased metabolic rate which result in adverse effect on many organs and system activities **(Golden *et al*., 2009).**

Recently there is an increased demand for using plants in therapy "back to nature" instead of using synthetic drugs which may have adverse effects that may be more dangerous than the disease itself. *Nigella sativa is* found in Southeast Asia and in Mediterranean countries, and South of Algeria **(Winkler *et al*., 2005).** There is a belief in Islamic Arabic countries that black seed is a universal healer that treats all illnesses but is not capable to protect people against aging or death. Its oil includes over 100 components: protein, carbohydrates, vegetable oil and fats, Omega 3 and Omega 6, essential oil, and other trace elements **(Ali *et al*., 2012).**

Nigella sativa Linn: Nigella sativa Linn (*N. S.*) which is also called the Black seeds were obtained from the local herb store in Jeddah, Saudi Arabia (AL Gassim). The seed oil of NS was found to be rich in polyphenols and tocopherols **(Meziti *etal.,* 2012*).*** The seeds contain 36–38% fixed oils, 0.4–2.5% essential (volatile) oil, proteins, alkaloids, and saponins (**Ali and Blunden, 2003**). The fixed oil is composed mainly of fatty acids, namely, linoleic, oleic, palmitic and stearic acids (**Parakh, 2010).** Thymoquinone (TQ) which is the most active ingredient (30–48%), together with its derivatives such as dithymoquinone, thymohydroquinone, and thymol **(Muhtasib *et al*., 2006).**

Selenium, DL-α-tocopherol, DL-γ-tocopherol, all-*trans*-retinol, thymoquinone and thymol contents were evaluated by **Al-Saleh *et.al.* (2006**). this plant has been a great focus of research and has several traditional uses and consequently has been

extensively studied for its chemical constituents and biological activities (**Middha *et al*., 2009**). Studies on animals have already been done to determine the effects of *N.S* on different components of the metabolic syndromes (**Winkler *et al*., 2005; Najmi *et al*., 2008**). The present study was designed to investigate if PTU induced hypothyroidism in rat can be reversed by administration of *Nigella sativa* oil. Both biochemical and histological studies were carried out to evaluate such possible effect.

2. Materials and Methods

Animals

Adult male Wister rats (n=30) with average weight 180-200g were obtained from the Animal House Unit in king Fahad Medical Research Center (KFMRC). The rats were placed in plastic cages (42x26.5x15cm), 5 in each cage, and kept at 22±1oC and 60% humidity. They were maintained on commercial food and libitum consisting of standard laboratory rat chow (Grain Silos and Flour mills organizationl laboratory animal, F- 648 net weight 50 kg) and had free access to drinking water. All animals received care according to the method approved under institutional guidelines for care and use of laboratory animals in KFMRC.

Animals were randomly divided into 6 groups 5 animals each as follows:

1. **HT**: Hypothyroid group: This group had received PTU for six weeks by intragastric intubation (6mg/kg/BW).
2. **HT-NSO**: This group had received same dose of PTU for six weeks then treated by oral NSO (400mg/kg/BW) for one month.

3-**HT-NSO-PTU**: Hypothyroid group treated with *N.S* (400mg/kg/BW) but still receiving PTU for one month.

4-**HT-T4**: Hypothyroid group treated with thyroxin in the form of Levothyroxine sodium (10µg/100gm/BW) for similar period.

5- **NSO**: receiving NSO (400mg/kg/BW) for one month.

6- **Control group**: receiving normal slandered rat pellets.

Animals were allowed for free access to water and observed daily for any clinical changes. Body weight, food and water intake were recorded for all animal groups. At the end of the experiment, animals were sacrificed and blood samples were processed for both biochemical and histological studies.

Preparation of *Nigella sativa* oil

The fixed oil from *N. S.* (NSO) crushed seeds was extracted as described by **Houghton *et al.* (1995**). Briefly, the seeds of *N. S.* (500g) were soaked in light petroleum ether overnight at room temperature (22-25 ^{o}C) with continuous shaking at 200 rpm (Queue orbital shaker, USA). The filtrate (organic phase) was collected by passing through filter paper to eliminate fine particles of the crushed seeds. Subsequently, a suitable quantity of dried magnesium sulphate was added to eliminate the water presented in the filtrate. The clear supernatant was then collected again using filter paper. Next, the filtrate was concentrated using a rotating evaporation (Rotavapor BüCHI, Switzerland) system at 40^{o}C until all the petroleum ether was evaporated. The volume of the oil extracted from 500gm *N.S* seeds was 50ml (45gm) and the yield of the extract was 9% (w/w). The oil was kept at 2-8^{o}C until needed.

Preparation of Propylthiouracil (PTU):

PTU was purchased from Sigma Aldrich lnc, USA. It was reported to decrease circulating T3 and T4 and increase in serum TSH resulting in a hypothyroid state in rats (**Maria, *et al*., 2005**). In the present study PTU were prepared by dissolving 6mg/kg/BW in warm water and administrated by intragastric intubation (**Suzki and O'neal, 1967).**

The kits (for T4, T3 and TSH) were purchased from Biosystem cadama USCN, UK. The assay employed a competitive inhibition enzyme immunoassay technique.

Electrolytes concentrations were determined by Dimension Rxlmax clinical chemistry analyzers, USA: high complexity was used.

For biochemical studies rats were anaesthetized with ether in the anesthetic jar. Blood was collected from intraorbital sinus (**Parasuraman *et al*., 2010**) using a capillary tube (5 ml, BD vacutainer, SST/Advance, UK). Serum was prepared by centrifugation of the blood for 15 min at 3000g at 4^{o}C (Hettich, Roto silent A/K, Western Germany) and kept at -80 C till analyzed.

Serum electrolytes and thyroid functions tests (T3, T4 and TSH) were estimated using the previous mentioned kits. For histological study ,the front of the neck were dissected to extract th***e*** thyroid gland The organ was immersed immediately in 10% neutral buffered formalin pH 7.4 (**Bancroft, 2008**). Further re-fixation in fresh neutral buffered formalin were done and after 24 hours, the samples were dehydrated in graded ethanol and embedded in paraffin. Five micron thick longitudinal sections of the thyroid were mounted on glass slides. Then the sections were subsequently stained with hematoxylin and eosin, to study the general structure and pathological changes. Photographs were taken using light microscope provided with digital camera.

Morphometric studies were done using the Soft Imaging System (SIS) Olysia, Bioreport Germiny version 3.2. Three slides from 3 different animals in each group (5-6 fieds/slids) were scanned in each

sample. The following parameters were measured: Area occupied by thyroid lobes (μm^2), number of follicles containing colloids and height of follicular cells (μm).

3. Results

In the present study, hypothyroidism (HT) was successfully induced by administration of PTU via intra-gastric route and confirmed by thyroid enlargement and biochemical analysis of thyroid functions. The effect of NSO in controlling hypothyroidism was compared with traditional treatment using L –thyroxin (T4).

1- Body weight, food and water intake

Table (1) showed the effect of PTU induced hypothyroidism on body weight, food and water intake and the effect of treatment by NSO. It was observed that HT rat group significantly gained weight after 6 weeks (56% increase), compared to the initial weight. However, weight gain was less compared to CTL animals (83% increases). The weight of HT-NSO, HT-T4 and CTL-NSO groups showed increase in the average mean weight by 13 %, 22% and 46 % respectively while the weight of HT-NSO-PTU group showed insignificant increase Food intake in HT group was significantly less than the control *(P<0.01)* The amount of food intake by hypothyroid animal group treated with NSO (HT–NSO) was significantly decrease while it was not significantly decrease in HT- T4 group compared to non treated group (HT) *(P<0.01)* but still higher than the HT-NSO-PTU group. Food conversion efficiency of rats treated with either *N. S.* or T4 was higher than the HT group. The amount of water intake by HT group was significantly less than the control *(P<0.01)* by 15 %**.**The amount of water drinking by the HT-NSO and HT-T4 group were significantly decrease by 20% and 21 % as compared to CTL group *(P< 0.01)* and non significantly decrease compared to HTgroup but still more than HT-NSO-PTU group .

2-Serum electrolyte

Regarding the effect of PTU induced hypothyroidism on blood electrolyte Table (2) demonstrated that the concentration of Ca^{++}, Mg^{++}, K^{+}, Cl^{-}, Na^{+},in all experimental animals were not changed when compared with that of the control.

3- Thyroid functions tests*:*

In PTU (6mg/kg/BW) treated animals, both T4 and T3 levels were significantly decreased in HT rats, indicating the induction of chemically induced hypothyroidism Significant differences *(P<0.001)* were found in TSH level between HT and TH-NS-PTU compared to control. Thyroid function tests of hypothyroid rats become more or less similar to that of control in animals administrated NSO in a daily dose of 400mg/kg/BW for 30 days. HT rats treated with T4 in a dose of 10μg/100gm/BW has significantly higher T4 level *(P<0.0001)* than untreated rats. On the other hand, NSO failed to reverse or protect against HT status with continuous PTU administration (Table 3).

4-Histological study

Gross picture:

Hypothyroidism induced by PTU result in thyroid enlargement (Goiter) (Figures 1 HTand 2B).

Light microscopic features:

Histological examination showed distortion of normal architecture and follicular structure. Hyperplasia and hypertrophy of the lining follicular cells and marked decrease or absence of colloidal content were observed in most follicles compared to control (Figures.3C and D). Glands of rats still receiving PTU with NOS (showed no improvement) (Figures 1HT-NSO-PTU and 4A). After treatment of hypothyroid rats by NSO for 4 weeks thyroid lobes returned more or less to normal size (Figures 1HT – NSO and 2C). The architecture of thyroid follicular arrangement appeared normal. Follicles are lined with high cubical cells. More follicles showed normal colloidal content (Figures 4B and C). NO change in thyroid gland was observed in rats receiving NSO (Figure 1NSO, 2D and 5A and B).

Morphometric studies

Morphometric studies confirm the above results (Table-4). Significant increase in lobe area and follicular cell height and decrease in number of acini containing colloid were observed in hypohyroid group *(P<0.005).* On the other hand, significant decrease in lobe area and cell height and increase in number of follicles containing colloid were observed in thyroid treated with NSO. Thyroid glands of hypothyroid animals treated with NSO but still receiving PTU did not show significant improvement in either cell height or follicular colloid content.

4. Dissusion

In the present study, PTU was used to induce hypothyroidism in rat. Propylthiouracil (PTU) is the U.S. Food and Drug Administration (FDA)-approved thionamide that inhibit thyroid hormone synthesis by interfering with thyroid peroxidase–mediated iodination of tyrosine residues in the thyroid gland at the steps of iodine organification and iodotyrosine coupling and also inhibit the conversion of T4 to T3 in extra thyroidal tissues (**Nayak, and Burman, 2006**).

Table 1: Effect of Nigella sativa oil on general features of Control (CTL), Control treated with Nigella sativa oil (NSO), Hypothyroid (HT), Hypothyroid treated with Nigella sativa oil (HT-NSO), Hypothyroid treated with Nigella sativa oil but still receiving propylthiouracil (HT-NSO-PTU), Hypothyroid treated with thyroxin (HT-T4).

Parameters	Study groups					
	CTL	HT	HT-T4	HT-NSO-PTU	HT-NSO	NSO
Initial body weight (g)	199±7.00	194±4.73	233±20.7	218±18.1	228±29.9	192±8.74
Final body weight (g)	365±41.0*	305±32.8*	285±38.6*	217±30.4	260±40.5*	281±24.6
Percentage change	↑83.42%	↑56.68%	↑22.57%	↓0.09%	↑13.74%	↑46.45%
food intake (g/day)	25.8±7.90	21.9±5.06[b]	20.0±4.04[b]	14.2±5.85[ab]	18.8±4.25[ab]	24.5±4.97[a]
Food conversion efficiency	0.20	0.19	0.38	1.4	0.58	0.30
water intake (ml/day)	32.6±6.90	27.2±6.70[b]	25.5±5.37[b]	22.1±8.29[ab]	25.0±4.83[b]	33.1±12.0[a]

Mean ±SEM from number of samples indicated (n=5). *: significance between initial and final weight using paired student "t" test $P < 0.05$.
a: significance versus HT group using paired student *"t"* test $P < 0.05$. b: significance versus CTL group using paired student *"t"* test $P < 0.01$.

Table 2: A comparison between electrolyte concentration in Control (CTL), Control treated with Nigella sativa oil (NSO), Hypothyroid (HT), Hypothyroid treated with Nigella sativa oil (HT-NSO), Hypothyroid treated with Nigella sativa oil but still receiving propylthiouracil (HT-NSO-PTU), Hypothyroid treated with thyroxin(HT-T4).

Parameters	Study groups					
	CTL	HT	HT-T4	HT-NSO-PTU	HT-NSO	NSO
Na^+ (mmol/L)	142 ±0.69	143±0.05	151±2.03[a]	150±0.88	144±0.37	141±0.80
K^+ (mmol/L)	4.45±0.15	4.18±0.12	4.80±0.36	4.41±0.09	4.67±0.05	4.40±0.09
Cl^- (mmol/L)	100±0.30	101±0.51	109±3.11	106±0.50	101±0.37	99.3±0.37[a]
Ca^{++} (mmol/L)	2.60±0.03	2.60±0.02	2.56±0.19	2.47±0.01[a]	2.61±0.13	2.49±0.07
Mg^{++} (mmol/L)	0.92±0.08	0.98±0.02	0.94±0.11	0.96±0.01	0.98±0.12	0.94±0.01

Mean ±SEM from number of samples indicated (n=5). Sodium: (Na^+), Potasium: (K^+), Calcuim: (Ca^{++}), Cholride: (Cl^-), Mganisium: (Mg^{++})
Comparison between groups using one way ANOVA test $P < 0.05$. a: significance versus CTL group.

Table-3: The effect of *Nigella sativa* on T3, T4 and TSH levels in control (CTL), Control treated with *Nigella sativa* oil (NSO), Hypothyroid (HT), Hypothyroid treated with *Nigella sativa* oil (HT-NSO), Hypothyroid treated with *Nigella sativa* oil but still receiving propylthiouracil (HT-NSO-PTU), Hypothyroid treated with thyroxin(HT-T4).

Parameters	Study groups					
	CTL	HT	HT-T4	HT-NSO-PTU	HT-NSO	CTL-NSO
TSH (mIU/L)	3.57±1.52	19.6±3.08*	1.83±0.26[a#]	12.0±2.22[ab*]	2.51±0.54[a]	3.60±0.79[a]
T3 (pg/ml)	3.79±0.67	0.61±0.19*	2.31±0.17[ab*]	0.71±0.16[b*]	3.25±0.08[a*]	4.04±0.25[a]
T4 (pg/ml)	27.19±1.32	0.32±0.06*	33.3±1.10[a]	0.30±0.07b*	32.3±1.44[ab*]	29.1±1.14[a]

Mean ±SEM from number of samples indicated (n=5). Using paired student "*t*" test *: significance versus CTL group $P < 0.001$.
a: significance versus HT group $P < 0.0001$. b: significance versus CT-NSO group $P < 0.0001$.

Table-4: Effect of Nigella sativa oil on morphometric features of Control (CTL), Control treated with Nigella sativa oil (NSO), Hypothyroid (HT), Hypothyroid treated with Nigella sativa oil (HT-NSO), Hypothyroid treated with Nigella sativa oil but still receiving propylthiouracil (HT-NSO-PTU), Hypothyroid treated with thyroxin(HT-T4).

Parameters	Study group				
	CTL	HT	CTL-NSO	HT-NSO	HT-NSO-PTU
Lobe area (μm^2)	7.81±0.35	18.0±0.75*	9.85±0.39*	14.2±0.43#*	16.0±0.81*
Number of acini containing colloid	18.5±0.76	1.33±0.33*	19.5±1.02	15.0±0.36#*	1.33±0.24*
Height of follicular cells (µm)	3.17±0.22	23.5±2.05*	10.9±0.60*	10.7±0.30#*	30.0±0.29*

Using one way ANOVA test P<0.005 *: significance versus CTL group. #: significance versus HT group

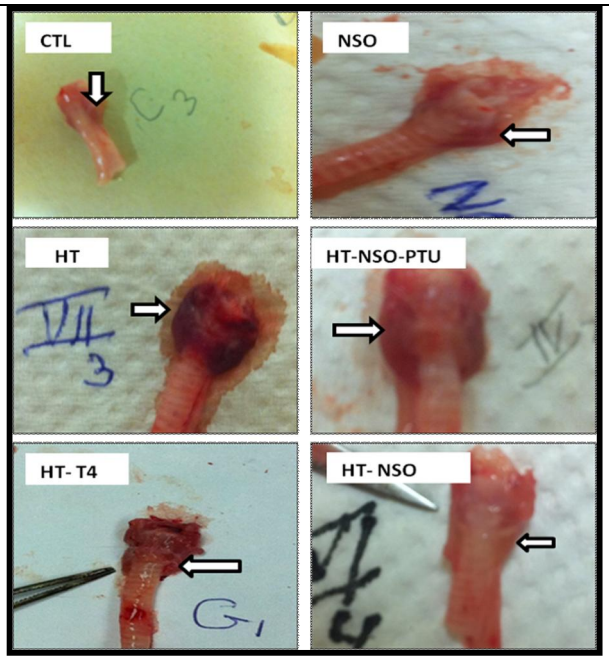

Figure 1:- Gross morphology of rat thyroid gland showing the anti goiterogenic effect of NSO CTL:control with normal size, NSO:N*igella sativa* oil with normal size. HT:hypothyroid enlarged thyroid, HT-NSO-PTU:hypothyroid treated with *Nigella sativa* oil but still receiving PTU still showed enlargement. HT-T4:Hypothroid treated with thyroxin showing normal size and HT-NSO: hypothyroid treated with *Nigella sativa* oil. Notice the return of thyroid gland to its normal size.

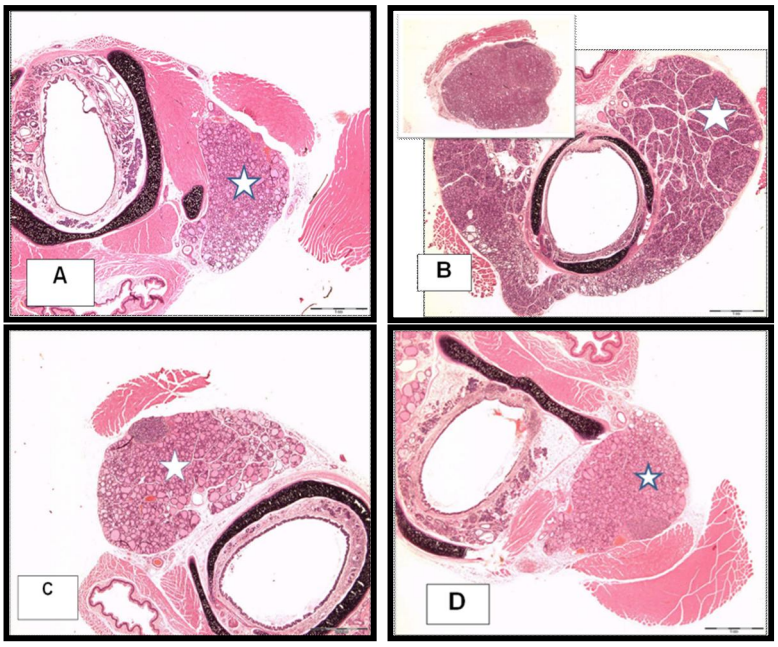

Figure 2:- Low power of thyroid gland of controls (A), Hypothyroid gland (B), Hypothyroid treated with *Nigella sativa* oil but still receiving PTU (insert). Hypothyroid treated with *Nigella sativa* oil (C), *Nigella sativa* only (D), Notice the size of thyroid lobe (star) in each case (Scale bar=1mm).

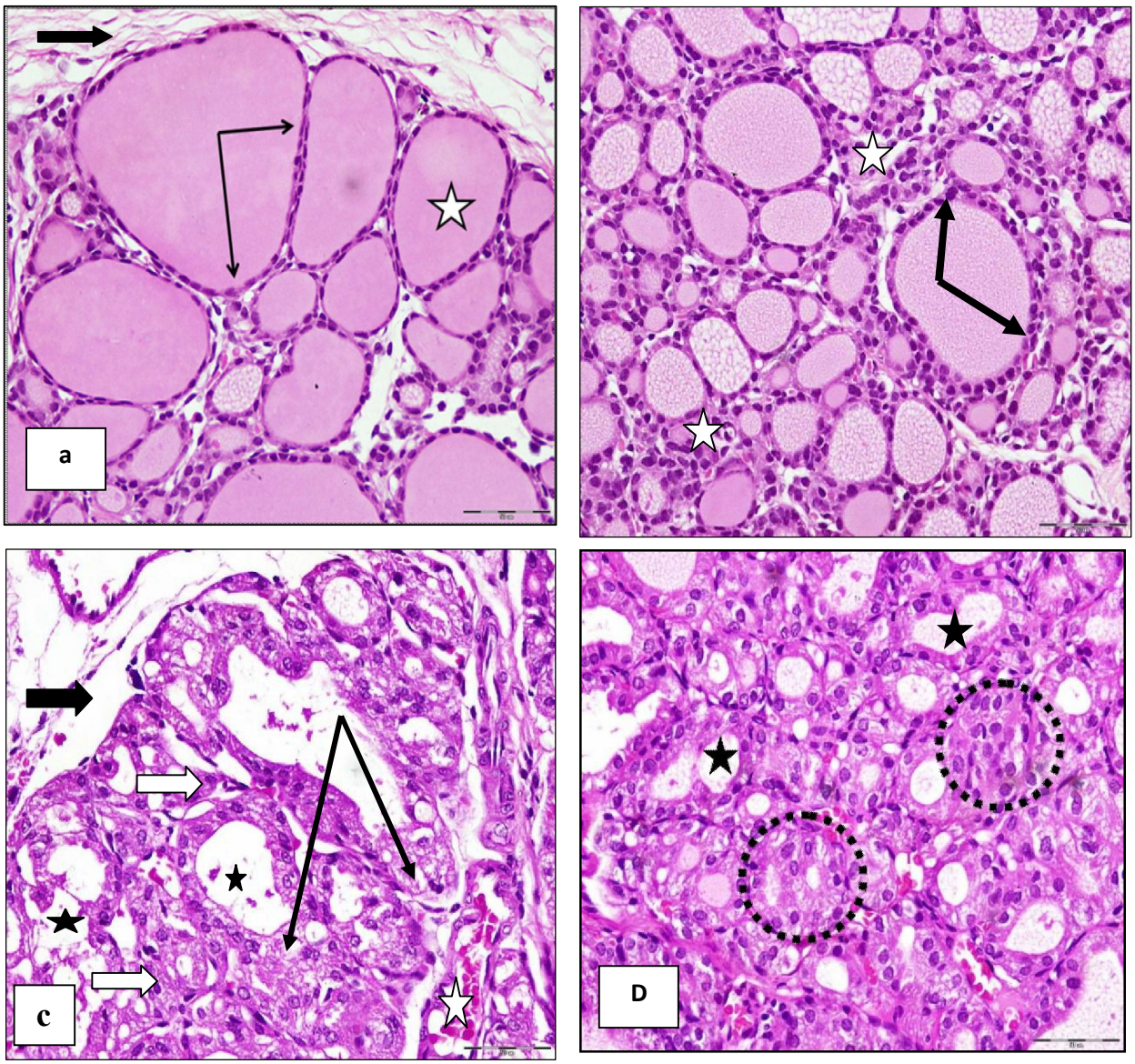

Figure 3:- Showing sections from the peripheral (A) and central (B) regions of control rat thyroid gland Notice the normal architecture of the follicles. Most are filled with homogenous colloidal material (star). The follicular cells are of low cubical shape (thin black arrows). Inter-follicular cells are seen (star). (C|) peripheral and (D) central regions of rat thyroid after 6 weeks of PTU induced hypothyroidism (HT) (6 mg/kg/BW). Notice the marked distortion of normal architecture and follicular arrangement compared to control (insert). The lumina of most follicles are obliterated by hyperplasia and hypertrophy of lining epithelium (dotted circle). Few follicles showed small lumina (black stars) with little colloidal material compared to control (white star insert) (Scale bar=50 μm) (Hx&E).

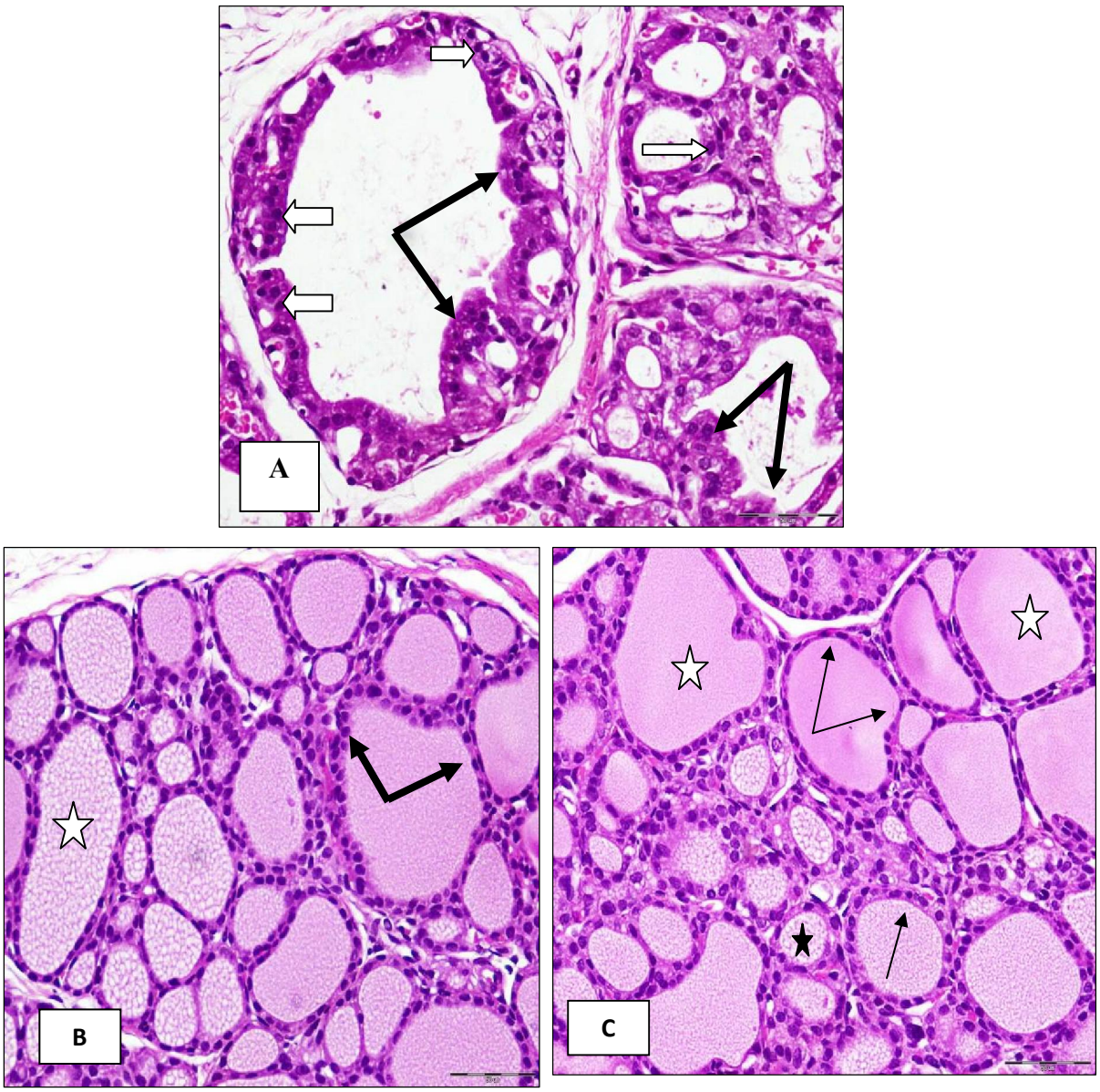

Figure 4 :- Showing A. section of thyroid (central part) of hypothyroid rat treated with Nigella sativa oil together with PTU(HT-NSO-PTU) still having distortion of follicular shape and papillary hypertrophy (black arrows). The lining cells showed degenerative changes such as cell vacuolation and dark nuclei (white arrows) due to apoptotic changes. (Scale bar=50 µm) (H&E). (B) Peripheral and C –central regions of thyroid gland of hypothyroid rat (HT) treated for one month with *Nigella sativa* oil(400mg/kg /4 weeks) showing more or less normal follicles, but smaller in size compared to control .The lining epithelium is cubical (arrows), and the colloid content appeared somewhat reticulated (stars) compared with the control.(Scale bar=50 µm) (H&E).

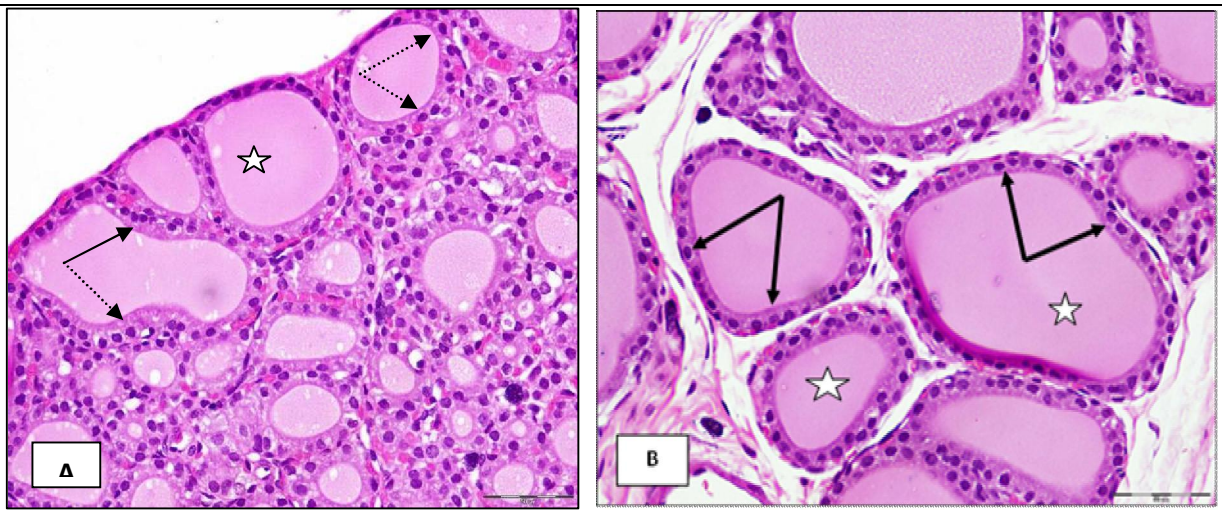

Figure 5. Sections from the periphery (A) and central (B) pats of rat thyroid receiving oral Nigella sativa oil (400mg/kg /4 weeks) are showing follicles with preserved normal shape. The lining epithelium is clearly cuboidal (arrows). Most follicles contain homogenous acidophilic colloid (white star) (Scale bar=50 µm) (H&E)

Hypothyroidism is a metabolic disorders which is usually associated with disturbance in food and water intake which are reflected on body weight of individual (**Cadnapaphornchai *et al.*, 2003**). In the present study the amount of water intake and the body weight of hypothyroid rats were less than the control group. Such findings are in line with the finding of **Badaue-Passos *et al.* (2001).** Furthermore, the body weight gain as reported by the same authors was lower 10% than control rats.. Because the food conversion efficiency in the present study was lower in PTU induced- hypothyroid rats than those treated by either *N. S.* or T4, the percentage of weight gain in untreated rats was higher than the treated rats. The results were due to impairment in energy metabolic processes along with a decrease in the basal metabolic rate (BMR), as also supported by **Wada and King (1986). Jin-Song *et al.* (2006)** proposed that rats fed on thyroxine showed an increase in BMR and had a lower body weight because of mitochondrial respiration, stimulation, and enzyme activities associated with aerobic metabolism. In this study,. Furthermore**, Park *et al.* (2001)** stated that the outcome of the treatment with thyroxine is a decrease in the average means of the body weight in the hypothyroid group when compared with the untreated group. *N. S.* seemed to match the effect of thyroxin to increase BMR and cause a reduction in weight.

Biochemical studies done in the present study revealed that there was no change in the serum concentration of Ca^{++}, Mg^{++}, K^{+}, Cl^{-}, and Na^{+} of hypothyroid rats as compared to the untreated group. This result correlates with what was reported by **Valizadeh (2009).** In the present study both histological and biochemical studies were used to evaluate the role of NSO as therapeutic herbal for PTU induced hypothyroidism.

Histological studies of thyroid gland showed that hypothyroidism was effectively induced by PTU. High doses given for prolonged periods could result in marked inhibition of thyroid hormone synthesis with subsequent increase in TSH and TSH which in turn was known to work on thyroid gland resulting in hyperplasia and hypertrophy of follicular cells, a condition known as nodular goiter **(Stelios *et al.*, 2007 and Zbucki *et al.*, 2007*)*** PTU-induced hypothyroidism was reported by **Bhanja and Chainy (2010)** to induce oxidative stress in rat cerebellum that resulting in tissue damage and apoptosis. **Poncin *et al.* (2010)** reported that oxidative stress is required to induce cell proliferation in thyroid gland resulting in goiter or thyroid enlargement. Biochemical studies, confirmed the hypothyroid status as the level of serum T3 and T4 concentration was significantly decreased while the TSH level significantly increased in the PTU-induced hypothyroid rat compared to control. The results of the present study are in line with those observed by **(Zbucki *et al.*, 2007)** who showed a significant decrease in the plasma concentration of T3 and T4 of hypothyroid rats.Whereas, that of TSH was significantly increased compared to control rats. It also agreed with results of **Haiying *et al.* (2006)** who found that hypothyroids subjects were diagnosed with biochemical parameters of T3 and T4 below the normal ranges, and TSH above the normal range.

In the present study, the administration of thyroxin (in the form of Levothyroxine sodium, 10µg/100gm/BW) to the hypothyroid rats, results in a significant increase in the T4 levels. These results are in agreement with previous studies by **Woeber (2002)** who indicated that the hypothyroid patients receiving T4 replacement therapy have demonstrated T3 levels lower than those of euthyroid subjects, even though the T4 levels are in the high range and TSH is suppressed. *Nigella sativa oil (NSO)* given orally significantly increases the concentration of T4 and T3 and decreases the TSH in hypothyroid rats as compared to untreated rats. It is apparent that recovery of thyroid parenchyma is related to protection offered by NSO against hyperplasic changes well known to be associated with hypothyroid status **(Stelios *et al.*, 2007).** The therapeutic effect of NSO against PTU induced hypothyroidism was most probably related to its antioxidant effect proved by many investigators (**Mahmoud *et al*., 2002, Nayak, and Burman, 2006, AlWafai 2013, Leong *et al.*, 2013**). **Sharif *et al.*, (2012**) reported that *Nigella sativa* ethanolic extract increase T3 serum concentration in diabetic rat. *N. S.* may also repair the thyroid gland and resynthesize the thyroid hormone. **Ismail *et al*., (2003)** concluded that *N.S.* could raise the lowered serum triiodothyronine concentration without changing the concentration of serum TSH.. Thus it could be suggested that the mechanism of action could be in part due to anti-oxidant defense system (**Meral *et al*., 2001**) that may protect the gland against PTU toxicity.

Further studies is needed to test the therapeutic effect if its direct or indirect using in vitro using thyroid cell line in relation to thyroid regulating hormones such as TSH, T4 and T3 to confirm and understand the exact mechanism. Pharmacokinetics of NSO and its pathway and effect on pituitary-thyroid axis could add much to understand suggested interaction of *N.S.* products on thyroid hormone synthesis and function.

Conclusion:

The results obtained in this investigation highly recommend the use of NSO as a combination therapy with antithyroid drugs to achieve a very ideal and effective treatment of such metabolic disorders concerning thyroid gland . Histological studies proved significant therapeutic effect against PTU induced hypothyroidism provided stopping or adjusting drug administration with reverse of hypothyroid changes in follicular cells and colloidal content in hypothyroid rats into normal status. However, it could not be used as a protective agent if PTU was to be continued.

Acknowledgement

I would also like to express my gratitude to King Abdulaziz City for Science and Technology for funding this project 27/15/58960.

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3/12/2013