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Der Pharmacia Lettre, 2018, 10 [3]: 91-102
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ISSN 0975-5071
USA CODEN: DPLEB4

Potential Impact of Talbina on Pituitary-Adrenal-Gonadal Disorders in Hypothyroid Adult Female Rats

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ABSTRACT

Hypothyroidism can impair pituitary-gonadal-adrenal function in adult female rats. Purpose: This study is designed to investigate the possible ameliorating effect of talbina on pituitary-gonadal-adrenal disorders as well as the oxidative stress which induced by hypothyroidism in adult female rats. Methods: Adult female albino rats were divided into 4 groups; Euthyroid intact control group, Euthyroid group orally administered talbina (100 mg.kg^{-1}.day^{-1}), hypothyroid group (received daily 5.0 mg kg^{-1} NeoMercazole®) and hypothyroid +talbina group (orally co- administered 5.0 mg.kg^{-1} plus 100 mg.kg^{-1} talbina). Treatment with talbina started after one month from hypothyroid induction for consecutive 30 days. Results revealed that NeoMercazole® exhibited a state of hypothyroidism, induced significant depletion in serum estradiol; follicle-stimulating hormone levels in contrast a significant elevation in corticosterone and prolactin, elevation in serum extracellular regulate kinase 1/2 (ERK1/2) and evoked an oxidative stress status by elevating serum 8-hydroxy guanosine which initiated apoptosis by elevating serum apoptotic marker Caspase-3, with respect to control group. The treatment with talbina showed a significant ameliorative effect, which reversed the exacerbated effect of hypothyroidism by alleviating the disruption of thyroid, gonadal and adrenal functions and enhanced the protection against oxidative stress and apoptosis. Conclusion: these results evidenced that talbina acted on multi arrays for protecting the physiological functions against hypothyroid adverse impact.

Keywords: Hypothyroidism, Endocrine disorders, Oxidative stress, Talbina.

INTRODUCTION

Homeostasis of thyroid hormone is important for regulation of vital process and any disturbances elicit physiological and behavioral disorders [1,2]. Thyroid disorders associated with disruption in skeletal muscle development, osteoporosis, cardiovascular disease, alteration in brain structure and function as well as dyslipidemia, [3,4]. Hypothyroidism is chronic thyroid

gland dysfunction, more prevalent in women due to their susceptibility to the goitrogenic effect of iodine and iron deficiency [5,6]. It induces adverse impact on reproductive function such as a disturbance in menstrual cycle; reduce fertility, induction hyperprolactinemia, and diminishing estrogen levels [7,8]. The hormone replacement therapy is the principle rout for hypothyroid treatment; this treatment is accompanied with adverse effects due to the difficulty in regulating the level of thyroid hormones through the use of drugs or an exogenous source of thyroid hormone. As a result, patients often experience only partial relief of the symptoms. So, it is useful to have a new treatment which control hypothyroid state and its associated side effect.

A mount of evidences revealed that food intake enriched with whole grain reduces the susceptibility of incidence of many chronic diseases. Barley (*Hordeum vulgare*) is an architecture crop deemed to be sustaining food source available for all disparate social classes of humankind. Arabian folk remedies used talbina (water soluble barley) in treatment of many visceral disorders and sore throat, also the cooked talbina used for alleviating a severity of rheumatoid and arthritis [9-12]. This valuable crop directed the scientists to scrutinize the bioactive component for optimal use as safe and effective medicine. Talbina has found to be enriched with valuable minerals (iron, selenium, potassium, calcium, phosphorous; zinc), phytoestrol (β-sitosterol, campesterol, stigmasterol), polyphenol (Ferulic, p-coumaric, sinapic, vanillic and p-hydroxybenzoic acids, cinnamic acid derivatives, proanthocyanidins, quinines, flavonols, chalcones, flavones, flavanones, and amino phenolic compound), water soluble vitamins (C; B1; B2; folic acid and B12), β-glucan, dietary soluble fiber, wide spectrum of amino acids and enzymes [9,13-18]. The diverse phytonutrient of talbina implicate its protection activity against certain types of cancers, cardiovascular disease, arthritis, diabetic, and hypercholesterolemia. It also increases a cellular energy to sustain the body homeostasis [19,20].

Bawazir [21] investigated the impact of talbina on neurotransmitters, adrenal and gonadal hormones in adult male rats; based on phytoestrol content the author attributed the modulating and improving effect on endocrine in addition neurotransmitter function.

From the previous scientific researches we conducted this study to address the possible ameliorative effect of talbina on the adrenal, gonadal, pituitary hormone dysfunction and oxidative stress status induced in hypothyroid female rats.

MATERIALS AND METHODS

Animals

Adult female Wistar albino rats, weighing approximately 180-200 g in regular estrous cycle, were provided from the animal house of National Organization for Drug Control and Research (NODCAR), Egypt. Animals were housed at 23 ± 2°C and 55 ± 5% humidity with 12 h light/dark cycle, free access to feed on pellet rodent chow and water ad labellum. Animals were acclimatized for one week before conducting the experiment. The protocol of the study was approved by the Institutional Animal Care and Utilization Committee (IACUC) at the National Herpetology and Tropical Medicine Research Institute, Egypt.

Preparation of talbina

Talbina was provided as barley grain powder, emulsion with water (one gm. talbina powder in 10 ml of distilled water) was given daily per OS [21].

Preparation of neo-mercazole

Carbimazole in the form of commercial tablets (Neo-Mercazole) obtained from AFT Pharmaceuticals was used. Each tablet contained 5 mg of the active principle, carbimazole, to prepare a suspension of drug 10 tablets were grinded and suspended in 100 ml distilled water to obtain a dose of 5 mg carbimazole/Kg bwt/10 ml dist. water.

Experimental design

Regulation of estrus cycle

Daily vaginal smears were obtained from each animal and examined for estrous cycle regularity. The vaginal smears were classified as proestrus (predominance of nucleated epithelial cells), estrus (predominance of cornified epithelial cells), metaestrous (diestrus I) (leukocytes, cornified, and nucleated epithelial cells) and diestrus (predominance of leukocytes). Animals showed cycle consists of one day of proestrus, two days of estrus and two days of diestrus (I&II) considered regular cycled. Animals with regular cycle were selected, and the treatment started from proestrus phase [22].

Induction of hypothyroidism

NeoMercazole® was found to be a least toxic anti-thyroid agent within therapeutic dose ranges [23] therefore; it is selected in our study for hypothyroid induction.

A total number of 20 adult female rats allocated equally in 4 cages (5 animal/cage), the animals were orally administered a daily dose of 5.0 $mg.kg^{-1}$ NeoMercazole® for one month. Hypothyroid was manifested by the increased level of serum TSH associated with low level of fT4. A number of 14 hypothyroid animals were selected for proceeding study [24].

Treatment plan

A number of 14 Euthyroid animals were equally allocated into two groups (7/group), first group was intact control group (CO), and second group was orally administered talbina (100 mg/kg bwt) (T). A number of 14 hypothyroid animals were allocated into two groups (7/group): hypothyroid (H) group was orally administered NeoMercazole® (5.0 mg/kg bwt) and hypothyroid plus talbina (HT) group was orally co-administered NeoMercazole® (5.0 mg/kg bwt) with talbina (100 mg/kg bwt).

All treatment was carried out daily for consecutive 30 days (total time of experiment 60 days from hypothyroid induction).

Blood collection

At the end of treatment the animals fasten overnight and each animal was rapidly decapitated and trunk blood was collected into serum preparation tube [25]. The separated serum was collected and divided into aliquots, stored at -20°C for further hormones assay.

Hormonal assay

Thyroidfunction

Thyroid hormones (TSH, fT4 and fT3), were determined in a serum samples using immunoassay technique specific for rat. All determinations were carried out according to the manufacture's instruction (WKEA, WKEA medical supplier co. China).

Adrenal and pituitary functions

Serum corticosteroid hormone was measured using immunoassay technique according to the manufacture's instruction (AccuBind- Monobind Inc, USA). Prolactin (PRL), FSH and LH were measured in serum samples using

immunoassay technique according to the manufacture's instruction (GSCIENCE, Glory Science Co., Ltd. USA), ELISA kits were rat specific.

Gonadal hormone

Serum 17-β-estradiol (E2) was determined using ELISA kit, according to the manufacture's instruction (Immnunospec, Immnunospec Co., USA), serum progesterone (P) was determined using ELISA kit according to the instruction of CUSABIO Co. (Baltimore, USA), and serum testosterone (Tes) was determined using ELISA kit according to the instruction of BioCheck (BioCheck Co., Ltd. USA). All ELISA kit specific for rat species. Ratio of estradiol to testosterone was estimated as a marker of aromatase activity [26].

Extracellular Signal Regulated Kinase (ERK1/2)

Serum rat ERK1/2 was determined, as marker of phosphorylated cellular protein modulating estradiol progesterone synthesis, using ELISA kit specific for rats according to the instruction of GSCIENCE (Glory Science Co., Ltd. USA).

Oxidative and apoptotic markers assay

Serum 8-hydroxy-guanosine (8-OHdG) as an oxidative marker and Casp-3 as apoptotic marker were determined using ELISA kit specific for rats according to manufacturer's instruction (GSCIENCE, Glory Science Co., Ltd. USA).

Statistical analysis

The statistical analysis for the biochemical data was performed using SPSS version 22. Data were expressed as mean ± S.D. Statistical differences between groups were performed using ANOVA test with significance level $P<0.05$, the mean significant difference between groups was carried out using Bonferroni`s test.

RESULTS

Thyroid hormone level

Figure 1 (A-C) Depicts the treatment effect on thyroid hormone levels TSH, fT4 and fT3 levels (Fig. 1A, $F(3,24)=9.185$; $p<0.001$),(Fig.1B, $F(3,24)=61.09$; $p<0.001$) and no treatment effect on fT3 level (Fig.1C, $F(3,24)=1.588$; $p=0.053$). The treatment with NeoMercazole® (NM) induced a state of hypothyroidism which proofed by increased serum TSH levels ($p<0.01$) and decrease in serum fT4 ($p<0.001$), compared to control group. Thyroid hormones levels were ameliorated to reach control levels in hypothyroid group treated with talbina (HT), it was observed by significant increase in serum fT4 ($p<0.0001$), accompanied with decrease in TSH ($p<0.05$), compared to hypothyroid group. Individual treatment with talbina unaltered thyroid hormone compared to control group.

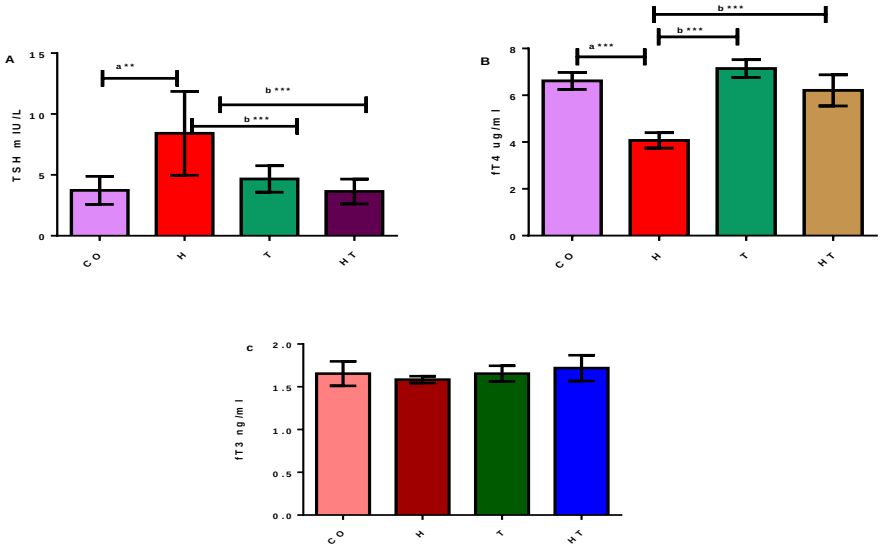

Figure 1: (A) Statistical significance of serum TSH mIU/L, (B) serum fT4 µg/ml, and (C) serum fT3 in control (CO) group, hypothyroid (H) group, talbina-treated (T) group and hypothyroid-talbina-treated group (HT). All data were represented as mean ± SD, n=7. *indicates a significant difference in p value <0.05, **indicates a significant difference in p value <0.01 and ***indicates a significant difference in p-value <0.001.

Adrenal and pituitary hormones

The treatment of hypothyroid group with talbina (HT) reverse the elevation of corticosterone, prolactin and depletion in FSH level in hypothyroid group (H) to reach control levels in control (CO) and talbina (T) treated groups (Fig 2A, $F(3,24)=9.460$; $p<0.001$) and (Fig 2B, $F(3,24)=21.58$; $p<0.05$) (Fig 2C, $F(3,24)=11.88$; $p<0.001$). No treatment effect on LH (Fig 2D, $F(3, 24)=3.896$; $p=0.064$) with no significant post hoc between groups has observed.

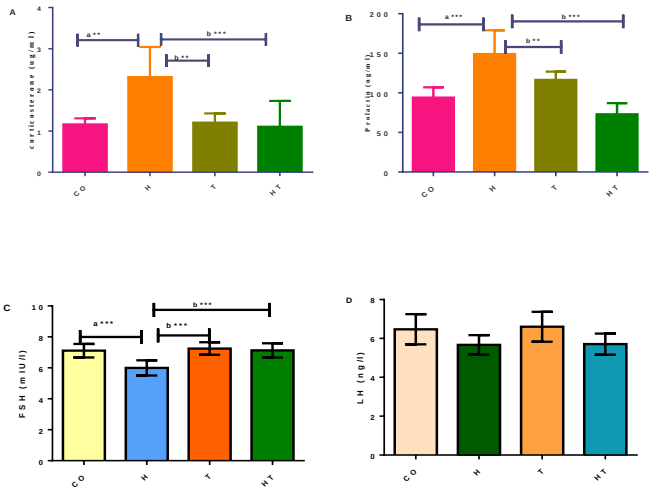

Figure 2: Statistical significance of serum corticosterone (ug/dl), (A), Serum prolactin (ng/ml) (B), Serum FSH (mIU/ml) (C) and serum LH (ng/l) (D) in control (CO) group, hypothyroid (H) group, talbina-treated (T) group and hypothyroid-talbina-treated group (HT). All data were represented as mean ± SD, n=7. *indicates a significant difference in p value <0.05, ** indicates a significant difference in p value <0.01 and ***indicates a significant difference in p value <0.001.

Effect of hypothyroidism status on gonadal hormones

Figure 3 (A-C) reveals the treatment impact on gonadal hormone, it induced a potent impact on estradiol (E2) levels (Fig 3A, F (3,24)=22.86; p<0.001), go with marked treatment effect on progesterone (P) levels (Fig 3B, F(3,24)=5.255; p<0.01), and no treatment effect noticed in testosterone (Tes.) level (Figure 3C, F(3,24)=1.872; p=0.1613). Hypothyroid female group (H) showed depletion in E2 (p<0.01) (p<0.001) levels with elevation in P (p<0.05) level, compared to control groups. Co-administration of talbina with neomercazol (HT) improves the gonadal hormones levels to reach control hormonal levels. Treatment with talbina (T) unaltered gonadal hormones compared to intact control group.

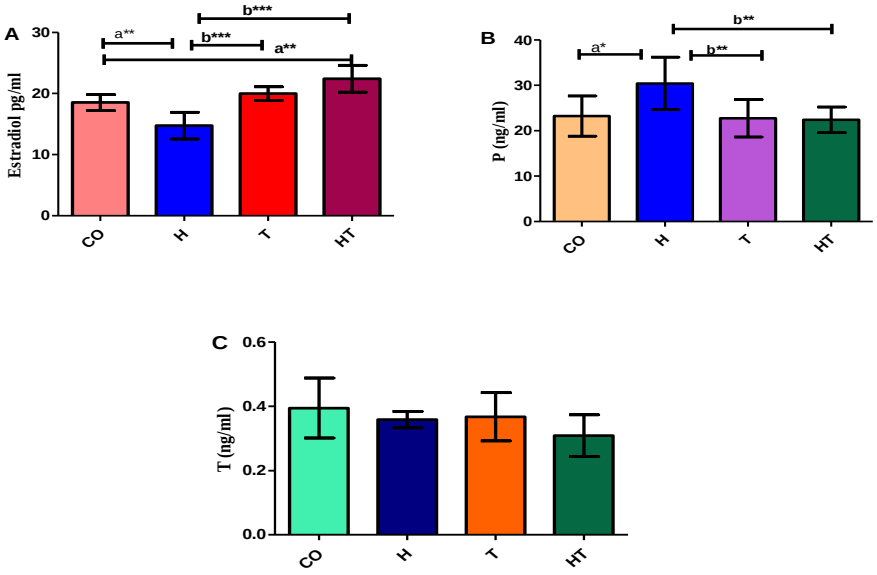

Figure 3: (A) Statistical significance of serum Estradiol (pmol/L), (B) serum P (ng/ml) and (C) serum T (ng/ml), in control group (CO), hypothyroid group (H), talbina-treated group (T) and hypothyroid-talbina-treated group (HT). All data were represented as mean ± SD, n=7. *indicates a significant difference in p value <0.05, ** indicates a significant difference in p value <0.01 and *** indicates a significant difference in p value <0.001.

Effect of hypothyroidism on E2/Tes ratio and extracellular signal-regulated protein kinases (ERK1/2)

Ratio of estradiol/ testosterone was significantly decreased (p<0.01) along with significant increase in extracellular signal-regulated protein kinases 1/2 (ERK1/2) in hypothyroid group (H) as compared to control. Treatment of hypothyroid group with talbina (HT) has normalized E2/Tes ratio and ERK1/2 level compared to control (CO) and talbina (T) groups (Figure 4A, F (3,24)= 9.277; p<0.05), (Figure 4B, F (3,24)= 10.33; p< 0.001)

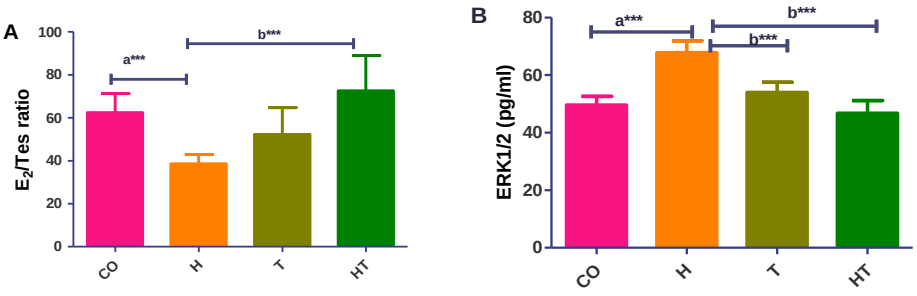

Figure 4: Statistical significance of **(A)** serum E_2/Tes ratio **(B)** serum ERK 1/2 (pg/ml) in control group (CO), group hypothyroid (H), talbina-treated group (T) and hypothyroid-talbina-treated group (HT). All data were represented as mean ± SD, n=7. *indicates a significant difference in p value <0.05, **indicates a significant difference in p value <0.01 and ***indicates a significant difference in p-value <0.001.

Oxidative and apoptotic markers

Post Hoc analysis revealed that the hypothyroidism induced significant increase in serum 8-OH-Guanosine level (p<0.01) and Caspase-3 activity (p<0.001), in respect to control group. Talbina (T) has similar effect as intact control group on both oxidative and apoptotic markers. Talbina treated hypothyroid group (HT) reversed the effect of hypothyroid status to reach base line levels. (Fig 5A, (F (3,24) = 6.501; p< 0.01); (Fig 5B, F (3,24)=72.01; p< 0.001)

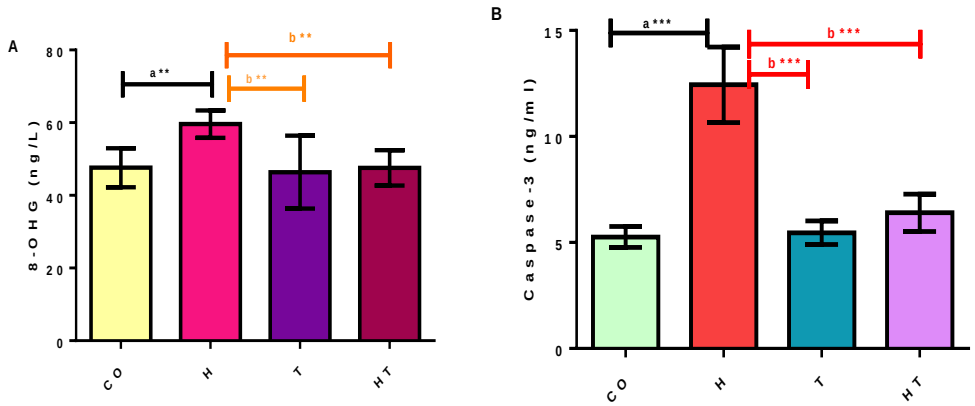

Figure 5: Statistical significance of serum 8-hydroxy guanosine (8-OHG) (ng/L) (A), Caspase-3 (ng/ml) (B) in control group (CO), hypothyroid group (H), talbina-treated group (T) and hypothyroid-talbina-treated group (HT). All data were represented as mean ± SD, n=7. *indicates a significant difference in p value <0.05, **indicates a significant difference in p value <0.01 and ***indicates a significant difference in p value <0.001.

DISCUSSION

The present study was conducted to address the potential ameliorative effect of talbina on the disturbance in adrenal pituitary gonadal hormones, as well as oxidative stress following hypothyroid induction in adult female albino rats. Present data showed that hypothyroism induced by NeoMercazole® drug caused disturbances in adrenal, pituitary and gonadal hormones. Our study indicated that talbina reversed the effect of antithyroid drug on the levels of thyroid hormones (TSH, fT4). These results may be attributed to a high iron (Fe) content in talbina which plays a crucial role in modulating thyroid peroxidase (TPO) enzyme activity [27-30], our data is supported by several studies which demonstrated that iron deficiency considered a cause of lowered thyroid hormone levels and TPO activity which reversed after iron treatment [28,31,32]. Triggiani [33] reported that herbal preparation enriches with Fe could either stimulate the thyroid or increase peripheral conversion from T4 to T3. Zimmermann et al. [34] studied the effect of iron supplementation on goitrous; iodine deficient patient and iodine deficient anemic patient; the authors reported that the supplementation of iron modulated the efficacy of iodide oil supplementation in iodine deficient anemic patient.

Our results revealed that hypothyroidism was associated with hyper corticosterone level. Walter and [35] attributed the elevation of serum corticosterone levels as a result of the decrease in its clearance and diminished negative feedback of thyroid-adrenal axis as a result of increasing serum TSH levels. As treatment with talbina normalized the TSH level that may activate the negative feedback of thyroid-adrenal axis, thus improve the level of corticosterone to reach the control level.

In our study, the hyper TSH level (hypothyroid group) stimulated the synthesis of corticosterone, and generated a state of oxidative stress, the latter inhibited the pituitary gonadotropin [36-38], this could imply the cause of FSH depletion with non-significant decrease of LH levels in hypothyroid group.

Lower level of estradiol in hypothyroid group associated with high progesterone level could be attributed to high ERK 1/2 level. Several researchers documented the role of ERK1/2 in modulating the LH to stimulate CYP17 gene and activated the steroidogenic acute regulatory (StAR) protein in controlling step for steroid production, to start progesterone synthesis [38-41]. Synthesis of progesterone was manifested by the low E2/Tes ratio (measure of aromatase activity) and FSH level, which failed to stimulate the granulosa cell to start estrogen synthesis [42,43], that may implicated in suppression of estradiol synthesis and maintained the hypothyroid female rats in pseudopregenancy state which is confirmed by hyperprolactinemia estimated. Pseudopregenancy state is a marker of hypothyroid female patient [5,7,44-47].

Phytoestrol compounds as β-sitosterol, campesterol, stigmasterol with estrogenic activity as agonist to estrogen receptor ER-α, β subsequently mimic estrogenic effect, several studies revealed that phytoestrol modulated steroidogenesis in adrenal gland, in addition phytoestrol could have protective effect against incidence of breast cancer via modulation of ER-α, β expression to act as tamoxifen (estrogen replacement anticancer therapy) [48-50]. In line with the previous studies we could develop general perception, that talbina, with its high content of phytoestrol, could modulate ER-α, β expression, augmented estradiol levels, in turn led to activate negative feedback mechanism of pituitary gonadal adrenal axis function and renormalize the disturbances of mentioned endocrine gland elicited by hypothyroid status.

Oxidative stress is related to hormonal disorders in a reciprocal way. Hypothyroidism is associated with oxidative stress in animal and human [51-53]. In addition to oxidative stress, it was documented that TSH is a robust activator to apoptotic process [52,54] klatka, [55] confirmed high TSH activity resulted in increase the percentage of lymphocyte apoptotic cells. The abovementioned researches is in line with our results, which reveal that hypothyroidism was associated with high significant increase in serum 8-hydroxyguanosine (an oxidative stress markers), together with marked elevation in Caspase-3 (an apoptotic marker). Treatment with talbina attenuated the oxidative stress status induced by hypothyroid status; it significantly decreased 8-OH guanosine levels and caspase-3 activity. This antioxidant activity of talbina could be attributed to ferulic, sinapic and ß-

hydroxy acids (BHA) content, the major predominant polyphenol, in talbina with their potent free radical scavengers [21,56,57]. Scientific researches implied the free radical scavenging properties of polyphenol compounds to their hydrogen-electron donating ability and the stability of the resulting phenoxyl radical, there was other postulated mechanism based on the chelation bond formed between polyphenol compound with transition mineral which catalyzed the oxidative stress induction via inhibiting antioxidant enzyme activities or modulating the expression of gene encoding anti-oxidative and phase II detoxification enzymes [58-60].

CONCLUSION

From our study we can conclude that, talbina encompassed profound effect not limited to oxidative stress response but extended over pituitary adrenal gonadal axis function. Talbina had this intricate contribution in modulating the exacerbate side effect induced by hypothyroidism via multiple arrays. It could act as an external iron supply which activated the TPO recovering subsequent thyroid hormone changes. The other array was sustaining cellular energy due to the antioxidant property of talbina, improved an endocrine function. The final array was modulation in ovarian function via phytoestrol content, increased aromatase activity and modulated the ovarian estradiol synthesis to establish normal homeostasis in pituitary-adrenal-gonadal hormone axis.

ETHICAL APPROVAL

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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