Chapter

Potential Role of Plants *Hordeum vulgare* L. and *Panax ginseng* L. in Resolving the Fertility Disorders and Stress-Induced Oxidative Stress Arises from Hypothyroidism in Adult Female Rats

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Abstract

Hordeum vulgare (Barley) and *Panax ginseng* have antioxidant activity referring to their diverse phytonutrient. Hypothyroidism in adult female rats was induced by pituitary-gonadal-adrenal disturbance, depleting the serum FSH levels with the elevation of corticosterone, prolactin, progesterone and testosterone hormones as well as (ERK1/2). Hypothyroidism evoked an oxidative stress status by increasing 8-hydroxy guanosine, which initiated apoptosis by uplifting apoptotic marker Caspase-3 both in serum and brain tissues. This is confirmed by the increase in the percentage of DNA-damage in the brain tissues. Significant decrease in all monoamines' levels in different brain areas, downregulation of dopamine and 5-hydroxytryptamine receptors transcription, with a significant increase in excitatory amino acids was noted. Barley and ginseng renormalized cortisol and oxidative stress markers by increasing cellular resistance to stress and potentiated the role of the immune system through phytosterol and ginsenosides, so they considered potent free radical scavengers. Barley and *Panax ginseng* ameliorate the hormonal and neural dysfunction resulting from hypothyroidism, so they are recommended for relieving stress and improving mood and depression.

Keywords: barley, *Panax ginseng*, oxidative stress, antioxidant, hypothyroidism: gonadal-neural dysfunction

1. Introduction

There are many evidences revealed that food intake enriched with whole grain reduces the susceptibility of the incidence of many chronic diseases. Barley (*Hordeum vulgare*) is a food source deemed to be available for all disparate social classes of humankind.

In the Arab culture, *Hordeum vulgare* or barley syrup is used to relieve depression. It is categorized into the spring and winter types, which are considered two-rowed or six-rowed depending on the number of seed rows on each spike. Based on its grain composition, barley is further classified into normal, waxy or high amylose starch type [1].

Barley 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, vitamin E; nicotinic acid; pyridoxine; folic acid; essential amino acids, such as tryptophan and phenylalanine; neutral amino acids (LNAA), such as the three branched-chain aromatic amino acids leucine, isoleucine, and valine [2, 3]. So, barley grain exhibits potential antioxidant and antiproliferative actions because its powerful phytochemical compounds that have been shown to lower the risk of many diseases [4, 5]. The diverse phytonutrient of barley implicates its protection activity against certain types of cancers, cardiovascular disease, arthritis, diabetic, and hypercholesterolemia. It also increases cellular energy to sustain the body homeostasis [6, 7] and modulating endocrine and neurotransmitters functions [8].

Red ginseng represents an important position as a health functional food**.** It belongs to the Panax genus of the *Araliaceae* family, ginseng characterized by a complex activity profile that includes antioxidant, anti-inflammatory, anti-apoptotic, and immune-stimulatory properties and has the effects of stabilizing and balancing the entire physiology [9]. So, in Asian countries; Korea, China, and Japan, ginseng used as a therapeutic agent for a variety of diseases [10].

The major active ingredients of *Panax ginseng* are saponins, which are triterpene glycosides called "ginsenosides". Other active components include proteins, peptides, and alkaloids, which are nitrogenous compounds; polyacetylene, which is a fat-soluble component; polysaccharides and other flavonoids; fatty acids, organic acids, vitamins, sugars, inorganic salts, sterols, oligopeptides [11, 12]. Ginsenosides can be classified into three categories: the panaxadiol group (e.g. Rb1, Rb2, Rb3, Rc, Rd., Rg3, Rh2, Rs1), the panaxatriol group (e.g. Re, Rf, Rg1, Rg2, Rh1), and the oleanolic acid group (Ro) [13, 14].

Ginsenosides are lipophilic compounds so they can pass easily through the cell membrane by simple diffusion and bind to its intracellular target proteins in the cytoplasm and nucleus. Ginseng also contains more than 10 phenolic compounds that possess antioxidant biological properties that have ability to lower the effect of oxidative stress [15]. Phytoestrogens, such as genistein is an important component of ginseng, have shown protective effects on conditions related to decreased estrogen, including menopause, osteoporosis, and cognitive disorders [13, 16].

Thyroid-stimulating hormone (TSH) is synthesized and secreted by the adenohypophysis lobe and exerts its effect by binding to the cognate thyrotropin receptor (TSHR) to stimulates the production of thyroglobulin and thyroid peroxidase proteins, which are essential for the synthesis and secretion of thyroid hormones (THs) [17]. THs bind their nuclear receptors (TRs), which are present in many tissues and organs in the human body and hence regulate their functions [18].

Thyroid hormones influence a wide range of brain developmental processes, such as myelination, neuronal and glial cell differentiation by regulating the gene involved in these processes thus hypothyroidism may reduce axonal growth and dendritic arborization in the cerebral cortex, visual cortex, auditory cortex, hippocampus, and cerebellum, as well as impaired memory, cognitive function and attentiveness [3, 19].

THs plays a role in neurotransmitter release from their storage vesicles such as norepinephrine (NE), epinephrine (E), serotonin (5-HT) and dopamine (DA) and hence maintaining good mental state, mood regulation, modulating post-receptor signal transduction, gene expression and preventing depression [19]. So, hypothyroidism is a highly prevalent condition that impairs learning, memory, induce delayed skeletal development, cardiovascular diseases, secondary hypertension, the deterioration of human reproductive health and brain dysfunction [20].

2. Materials and methods used in the research

2.1 Animals

The study was carried out by using adult female Wistar albino rats weighing 180–200 g. Animals were housed at 23 ± 2°C and 55 ± 5% humidity with a 12 h light/dark cycle rats were provided a standard diet and water *ad libitum*.

2.2 Preparation of *Hordeum vulgare* (barley)

Barley was prepared as an emulsion in water (1 g ground barley soaked in 10 ml of distilled water) and administered daily *per* [21]. The nutritional facts of barley per 100 g are presented in **Table 1**.

2.3 Preparation of *Panax ginseng*

Dried roots of the Korean *Panax ginseng* were obtained as a brown powder and dissolved in distilled water. Animals received a daily oral dose of 1.8 mg/200 g body weight (equivalent to the therapeutic dose [22]) for 30 days.

2.4 Induction of hypothyroidism

Neo-Mercazole is the least toxic anti-thyroid agent within therapeutic dose ranges [23] therefore it was selected for hypothyroidism induction. The animals were orally administered a daily dose of 5.0 mg.kg—of Neo-Mercazole for 1 month [24]. Hypothyroidism was manifested by the increased level of serum TSH associated with low level of f T4.

Carbohydrates	(78.2 g)	Vitamin B6	(0.29 μg)	Choline	(38 mg)
Fibers	(15.5 g)	Vitamin K	(2.5 μg)	Riboflavin (B2)	(0.124 μg)
Energy	(350 kcal)	Niacin (B3)	(4.8 μg)	Calcium	(30 mg)
Fat	(1.2 g)	Pantothenic acid (B5)	(0.29 μg)	Iron	(3.5 mg)
Protein	(10 g)	Thiamine (B1)	(0.2 μg)	Magnesium	(80 mg)
Vitamin A	(15 μg)	Folic acid	(25 μg)	Phosphorus	(200 mg)
Zinc			(2.5 mg)		
Potassium			(250 mg)		

Table 1.
Nutrition facts in barley/100 g.

2.5 Experimental design of barley work

Animals randomly divided into equally four-treatment groups. Except for euthyroid animals (EU) (groups 1&2), hypothyroid animals (H) (group 3&4) were orally administered 5.0 mg kg^{-1} bwt Neo-Mercazole until the end of the study. Following 30 days of Neo-Mercazole administration, groups 2 and 4 orally administered 100 mg kg^{-1} bwt barley (B) [21] water suspension for 4 weeks. The four groups named: EU; EU + B; H; H + B.

2.6 Experimental design of *Panax ginseng* work

Rats were divided equally into four groups. First group was an intact control group that received distilled water. The second group was the hypothyroid group (H group) which orally treated with 5 mg kg^{-1} body- weight Neo-Mercazole for 30 days for induction of hypothyroidism. The third group was orally administered *Panax ginseng* (G group) in a daily oral dose of 1.8 mg/200 g body weight for 30 days. The fourth group receiving both Neo-Mercazole for 30 days and followed by *Panax ginseng* (H + G group) for another 30 days.

2.7 Blood and tissue collection

At the end of treatment, the animals were anesthetized with 1% isoflurane followed by decapitation [25], blood was collected into serum preparation tube and the separated serum was collected and divided into aliquots, stored at −20°C for further hormones assay. The whole brains were removed from 10 rats from each group and the hypothalamus, hippocampus, cerebral cortex, midbrain, and cerebellum were dissected using a sharp blade. From another 10 rats' whole brain and thyroid gland were immediately removed and stored in ice-cold saline at −20°C for further biochemical and comet assay.

2.8 Methods

Levels of f T3, f T4, ERK1/2, 8-hydroxy-2′-de-oxyguanosine (8-OhdG) and apoptotic marker Caspase-3 were determined using ELISA kit specific for rats according to manufacturer's instruction (Glory Science Co., Ltd., USA). Serum corticosteroid and gonadal hormones were determined using the ELISA kit according to the instruction of BioCheck (BioCheck Co., Ltd., USA). DNA degradation in brain and thyroid homogenates was determined by using the Comet technique according to the method [26]. Determination of monoamines in brain areas were carried according to methods of [19, 27] while free amino acids were done according to the method of [28] using the precolumn phenylisothiocyanate (PTC) derivatization technique.

3. The antioxidant effect of barley on fertility disorders and oxidative stress induced by hypothyroidism

The study was conducted to address the potential ameliorative effect of barley on the disturbance in adrenal pituitary-gonadal hormones, as well as oxidative stress following hypothyroid induction. Hypothyroidism induction caused disturbances in adrenal, pituitary and gonadal hormones (**Figures 1**–**3**). Barley reversed the effect of the antithyroid drug on the levels of thyroid hormones (TSH, f T4) and their transporting proteins (TBG, TTR) as shown in **Table 2** due to its higher iron (Fe) content which plays a crucial role in modulating thyroid peroxidase

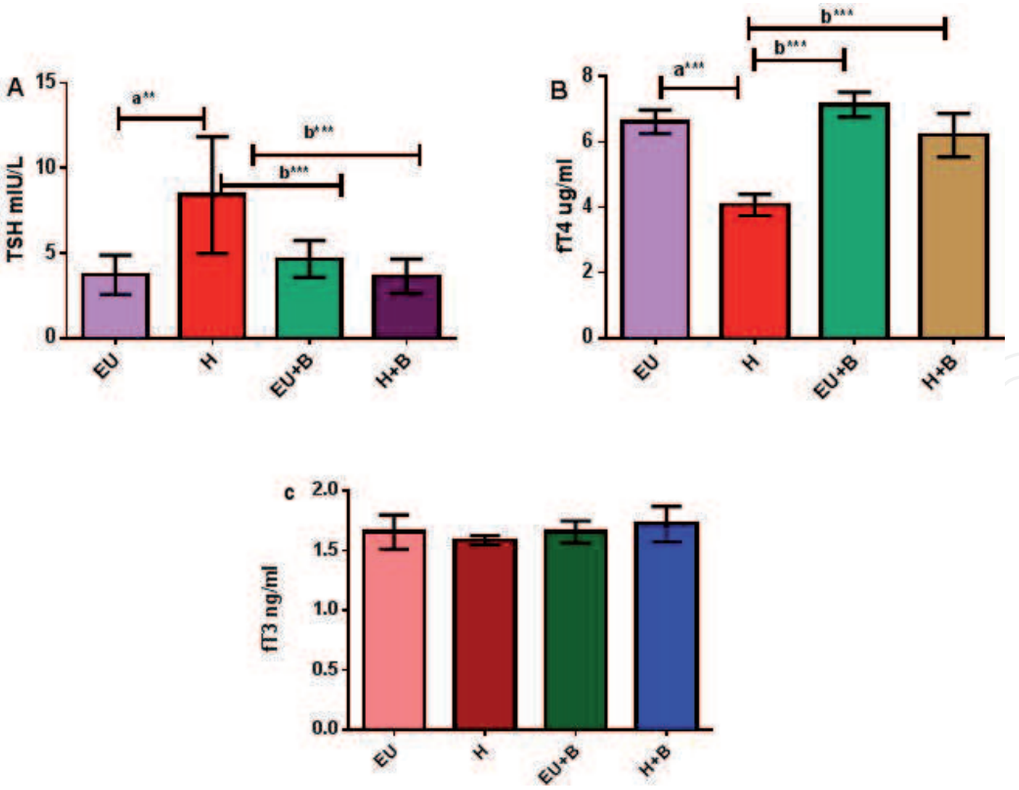

Figure 1.
(A) Serum TSH mIU/L, (B) serum fT4 μg/ml, and (C) serum f T3 in control (CO) group, hypothyroid (H) group, barley treated (T) group and hypothyroid-barley-treated group (HT).

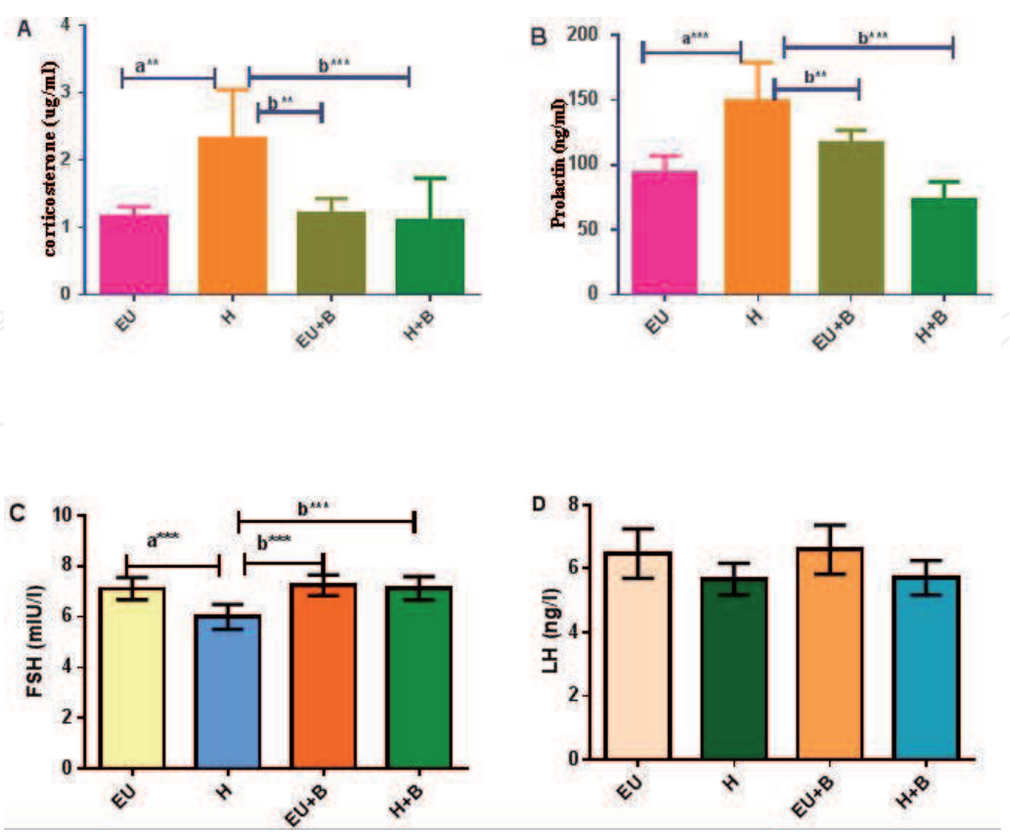

Figure 2.
Serum corticosterone (ug/dl) (A), serum prolactin (ng/ml) (B), serum FSH (mIU/ml) (C) and serum LH (ng/l) (D) in control (CO) group, hypothyroid (H) group, barley-treated (T) group and hypothyroid-barley-treated group (HT).

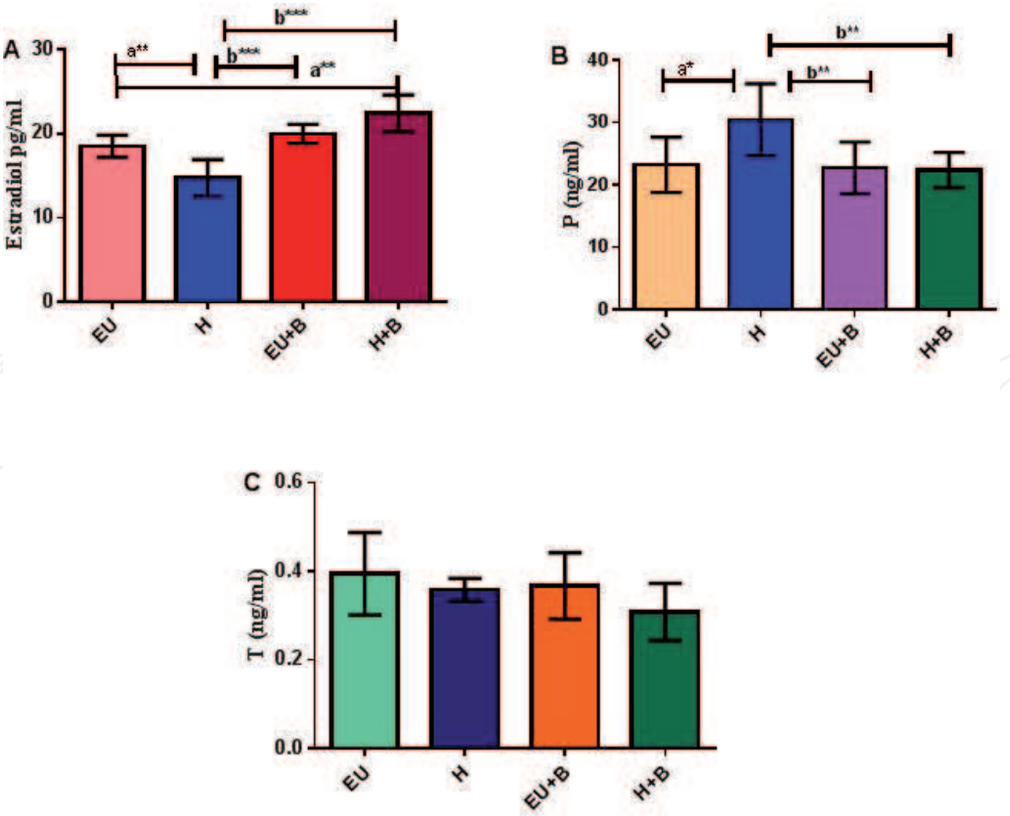

Figure 3.
(A) Serum estradiol (pmol/L), (B) serum P (ng/ml) and (C) serum T (ng/ml), in control group (CO), hypothyroid group (H), barley-treated group (T) and hypothyroid-barley-treated group (HT).

	EU	EU + B	H	H + B
f T4 (µg/ml)	4.43 ± 0.11	4.21 ± 0.14	3.09 ± 0.25 (a*)	4.46 ± 0.19(b**)
TSH (mIU/ml)	12.47 ± 0.42	12.73 ± 0.62	15.97 ± 0.26 (a*)	12.53 ± 0.29 (b*)
TTR (ng/ml)	51.23 ± 2.39	45.64 ± 6.53 (a*)	16.85 ± 1.88 (a***)	34.25 ± 1.65 (a** b***)
TBG (pg/ml)	1.58 ± 0.04	2.35 ± 0.16 (a**)	2.09 ± 0.04 (a*)	2.57 ± 0.14 (a***b*)
ERK1/2 (pg/ml)	43.67 ± 1.53	46.33 ± 1.53(a*)	33.19 ± 2.16 (a**)	47.94 ± 0.60(b**)

All data in tables represented by mean ± SD, n = 10 animals.
**p < 0.05, **p < 0.01 and ***p < 0.001.*
a: mean significance difference from control group. B: mean significance difference from hypothyroid group.

Table 2.
Effect of barley on THs, TTR and TBG in brain tissue of EU- and H-groups.

(TPO) enzyme activity [2, 29]. Oxidative stress is related to hormonal disorders in a reciprocal way so in our study, the hyper TSH level stimulated the synthesis of corticosterone, and generated a state of oxidative stress which inhibited the pituitary gonadotropin [30, 31], and cause FSH depletion with the non-significant decrease of LH levels in the hypothyroid group. Also, lower levels of estradiol in the hypothyroid group associated with high progesterone and prolactin levels could be attributed to high ERK1/2 level (**Figure 4**). Barley, with its high content of phytosterol, could modulate ER-α, and β expression, augmented estradiol levels, in turn, led to activate negative feedback mechanism of pituitary-gonadal adrenal axis function and renormalize the disturbances of endocrine gland elicited by hypothyroidism.

Oxidative stress (ROS) is an imbalance between the production of pro-oxidant substances and antioxidant defense. Hypothyroidism augments the oxidative

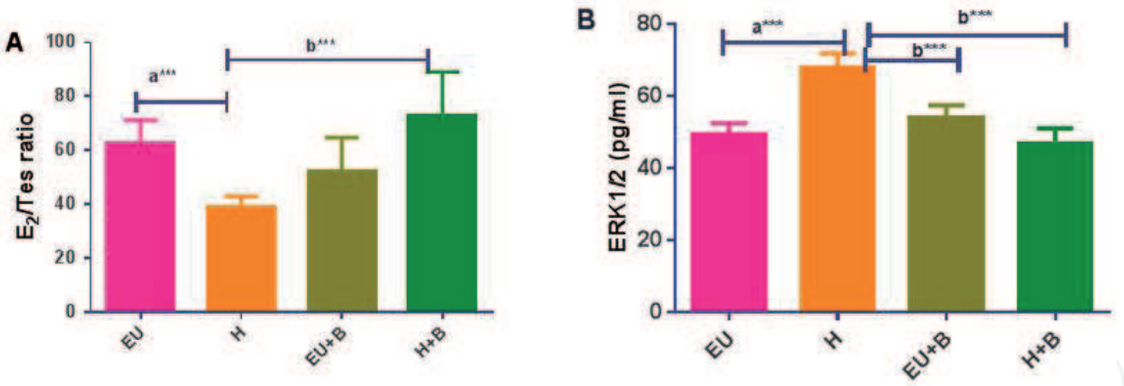

Figure 4.
(A) Serum E2/T ratio (B) serum ERK1/2 (pg/ml) in control group (CO), group hypothyroid (H), barley-treated group (T) and hypothyroid-barley-treated group (HT).

insult, impairing the brain by increasing nitric oxide (NO) and NO synthase (NOS) levels in the hippocampus, which affects the lipid composition of rat brain tissues and induces DNA damage [32, 33]. In present work, hypothyroidism was associated with the high significant increase in 8-hydroxyguanosine (an oxidative stress marker), together with marked elevation in Caspase-3 (an apoptotic marker) in serum and brain tissue (**Figures 5** and **6**). These findings were confirmed by alkaline comet assays of thyroid and brain tissue homogenates. As DNA was degraded, it converted from a supercoiled form to a comet-like shape with a measurable tail length so our study revealed that the hypothyroid status induced a significant increase in the tail length, in the thyroid and brain tissues. Treatment with barley attenuated the oxidative stress status induced by hypothyroid status; it significantly decreased 8-OH guanosine levels and Caspase-3 activity. This antioxidant activity of barley could be attributed to flavonoids, ferulic, sinapic and ß- hydroxy acids (BHA) content, the major predominant polyphenol, in barley with their potent free radical scavengers by absorbing and neutralizing oxygen radicals [21, 34, 35]. Also, DNA damage was repaired by the antioxidant activity of barley as illustrated in **Figures 7** and **8**, which refer to vitamins A and E that essential for nucleotide, DNA biosynthesis, DNA repair, and methylation [1, 2, 19, 36]. Additionally, zinc protects against oxidative stress by stabilizing membranes through the inhibition of the enzyme nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) and the stimulating of the synthesis of metallothioneins, which reduce the levels of hydroxyl radicals and sequester ROS produced in response to hypothyroidism.

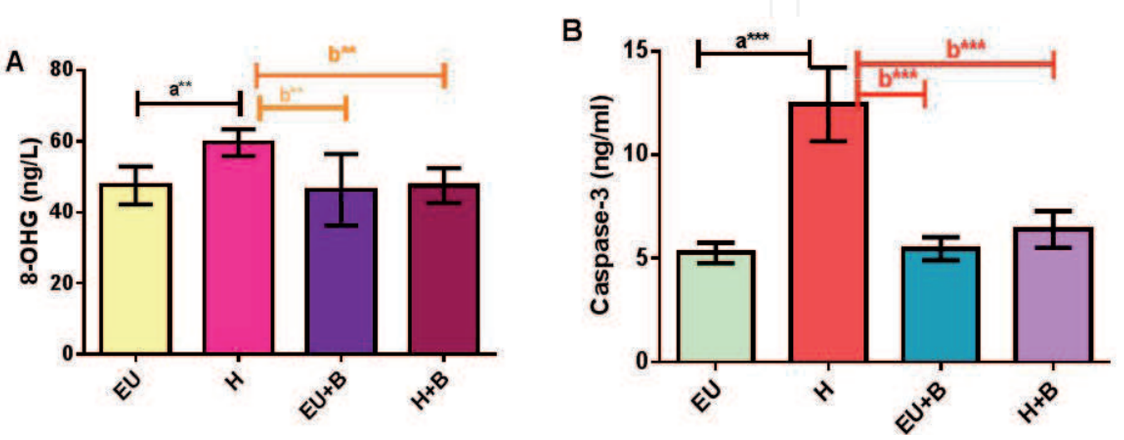

Figure 5.
Serum 8-hydroxy guanosine (8-OHG) (ng/L) (A), Caspase-3 (ng/ml) (B) in control group (CO), hypothyroid group (H), barley-treated group (T) and hypothyroid-barley-treated group (HT).

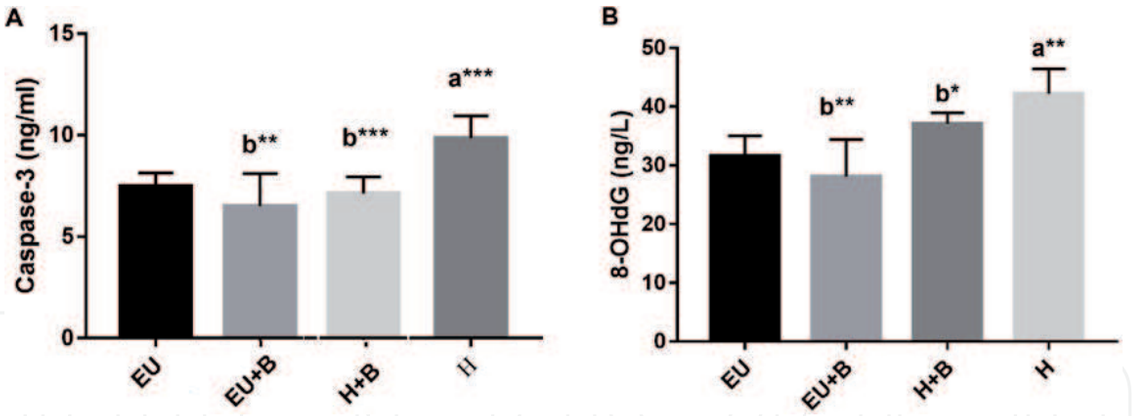

Figure 6.
Effect of barley on caspase-3 (ng/ml) and 8-OhdG (ng/ml)in brain tissue.

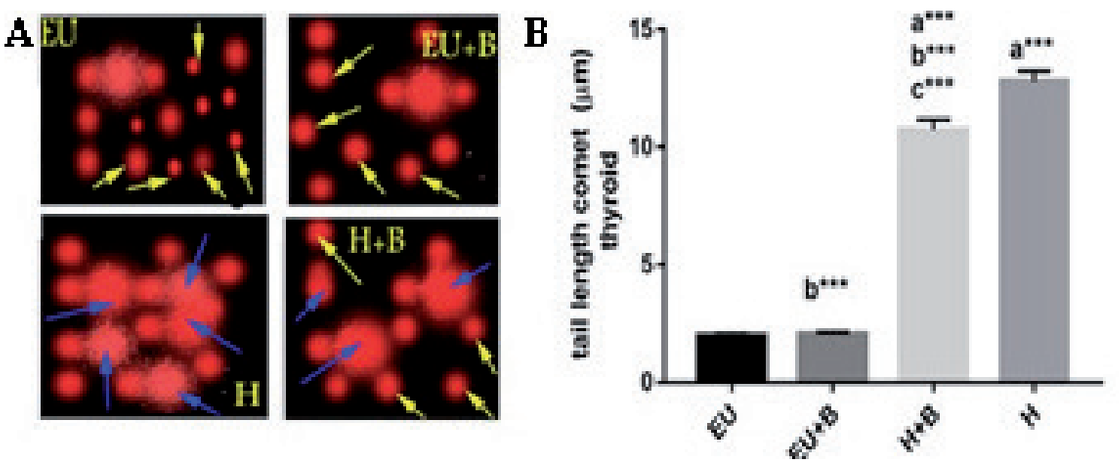

Figure 7.
Effect of barley on DNA damage in thyroid tissue (A) fluorescence photomicrograph showing comets in EU-, H-, EU + B and H + B-groups. The → indicated the intact DNA and → indicated the degree of damaged DNA (B) tail length expressed in µm in thyroid tissue of all treated groups.

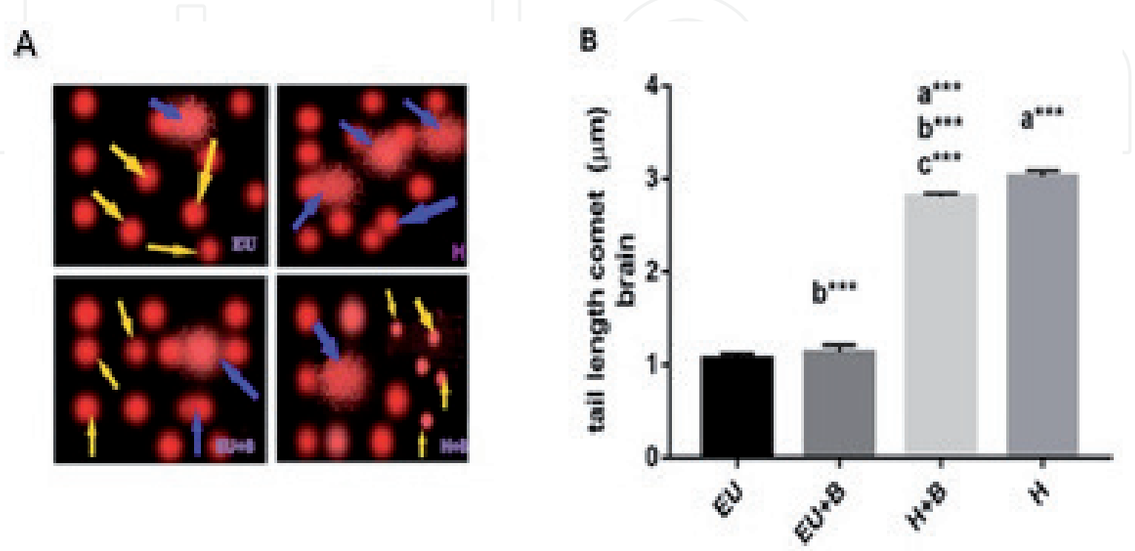

Figure 8.
Effect of barley on DNA damage in whole brain tissue (A) fluorescence photomicrograph showing comets in EU-, H-, EU + B and H + B-groups. The → indicated the intact DNA and → indicated the degree of damaged DNA (B) tail length expressed in µm in whole brain tissue of all treated groups.

Neurotransmitters NE, DA, and 5-HT levels were significantly reduced in all brain areas (cerebellum, midbrain, cerebral cortex, hypothalamus, and hippocampus) with hypothyroidism induction as shown in **Table 3**, these could be attributed to the reduced oestradiol level as mentioned above [37, 38]. The administration of *Hordeum vulgare* (barley) improved the disturbances in the dopaminergic, serotonergic and noradrenergic pathways via two different mechanisms; first, the high phytosterol content modulates estrogen receptors (Erα and β) expression and elevate oestradiol levels. Second, barley is enriched with tryptophan and phenylalanine and could regulate the synthesis of 5-HT, DA and NE through the conversion of tryptophan to 5-hydroxytryptophan (5-HTP) to 5-HT and hydrolysis of phenylalanine to generate tyrosine that ultimately produces DA and NE [2, 36, 39, 40]. In the present study, hypothyroidism induced a significant increase in inhibitory amino acid, including GABA and histidine, which is an excitatory amino acid (**Figures 9** and **10**).

These results could explain the increase in Caspase-3, which may be attributed to reduced blood oxygen–glucose levels in several brain regions as a result of increased GABA levels. The study also revealed an increase in dopamine receptors, whereas serotonin receptors were significantly decreased. The *Hordeum vulgare* (barley) treatment in the present study caused a renormalization the observed disturbances in the amino acid and neurotransmitter levels because it is enriched with folic acid, which is involved in the synthesis of monoamine neurotransmitters and modulate serotonergic, dopaminergic and noradrenergic systems by acting as a cofactor for enzymes that convert tryptophan to 5-HT and enzymes that convert tyrosine to noradrenaline [19]. The alteration in serotonin receptor densities was restored by the barley administration, due to its enriched levels of tryptophan, which is metabolized to serotonin [36, 40] and activates these receptors (**Figure 11**).

	Frontal cortex	Hippocampus	Hypothalamus	Mid brain	Cerebellum
NE (μg g−1 tissue)					
EU	0.52 ± 0.03	0.69 ± 0.02	0.40 ± 0.01	0.70 ± 0.01	0.58 ± 0.01
EU + B	0.45 ± 0.02	0.65 ± 0.01	0.40 ± 0.01	0.69 ± 0.01	0.60 ± 0.01
H	0.21 ± 0.09a***	0.31 ± 0.01a***	0.13 ± 0.01a***	0.35 ± 0.01a***	0.29 ± 0.01a**
H + B	0.35 ± 0.01a**b*	0.46 ± 0.01a**b**	0.22 ± 0.01a**b**	0.48 ± 0.01a**b**	0.40 ± 0.01a**b**
DA (μg g−1 tissue)					
EU	0.59 ± 0.02	2.40 ± 0.07	1.473 ± 0.10	1.31 ± 0.01	0.60 ± 0.01
EU + B	0.55 ± 0.01	2.39 ± 0.09	1.32 ± 0.06	1.32 ± 0.01	0.60 ± 0.01
H	0.26 ± 0.01a***	0.93 ± 0.03a***	0.90 ± 0.03a*	0.85 ± 0.01a**	0.27 ± 0.05a***
H + B	0.35 ± 0.01a**b**	1.17 ± 0.01a**b**	1.07 ± 0.02a*b*	1.0 ± 0.01a*b*	0.40 ± 0.01a**b**
5-HT (μg g−1 tissue)					
EU	0.57 ± 0.01	0.38 ± 0.01	0.78 ± 0.01	0.72 ± 0.01	0.47 ± 0.01
EU + B	0.56 ± 0.02	0.38 ± 0.01	0.76 ± 0.02	0.65 ± 0.05	0.50 ± 0.01
H	0.23 ± 0.01a***	0.11 ± 0.01a***	0.45 ± 0.01a**	0.30 ± 0.01a ***	0.18 ± 0.01a***
H + B	0.35 ± 0.01a**b**	0.18 ± 0.01a**b*	0.54 ± 0.01a*b*	0.41 ± 0.01a**b**	0.28 ± 0.01a**b**

All data in tables represented by mean ± SD, n = 10 animals.
**p < 0.05, **p < 0.01 and ***p < 0.001.*
a: Mean significance difference from control group. b: Mean significance difference from hypothyroid group.

Table 3.
Effect of barley on neurotransmitters level in discrete brain regions in control and treated groups.

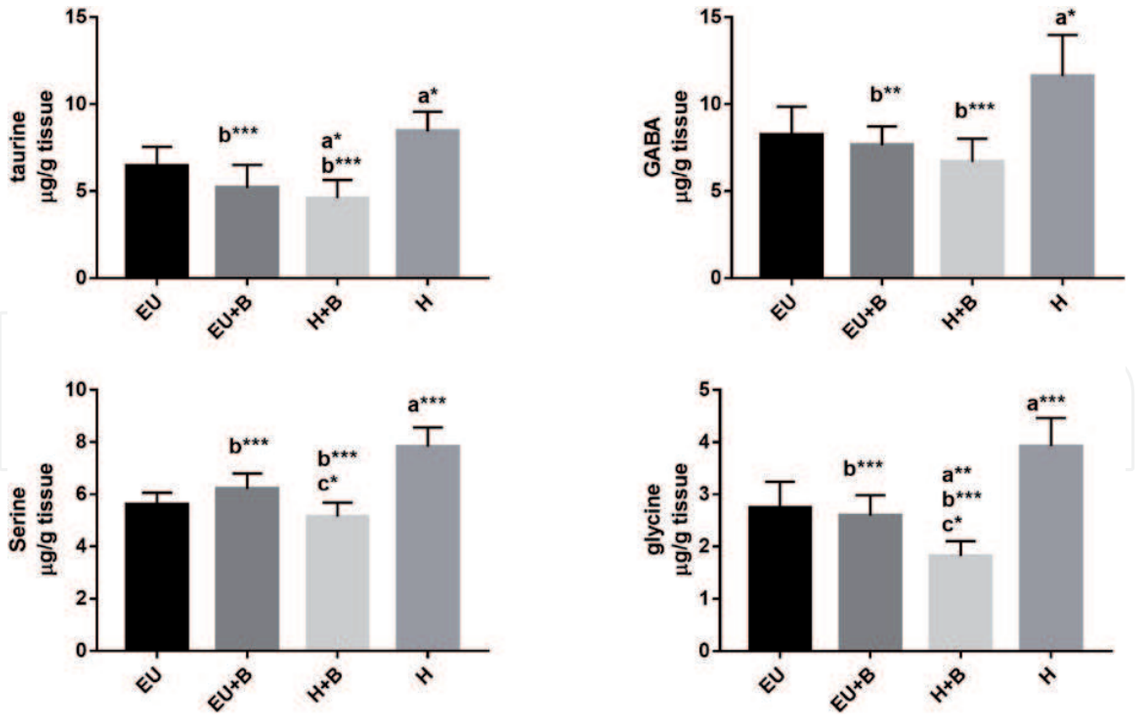

Figure 9.
*Effect of barley on inhibitory amino acids in EU and H groups. All data represented by mean ± SD, n= 10 animals, *p<0.05, **p<0.01 and ***p<0.001, (a) mean significance difference from control group, (b) mean significance difference from hypothyroid group. (C) mean significance difference from EU+B.*

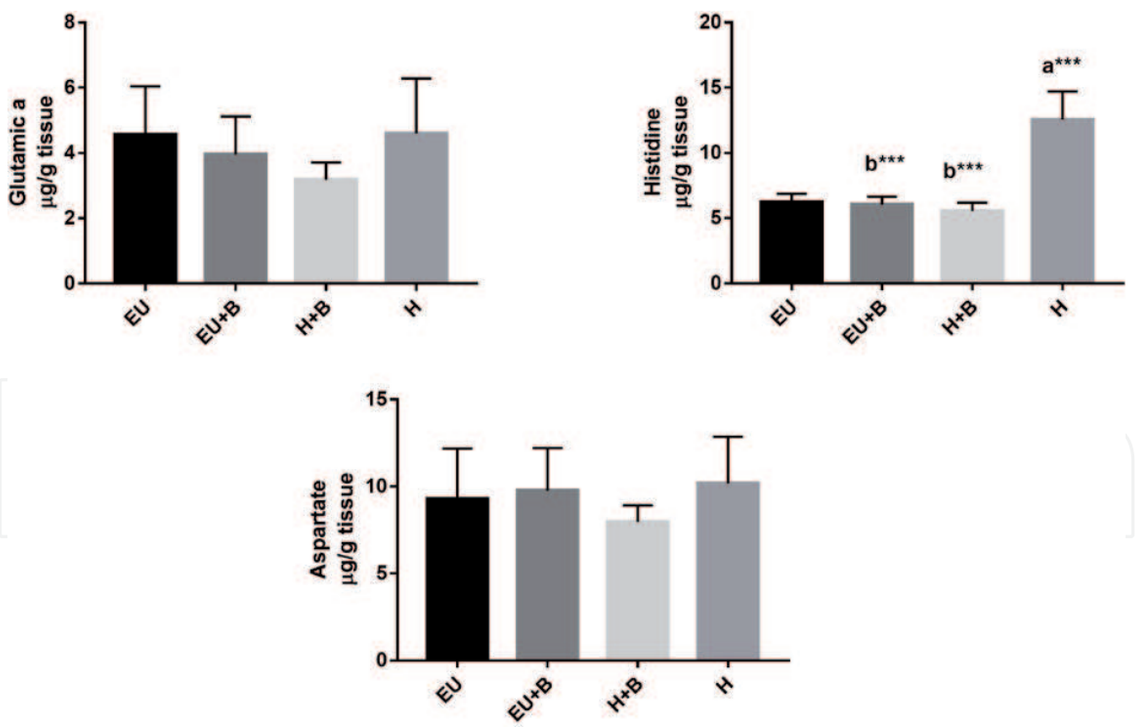

Figure 10.
*Effect of barley on excitatory amino acids in EU and H groups. All data represented by mean ± SD, n= 10 animals, *p<0.05, **p<0.01 and ***p<0.001, (a) mean significance difference from control group. (B) mean significance difference from hypothyroid group. (C) mean significance difference from EU+B.*

The elevation in serotonin levels after barley administration in the present study also resulted in ERK1/2 improvement in brain tissue, which was reduced by hypothyroidism induction. The binding of serotonin to 5-TH2 receptors stimulates ERK1/2 phosphorylation via the release of epidermal growth factor (EGF) agonist

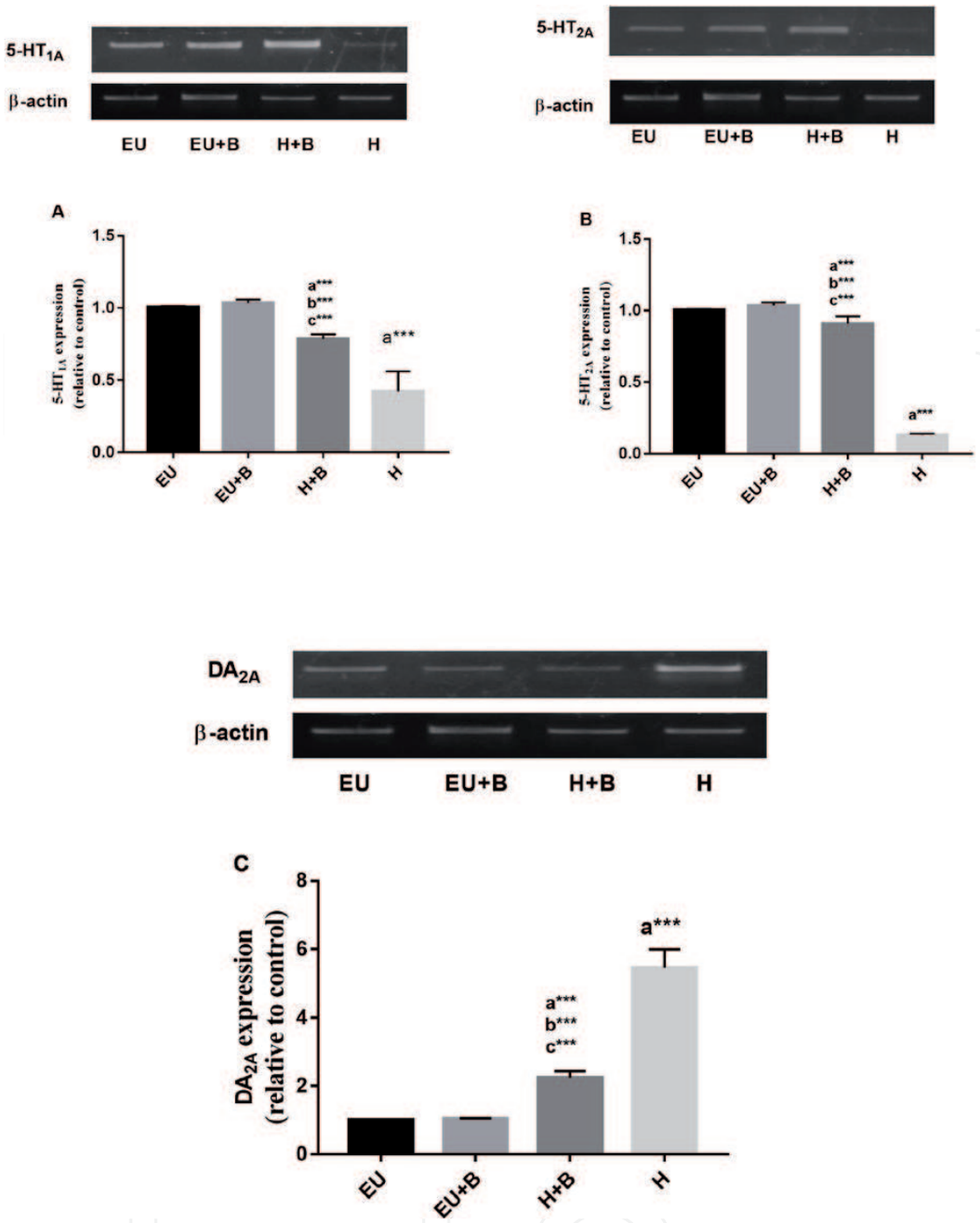

Figure 11.
Effect of barley on relative expression of (A) mRNA 5-HT1A, (B) mRNA 5-HT2A and (C) mRNA DA2A.

and transactivation of (EFG) receptors [41]. The improvement of serum oestradiol in hypothyroid-barley-treated groups as mentioned before could explain the positive effect of barley on restoring dopamine levels in brain tissues because the reproductive hormones, estrogen and progesterone, modulate the dysregulated serotonergic, dopaminergic, and glutamatergic neurotransmission by regulating the expression of receptors, the synthesis, reuptake, and release of the neurotransmitter serotonin and dopamine, which interact with dopaminergic neurons directly to downregulate D2 autoreceptors and indirectly by inhibiting GABAergic transmission [42].

Based on the above findings, we conclude that barley (*Hordeum vulgare*) is a nutritious food with high carbohydrate, zinc, magnesium content, and a high amino acids Trp:BCAA ratio has a positive effect on ameliorating the neural dysfunction induced by hypothyroidism and recommended for relieving stress, improving mood and depression.

4. The antioxidant effect of *Panax ginseng* on fertility disorders and oxidative stress induced by hypothyroidism

The present study revealed a reduction in fT3, fT4, and elevation in TSH levels as well as deterioration in THs transporting protein (TBG and TTR) in brain tissues of hypothyroid rats as shown in **Table 8**. This disturbance attributed to Neo-Mercazole which is an antithyroid agent that blocks thyroid hormonogenesis by inhibiting thyroid peroxidase (TPO) activity and preventing the formation of thyroglobulin from tyrosine [43, 44]. Hypothyroidism also causes elevation of cortisol that leads to inhibition of the deiodinase enzyme type 2 (D2) enzyme, responsible for the conversion of T4 into T3 [45]. Ginseng treatment improves the levels of thyroid hormones in serum and brain tissues through restoration of the impairment transporting protein (TTR & TBG) as shown in **Tables 4** and **8**. Moreover, ginseng boosts the activity of the enzyme responsible for converting T4 to active T3 and reduces thyroid hormone-blocking reverse T3 (rT3) which inhibits active T3 from binding to its functioning T3 receptors [46, 47].

The sexual dysfunction may be developed from psychological stress state exerted by hypothyroidism induction and this confirmed the role of the pituitary–adrenal gonadal axis (HPA) as a defense mechanism carried out by the organism against stress event [48]. So the reduction in trophic hormone (FSH & LH) associated with hypothyroidism led to decreasing the E2 hormone level and elevation of progesterone and testosterone. The inhibition in gonadal activity in hypothyroid rats in the present model as documented in **Table 5** was confirmed by the lowering of E2/T (**Table 6**) ratio which is a marker of the aromatase enzyme activity

Parameter	Control	G	H	H + G
f T3 (ng/ml)	1.99 ± 0.06	1.54 ± 0.05	1.59 ± 0.02	1.80 ± 0.04 (b*)
f T4 (μg/ml)	6.61 ± 0.14	7.32 ± 0.36	4.07 ± 0.12 (a*)	6.78 ± 0.34 (b**)
TSH (mIU/ml)	12.31 ± 0.62	12.81 ± 0.69	14.09 ± 0.42 (a*)	10.75 ± 0.26 (b**)
TTR (ng/ml)	32.56 ± 2.04	36.93 ± 2.95	44.19 ± 3.34 (a*)	27.41 ± 1.96 (b*)
TBG(pg/ml)	2.54 ± 0.19	2.49 ± 0.06	2.26 ± 0.07	2.37 ± 0.13

All data in tables represented by mean ± SD, n= 10 animals.
**p<0.05, **p<0.01 and ***p<0.001.*
a: mean significance difference from control group. B: mean significance difference from hypothyroid group.

Table 4.
Thyroid hormones and their carrying proteins levels of in the studied groups.

Parameter	Control	G	H	H + G
FSH (mIU/L)	7.11 ± 0.16	7.98 ± 0.14	5.99 ± 0.18 (a*)	7.03 ± 0.12
LH (ng/L)	6.47 ± 0.29	7.38 ± 0.38	4.35 ± 0.19 (a**)	6.73 ± 0.28 (b**)
E2 (Pg/ml)	20.76 ± 0.64	20.3 ± 0.39	14.7 ± 0.8 (a*)	18.00 ± 0.77 (a*,b**)
P (ng/ml)	23.24 ± 1.66	20.66 ± 1.51	30.41 ± 1.18 (a*)	20.56 ± 1.67 (b*)
T (ng/ml)	0.32 ± 0.06	0.35 ± 0.03	0.39 ± 0.07 (a*)	0.35 ± 0.04
PRL (ng/ml)	94.06 ± 4.89	104.12 ± 4.41	149.11 ± 11.33 (a**)	101.02 ± 6.58 (b*)

All data in tables represented by mean ± SD, n= 10 animals.
**p<0.05, **p<0.01 and ***p<0.001.*
a: mean significance difference from control group. B: mean significance difference from hypothyroid group.

Table 5.
Serum fertility hormones levels in hypothyroid and treated adult female albino rats.

(estrogen synthase, CYP19A1), this is a key enzyme converted testosterone into estrogen in granulosa cell [49]. Hyperprolactinemia, as recorded in the present study, negatively affected the activity of aromatase enzyme and this lead to hypoestrogenism, inhibited the release of gonadotrophic hormones (LH & FSH) from the pituitary gland and potentiated the inhibitory action of inhibin hormone that stimulated negative feedback and lowered estradiol level [50]. The administration of *Panax ginseng* was ameliorated these changes in trophic and steroidal gonadal hormones due to the triterpenoid saponins which steroidal in nature and considered the precursors of steroidal hormones in both plants and animals [51].

The current study exhibited a significant increase in serum corticosterone hormone. Also, the elevation of 8-hydroxyguanosine, an oxidative stress marker, and Caspase-3, an apoptotic marker in serum and brain tissues (**Table 7** and **Figure 12**). The administration of *Panax ginseng* was renormalized the cortisol and oxidative stress markers by increasing the cellular resistance to stress and potentiated the role

Parameter	Control	G	H	H + G
ERK1/2 (pg/ml)	47.38 ± 1.93	50.17 ± 2.57	63.17 ± 3.13 (a*)	42.26 ± 1.7 (a*b**)
E2/T ratio	64.87 ± 10.3	58.0 ± 13.0	37.6 ± 11.4(a***)	51.4 ± 19.25 (b*)
Corticosterone (μg/dl)	1.8 ± 0.06	1.7 ± 0.11	2.82 ± 0.21 (a**)	1.73 ± 0.10 (b*)

All data in tables represented by mean ± SD, n= 10 animals.
**p<0.05, **p<0.01 and ***p<0.001.*
a: mean significance difference from control group. B: mean significance difference from hypothyroid group.

Table 6.
ERK1/2, E2/T ratio and cortisol level in serum of hypothyroid and treated female albino rats.

Parameter	Control	G	H	H + G
8-OH Guanosine (ng/L)	48.46 ± 2.21	32.79 ± 0.05	57.99 ± 0.02 (a*)	48.63 ± 0.08 (b*)
Caspases (ng/ml)	5.26 ± 0.20	5.94 ± 0.15	12.44 ± 0.67 (a***)	5.48 ± 0.14 (b***)

All data in tables represented by mean ± SD, n= 10 animals.
**p<0.05, **p<0.01 and ***p<0.001.*
a: mean significance difference from control group. B: mean significance difference from hypothyroid group.

Table 7.
Oxidative stress markers in serum of hypothyroid and ginseng-treated female albino rats.

	Control	H	G	H + G
f T4 (μg/ml)	4.44 ± 0.11	3.97 ± 0.10 (a*)	3.65 ± 0.34 (a*b*)	4.08 ± 0.58 (c*)
f T3 (μg/ml)	1.62 ± 0.08	1.40 ± 0.08 (a*)	1.49 ± 0.12 (a*b*)	1.51 ± 0.11 (a*b*)
TSH (mIU/ml)	12.47 ± 0.42	15.81 ± 0.53 (a**)	10.87 ± 0.48 (a***b***)	13.57 ± 0.87 (b***c***)
TTR (ng/ml)	51.23 ± 2.39	15.07 ± 0.14 (a***)	42.1 ± 2.88 (a**b***)	33.28 ± 1.88 (a***b***c**)
TBG (pg/ml)	1.58 ± 0.04	2.09 ± 0.08 (a*)	1.75 ± 0.39 (a*b*)	1.73 ± 0.35 (a*b*)

All data in tables represented by mean ± SD, n= 10 animals.
**p<0.05, **p<0.01 and ***p<0.001.*
a: mean significance difference from control group. B: mean significance difference from hypothyroid group.

Table 8.
Effect of ginseng on f T3, f T4, TSH, TBG and TTR in brain tissue of control and hypothyroid-treated rats.

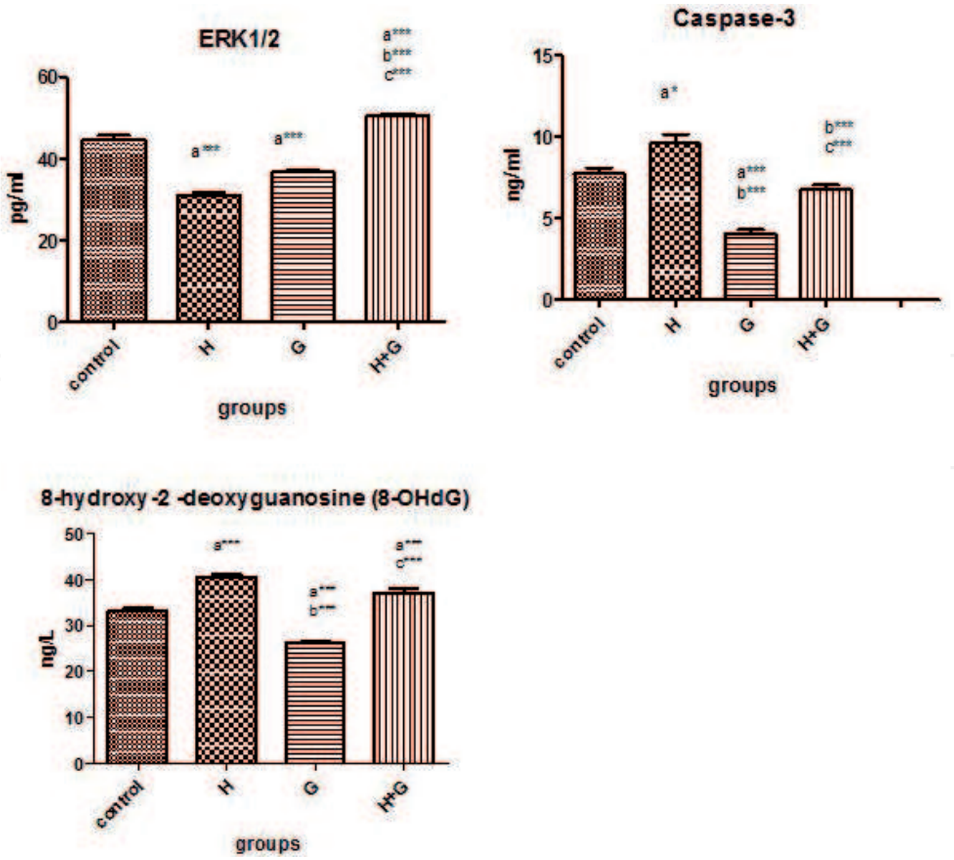

Figure 12.
Effect of ginseng on ERK1/2, Caspase-3 and 8-OHdG in all studied groups.

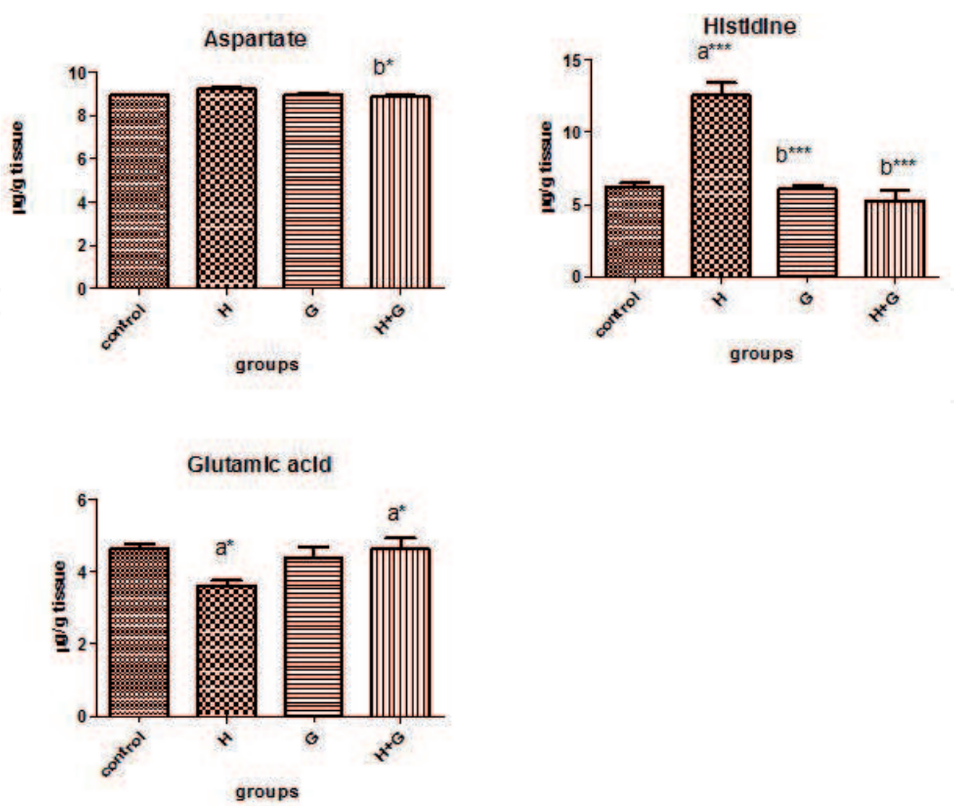

Figure 13.
Effect of ginseng on excitatory amino acids in all studied groups.

of the immune system through triterpenes of ginsenosides [48, 52]. Serum ERK1/2 was activated in response to the elevation of oxidative stress and cell death apoptotic markers as underlined in the present study. Treatment with ginseng ameliorates 8-OHdG, Caspase-3 and ERK1/2 levels referring to its neuroprotective effect and retrieval homeostasis. Also, ginsenosides which is the pharmacologically active constituents with its adaptogenic, powerful antioxidant and radical scavenging activities, regulate the function of HPA, support neurogenesis, synaptogenesis, neuronal growth, and neurotransmission, in turn, protect the central nervous system [53–56]. The study of [57] showed that the panaxatriol group of ginsenosides blocked Caspase-3 expression and increased anti-apoptotic Bcl-2 and p53, indicating that RG repressed cellular apoptosis otherwise, ginsenosides Rd and Re have neuroprotective properties by modulation of ERK1/2 signaling pathway [58]. The elevation of Caspase-3 in the present hypothyroid modal was confirmed by studying the Comet tailed DNA damage of the brain and thyroid tissues as illustrated in **Figures 15** and **16**. Ginseng treatment repair DNA damage in brain and thyroid tissues, this denoted to its highest content of phenolic compounds which act as antioxidants.

Thyroid hormones control the levels of these neurotransmitters which are responsible for maintaining a good mental state and preventing depression [19]. THs regulate both the release of the neurotransmitters and their post-receptor signaling to promote mood stabilization so, their deficiency may weaken the neurogenesis, maturation and synaptic transmission (**Figures 13** and **14**).

The present data, exhibited that the induction of hypothyroidism resulted in a significant decrease of NE, DA and 5-HT concentrations in all studied brain areas

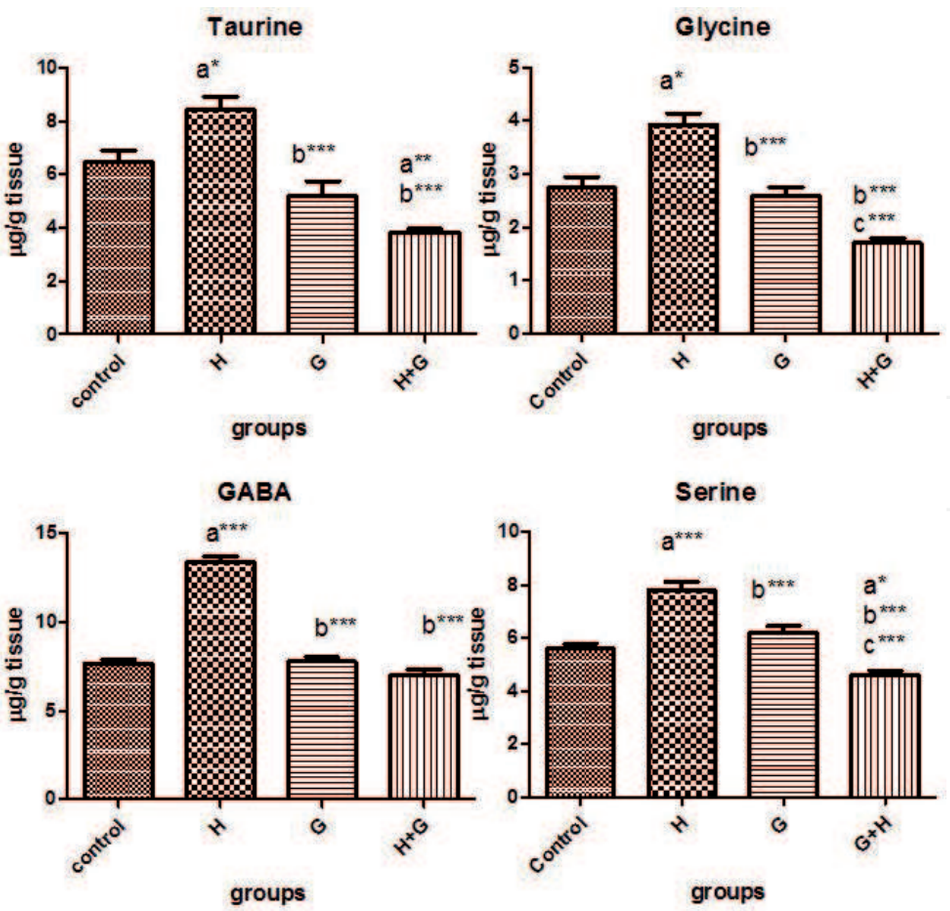

Figure 14.
Effect of ginseng on inhibitory amino acids in all studied groups.

	Frontal cortex	Hippocampus	Hypothalamus	Mid brain	Cerebellum
DA (μg g−1 tissue)					
Control	0.59 ± 0.04	2.39 ± 0.18	1.47 ± 0.27	1.31 ± 0.01	0.60 ± 0.02
H	0.25 ± 0.02 a***	0.93 ± 0.07 a***	0.89 ± 0.08 a***	0.89 ± 0.03 a***	0.27 ± 0.01 a***
G	0.58 ± 0.01b***	2.74 ± 0.08b***	1.69 ± 0.17b***	1.31 ± 0.01b**	0.59 ± 0.01b***
H + G	0.44 ± 0.02a***b**c***	1.67 ± 0.07a***b***c***	1.12 ± 0.04a**c***	1.18 ± 0.01a***b***c***	0.50 ± 0.01a**b***c**
NE (μg g−1 tissue)					
Control	0.51 ± 0.07	0.68 ± 0.04	0.39 ± 0.01	0.70 ± 0.01	0.58 ± 0.03
H	0.21 ± 0.02 a***	0.31 ± 0.02 a***	0.13 ± 0.02 a***	0.35 ± 0.02 a***	0.29 ± 0.01 a***
G	0.56 ± 0.08b***	0.67 ± 0.06 b***	0.38 ± 0.02b***	0.69 ± 0.01b***	0.6 ± 0.01b***
H + G	0.35 ± 0.03a***b**c***	0.54 ± 0.03a***b***c***	0 .29 ± 0.01a***b***c***	0.56 ± 0.03a***b***c***	0.49 ± 0.01a***b***c***
5-HT (μg g−1 tissue)					
Control	0.57 ± 0.03	0.38 ± 0.02	0.78 ± 0.03	0.72 ± 0.03	0.47 ± 0.02
H	0.23 ± 0.01 a***	0.11 ± 0.01 a***	0.45 ± 0.02 a***	0.29 ± 0.01 a***	0.18 ± 0.01 a***
G	0.57 ± 0.03b***	0.39 ± 0.01b***	0.77 ± 0.02b***	0.70 ± 0.03b***	0.47 ± 0.01b***
H + G	0.41 ± 0.02a***b***c***	0.29 ± 0.01a***b***c***	0.56 ± 0.02a***b***c***	0.5 ± 0.01a***b***c***	0.34 ± 0.01a***b***c***

Data in tables given are mean ± S.D. the number of animals was 10 in each group.
***Significant at p 0.01 and ***significant at p 0.001.*
(a) Significant versus control group; (b) significant versus hypothyroid (H) group and (c) significant versus ginseng (G) treated group.

Table 9.
Effect of ginseng on monoamines levels of discrete brain regions in control and hypothyroid-treated rats.

(frontal cortex, hippocampus, hypothalamus, midbrain and cerebellum) as shown in **Table 9**. Monoamines reduction after hypothyroidism refereed to the disturbance in the synthesis and release of these amines from impairment neurons or may be due to an alteration pattern of their synthesizing and/or degradative enzymes [59]. Ginseng treatment ameliorate the reduced monoamine levels of hypothyroid rats and this refers to its powerful ability to maintain homeostasis and modulating neurotransmitter levels hence can amend the neurodegenerative diseases [54, 60]. Also, ginseng saponins modulate dopaminergic activity at both presynaptic and postsynaptic receptors [54, 55].

Improvement of monoamines after ginseng treatment refer also to gintonin which is one of the important ginseng constituents that increased the expression of learning and memory and modulate cholinergic, glutaminergic and other molecular signaling pathways that are vital for cognitive activity as stated in [16]. Ginseng which considered a potential phytoestrogen exhibits antidepressant so, ginsenosides Rb1 enhances the serotonergic system by increasing 5-HT synthesis,

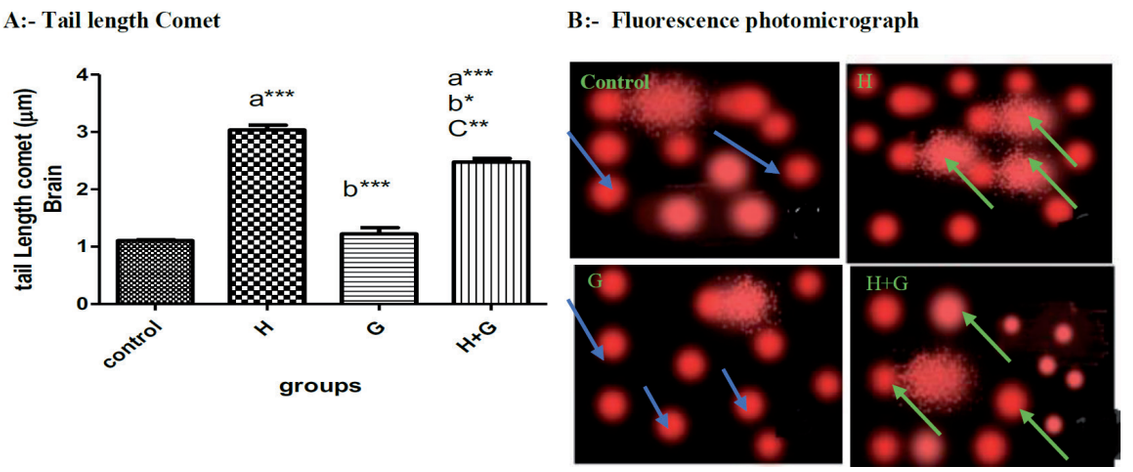

Figure 15.
*Effect of ginseng on DNA damage in whole brain tissue, (A) Tail length expressed in µm in brain tissue of all treated groups. *Significant at p. 0.05, **significant at p. 0.01 and ***significant at p. 0.001. (a), significant versus control group, (b) significant versus hypothyroid, (H) group and (c) significant versus ginseng, (G) treated group, (B) Fluorescence photomicrograph showing comets in control, H, G and H+G -groups. (blue arrows) indicated the intact DNA and (green arrows) indicated the degree of damaged DNA (tail).*

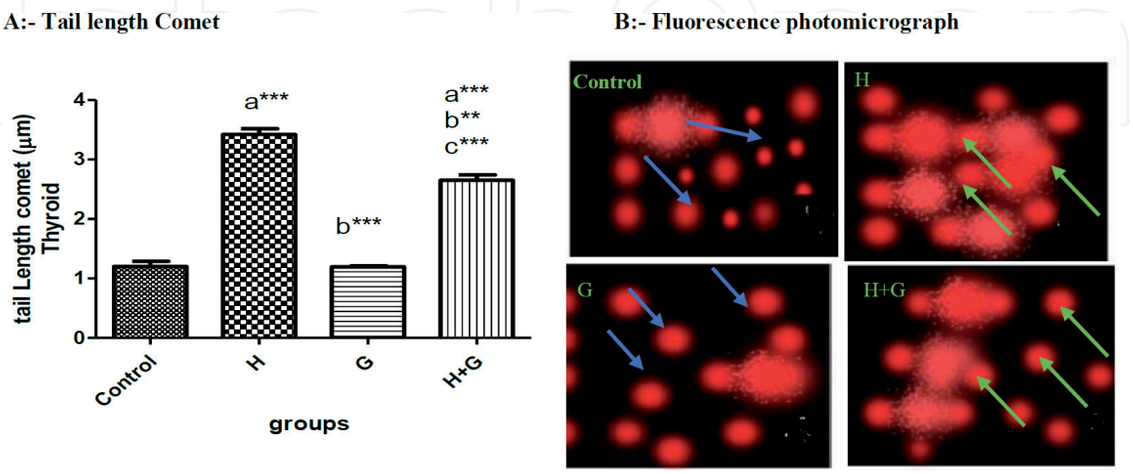

Figure 16.
*Effect of ginseng on DNA damage in thyroid tissue. (A) Tail length expressed in µm in thyroid tissue of all treated groups. ** significant at p 0.01 and *** significant at p 0.001. (a) significant versus control group, (b) significant versus hypothyroid, (H), group and (c), significant versus ginseng (G), treated group (B), Fluorescence photomicrograph showing comets in control, H, G and H+G -groups. (blue arrows) indicated the intact DNA and (green arrows) indicated the degree of damaged DNA (tail).*

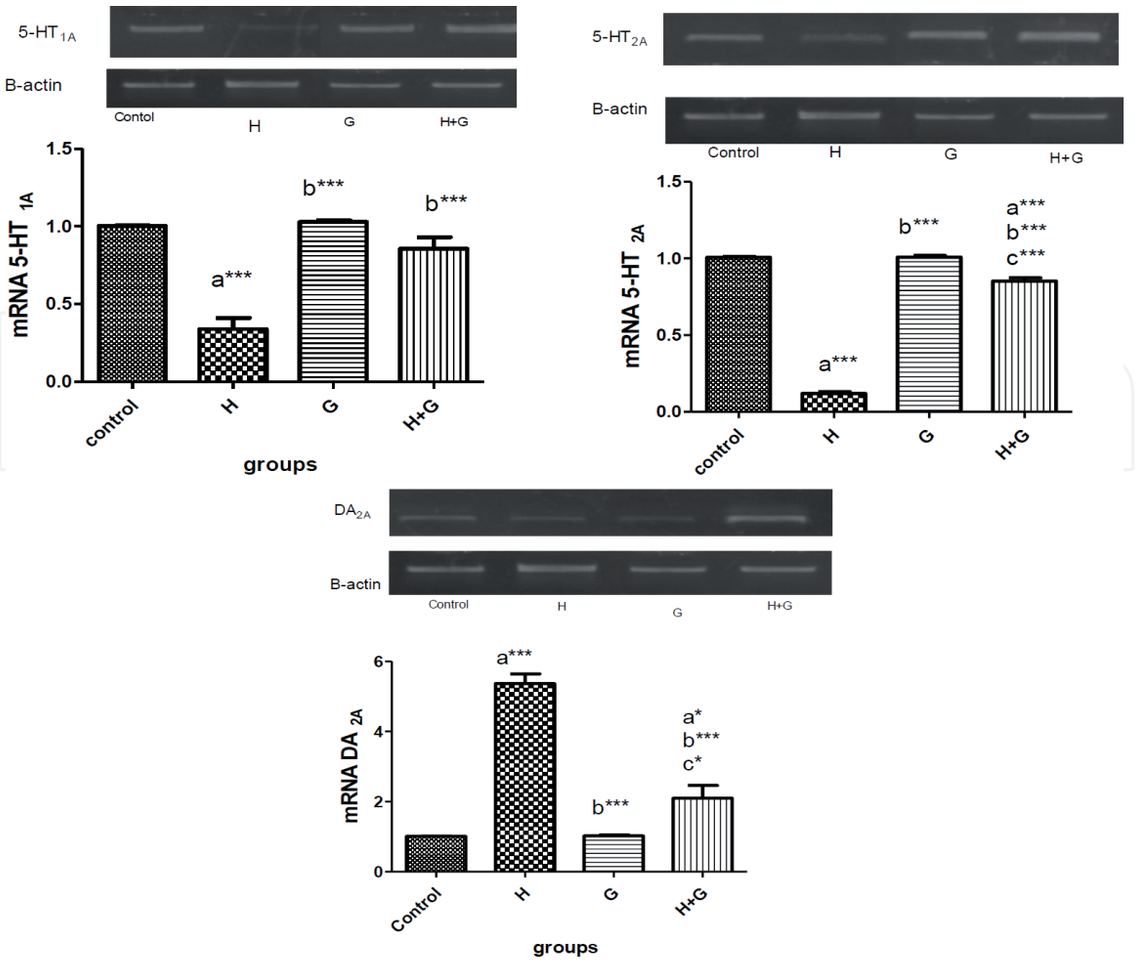

Figure 17.
*Effect of ginseng on expression of mRNA 5-HT1A, mRNA 5-HT2A and mRNA DA2A. *Significant at p 0.05, and ***significant at p 0.001. (a) Significant versus control group (b) significant versus hypothyroid (H) group and (c) significant versus ginseng (G) treated group.*

decreasing 5-HT degradation, stimulating 5-HT2A receptor and suppress the activity of the inhibitory 5-HT3A receptor in the brain. Also, effect via increasing 5-HT activity. This effect is mediated by the activation of estrogen receptor [61, 62]. In the present study, hypothyroidism induction by Neo-Mericazole lead to a significant increase of excitatory amino acid, histidine, and all inhibitory amino acids while excitatory glutamic acid was significantly decreased in brain tissues (**Figures 15** and **16**). *Panax ginseng* with its powerful components ameliorates the disturbance of amino acids and in turn monoamines. The induction of hypothyroidism revealed an elevation in the concentration of dopamine receptors and the reduction of serotonin receptors density (**Figure 17**). Treatment with ginseng restores the level of dopamine and serotonin receptors density towards the control value. This refers firstly to the genomic pathway of ginsenosides which bind to intracellular nuclear hormone receptors like androgen receptor (AR), estrogenic receptor (ER) and progesterone receptor [13].

5. Conclusion

In conclusion, the present study is pointed out to the pituitary-gonad-adrenal disturbances aroused from the hypothyroidism induction by Neo-Mericazole and how ginseng, one of the most Asian medicinal traditional plants, significantly normalized the fertility disorders and stress by acting as free radicals' scavenger.

Conflict of interest

The authors declare no conflict of interest.

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