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IMPACT OF FOLIC ACID ON THE NEUROTRANSMITTERS AND OXIDANT-ANTIOXIDANT BALANCE IN HYPOTHYROID AND HYPERTHYROID RATS

HEBA BARAKAT* AND SHIMAA EL-MASRY

Department of Biochemistry and Nutrition, Faculty of Women for Arts, Science and Education Ain Shams University, Cairo, Egypt

ABSTRACT

Thyroid hormones status plays an important role in various body activities. Thus, this study aimed to clarify the effect of hypothyroidism and hyperthyroidism in rats on the serum neurotransmitters, oxidative stress, total homocysteine and antioxidant defense system in relation to folic administration. The adult male rats were administered carbimazole and eltroxin to induce hypo and hyperthyroidism, respectively. In hypothyroid group, a marked depletion was observed in serum free thyroxine and free triiodothyronine with a significant increase in the thyroid stimulating hormone levels. The reverse pattern was recorded in hyperthyroid group. All of these variations were modulated by folic acid. The neurotransmitters were greatly disturbed; however folic acid administration exerted beneficial effects. In both of the thyroid statuses, serum oxidative stress markers were increased but glutathione reductase was inhibited. Folic acid administration lowered the serum total homocysteine level in both of the hypothyroid and hyperthyroid.

KEYWORDS: **Thyroid, folic acid, neurotransmitters, oxidative stress, homocysteine**

*Corresponding author

HEBA BARAKAT
Department of Biochemistry and Nutrition, Faculty of Women for Arts, Science and Education
Ain Shams University, Cairo, Egypt

1. INTRODUCTION

Thyroid hormones, including tri-iodothyronine (T_3) and tetra-iodothyronine (T_4) are critical in regulating the growth and differentiation of many tissues and organs[1]. They have also shown to play a crucial role in the development and physiological functioning of the central nervous system (CNS), not only during brain maturation but also in adult vertebrate brain[2]. Alterations in thyroid hormone normal levels cause some biochemical and clinical abnormalities such as hypothyroidism (Hypo) and hyperthyroidism (Hyper)[3]. Thus any disturbance during the developmental period may result in an irreversible impairment, morphological and cytoarchitecture abnormalities, disorganization, mal-development and physical retardation that are permanent[4]. These effects may be responsible for the loss of neural vital functions and may lead, in turn, to biochemical dysfunctions[5]. Moreover, adult-onset thyroid dysfunction is also associated with neurological abnormalities[6].It has been suggested that thyroid dysfunction may be linked with abnormalities in central noradrenergic neurotransmission[7]. Several lines of evidence also support a relationship between thyroid hormones and serotonergic transmission in the brain[8]. Hence, the alterations in these hormones affect the characteristics of various neurotransmitters and neurotransmission in brain of mammals[9]. Folic acid is a water-soluble vitamin, which is essential in our life. It is essential to numerous body functions ranging from nucleotide biosynthesis to the remethylation of homocysteine (Hcy)[10]. Folic acid can be used to help in Alzheimer`s disease treatment, depression, anemia and certain types of cancers[11]. Folic acid status is affected by Hypo. In healthy individuals, folic acid is converted to L-methyl folate, which is the biological active form of the vitamin. Hypo causes a decrease in the activity of the enzyme methylenetetrahydrofolate reductase, which is responsible for producing L-methyl folate in the liver[11-14]. On the other hand, Hyper in man is associated with depletion of folate stores and subclinical deficiency of this vitamin. This is attributed to an increased demand for folic acid in the hyper-metabolic state[15]. There is a little information about the effect of folic acid administration on neurotransmission in cases of thyroid abnormalities. So, this study aimed to investigate the effect of dietary folic acid on the changes of neurotransmitters and oxidant-antioxidant balance of the brain in both of the Hypo- and Hyper- thyroid rats.

2. MATERIALS AND METHODS

2.1. Experimental animals

The present study was carried out on sixty adult male albino rats "Sprague-Dawely strain" weighing 180-200 g. They were obtained from the Medical Research Center, Faculty of Medicine, Ain Shams University. The rats were kept in animal house for 4 days for acclimatization. They were housed in stainless steel cages at normal atmospheric temperature and normal daily light/dark periods of 12 h each. They were fed on a purified standard diet prepared according to AIN[16]and tap water was used for drinking *ad-libitum*.

2.2. Experimental Schedule

The rats were allocated into 5 groups as follows Group (1): Control group (CO): Rats were provided with the standard diet and tap water. Group (2): Hypothyroid group (Hypo): Rats were rendered hypothyroid by administration of antithyroid agent, carbimazole (Chemical Industries Development, Giza, Egypt), an inhibitor of triiodothyronine (T_3) and thyroxine (T_4) synthesis[17], in drinking water at concentration of 0.02% (weight per volume; w/v)[18].Group (3): Hypothyroid group administered folic acid (Hypo+Folic): Rats received 0.02% carbimazole in drinking water and fed on a purified standard diet supplemented with 0.006% folic acid[19] (Nile Company for Pharmaceutical and Chemical Industries, Egypt). Group (4): Hyperthyroid group (Hyper): Rats were rendered hyperthyroid by administration of exogenous T_4, eltroxin (Glaxowellcome, Germany)[15], in drinking water at concentration of 100µg/Kg body weight intragastrically. Group (5): Hyperthyroid group administered folic acid

(Hyper+Folic): Rats received 100µg/Kg eltroxin in drinking water and fed on a purified standard diet supplemented with 0.006% folic acid.

2.3. Body weight measurement

Body weight was recorded weekly beginning on zero time (the time prior to treatment) and continued until the end of the treatment (4 weeks).

2.4. Handling of tissue and serum

Blood samples were collected from rats and allowed to coagulate at room temperature. All samples were centrifuged at 2000rpm for 15 min to separate serum. The brain was immediately removed and washed with ice-cold phosphate buffered saline, then plotted on filter paper to remove excess fluid, finally weighed and stored.

2.5. Determination of thyroid hormones level

Serum free T_3 (FT_3) and T_4 (FT_4) levels were determined using (Acculite) kit by competitive chemiluminscence immunoassay analog method according to Verheecke[20] and Midgalsy-John[21], respectively. Serum thyroid stimulating hormone (TSH) level was determined by kit according to Wada et al.[22]

2.6. Determination of monoamines level

The values of neurotransmitters; dopamine (DO), serotonin (SE) and noradrenaline (NA) were determined in serum samples according to the methods of Kim et al.[23]; Trofs et al.[24]and Westermann et al.[25], respectively.

2.7. Determination of oxidative stress markers and antioxidant defense system levels

Serum peroxidation index was determined as malondialdehyde (MDA) and nitric oxide (NO) by using colorimetric methods according to Draper and Hadley[26]and Berkels et al.[27], respectively. Also the serum non-enzymatic antioxidant, reduced glutathione (GSH) was determined according to Oberley and Oberley[28].

2.8. Determination of serum total homocysteine (t-Hcy) level

Serum t-Hcy was determined using HPLC technique with the following condition; flowrate 1/min; Agilent 1100 series (Waldborn, Germany), quaternary pump (G1311A), Degasser (G1322A), Thermostated Autosamples (G1329A), variable wave length detector (G1314A); and column: Zorbax 300SB C18 column (Agilent Technologies, USA). Injection was carried out at wave lengths 254 nm for separation according to the method of Jacobsen et al.[29].

2.9. Statistical analysis

The statistical analysis for the biochemical data was performed using SPSS version 9.0. Data were expressed as mean ±S.D. Statistical differences between groups were performed using student t-test, differences considered significant when $P<0.05$.

3. RESULTS

3.1. Effect of thyroid status on body weight

Table (1) shows that at the beginning of the experiment, there was no significant difference between all the experimental groups ($P>0.05$). At the end of the 4 weeks, it is clear that there was a significant difference in the body weight between the control group and each of the Hypo and Hyper groups ($P<0.05$). That the Hypo rats recorded an increase in the body weight values contrasting to the Hyper rats. However, there was no significant effect of the dietary folic acid administration of either the Hypo or Hyper groups.

Table 1
Initial, final and change in body weight values of adult male albino rats classified on different experimental designs

Group	Initial body weight (g)	Final body weight (g)	Change body weight (g)
Control	200.6 ± 17.04	269.0 ± 17.90	68.4 ± 11.76
Hypo	204.0 ± 8.86	217.6 ± 7.44	13.6 ± 3.91
Hypo + folic acid	201.0 ± 11.66	209.6 ± 12.03	8.6 ± 0.89
Hyper	203.0 ± 16.29	188.6 ± 19.96	-14.4 ± 4.34
Hyper +folic acid	197.8 ± 11.69	196.0 ± 4.53	13.52 ± 30.53

Values are the mean ± S.D., n = 12.

3.2. Effect of thyroid status on thyroid hormone levels

Hypo- and Hyper-thyroidism induced by oral administration of carbimazole and eltroxin, respectively were verified by measuring FT_3 , FT_4 and TSH levels in serum control, Hypo and Hyper groups. As well as for the folic acid treated groups to follow up its beneficial effect. As noticed from table (2), the lowest levels of serum FT_3 and FT_4 was recorded in the Hypo rats, whereas the highest values were for the Hyper rats compared with the other groups. The administration of dietary folic acid alleviates these variations with no significant difference between both of the treated groups ($P>0.05$). As expected the serum TSH level was in an inversely relation with the levels of both T_3 and T_4 for the different groups. Moreover, there was no significant difference between the serum TSH levels of both the folic acid treated groups ($P>0.05$).

Table 2
Serum free T3, T4 and TSH values of adult male albino rats classified on different experimental designs

Group	Free T3 (Pg/ml)	Free T4 (ng/dL)	TSH (µIU/ml)
Control	2.77 ± 0.191	1.86 ± 0.182	$0.146 \pm 2.79 \times 10^{-2}$
Hypo	$1.13 \pm 4.32 \times 10^{-2}$	$0.776 \pm 9.56 \times 10^{-2}$	$0.342 \pm 4.87 \times 10^{-2}$
Hypo + folic acid	1.42 ± 0.109	1.28 ± 0.138	$0.242 \pm 2.86 \times 10^{-2}$
Hyper	3.19 ± 0.464	5.60 ± 0.560	$8.6 \times 10^{-2} \pm 1.67 \times 10^{-2}$
Hyper +folic acid	1.84 ± 0.241	3.16 ± 0.345	$0.196 \pm 2.07 \times 10^{-2}$

Values are the mean ± S.D., n = 12.

3.3. Effect of thyroid status on monoamine levels

From table (3), it is obvious that, DO levels in serum of rats of either Hypo or Hypo+ folic acid groups were largely affected by a significant decrease ($P<0.05$) compared with the control group. However, the hyperthyroidism treatments did not differ significantly ($P>0.05$) with the control group. Also, it is clear that there is no significant difference between the serum SE levels of the experimental groups as compared with the control group. Contrasting to SE, the serum NA levels of the different experimental groups recorded a significant difference ($P<0.05$) as compared with the level of the control group.

Table 3
Serum monoamine neurotransmitter values of adult male albino rats classified on different experimental designs

Group	Dopamine (ng/ L)	Serotonin (ng/ L)	Noradrenaline (ng/ L)
Control	34.25 ± 7.82	230.08 ± 11.26	204.39 ± 5.12
Hypo	24.94 ± 3.31	224.44 ± 11.72	113.80 ± 12.34
Hypo + folic acid	18.60 ± 5.41	197.58 ± 19.90	179.96 ± 12.17
Hyper	36.10 ± 2.56	202.12 ± 12.51	268.32 ± 13.48
Hyper +folic acid	31.30 ± 2.22	190.94 ± 10.32	242.00 ± 8.24

Values are the mean ± S.D., n = 12.

3.4. Effect of thyroid status on oxidative markers and antioxidant levels

Table (4) showed that the rats of the Hypo group had the highest significant ($P<0.05$) serum level of tHcy among all the experimental groups. However this elevation was alleviated by the administration of folic acid for 4 weeks. The serum tHcy of the Hyper group was lower than that of the control group. Moreover the Hyper+ folic acid group recorded the lowest serum tHcy value. The results of serum MDA and NO as markers of lipid peroxidation were affected similarly. The induction of either hypo- or hyper-thyroidism in rats elevated significantly ($P<0.05$) the serum peroxidation levels of MDA and NO compared to the levels of control group. However these elevations were modulated by the administration of folic acid, that there was no significant difference between the two folic acid supplemented groups compared by the control group. On the other hand, the serum non enzymatic antioxidant GSH levels of either the Hypo or Hyper rats recorded no significant difference ($P>0.05$) compared with the level of the control group. Whereas, the Hypo or Hyper groups administered folic acid were decreased significantly ($P<0.05$) comparing with either the level of control or the non-administered groups.

Table 4
Serum total homocysteine, malondialdehyde, nitric oxide and reduced glutathione values of adult male albino rats classified on different experimental designs

Group	Total homocysteine (mg/ml)	Malondialdehyde (nmol/ml)	Nitric oxide (µmol/L)	Reduced glutathione (mg/dL)
Control	1.87 $X10^{-4}$	18.00 ± 1.30	1.61 ± 0.14	3.70 ± 0.50
Hypo	2.14 $X10^{-4}$	29.19 ± 1.45	2.56 ± 0.13	3.37 ± 0.42
Hypo + folic acid	1.27 $X10^{-5}$	23.08 ± 1.43	1.80 ± 0.14	2.59 ± 0.19
Hyper	6.26 $X10^{-5}$	31.79 ± 1.33	2.80 ± 0.10	3.19 ± 0.14
Hyper +folic acid	9.18 $X10^{-5}$	21.54 ± 1.62	1.81± 9.83$X10^{-2}$	1.68 ± 0.33

Values are the mean ± S.D., n = 12: except the values of total homocysteine represents as values.

4. DISCUSSION

4.1. Effect of thyroid status on thyroid hormone levels

In the present study, hypothyroidism was successfully induced in adult rats by oral administration of carbimazole as indicated by the significant reduction in serum FT_3 and FT_4 levels and the significant elevation in serum TSH level as compared to control group. In addition, the folic acid group showed an improvement in these results. These findings were similar to those obtained by Ibrahim et al.[30] and Hassan et al.[6].These results may be due to that carbimazole reduces circulating levels of thyroid hormones by inhibiting thyroperoxidase activity within the thyroid gland leading to block the organification of thyroglobulin and thus block thyroid hormones synthesis. This in turn leads to increased TSH

level due to the lack of negative feedback from the thyroid hormones. From the results, rats administered with folic acid showed great improvement in serum FT_3, FT_4 and TSH values. These results disagreed with Ibrahim et al.[30] who remarked that, plasma TSH concentration showed dramatic elevation in hypothyroid rats co-treated with folic acid than the values in rats were administered with propylthiouracil. These findings were explained by Tousson et al.[9]as the increase in TSH can be explained by decreased production of T_3 from the thyroid gland that minimizes TSH feedback inhibition of the interior pituitary gland. In order to ensure the hyperthyroid state, serum FT_3, FT_4 and TSH levels were evaluated at the end of the experimental period where serum FT_3, FT_4 levels are increased and serum TSH levels aredecreased in rats received eltroxin indicating the induction of hyperthyroid state. The increase in serum T_3 level in hyperthyroid rats may arise from alteration in the monodeiodination pathway[18]. Moreover, Wemeau[31]reported that hyperthyroidism leads to an overproduction of thyroid hormones, which determine diverse expressions of thyrotoxicosis. Administration of folic acid to the hyperthyroid rats, exert an improving effect in lowering both of the thyroid hormones and elevating the TSH level. This may be explained by the effect of folic acid on TSH secretion which in turn induces a feedback on the lowered production of the thyroid hormones.

4.2. Effect of thyroid status on body weight

From the present study, it could be noticed that the hypothyroid rats had an increase in body weight at the end of 4 weeks. This increase in body weight during hypothyroidism is presumably a consequence of increased energy availability associated with a reduction in the basal metabolic rate[32]. The present study also demonstrated that hyperthyroidism caused a decrease in body weight. These results are consistent with those obtained by other investigators who revealed that hyperthyroidism resulted in a significant decrease in body weight[6,32]

4.3. Effect of thyroid status on monoamines

Interaction of the thyroid and monoamine neurotransmitters (DO, SE and NA) systems has been suggested as a potential underlying mechanism of action. Thyroid hormones are known to modulate a number of neurotransmitter system[34].The effects of hypothyroidism were investigated on the serum levels of some neurotransmitters. The present data revealed that administration of carbimazole to adult rats provoked a significant reduction in serum DO, SE and NA. The administration of folic acid did not exert any beneficial effect in either DO or SE in contrast to NA which is increased compared with the hypothyroid rats. These changes are probably related to hormonal influences in the induction and activities of enzymes involved in biosynthesis metabolism of neurotransmitters. These observations are in concurrence with many other publications indicating that discrete brain regions of adult rats[34,35]. More recently, Tousson et al.[36]observed a significant decrease in NA and SE concentrations in hypothalamus and cortex of hypothyroid rats. Tan et al.[37]reported that dopaminergic dysfunction is associated with thyroid deficiency. Moreover, the reduced levels of thyroid hormones appear to slow serotonergic neurotransmission in the brain[35] this effect was associated with low mood. Furthermore, Hassan et al.[6]reported that depression causes an inhibition of type II deiodinase enzyme that leading to a decrease in the brain level of T_3 and consequently contributing to the decrease of SE in depressive picture. The results obtained from the current study had shown that hyperthyroidism provoked a marked increase in the serum levels of NA and DO with an unexpected decrease in the serum SE level. On the other hand, folic acid administration to the hyperthyroid rats exerts an overall decrease in all of the three monoamines.These results may be due to that, hyperthyroidism affect positively on the receptors of NA and DO however that its effect was negatively on SE. The results of NA and DO were correlated to many previous studies[6,38,39]. The increment in these monoamines was explained by Chaube and Joy[40]as an elevation in tyrosine

hydroxylase activity, the first and rate-limiting enzyme in the synthetic pathway of catecholamine following hyperthyroidism.

4.4. Effect of thyroid status on oxidative markers and antioxidant levels

The present study declared an increase in serum tHcy level in hypothyroid group when compared to the control group. This finding is correlated to the results of Orzechowska-Pawilojc et al.[41]and Ibrahim et al.[30]The pathogenesis of elevated tHcy in hypothyroidism can be explained by the fact that hypothyroidism markedly affects riboflavin metabolism, mainly by reducing the activity of flavokinase and thereby the synthesis of FMN and FAD which serve as cofactors for flavoprotein methylenetetrahydrofolate reductase (MTHFR)[41,42]. It is suggested that changes in folate level[43] or in activities of methionine synthase and cystathionine-β-synthase not only MTHFR[44] may be responsible for the increased serum Hcy level in patients with hypothyroidism. An alternative explanation of this effect could be attributed to the reduced glomerular filtration rate in hypothyroidism which is linked to impaired renal Hcy clearance and hyperhomocysteinemia[45]. On the other hand, in the folic acid administered hypothyroid rats there was a great decrease in serum tHcy level compared to the Hypo group. This finding suggests the role of folic acid supplementation to decrease Hcy level as reported by Diekman et al.[44] and Clarke et al.[46]. The induction of hyperthyroidism in rats reduced the serum tHcy compared to the control group as expected and referenced in the previous studies. Moreover, the Hyper group supplemented with folic acid had more reduction in tHcy level compared to the Hyper group. Nedrebo et al.[47]reported that, the methylenetetrahydrofolate reductase is increased in hyperthyroidism and decreased in hypothyroidism which may be relevant for the relation between the tHcy level and thyroid status. This enzyme is responsible for the formation of 5-methyltetrahydrofolate, which functions as a methyl donor during remethylation of homocysteine to methionine. Indeed, there is an increase in blood folate observed during the hyperthyroidism which could account for the reduction in Hcy, but folate increases were offset by a decrease in vitamin B_6, a necessary cofactor for the transsulfuration pathway of Hcy metabolism[48]. But that hypothesis may not take place in this study due to that rats were fed on a standard purified diet which contained DL-methionine as a separated component and vitamin mixture composed of all the necessary vitamins for optimum wellness and health. This prediction may explain the increased reduction in the Hyper group ingested folic acid, which may counteract the disturbance in B-vitamins as a result of the hyperthyroidism condition. Oxidative stress is a condition which can be defined as a serious imbalance between production of reactive species and antioxidant defense, and could result from diminished levels of antioxidants and/or increased production of reactive species[49]. Hypothyroidism induces selective oxidative stress in both the hippocampus and amygdala, where the nitrergic system is involved[50]. The results of the present study agreed with that where, MDA- the end product of lipid peroxidation- the most oxidative stress parameter affected by hypothyroidism. The enhanced oxidative stress in hypothyroidism is suggested by Hoch[51] to develop due to oxidation of membrane lipids of cells by hypothyroidism. Furthermore, it is suggested to be associated with the observed hyperhomocysteinemia[52] as represented in the present study by the significant correlation between tHcy and serum MDA. Another explanation of this enhanced oxidative stress could be attributed to folate deficiency associated with hypothyroidism as reported by Diekman et al.[44]. In the folic acid administered rats, results showed that lipid peroxidation significantly decreased in serum as compared to hypothyroid group. This reflects the antioxidant power of folic acid against free radicals[53]. The current study showed that serum total NO was significantly higher in the hypothyroid group when compared to the respective control. This is consistent with the findings of Virdis et al.[54]This finding may be due to increased vascular oxidative burden

associated with homocysteinemia that induces NADPH oxidase and inducible nitric oxide synthase activity, contributing to increased superoxide radicals production in rat vessels. Furthermore, Hcy is closely associated with endothelial dysfunction through its impact on eNOS coupling[55,56]. These superoxide radicals react with nitric oxide to for peroxynitrite radicals. Leading to low endothelial NO bioavailability and endothelial dysfunction. This assumption is confirmed by the significant positive correlation between tHcy and NO presented in the present study. On the other hand, serum NO was decreased in the folic acid administered rats compared with the Hypo rats. This finding can be explained by the ability of folic acid to prevent peroxynitrite-mediated tetrahydrobiopterinoxidation and improve eNOS coupling and dimerization[57]. The significant decrease in serum GSH level in the hypothyroid group corroborates the role of thyroid hormones in triggering the biosynthesis of GSH[58].On the other hand the folic acid-administered hypothyroid group had lower serum GSH level than both of the control and the Hypo groups.

CONCLUSION

Thyroid hormones disturbance had a great effect on the physiological functioning of the CNS and the oxidant-antioxidant balance of the adult male albino rats. However, the dietary folic acid administered to both of the hypo- and hyper-thyroid rats exerted noticeable beneficial effects on almost the examined markers.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFRENCES

1. Sigrun H and Heike H, Review: thyroid hormone action during brain development: more questions than answers. Molecular and Cellular Endocrinol., 315: 19-26 (2010).
2. Anderson GW, Commentary: thyroid hormones and the brain. Frontiers in Neuroendocrinology, 22: 1-17 (2001).
3. Wiens SC and Trudeau VL, Review: Thyroid hormone and γ-aminobutyric acid (GABA) interactions in neuroendocrine systems. Comparative Biochem. Physiol., A144: 332-344 (2006).
4. Argumedo GS, Sanz CR and Olguin HJ, Experimental models of developmental hypothyroidism. Hormone and Metabolic Res., 44(2): 79-85 (2012).
5. Ahmed OM, Ahmed RG, El-Gareib AW, El-Bakry AM and Abd El-Tawab SM, Effects of experimentally induced maternal hypothyroidism and hyperthyroidism on the development of rat offspring: II. The development of pattern of neurons in relation to oxidative stress and antioxidant defense system. Int. J. Devel. Neurosci., 30 (6): 517-537 (2012).
6. Hassan WA, Alt MS, Abdel Rahman T and Shahat AS, Impact of experimental hypothyroidism on monoamines level in discrete brain regions and other peripheral tissues of young and adult male rats. Int. J. Devel. Neurosci., 31: 225-233 (2013).
7. Saravanan P, Visser TJ and Dayan CM, Psychological weak-being correlates with free thyroxine but not free 3,5,3`-triiodothyronine levels in patients on thyroid hormone replacement. J. Clin. Endocrinol. And Metabolism, 91(9): 3389-3393 (2006).
8. Lifschytz T, Segman R, Shalom G,Lerer B, Gur E, Golzer T and Newman ME, Basic mechanisms of augmentation of antidepressant effects with thyroid hormone. Current Drug Targets, 7: 203-210 (2006).
9. Tousson E, Beltagy DM, El-Gerbed MSA, Abo Gazia M and Akela MA, The ameliorating role of folic acid in rat hippocampus after propylthiouracil-induced hypothyroidism. Biomedicine and Aging Pathol., 2: 104-110 (2012).

10. Matte C, Scherer EB, Stefanello FM, Barschak AG, Vargas CR and Netto CA, Concurrent folate treatment prevents Na^+, K^+-ATPase activity inhibition and memory impairments by chronic hyperhomocysteinemia during rat development. Inter. J. Devel. Neurosci., 25: 542-552 (2007).
11. Tousson E, Ali EM, Ibrahim W and Mansour MA, Treatment with folic acid ameliorated the histological alterations caused by propylthiouracil-induced hypothyroid rat testes. Toxicol. Ind. Health, 28: 566-576 (2012a).
12. Ibrahim W, Abo Gazia ME, El-Atrash A, Massoud A, Massoud A, Tousson E and Abou-Hagar H, Ameliorative effect of folic acid on propylthiouracil-induced histology and immunohistochemical alterations in aorta of albino rats. J. Am. Sci., 8: 155-159 (2012).
13. Ibrahim W, Tousson E, El-Masery T, Arafa N and Akela M, The effect of folic acid as an antioxidant on the hypothalamic monoamines in experimentally induced hypothyroid rat. Toxicol. Ind. Health, 28: 253-261(2012a).
14. Tousson E, Ibrahim W, Arafa N and Akela MA, Histopathological changes in rat hypothalamus after propylthiouracil induced hypothyroidism and the protective role of folic acid. J. Neurol. Sci., 29: 705-713 (2012b).
15. Tousson E, Hafez E,Massoud AA, Sweef O and Atta N, Protective role of folic acid in thyroxine-induced cardiac hypertrophy in hyperthyroid rat. Biomedicine and Aging Pathol., 3: 89-95 (2013).
16. AIN by Reeves PG, Nielsen FH and Fahly GC, AIN-93 Purified diets for laboratory rodents: Final report of the American Institute AdHoc writing committee on the reformulation of the AIN-76 A rodent diet. J. Nutr. 123: 1939-1951 (1993).
17. Zaidi TM, Khan AA, Haan BM and Faruqi AN, Carbimazole induced thyroid histology in albino rats during development. J. Anat. Soc. India, 53(2): 14-17 (2004).
18. Ahmed OM, Abd El-Tawab SA and Ahmed RG, Effects of experimentally induced maternal hypothyroidism and hyperthyroidism on the development of rat offspring: I. The development of the thyroid hormones- neurotransmitters and adenosinergic system interactions. Int. J. Devel. Neurosci., 28: 437-454 (2010).
19. Li M, Chen J, Li Y, Feng Y and Zeng Q, Folic acid reduces chemokine MCP-1 release and expression in rats with hyperhomocysteinemia. Cardiovascular Pathology, 16: 305-309 (2007).
20. Verheecke, P, Free triiodothyronine concentrations in serum of 1050 euthyroid children is inversely related to their age. Clin. Chem., 43: 963-967 (1997).
21. Midgalsy-John EM, Direct and indirect free thyroxine assay methods. Theory and practice, Clin. Chem., 47: 1353-1363 (2001).
22. Wada GH, Danisch RJ, Baxter SR, Federici MM, Fraser RC, Browmmiller LJ and Lankford JC, Enzyme immunoassay of the glycoprotein tropic hormones-choriogonadotropin, lutropin, thyrotropin-with solid-phase monoclonal antibody for the α-subunit and enzyme-coupled monoclonal antibody specific for the β-subunit.Clin. Chem., 28 (9): 1862-1866 (1982).
23. Kim J, Jeon M, Paeng K and Paeng IR, Competitive enzyme-linked immunosorbent assay for the determination of catecholamine, dopamine in serum. Anal. Chimica Acta, 619: 87-93 (2008).
24. Torfs SC, Maes AM, Delesalle CJ, Deprez P and Croubels SM, Comparative analysis of serotonin in equine plasma with liquid chromatography-tandem mass spectrometry and enzyme-linked immunosorbent assay. J. Vete. Diagn. Invest., 24(6): 1035-1042 (2012).
25. Westermann J,Hubl W, Kaiser N and Salewski L, Simple, rapid and sensitive determination of epinephrine and norepinephrine in urine and plasma by non-competitive enzyme immunoassay,

compared with HPLC method. Clin. Lab., 48:6171 (2002).
26. Draper HH, and Hadley M, Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol. 186: 421-431 (1990).
27. Berkels R,Purol-Schnabel S and Roesen R, Measurement of nitric oxide by reconversion of nitrate/nitrite to NO. J. Human Press., 279: 1-8 (2004).
28. Oberley TD and Oberley LW, Antioxidant enzyme levels in cancer. Histol. Histopathol.; 12, 525-535 (1997) .
29. Jacobsen DW, Gatautis VJ, Green R, Robinson K, Savon SR, Secic M, Ji J, Otto JM and Taylor LM, Rapid HPLC determination of total homocysteine and other thiols in serum and plasma: sex differences and correlation with cobalamin and folate concentrations in healthy subjects. Clin. Chem., 40(6): 873-881 (1994).
30. Ibrahim W, Tousson E, Ali EMM and Mansour MA, Folic acid alleviates oxidative stress and hyperhomocysteinemia involved in testicular dysfunction of hypothyroid rats. General and Comparative Endocrinol., 174: 143-149 (2011).
31. Wemeau JL, Hyperthyroidism: current concepts and managemen. Rev. Prat., 55(2): 149-157 (2005).
32. Thrift TA, Bernal A, Lewis AW, Neuendroff DA, Willard C and Randel RD, Effects of induced hypothyroidism on weight gains, lactations and reproductive performance of primiparous Brahman cows. J. Animal Sci., 77(7): 1844-1850 (1999).
33. Tikhonova MA, Kulikov AV, Lebedeva EI, Barykina NN,Amstislavskaya TG and Popva NK, On association between cortical 5-Ht_{2A} receptors and behavior in rats with experimental thyroid disturbances. Pharmacol. Biochem. Behavior, 82: 506-514 (2005).
34. Ahmed OM, El-Gareib AW, El-Bakry AM, El-Tawab SMA and Ahmed RG, Thyroid hormones states and brain development interaction. Int. J. Devel. Neurosci., 26: 147-209 (2008).
35. Bauer M, Heinz A and Whybrow PC, Thyroid hormones, serotonin and mood: of synergy and significance in the adult brain. Molecular Psychiatry, 7: 140-156 (2002).
36. Tousson E, Ibrahim W, Arafa N and Akela MA, Monoamines concentrations changes in the PTU-induced hypothyroid rat brain and the ameliorating role of folic acid. Human and Exper. Toxicol. 31(3): 282-289 (2012c).
37. Tan EK, Ho SC, Eng P, Loh LH, Koh L, Lum SY,Teoh ML, Yih Y and Khoo D, Restless legs symptoms in thyroid disorders. Pakinsonism and related disorders, 10: 149-151 (2004).
38. Gur E,Lifschytz T, Lerer B and Newman ME, Effects of triiodothyronine and imiparamine on basal 5-HT levels and 5-HT_{1A}autoreceptor activity in rat cortex, Europ. J. Pharmacol. 457: 37-43 (2002).
39. Lifschytz T, Gur E, Lerer B and Newman ME, Effect of triiodothyronine and fluoxetine on 5-HT_{1A} and 5-HT_{1B}autoreceptor activity in rat brain: regional differences. J. Neurosci. Methods, 140: 133-139 (2004).
40. Chaube R and Joy KP, Thyroid hormone modulation of brain in vivo tyrosine hydroxylase activity and kinetics in the female catfish Heteropneustesfossilis. J. Endocrinol., 179: 205-215 (2003).
41. Orzechowska-Pawilojc A SworczakK, Lewczuk A and Babinska A, Homocysteine, folate and cobalamin levels in hypothyroid women before and after treatment. Endocrinol. J., 54(3): 471-476 (2009).
42. Cakal B, Cakalm B and Demirbasm B, Homocysteine and fibrinogen changes with L-thyroxine in subclinical hypothyroid patients. J. Korean Med. Sci., 22(3): 431-435 (2007).
43. Lien EA, Nedrebo BC, Varhau JE, Nygard O, Vakvaag A and Ueland PM, Plasma total homocysteine levels during short term iatrogenic hypothyroidism. J. Clin.

Endocrinol. Metab., 85: 1049-1053 (2000).
44. Diekman MJ, van der Put NM et al, Determinants of changes in plasma homocysteine in hyperthyroidism and hypothyroidism. Clin. Endocrinol., 54: 197-204 (2001).
45. Turhan S, Sezer S and Erden G, Plasma homocysteine concentrations and serum lipid profile as atherosclerotic risk factors in subclinical hypothyroidism. Ann. Saudi. Med., 28(2): 96-101 (2008).
46. Clarke ZL, Moat SJ and Miller AL, Differential effects of low and high dose of folic acid on endothelial dysfunction in a murine model of mild hyperhomocysteinemia. Eur. J. Pharmacol., 551: 92-97 (2006).
47. Nedrebo BG, Ericsson UB,Nygard O, Refsum H, Ueland PM, Aakvaag A, Aanderud S and Lien EA, Plasma total homocysteine levels in hyperthyroid and hypothyroid patients, Metabolism. 47(1): 89-93 (1998).
48. Colleran KM, Romero LA, Upton DA and Burge MR, Methimazole induced hypothyroidism paradoxically decreases homocysteine. Metabolism Clin. Exper., 54: 460-465 (2005).
49. Halliwell B, Oxidative stress and neurodegeneration: where are we now? Neurochem., 97: 1634-1658 (2006).
50. Cano-Europea E, Perez-Severiano F, Vergara P, Ortiz-Bution R, Rios C and Segovia J, Hypothyroidism induces selective oxidative stress in amygdala and hippocampus of rats. Metab. Brain Dis., 23(3): 275-287 (2008).
51. Hoch FL, Lipids and thyroid hormones. Prog. Lipid Res., 27: 199-270 (1988).
52. Das UN, Folic acid says to vascular diseases. Nutrition, 19: 686-692 (2003).
53. Patro BS, Adhikari S, Mukherjee T and Chattopadhyay S, Folic acid as a Fenton-modulator: possible physiological implication. Med. Chem., 2: 407-413 (2006).
54. Virdis A, Colucci R, and Forrai M, Inducible nitric acid synthase is involved in endothelial dysfunction of mesenteric small arteries from hypothyroid rats. Endocrinology, 150(2): 1033-1042 (2009).
55. Rabelink T and van Zonneveld AJ, Coupling eNOS uncoupling to the innate immune response. Arterioscler. Thromb. Vasc. Biol., 26: 2585-2587 (2006).
56. Shirodaria C, Antoniades C and Lee J, Global improvement of vascular function and redox state with low-dose folic acid: implications for folate therapy in patients with coronary artery disease. Circulatory, 115: 2262-2270 (2007).
57. Moens AL, Champion HC and Claeys MJ, High-dose folic acid pretreatment blunts cardiac dysfunction during ischemia coupled to maintenance of high-energy phosphates and reduces post reperfusion injury. Circulation, 117: 181 0-1819 (2008).
58. Sahoo D, Roy A, Bhanja S and Chainy G, Hypothyroidism impairs antioxidant defense system and testicular physiology during development and maturation. General Comp. Endocrinol., 56: 63-70 (2008).