

Effect of L-theanine and Citicoline on the levels of Sex hormones and Interleukines (IL-4 & IL- 6) in albino male rats exposed to oxidative stress

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Abstract:

The purpose of present study was to investigate the effect of L-theanine and citicoline as antioxidant to protect the male reproductive system and immune system from oxidative stress produced by treatment with H_2O_2 (0.5%) in drinking water for (30) days , and determine the concentration of Testosterone, Follicle stimulating hormone (FSH) Luteinizing hormone (LH), prolactin (PRL) and interleukins (IL4&IL6).

Albino male rats (*Rattus norvegicus*) (3-4) month of age and weighing (250-350 g) were used in the experiment. They were randomly divided to (7) groups : Ist group was conserved as control , second group was treated with H_2O_2 (0.5%), third group treated with L-theanine (6 mg/ kg) , fourth group treated with L-theanine (6mg/kg) + H_2O_2 , fifth group treated with citicoline (20mg/kg) , sixth group treated with citicoline (20mg/kg)+ H_2O_2 and seventh group treated with L-theanine (6mg/kg) +citicoline (20mg/kg) + H_2O_2 . At the end of (30) day of handling period the animals were fasted overnight and sacrificed by decapitation under chloroform anaesthesia and the blood (5ml) was collected by cardiac puncture , and the serum obtained was preserved at (-20)°C until the tests.

The results revealed that induction of oxidative stress in rats lead to a significant ($p \leq 0.05$) decrease in the concentration of Testosterone, FSH, and LH. While the significant increase showed in PRL concentration and non significant differences in (IL-4) level when compared with control group . At the same time it was found the treatment with L- theanine, citicoline alone or together lead to significant increase in the levels of Testo., FSH, LH and decreasing PRL and IL-6.

The study revealed an important effect of L-theanine similar to citicoline effect as antioxidant by reduction the harmful effects of free radicals through improvements was observed in the productivity hormones levels and decreasing of inflammatory interleukin (IL-6) level .

Key words: L-theanine, Citicoline, Testosterone, FSH, LH, Interleukin, Oxidative stress.

Introduction:

Free radicals (FR) are atoms or molecules which contain a single electron or more than one electron in their external orbit , they are produced in living cells especially in mitochondria and they are either Reactive oxygen species or Reactive nitrogen species [1], while oxidative stress it represents a situation that increases the production of free radicals and oxidants in cells so that exceeds the ability of antioxidants to remove or minimize their effects, leading to the formation of oxidative damage to cells and tissues and thus lead to initiation of various diseases in humans, such as cancer , diabetes , cardiovascular diseases and arthritis [2]. To reduce free radicals activity and damages caused by them there are complex systems of multiple models for antioxidants maintained by human and animals , some of them are exogenous and some endogenous. They are a reducing substances by inhibition or ending reactions that electrons moved from a certain material to oxidant factor and that leading to production of intermediate free radicals [3]. Examples to the important antioxidants are amino acid (L-theanine) which is primarily found in green tea [4] it works to reduce the level of cholesterol in the blood prevents oxidation of LDL cholesterol in the arteries helps to lower blood pressure, protects against diabetes [5] and has a role in inhibition of transport of Glutamate to tumor cells and thus leading to prevent the decreasing of Glutathione levels *in vivo*. It also has a major role

in inhibition of efflux of Chemotherapy factors outside of tumor cells, leading to accumulation in the tumor cells as well as its role in protecting normal cells from chemotherapy damage by its antioxidant activity and that maintaining the level of cellular Glutathione [6].

The Citicoline is a complex organic molecule as an intermediate in biosynthesis of phospholipids of cell membrane and also known as Cytidine diphosphate choline (CDP- Choline) [7]. It belongs to a group of biomolecules called nucleotides which plays an important role in cellular metabolism and working to stimulate acetyl choline esterase [8] and it is also used in the treatment of stroke patients by reducing the building of free fatty acids in the site of clot and also serves on the reducing the stimulation of phospholipase [9].

The present study aimed to find out the activity of L-theanine and Citicoline as antioxidants to protect male reproductive system and immune system from free radicals effects by determination of the concentration of Testosterone , FSH, LH , Prolactin and Interleukins (IL-4,6) in adult male albino rats exposed to oxidative stress by hydrogen peroxide in drinking water.

Materials and Methods

Animals:

This study used male albino rats (*Rattus norvegicus*): (250-285)g weights and age ranges between (3-

4)months obtained from the national center for biological research and drug control /Baghdad. The animals were housed in metal cages with dimensions(60x40x40) cm ,with ground furnished of wood shavings. The cages were cleaned daily and water and feed were changed while maintaining sterility, They were housed at ambient temperature of $(25\pm 2)^{\circ}\text{C}$ and (45-55%) humidity, with normal light cycle (12 h light and 12 h dark) and stayed for 2 weeks to adapte the place and to make sure they were free of disease before beginning the experiment. They were fed with standard laboratory diet [10]. Animals were fed diet and water was given *ad libitum* during the experiment period from September (2012) to December(2012).

Experimental Design :

In the present experiment (35) rats were used and divided into (7) groups,(5 per group) were. Equal weights of each group was taken into consideration as much as possible before the start of the study :

Group1 :(Control group) rats were given water and food for(30) days.

Group 2: (Hydrogen peroxide H_2O_2 group) : rats of this group were given(0.5%) of H_2O_2 in drinking water daily for 30 days with replacement of H_2O_2 each two days to maintain the concentration of it.

Group3: (L-theanine group 6mg/kg): rats of this group were orally administrated (pipe feeding) with (6mg/kg)of Theanine for(30) days.

Group4: (L-theanine 6mg/kg + H_2O_2) : rats of this group were orally administrated (pipe feeding) with (6mg/kg)of Theanine plus (0.5%) of hydrogen peroxide for(30) days .

Group5: (Citicoline 20mg/kg): rats of this group were orally administrated (pipe feeding) with (20mg/kg)of Citicoline for(30) days.

Group6:(Citicoline 20mg/kg+ H_2O_2): th rats of this group were orally administrated (pipe feeding) with (20mg/kg)of Citicoline plus (0.5%) of hydrogen peroxide for(30) days .

Group7:(L-theanine 6mg/kg+ Citicoline 20mg/kg+ H_2O_2): rats of this group were orally administrated (pipe feeding) with (6mg/kg)of Theanine (20mg/kg) of Citicoline and(0.5%) of hydrogen peroxide for (30) days .

Materials used in study :

Equipped L-theanine was provided by SOURCE NATURALS, INC. company as a capsule form contain(200 mg) L- theanine and it was used as dose(6 mg/kg) . Citicoline was provided by Spanish Ferrer international ,S.A. company and each ml contain(100 mg) of Citicoline and it was used as dose (20 mg/kg) while hydrogen peroxide provided by British Chemical company GAINDLAND at concentration (35%) and prepared at(0.5%) and was given to animals in drinking water .

Blood Sampling :

At the end of 30 days of the treatment and administration period, the animals were fasted overnight then were painlessly sacrificed by

decapitation under chloroform anaesthesia and the blood (5 mls)were collected by cardiac puncture . Blood was put in tubes without anticoagulant and left in the laboratory temperature for (15) minutes then the serum was separated by centrifuge and save at degree $(-20)^{\circ}\text{C}$ until used for hormonal and immune tests .

Hormonal Assay:

1- Determination of Testosterone conce.: the concention of testosterone was estimated depending on the steps came with kits of American Monobind company and as instruction of the manufacturer's own technology ELISA [11] .

2- Determination of prolactin (PRL) concentration : the concention of Prolactin was estimated depending on the steps came with kits of American Monobind company and as instruction of the manufacturer's own technology ELISA [11] .

3- Determination of FSH concentration : the concention of FSH was estimated depending on the steps came with kits of American Monobind company and as instruction of the manufacturer's own technology ELISA [12] .

4- Determination of LH concentration : the concention of LH was estimated depending on the steps came with kits of American Monobind company and as instruction of the manufacturer's own technology ELISA[13].

Immunological assay :

ELISA technique was used to determine the concentration of (IL-4,IL-6) in sera , these concentrations were estimated using several kits provided by the American Ray Bio company and following the steps came with kits [14].

Statistical analysis:

Descriptive statistics including the mean, standard error, median and rangewere calculated for all variables. The one-way (ANOVA) followed by Duncun multiple range test were used for comparison of different parameters. The data were analyzed by (SAS,2001) software, and($p\leq 0.05$) was accepted as statistically significant[15].

Results and discussion :

The results(table-1) indicate a significant($p\leq 0.05$) decreasing in the concentration of testosterone , FSH and LH in serum of animals exposed to oxidative stress by hydrogen peroxide as compared with normal control group and this is related to harmful effect of hydrogen peroxide on pituitary gland which negatively reflect on their secretion of gonadotrophic hormones (GnH)and play an important role in Spermatogenesis, LH affect on leydig's cells which leading to increasing of testosterone secretion and as known the last one stimulate sertoli cells and that leading to increasing of Spermatogenesis [16].

It was also shown that reactive oxygen species destruct sertoli cells in testis which responsible for secretion of testosterone that responsible for growth and development of the gland.

The administration of treated animals by hydrogen peroxide leading to oxidative stress and the results are many types of reactive oxygen species [17,18] causing pathological changes in cells and tissues as testis and sexual glands [19].

These effects are diverse may inhibit Spermatogenesis [20] or work on breaking down of proteins and rearrangement causing lack of sperm enzymes activity as glutathione peroxidase (GSH-px) and the last is antioxidant enzymes which protect the

body from oxidative stress [17]. And association with this aspectm [19] noticed that reactive oxygen species work on destruction of Leydig cells which responsible for secretion of testosterone in testis and that's leading to decreasing of the level of hormone responsible for prostate and seminal vesicle function and decreasing in the number of sperms in addition of destruction of cells lined with seminal tubules (sertoli cells and spermatogenic cells) and finally leading to interferes sperms maturation [21,22]

Table (1):Effect of Citicoline and Theanine on the levels of Testosterone , Prolactin , FSH and LH in male albino rats exposed to oxidative stress.

Groups	Testosterone (ng/ ml)	Prolactin (ng/ ml)	FSH (mIU/ ml)	LH (mIU/ ml)
Control	5.7 + 0.3 c	7.1 + 0.2 b	6.2 + 0.4 d	7.3 + 0.2 a
H ₂ O ₂	2.0 + 0.1 e	12. + 0.8 a	4.3 + 0.3 e	4.1 + 0.2 d
L-theanine	5.8 + 0.6 c	6.2 + 0.4 c	8.8 + 0.4 a	6.6 + 0.4 b
L- theanine+ H ₂ O ₂	5.2 + 0.4 c	5.9 + 0.7 c	6.9 + 0.3 c	7.4 + 0.3 a
Citicoline	10.4 + 0.9 a	5.3 + 0.3 d	7.1 + 0.2 c	5.9 + 0.4 c
Citicoline+ H ₂ O ₂	8.6 + 0.7 b	4.5 + 0.5 e	7.8 + 0.4 b	5.7 + 0.3 c
L- theanine + Citicoline+ H ₂ O ₂	8.7 + 0.5 b	6.3 + 0.6 c	7.5 + 0.3 b	6.4 + 0.5 b

The significant increase in the concentration of testosterone , LH and FSH in animals treated with citicoline as compared with H₂O₂ group (table- 1) may be related to the role of citicoline to repair the neurological membrane where citicoline enter multiple metabolic pathways : repairing neurological membrane by increasing formation of phosphatidylcholine , repairing damage choline neurons by potential production of acetyl choline and decreasing in free fatty acids building in the site of clot which induced from neuron damage in addition to the profit of phosphatidylcholine from citicoline as intermediate in the building of sphingomyelin the another component of phospholipids of neurological membrane . the ischemic ability of citicoline to preservation on sphingomyelin illustrated after infection of post-perfusion [9] or may related to antioxidant role of citicoline by synthesis of glutathione .Dela Cruz *et al* [23] indicated that choline inter in structure of citicoline may convert to glutathione (GSH) by S-adenosyl-L-methionine (AdoMet) pathway , so that exogenous citicholine participate in the levels of glutathione reductase (GSH -rd).Adibhatla *et al* [24] found the role of citicoline in increasing glutathione reductase level in culture of nerve cells and decreasing caspase-3 that intermediate apoptosis of nerve cells by supporting the antioxidant glutathione system [25], thus participate in reducing the destructive damage of free radicals on pituitary gland and this it reflect positively on their secretion and give a strong reason to physiological balance of LH and elevation of its concentration in serum and testes and the most important antioxidants in reproductive

system and seminal fluid superoxide dismutase (SOD), Catalase (CAT) and sometimes glutathione reductase (GSH-rd [26].

The results also illustrated a significant increasing in concentration of testosterone , LH and FSH in animals treated with theanine as compared with H₂O₂ group , reasons related to that if may convert *in vivo* to glutamate and the last is one of glutathione component which is a peptide consist of three amino acids Cysteine, Glutamat and Glycine [27] and[28] indicated the role of theanine in elevation of levels of enzymatic antioxidants (SOD) and non-enzymatic antioxidants (GSH)in sera of hydrogen peroxide animals .

The results (table -1) also indicated a significant increasing in concentration of prolactin in hydrogen peroxide group , this reasons may be related to oxidative stress resulted by H₂O₂ , [29] indicated that exposure ot oxidative stress increase the level of Glucocorticoids from zona fasciculate of adrenal cortex and it play arole in stimulating the anterior lobe of pituitary gland to secrete prolactin thus leading to elevation prolactin level in blood .and the significant decreasing in gonadotrophin in this group, the significant increasing in prolactin which was recorded in current study concentrations because the increasing in the last one suppress secretion of gonadotrophic releasing hormones from hypothalamus and that leads to inhibition of the secretion of LH and FSH from Anterior lobe of pituitary gland by negative feedback mechanism thus leading to decreasing of gonadotrohins in blood [30]. The association of damage of immune system table-2 illustrated a significant increase in IL-6 concentration

in hydrogen peroxide treated animals as compared with control groups and this increasing may related to inflammation occurring in body and production of this cytokin especially by WBC [31]

Wich was indicated by a significant increase in differential ccount in WBCs in H₂O₂ treated group as compared with control group , there are an increase in lymphocytes that was related to oxidative stress stimulate immune system to active defense cells, thus leading to elevation of WBCs especially T lymphocytes and macrophage as responses to inflammatory state [32] and play a vital role in inflammation and autoimmune diseases as

atherosclerosis, diabetes, prostate cancer and rheumatoid arithritis [33].

Results (table-2) indicated a significant decrease in IL-6 in groups of citicoline and theanine either alone or with H₂O₂ compared with H₂O₂ treated group , and this was related to the antioxidant roles of both by reducing inflammation resulted from oxidative stress which was caused by reactive oxygen species . [28] indicate that the roles in reducing inflammation resulted from oxidative stress by reducing total WBCs of animals treated with citicoline and theanine compared with H₂O₂ group.

Table (2):effect of Citicoline and theanine on the levels of interleukins (4-6) in male albino rats exposed to oxidative stress.

Groups	IL-4 (pg/ ml)	IL- 6 (pg/ ml)
Control	50 + 5 a	258 + 12 b
H ₂ O ₂	49 + 3 a	312 + 15 a
L-theanine	50 + 6 a	268 + 10 b
L-theanine+ H ₂ O ₂	47 + 4 a	262 + 16 b
Citicoline	53 + 5 a	255 + 13 b
Citicoline+ H ₂ O ₂	50 + 3 a	243 + 15 c
L-theanine + Citicoline+ H ₂ O ₂	50 + 7 a	262 + 11 b

The with association with IL-4 (table-2) no significant difference in its concentration in appeared sera of all animals and because of a limited information about this result , that IL-4 doesn't intermediate inflammatory state occurring in body after exposure to H₂O₂ , IL-4 secreted by CD4 T cells which work oplay arole in proliferation of B

lymphocytes which produce Antibody IgE that intermediate allergy [34].

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تأثير الثيانين L-theanine والسيتي كولين Citicoline في مستويات الهرمونات الجنسية والانتروكينات (4و6) في ذكور الجرذان البيض المعرضة للإجهاد التأكسدي

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الملخص

الغرض من الدراسة الحالية اختبار فاعلية الحامض الأميني الثيانين L-theanine والسيتي كولين citicoline كمضادين للأكسدة لوقاية الجهاز التناسلي والجهاز المناعي من تأثير الاجهاد التأكسدي الناتج من المعاملة ببيروكسيد الهيدروجين H_2O_2 (0.5%) في ماء الشرب لمدة 30 يوما من خلال تقدير تراكيز هورمون الشحمون الخصوي Testosterone والهورمون المحفز للجريبات FSH والهورمون اللوتيني LH والانتروكينات Interleukin (IL-4,6) في ذكور الجرذان البيض البالغة *Rattus norvegicus* التي تراوحت اعمارها من (3-4) اشهر واوزانها من (250-285) غم والتي تم الحصول عليها من المركزى الوطني للبحوث والرقابة الدوائية/ بغداد استعملت في الدراسة (35) جرذا وقسمت عشوائيا الى سبعة مجاميع بواقع (5) جرذان لكل مجموعة وعلى النحو الاتي:

مجموعة السيطرة، المجموعة المعاملة ببيروكسيد الهيدروجين H_2O_2 ، المجموعة المعاملة بالثيانين (6ملغم/كغم/وزن الجسم) ، المجموعة المعاملة بالثيانين (6 ملغم/كغم/وزن الجسم) + H_2O_2 ، المجموعة المعاملة بالسيتي كولين (20ملغم/كغم/وزن الجسم) ، المجموعة المعاملة بالسيتي كولين (20ملغم/كغم/وزن الجسم) + H_2O_2 ، المجموعة المعاملة بالثيانين (6ملغم/كغم/وزن الجسم) و المجموعة المعاملة بالسيتي كولين (20 ملغم/كغم/وزن الجسم) + H_2O_2 وبعد مرور 30 يوما على معاملة الجرذان صومت لمدة 24 ساعة وخدرت بالكلوروفورم ومسحب مايقارب (5) مل من الدم من القلب مباشرة بطريقة الطعنة القلبية cardiac puncture وفصل المصل واجريت عليها الفحوصات المطلوبة .

واظهرت النتائج انخفاضاً معنوياً في المجموعة المعاملة ببيروكسيد الهيدروجين في تراكيز هورمونات الشحمون الخصوي والهورمون المحفز للجريبات والهورمون اللوتيني وارتفاعاً معنوياً في تركيز البرولاكتين والانتروكين -6 وعدم وجود فرق معنوي في تركيز الانتروكين -4 مقارنة بمجموعة السيطرة في حين ادت المعاملة الحيوانات المعرضة للاجهاد التأكسدي بالثيانين اوالسيتيكولين او بالثيانين والسيتيكولين معا الى ارتفاع معنوي في تراكيز هورمونات الشحمون الخصوي والهورمون المحفز للجريبات والهورمون اللوتيني وانخفاض معنوي في تركيز البرولاكتين البرولاكتين-6 وعدم وجود فرق معنوي في تركيز الانتروكين -4 مقارنة مع مجموعة ببيروكسيد الهيدروجين. تشير الدراسة الحالية الى الدور المهم للثيانين كمضاد للأكسدة لا تقل عن اهمية السيتيكولين في النقيض من التأثيرات الضارة للجنور الحرة الناتجة من المعاملة ببيروكسيد الهيدروجين من خلال زيادة مستوى هورمونات الخصوبة وخفض ومستوى انتروكين -6 .