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THE PROTECTIVE EFFECTS OF THE ORGANIC SELENIUM (HIGH-SELENIUM YEAST) ON THE OXIDATIVE STRESS CAUSED BY METHANEDIENONE ON THE BRAIN OF MALE RABBITS

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Abstract

The aim of this study is to investigate the protection role of the organic selenium (high-selenium yeast) in adult male rabbits treated with Methandienone and combination with selenium. A total of 20 intact male rabbits were divided into four equal groups: control group receiving distal water, (T1) receiving Methandienone (oral dose of 0.35 mg / kg.),(T2) receiving high-selenium yeast (3 µg/ kg orally)and (T3) receiving both methandienone and selenium. A significant decrease in GSH with an increase in SOD activity were found and a significant increase in the oxidative markers MDA and PCCin serum (0.97+0.021)and in brain tissue (1.01+0.058) in T1 compared with other groups (control, T2 and T3). On the other hand, T3 group shows an increase in serum GSH and a decrease in SOD, MDA and PCC in serum and brain tissue. These changes are significant compared to AAS group (T1). Moreover, there is a significant increase in reverse transcription (mRNA) of Nrf2,TSPO and CYP19A1genes in AAS group(T1) as compared with other groups (T2 and T3). In addition, we showed a significant decrease in levels Nrf2, TSPO, and CYP19A1 mRNA in the selenium-yeast group compared to the AAS group (T1). In conclusion, these results showed that organic selenium (high selenium yeast) has a positive effect (protective role) against oxidative damage to the brain caused by AAS.

Keywords: Methandienone, selenium, PCC, Nrf2,TSPO, and CYP19A1.

Introduction

Anabolic androgenic steroids (AAS) are synthetic derivatives of the male sex hormone testosterone, developed to increase bioavailability and reduce adverse androgenic properties, while maximizing anabolic effects. Nowadays, AAS are clinically used in hormone replacement therapies, including hypogonadism and aging and to treat muscle wasting due to cancer(S. M. Choi & Lee, 2015). In this experiment, we used a compound of the third class AAS (Methandienone), which is alkylated at C17. Since alkylation delays metabolism in the liver, this AAS group is orally active (Basaria *et al*., 2001). It is used in treating postmenopausal osteoporosis and pituitary deficiency dwarfism (Llewellyn, 2011). Methandienone is still produced today, but usually in countries that have strict rules for prescription drugs and companies that still prefer to serve the underground sports market.

Overuse of AASs causes serious side effects that involve the cardiovascular system, mood disorders ,the reproductive system and the liver (van Amsterdam *et al*., 2010).At present, neurobiological mechanisms and sites of action of AASs are unclear. In vitro, low AAS concentrations increase the death of excitotoxi cneurons (Orlando *et al*., 2007).In normal male volunteers, high doses of AAS cause cognitive impairment (Daly *et al*., 2003).The neurotoxicity induced by AASs can be complicated by the induction of excitotoxicity effect. This phenomenon occurs after a massive release of glutamate, However, over activation of NMDA and AMPA receptors by glutamate results in mitochondrial dysfunction, redox imbalance and pro-apoptotic pathways (Prentice *et al*., 2015). AASs have been shown to cause a wide range of toxic effects in the brain, such as direct action on GABA receptor, an excessive increases in cytosolic Ca^{2+}, calcium overload, ATP depletion, neuronaloxidative stress and cell death (M Vicencio *et al*., 2011; Oberlander *et al*., 2012; Basile *et al*., 2013). The objective of this experiment is to determine the AAS-induce oxidative stress in brain and prophylactic role of organic selenium to reduce this effect.

Little is known about the role of AASs in the regulation of oxidative status in the brain. Oxidative stress is characterized by an imbalance between the production of reactive oxygen species (ROS), reactive nitrogen species(RNS) and their elimination (Birben *et al*., 2012). Protein carbonyl content (PCC), which is the most widely used marker of oxidative protein modification (Handayani *et al*., 2019). Nuclear factor erythroid 2 related factor 2 (Nrf2) a sensory regulator of redox homeostasis, Under normal conditions, Nrf2 is detected at very low levels in the cell cytoplasm due to constant degradation of the proteosomes. Nrf2 levels increase with a quick response to a changing intracellular environment, which allows it to be transported into the inner nucleus and increases the expression of these protective genes (more than 100 genes) in order to maintain redox homeostasis in mammalian cells (Tonelli *et al*., 2018). Translocator Protein (TSPO) is a protein expressed at very low levels in the brain, this protein is found in the outer membrane of mitochondria, but with damage or inflammation of the brain, its expression is enhanced in areas of the brain that are specific for damage. TSPO is mainly expressed in two types of cells, microglia and astrocytes (Loth, 2018).

Aromatase(CYP19A1) is the enzyme responsible for all endogenous 17β-estradiol (E2) production in both females and males. Importantly, It' sexpressed in the brain as peripheral organs (i.e., gonads and adipose tissue) (Hara *et al*., 2015).The expression of CYP19A1 correlates with neuroprotection. Indeed, weak neurodegenerative stimuli cause severe neuro degeneration when the aromatase is

genetically or pharmacologically inhibited(Garcia-Segura *et al*., 2003).

The second partition of this research, we used selenium (selenium-enriched yeast) as protection against AAS-induced oxidative stress in brain. Selenium has been found in many studies due to its antioxidant and anti-cancer properties (Ramoutar, 2009; Alqayim, 2019). In cells, selenoproteins have important antioxidant activity and protect microchondria, the plasma membrane and DNA from the oxidative damage of ROS (Zoidis *et al*., 2018). Imam *et al*. (1999) showed the role of selenium in improving against methamphetamine-induced neurotoxicity. Moreover, The positive responses obtained during selenium therapy for neurodegenerative diseases are providing evidence of the important role of reactive oxygen species and oxidative stress in pathological processes (Santamaría *et al*., 2003; Dominiak *et al*., 2016).

Materials and Methods

Animals and experimental design

We used 20 male adult rabbits (8-12 weeks old; body weight 820-1050 g).The animals were divided into four equal groups as follows: Control took 1 ml / kg of distal water orally, T1 received an oral dose of 0.35mg/ kg B.Wt of Methandienone provided by Black dragon pharma (Thailand).T2 was treated with 3 µl / Kg body weight of Se-yeast provided by 21st Century® *(USA*). T3 received both Methandienone and Se-yeast orally (0.35mg/ kg and 3 µl / Kg, respectively). All animals received treatment every day for 60 days by oral administration. After the experiment, animals were anesthetized by double dose anesthesia and blood samples were taken for biochemical analysis and brain tissues for RNA isolation.

Biochemical analyses:

Reduced Glutathione (GSH) was evaluated using a modified method (Burtis *et al*., 2012). Malondialdehyde (MDA) Assay was determined by colorimetric method(TBARS)used by (Tateishi *et al*., 1987). Total-Superoxide Dismutase Activity (SOD) activity was determined as previously described by Fernandez *et al*. (2009) using SOD kit (Elabscience Biotech. Inc. china). Protein carbonyl content (PCC) in both serum and brain tissue was determined by colorimetric method (Levine *et al*., 2000)using Protein Carbonyl Colorimetric Assay Kit (Elabscience Bio.tech. Inc. china).

Gene expression analysis

Total RNA was isolated using the magnatic nanosorbent kit ((RealBest Extraction 100, Vector-Best, Novosibirsk, Russia)) according to the protocol of the manufacturer. The RNA integrity and purity were measured using a spectrophotometer . Afterwards, isolated RNA was then reverse transcribed into cDNA using M-MLV Reverse Transcriptase (*Synthol*, *Russia*) according to the manufacturer's instructions. as a template for the real-time (RT) polymerase chain reaction (PCR) (*EvaGreen*RT-PCR kit; *Synthol*, *Russia*)) with specific primers. All primers were obtained from Bioneer (Korea), The primer sequences are shown in the Table (1.1) below. The expression of these genes (genes of interest) was analysed, and GAPDH was used as a reference housekeeping gene. For RT-PCR, start Exicycler™ 96 Real-Time Quantitative Thermal Block instrument and loaded the following Program was used with the following PCR program as follows: reverse transcription step (50 °C,1hour), PCR initial activation step (95 °C, 5 min), two steps cycling (95 °C, 20 s and 60 °C, 45 s respectively), repeated 45 times. Melting curve analysis was performed to check the specificity of PCR products. The delta-delta CT method was used for relative quantification of expression of particular gene as described elsewhere (Livak & Schmittgen, 2001).

Table 1.1 : Primer names and their sequence

Primer		Sequence
Nrf2 (Nuclear factor erythroid 2 related factor 2)	F	CCCACACAAGGTTCGGCATCAC
	R	TGGCGATTCCTCTGGCGTCT
TSPO (Translocater protein)	F	GTGGACCTCCTGCTCCTCAC
	R	ACGCCATGTAAGGGTAGAGC
CYP19A1 (Aromatase enzyme)	F	GGAAGAATGCATCGACTTGAGTT
	R	GGGCCCAAAACCAAATGGT
GAPDH (glyceraldehyde-3-phosphate dehydrogenase)	F	ATGCCCCCATGTTTGTGATG
	R	AGGATGCGTTGCTGACAATC

R: Reverse
F: Forword

Statistical Analysis:

Data have been analyzed statistically using (SPSS) version 24. Statistical analysis of data was conducted on the basis of (One-way) Analysis of Variance (ANOVA) utilizing a least significant differences (LSD) as portrayed by (Larsen *et al*., 1973). A level of $p<0.05$ was defined as statistically significant.

Results

The effect of Methandienone, Selenium and both on the oxidative markers

According to the results which are illustrated in table and figure (1). There was a significant decrease ($p > 0.05$) in serum GSH concentration in T1 group when compared with other groups. There was a significant increase ($p < 0.05$) in serum MDA concentration in T1 when compared with control ,T2 and T3 groups. At the same time the T1 group (AAS) showed a significant increase ($p<0.05$) of serum SOD activity as compared with other groups. Also, we observed a signficant increase ($p<0.05$) of SOD in T3 (Metha. + Se-yeast) compared to control and T2. The result also appeared a significant increase of protein carbonyl content (PCC) in serum and brain tissue of T1 as compared with other groups(control,T2 and T3). while, Metha. + Se-yeast group (T3) showed a significant increase ($p<0.05$) serum PCC concentration in comparable to control and Se-yeast groups.

Table 1 : The oxidative stress markers (GSH, MDA, SOD and PCC) in serum and PCC activity in brain tissue of different groups.

Groups / Parameter	Control	Methandienone (T1)	Se-yeast (T2)	Metha. + Se-yeast (T3)	LSD
GSH (µmol/l)	1.56±0.057 A	0.60±0.020 C	1.11±0.072 B	1.21±0.130 B	0.3
MDA (µmol/l)	0.14±0.005 B	1.28±0.018 A	0.12±0.003 B	0.20±0.011 B	0.09
SOD Activity (U/ml)	19.62±0.288 C	34.11±0.360 A	19.37±0.389 C	28.14±0.349 B	1.33
PCC (serum) (nmol/mgprot)	0.52±0.023 C	0.97±0.021 A	0.25±0.011 D	0.73±0.145 B	0.12
PCC (brain tissue) (nmol/mgprot)	0.40±0.057 B	1.01±0.058 A	0.43±0.033 B	0.46±0.033 B	0.15

Value express as mean ± SE, Number of animals per each group (5).
The different capital letters refer significant differences between groups within one row at (P≤0.05)

Control group received 1 ml / kg of distal water orally for 60 days. T1: Animals received an oral dose of 0.35 mg / kg of Methandienonefor 60 days.T2:seleniun was treated with 3 µl / Kg body weight of selenium (High Selenium yeast). for 60 days.T3: The combined group received both Methandienone and selenium-yeast orally (0.35 mg / kg and 3 µl / Kg body weight, respectively). for 60 days.

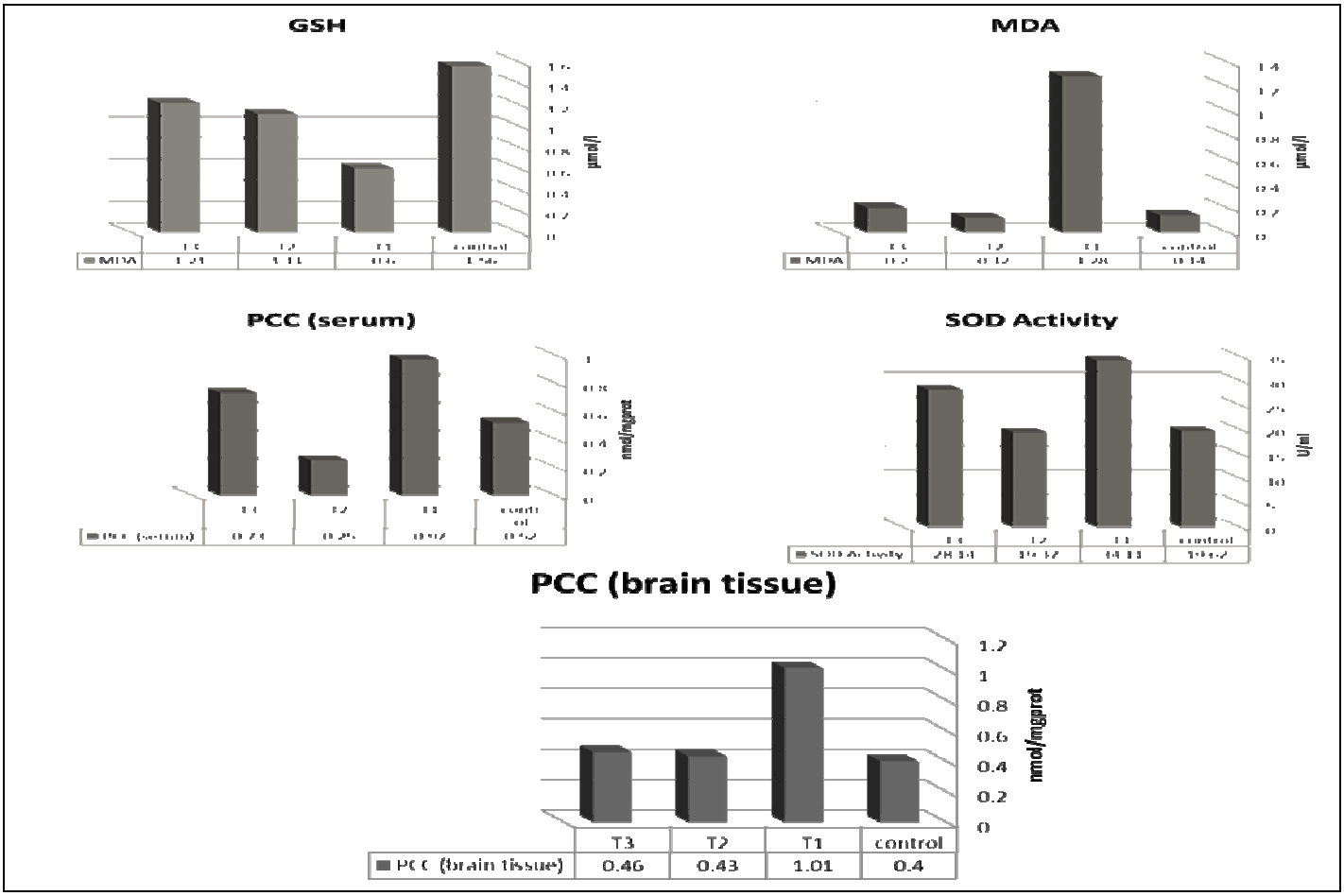

Fig. 1 : The variation of the oxidative stress markers (GSH, MDA, SOD and PCC activity in serum and PCC brain tissue of different groups.

The effect of Methandienone, Selenium and both on the Relative genes expression

The results of relative gene expression in (Nrf2) gene appeared clear difference in fold change of gene expression levels between control and treated groups. Where, T1 group (AAS) up regulation at (19.46±0.384), T2group (Se-yeast) showed low change at (6.10±0.378), T3 group (Metha. + Se-yeast) showed up regulation at (14.20±0.152) relative to T2 and control group that is equal to 1 fold change of gene expression levels (table 2 and figure2). Moreover, we observed a significant increase of mRNA of TSPO gene in T1group (methan.) as compared with other groups (T2 and T3). On other side, the T3 group (Metha. + Se-yeast) appear up regulated of expression of TSPO gene as compared with T2 group (Se-yeast).

In the same table and figure, we demonstrated high level of revers transcription of Cyp19A1 gene in T1 group compared with T2 and T3 groups. Also,we see increased mRNA level in T3 group compared to T2.The statistical analysis of relative genes expression (Nrf2,TSPO and CYP19A1) found significant differences in AAS (methan.) group compared with other groups groups at level P≤0.05.

Table 2 : The relative gene expression analysis of Nrf2, TSPO and CYP19A1 gene in brain of different groups.

Groups / Parameter	Control	Methandienone (T1)	Se-yeast (T2)	Metha. + Se-yeast (T3)	LSD
Fold change (2^-ΔΔCT) of Nrf2 gene	1.00±0.00 D	19.46±0.384 A	6.10±0.378 C	14.20±0.152 B	0.9
Fold change (2^-ΔΔCT) of TSPO gene	1.00±0.00 D	22.88±0.646 A	4.10±0.208 C	6.41±0.145 B	1.13
Fold change (2^-ΔΔCT)of CYP19A1 gene	1.00±0.00 D	6.20±0.141 A	2.11±0.106 C	3.59±0.095 B	0.32

Value express as mean ± SE, Number of animals per each group (5).

The different capital letters refer significant differences between groups within one row at (P≤0.05) control group received 1 ml / kg of distal water orally for 60 days. T1: Animals received an oral dose of 0.35 mg / kg of Methandienonefor 60 days.T2:seleniun was treated with 3 µl / Kg body weight of selenium (High Selenium yeast). for 60 days.T3: The combined group received both Methandienone and selenium-yeast orally (0.35 mg / kg and 3 µl / Kg body weight, respectively). for 60 days.

Nrf2 (Nuclear factor erythroid 2–related factor 2), TSPO (Translocator Protein), Cyp19A1 (Aromatase).

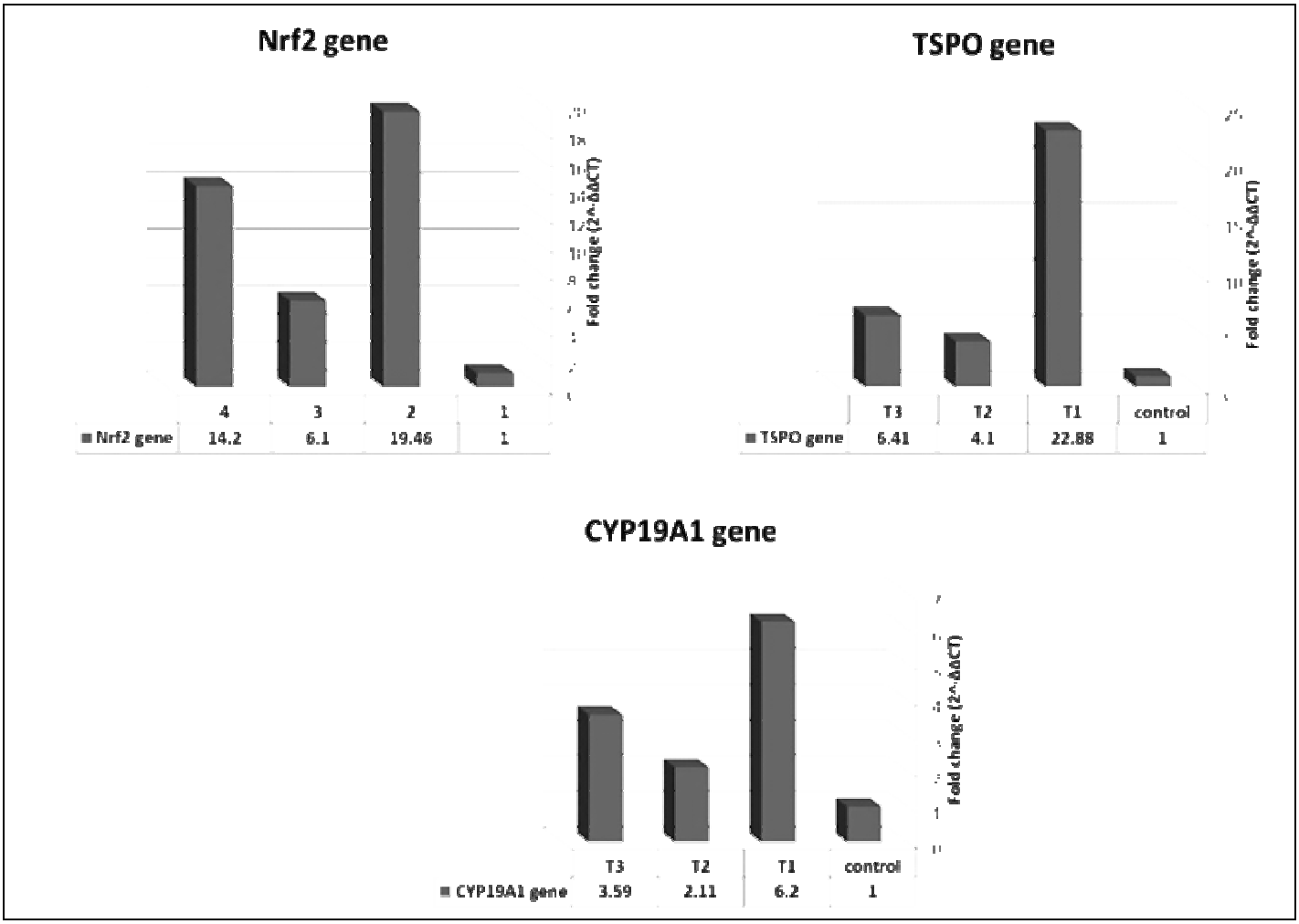

Fig. 2 : The variation in genes expression (Nrf2,TSPO and CYP19A1) in brain of different groups.

Discussion

The oxidative stress state increases the oxidation of GSH to the binary sulfuric form of GSSG by inhibiting the pentose phosphate shunt pathway, which determines the NADPH production necessary for the activity of the glutathione reductase enzyme to recreate its oxidized form GSH (Hansen *et al*., 2019). A decrease in GSH leads to an increase production in ROS due to AAS-induced oxidative stress.

In addition to depletion of GSH, lipid peroxidation (damage in cell compartments) occurs during the administration of AAS. Thus, measuring serum MDA levels

provides a convenient indicator of lipid peroxidation and is a non-invasive oxidative stress biomarker (Janicka *et al*., 2010). In this study, MDA increased significantly after administration of AAS alone and plus with Se-yeast compared to control.

This result is consistent with Camiletti-Moirón (2016), which reported a significant increase in plasma MDA concentration and the harmful effects caused by the administration of AASs during the oxidation of lipids and proteins in the kidneys.

The importance of SOD activity comes from its high effectiveness as an antioxidant as it blunts cascading reactions caused by superoxide radical, thus it is considered a major antioxidant and the first defense (Buettner, 2011). Androgen (testosterone) has been reported to increase the production of ROS and oxidative effect in cardiomyocytes by transmitting signals through the androgen receptor (AR) through genomic and non-genomic mechanisms, activated androgen receptor translocates in the nucleus and increases expression of prooxidant enzymes genes (e.g.NAD (P) H oxidase cyclooxygenase 2 (COX-2) and xanthine oxidase) which, in turn, enhances the activation of pro-oxidant enzymes and elevate production ROS by changing the function of mitochondria (Cruz-Topete *et al*., 2020).This explains the significant increase activity of SOD after administration of AAS in male rabbits. In addition, ROS leads to protein damage, measured by protein carbonyl contents (PCC), direct damage to proteins or a chemical change in their amino acids as a result of oxidative stress can increase PCC, protein oxidation leads to the loss of sulfhydryl groups and changes in the resonant structure of amino acids that change the function of the protein and therefore body integrity (Parvez & Raisuddin, 2005).

The present study showed that treatment with AAS leads to a raise in PCC in brain tissue. Turillazzi *et al*. (2016) mentioned the metabolic activation of androgen (testosterone) derivatives leads to the release of free and caused oxidative effect that observed in all areas of the brain caused by prolonged administration of AAS (nandrolone). Secondary reaction with product of lipid peroxidation (e.g. HNE) with certain amino acid residues that cause elevated of protein oxidation (Hall *et al*., 2016). As well as, the classical intracellular androgen receptor via, AASs can exertan apoptotic effect through a non-genomic pathway **(**M Vicencio *et al*., 2011).

On the other hand, we use the amerative effect of selenium-enriched yeast on AAS-inducing oxidative stress that caused improvement of antioxidant defense and reduction of oxidant markers. The type of Se supplementation used in this study was Se-Yeast, which is a spray dried Se yeast formulation (Saccharomyces cerevisiae) and a common form of Se used for supplementation of the dietary intake (Griffiths *et al*., 2006; Aremmt *et al*., 2019). The organic Se content in Se-Yeast is equal to or greater than 98% of the total Se content (Griffiths *et al*., 2006). El-Demerdash & Nasr (2014)reported that selenium is crucial in several enzymes with physiological antioxidant properties, including GPx and thioredoxin reductase.GPx scavenges H_2O_2 and lipid hydroperoxides, using reducing equivalents from glutathione and protecting membrane lipids and macromolecules from oxidative damage (Zoidis *et al*., 2018).

Brigelius-Flohé & Maiorino in (2013) menioned that enzymes responsible for reduction of two-electron of hydroperoxides are selenium-dependent glutathione peroxidases, (GPx) and selenoprotein P (SeP). Thus, in this study, we observed the role of yeast supplementation in reducing the effect of oxidative damage caused by AAS in the brain.

The second partition in this research, we used molecular bioactive markers to detect brain injury, including transcription of Nrf2, TSPO, and CYP19A1 mRNA. (Real-time PCR). In addition to increasing performance and muscle building properties, AASs is associated with a wide range of symptoms, including hardness, aggression and impulsive behavior (Pagonis *et al*., 2006). While the positive effects of androgenic steroids on mood, such as euphoria and hypomania, have been reported at the beginning of the use of (Thiblin & Petersson, 2005), impulsivity, anxiety, aggression and severe irritability usually occur after prolonged use of anabolic steroids (Pagonis *et al*., 2006). Supraphysiological doses of AASs can cause apoptotic effects on various types of cells, including neurons(nerve cells) (Basile *et al*., 2013).

This study clearly shows that the level of Nrf2 gene expression in brain tissue is significantly higher compared to the Se and Se plus AAS groups. In response to oxidative stress, cells have various protective systems to regulate damage caused by oxidative stress. The antioxidant phase II reaction is considered an important protective pathway present in cells.It is regulated by the transcription factor Nrf2; the antioxidant pathway Nrf2 is an emerging master of the defense against oxidative effect (Buendia *et al*., 2016).The difficulty of the mechanisms of AASs-induced neurotoxicity affects oxidative stress, since apoptosis itself can be caused by oxidative stress (Pomara *et al*., 2015). Thus, the increase in Nrf2 mRNA in this study in animals treated with AAS may be associated with oxidative stress caused by AAS.

On the other hand, we found an increase in the expression of TSPO and CYP19A1 (aromatase) genes, which are also regarded as a biomarker of brain inflammation or injury after administration of AAS. TSPO is present both in neurons and activated glial cells (Maeda *et al*., 2007) and also microglia which is activated by TSPO ligands (Choi *et al*., 2011). TSPO up-regulation in glia is a major hallmark of brain injury, inflammation and neuro degeneration (Girard *et al*., 2012). Where it is expressed at very low levels expression in a normal healthy brain, TSPO is activated in disease conditions associated with the activation of glial cells. A quantitative assessment of its expression in neurological diseases has confirmed that it is most consistent with the presence of activated microglia cells (Kannan *et al*., 2009).

In this study, increased regulation of TSPO mRNA may be associated with AAS-induced oxidative effect in the brain tissue, and it is suggested that the 18 kDa translocator protein (TSPO) can protect cells from damage caused by free radicals. Another side, In light of the discovery that the brain has the ability to make steroids locally to regulate neuronal function (Schumacher *et al*., 2000), and with the knowledge that the synthesis of steroids in the brain is not regulated by any known hormone. Induction of neurosteroid production in the brain using the ligands of the drug TSPO is used to attenuate the symptoms of neuropsychiatric diseases

(Rupprecht *et al*., 2010). For example, estrogens. In neurons, estrogens prevent the death of neurons by raising the endogenous synthesis of anti-apoptotic molecules (Meda *et al*., 2000).

Because aromatase expression (CYP19A1) correlates with neuro protection. In fact, weak neurodegenerative stimuli cause strong neuro degeneration(inflammation when the enzyme is genetically or pharmacologically inhibited (Garcia-Segura *et al*., 2003), possibly because aromatase is used for local production of neuroprotective estrogens (Pareto *et al*., 2013). Garcia-Segura *et al*. (1999) found that although astrocytes usually do not express aromatase, enzyme expression in these glial cells is induced by various types of brain damage.

The results obtained indicate the vital role of local formation of astroglial estrogen in brain restoration. Thus, we observed increased regulation of CYP19A1 (aromatase) in the AAS group to reduce the adverse effects of AASs on the brain. In addition, the function of TSPO for steroidogenesis in the brain. On other side, AAS can also be aromatized to estrogens and interface with both estrogen receptor alpha and beta (ERα, ERβ).

On the other side, we observed that the protective role of Se (T3 group) is manifested in a significant decrease in molecular bioactive markers involved in the level of expression of the Nrf2, TSPO, and CYP19A1 genes in brain tissue.The recommended daily dose – 55 µg/day – is based on the amount needed to maximize the activity of the selenium-dependant master antioxidant enzyme glutathione, selenium also binds to the sulfur-containing amino acid cysteine and forms antioxidant selenoproteins in the body, selenoproteins carry selenium to the tissues, reduce inflammation, support a function of immune system and thyroid gland (Rocourt & Cheng, 2013). Wrobel *et al*. (2013) explained in his study the main effect of different selenium compounds such as synthetic L-selenomethionine, inorganic sodium selenite, and organic high-selenium yeast on the development of metastatic brain lesions have shown that the selenium-enriched yeast formulation resulted in a higher survival rate and in decreased tumor growth as compared to controls.

Another study shows the comparative effects of two kinds of selenium supplement selenomethionine and high-selenium yeast supplements has showed a decrease in oxidative stress biomarkers after supplementing with the organic high-selenium yeast compared to selenomethionine supplementation (Richie *et al*., 2014). Thus, Se is very important for your brain health. Low Se levels are linked to poor cognition, memory problems, and low neurotransmitter levels in the brain. Alzheimer's patients had only 60% of the brain selenium levels of healthy people in one study (Cardoso *et al*., 2013). So that in this study we found the Se-yeast reduction of damages induction by AAS.

References

Alqayim, M.A.J. (2019). Biosynthesis, Characterization and Bioactivity of Selenium Nanoparticles Synthesized by Propolis. The Iraqi Journal of Veterinary Medicine, 43(1): 197–209.

Aremmt, M. K.; Mohammed, T. R. and Alrawi, S.T.J. (2019). Effect of Yeast (Saccharomyces cerevisiae) Supported by Selenium and Zinc on Lipid Profile of Local Sheep Males. Al-Anbar Journal of Veterinary Sciences, 12(1): 89–96.

Basaria, S.; Wahlstrom, J. T. and Dobs, A. S. (2001). Anabolic-androgenic steroid therapy in the treatment of chronic diseases. The Journal of Clinical Endocrinology & Metabolism, 86(11): 5108–5117.

Basile, J. R.; Binmadi, N. O.; Zhou, H.; Yang, Y.; Paoli, A. and Proia, P. (2013). Supraphysiological doses of performance enhancing anabolic-androgenic steroids exert direct toxic effects on neuron-like cells. Frontiers in Cellular Neuroscience, 7: 69.

Birben, E.; Sahiner, U. M.; Sackesen, C.; Erzurum, S. and Kalayci, O. (2012). Oxidative stress and antioxidant defense. World Allergy Organization Journal, 5(1): 9–19.

Brigelius-Flohé, R. and Maiorino, M. (2013). Glutathione peroxidases. Biochimica et Biophysica Acta (BBA)-General Subjects, 1830(5): 3289–3303.

Buendia, I.; Michalska, P.; Navarro, E.; Gameiro, I.; Egea, J. and Leon, R. (2016). Nrf2–ARE pathway: an emerging target against oxidative stress and neuroinflammation in neurodegenerative diseases. Pharmacology & Therapeutics, 157: 84–104.

Burtis, C. A.; Ashwood, E. R. and Bruns, D. E. (2012). Tietz textbook of clinical chemistry and molecular diagnostics-e-book. Elsevier Health Sciences.

Camiletti-Moirón, D. (2016). Consequences of high-protein diets consumption, anabolic androgenic steroids administration and high-intesity exercise on brain and kidney oxidative stress markers in wistar rats.

Cardoso, B. R.; Cominetti, C. and Cozzolino, S. M. F. (2013). Importance and management of micronutrient deficiencies in patients with Alzheimer's disease. Clinical Interventions in Aging, 8: 531.

Choi, J.; Ifuku, M.; Noda, M. and Guilarte, T. R. (2011). Translocator protein (18 kDa)/peripheral benzodiazepine receptor specific ligands induce microglia functions consistent with an activated state. Glia, 59(2): 219–230.

Choi, S. M. and Lee, B.-M. (2015). Comparative safety evaluation of selective androgen receptor modulators and anabolic androgenic steroids. Expert Opinion on Drug Safety, 14(11): 1773–1785.

Cruz-Topete, D.; Dominic, P. and Stokes, K. Y. (2020). Uncovering sex-specific mechanisms of action of testosterone and redox balance. Redox Biology, 101490.

Daly, R. C.; Su, T.-P.; Schmidt, P. J.; Pagliaro, M.; Pickar, D. and Rubinow, D. R. (2003). Neuroendocrine and behavioral effects of high-dose anabolic steroid administration in male normal volunteers. Psychoneuroendocrinology, 28(3): 317–331.

Dominiak, A.; Wilkaniec, A. and Adamczyk, A. (2016). Selenium in the therapy of neurological diseases. Where is it going? Current Neuropharmacology, 14(3): 282–299.

El-Demerdash, F. M. and Nasr, H. M. (2014). Antioxidant effect of selenium on lipid peroxidation, hyperlipidemia and biochemical parameters in rats exposed to diazinon. Journal of Trace Elements in Medicine and Biology, 28(1): 89–93.

Fernandez, C.; San Miguel, E. and Fernandez-Briera, A. (2009). Superoxide dismutase and catalase: tissue activities and relation with age in the long-lived species

Margaritifera margaritifera. Biological Research, 42(1): 57–68.

Garcia-Segura, L. M.; Veiga, S.; Sierra, A.; Melcangi, R. C. and Azcoitia, I. (2003). Aromatase: a neuroprotective enzyme. Progress in Neurobiology, 71(1): 31–41.

Garcia-Segura, L. M.; Wozniak, A.; Azcoitia, I.; Rodriguez, J. R.; Hutchison, R. E. and Hutchison, J. B. (1999). Aromatase expression by astrocytes after brain injury: implications for local estrogen formation in brain repair. Neuroscience, 89(2): 567–578.

Girard, C.; Liu, S.; Adams, D.; Lacroix, C.; Sineus, M.; Boucher, C. and Groyer, G. (2012). Axonal regeneration and neuroinflammation: roles for the translocator protein 18 kDa. Journal of Neuroendocrinology, 24(1): 71–81.

Griffiths, J. C.; Matulka, R. A. and Power, R. (2006). Genotoxicity studies on Sel-Plex®, a standardized, registered high-selenium yeast. International Journal of Toxicology, 25(6): 477–485.

Hall, E. D.; Wang, J. A.; Bosken, J. M. and Singh, I. N. (2016). Lipid peroxidation in brain or spinal cord mitochondria after injury. Journal of Bioenergetics and Biomembranes, 48(2): 169–174.

Handayani, N.; Sujuti, H.; Permatasari, N. and Rudijanto, A. (2019). Niacin Regulates Glucose Reactive Protein (GRP78), Protein Carbonyl Content (PCC) and Malondialdehyde (MDA) in the Hyperglycemic Human Lens Epithelial Cells. Pharmacognosy Journal, 11(1).

Hansen, J.; Jones, D. P. and Harris, C. (2019). The redox theory of development. Antioxidants and Redox Signaling, (ja).

Hara, Y.; Waters, E. M.; McEwen, B. S. and Morrison, J. H. (2015). Estrogen effects on cognitive and synaptic health over the lifecourse. Physiological Reviews, 95(3): 785–807.

Imam, S. Z.; Newport, G. D.; Islam, F.; Slikker Jr, W. and Ali, S. F. (1999). Selenium, an antioxidant, protects against methamphetamine-induced dopaminergic neurotoxicity. Brain Research, 818(2): 575–578.

Janicka, M.; Kot-Wasik, A.; Kot, J. and Namieśnik, J. (2010). Isoprostanes-biomarkers of lipid peroxidation: their utility in evaluating oxidative stress and analysis. International Journal of Molecular Sciences, 11(11): 4631–4659.

Kannan, S.; Balakrishnan, B.; Muzik, O.; Romero, R. and Chugani, D. (2009). Positron emission tomography imaging of neuroinflammation. Journal of Child Neurology, 24(9): 1190–1199.

Larsen, I. L.; Hartmann, N. A. and Wagner, J. J. (1973). Estimating precision for the method of standard additions. Analytical Chemistry, 45(8): 1511–1513.

Levine, R. L.; Wehr, N.; Williams, J. A.; Stadtman, E. R. and Shacter, E. (2000). Determination of carbonyl groups in oxidized proteins. In Stress Response (pp. 15–24). Springer.

Livak, K. J. and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods, 25(4): 402–408.

Llewellyn, W. (2011). Anabolics. Molecular Nutrition LLC.

Loth, M. K. (2018). Translocator Protein 18 kDa: from Biomarker to Function. Columbia University.

M Vicencio, J.; Estrada, M.; Galvis, D.; Bravo, R.; E Contreras, A.; Rotter, D.; … Jaimovich, E. (2011). Anabolic androgenic steroids and intracellular calcium signaling: a mini review on mechanisms and physiological implications. Mini Reviews in Medicinal Chemistry, 11(5): 390–398.

Maeda, J.; Higuchi, M.; Inaji, M.; Ji, B.; Haneda, E.; Okauchi, T. and Suhara, T. (2007). Phase-dependent roles of reactive microglia and astrocytes in nervous system injury as delineated by imaging of peripheral benzodiazepine receptor. Brain Research, 1157: 100–111.

Meda, C.; Vegeto, E.; Pollio, G.; Ciana, P.; Patrone, C.; Pellicciari, C. and Maggi, A. (2000). Oestrogen prevention of neural cell death correlates with decreased expression of mRNA for the pro‐apoptotic protein nip‐2. Journal of Neuroendocrinology, 12(11): 1051–1059.

Oberlander, J. G.; Penatti, C. A. A.; Porter, D. M. and Henderson, L. P. (2012). The buzz about anabolic androgenic steroids: electrophysiological effects in excitable tissues. Neuroendocrinology, 96(2): 141–151.

Orlando, R.; Caruso, A.; Molinaro, G.; Motolese, M.; Matrisciano, F.; Togna, G. and Bruno, V. (2007). Nanomolar concentrations of anabolic–androgenic steroids amplify excitotoxic neuronal death in mixed mouse cortical cultures. Brain Research, 1165: 21–29.

Pagonis, T. A.; Angelopoulos, N. V, Koukoulis, G. N.; Hadjichristodoulou, C. S. and Toli, P. N. (2006). Psychiatric and hostility factors related to use of anabolic steroids in monozygotic twins. European Psychiatry, 21(8): 563–569.

Pareto, D.; Biegon, A.; Alexoff, D.; Carter, P.; Shea, C.; Muench, L. and Logan, J. (2013). In vivo imaging of brain aromatase in female baboons:[11C] vorozole kinetics and effect of the menstrual cycle. Molecular Imaging, 12(8): 2013–7290.

Parvez, S. and Raisuddin, S. (2005). Protein carbonyls: novel biomarkers of exposure to oxidative stress-inducing pesticides in freshwater fish Channa punctata (Bloch). Environmental Toxicology and Pharmacology, 20(1): 112–117.

Pomara, C.; Neri, M.; Bello, S.; Fiore, C.; Riezzo, I. and Turillazzi, E. (2015). Neurotoxicity by synthetic androgen steroids: oxidative stress, apoptosis, and neuropathology: A review. Current Neuropharmacology, 13(1): 132–145.

Prentice, H.; Modi, J.P. and Wu, J.-Y. (2015). Mechanisms of neuronal protection against excitotoxicity, endoplasmic reticulum stress, and mitochondrial dysfunction in stroke and neurodegenerative diseases. Oxidative Medicine and Cellular Longevity, 2015.

Buettner, G. (2011). Superoxide dismutase in redox biology: the roles of superoxide and hydrogen peroxide. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 11(4): 341–346.

Ramoutar, R. (2009). Understanding the antioxidant mechanism of inorganic selenium, oxo-sulfur, and polyphenol compounds, and the biological implications of functionalized nanoparticles.

Richie, J.P.; Das, A.; Calcagnotto, A.M.; Sinha, R.; Neidig, W.; Liao, J. and Ciccarella, A. (2014). Comparative effects of two different forms of selenium on oxidative stress biomarkers in healthy men: a randomized clinical

trial. Cancer Prevention Research, 7(8): 796–804.

Rocourt, C. R. B. and Cheng, W.-H. (2013). Selenium supranutrition: are the potential benefits of chemoprevention outweighed by the promotion of diabetes and insulin resistance? Nutrients, 5(4): 1349–1365.

Rupprecht, R.; Papadopoulos, V.; Rammes, G.; Baghai, T. C.; Fan, J.; Akula, N.; … Schumacher, M. (2010). Translocator protein (18 kDa)(TSPO) as a therapeutic target for neurological and psychiatric disorders. Nature Reviews Drug Discovery, 9(12): 971.

Santamaría, A.; Salvatierra‐Sánchez, R.; Vázquez‐Román, B.; Santiago‐López, D.; Villeda‐Hernández, J.; Galván‐Arzate, S. and Ali, S. F. (2003). Protective effects of the antioxidant selenium on quinolinic acid‐induced neurotoxicity in rats: in vitro and in vivo studies. Journal of Neurochemistry, 86(2): 479–488.

Schumacher, M.; Akwa, Y.; Guennoun, R.; Robert, F.; Labombarda, F.; Désarnaud, F. and Baulieu, E.-E. (2000). Steroid synthesis and metabolism in the nervous system: trophic and protective effects. Journal of Neurocytology, 29(5–6): 307–326.

Tateishi, T.; Yoshimine, N. and Kuzuya, F. (1987). Serum lipid peroxide assayed by a new colorimetric method. Experimental Gerontology, 22(2): 103–111.

Thiblin, I. and Petersson, A. (2005). Pharmacoepidemiology of anabolic androgenic steroids: a review. Fundamental & Clinical Pharmacology, 19(1): 27–44.

Tonelli, C.; Chio, I. I. C. and Tuveson, D. A. (2018). Transcriptional regulation by Nrf2. Antioxidants & Redox Signaling, 29(17): 1727–1745.

Turillazzi, E.; Neri, M.; Cerretani, D.; Cantatore, S.; Frati, P.; Moltoni, L. and Fineschi, V. (2016). Lipid peroxidation and apoptotic response in rat brain areas induced by long‐term administration of nandrolone: the mutual crosstalk between ROS and NF‐kB. Journal of Cellular and Molecular Medicine, 20(4): 601–612.

van Amsterdam, J.; Opperhuizen, A. and Hartgens, F. (2010). Adverse health effects of anabolic–androgenic steroids. Regulatory Toxicology and Pharmacology, 57(1): 117–123.

Wrobel, J. K.; Seelbach, M. J.; Chen, L.; Power, R. F. and Toborek, M. (2013). Supplementation with selenium-enriched yeast attenuates brain metastatic growth. Nutrition and Cancer, 65(4): 563–570.

Zoidis, E.; Seremelis, I.; Kontopoulos, N. and Danezis, G. P. (2018). Selenium-dependent antioxidant enzymes: Actions and properties of selenoproteins. Antioxidants, 7(5): 66.