Journal of Applied Biological Sciences
E-ISSN: 2146-0108 17(3): 432-439, 2023
DOI: https://doi.org/10.71336/jabs.1237
Research Article

SHORT-TERM EFFECTS OF OVARIOHYSTERECTOMY ON SOME ANTIOXIDANT PARAMETERS IN CATS

Ahmet Gozer[1,*], Onur Bahan[2], Ishak Gokçek[3],
Ufuk Kaya[4], Gokhan Uyanık[5], Gokhan Dogruer[1]

[1]*Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, Hatay, Turkey*
[2]*Yozgat Bozok University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, Yozgat, Turkey*
[3]*Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Physiology, Hatay, Turkey*
[4]*Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Biostatistics, Hatay, Turkey*
[5]*Erciyes University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, Kayseri, Turkey*

**Corresponding Author:*
E-mail: ahmetgozer@gmail.com

(Received 06th June 2023; accepted 02nd October 2023)

ABSTRACT. This research aimed to study the short-term effect of ovariohysterectomy on oxidant/antioxidant status in healthy cats. Twenty-two female cats were allocated to the present study. All the cats were anesthetized with the xylazine-ketamine and spayed. Blood samples were collected before (D0) and three and ten days after the ovariohysterectomy to determine serum malondialdehyde, glutathione, catalase, and glutathione peroxidase concentrations. There was a statistical increase in MDA concentrations on Day 3 compared to Day 0 ($p<0.05$). There were no statistical differences in the MDA concentrations between Day 3 and Day 10. Catalase activity showed a remarkable increment on Day 3 compared to Day 0 and then a declination on Day 10 compared to Day 3 ($p<0.05$). Glutathione activity was greater on Day 3 and Day 10 compared to Day 0. Glutathione peroxidase activity was decreased on Day 3 compared to Day 0 and then increased on Day 10. Nevertheless, these increases were not significant. In conclusion, this study clearly showed that ovariohysterectomy surgery resulted in oxidative stress within the first three days after ovariohysterectomy and oxidative stress disappeared ten days after ovariohysterectomy.

Keywords: *Cat, ovariohysterectomy, oxidative stress.*

INTRODUCTION

Ovariohysterectomy is defined as removal of the both the ovary and uterus through a midline or flank laparotomy [1]. It is one of the most performed surgeries by veterinarians for preventive and therapeutic purposes such as mammary and uterine tumors [2, 3, 4]. Ovariohysterectomy is also the most radical treatment option for many diseases such as pyometra, ovarian and uterine tumors, ovarian cysts, and ovarian remnant syndrome [5, 6, 7]. In addition, ovariohysterectomy is one of the practices for preventing the

overpopulation of stray animals which are the important carrier of zoonoses and pose a threat to public health [8, 9].

Surgical operations lead to surgical stress response in which endocrine, humoral, immunological, local, and systemic inflammatory systems are activated. Activation of the inflammatory system includes the production of cytokines and provoking the blood cell such as mast cells, platelets, and macrophages. This situation leads to the production of reactive oxygen species (ROS) including superoxide ($O_2^{\cdot}$), peroxyl ($RO_2^{\cdot}$), hydroperoxyl ($RO_2^{\cdot}$), hydrogen peroxide (H_2O_2), ozone (O_3), and hypochlorous acid (HOCl). Under physiological circumstances, ROS were eliminated by means of antioxidant enzymes [10, 11]. But, in the case of excessive production of ROS, depletion of the antioxidant reserve may occur and this situation may cause the accumulation of excessive ROS, therefore resulting in oxidative stress [12, 13]. Oxidative stress is explained as a situation in which excessive reactive oxygen species production surpasses the capacity of the living organism to detoxify ROS [14, 15]. Reactive oxygen species induce peroxidative damage to macromolecules such as lipids and proteins, consequently leading to immunosuppression and deterioration of the many metabolic and physiological processes [13, 16].

Despite its many therapeutic and preventive purposes, ovariohysterectomy surgery causes oxidative stress during the operation and postoperative period [17, 18, 19, 20, 21]. Even though there are numerous studies regarding the effect of ovariohysterectomy on the oxidative stress parameters in various species [17, 21], little is known about whether OHE induces oxidative stress in domestic cats [22, 23]. The aim of present study was to evaluate the effect of ovariohysterectomy on oxidant/antioxidant status in domestic queens.

MATERIALS AND METHODS

Ethics

This research was approved by Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee with the decision numbered 2022/08-15.

Animals

The study consisted of 22 non-pregnant healthy cats (aged from 5 months to 4 years; body weights from 2.0 to 4.0 kg) admitted for spaying surgery at the Hatay Mustafa Kemal University Veterinary Health Application and Research Hospital Directorate. Prior to surgery, routine hematological examinations were performed.

Ovariohysterectomy (OHE) Operation

Ovariohysterectomy was performed according to Coe et al. [1]. Xylazine hydrochloride (1 mg/kg, IM, Basilazine 2%, Bavet, Turkey) and ketamine hydrochloride (10 mg/kg, IM, Ketasol 10%, Interhas, Turkey) was used as anesthetic medication. After induction of anesthesia, cats were laid in dorsal recumbency. A 0.2 mg/kg dose of meloxicam (Maksikam, Bavet, Turkey) was administered subcutaneously as an analgesic close to the completion of the operation. To prevent postoperative infections, cats received 20 mg/kg of cefazolin sodium (Cefazol, Mustafa Nevzat, Turkey) twice a day for 5 days. Sutures were removed on Day 10.

Blood Samplings and Analyses

Blood samples were collected into the tubes without anticoagulants for the measurements of the malondialdehyde (MDA), catalase (CAT), glutathione (GSH), and glutathione peroxidase (GSH-Px) before the surgery (Day 0) and at 3 (Day 3) and 10 days (Day 10) after OHE. Sera were obtained by centrifuging the blood at 5000 rpm for 5 minutes and stored at -20 ºC until the analyses.

Malondialdehyde Measurement: Malondialdehyde concentrations were measured according to the concentration of thiobarbituric acid reactive substances. The amount of MDA produced will be used as an index of lipid peroxidation. The MDA level was measured at 532 nm and expressed in nanomoles [24].

Catalase Measurement: Catalase was measured according to Goth [25]. A 0.2 mL serum plasma sample was taken and incubated in 1.0 mL substrate at 37 °C for 60 seconds. This enzymatic reaction was with 32.4 mM ammonium molybdate and the measurement was made against the blank at 405 nm in the spectrophotometer. Catalase activity was expressed as kU/L.

Glutathione Measurement: Glutathione concentrations were measured using Sedlak and Lindsay's method [26]. Sera were precipitated by the 50% trichloroacetic acid (TCA). The supernatant of the sediments was separated. Then, 0.5 mL of the supernatant was mixed with 2 mL of Tris-EDTA buffer (0.2 M, pH=8.9) and 0.1 mL of 0.01 M 5,5'-dithiol-bis-2-nitrobenzoic acid. This mixture was incubated at 25 °C for five minutes. The results of the reaction were measured by the 412 nm wavelength.

Glutathione Peroxidase Measurements: Glutathione Peroxidase measurements were performed according to the method described by Lawrence and Burk [27]. A solution including 1 mM EDTA, 1 mM sodium azide, 0.2 mM reduced NADPH, 1 IU/mL oxidized glutathione reductase (GSSG), 1 mM GSH, and 0.2 mM H_2O_2 and 50 mM potassium phosphate buffer was prepared. 0.1 mL of sera and 0.8 mL of solution were mixed. Then, 0.1 mL of peroxide solution was added and incubated at 25 °C. Results were expressed as IU/L by measuring their absorbance at 340 nm within 5 minutes.

Statistical Analyses

Before performing the statistical analysis, data were examined for parametric test assumptions. Descriptive statistics for each variable were calculated and presented as "mean ± standard error of the mean". To test the differences in MDA, GSH, catalase, and GSH-Px between sampling days, general linear models with repeated measures design were used. When a significant difference was revealed, pairwise comparisons were determined with Bonferroni correction. $P<0.05$ was considered significant in all analyses. IBM SPSS Statistics Software Version 23.0 was used in the analysis of the data.

RESULTS AND DISCUSSION

The results of the mean hematological parameters were summarised in Table 1.

Table 1. *Mean hematological results of the cats (n=22) in the study.*

Parameters	**Mean± SD (Min-Max)**	**Reference Range**
WBC (m/mm^3)	9.90 ± 2.77 (5.76-15.90)	5-19.5
LYM (%)	39.06 ± 15.42 (10.30-67.5)	12-45
LYM (#)	3.80 ± 1.67 (0.90-6.80)	1-7
MON %	5.71 ± 1.37(3.70-7.40)	2-9
MON (#)	0.57 ± 0.25 (0.30-1.20)	0.07-1.9
GRAN %	53.35 ± 16.04 (24.50-85.10)	25-85
GRAN (#)	5.34 ± 2.35 (2.10-9.50)	2-15
EOS (%)	2.61 ± 2.23 (0.70-7.40)	0-4
RBC (m/mm^3)	9.92 ± 1.26 (6.55-11.90)	4.6-10
MCV (fL)	40.52 ± 4.02 (35.70-47.0)	39-52
HCT (%)	40.02 ± 5.18 (30.5-48.70)	28-49
MCH (pg)	12.32 ± 0.96 (11.20-14.0)	13-21
MCHC (g/dl)	30.58 ± 1.54 (28.70-33.10)	30-38
RDW (%)	13.75 ± 2.46 (10.8-18.40)	14-18
HGB (g/dl)	12.31 ± 1.65 (9.20-14.80)	8-16
THR (m/mm^3)	300.31 ± 133.20 (48-520)	100-514
MPV(fL)	9.98 ± 0.98 (8.10-11.50)	9-18
PCT (%)	0.29 ± 0.14 (0.04-0.50)	0.14-0.62

White blood cell (WBC), lymphocyte (LYM), monocyte (MON), granulocyte (GRAN), eosinophil (EOS), red blood cell (RBC), mean corpuscular volume (MCV), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), hemoglobin (HGB), mean platelet volume (MPV), platelet distribution width (PDW), platelet (PLT), platelet crit (PCT)

In various surgical operations, the occurrence of oxidative stress is a common phenomenon. Surgical operations reduce the lipid peroxide concentration and increased the free radicals, consequently resulting in oxidative stress [11, 28, 29, 30]. This study was carried out to indicate the effect of ovariohysterectomy on oxidative stress parameters (serum malondialdehyde, glutathione peroxidase, glutathione, and catalase) in domestic queens. In the current study, there was a statistical increase in the MDA concentration on Day 3 compared to Day 0 ($p<0.05$). There were no statistical differences in the MDA concentration between Day 10 and Day 3. Catalase activity increased on Day 3 compared to Day 0 and then decreased on Day 10 compared to Day 3 ($p<0.05$). Glutathione activity was greater on Day 3 and Day 10 compared to Day 0. Glutathione peroxidase activity was decreased on Day 3 compared to Day 0 and then increased on Day 10. However, these alterations were not significant. Table 2 illustrated the serum MDA concentrations and GSH, Catalase, and GSH-PX activity in cats before and after ovariohysterectomy.

Table 2. *Serum MDA* concentrations *and Catalase, GSH, and GSH-PX activity.*

Parameters	Samplings			P value
	Before the OHE (Day 0)	3 days after OHE (Day 3)	10 days after OHE (Day 10)	
MDA (nmol/mL)	46.655 ± 1.004^{b}	50.618 ± 1.232^{a}	48.439 ± 1.174^{ab}	0.048
Catalase (kU/mL)	344.653 ± 38.344^{b}	533.702 ± 54.788^{a}	301.805 ± 38.703^{b}	0.001
GSH (IU/L)	3.396 ± 0.07	3.584 ± 0.087	3.573 ± 0.098	0.211
GSH-PX (IU/L)	542.869 ± 110.38	356.748 ± 28.269	587.137 ± 75.233	0.084

^{a,b} Values within a row with different superscripts differ significantly at $P<0.05$

As mentioned in the literature review, ovariohysterectomy results in oxidative stress in the laboratory and domestic animals. Anadol et al. [20]. showed that ovariohysterectomy caused a substantial increase in the MDA concentrations 24 h after OHE in rats. Serin et al. [17] indicated that MDA concentrations increased 24 h after OHE with performed by xylazine-ketamine anesthetic combination. It has been reported that MDA concentrations increase 72 h after OHE in the bitch [21,31] and returned to the preoperative days levels 14 days after OHE in bitches [21]. On the other hand, many reports have indicated that ovariohysterectomy has no significant effect on the MDA concentrations in dogs and cats [22, 23, 33]. In the current study, serum MDA concentration increased significantly 3 days after the OHE ($p<0.05$) whereas it returned to preoperative levels on Day 10 (Table 2). The results of this study were compatible with the previous studies [17, 20, 21, 31]. It has been stated that oxidative stress induced by the surgical operation is related to the surgical technique, anesthetic agents (xylazine-ketamin), the duration of the surgery, hypothermia, ischemia, inflammation, and postoperative pain [17, 30, 34, 35]. In the current study, it seems hard to explain to what extent the abovementioned mechanisms were involved in the formation of oxidative stress. It was assumed that all the factors may contribute to oxidative stress occurrence within the first 3 days after ovariohysterectomy. All the parameters evaluated remained the same at the 10 days after ovariohysterectomy. Therefore, the current study indicated that ovariohysterectomy causes oxidative stress in the first three days and oxidative stress disappeared at 10 days after ovariohysterectomy in domestic cats.

It has been reported that ovariohysterectomy has no remarkable effect on catalase activity in dogs and cats [21, 23]. On the contrary, Azevedo et al. [37] stated that ovariectomy diminished the catalase activity in the intraperitoneal macrophages in rats 1 month after ovariectomy. It was reported that catalase is the most adaptive antioxidant enzyme in normal conditions [38] and antioxidant enzyme activity adapts according to the quantity of ROS production [39]. Continuous scavenging of ROS by the antioxidant enzymes may result in the depletion of the antioxidant reserves [40]. In this study, the increase in the catalase activity on day 3 may be a compensatory mechanism for eliminating oxidative stress. Decreased activity of catalase on Day 10 may be related to the depletion of the antioxidant enzymes.

Glutathione (GSH) is an antioxidant enzyme containing glycine, glutamic acid, and cysteine [41]. It has higher intracellular levels and remarkable electron-donating ability. It detoxifies the free radicals and protects the DNA, proteins, and lipids against oxidative stress, thereby sustaining the equilibrium of the oxidant/antioxidant system [42]. It has

been stated that ovariectomy caused a reduction in GSH activity [17, 43]. On the contrary, Yılmaz et al. [33] concluded that ovariohysterectomy has no effect on the GSH activity in bitches. In the present study, although not significant, there were a slight increase in the GSH activity, which may be due to the oxidative stress after ovariohysterectomy.

It has been stated that the activity of the GSH-Px decreased one day after ovariohysterectomy and returned to preoperative levels ten days after ovariohysterectomy in the rats [20]. Similarly, Torabi et al. [23] stated that ovariohysterectomy did not cause significant alterations in GSH-Px activity one month after ovariohysterectomy in cats. On the contrary, Szczubial et al. [19] also indicated that GSH-Px activity increased at 14 days after OHE in bitches. In our study, although not significant, GSH-Px activity decreased on Day 3 and increased on Day 10 compared to Day 0. The severity of declination in the activity of the GSH-Px depends on the duration of the surgery and ischemia-reperfusion syndrome [30, 34]. Xylazine-ketamine anesthesia also caused a crucial increase in oxidative stress and a decrease in the GSH-Px after ovariohysterectomy. The results of the current study were in line with the previous studies [19, 20, 23].

CONCLUSION

In conclusion, ovariohysterectomy causes oxidative stress within the first three days after surgery. After completion of the recovery period, the oxidant-antioxidant balance returned to the preoperative levels in domestic cats. This study indicated that oxidative stress disappeared ten days after ovariohysterectomy.

Conflict of Interest. The authors declared that there is no conflict of interest.

Authorship Contributions. Concept: A.G., I.G., Design: A.G., I.G., Data Collection or Processing: A.G., O.B., G.U., Analysis or Interpretation: A.G., I.G., Literature Search: H.E., M.Y., Writing: A.G., I.G., G.,D.

Financial Disclosure. This research received no grant from any funding agency.

REFERENCES

[1] Coe, R.J., Grint, N.J., Tivers, M.S., Hotston Moore, A., Holt, P.E. (2006): Comparison of flank and midline approaches to the ovariohysterectomy of cats. Veterinary Record 159(10): 309-313.

[2] Howe, L.M. (2006): Surgical methods of contraception and sterilization. Theriogenology 66(3): 500-509.

[3] Kustritz, M.V.R. (2007): Determining the optimal age for gonadectomy of dogs and cats. Journal of the American Veterinary Medical Association 231(11): 1665-1675.

[4] Brodey, R.S., Roszel, J.F. (1967): Neoplasms of canine uterus vagina and vulva – a clinicopathologic survey of 90 cases. Journal of American Veterinary Medical Association 151: 1294.

[5] Gharagozlou, F., Youssefi, R., Akbarinejad, V., Sasani, F., Taghizadeh-Jahed, M., Shahpoorzadeh, T., Atashbaste, M. (2014): Evaluation of serum anti-Müllerian hormone (AMH) in a Persian queen cat with bilateral cystic ovarian disease. Comparative Clinical Pathology 23: 237-239.

[6] Hagman, R. (2018): Pyometra in small animals. Veterinary Clinics: Small Animal Practice 48(4): 639-661.

[7] Gozer, A., Bahan, O., Dogruer, G., Kutlu, T. (2023): Serum antimüllerian hormone concentrations in female cats. Relation with ovarian remnant syndrome, ovarian cysts and gonadectomy status. Theriogenology 200: 106-113.
[8] Reichler, I.M. (2009): Gonadectomy in cats and dogs: a review of risks and benefits. Reproduction in Domestic Animals 44(2): 29-35.
[9] Taetzsch, S.J., Bertke, A.S., Gruszynski, K.R. (2018): Zoonotic disease transmission associated with feral cats in a metropolitan area: A geospatial analysis. Zoonoses and Public Health 65(4): 412-419.
[10] Bayr, H. (2005): Reactive oxygen species. Critical Care Medicine 33(12): 498-501.
[11] Kucukakin, B., Gogenur, I., Reiter, R.J., Rosenberg, J. (2008): Oxidative stress in relation to surgery: is there a role for the antioxidant melatonin?. The Journal of Surgical Research 152: 338–347.
[12] Gutteridge, J.M. (1995): Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clinical Chemistry 41(12): 1819-1828.
[13] Berlett, B.S., Stadtman, E.R. (1997): Protein oxidation in aging, disease, and oxidative stress. Journal of Biological Chemistry 272(33): 20313-20316.
[14] Sies, H. (1997): Oxidative stress: oxidants and antioxidants. Experimental Physiology 82(2): 291–295.
[15] Yoshikawa, T., Naito, Y. (2002). What is oxidative stress?. Japan Medical Association Journal 45(7): 271-276.
[16] Halliwell, B., Matthew, W. (2004): Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean?. British Journal of Pharmacology 142(2): 231-255.
[17] Serin, G., Kiral, F., Serin, I. (2008): Acute effect of ovariohysterectomy on lipid peroxidation and some antioxidant levels in dogs. Bulletin of the Veterinary Institute in Pulawy 52: 251-253.
[18] Gunay, A., Gunes, N., Gunay, U. (2011): Effect of Ovariohysterectomy on Lipid Peroxidation and Levels of Some Antioxidants and Biochemical Parameters İn Bitches. Bulletin of the Veterinary Institute in Pulawy 55: 695-698.
[19] Szczubial, M., Kankofer, M., Bochniarz, M., Dąbrowski, R. (2015): Effects of ovariohysterectomy on oxidative stress markers in female dogs. Reproduction in Domestic Animals 50(3): 393-399.
[20] Anadol, E., Yarim, G.F., Gültiken, N., Kazak, F. (2016): Effect of ovariohysterectomy on some oxidative stress markers in the rat. Harran Üniversitesi Veteriner Fakültesi Dergisi 5(2): 124-128.
[21] Sakundech, K., Chompoosan, C., Tuchpramuk, P., Boonsorn, T., Aengwanich, W. (2020): The influence of duration on pain stress, oxidative stress, and total antioxidant power status in female dogs undergoing ovariohysterectomy. Veterinary World 13(1): 160-164
[22] Teixeira, L.G., Martins, L.R., Schimites, P.I., Dornelles, G.L., Aiello, G., Oliveira, J.S., da Silva, F.C., Brum, B.T., Walter, T.M., Andrade, C.M., Soares, A.V. (2020). Evaluation of postoperative pain and toxicological aspects of the use of dipyrone and tramadol in cats. Journal of Feline Medicine and Surgery 22(6): 467-475.
[23] Torabi, A.M., Yasini, S.P., Shirazi, Beheshtiha, S.H. (2022). Evaluation of antioxidant enzymes and lipid peroxidation before and after ovariohysterectomy in queen. Iranian Veterinary Journal 18(1): 71-76.
[24] Placer, Z.A., Cushman, L.L., Johnson, B.C. (1966). Estimation of product of lipid peroxidation (malonyldialdehyde) in biochemical systems. Analytical Biochemistry 16: 359-364.
[25] Goth, L.A. (1991): Simple method for determination of serum catalase activity and revision of reference range. Clinica Chimica Acta 196: 143-152.
[26] Sedlak, J., Lindsay, R.H. (1968): Estimation of total, protein bound and nonprotein sulfhydryl groups in tissue with Ellman'sreagent. Analytical Biochemistry 25: 192-205.

[27] Lawrence, R.A., Burk, R.F. (1976): Glutathione peroxidase activity in selenium-deficient rat liver. Biochemical and Biophysical Research Communications 71(4): 952-958.
[28] Mudron, P., Herzog, K., Höltershinken, M., Rehage, J. (2007): Effects of abdominal surgery on thiobarbituric acid reactive substances and plasma anti-oxidative capacity in dairy cows. Journal of Veterinary Medicine. A, Physiology, Pathology, Clinical Medicine 54(8): 441-444.
[29] Rosenfeldt, F., Wilson, M., Lee, G., Kure, C., Ou, R., Braun, L., de Haan, J. (2013): Oxidative stress in surgery in an ageing population: pathophysiology and therapy. Experimental Gerontology 48(1): 45-54.
[30] Koźlik, J., Przybyłowska, J., Mikrut, K., Żukiewicz-Sobczak, W.A., Zwoliński, J., Piątek, J., Sobcza, P. (2015): Selected oxidative stress markers in gynecological laparoscopy. Videosurgery and Other Miniinvasive Techniques 10(1): 92-100.
[31] Salavati, S., Mogheiseh, A., Nazifi, S., Amiri, A., Nikahval, B. (2021): The effects of melatonin treatment on oxidative stress induced by ovariohysterectomy in dogs. BMC Veterinary Research 17(1): 1-8.
[32] Haliwell, B., Chirico, S. (1993): Lipid peroxidation: Its mechanism, measurement, and significance. American Journal of Clinical Nutrition 57: 7155-7255.
[33] Yilmaz, O., Korkmaz, M., Jaroszewski, J.J., Yazici, E., Ulutas, E., Saritas, Z.K. (2014): Comparison of flunixin meglumine and meloxicam influence on postoperative and oxidative stress in ovariohysterectomized bitches. Polish Journal of Veterinary Sciences 17(3): 493–499.
[34] Anup, R., Aparna, V., Pulimood, A., Balasubramanian, K.A. (1999): Surgical Stress and The Small Intestine: Role of Oxygen Free Radicals. Surgery 125: 560-569.
[35] Kehlet, H., Holte, K. (2001): Effect of postoperative analgesia on surgical outcome. British Journal of Anaesthesia 87(1): 62-72.
[36] Nandi, A., Yan, L.J., Jana, C.K., Das, N. (2019): Role of catalase in oxidative stress-and age-associated degenerative diseases. Oxidative Medicine and Cellular Longevity 2019: 9613090.
[37] Azevedo, R.B., Lacava, Z.G.M., Miyasaka, C.K., Chaves, S.B., Curi, R. (2001). Regulation of antioxidant enzyme activities in male and female rat macrophages by sex steroids. Brazilian Journal of Medical and Biological Research 34: 683-687.
[38] Matés, J.M., Pérez-Gómez, C., De Castro, I.N. (1999): Antioxidant enzymes and human diseases. Clinical Biochemistry 32(8): 595-603.
[39] Franco, A.A., Odom, R.S., Rando, T.A. (1999): Regulation of antioxidant enzyme gene expression in response to oxidative stress and during differentiation of mouse skeletal muscle. Free Radical Biology and Medicine 27(9-10): 1122-1132.
[40] Arora, K.S., Gupta, N., Singh, R.A., Nagpal, S., Arora, D. (2009): Role of free radicals in menopausal distress. Journal of Clinical Diagnosis Research 3: 1900–1902.
[41] Pérez-Torres, I., Guarner-Lans, V., Rubio-Ruiz, M.E. (2017): Reductive stress in inflammation-associated diseases and the pro-oxidant effect of antioxidant agents. International Journal of Molecular Sciences 18(10): 2098.
[42] Li, Y., Wei, G., Chen, J. (2004): Glutathione: a review on biotechnological production. Applied Microbiology and Biotechnology 66(3): 233-242.
[43] Luo, J.L., Hammarqvist, F., Andersson, K., Wernerman, J. (1996): Skeletal muscle glutathione after surgical trauma. Annals of Surgery 223(4): 420.