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Research Article

The Possible Toxic Effects of Glutathione Intramuscular Injection on Ovaries of Female Albino Rats (Experimental Study)

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Abstract

Background: Glutathione can alter the depigmentation of skin and convert eumelanin to pheomelanin during melanogenesis. As a result, glutathione has become one of the most used skin-whitening agents, and women have become obsessed with it. So, the purpose of this study was to see how intramuscular glutathione injection can affect the ovaries of female albino rats. **Methods:** 50 females albino rats were involved. The animals were divided into two groups; A: the control group and B: the glutathione-injected group, which received glutathione intramuscularly three times per week for three consecutive weeks at a dose of 30 mg/day. The injected group was subdivided into four subgroups based on how soon after the last dose the rats were sacrificed (B1: immediately, B2: after 24h, B3: after one week and B4: after two weeks). Ovarian tissue samples were collected from all groups. Each sample was subjected for histopathological examination and estimation of estrogen and progesterone by ELISA. Malondialdehyde (MDA) and reduced glutathione (GSH) levels also were detected. **Results:** MDA level was significantly higher in subgroups of group B than group A, while GSH level was significantly reduced. The histopathological examination revealed that glutathione injections led to edematous stroma and luteoma. As regard hormones levels, there was significant increase in all (B) subgroups compared to control group. In this study, ovarian changes caused by the prooxidant behavior of glutathione, were reduced partially after two weeks. **Conclusion:** External glutathione may function as a pro-oxidant and have negative effect on the ovaries.

Keywords: Estrogen, Progesterone, ELISA and MDA

Introduction

Glutathione can influence directly or indirectly pigmentation of skin by preventing tyrosinase action during melanogenesis. Glutathione alters the depigmentation of melanocytotic substances and converts eumelanin to pheomelanin[1].

Glutathione, as skin lightening compound, is widely available in the market in topical, oral, inhaled or injectable formulations. Oral supplements are less effective than injections due to their low bioavailability[1]. So, injections are considered to be more demanded.

The Philippine Food and Drug Administration has documented adverse effects from parenteral glutathione injection including kidney dysfunction, abdominal pain, skin diseases, and the consequences of unskilled injection methods. The safety profiles of parenteral glutathione, as skin lightening component, are still unknown and researches on its impact on different body organs are limited[2].

It is also known that women are more interested and the main users of these supplements. Thus, it is important to demonstrate if these supplements can affect their reproductive life[3]. The objective here was to investigate the impacts of glutathione intramuscular injection on the ovaries.

Material and Methods

Animals:

Fifty female albino rats weighing 180-220 gm on average (at 9 weeks of age) were involved. They were obtained from the university's growth facility for research animals in Minia, Egypt. Experimental study was conducted in accordance with the recommendations of care and use of laboratory animals approved by ethical committee of Faculty of Medicine, Minia university, approval No 208:2022.

The animals were housed in separated clean plastic cages with good ventilation. They were provided with a well-balanced standard diet and tap water. They were maintained at a consistent temperature. This study was done during the period from 1st of March to 5th of April 2022.

Chemicals:

Glutathione IM, Spanish product was obtained from Cosmo Medica Company, Nasr City, Cairo Governorate. The vial is about 10 ml. each contains 2400 mg glutathione.

Experimental design:

Rats were classified into two groups, ten rats in group (A) and forty rats in group (B). They were arranged as follows:

Group (A): served as control. 10 rats were given saline by intramuscular method to exclude stressful injection conditions.

Group (B): 40 rats injected intramuscularly, at 10 a.m., with a dose of 30 mg/ day. It was given three times per week for three consecutive weeks.

Group B was divided into four subgroups in separate cages, 10 rats per each subgroup as follows:

Subgroup (B1): 10 rats were sacrificed immediately after the last dose to study the glutathione effect on the ovaries.

The rest 30 rats were kept without any injections and subjected to the same previous living conditions to study the extent of glutathione effect (if any in subgroup B1). Then rats were sacrificed as the follows:

Subgroup (B2): 10 rats were sacrificed one day after glutathione last dose.

Subgroup (B3): 10 rats were sacrificed one week after the last dose.

Subgroup (B4): last rats were sacrificed two weeks after the last dose.

The rats were slaughtered by cervical dislocation while exposed to ether inhalational anesthesia. the ovaries were dissected and processed for histopathological study after staining by H&E, biochemical analysis using the ELISA method and oxidative markers detection.

Biomarkers:

Reduced glutathione (GSH):

According to **Turgut et al., (2006)[4],** GSH estimation accomplished through a modification of the Moron et al. procedure in 1979, using dithio-bis-nitrobenzoic acid (DTNB). The unit of measurement is nmol/g.

Malondialdehyde (MDA):

According to **Aly (2020)[5],** its measurement in tissues is relied on thiobarbituric acid reaction (TBARS). The unit of measurement is nmol/g.

Histopathology:

The ovarian sections stained with hematoxylin and eosin (H&E) then examined using a light microscope with an attached camera to photograph these sections (Olympus BX51, Tokyo, Japan), in Pathology department, Faculty of Medicine, Minia University.

Kits for biochemical analysis:

Kits were obtained from BioKits, 27 Mohamed Naguib Serry St.,Off El Gomhoureya St., - Assiut – Egypt. The **Estrogen** ELISA kits, Bioassay Technology Laboratory, USA (Catalog No: E0176Ra). The measurement unit is ng/L. The **Progesterone** ELISA kits, FineTest, USA (Catalog No: ER1255). The unit of measurement is ng/ml.

Statistical analysis:

The collected data were coded, tabulated, and analyzed using (SPSS) program software, version 25. Graphical Presentations were done using Microsoft office Excel 365, version 2016. Level of significance was considered at P value ≤ 0.05.

Results

Oxidative stress markers:

Ovarian MDA:

There is statistically significant increase in all subgroups (B1, B2, B3 and B4) compared with that of the control group. Its level increases through time among the four glutathione subgroups. There is a significant difference *(P < 0.001)* in ovarian MDA levels between B1 and B2. Difference in subgroups (B2 vs. B3) and (B3 vs. B4) is insignificant (Table 1).

Ovarian GSH:

There is statistically significant decrease in GSH level in all subgroups (B1, B2, B3 and B4). Level of ovarian GSH decreases through time among the four glutathione subgroups. There is a significant difference *(P < 0.001)* in GSH levels between B1 and B2. Difference in subgroups (B2 vs. B3) and (B3 vs. B4) is insignificant (Table 2).

According to (chart 1), In ovarian tissues, GSH and MDA parameters are strongly inversely correlated.

Histopathology results:

Ovarian tissue sections obtained from rats in group A, shows multiple follicles at different stages of development within normal stroma (Figure1). While ovarian tissue sections obtained from rats in subgroup B1, shows enlarged graafian follicle with marked stromal edema (Figure2). In subgroup B2, there is marked ovarian stromal edema with graafian follicles at various stages of development and large luteoma (increased cell number and size of corpus luteum) (Figure 3A& 3B).

As regard ovarian sections obtained from rats in subgroup B3, shows large graafian follicles with moderate stromal edema (Figure 4). While ovarian sections obtained from rats in subgroup B4, shows mild stromal edema with few luteal cells (Figure 5).

Biochemicals:

Estrogen and Progesterone:

As regard hormonal levels, ANOVA test reveals statistically significant (*P < 0.001*) rise in all subgroups (B1, B2, B3 and B4) when compared with group A. There is a statistically significant decrease (*P < 0.001*) in both estrogen and progesterone levels in subgroups B3 and B4 compared with B1 and B2 (Table 3, 4).

Table (1): Ovarian MDA level changes between the examined groups

	Group A Control	**Group B1 Immediate**	**Group B2 After 24 h**	**Group B3 After 1 week**	**Group B4 After 2 weeks**
	N=10	**N=10**	**N=10**	**N=10**	**N=10**
Ovarian MDA (nmol/g) *Range* *Mean ± SD*	 (0.21-0.21) 0.21±0.004	 (0.24-0.33) 0.28±0.024	 (0.36-0.39) 0.37±0.01	 (0.38-0.40) 0.39±0.003	 (0.39-0.41) 0.40±0.006
Control (A)		***<0.001****	***<0.001****	***<0.001****	***<0.001****
Immediate (B1)			***<0.001****	***<0.001****	***<0.001****
After 24 h (B2)				***0.068***	***<0.001****
After 1 week (B3)					***0.112***

One Way ANOVA test for quantitative data between the five groups followed by post Hoc Tukey's analysis between each two groups

- ****: Significant level at P value < 0.05***

Table (2): Ovarian GSH level changes between the examined groups

	Group A Control	**Group B1 Immediate**	**Group B2 After 24 h**	**Group B3 After 1 week**	**Group B4 After 2 weeks**
	N=10	**N=10**	**N=10**	**N=10**	**N=10**
Ovarian GSH (nmol/g) *Range* *Mean ± SD*	 (0.67-0.71) 0.69±0.01	 (0.59-0.63) 0.61±0.01	 (0.37-0.42) 0.40±0.02	 (0.35-0.42) 0.38±0.02	 (0.31-0.42) 0.37±0.04
Control (A)		***<0.001****	***<0.001****	***<0.001****	***<0.001****
Immediate (B1)			***<0.001****	***<0.001****	***<0.001****
After 24 h (B2)				***0.505***	***0.015****
After 1 week (B3)					***0.438***

One Way ANOVA test for quantitative data between the five groups followed by post Hoc Tukey's analysis between each two groups
****: Significant level at P value < 0.05***

Table (3): Estrogen level changes between the examined groups

	Group A Control	**Group B1 Immediate**	**Group B2 After 24 h**	**Group B3 After 1 week**	**Group B4 After 2 weeks**
	N=10	**N=10**	**N=10**	**N=10**	**N=10**
Estrogen (ng/L) *Range* *Mean ± SD*	 (2.89-2.98) 2.94±0.03	 (3.08-3.19) 3.12±0.03	 (3.95-4.06) 4.00±0.04	 (3.78-3.96) 3.87±0.06	 (3.13-3.29) 3.21±0.06
Control (A)		***<0.001****	***<0.001****	***<0.001****	***<0.001****
Immediate (B1)			***0.001****	***<0.001****	***<0.001****
After 24 h (B2)				***<0.001****	***<0.001****
After 1 week (B3)					***<0.001****

- ***One Way ANOVA test for quantitative data between the five groups followed by post Hoc Tukey's analysis between each two groups***
- ****: Significant level at P value < 0.05***

Table (4): Progesterone level changes between the examined groups

	Group A Control	**Group B1 Immediate**	**Group B2 After 24 h**	**Group B3 After 1 week**	**Group B4 After 2 weeks**
	N=10	**N=10**	**N=10**	**N=10**	**N=10**
Progesterone (ng/mL) *Range* *Mean ± SD*	 (0.9-0.99) 0.95±0.03	 (1.01-1.06) 1.03±0.02	 (1.32-1.36) 1.34±0.01	 (1.26-1.33) 1.29±0.02	 (1.04-1.11) 1.076±0.02
Control (A)		***<0.001****	***<0.001****	***<0.001****	***<0.001****
Immediate (B1)			***<0.001****	***<0.001****	***<0.001****
After 24 h (B2)				***<0.001****	***<0.001****
After 1 week (B3)					***<0.001****

- ***One Way ANOVA test for quantitative data between the five groups followed by post Hoc Tukey's analysis between each two groups***
- ****: Significant level at P value < 0.05***

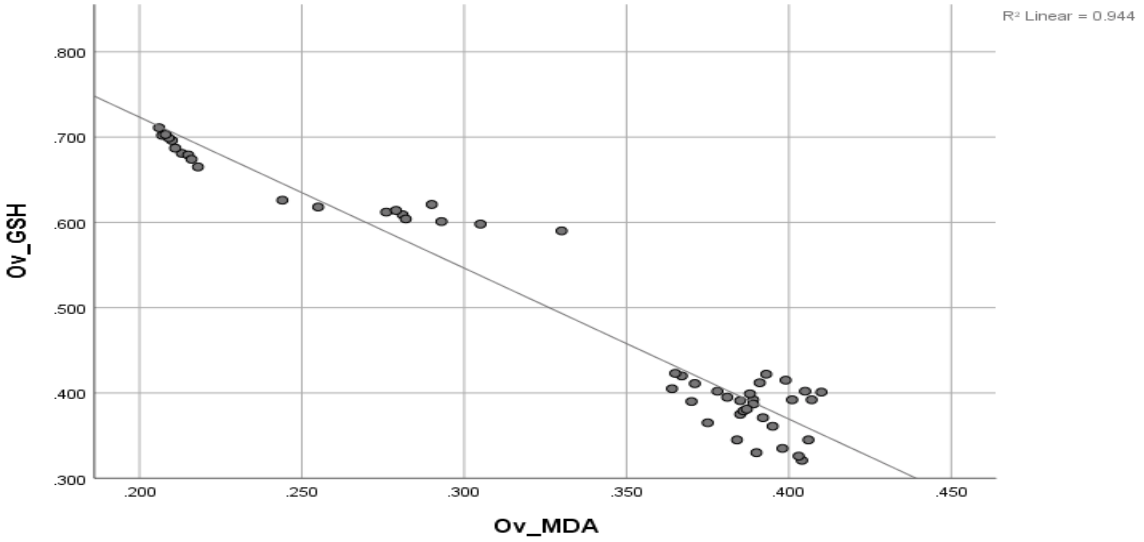

Chart (1): showing correlation between levels of ovarian MDA and GSH

Histopathology results:

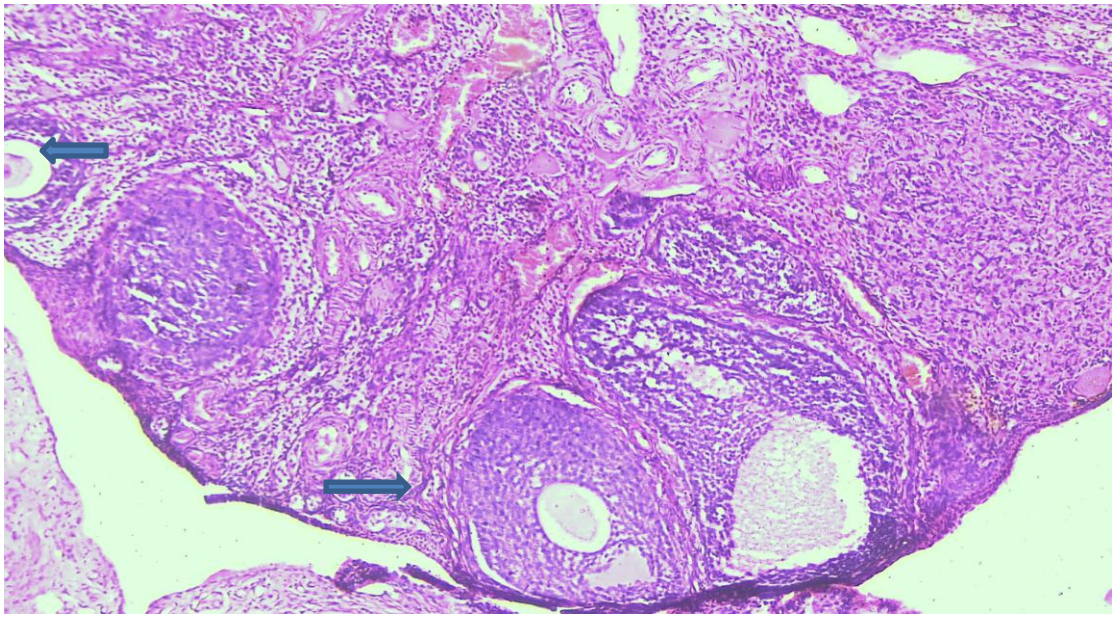

Figure (1): A photomicrograph of rat ovarian section of the control group A: showing multiple follicles at different stages of development (arrows) within normal stroma (H&E stain X4)

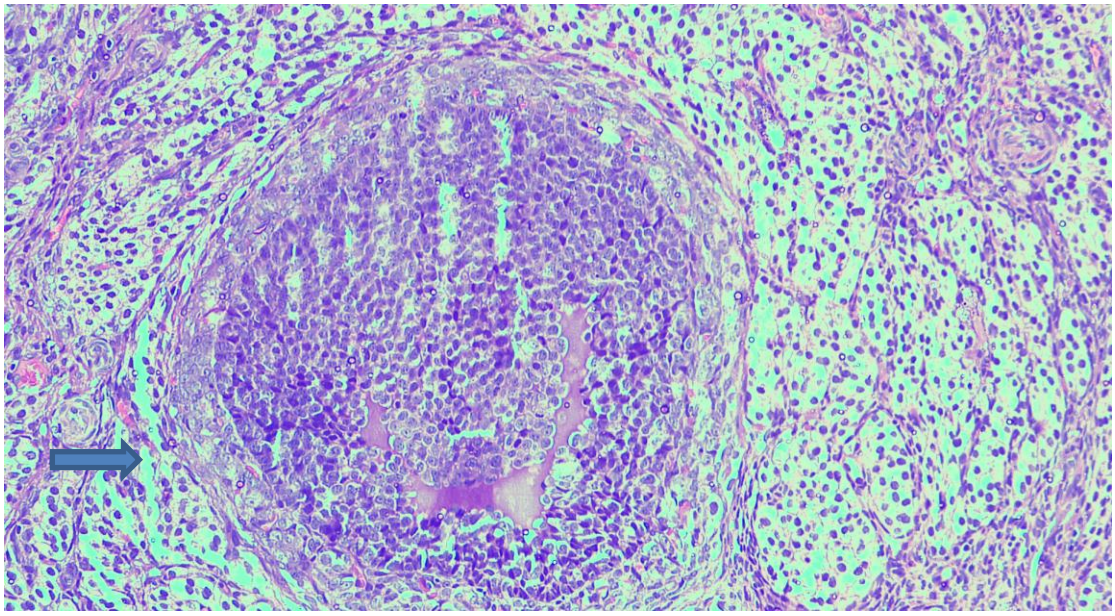

Figure (2): A photomicrograph of rat ovarian section of subgroup B1: showing enlarged graafian follicle with marked stromal edema (H&E stain X10)

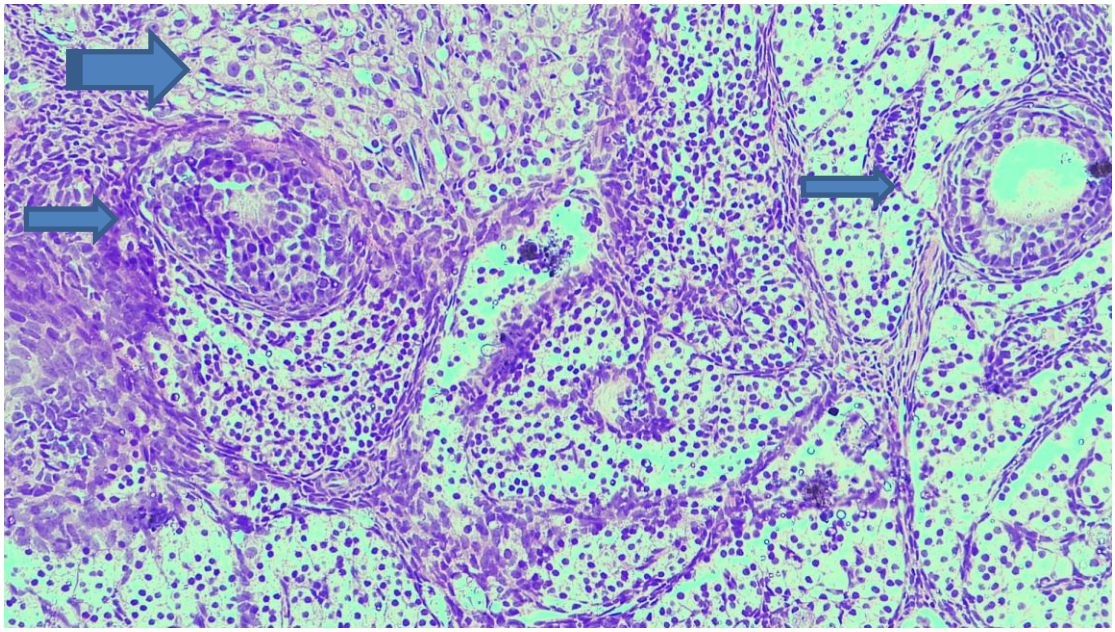

Figure (3A): A photomicrograph of rat ovarian section of subgroup B2: showing marked ovarian stroma edema with graafian follicles at different stages of development (small arrows) and luteoma (Large arrow) (H&E stain X10)

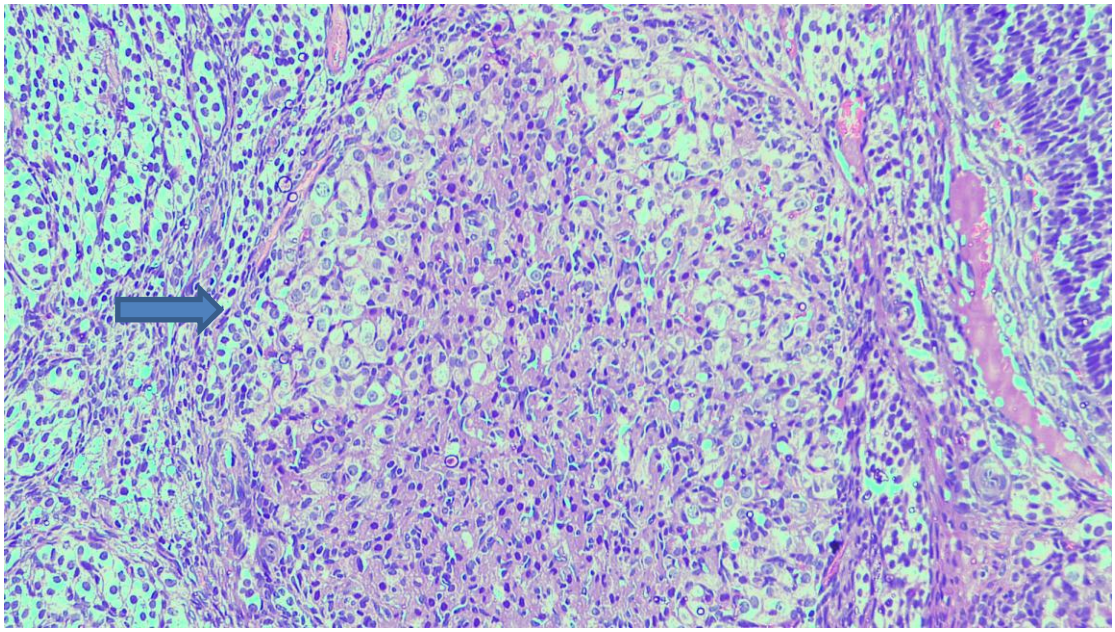

Figure (3B): A photomicrograph of rat ovarian section of subgroup B2: showing severe ovarian stromal edema and large luteoma (arrow) (H&E stain X10)

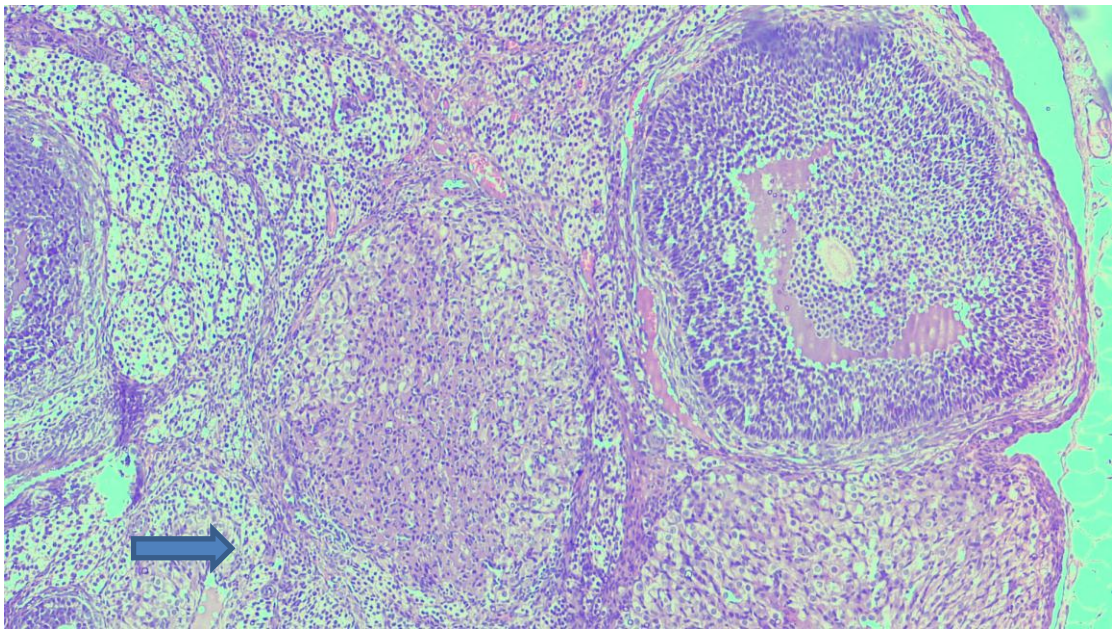

Figure (4): A photomicrograph of rat ovarian section of subgroup B3: showing large graafian follicles with moderate stromal edema (H&E stain X4)

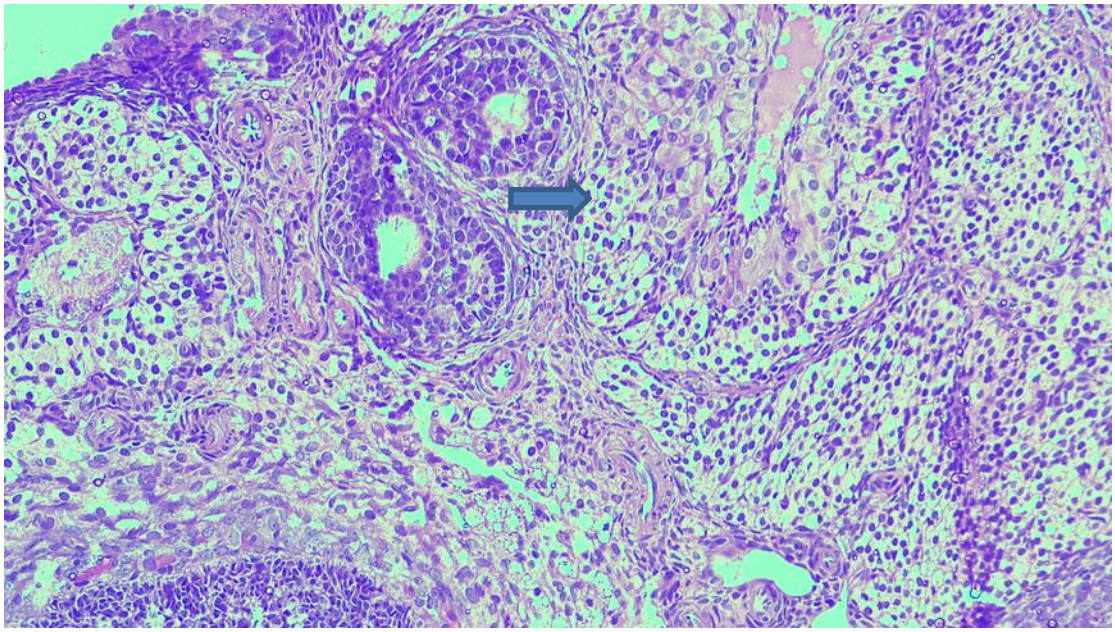

Figure (5): A photomicrograph of rat ovarian section of subgroup B4: showing mild ovarian stromal edema with few luteal cells (arrow) (H&E stain X10)

Discussion

Glutathione, as whitening agent, is commonly accessible in markets as topical, oral, inhaled or injectable formulations. Although several studies have shown positive effects of glutathione supplements on metabolic variables, researches on safety profiles of glutathione injection and its effects on various body systems are not enough[6].

However, in the year of 2010, Ramirez et al.,[7] found that oral and intravenous glutathione caused abnormal thyroid function, and subclinical hyperthyroidism was present in all cases included in the study. In 2016, Zubair et al.,[8] also revealed that parenteral glutathione could lead to abnormal liver function and anaphylactic shock. The purpose of this study was to evaluate the burden of glutathione injections on ovaries as women are the most concerned about cosmetics.

When injected rats' ovaries were compared to the control group, MDA level was significantly higher and GSH level was significantly lower in all subgroups. In terms of significant differences between subgroups, B1 and B2 subgroups showed a significant difference, but the difference between (B2 vs. B3) and (B3 vs. B4) was insignificant.

Unexpectedly, the findings in this study suggested that three-week glutathione injection was linked to high MDA levels and low GSH levels, which may be explained by the possibility that external glutathione could have pro-oxidant effects and affect cellular redox potential[9].

That are consistent with Sagristá et al., 2002[10] who demonstrated that thiol-reactive radical interaction can result in the formation of thiyl radicals, which could possess pro-oxidant properties. This was determined by the nature of the radicals and the iron/H_2O_2 -dependent oxidative actions.

Also, in agreement with Pompella& Corti, 2015[11] who shown that GSH, and particularly its catabolites, can support oxidative processes by taking part in metal ion-mediated reactions that eventually led to release of reactive oxygen species and free radicals.

These also accordance with Dewi et al., 2020[12] who evaluated the effect of glutathione (1.5

milligram intramuscular /day for two weeks) on MDA level in albino rat retina. They found that MDA levels in injected rats with glutathione were in higher level than in the control group. That was explained through pro-oxidant action of glutathione.

On the other hand, these results don't agree with many previous studies which proved that glutathione has antioxidant action. Cheraghi et al., 2019[13] study which included 50 coronary disease patients. MDA and GSH levels were measured to compare between healthy and diseased patients. They illustrated that glutathione has protective effect on heart against MDA.

In addition, Ahmadvand et al., 2019[14] study demonstrated that on antioxidant (gallic acid), intra peritoneal injection in a dose of 100 mg/kg to renal ischemic rats, the treated group had lower MDA levels than the control.

In the current study GSH and MDA levels didn't change significantly at subgroups B3 and B4, this may be because no further free radicals were released on cessation of injections. GSH level also was negatively related to MDA level, which could be explained by the fact that GSH (the reduced form) was consumed to neutralize free radicals, resulting in lipid peroxidation and high MDA levels[15].

This is in conjunction with Surapaneni, 2007[16] who studied the relation between lipid peroxidation and antioxidant system. He found that increased oxidative stress markers such as MDA was accompanied with drop in antioxidant concentration such as GSH.

As regard the histological findings in the injected subgroups compared to the control group, ovarian tissues of B1 rats revealed marked stromal edema and Subgroup B2 ovarian sections demonstrated marked ovarian stromal edema and a large luteoma. While edema and the number of luteal cells decreased in subgroups B3 and B4.

This is in conjunction with Alchalabi et al., 2016[17] study which revealed that oxidative stress, caused by radiation exposure for one to two months, could lead to hypertrophy of corpus luteum and ovarian congestion in female rats. According to Firouzabadi et al., 2022[18], disruption at antioxidant / ROS balance leads to oxidative stress which in turn can cause problems such as infertility.

However, in Li et al., 2021[19] study human ovarian tissue was frozen and xenografted into mice and glutathione supplement was given intravenously once per day for one week. They showed that GSH supplement in the hosts ovarian grafts improved follicular survival rate.

When it comes to ovarian biochemistry, estrogen and progesterone levels significantly increased in all subgroups that received glutathione. Levels of hormones increased immediately after the last dose and through the next 24 hours. This was followed by gradual decrease over the next two weeks.

The oxidative stress caused by glutathione injection increased the levels of ovarian hormones. In the long run, this can affect ovulation and pregnancy[20].

That agrees with Vahid et al., 2012[21] who conducted an experiment in which mobile waves were shown to have oxidative stress effects. These effects included increase in ovarian follicle size and significant increase in FSH, estrogen and progesterone hormones secretion. Also, Dirik & Kömüroğlu, 2021[22] discovered that the increasing MDA parameters in rats' tissue in the ovarian hyperstimulation syndrome group was associated with higher levels of estrogen.

However, Elkady et al., 2019[23] illustrated how antioxidants, in appropriate dose and type, could be used to reverse ovarian toxicity and premature ovarian failure brought on by oxidative stress and inflammation (induced by cyclophosphamide).

Thus, external parenteral glutathione, as skin whitening agent, in such dose can alter redox status and can lead to oxidative stress impact on ovarian tissues and hormones.

Literatures on the impact and mechanism by which intramuscular glutathione injection can affect ovaries are scarce. This might be because of the novelty of the study topic and the scant research.

References

1. Weschawalt S, Tho.ngthip S, Phut rakool. P and Asawa.nonda P. Glutathione and its antiaging and antimelanogenic effects. Clinical, Cosmetic and Investigational Dermatology. 2017; 10:147-153.
2. Podder I and Sar.kar R. Systemic therapy for melasma. Exploring newer options− A comprehensive review. Pigment International. 2017; 4(2): 78 -84.
3. Blay Y. Skin bleaching and global white supremacy. The Journal of Pan African Studies. 2011; 4(4): 4-46.
4. Turgut G, Yaşar EN, Kaptan oğlu B, Turgut S and Osman GE. Changes lev of MDA & GSH. Eastern Journal of Medicine. 2006; 11(1): 7-12.
5. Aly AA, Sayed SM, Abdel hafez ES, Abdelhafez SM, Abdelzaher WY, Raslan MA, et al. New quinoline-2-one/pyrazole derivatives and caspase-3 inhibition assay. Bioorganic Chemistry.2020;94:1-13.
6. Mahmood M. The Effectiveness of Glutathione on Skin Lightening. International Journal of Medical Sciences. 2022; 5(2): 5-16.
7. Ramirez DJ, Vergara-Villaluz JC, Pilar M, Jasul GV and Añel-Quimpo JS. Prevalence of thyroid dysfunction among individuals taking glutathione supplementation: a cross-sectional study. Philippine Journal of Internal Medicine. 2010;48(3):1-6.
8. Zubair S, Hafeez S and Mujtaba G. Efficacy of intravenous glutathione vs. placebo for skin tone lightening. Journal of Pakistan Association of Dermatologists. 2016; 26(3): 177-181.
9. Rebrin I and Sohal RS. Pro-oxidant shift in glutathione redox state during aging. Advanced drug delivery reviews. 2008; 60(14): 1545-1552.
10. Sagristá ML, GarcÍa AF, De Madariaga MA and Mora M. Antioxidant and pro-oxidant effect of the thiolic compounds N-acetyl-L-cysteine and glutathione against free radical-induced lipid peroxidation. Free Radical Research.2002;36(3):329-340
11. Pompella A and Corti A. The changing faces of glutathione, a cellular protagonist. Frontiers in pharmacology.2015;6:98- 104.
12. Dewi PK, Cahyono M, Prih atningtias R, Ekowati L and Wildan A. The Effect of Glutathione on Serum Malondialdehyde (MDA) Level in Retinopathy of Prematurity Rat Models. Journal of Biomedicine and Translational Research. 2020; 6(3): 86-88.
13. Cheraghi M, Ahmadvand H, Maleki A, Babaeenezhad E, Shakiba S and Hassanzadeh F. Oxidative stress status and liver markers in coronary heart disease. Reports of Biochemistry & Molecular Biology. 2019; 8(1): 49- 55.
14. Ahmadvand H, Yalameha B, Adibh esami G, Nasri M, Naderi N, Babaeenezhad E, et al., The protective role of gallic acid pretreatment on renal ischemia-reperfusion injury in rats. Reports of Biochemistry & Molecular Biology. 2019; 8(1): 42-48.
15. Tsikas D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. Analytical Biochemistry. 2017;524: 13-30.
16. Surapaneni KM. Status of Lipid Peroxidation and Antioxidants in Schizophrenic. Journal of Clinical Diagnosis and Research. 2007; 1(2): 39-44.
17. Alchalabi AS, Rahim H, Aklilu E, Al-Sultan II, Malek MF, Ro.nald SH, et al. Histopathological changes associated with oxidative stress induced by electromagnetic waves in rats ovarian and uterine tissues. Asian Pacific Journal of Reproduction. 2016; 5(4): 301-310.
18. Firouzabadi AM, Imani M, Zakizadeh F, Ghaderi N, Zare F, Yadegari M, et al. Evaluating effect of acrylamide and ascorbic acid on oxidative stress and apoptosis in ovarian tissue of wistar rat. Toxicology Reports. 2022; 9: 1580-1585.
19. Li Y, Hu Y, Zhu S, Tuo Y, Cai B, Long T, et al. Protective Effects of Reduced Glutathione and Ulinastatin on Xenotransplanted Human Ovarian Tissue Against Ischemia and Reperfusion Injury. Cell Transplantation. 2021; 30: 1-12.
20. Özdemir AZ, Karli P and Gülümser Ç. Does high estrogen level negatively affect pregnancy success in frozen embryo transfer?. Archives of Medical Science. 2022; 18(3): 647- 651.
21. Vahid HJ, Khatereh D, Esmaeal F, Maryam N and Mohammad F. The effects of mobile phone waves on the reproductive physio logy in adult female rats. Advances in Environmental Biology. 2012; 2735-2742.

22. Dirik D and Kömüro ğlu AU. The Effect of Infliximab on Oxidative Stress in Ovarian Tissue of the Rat with Ovarian Hyper stimulation Syndrome. Eastern Journal of Medicine. 2021; 26(3): 475-480.
23. Elkady MA, Shalaby S, Fathi F and El-Mand ouh S. Effects of quercetin and rosuvastatin each alone or in combination on cyclophosphamide-induced premature ovarian failure in female albino mice. Human & Experimental Toxicology. 2019; 38(11):1283-1295.