



The effect of vitamin C on ovary of female white rats treated with kmno4. Histological & physiological study

Sami Raheem Al-Katib* Ahmed H. A. Al Azam**
Sana Abadi Habeab***

*College of Medicine, University of Kufa

**College of Veterinary Medicine, University of Kufa

***College of Science, University of Kufa

Abstract:

The present study was conducted to verify the oxidative stress status by using different doses of KMnO₄ (100,200,300) mg /70kg b.w and role of antioxidant agents at different concentrations of Vitamin C (1000, 1500, 2000) mg /70kg b.w on female infertility. To achieve this aim, 72 female rats with ages of 2-3 months, weighing (180g-250g) were enrolled. To compare the results, 24 healthy individuals of ages (2-3 months) were also studied which divided to three chief groups: treated with KMnO₄, Vitamin C and combination of (KMnO₄+Vitamin C). Serum Vitamin C and ovaries were dissected for histopathological study. The results indicated a significant decrease ($p<0.05$) of Vitamin C values in sera of groups that treated with (KMnO₄) alone, when compared with the control group, while Vitamin C levels showed a significant ($p<0.05$) increase in Vitamin C treated groups in respect with those of the control group. The highest levels of Vitamin C values were found in the groups that combined of treated with (KMnO₄+Vitamin C). There was a normal histological appearance of ovary in control group, the treatment with 1000 mg/70kg vitC. b wt showed an increase in the blood flow within the parenchyma of ovary representing congestion of blood vessels and numerous of growing follicles and huge graffian follicles. While treatment with different doses of KMnO₄ resulted in appearance of few number of growing follicles and huge graffian follicles. These results highlighted the involvement of the oxidative stress in female infertility and confirmed the benefits of the use of antioxidants in the medication of this condition, not only to improve the environment of follicles, but also to modulate female infertility hormonal profile.

دراسة نسيجية وفسلجية لتأثير فيتامين ج على المبيض في أنثى الجرذان البيضاء

سامي رحيم الكاتب* أحمد حميد العزام**
سنا عبادي حبيب***

*كلية الطب، جامعة الكوفة
**كلية الطب البيطري، جامعة الكوفة
***كلية العلوم، جامعة الكوفة

الخلاصة:

تم تصميم الدراسة الحالية للتحقق من تغيرات الإجهاد التأكسدي ودراسة تأثير فعل مضادات التأكسد في الجهاز النكاثري الأنثوي للجرذان المختبرية، وللتحقق هذا الهدف تم اختيار 72 جرذ، استعمل كمجاميع تجريبية و 24 جرذ استعمل كمجموعة سيطرة وتراوحت أعمارها (2-3 شهر) وتراوحت أوزانها (180-250 غم). استعملت مادة برمنغنات البوتاسيوم كمادة مؤكسدة بتركيزات مختلفة (100,200,300) ملغم/70 كغم. وفيتامين ج كمادة مضادة للتأكسد بتركيزات مختلفة (1000,1500,2000) ملغم/70 كغم. قسمت مجاميع المعاملة الى ثلاثة: مجموعة تعامل بمادة برمنغنات البوتاسيوم فقط، و مجموعة تعامل بفيتامين ج فقط، و مجموعة تعامل بالمادتين سوياً. إضافة الى مجموعة السيطرة. أذ تم قياس مستويات فيتامين ج وكافة المجاميع، بالإضافة الى دراسة المقاطع النسيجية للرحم وكانت النتائج كما يلي:-

1. حدوث زيادة معنوية ($p < 0.05$) في معدل تركيز فيتامين ج في المجموعة المعاملة بفيتامين ج وبكافة التراكيز مقارنة بمجموعة السيطرة.
2. حصول نقصان معنوي ($p < 0.05$) في معدل تركيز فيتامين ج في المجموعة المعاملة ببرمنغنات البوتاسيوم فقط، مقارنة بمجموعة السيطرة.
3. حدوث زيادة معنوية ($p < 0.05$) في معدل تركيز فيتامين ج في المجموعة المعاملة بفيتامين ج و برمنغنات البوتاسيوم عند التركيز (1500+200)، مقارنة مع مجموعة السيطرة والمجموعتين الباقيتين.
4. وأخيراً حدوث تغيرات في المقاطع النسيجية المأخوذة أذ كان التركيب النسيجي طبيعي للمبيض في المجموعة المعاملة ب(فيتامين ج وبرمنغنات البوتاسيوم) ومجموعة فيتامين ج عند التركيزين 1000 و 2000 ملغم/70 كغم. ولوحظ تحسن في التركيب النسيجي للمبيض وزيادة عدد الحويصلات المبيضية عند التركيز 1500 ملغم/70 كغم. أما عند المعاملة ببرمنغنات البوتاسيوم بمختلف التراكيز، فقد لاحظت الدراسة وجود عدد قليل من الحويصلات المبيضية والناضجة.

Introduction:

Vitamin C (ascorbic acid, ascorbate, AA) ($C_6H_8O_6$) is a water soluble organic compound involve in many Biological process. AA plays crucial roles in electron transport, hydroxylation reactions and oxidation catabolism of aromatic compound in animal metabolism (1). Although all the functions of AA are not fully explain, it is also involved in maintaining the reduced state of metal cofactors, for example at monooxygenase (Cu^+) and dioxygenase (Fe^{2+}) (2). In cells the other role of AA is to reduce hydrogen peroxide (H_2O_2), which preserves cells against reactive oxygen species (3,4,5). An oxidation cycle of ascorbic acid to dehydroascorbic acid is shown in figure 1. The details about ascorbic acid antioxidant system cooperated with glutathione was described by Meister (6). Besides this primates and several other mammals are not able to synthesize ascorbic acid (5). the animal species, which able to produce this molecule, biosynthesis AA from glucose catalyzed L-gulonolactone oxidase (1,2). In spite of the ability to synthesize this molecule both groups of animal species suffer from deficiency of AA (1,2).

Daily needs of vitamin C, by only way human uptake ascorbic acid is via food (7) but the Daily needs of vitamin C for a human are not clear yet. Linus Pauling postulated that people needs for vitamin and other nutrients vary markedly and that to maintain good health, many people need amounts of nutrients much greater than recommended doses. According to his suggestions, daily uptake of vitamin C has to be within units of gram of AA to reduce the incidence of colds and other diseases. These "huge" amount of AA have not been ever proved as the reason for large reducing of the of the illness. Nowadays estimated ovary requirement and recommended dietary allowance of ascorbic acid are 100mg per day and 120mg per day, respectively (8,9).

2. Material and method:**2.1 Preparations real samples:****2.1.1 Animal Preparation:**

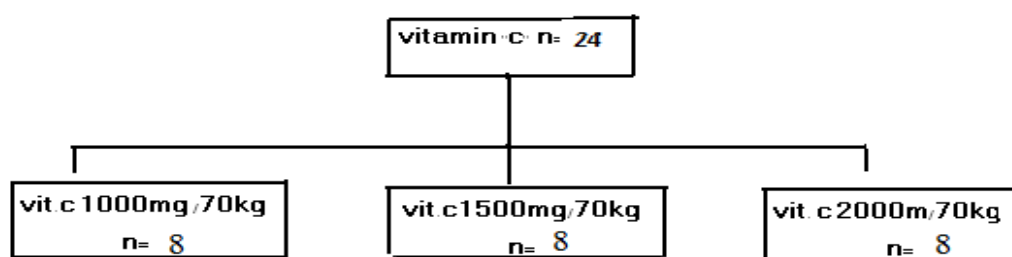
Experiments were performed on adult female Wistar (*Rattus norvegicus*) rats. Weighing 180-250g. Rats were housed in group of four per cage with free access to food and water under a: 12:12h light- dark cycle, with lights on at 8:00am.

To vaginal smears (10, 11). Which were collected on glass slides and stained with Giemsa (12). Vaginal smear cytology is used for the determination of the estrous cycle phases (13) all experimental rats in

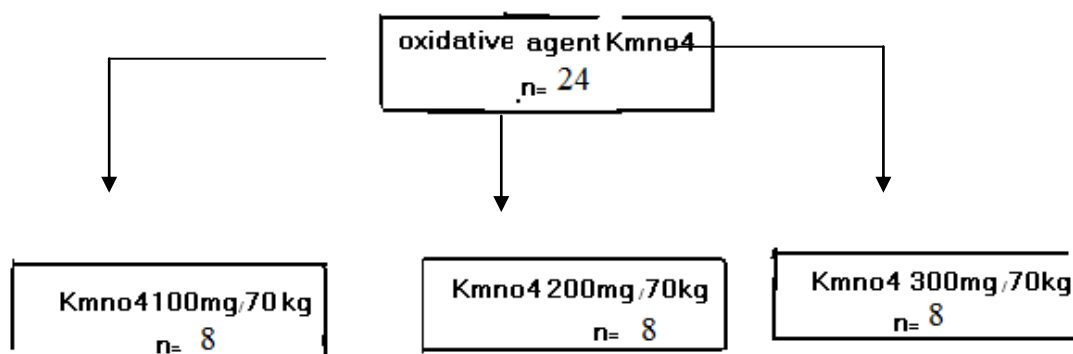
metestus were used for determination of concentration of vitamins "C" in plasma and to know the role activity in uterus and ovary. During the sexual cycle in healthy circumstances.

This experiment was designed as :-

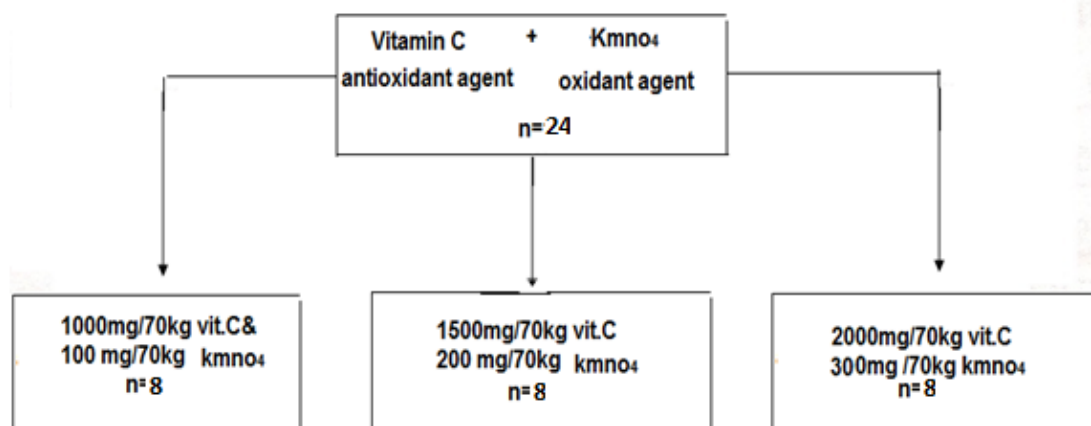
1- Group -1- for determination of vitamin "C" (n=24).



2-Group -2-for determination of oxidative agent ,Kmno4 (n=24).



3- Group-3- for determination of Kmno4 & vitamin "C" (n=24).



4- Group-4-control (n=24).

And after 10 days (toward period of estrus cycle) long enough to get a complete depletion of endogenous hours.

Female reproductive tract morphology

An important requirement for experiments is the ability to assess the effects of drugs (oxidant & antioxidant agent) on specific organs. It was essential to understand the features of entire female reproductive tract morphology in the present study. This will enable to provide strong explanations for the functional significance of variation in ovary morphology between different treatment groups. Hence, intact were dissected from control, and other groups for observation. They were cleaned and photographed using Real DV MPEG4 camera.

Biochemical measurements

Photometric Method for the determination of Ascorbic Acid :-(14)

Principle:

Ascorbic acid in plasma is oxidized by Cu^{+2} to form dehydroascorbic acid 2,4-dinitrophenylhydrazine to form a red bis-hydrozone, which is measured at A520.

Specimen:

Collect heparinized blood and centrifuge to obtain plasma, which should be analyzed immediately, or not later than 3h if specimen is refrigerated.

Reagents:

1. Metaphosphoric acid solution, 6.0 g/dL. Dissolve 30.0 g of metaphosphoric acid (HPO_3) in distilled water and bring to a final volume of 500mL. Prepare immediately before use.

2. Sulfuric acid, 4.5mol. Add slowly 250 ml of concentrated sulfuric acid, reagent grade, to 500mL of cold water in a 1-L flask and fill to mark with distilled water. Caution: Because significant heat is generated when concentrated sulfuric acid is diluted, the flask should be placed in an ice bath. The concentrated acid should be added slowly and the resulting solution mixed constantly.

3. Sulfuric acid, 12mol /L. Add 650mL of concentrated sulfuric acid to 300 mL of cold water in a 1-L flask cool and fill to mark with distilled water. Refrigerate

4. 2,4- Dinitrophenylhydrazine reagent, 2.0 g/dL in sulfuric acid, 4.5 mol/L. Dissolve 10 g to 2,4--Dinitrophenylhydrazine in sulfuric acid, 4.5 mol /L, and dilute to a final volume of 500 mL. Let stand in the refrigerator overnight, and then filter.

5. Thiourea solution, 5.0 g / dL. Dissolve 5g of thiourea in glass-distilled water and dilute to a final volume of 100 mL. This reagent is stable for 1 mol at 4 C.

6. Copper sulfate solution, 0.6 g / dL. Dissolve 0.6 g of anhydrous copper sulfate corin glass-distilled water and dilute to a final volume of 100 mL.

1mL of the thiourea solution, 5mL of the copper sulfate solution, and 100 mL of the 2,4- dinitrophenylhydrazine reagent. Store in a bottle at 4 C for a maximum of 1wk.

8. Calibrators. All ascorbic calibrators should be prepared daily.

a. Ascorbic acid stock calibrator, 50.0mg /dL. Dissolve 50 mg ascorbic acid in metaphosphoric acid (6.0 g /dL) and bring to a final volume of 100mL with metaphosphoric acid.

b. Intermediate ascorbic acid calibrator, 5.0 mg /dL. Pipette 10.0mL of stock calibrator into a 100-mL volumetric flask and dilute to mark with metaphosphoric acid (6.0 g/dL).

c. Working calibrators. In a series of 25-mL volumetric flasks, pipette the following amount of intermediate calibrator: 0.5, 2.0, 4.0, 6.0, 10.0, 15.0 and 20.0mL. Bring to a final volume of 25mL with metaphosphoric acid (6.0 g /dL) to yield working calibrators of 0.10, 0.40, 0.80, 1.20, 2.00, 3.00, and 4.00mg /dL.

Procedure:

1. Add 0.5 mL of heparinized plasma to 2.0 mL of freshly prepared metaphosphoric acid in a 13 *10 mm test

tube, and mix in avortex mixer. Centryfuge the plasma - metaphosphoric acid mixture for 10 min at 2500*g .pipe 1.2 mL of the clear supernatant into a 13*100mm Teflon –lined ,screw –cap test tube .

2. Add 1.2 mL of each concentration of working calibrator into 13*100 mm screw –cap test tubes. Add 1.2 mL of metaphosphoric acid to tow tubes for use as blabks.

3. Add 0.4mL of DTCS reagent to tubes .Cap tubes, misx content , and incubate the tubes in a water bath at 37C for 3h.

4. Rrmove the tubes from the water bath and chill for 10 min ice bath .While mixing ,slowly add to all tubes 2.0mLof cold sulfuric acid , 12mol /L, cap ,and mix in a vortex mixer .(The temperature of mixture must not exceed room temperature).

5. Adjust the spectrophotometer with the blank to read zero A at 520 nm, and the calibrators and unknowns. Plot the concentration of each working calibrator versus absorbance values. The calibration curve obeys beer, slaw up to ascorbic acid concentration of 2.0 mg /dL .

Calculation:

The concentration of the samples is obtained from the calibration curve and is multiplied by 5 (to correct for dilution of the plasma by metaphosphoric acid) to give the concentration of ascorbic acid per dL of plasma.

Statistical analysis:

The values are expressed as mean $\pm$ standard deviation (SD). The results were evaluated by using the SPSS (version 12.0) and Origin 6 software and valuated by one-way ANOVA followed by Bonferroni t-test. Statistical Significance was considered when value of P was < 0.5

Results:

Effect of vitamin C&KMno4 on certain parameters of ovary:-

1. Diameter of ovary:-

There was a significant increase ($p < 0.05$) by administration of 1000mg/70kg vit.C ,but not for1500& 2000mg /70 kg b.w vit.C as compared with control group, the concentration of KMno4 groups at 100 mg /70kg showed a significant decrease ($p < 0.05$) as compared with control group & others groups , Table (1). In third group, revealed no significant different ($p > 0.05$), in all groups as compared with control group. Table (2).

2. Diameter of corpus luteum:-

Statically analysis for the results of diameter of corpus luteum showing a significant increase ($p < 0.05$) at 1000mg /70kg Vit.C group as compared with control group & others groups(Table 1).. And only a significant decrease ($p < 0.05$) in 100mg/70kg b.w KMno4 group as compared with control group and 200 &300 mg/70kg b.w KMno4 groups. In third group, there was a significant increase at (1500+200) (mg/70kg b.w) of KMno4 + vitamin C group as compared with control group& others groups . (Table 2).

3. Diameter of Graffian follicle:-

The result showed a significant increase ($p < 0.05$) at (1000&1500) mg /70 kg concentration of vitamin C groups as compared with control group&2000(mg/70kg b.w),Table (1).Only a significant decrease ($p < 0.01$) at300mg/70kgb.w of KMno4 group, not significantly changes for other two groups as compared with control group. In third group, there was a significant increase ($p < 0.05$) at (2000+300) (mg/70kg b.w) of KMno4+ vitamin C group as compared with control group & others groups. (Table 2).

4. Weight of ovary:-

Table (1) revealed a significant increase ($p < 0.05$)in Weight of ovary at2000(mg/70kgb.w vit.C) but not for 1000 &1500mg/vit.C, as compared with control group. But a significant decrease ($p < 0.05$) at 300 &200mg /70kg b.w in

KMno₄ group, as compared with control & 100(mg/70kg b.w) group. In third group, significantly increase ($p < 0.05$) at (1000+100) (mg/70kg b.w) of KMno₄+ vitamin C group as compared with control

group, there is no different alteration at (1500+200) and (2000+300) (mg/70kg b.w) of KMno₄+ vitamin C group as compared with control group. Table (2).

Table (1): Effect of (vitamin C & KMno₄) on ovary (mean $\pm$ SD)

Parameter	Control	Vitamin C mg/70kg			control	KMno ₄ mg/70kg		
		1000	1500	2000		100	200	300
Diameter of ovary (mm)	4.82 $\pm$ 0.62 a	5.41 $\pm$ 1.58* b	4.72 $\pm$ 0.72 a	4.74 $\pm$ 0.19 a	4.38 $\pm$ 0.50 a	3.31 $\pm$ 0.49* b	3.85 $\pm$ 0.59 a	3.87 $\pm$ 0.74 a
Diameter of corpus luteum (mm)	0.90 $\pm$ 0.52 a	1.22 $\pm$ 1.94* b	1.13 $\pm$ 2.56 b	1.06 $\pm$ 3.18 a	0.95 $\pm$ 0.50 a	0.76 $\pm$ 1.31* b	0.84 $\pm$ 1.84 a	0.85 $\pm$ 0.50 a
Diameter of Graffian follicle (mm)	1.15 $\pm$ 0.46 a	1.72 $\pm$ 1.41* c	1.92 $\pm$ 0.47* b	1.46 $\pm$ 1.21 a	1.05 $\pm$ 0.55 a	1.00 $\pm$ 1.00 a	0.90 $\pm$ 1.70 a	0.70 $\pm$ 2.21* b
Weight of ovaries(g)	2.26 $\pm$ 0.19 a	2.12 $\pm$ 0.51 a	2.98 $\pm$ 0.17 a	3.38 $\pm$ 0.08* b	2.42 $\pm$ 0.22 a	1.72 $\pm$ 0.18 a	1.46 $\pm$ 0.12* b	1.26 $\pm$ 0.24* c

* means significant difference at ($p < 0.05$) at same group

** means significant difference at ($p < 0.01$) at same group

a,b,c means significant difference at ($p < 0.05$) between group

Table (2): Effect of (vitamin C & KMno₄) on ovary (mean $\pm$ SD)

Parameter	control	KMno ₄ + Vitamin C mg/70kg		
		1000+100	1500+200	2000+300
Diameter of ovary (mm)	4.92 $\pm$ 0.68 a	4.66 $\pm$ 0.48 a	4.22 $\pm$ 0.74 a	4.63 $\pm$ 0.24 a
Diameter of Graffian follicle (mm)	1.50 $\pm$ 0.60 a	1.58 $\pm$ 0.89 a	1.50 $\pm$ 0.65 a	1.94 $\pm$ 0.32b*
Diameter of corpus luteum (mm)	0.74 $\pm$ 0.44	0.83 $\pm$ 1.00	1.22 $\pm$ 1.02*	0.94 $\pm$ 1.82*
Weight of ovaries(g)	2.38 $\pm$ 0.18	3.83 $\pm$ 0.09*	2.1 $\pm$ 0.20	1.5 $\pm$ 0.12

* means significant difference at ($p < 0.05$), at same group

** means significant difference at ($p < 0.01$) at same group

a,b,c means significant difference at ($p < 0.05$) between group

Determination of Vitamin C concentration (mg/ml) in serum blood for study groups:-

The result of experiment showed a significant increase ($p < 0.05$) in vitamin C level by administration of 1500 mg/70kg & 2000 mg/70kg groups as compared with control group & other groups but not at 1000

mg/70kg (Fig.1). Also no significant increase ($p > 0.05$) of vitamin C concentration revealed at 100 mg/70kg as compared with control group & other groups (Fig.2). In third group, no different at first & third groups, second group showed a significant increase ($p < 0.05$) in vitamin C level as compared with control group & other groups (Fig.3).

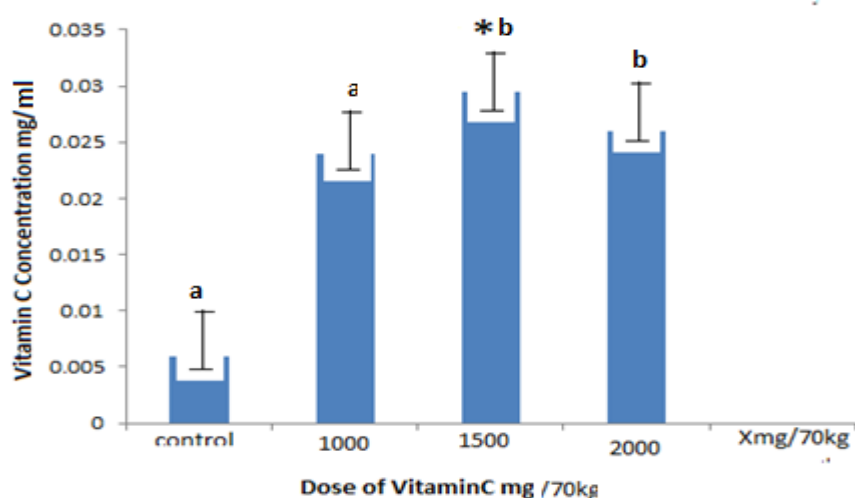


Figure (1): Determination of Vitamin "C" concentration (mg/ml) for vitamin C treated groups.
* means significant difference at ($p < 0.05$), at same group
a, b, means significant difference at ($p < 0.05$) between group

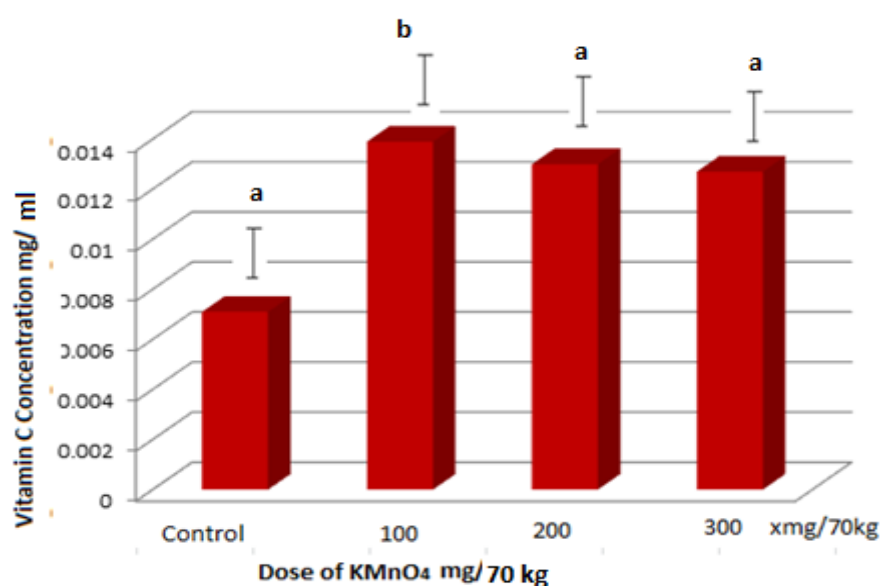


Figure (2): Determination of Vitamin "C" concentration (mg/ml) for KMnO4 treated groups.
a b, means no significant difference at ($p > 0.05$) between groups

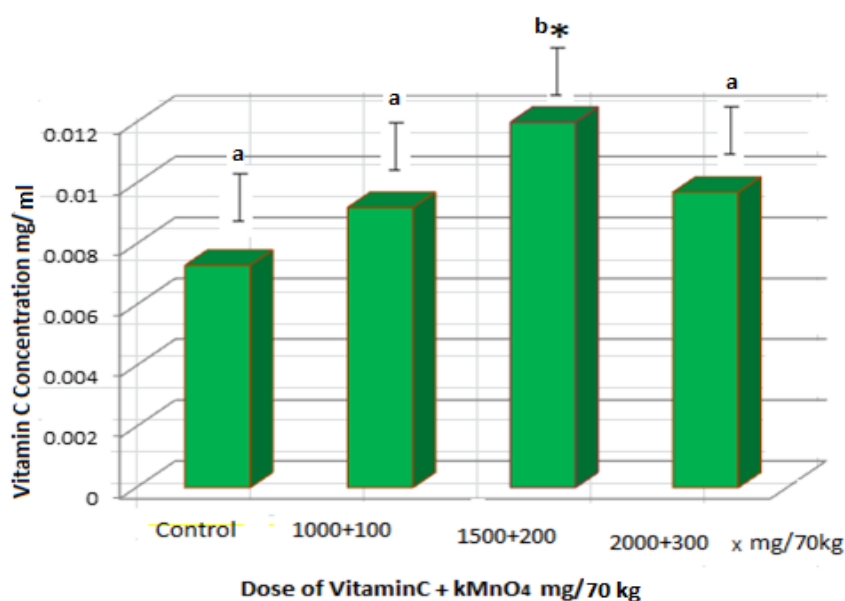


Figure (3): Determination of Vitamin "C" concentration (mg/ml) of vitamin C+KMno4 treated groups.

* means significant difference at ($p < 0.05$), at same group.

a,b, means significant difference at ($p < 0.05$) between groups.

Histological study:

The results of histological study reveal there was a normal histological appearance of ovary in control group, representing by presence of usual number of no growing follicle with one or two growing follicle, (Fig.6), while treatment with different doses of KMno4 resulted in appearance of few number of owing follicles and huge graffian follicle (mature follicle), (Fig4,8,13).

In contrast, the treatment with 1000 mg/70kg vit.C body weight showed an

increase in the blood flow within the parenchyma of ovary representing congestion of blood vessels (fig.11). A number of growing and graffian follicles within parenchyma of ovary especially after treatment with 1500 & 2000 mg/70kg vit.C body weight were also noted. (Fig.9, 12). In treatment groups with combination of KMno4 with vitamin C. There was increase number of growing follicle (Fig.5, 7, 10).

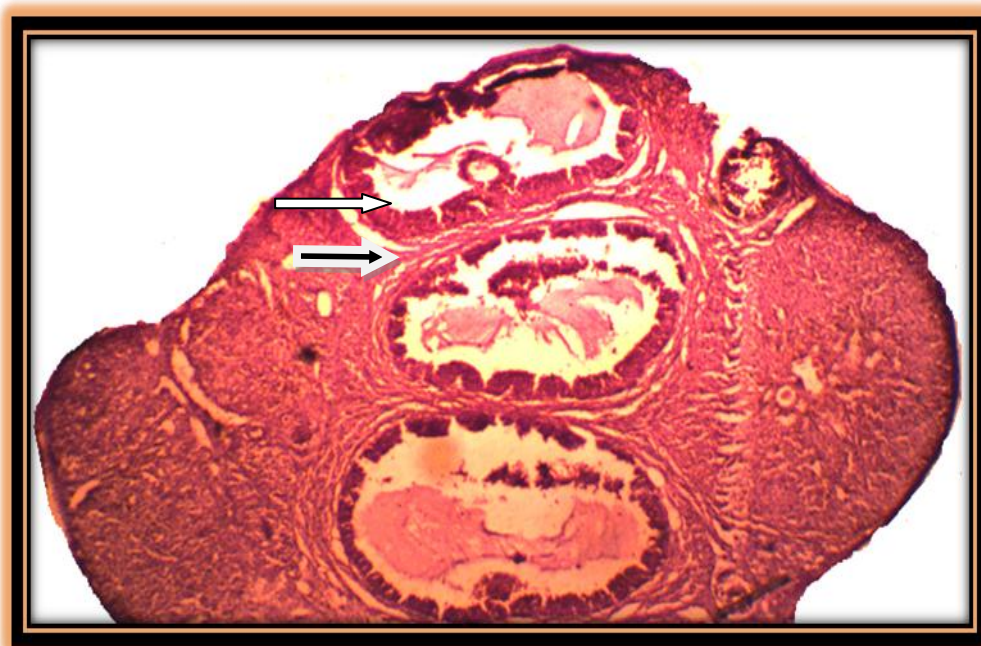
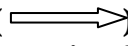


Figure 4:- Section of ovary of rat treated by (Kmno4:100 mg/70 kg b.w). Revealed number of growing follicles () are seen in parenchyma of ovary with ovulation of one follicle. () H&E stain,100

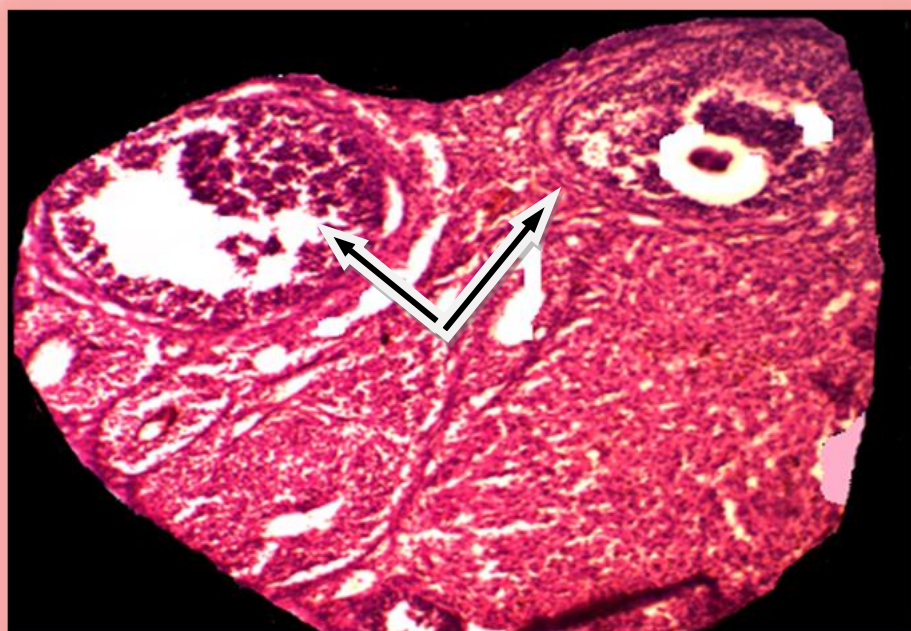


Figure 5:- Section in ovary of rat treated by (2000 Vit C+ 300mg Kmno4/70 kg): revealed number of growing follicles with a huge graffian follicle. () H&E stain, 100X

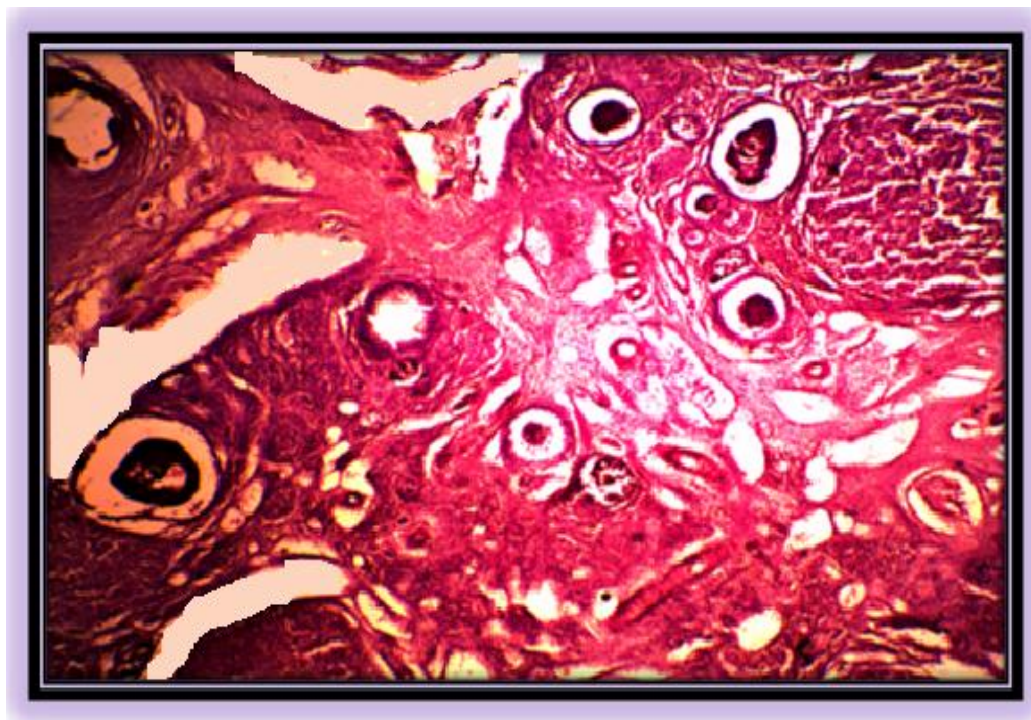


Figure 6:- Section in ovary of control group revealed number of primary & secondary follicles (H&E stain, 200X)

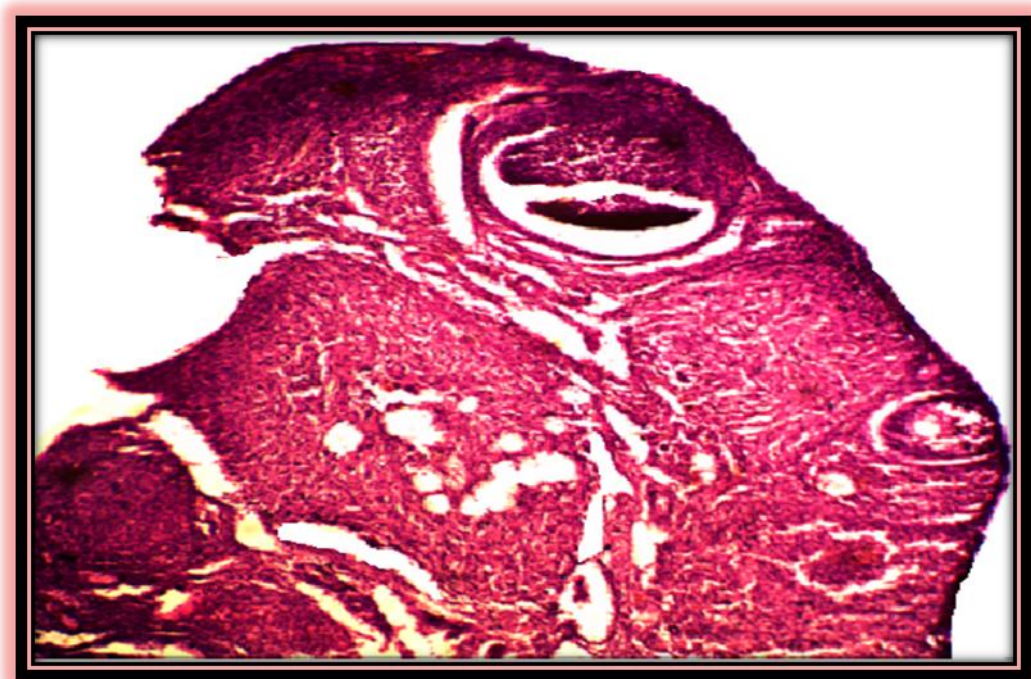


Figure 7:- Section in ovary of rat treated by (200 KMnO_4 + 1500 vit.C) mg/70 kg: revealed number of growing follicles (H&E stain, 200X).

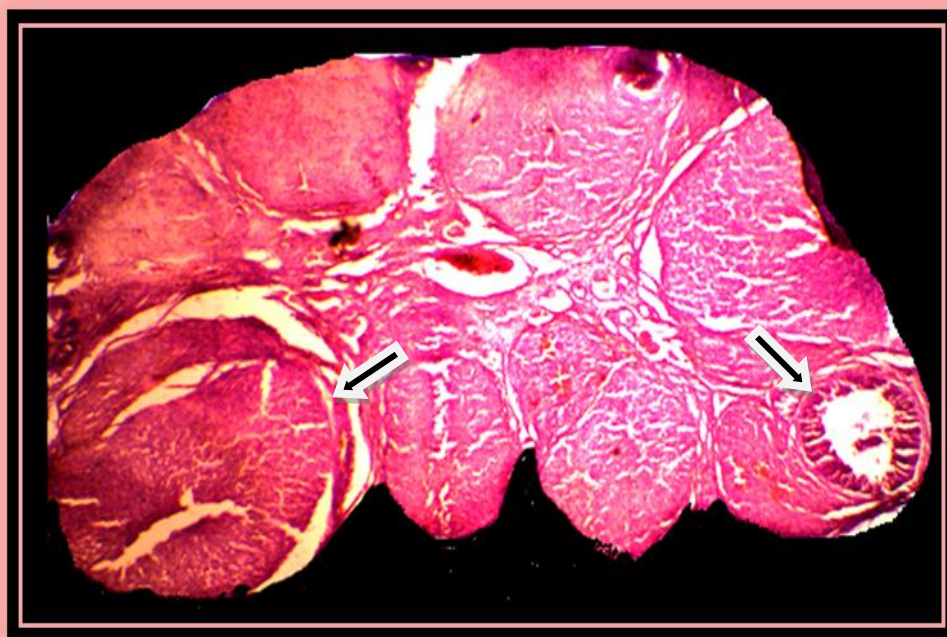


Figure 8:- Cross section in ovary of rat treated by ($\text{KmnO}_4=200\text{mg}/70\text{ kg b.w}$) revealed one follicle and one growing follicle (arrow) . H&E stain, 100X

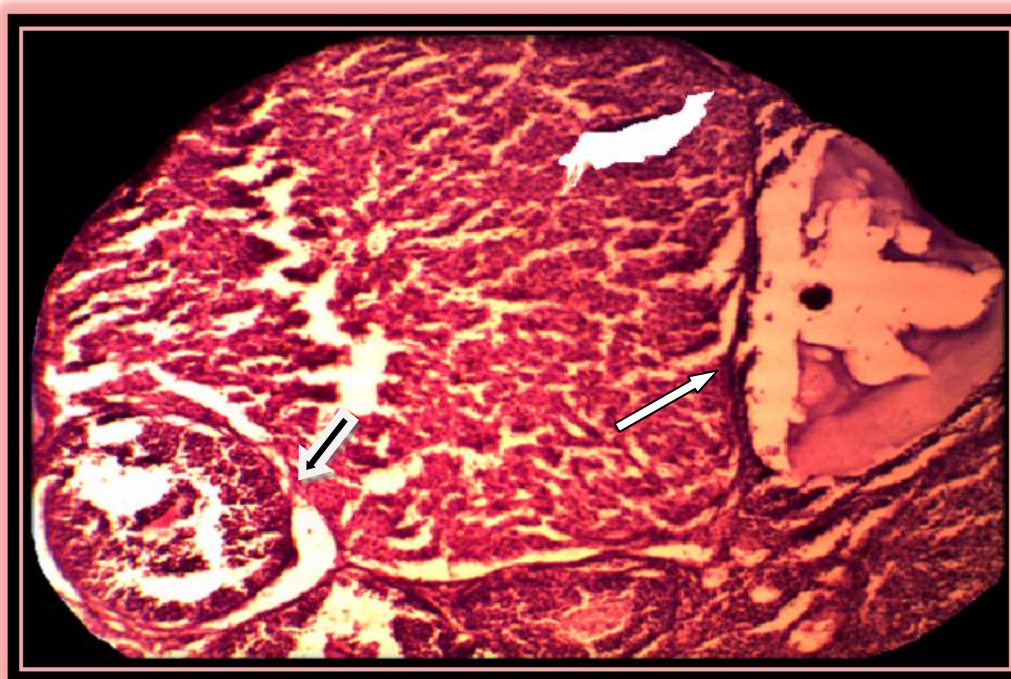


Figure 9:- Cross section in ovary of rat treated by (Vit.C 1500mg /70 kg) revealed marked huge griffin follicle ($\Rightarrow$) with flout ovum in the antrum. ($\Rightarrow$) H&E stain, 200X

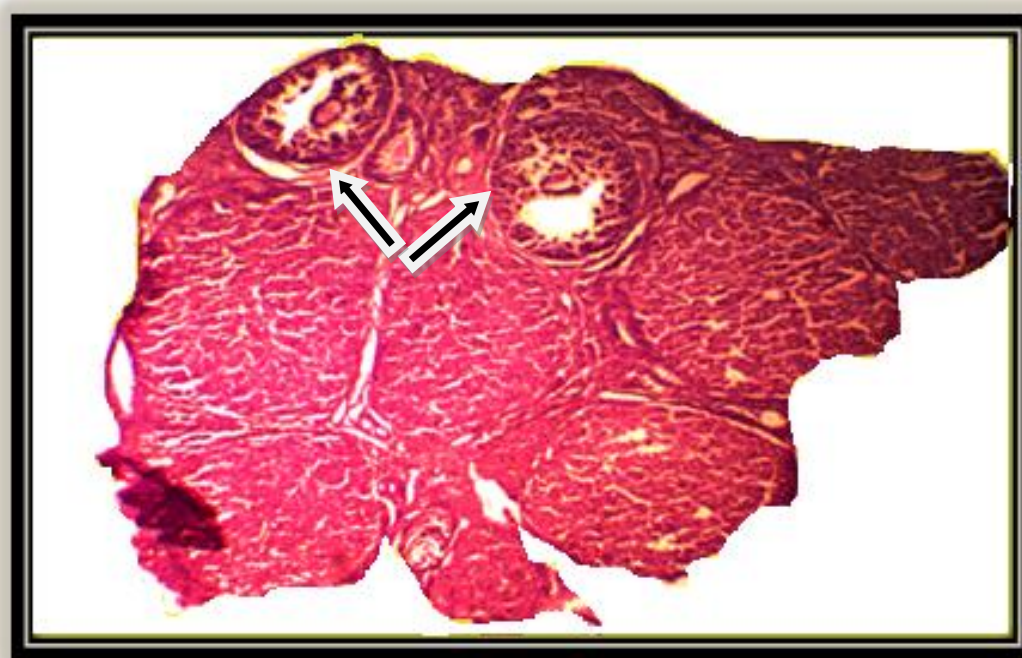


Figure 10:- Cross section in ovar of rat treated by(Kmno4: 100 +1000VitCmg/70kg b.w) marked to two growing follicles () H&E stain,100X

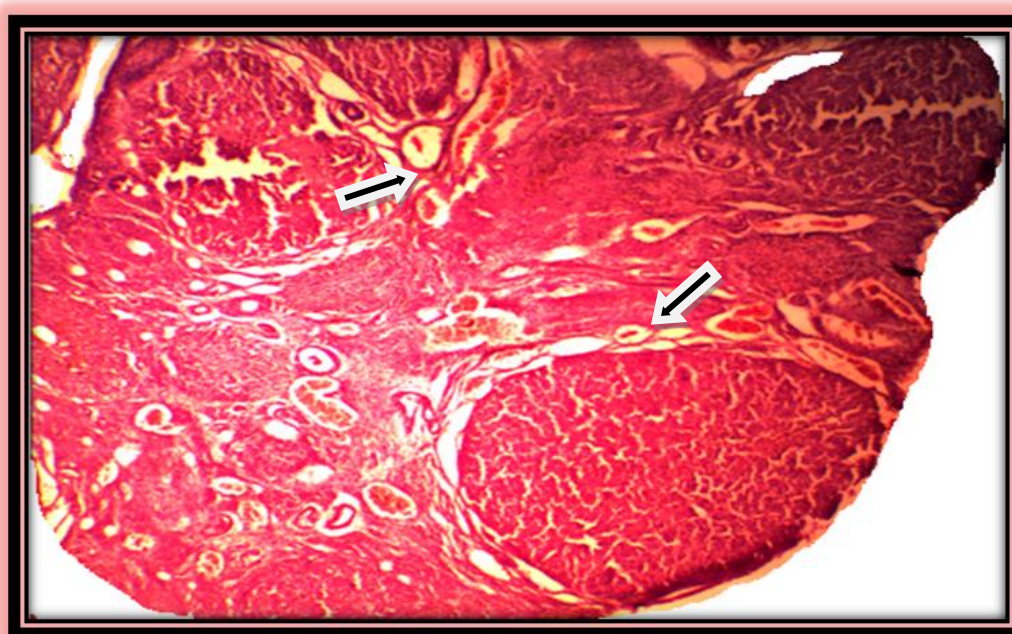


Figure 11:- Cross section in ovary of rat treated by (vit. C:1000mg /70kg b.w) revealed number of congested blood vessel, () within the parenchyma of ovary H&E stain,200X .

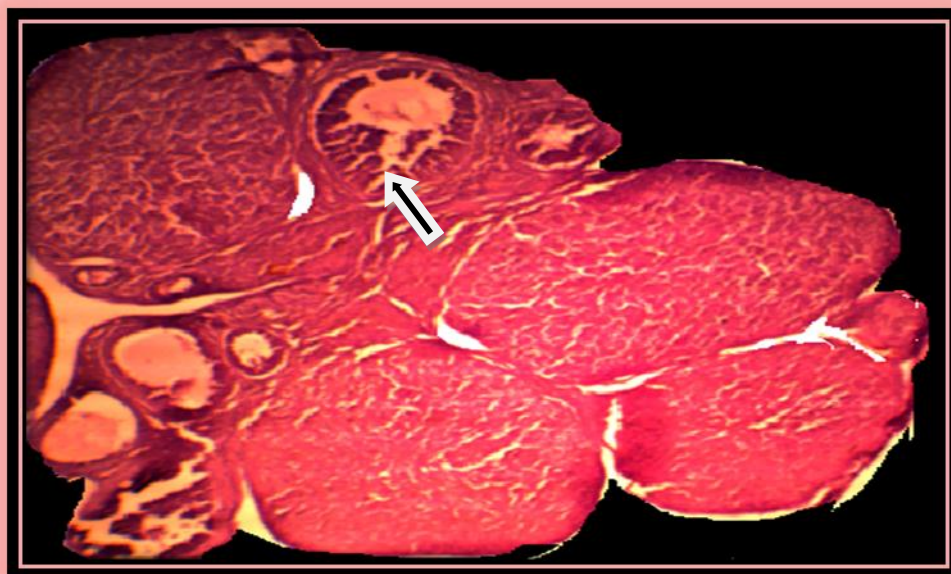


Figure 12:- Cross section in ovary of rat treated by (vit. =2000mg/70 kg b.w) revealed growing and graffian follicle . number of growing and single graffian follicle.($\Rightarrow$) H&E stain,100X

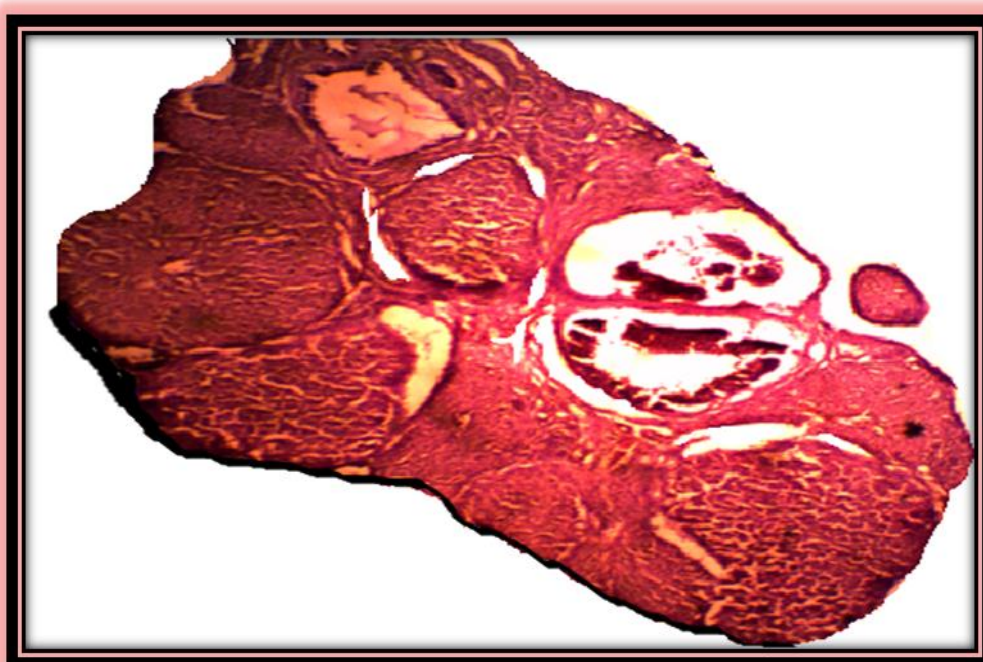


Figure13:- Cross section in ovary of rat treated by (KmnO₄:300mg/70kg b.w) showed atresia of large follicle H&E stain, 100X

Discussion:**Effect of vitamin C & KMnO₄ on serum****Vitamin C concentration**

Vitamin C is a known free-radical scavenger and has been shown to inhibit lipid peroxidation in liver (15). As (fig 1), there was a significant increase at 1000 & 1500 mg /70kg that vitamin C administration plays an important role in increasing the absorption rate of ascorbate to reach 98% and this shows a high plasma level but the gradual accumulation in the tissues and its plasma level may reach to the renal reabsorption threshold (1.5mg/dl) in women. The surplus is rapidly excreted in the urine and this agrees with many results (16) and (17), (18) & (19). Or may indicate that concentrations of vitamin C in the plasma were increased with prolongation of the treatment period (20).

The decrease in serum levels of vitamin C as (fig 2) at 200 & 300 mg /70kg may be due to the human body subjected to mild oxidative stress and vitamin C protects against oxidation when it is converted to its oxidized form (DHA). DHA leads to sparing ascorbate due to the fact that the human body loses the ability to synthesize vitamin C because it lacks L-gulonolactone oxidase enzyme which is required in the last step of vitamin C synthesis contribute to the decrease of its level which agrees with many researchers (21) and (22).

But in (fig 3), Vitamin C plays as a scavenger for generated ROS from KMnO₄, likewise we, other studies indicate vitamin C might have significant chelation capacity for lead. One rat pharmacokinetic study found intravenously administered vitamin C lowered lead tissue levels in rats that were continuously administered lead. (48) A human study, evaluating blood lead levels in pregnant women, found that 1,000 mg vitamin C per day, in addition to a prenatal multivitamin supplement, significantly lowered blood lead levels from a mean of 5.1 to 1.1 µg/dL during the course of pregnancy. (49) The safety of chelating

pregnant women, however, and potential exposure of the fetus to lead (23).

Effect of vitamin C & KMnO₄ on ovary

Table (1) & (2) revealed significant increase in weight of ovary at 2000 mg /70kg b.w Vitamin C in contrast for KMnO₄ groups which depletion at 100 mg /70kg b.w. And all parts of these organs affected by these different treatments also there was a significant increase of graafian follicles at (1000 & 1500) mg /70kg b.w Vitamin C, a significant decrease at 300 mg /70kg b.w of KMnO₄. Noticed no change neither weight of ovary or graafian follicles for the third groups. Because the ovary and all parts of these organs histopathologically affected greatly by the ovarian hormones as induced for antioxidants like Vitamin C, E which may increase proliferation of mucosa within the parenchyma of ovary as (fig. 9, 12) or congested blood vessels (fig. 11) (24).

A marked decrease in ovarian weights and an increase in atresia of large follicles, (fig. 13), decrease in newly formed corpus luteum were detected in ovary as KMnO₄ treatment related changes. The follicles are sensitive to KMnO₄ doses dependently (fig. 4, 8) as compared with control (fig. 3a). Subsequent increase in serum FSH and LH suggests that both smaller and larger follicles are equally important in the regulation of serum gonadotropins. Secretion of estradiol is indicative of functional ovulatory follicles, which triggers the preovulatory surge of gonadotropins; an important intraovarian growth, differentiation and survival factor (25). In addition, estradiol is known to suppress apoptosis of granulosa cells, inhibit atresia and help maturation of large follicles. Progesterone plays a functional role in the luteinization process triggered by the LH surge, thus playing a critical role in ovulation, (26). There was a decrease in ovary weight; and histopathologically, a decrease in the number of follicles and effect on corpora lutea was also observed in the present study. The incidence and

severity of these changes in ovarian histology is KMno4 destroys developing follicles by attacking rapidly dividing granulosa cells, reducing their steroid secretion and increasing gonadotropins. . Moreover, in the present study Vitamin C levels were found to correlate with histological finding In groups that treated with (Vitamin C +KMno4) as (fig 5, 7, 10) illustrated no change of ovary weight, may explain as vitamins C and E may play an important role in scavenging ROS (antioxidant function), ascorbic acid is a required cofactor in the synthesis of collagen in the luteal extracellular matrix²⁶. ROS are produced during luteal regression, in part though cytochrome P450 enzymes which are necessary for the first step of steroidogenesis (27).

References:

- 1.Velsek, j. ;Cejpek, K.Biosynthesis of foo constituents: Vitamins. 2.Water – soluble vitamins:part 1-areview. *Czech .J.Food Sci.*2007, 25, 49-64.
2. Linster ,C.L.; Van schaftingen, E. Vitamin C Biosynthesis, recycling and degradation in mammals .*Febs j.*2007 ,274,1-22.
3. Davey , M.W.; Van Montagu ,M.;Inze, D.;Sanmartin ,M.;Kanellis, A.;Smirnoff, N.; Benzie I. J.J.; Strain, J.J.;Favell,D.; Fletcher ,J. Plant L-ascorbic acid :chemistry ,function, metabolism ,bioavailability and effects of processing .*J Sci.Food Agric .* 2000,80, 825-860
4. G.;Foyer, C.H.Ascorbate and glutathione :Keeping active oxygen under control .*Annu.Rev.Plant Physiol.Plant Molec.Biol.*998,49,294-279.
- 5.Kleszczewska,E. L-Ascorbic acid – clinical use toxicity ,properties ,method of determination and application in chemical analysis .*Pharmazie* 2000,55,640-644.
6. Meister, A.GlutathioneAscorbic Acid Antioxident Systemin Animals.*J.Biol. Chem.*1994, 269,9397-9400.
- 7.Englard ,S.; Seifter ,S.The Biochemical Function of Ascorbic –Acid. *Annu.Rev.Nutr.*1986, 6,365-406.
- 8.Leving,M.; Rumcy, S.C.;Daruwalas, R.;Park,J.B.; Wang,Y.H.Criteria and recommendation for vitamin Citake.*JAMA-J.Am.Med.Assoc.*1999,281, 1415-1423.
9. Gomea-Romero, M.;Arraez- Roman D.; Segura- Carretero,A.;Fernandez-Gutierrez,A.;Analytical determination of antioxidant in tomato:Typical compounds of the Mediterranean diet.*J.Sep.Sci.*2007,30,452-561.
- 10.Deicher ,R.;Horl, W.H. Vitamin Cin chronic kidney disease and hemodialysis patients .*Kidney Blood Pressure Res .* 2003, 26,100-106.
- 11.Ogriri, Y.;Sun, F.;Hayami ,S.;Fujimura, A.;Yamamoto,K.;Yaita,M.;Kojo,S.Very low vitamin C of orally administered L-dehydroascorbic acid .*J.Agric .Food Chem .*2002,50,227- 229.
- 12.Yassen ,N.;Y.Cytogenetic studyo on human colorectal cancer cells.1990 ,PhD.Thesis .Univ. Shefhidd.
13. Hoar ,W.; &Hichman , C.P. Ovariectomy and the estrus cycle of the rat. In. W. Hoar &C.P.Hichman (eds) , General and comparative physiology .2. ed. 1975.Prentice –Hall,New jersy,pp.260-265.
14. Burtis ,Carl A. ;& Ashwood , Edward R. Tietz –Textbook of clinical chemistry .3rd ed.vol. 2.1999 .W.B.Saunders company. P:1025.

15. Patrick Lyn, ND (2006): Lead Toxicity Part II: The Role of Free Radical Damage and the Use of Antioxidants in the Pathology and Treatment of Lead Toxicity Alternative Medicine Review 11.
16. Leving,M.; Rumcy, S.C.;Daruwalas, R.;Park,J.B.; Wang,Y.H. (1999):Criteria and Recommendation for Vitamin Citake.JAMA-J.Am.Med.Assoc. 281, 1415-1423.
17. Hedgier and Mathias, A., (2002): New View at Vitamin NaturMedicine, 8: (5) 445-6.
18. Dingchao, H., Zhiduan, Q., Liye, H. and Xiaodong, F. (1994): The Protective Effects of High-Dose Ascorbic Acid on Myocardium Against Reperfusion Injury During and After Cardiopulmonary Bypass. Thorac. Cardiovasc. Surg., 42, 276–278.
19. Nakamura, M., Ozaki, M., Fuchinoue, S., Teraoka, S. and Ota, K. (1997): Ascorbic acid Prevents Ischemia-Reperfusion Injury in the Rat Small Intestine. Transpl. Int., 10, 89–95.
20. AL–Gnami, S. A.; Al – Katib, S. R.; and Al – Quraishy, I. A. (2004): Effect of Vitamin C on Some physiological and Biochemical parameters of blood in females rabbits. M. sc. Thesis in animal physiology, College of Education, Al – Qadisiya University.
21. Nualart, F.; Rivas, C.; Monteinos, V.; Godoy, A.; Guaiquil, V.; Goldie, D. and Veraj, (2003): Recycling of Vitamin C by Stander Effect. J. Biol. Chem., 278(12): 10128-33.
22. Monte Hagen, A.; Kinet, S.; Manel, N.; Mongellaz, C.; Prohaska, R.; Battini, J.L.; Delaunay, J. and Sitbon, M. *et al.*, (2008): Erythrocyte Glut Triggers Dehydroascorbic Acid Uptake in Mammals Unable to Synthesis Vitamin C. Cell, 132(6): 1039-48.
23. Gurer,H.& Ercal,N.C.(2000): An Antioxidants be Beneficial in the Treatment of Lead Poisoning? Free Radic BiolMed;29:927-945.
24. Ikeda Y, Nagai A, Ikeda MA, Hayashi S.(2001):Neonatal estrogen exposure inhibits steroidogenesis in the developing rat ovary. Dev Dyn; 221:443–453.
25. Natraj U, Richards JS. (1993): Hormonal Regulation, Localization, and Functional Activity of the Progesterone Receptor in Granulosa Cells of Rat Preovulatory Follicles. Endocrinology; 133: 761–769.
26. Quirk, S.M. Cowan, R.G. Harman, R.M.Hu, C.L. Porter, D.A. (2004): Ovarian Follicular Growth and Atresia: the Relationship Between Cell Proliferation and Survival. J Anim Sci; 82(E-Suppl): E40–E52.
27. Luck MR, Zhao Y. (1993): Identification and Measurement of Collagen in the Bovine Corpus Luteum and its Relationship With Ascorbic Acid and Tissue Development. J Reprod Fertil.;99:647–652.
- 28.Behrman,H.R.;Kodaman,P.H.;Preston,S. L. *et al* (2001):Oxidative Stress and the Ovary.J.Soc.Gynecol Investig ;8:540-542.