

Effect of Clomiphene Citrate on The Kidney of Female Albino Rat and The Possible Protective Effect of Ascorbic Acid

(Light and Electron Microscopic Study)

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ABSTRACT

Background: Clomiphene Citrate (CC), a selective estrogen-receptor modulator is used to manage polycystic ovary syndrome, but it has harmful implications.

Aim of the work: To identify the histological results of CC on the kidneys of female albino rats and the protective effects of ascorbic acid.

Patients and Methods: Forty-eight adult female albino rats were selected for this 21-days experiment. They were randomly divided into equal four groups. Group (I): control group, Group (II): given Ascorbic acid (25mg per Kg. BW) daily for 21 days, Group (III): given orally Clomiphene Citrate (1.2 mg per Kg. BW) daily for 21days, Group (IV): CC plus Ascorbic acid. Retroocular Blood samples for renal function tests Specimens from the kidneys of every rat were taken for histological examination by the LM & EM.

Result: Degenerative changes in the kidneys of group III, in the form of vacuolation in the cells of the proximal and distal convoluted tubules (PCT&DCT), Disappearance of the brush border of the (PCT), Congestion of glomerular capillary tufts and change of the Bowman's space. The podocyte's pedicle appeared degenerated. In group IV, vacuolation in (PCT&DCT) was mild. The proximal tubules appeared normal and their cells' brush borders were restored. The glomerular capillary tufts were similar as in group I, with good cellularity and Bowman's space. The podocyte's pedicle appeared to be almost intact.

Conclusion: CC has harmful effects on the components of the kidney. Ascorbic acid has a protective effect against the harmful effects of CC as restoring the normal appearance.

Keywords: Clomiphene citrate; Ascorbic acid; Kidney.

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INTRODUCTION

Anovulatory infertile women are treated with clomiphene citrate (CC), a selective estrogen-receptor modulator. It blocks the negative endogenous estrogen feedback on the hypothalamic-pituitary axis, resulting in increased FSH secretion, follicular development, and ovulation ¹.

Ovarian enlargement, vasomotor flashes, nausea, vomiting, breast pain, headache, excessive vaginal bleeding, visual signs, weight gain, and shortness of breath were all reported as side effects of clomiphene citrate usage. ²

CC caused many biochemical changes in rat's kidneys ³. In order to minimize side effects of Clomiphene citrate, we looked for antioxidant. Various studies assured the nephro-protective effects of ascorbic acid ⁴.

Ascorbic acid is a strong scavenger of activated oxygen species and other free radicals, neutralizing (ROS) and it reduces oxidative damage to the cell membranes. ⁵

The aim of this work was to identify the histological results of CC on the kidneys of female albino rats and the protective effects of ascorbic acid.

MATERIAL AND METHODS

Drugs used:

Clomiphene citrate tablets (Clomid 50 mg tab.) were used. **Ascorbic acid** Sachets (1000 mg Ascorbic acid, sachet) were used.

Dose of the used Drugs: The daily single oral dose of CC. (1.2mg / kg. body weight). The daily single oral dose of Ascorbic acid Sachets (25 mg Ascorbic acid /kg. body weight).⁶

Animals and experimental protocol:

Forty-eight adult female albino rats weighting (140 ± 20 g) were selected for this 21-days experiment. The animals were held in a clean, well-ventilated cages at a temperature of $25 \pm 2^\circ\text{C}$, with a 12-hour light/dark photoperiod and unlimited access to normal laboratory food and water.

Animal groups: The rats were randomly divided into four groups (12 rats each) and orally treated as follows:

Group (I): The control group in which rats were given 10 ml 0.9% Na CL orally daily for 21 days.

Group (II): Ascorbic acid group: in which rats were given the therapeutic dose of Ascorbic acid (25 mg./kg. body weight) orally dissolved in distilled water daily for 21 days according to *Paquet schedule*.

Group (III): Clomiphene citrate group: in which rats were given orally the therapeutic dose of CC. (1.2 mg./kg. body weight) dissolved in 0.9% Na CL daily.

Group (IV): CC. plus Ascorbic acid group: in which rats were given orally the therapeutic dose of (CC) plus Ascorbic acid daily for 21 days.

Blood samples for renal function tests were taken (Blood urea, Serum creatinine and Uric acid).

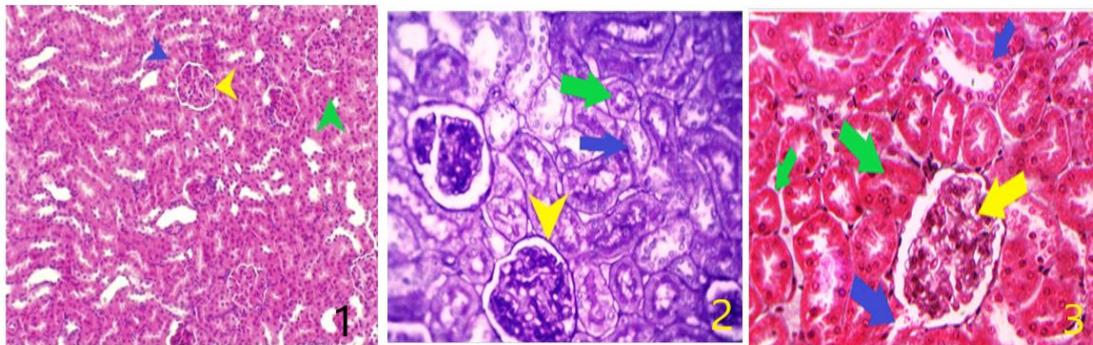
Specimens from the kidneys of the rats were taken for histological examination by:

Light microscope using: Hematoxylin-Eosin technique (H&E), Periodic Acid Schiff technique (PAS) and Masson's Trichrome technique.
Transmission electron microscopy.

RESULTS**Group I: (The control group):**

Hx &E stained sections of the kidneys of control rats showed normal structure of the renal corpuscles with good vascularity and Bowman's spaces. Proximal and distal convoluted tubules appear numerous with prominent vesicular nuclei of their epithelial cells (Pic.1). PAS stained sections of kidneys of the control rats showed positive PAS materials in the apical and basal membranes of the proximal and distal tubule cells. (Pic.2).

Masson's trichrome stained sections of kidneys of control rats showed normal distribution of collagen fibers in the capsular wall, peritubular and around the blood vessels. (Pic.3). Electron microscopic examination of the renal cortex of the control group showed the proximal and distal convoluted tubules with intact nuclei, thin basement membranes and numerous mitochondria (Pic.4). The renal corpuscles appeared normal containing glomerular capillaries that were lined with fenestrated endothelial cells and covered by the cell bodies of podocytes. The podocytes appeared normal with healthy filtration membranes (Pic.5).

**Fig. 1:**

Pic. 1: Renal cortex of rat of the control group showing the glomerular tuft of capillaries surrounded by Bowman's capsule (Yellow arrow). Renal tubules proximal (Green arrow) and distal (Blue arrow) appear normal with intact cells (**Hx &E stain X 200**).

Pic. 2: Renal cortex of rat of the control group showing PAS positive materials in glomerulus (Yellow arrow), renal tubules (Green arrow), distal tubules (Blue arrow) (**PAS stain X 400**).

Pic. 3: Renal cortex of rat of control group showing thin collagen fibers supporting the glomerulus, in Bowman's capsule (yellow arrow) and basement membrane of the cuboidal cells around renal tubules, the distal (blue arrows) and the distal (green arrows) (**Masson's trichrome stain X400**).

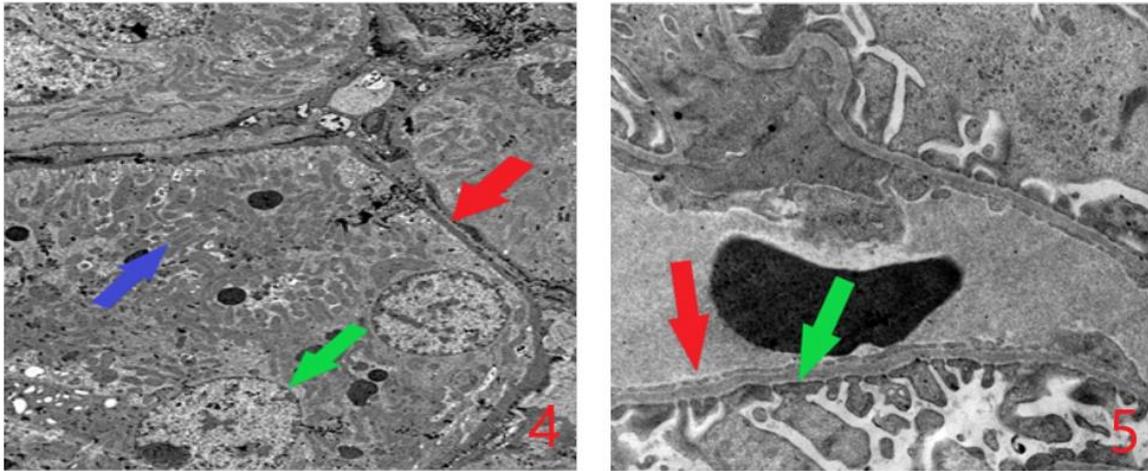


Fig. 2:

(Pic. 4): Photo electron micrograph of renal cortex of rat from the control group showing intact three proximal convoluted tubules with intact nuclei (green arrow), thin basement membrane (red arrow) and multiple mitochondria (blue arrow). **(X 10000).**

(Pic. 5): Photo electron micrograph of renal cortex of rat from the control group showing intact Podocyte's foot processes (green Arrow), capillary loops which lined by fenestrated endothelial membrane (red arrow). **(X 10000).**

Group II: (Ascorbic acid): Hx &E stained sections of kidneys of rats of group II showed no affection of glomeruli with normal widening of the Bowman's spaces (Pic. 6). PAS stained sections of this group showed positive PAS reaction in the apical membranes with adequate staining affinity in the basal membranes of the proximal and distal convoluted tubular cells. A moderate reaction in the distorted glomeruli was also observed (Pic.7).

Masson trichrome stained sections showed average collagen fibers around the renal corpuscles and the renal tubules as well as collagen fibers between the glomerular capillaries as compared with the control group (Pic.8). Electron microscopic examination of the renal cortex of group II showed normal appearance of the glomerular capillary basement membranes, capillary endothelial cells and Podocyte's pedicles (Pic. 9).

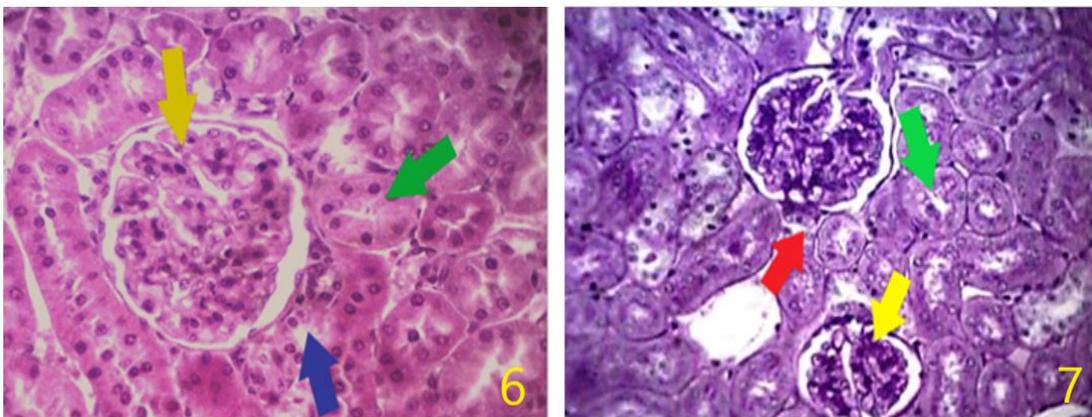


Fig. 3:

(Pic. 6): Renal cortex of rat of group II showing normal glomerulus (Yellow arrow), proximal tubules (Green arrow), distal tubules (Blue arrow) **(Hx&E stain X 400).**

(Pic. 7): Renal cortex of rat of group II showing more or less PAS +ve materials in the apical and basal membranes of the proximal (Green arrow) and distal tubule cells (red arrow) as well as in the glomerular tufts (Yellow arrow) comparable to the control **(PAS stain X400).**

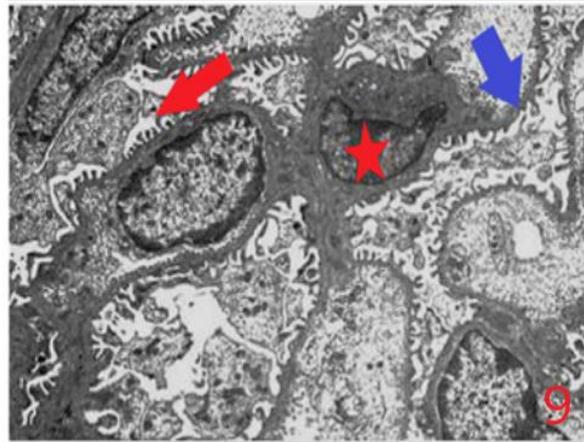
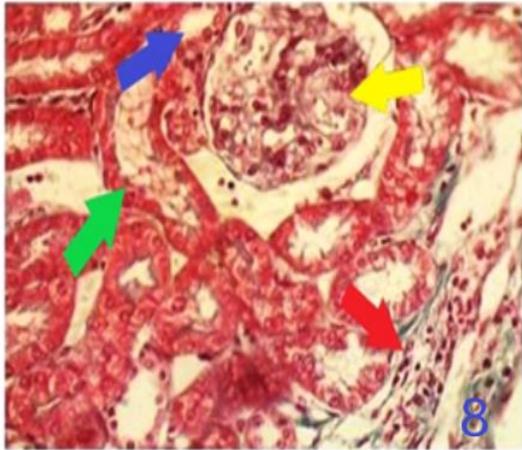


Fig. 4:

Pic. 8: Photomicrograph of renal cortex of rat of group II showing normal masson's trichrome stain affinity in the apical and basal membranes of the proximal (Green arrow) and distal tubule cells (red arrow) as well as in the glomerular tufts (Yellow arrow) comparable to the control (**Masson's trichrome stain X400**)

Pic. 9: Photo electro micrograph of renal cortex of rat of the group II showing Podocyte's with nearly regular foot processes (Red arrow) flat nucleus of capillary endothelium (Star) normal thickening of GBM (Blue arrow) (**X 5000**)

Group III: (Clomiphene citrate group): Hx&E stained sections of the renal cortex of group III rats showed marked structural changes in the renal corpuscles and some convoluted tubules (Pic.10). PAS stained sections of group III rats showed decreasing of PAS reaction with stained glomeruli (Pic. 11). Masson trichrome group showed increase of the collagen fibers around glomerulus and renal tubules within the arterial wall with partial destruction of glomerulus (Pic.12). Electron microscopic examination of group III showed

prominent glomerular changes. The glomeruli showed thickened (GBM) (Pic. 13). Also, capillary lumens were obliterated by end capillary hypercellularity and hypertrophy with fusion of secondary foot processes and mesangial hypercellularity (Pic. 13). The renal tubules revealed many changes. The lining cells of proximal convoluted tubules showed partial loss of microvilli, cytoplasmic vacuolation and few degenerated mitochondria and abnormal pyknotic nuclei (Pic. 14, 15).

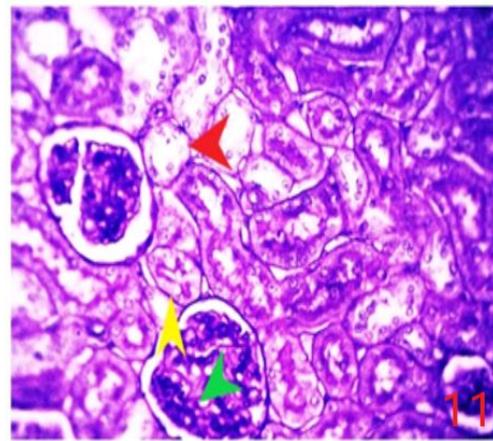
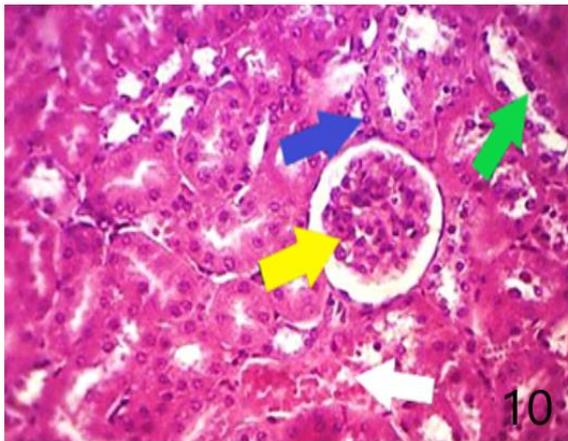


Fig. 5:

Pic. 10: Photomicrograph of renal cortex of rat of group III with mild degeneration of vascular tuft, widening of filtration space (yellow arrow) proximal, distal and collecting tubules show congestion and widening (blue and green arrows) (**Hx&E. X400.**)

Pic. 11: Photomicrograph renal cortex of rat of group III showing decreased PAS +ve materials of convoluted tubules (red and yellow arrow heads) with decreased stained glomeruli (green arrow heads) (**PAS stain X400**)

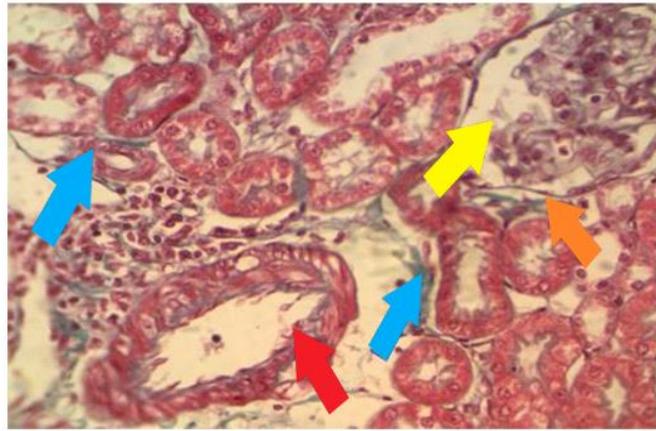


Fig. 6:

Pic. 12: Photo micrograph of renal cortex of rat group III showing prominent of the collagen fibers around glomerulus (orange arrow) and renal tubules (Blue arrow) and the arterial wall (Red arrow) Also, there is partial destruction of glomerulus (Yellow arrow) (**Masson's trichrome stain X400**).

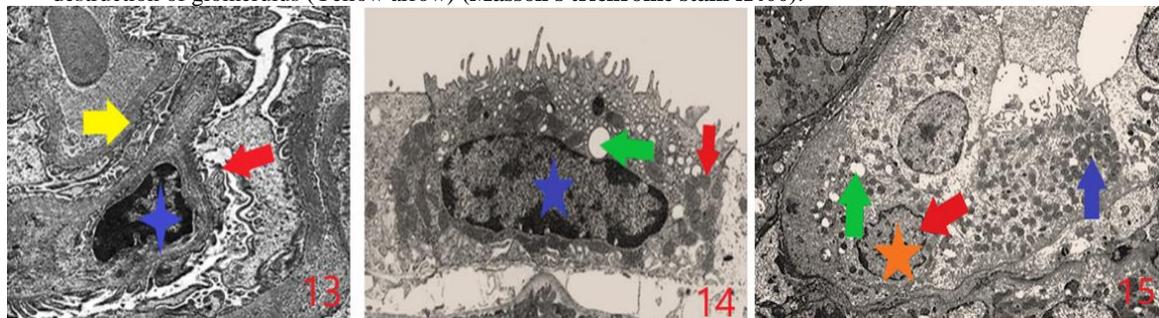


Fig. 7:

Pic. 13: Photo electron micrograph of renal cortex of rat from the group III showing destroyed Podocyte's primary and secondary processes (Red arrow) with thickened capillary basement membrane (Yellow arrow) and corrugated nuclear membrane of the endothelial lining (Star) (**X5000**).

Pic. 14: Photo electron micrograph of rat group III showing vacuolization of apical of proximal convoluted tubule part (green arrow) and the nucleus of convoluted tubules appeared with slightly corrugated membrane (blue star), globular shaped mitochondria (red arrow).

(**X 5000**)

Pic. 15: Photo electron micrograph of the renal cortex of rat of group III showing epithelial lining of the distal tubule with cytoplasmic vacuolization (Green arrow), deformed epithelial cell (Blue arrow) and abnormal nucleus (Star) with corrugated nuclear membrane (Red arrow) (**X 3000**)

Group IV: Clomiphene citrate group + ascorbic acid: Hx &E stained sections of the renal cortex of group IV: The proximal and distal convoluted tubules appear with intact cells with vesicular nucleus. The glomeruli showed normal capillary tuft and Bowman's capsule (Pic. 16). PAS stained sections of group IV rats showed more or less PAS reaction in the apical and basal membranes of the proximal and distal tubules cells as well as in the glomerular tufts comparable to the control group (Pic. 17). Masson's trichrome stained sections of the kidney cortex of group IV rats showed fine and scattered collagen fibers around the renal corpuscle

and the renal tubules can be hardly seen as comparable with the control group (Pic.18). Electron microscopic examination of the renal cortex of group IV showed well maintained ultrastructure of kidney. The glomerular basement membranes appeared thin and intact, the lining endothelium of capillaries and the Podocyte's pedicle appeared intact. PCTs displayed intact microvilli, mitochondria and nuclei. The DCTs had a conserved ultrastructure. The cytoplasm contains elongated mitochondria & small lysosomes also, lateral and basal cell membrane enfolding and vesicular euchromatic nucleus (Pic. 20).

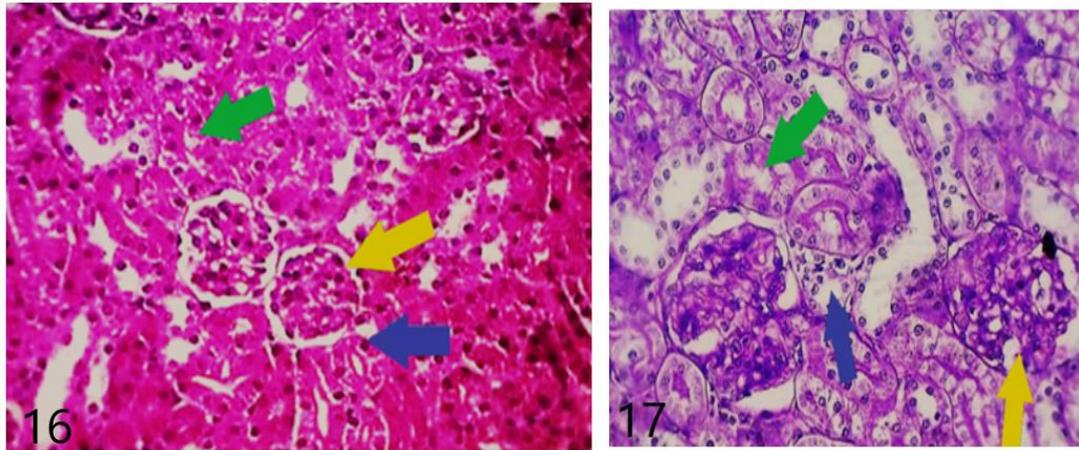


Fig.8:

Pic. 16: Photo micrograph of renal cortex of rat of group IV showing normal Bowman's capsule including glomerulus (Yellow arrow), but some of proximal (Green arrow) and distal (Blue arrow) convoluted tubules show mild vacuolization (**Hx &E stain X 400**).

Pic. 17: Renal cortex of rat from group IV showing PAS +Ve Bowman's capsule (yellow arrow). Renal tubules seen normal (green arrow for PCT. & blue arrow for DCT (**PAS. X400**))

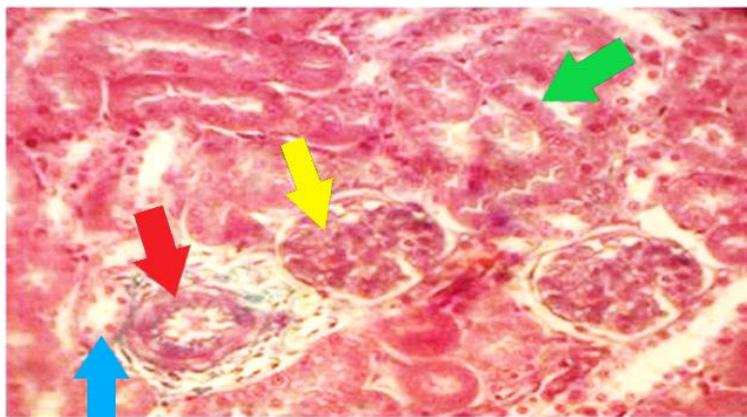


Fig. 9:

Pic. 18: Renal cortex of rat of group IV showing minimal amount of collagen in interstitial tissue (Red arrow), normal capillary tuft of glomerulus (Yellow arrow) with normal proximal (Green arrow) and distal tubules (Blue arrow) (**Masson's trichrome stain. X400**).

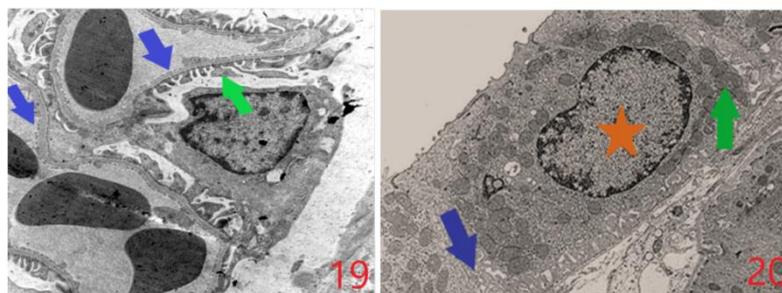


Fig. 10

Pic. 19: Photo electron micrograph of rat kidney of group IV intact filtration membrane of the glomerulus (blue arrows) the foot processes of the podocytes appear normal and intact. (**X5000**)

Pic. 20: Photo electron micrograph of rat kidney of group IV showing lining cells of distal convoluted tubules which are flattened, and the nucleus show increase in width (Star). The lateral intercellular spaces (Blue arrow) appear decreased in width as compared to spaces in normal tubules and mitochondria (Green arrow) look normal (**X4800**).

Biochemical effects:

Blood samples were taken from the rats of each group. Renal function tests were done to determine the effect of the drugs used in this experiment. The results showed normal values in group I (the control group) and group II (the ascorbic acid treated group) while in group III (CC treated group) marked renal toxicity was observed in the form of elevated levels of urea and creatinine and improvement of renal function in group IV (treated with clomid and ascorbic acid together) where p value < 0.01

Parameters	control	Vit. C	Clomiphene citrate	Vit. C+ C.C.
Creatinine	1.29± 0.21	0.93± 0.19	2.71± 0.43*	2.04±0.5 4
Urea	37.8± 0.87	34.37± 1.85	63.00± 1.42*	46.82 ± 87*
Uric acid	16.0± 0.58	14.50± 0.8	12.00± 0.16*	13.5± 0.29*

*P < 0.05

Table 1: Effect of clomiphene citrate and /or vit. C on the Renal biochemical Parameters of Rats. (mean ± S.E).

DISCUSSION

This Study proves that (CC), treated rats revealed numerous glomerular and tubular affection in the form of massive tubular vacuolization especially cells of the proximal and distal convoluted tubule. The lumen of some PCTs contained remnants of degenerated epithelial cells. Their nuclei showed dark staining, with different degrees of degeneration.

These changes were accompanied with partial affection of glomeruli associated with congestion of glomerular capillaries, Change of the glomerular capsular space.

Some of epithelial cells of the proximal and distal tubules show features of vacuolization. Also, there was thickening of vascular wall which filled with blood cells and debris. Obliteration of the capsular space or irregular outline of the glomerulus with pathological changes of some areas of the capsular space were seen in some corpuscles. Other corpuscles showed damage in the form of irregularity, shrinkage, and destruction of their vascular portion. This is in agreement with El-Gendy et al. (2019).

Changes in renal functions resulted in an increase in creatinine, urea and uric acid in the blood specimens taken, all of which are signs of renal failure. Prasad, et al. (2018) confirmed that Clomiphene citrate caused marked tubular degeneration, necrosis, tubular epithelial cell desquamation with cystic formation, interstitial cellular infiltration, large capsular space, and congested glomerular capillary tufts.

Kohler (2009) approved that necrotic and apoptotic changes of the renal tubular cells were due to the direct effect of intracellular Clomiphene citrate, which focused primarily on the convoluted tubules at

the cortico-medullary region, Glomerular affection (in Group III) included atrophy, destruction and irregular outline of the glomerulus with widening of some areas of the capsular space, with interstitial cellular infiltration and hemorrhage. This damage was observed in many corpuscles in the form of irregularity and shrinkage with loss of their vascular components. Also, the following results were noted, abnormal mitochondria (oval mitochondria having densely packed cristae, cristae reduplication) and mitochondrial swelling⁹.

In contrast, other researches,¹⁰ stated that CC appears to be generally well tolerated, with minimal nephrotoxicity reported in early studies due to the high safety profile and this does not agree with this study.

Brown (2018) stated that, after Clomiphene citrate intake for many months, there was minimal vacuolization in some cells of proximal and the distal tubules accompanied with well-preserved brush border of proximal tubule in the rest of the tubules

In this study, co-administration of (Ascorbic acid with Clomiphene citrate) improved the histopathological findings of Clomiphene citrate -induced renal toxicity, where only mild tubular degenerative changes were noticed. The beneficial renal protective effect of Ascorbic acid in Clomiphene citrate -treated rats might be due to its antioxidant and / or cyto-protective, anti-apoptotic effects¹²

Yousef, J. (2011) reported that treatment with IV Ascorbic acid with Clomiphene citrate administration protected the kidney against the induced nephrotoxicity, and protective effect of ascorbic acid was dose dependent.

CONCLUSION

In the present study, Clomiphene citrate causes nephrotoxicity. Co-administration of Ascorbic acid + CC improved the histopathological and biochemical findings of CC-induced renal toxicity. Clomiphene citrate nephrotoxic effects on renal structure can be avoided by co-administration of ascorbic acid.

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