Light and Transmission Electron Microscopic Study on the Effect of Contraceptive Pills on the Glomerulus and Juxtaglomerular Apparatus in Mice

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ABSTRACT

Light microscopic (LM) and transmission electron microscopic (TEM) observations were performed in the kidneys of oral contraceptive treated mice. Experimental groups were given 0.1 ml of olive oil containing 0.015 fraction of (Neogynon) tablet daily. Control groups were give olive oil alone. Five experimental and three control animals were scarified after 1, 3, 6, 9, and 12 estrus cycles. Glomerulosclerosis, hyaline arteriopathy associated with focal areas of interstitial fibrosis were observed in the first cycle. Mild hypertrophy of juxtaglomerular apparatus "JGA" appeared in the 3rd cycle with increased granularity of the JGCs. Proliferative-sclerosing stages of focal glomerulonephritis were observed and were more advanced in cycle twelve. By transmission electron microscope, there were slight changes in the glomeruli during cycle one. These evident gradually developed in cycle three, thus the glomeruli showed disordered capillary loop occlusion. In cycle six, capillary lumens were occluded by protuberance projection. There were hyperactivity and proliferation of mesangial cells and electron-dense material deposits in glomerular basal lamina; these deposits were accompanied with electron-lucent materials appeared in cycle 9 behind swelled mesangial cells indicating hyperactivity and proliferative cells which showed abundant RER and mitochondria. These lesions were correlated with hyper-granularity of JGCs and hypertrophied JGA. The JGGs appeared in cycle 12 as heterogenized size. These changes indicate that abuse of oral contraceptives is relatively unsafe, especially after long-term of high-dose usage.

KEYWORDS: LM, TEM, contraceptives, juxtaglomerular apparatus

INTRODUCTION

The introduction of oral contraceptives "O.C." in the 1960s was one of the major advances in obstetrics and gynecology in the last century (Burkman 1990). It has had profound social, economic, religious and political impact. The efficiency and ease use of O.C. has lead to their wide spread use throughout most of the world; it is estimated that 50-60 million women are users of O.C. and that 150 million women have used at some time (Dalen & Hickler, 1981).

Oral contraceptives appear to act by suppressing the mid-cycle peak of LH and FSH, thereby inhibiting ovulation; both estrogen and progesterone have this property; in addition these drugs alter the cervical mucous membrane making its thicker and more cellular thus inhibiting sperm transport (Chilvers 1994). Furthermore, they act to decrease endometrial glycogen production so that less energy is available for blastocyst to survive in the uterine cavity (Mitsumori et al. 1994).

The adverse effect of O.C. has been investigated by many authors; the longer use of pill containing Norgstrel and Levonogestrel was associated with higher risk of breast cancer, especially in older aged women (Matt & Leeuwen 1994). The risk of adenocarcinoma of uterine cervix was observed in the mucous epithelial lining of O.C. users and constitutes 10-15% of all cervical cancer (Ursin et al. 1994). The risks of cardiovascular disease associated with O.C. use has been

attributed to alteration in lipoprotein level and to change in platelets aggregation and coagulation factors, that are associated with increase in the dose of estrogen (Stenchever 1993). Hypertension and cardiovascular disease were the most important side effects for the users of Norgstrel and ethinyloestradiol (Fuchs et al, 1995). Significant reduction in the oral tolerance test was found in health women using single or combination O.C. (Rammoorthy et al. 1989). Light (Al-Shaik 1983) and electron (Husain & Al-Ani 1997) microscopic studies have demonstrated degeneration in the pancreatic B-cells of mice treated with O.C. Brandl et al (1992) observed significant increase in the endogenous creatinin clearance, potassium excretion and glomerular filtration rate in women taken oral contraceptive pills of combination preparation. Vasudevanlt et al (2005) showed that estrogen and testosterone administration influence the development of insulin resistance and hypertension in ganadectomized rats of either sex.

It is well known that JGA is the regulation monitoring part of the renin-angiotensin-aldosterone system (RAA-system). Renin is released from the myo-epithelial cells of the afferent arterioles and through the RAA-system causing vasoconstriction and stimulation of aldosterone release (Al-Ani 1991). Up to the authors' knowledge, there has been no published data concerning the effects of O.C. on the morphology of the kidney. The purpose of the present investigation is to study the histopathological and ultrastructural changes possibly occurring in glomeuli and JGAs in female mice after various cycles of receiving O.C.

MATERIALS AND METHODS

Twenty five female swiss albino mice weighing 25-30 gm and aged 7-8 weeks were given (0.1 ml) daily intraperitonial dose of olive oil containing Neogynon which is equal to 0.0037mg of Levonogestrel (progesterone) and 0.00075mg ethinyloestradiol (estrogen), this was calculated according to Fenichel et al (1969). Eight control mice were given 0.1 ml of olive oil alone. The schedule of treatment consisted of giving the drug for 5 days which is equal to one estrus cycle in mice (Rafferty, 1970) and giving a rest for one day for a total of 12 cycles. Five experimental animals and three controls were scarified after 1, 3, 6, 9 & 12 cycles of treatment. Abdomen of mice in each group were immediately opened and both kidneys were perfused with injection of one ml of 3% glutaraldehyde of phosphate buffer; then both kidneys were removed and small pieces of cortex were processed for light and electron microscopy. The experimental animal protocol was conducted in compliance with humane animal care standards outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals, the experimental study was with ethic number of Al-Nahrain Univ. 000 (M) 00.

RESULTS

Treatment of animals with O.C. showed mild changes in cycle one and increased progressively in cycle twelve. A focal glomerulosclerosis "FSGS" was noted in cycle one where the glomeruli showed lesions containing numerous hyaline eosinophilic deposits and mesangial cell proliferation (Fig.1). No significant changes were observed in the JGA.

During cycle three, the glomeruli showed disordered capillary loop occlusion and slight collapse caused by endothelial cell proliferation and thickening of basal lamina and the podocytes were edematous with vacuoles in their foot processes (Figs. 2a & 2b). There was an increase in the granules of the JGCs (Fig. 3).

During cycle six, there was a perivascular mononuclear infiltration (Fig. 4a), there was mild hypertrophy of the JGA (Fig. 4b) and the lumina of the glomerular capillaries had marked vascular congestion and there was a proliferation of mesangial cells (Fig. 5a). Electron microscopy showed the arteriosclerosis of the afferent arterioles was accompanied by bright, fibrinoid deposit and

the lumen capillaries were almost occluded by hyperactive-proliferated endothelial cells with excessive protuberances projecting into the capillary lumen. The podocytes looked edematous, the basement membrane showed thickness, with deposits of electron-dense material (Fig. 5b).

During cycle nine, there was more interstitial edema and mononuclear cell infiltration around blood vessels and glomeruli (Fig. 6a). The glomeruli showed proliferative-sclerosing stages of focal glomerulonephritis. The JGA showed signs of hypertrophy with an increase granulation of JGCs (Fig. 6b). Electron microscopy has showed destructive degenerative changes in the pedicles of podocytes (Fig.11). Endothelial cells were actively proliferating with papillary protuberances projecting into capillary lumen with a new formation of second basement membrane (Fig. 7b).

During cycle, the glomeruli showed mesangial hypertrophy and swelling endothelial cells with mononuclear cell infiltration (Fig. 8a). Electron microscopy demonstrated actively proliferated mesangial cells, the JGAs were hypertrophied with large heterogenized-sized JGCs (Fig.8b).

DISCUSSION

The mechanism for the development of overt hypertension due to the oral contraception ingestion remains unclear. Increase in body weight, plasma volume, exchangeable sodium, plasma insulin, insulin resistance and hepatic synthesis of angiotensinogen have been reported (August and Oparils 1999). Experimental evidence favors a role for the renin-angiotensin system (WHO, 1989). Administration of estrogen alone in rats caused hypertension and an increase in angiotensinogen and angiotensin II levels (Byrne et al. 1994).

The present light and electron microscopic study has shown that O.C. produce marked changes in glomerular membranes, podocytes, mesangial cells and JGA. During cycle one and three, there was a mild increase in granularity and slight hypertrophy of the JGA. The presence of numerous numbers of JGCs with large size granules may represent an active stage of JGC activity (Al-Ani 1980; Taugher & Metz 1986). Sommers & Melamed (1996) demonstrated a correlation activity of mature granules and the presence of organelles such as Golgi apparatus, ribosomes and mitochondria; the present studies demonstrated large number of JGCs containing large size granules associated with ribosomes and suggest that the increased granularity may reflect an increase metabolic activity, which may be associated with glomeruloseclerotic changes.

The present study showed significant changes of the basement membrane in four locations; the glomerular basement membrane, between glomerular basement membrane and the epithelial cells, between glomerular basement membrane and the endothelial cells and within the mesangial region. These locations showed excessive amount and distribution of electron dense deposits which might be account as result of mesangial proliferation that leads to collapse in capillary lumen. There is also mild appearance of formation of second basement membrane within the capillary lumen which progress to increase the thickness of basement membrane. This observation is in agreement with Dillard et al, (1975), who showed these deposits may caused by mesangial cells proliferation and compression of capillary lumen that indicate glomerulosclerosis (Tornorth et al. 1987). Our opinion is correlated with the results obtained by Lee et al (1990) who observed that 83-88% long duration users of Neogynon, showed an increased deposits of electron-dense material in glomerular basement membrane of kidney biopsies, which lead to sever glomerular lesions,, characterized by an irregular thickness of glomerular basement membrane with electron-dense deposits and electron-lucent areas in capillary lumen and mesangium.

During cycle six, there was hypergranularity in the JGCs with hypertrophy of JGA. These changes were more progressed during cycles nine and twelve. These changes were accompanied with thickening of the intema of the afferent arteriole and associated deposition of electron-dense material in the capillary wall. These results are in agreement with Rabinovitch et al (1986) who demonstrated by ultrastructural and clinical studies that Norgstrel caused raised diastolic blood pressure associated with a degree of afferent arteriole thickening and narrowing, these findings

are correlated with severe diffuse arteriolar sclerosis and vascular fibrinoid necrosis with active proliferative endothelial cells (Morita et al. 1995). More histological injury in the kidney and more prominent congestion in the capillary might demonstrate remarkably increased vascular congestion of the glomeruli (Nanth et al. 2005).

The present study showed that JGCs have shown a progressive-stage of maturation, the granules appear larger in size, irregular in shape, increased in number with excessive amount of organelles, especially number of ribosomes in the perivascular area of the JGGs. Skovits et al (1972) reported development of protogranules into mature granules in animals treated with steroids. Gaffieny & Panner (1981) recorded that large granules represent hyperactivity of JGCs to produce and secrete renin and related their observation to hypertension as they measured blood pressure and renin level in blood.

The present study showed that pedicles of podocytes became swollen and broke away from the underlying capillary basement membrane; these changes may be associated with edematous podocytes that started in cycle three and progressed forward leading to degenerative of edematous podocytes. These changes are in agreement with Sinaiko & Michael (1996) who recorded that these changes of degenerative podocytes lead to renal dysfunction and lesions in women using O.C. for a long duration time and this may develop to hypertension. Goldsland et al (1996) observed such changes and suggested that steroid drugs initiate excessive storing of protein and fluid in podocytes and their foot processes which become swollen and ruptured.

In cycles nine and twelve, mesangial cells exhibited chromatin material and mesangial matrix containing accumulation of organelles. This may indicate hyper-proliferating activity of mesangial cells leading progressive scaring stage of glomerulopathy. The presence of inclusion bodies within the mesangial matrix in cycle twelve may indicate degenerative-stag of mesangial cells. The presence of degenerative mesangial cells was considered as a clear sign of glomerulopathy and kidney-tumors (Lawler et al. 1980). Hawie (1986) reported, that steroid may induce hyper-metabolic activity, increased immunological and phagocytic response, in women using long-term of steroid drugs, that may lead to excessive of inclusion bodies within the affected tissue, therefore these bodies could be consider, either phagocytic cells or degenerative cells. The present changes in the mesangium of O.C. treated mice kidneys in late cycles may represent hyper-metabolic activity caused by the toxic effect after long-duration of using O.C. Further immunefluorescent studies are needed to study the effect of O.C. on JGA and to study the electron-dense materials found in the basement membrane and mesangium.

CONCLUSION

It appears that Neogynon stimulated glomerular lesions and arteriopathy, as well as mesangial proliferation and hypertrophy of the JGA. These changes are more apparent with high dose after long-duration of time.

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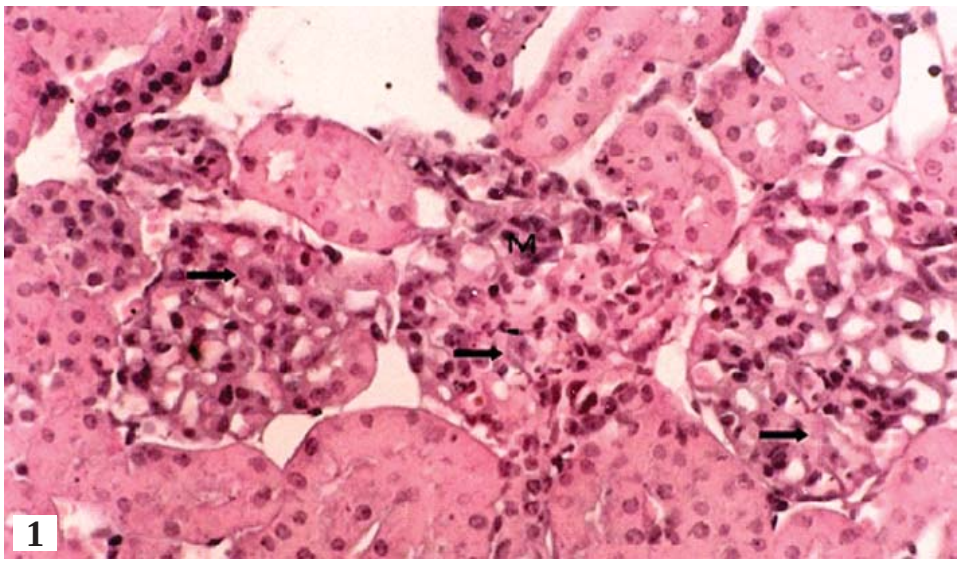

Fig.1. Cortex of mouse kidney, after one cycle of O.C. treatment. A focal glomerulosclerosis appear. Glomeruli showing numerous hyaline eosinophilic deposits (arrows). Cellular proliferation appears in mesangial cells (M). H & E, X 71.50

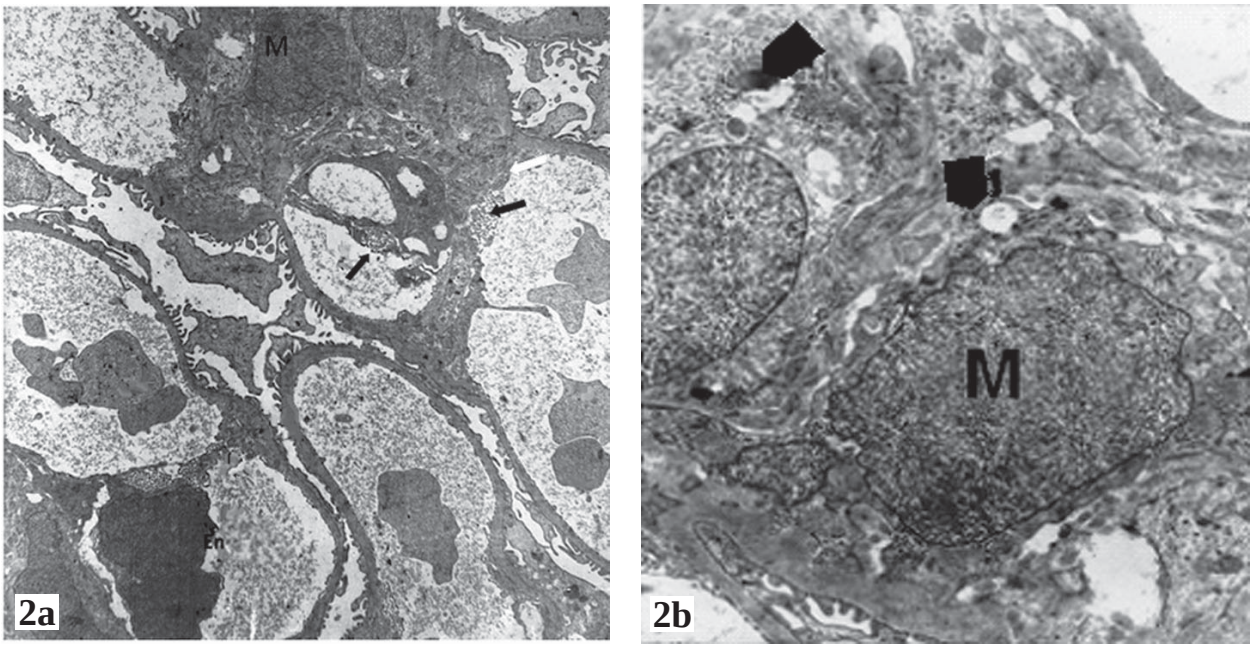

Fig.2. Mouse kidney of three cycles Neogynon treatment, a) showing proliferation of mesangial cells (M), glomerular capillary lumens are occluded by proliferating endothelial cells (E) with a formation of new second basement membrane (white arrow) and presence of capillary protuberance projecting into capillary lumen (black arrows). X. 34.00. b) showing proliferation of mesangial cells (M) with distributed chromatin material, degenerative cells "inclusion bodies" (arrow head) appear in mesangial matrix (arrows) with presence ribosomes. lead citrate and uranyl acetate. X. 7900.

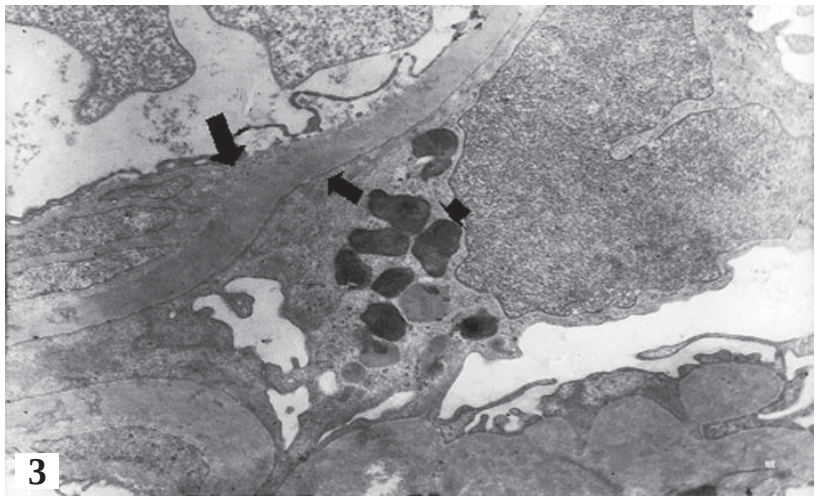

Fig. 3 Mouse kidney of three cycles Neogynon treatment, showing hypertrophy of the JGA (arrows) with mild increase in the granulation of the JGCs (arrowhead). Lead citrate and uranyl acetate. X. 5800.

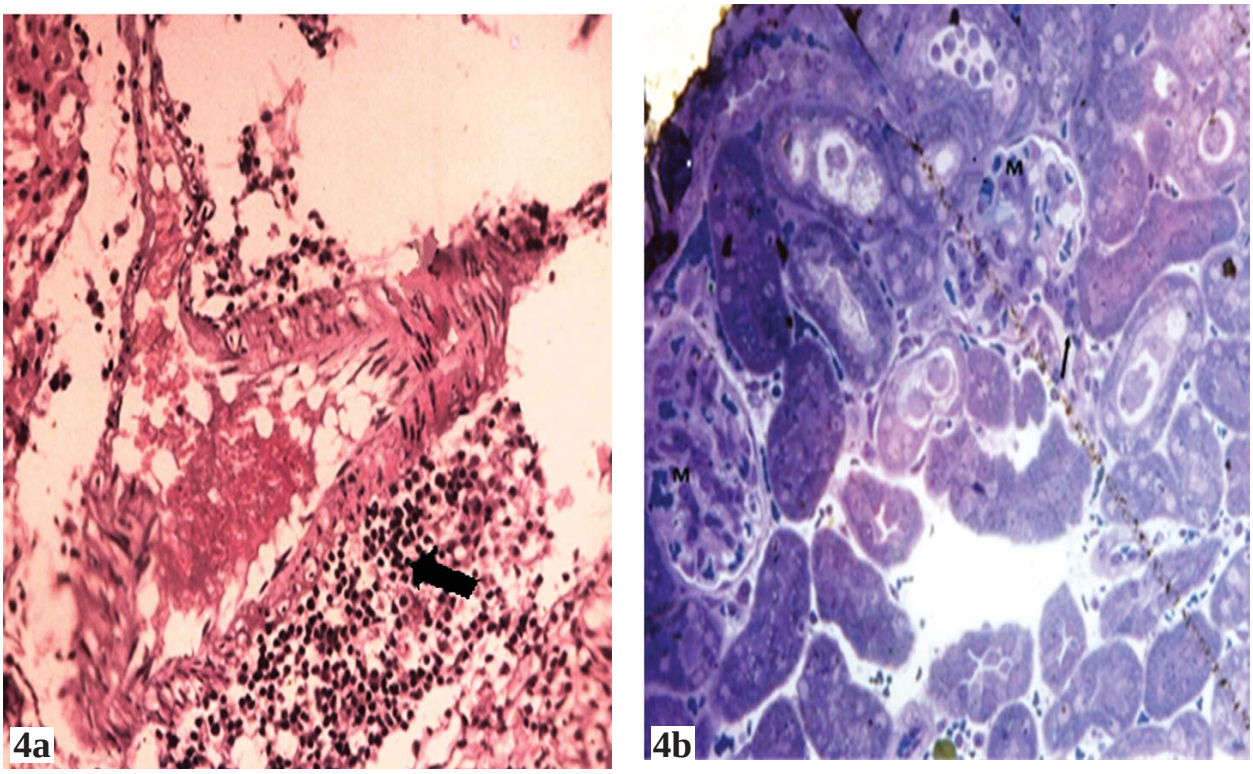

Fig.4. Mouse kidney of six cycles Neogynon treatment, a) showing thickened media of renal arteriole with perivascular mononuclear cells infiltration (arrow), H & E, X. 71.50. b) Showing proliferation of mesangial cells (M). JGA shows mild hypertrophy (J) with an increase in the granulation of the JGCs (arrow). Methylen blue, X. 71.50

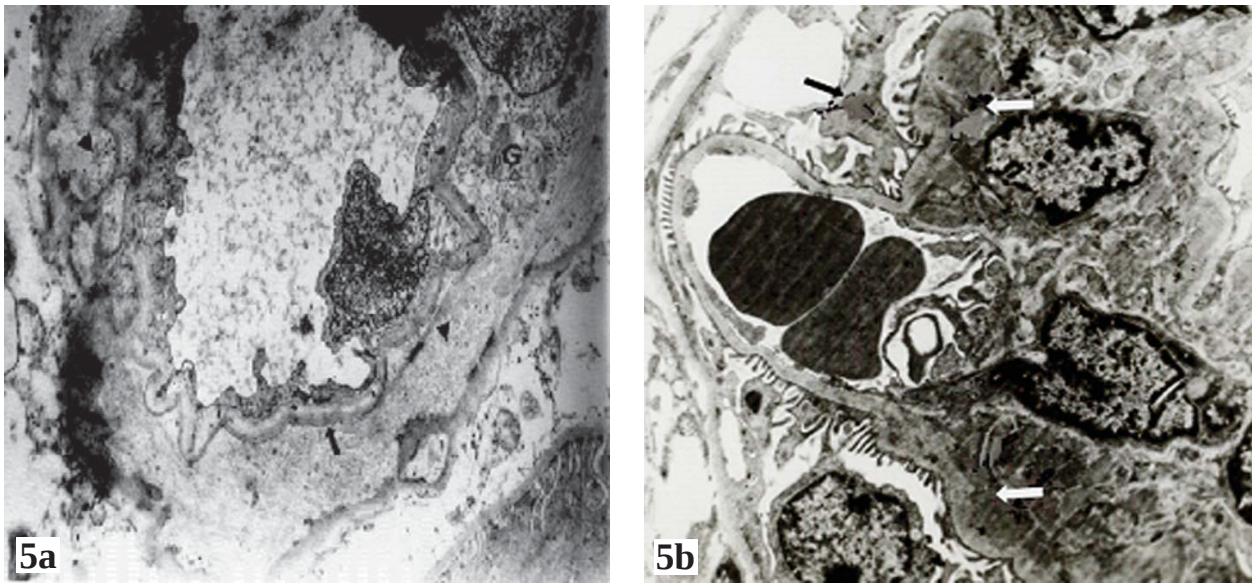

Fig.5. Kidney of six cycles Neogynon treated mouse, a) showing arteriosclerosis (arrow) of the afferent arteriole associated with deposition of collagen fibrils (arrow head), JGCs increase in number (G), X.4600. b) Showing variable thickness of glomerular basement membrane with deposition of electron-dense materials (white arrow), Edematous podocytes (black arrow). Lead citrate and uranyl acetate. X. 460.

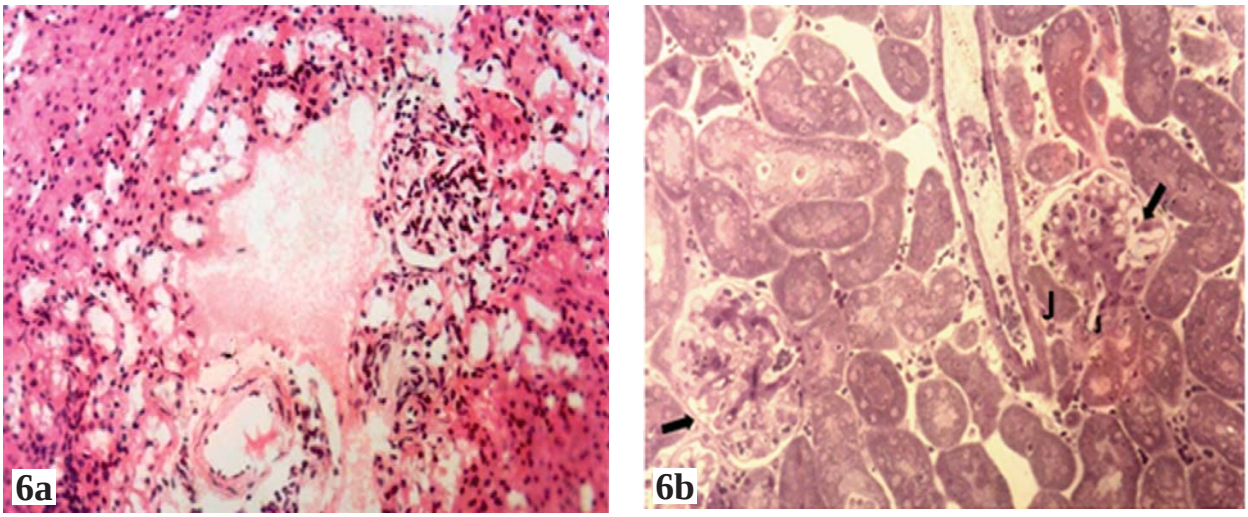

Fig.6. Kidney of nine cycles Neogynon treated mouse. a) showing a progressive-stage of arteriosclerosis associated with hyper-infiltration of mononuclear cells. Notice the proliferative activity of the mesangial cells. H&E, X. 71.50. b) showing glomerulonephritis with proliferative glomerular basement membrane (arrows). The JGA (J) is showing hypergranularity of the JGCs. Tulidin blue, X. 71.50.

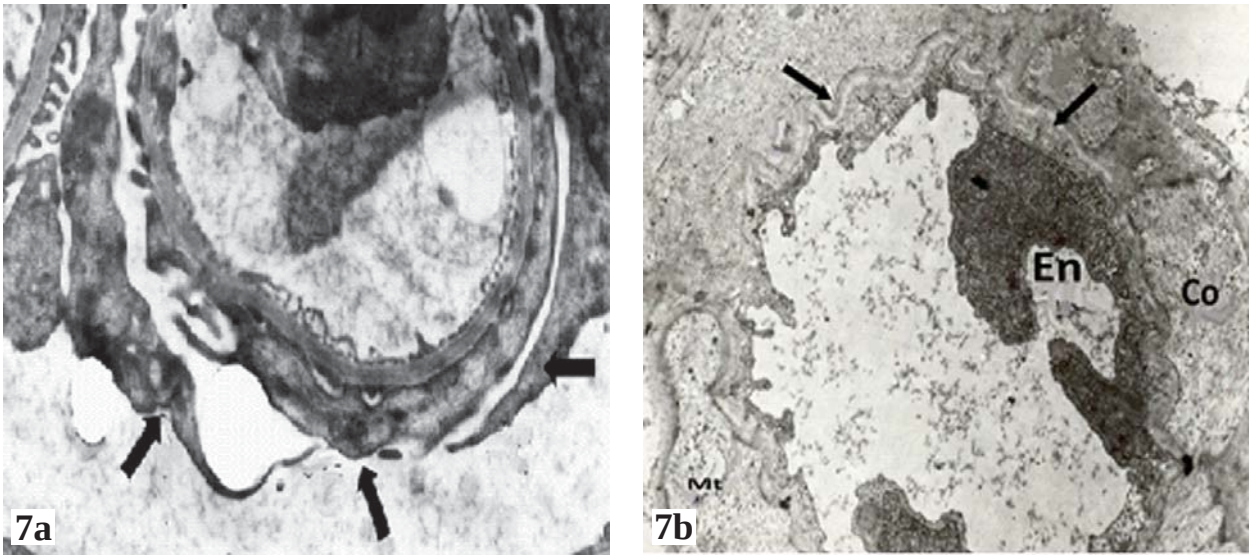

Fig.7. Kidney of nine cycles Neogynon treated mouse. a) showing swollen and ruptured pedicles of podocytes (arrows). X. 7900. b) showing arteriosclerosis (arrows) of the afferent arteriole with endothelial proliferation (En). Collagen-fibril material deposits and mitochondria (Mt) appear behind the afferent arteriole. Lead citrate and uranyl acetate. X. 4600.

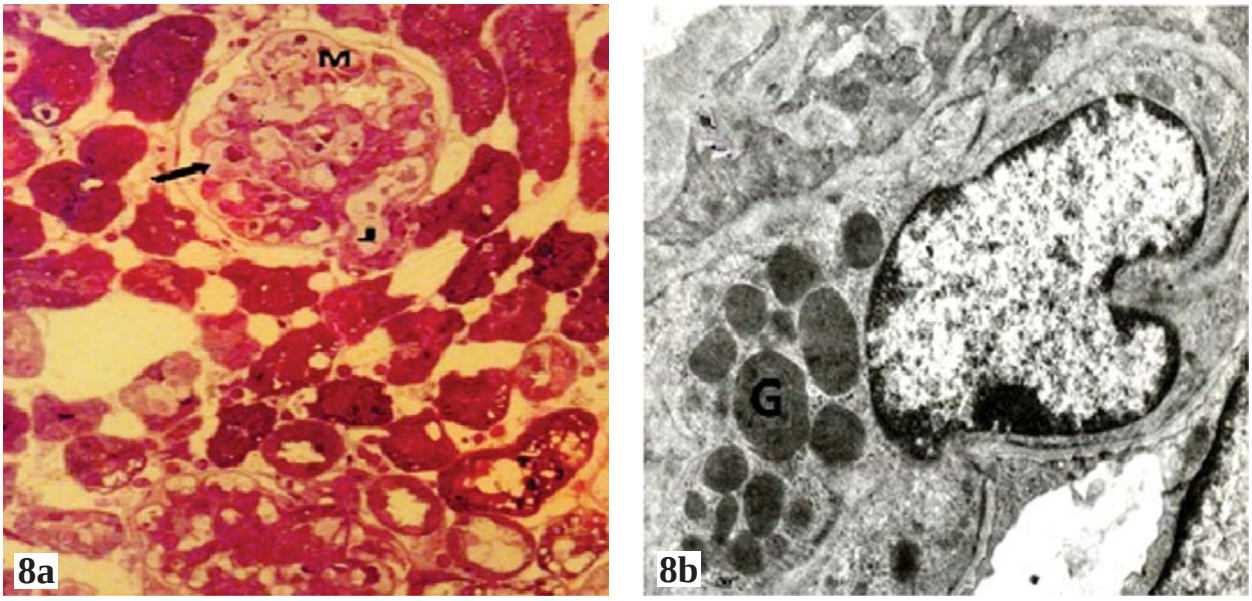

Fig.8. Kidney of twelve cycles Neogynon treated mouse. a) showing degenerated glomeruli with proliferative-endothelial cells (arrow) and hyper-proliferative mesangial cells (M). JGA (J) appears with hypergranularity, (Toulidin blue X. 71.50.) b) showing JGC with hypergranularity associated with abundant ribosomes. Lead citrate and uranyl acetate. X. 7900.