

# Carisoprodol (Somadril) Induced Histological and Histochemical Changes in Renal Cortex of Pregnant Rats and Their Fetuses

Original  
Article

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## ABSTRACT

**Background:** Carisoprodol is a generally prescribed skeletal muscle relaxant whose primary active metabolite is meprobamate, a substance with well-established abuse potential similar to that of benzodiazepines. A number of reports showed that carisoprodol has been abused for its anodyne and relaxant effects.

**Aim:** The diversion and misuse of carisoprodol and its adverse health effects appeared to be dramatically increased over the last several years. This study aimed to detect the histological and histochemical changes in the cortex of renal tissue of pregnant rats and their fetuses after treatment with carisoprodol.

**Materials and Methods:** Thirty pregnant female rats were randomly categorized into three groups (ten pregnant female rats in each group). The first was administered oral doses of distilled water and served as control. The other two groups were administered oral doses of carisoprodol (Somadril compound) in the distilled water equivalent to 10.8 and 21.6 mg/100g body weight/day respectively for 15 days from the 6<sup>th</sup> day to the 20<sup>th</sup> day of gestation.

**Results:** Maternal and fetal renal cortex tissue of both treated groups showed lots of degenerative changes post-treatment with carisoprodol. The severity of these changes was more obvious in fetal cortex of renal tissue of both groups this was accompanied with numerous histochemical changes.

**Conclusion:** Treatment of pregnant rats with carisoprodol led to numerous dystrophic changes in the maternal and fetal renal cortex tissue. These findings provide evidence to support current recommendations to avoid the use of carisoprodol in pregnant rats except if the potential benefit justifies the risk to the fetuses.

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**Key Words:** Carisoprodol, fetuses, histopathology, kidney, pregnant rats.

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## INTRODUCTION

Skeletal muscle relaxant (SMRs) is a set of antispasmodic factors directed to supply relief of worry linked with severe aching musculoskeletal cases<sup>[1]</sup>. It is also used off-brand for several pain cases, such as stress headache and fibromyalgia<sup>[2]</sup>. However, SMRs have limited confirmed efficacy and their side effect profile is wide. Carisoprodol is a centrally skeletal muscle relaxant, structurally and pharmacologically<sup>[3]</sup>. In the 1950, carisoprodol was introduced for comforting of back ache and muscle convulsion. It has weak anticholinergic, antipyretic and painkiller properties<sup>[4]</sup>. Subsequent ingestion of large dose of carisoprodol, death is credited to the central nervous system (CNS) depression with respiratory failing. Death of a 4 year old child has caused by ingestion of 3.5 g of carisoprodol<sup>[5]</sup>. Seizures and coma continually for 33h followed ingestion of carisoprodol up to 14.7 g in an adult<sup>[6]</sup>, whereas ingestion of 9.45 g has caused milder CNS effects<sup>[4]</sup>. The accurate mechanism by which carisoprodol acts is not fully unstated. However, the action of carisoprodol is believed to result from its central nervous

system calming effects rather than its role as a muscle skeletal relaxant<sup>[7]</sup>. Chan<sup>[8]</sup> reported that carisoprodol can alter pain perception by prevent interneuronal action in the descending reticular development and spinal cord in the animal. The same author added that some brands of carisoprodol are Soma, Vanadom, Carisoma and Somadril; most of carisoprodol is metabolized and only a trace amount is secreted without change. The same author also added that carisoprodol is metabolized into three main metabolites by hepatic biotransformation: hydroxycarisoprodol, hydroxy meprobamate and meprobamate which were suggested in the animal studies. The side effects and possible abuse of carisoprodol may be due to changing it to meprobamate or may be due to carisoprodol alone which is a major component in reliance and toxicity<sup>[8,9]</sup>. Carisoprodol has a great ability to change due to its aptitude to potentiate the effects of other illegal drugs<sup>[10]</sup>. High dosage of carisoprodol led to symptoms of tolerance, dependence and withdrawal in humans<sup>[11]</sup>. Drug Enforcement Administration (DEA)<sup>[12]</sup> showed that carisoprodol abuse has expanded in the last decade in the United States. DEA added that animal studies

managed under the directive of the National Institute on Drug Abuse (NIDA) demonstrated that particular effects of carisoprodol may be like to other central nervous system drugs such as meprobamate, chlordiazepoxide and pentobarbital; the beginning of action of carisoprodol is rapid and remains 4 to 6 hours; it is metabolized in the liver and secreted through the renal. Metabolic path of carisoprodol includes its alteration to meprobamate, a drug with substantial barbiturate-like biological actions<sup>[11,12]</sup>. Adverse reactions of carisoprodol included the central nervous system related effects such as dizziness, vertigo, drowsiness, tremor, ataxia, agitation, headache, irritability, depressive reactions, syncope and insomnia; carisoprodol may also harmfully affect on cardiovascular (postural hypotension, tachycardia and facial flushing), gastrointestinal (vomiting, nausea, hiccup and epigastric distress); it may cause idiosyncratic indications including transient quadriplegia, extreme weakness, difficulty in speech, double vision, temporary loss of vision, dilated pupils, euphoria, agitation, confusion and disorientation and overdose of carisoprodol may led to coma stupor, respiratory depression, jolt and death<sup>[11,12]</sup>. In liver and renal, carisoprodol is broken down so these organs seize the most stress as a result of its misuse<sup>[13]</sup>.

No obtainable studies were found which concerning the tissue changes in mammals post Somadril treatment. The present work designed to detect the influence of carisoprodol drug on rat's renal cortex of mothers and fetuses.

## MATERIALS AND METHODS

### *Carisoprodol Drug*

Carisoprodol drug (Somadril compound) was acquired by way of pills of a grouping product having 200mg carisoprodol, paracetamol 160mg and 32mg caffeine, this group was got from Mina Pharm for Pharmaceuticals and Chemical Industries, Cairo. The dose for rats were deliberated by method of Paget and Barnes<sup>[14]</sup>.

### *Experimental animals*

Thirty female of Albino *Rattus norvegicus* and 20 male, 150-200 g body weight were used. Rats nourished on normal food and certain vegetables. The experiment was performed on July and August 2016 and the approval was got from ethics committee of Faculty of Science, Al-Azhar University, Egypt.

### *Induction of pregnancy*

Female rats were mated with male (2:1) overnight. The vaginal smears were got to examine occurrence of spermatozoa. Presence of spermatozoa or vaginal plug in the vagina was considered day zero of gestation.

### *The experimental design*

Pregnant Albino rats stayed and categorized into 3 sets after coupling, each set included 10 pregnant rats, control group (C), gravid rats were administered oral dose of the

drug equal to 10.8 mg/100g body weight from the day 6 to the day 20 of gestation (S1), gravid rats were administered oral dose of drug equal to 21.6 mg/100g body weight from day 6 to day 20 of gestation (S2).

### *Histological and histochemical studies*

Small pieces of renal from the pregnant rats and their fetuses were fixed in 10% neutral buffered formalin and Bouin's solution, dehydrated in ascending grades of alcohol, cleared then embedded in paraffin. Paraffin sections of 5  $\mu$ m thickness were cut and stained with hematoxylin and eosin for general histological structure<sup>[15]</sup>, Mallory's trichrome stain<sup>[16]</sup> for collagen fibers, mercuric bromophenol blue technique for detecting total proteins<sup>[17]</sup>, polysaccharides and DNA content were detected respectively by periodic acid Schiff and Feulgen method<sup>[18]</sup> and Congo red technique<sup>[19]</sup> for detecting amyloid protein.

### *Quantitative histochemical analysis*

The optical density of histochemical stained sections in renal cortex tissue for total protein, DNA, PAS materials and amyloid- $\beta$  protein was documented by IPWIN 32 image analysis software.

### *Statistical analysis*

Statistical analysis were achieved by using analyses of variance (ANOVA) according to the method of Snedecor and Cochran<sup>[20]</sup>. Statistical Analysis for Social Science, version 8 was used to process and analyze the data. Student T-test was used to detect significant differences between groups. Data were offered as mean $\pm$ SD and statistically significant when *P* less than or equal 0.05.

## RESULTS

### *1. Results of pregnant rats*

#### *Histological observations*

A histological pattern of the renal cortex tissue of a control pregnant rats showed normal glomeruli, Bowman's space, proximal and distal convoluted tubules (Figure 1a). Renal cortex tissue of group S1 showed numerous degenerative changes included highly distorted and degenerated cells of the distal and proximal convoluted tubules which were surrounded by numerous fibrotic and hemorrhagic areas most of them lost their normal architecture, cellular detachment, thickening of arterial wall, edema between renal tubules, intertubular leukocytic infiltration and cellular debris in lumens of some renal tubules, most tubules were dilated, their cells had pyknotic nuclei, many of the glomerular tufts were lobulated or atrophied with wide urinary space and some were totally degenerated (Figure 1b-d). Renal cortex tissue of pregnant rats of group S2 showed many dystrophic changes included numerous degenerated tubules, congested and lobulated glomeruli with wide urinary space, prominent internal hemorrhagic areas. Signs of pyknosis, karyorrhexis and karyolysis were realized in nuclei of cells of the convoluted tubules and with cellular debris in the lumens of most

tubules, cellular detachment of the tubular cells, thickened of arterial wall with narrow lumen and intertubular leukocytic infiltration (Figure 1e and f).

The normal distribution of collagen was demonstrated in the normal group (Figure 2a). Highly increased collagen fiber was found in renal cortex tissue of S1 group, but fibrotic and hemorrhagic areas in the cortex acquired red coloration (Figure 2b). Also, highly increased collagen fibres were demonstrated in the renal cortex tissue of group S2 (Figure 2c).

#### ***Quantitative histochemical measurements***

A significant decrease in the mean value of total protein content was noted in S1 and S2 groups which reached  $0.60 \pm 0.16$  and  $0.56 \pm 0.16$  respectively (Figure 3b, c and Table 1) compared to the control group ( $0.96 \pm 0.11$  in Figure 3a). PAS +ve materials in the renal cortex tissue of groups S1 & S2 showed a significant decrease in the mean value which reached  $0.22 \pm 0.01$  and  $0.19 \pm 0.02$  respectively (Figure 4b, c, Table 1) compared to the control group ( $0.31 \pm 0.06$  in Figure 4a). A significant decrease in mean value of total DNA content were recorded in the groups of S1 and S2 which reached  $0.30 \pm 0.02$  and  $0.29 \pm 0.02$  respectively (Figure 5b, c, Table 1) compared to the control group ( $0.40 \pm 0.04$  in Figure 5a). A significant increase in the mean value of amyloid- $\beta$  protein content in the renal cortex tissue of groups S1 and S2 which reached  $0.89 \pm 0.13$  and  $1.00 \pm 0.13$  respectively (Figure 6b, c, Table 1) compared to the control group.

#### ***Histological observations***

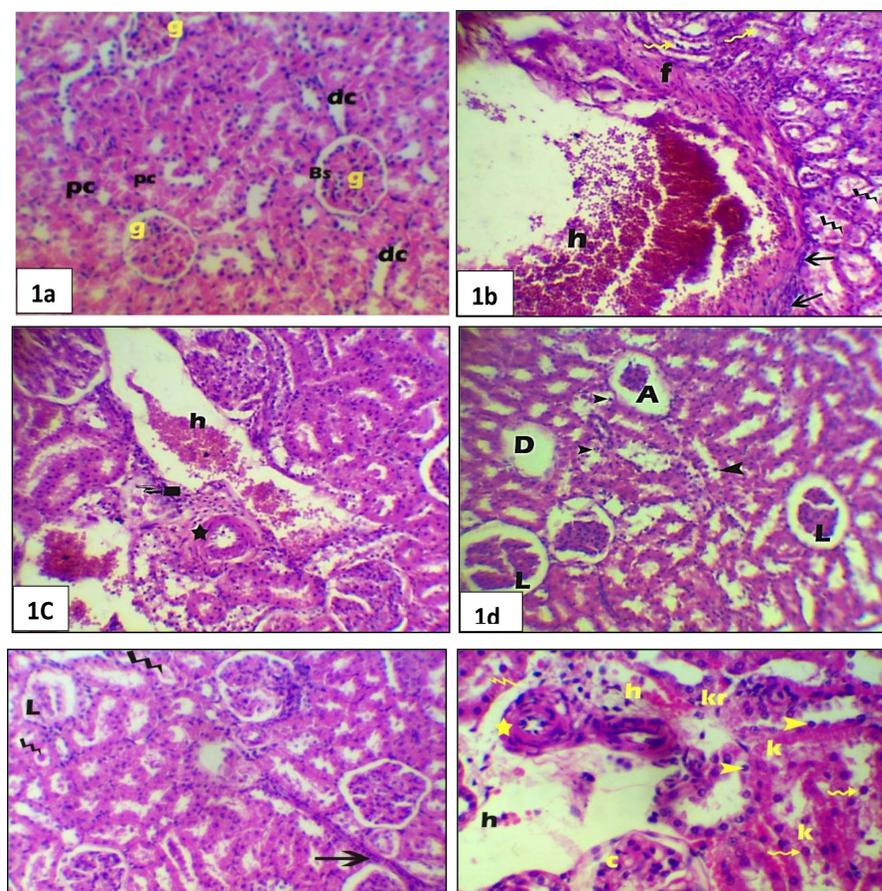
Fetal renal cortex of the control group showed normal glomeruli, proximal and distal convoluted tubules, the latter had wide lumens (Figure 7a). Fetal renal cortex of group S1 showed highly distorted and degenerated cells of the distal and proximal convoluted tubules which were surrounded by numerous fibrotic areas, the glomeruli showed many changes such as they were atrophied, lobulated, degenerated or elongated and lost their normal

architecture with numerous pyknotic nuclei (Figure 7b). Numerous dystrophic changes were observed in the fetal renal cortex of group S2. These changes included highly distorted and degenerated cells of distal and proximal convoluted tubules which lost their normal architecture and they were surrounded by numerous fibrotic areas this was accompanied by congested and degenerated glomeruli and tubular epithelial cytoplasm showed brown-colored pigment ( hemosiderin deposition) (Figure 7c and d).

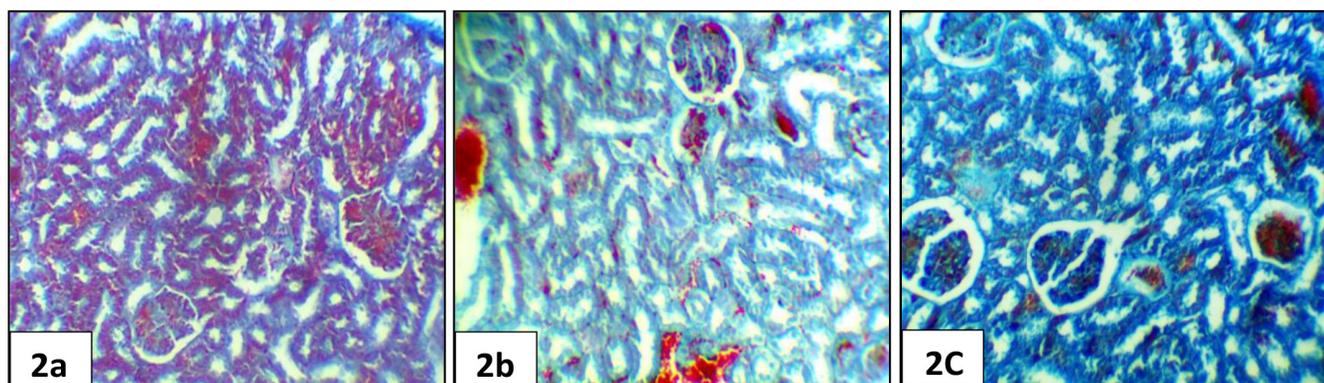
Normal distribution of collagen fibres were observed in the control group (Figure 8a). Highly increased collagen fibres were observed in the renal cortex of group S1 especially in the brush borders and in the basement membranes of the convoluted tubules and in Bowman's capsules (Figure 8b). Intensely stained collagen fibres was observed in the fetal renal cortex of group S2, most of the tubules and glomeruli were replaced by collagen fibres (Figure 8c).

#### ***Quantitative histochemical measurements***

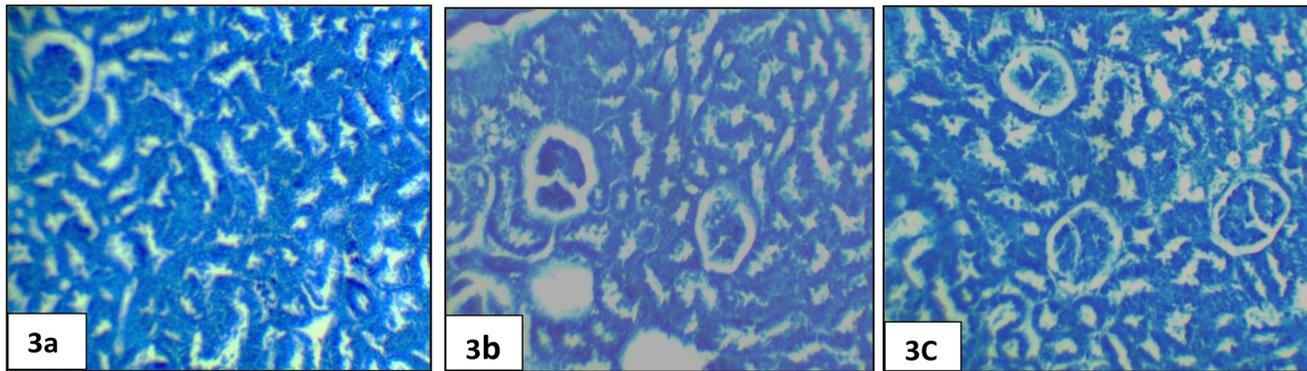
A significant decrease was detected in the mean value of total protein content in the fetal renal tissue in groups S1 and S2 which reached  $0.41 \pm 0.02$  and  $0.23 \pm 0.01$  respectively (Figure 9b, c, Table 2) compared to the control group ( $0.46 \pm 0.03$  in Figure 9a). PAS +ve materials in the fetal renal cortex tissue of groups S1 and S2 showed a significant decrease in the mean value which reached  $0.25 \pm 0.02$  and  $0.23 \pm 0.02$  respectively (Figure 10b, c, Table 2) compared to the control group ( $0.38 \pm 0.04$  in Figure 10a). A significant decrease was realized in the mean value of total DNA content in groups S1 and S2 which reached  $0.15 \pm 0.02$  and  $0.12 \pm 0.03$  respectively (Figure 11b and c and table 2) compared to the control group ( $0.23 \pm 0.05$  in Figure 11a). Amyloid  $\beta$  -protein in the fetal renal cortex tissue of groups S1 and S2 exhibited a significant increase in the mean value which reached  $1.12 \pm 0.16$  and  $0.85 \pm 0.11$  respectively (Figure 12b, c, Table 2) compared to the control group ( $0.21 \pm 0.02$  in Figure 12a).



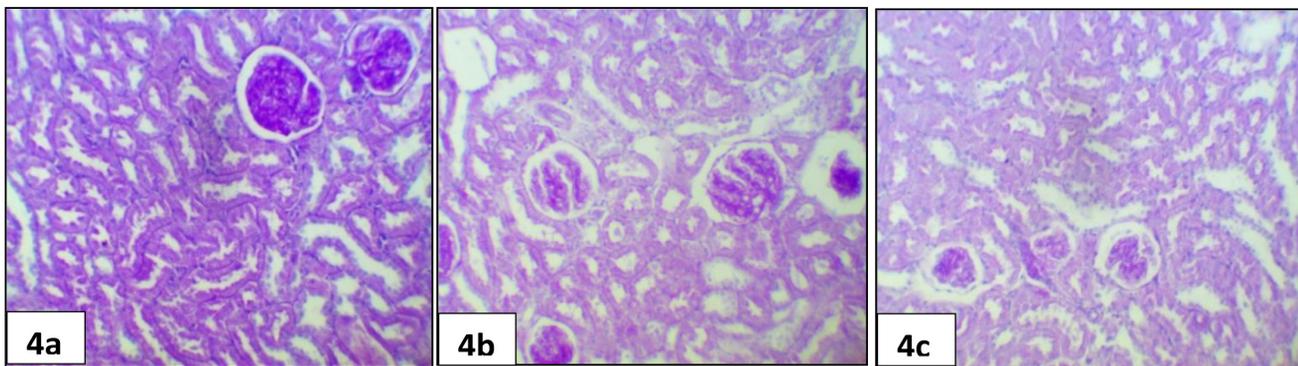
**Fig.1(a-f):** Photomicrographs of sections in kidney cortex tissue of pregnant rats stained with hematoxylin and eosin, (a) control group showing normal glomeruli(g),Bowman's space (Bs), proximal (pc) and distal (dc) convoluted tubules, (b-d) S1 group showing highly distorted and degenerated cells of the distal and proximal convoluted tubules which are surrounded by numerous fibrotic (f) and hemorrhagic (h) areas, cellular detachment (corrugated arrows), thickening of arterial wall (star), edema between renal tubules (hand), intertubular leukocytic infiltration (→) and cellular debris (Corrugated lines) in lumens of some renal tubules, most tubules are dilated, their cells have pyknotic nuclei (▶), many of the glomerular tuft are lobulated (L) or atrophied (A) with wide urinary space and some are totally degenerated (D), (e and f) S2 group showing numerous degenerated tubules, congested (C) and lobulated (L) glomeruli, pyknotic (▶), karyorrhexis (kr) and karyolytic (K) nuclei in the cells of tubules and glomeruli with cellular debris (Corrugated lines) in the lumens of most tubules, cellular detachment of the tubular cells (corrugated arrows), thickened arterial wall with narrow lumen (star) intertubular leukocytic infiltration (→).  
(Original magnification a-e X 200 & f X 400)



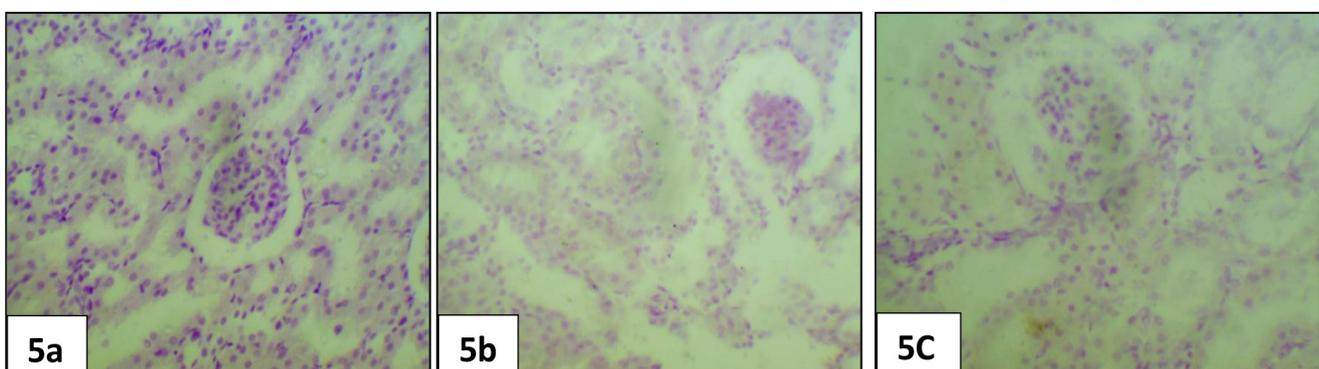
**Fig.2 (a-c):** Photomicrographs of sections in renal cortex tissue of pregnant rats stained with Mallory's trichrome stain showing: (a) the control group- normal distribution of collagen fibres, (b) S1 group - dense stain affinity of collagen fibres, but the fibrotic and hemorrhagic areas acquire bright red coloration,(c) S2 group -highly increased collagen fibres.  
(Original magnification X 200)



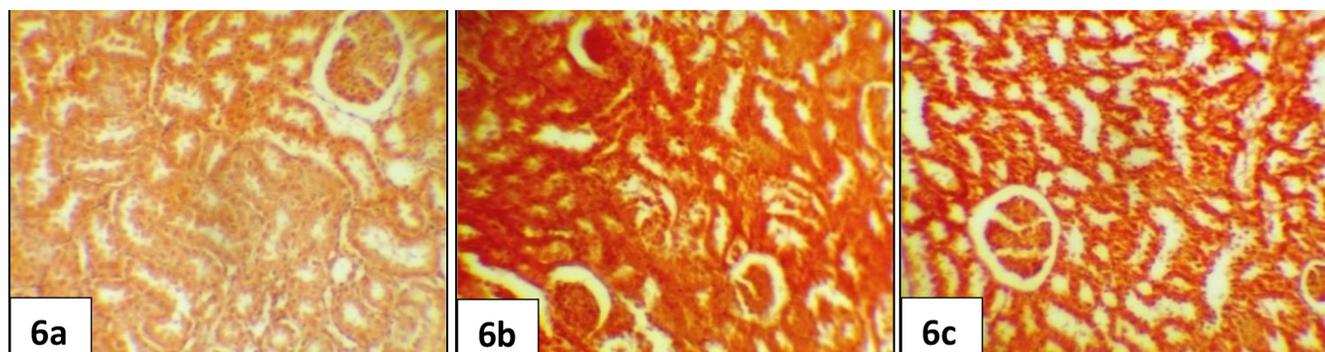
**Fig.3(a-c):** Photomicrographs of sections in renal cortex of the pregnant rats stained with mercuric bromophenol blue showing: (a) the control group - deeply stained glomeruli , cells of the distal and proximal convoluted tubules with less stained brush borders,(b) S1group - decreased staining affinity of total protein in the glomeruli and walls of the convoluted tubules with negatively stained degenerated areas , (c) S2 group - faintly stained total protein in the convoluted tubules and glomeruli. (Original magnification X 200)



**Fig.4(a-c):** Photomicrographs of sections in renal cortex of the pregnant rats stained with periodic acid Schiff technique showing: (a) the control group - deeply stained glomeruli , distal convoluted tubules , Bowman's capsules and brush borders of the proximal convoluted tubules,(b) S1 group -faintly stained PAS +ve materials,(c) S2 group - highly decrease staining affinity of PAS +ve materials. (Original magnification X 200)

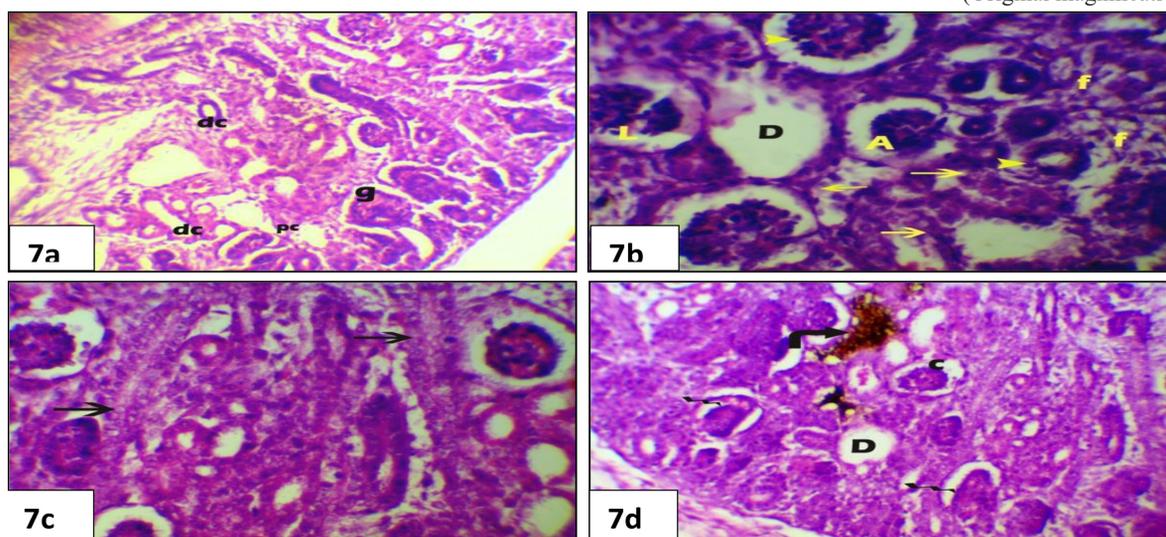


**Fig.5(a-c):** Photomicrographs of sections in kidney renal of the pregnant rats stained with Feulgen reaction showing :(a) the control group - normal distribution of DNA in glomerular capillaries and convoluted tubules ,(b) S1group - decreased total DNA content with faintly staining affinity in the glomeruli and in the epithelial cells of the proximal and distal convoluted tubules ,(c) S2 group - highly decreased total DNA content with faintly staining affinity in the glomeruli and in the epithelial cells of proximal and distal convoluted tubules. (Original magnificationX 400)



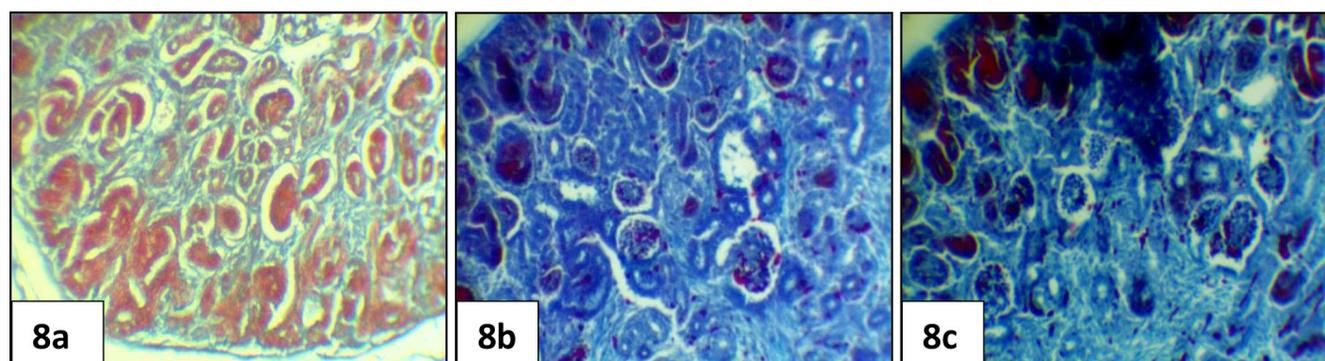
**Fig.6(a-c):** Photomicrographs of sections in renal cortex of the pregnant rats stained with Congo red showing : (a) the control group - faintly stained amyloid- $\beta$  protein, (b) S1 group - densely stained amyloid  $\beta$  -protein in the glomerular capillaries, especially in the basement membranes of some convoluted tubules and in hemorrhagic areas,(c) S2 group - densely stained amyloid  $\beta$  -protein.

(Original magnification X200)



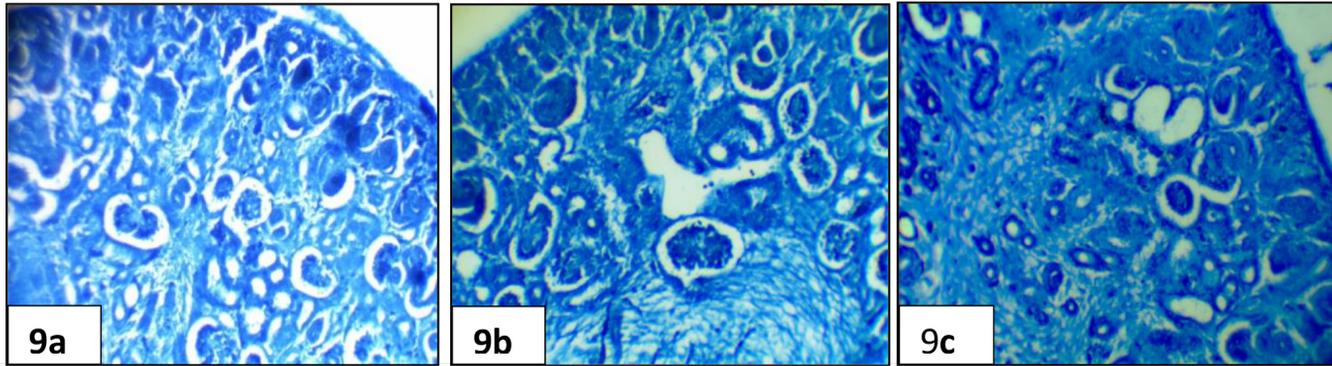
**Fig.7 (a-d):** Photomicrographs of sections in fetal renal cortex stained with hematoxylin and eosin,(a)control group shows normal glomeruli (g), proximal (pc) and distal (dc) convoluted tubules the later had wide lumens,(b)S1 group shows highly distorted and degenerated cells(→) of distal and proximal convoluted tubules which are surrounded by numerous fibrotic (f) areas, glomeruli are atrophied (A) , lobulated (L), degenerated (D), with numerous pyknotic nuclei (arrow head),(c&d) S2 group showing highly distorted and degenerated cells of the distal and proximal convoluted tubules(→) which lost their normal architecture and surrounded by numerous fibrotic areas. Glomeruli are congested (c), degenerated (D) or with abnormal shape (corrugated line). Tubular epithelial cytoplasm showing brown-colored pigment (hem siderin deposition) (curved arrow).

(Original magnification a&d X 200 - b &c X 400)

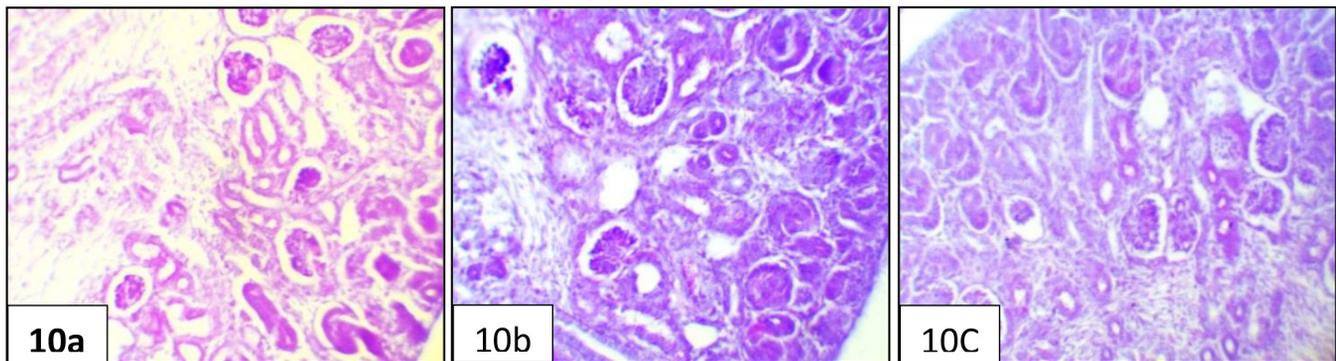


**Fig.8(a-c):** Photomicrographs of sections in fetal renal cortex tissue stained with Mallory's trichrome stain showing:(a) the control group - collagen fibres supporting the stroma, the capsule, walls of the convoluted tubules, brush borders of the proximal convoluted tubules and Bowman's capsules, (b) S1 group - highly increased collagen fibres especially in the brush borders and in the basement membranes of the convoluted tubules and in Bowman's capsules, (c) S2 group - intensely stained collagen fibres and most of the tubules and glomeruli are replaced by collagen fibers.

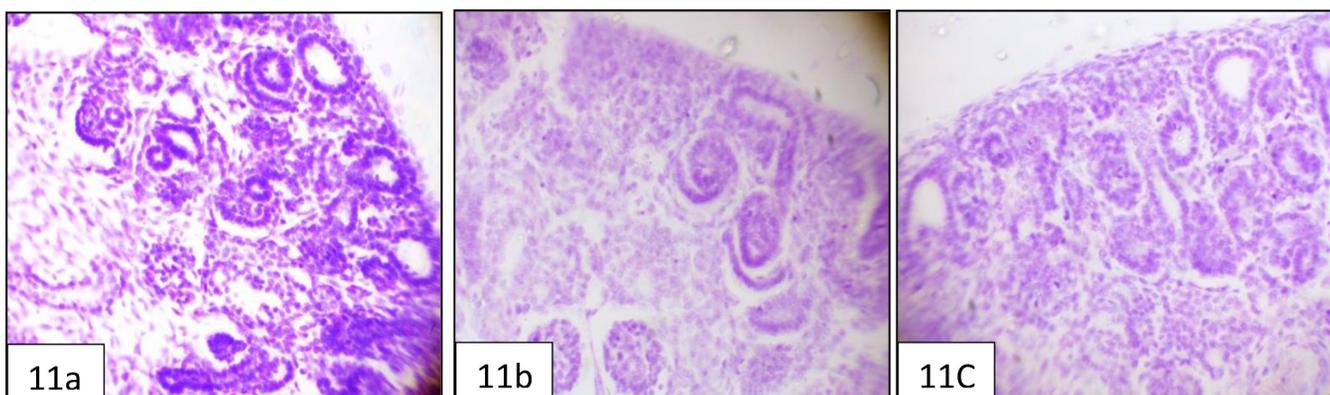
(Original magnification X200)



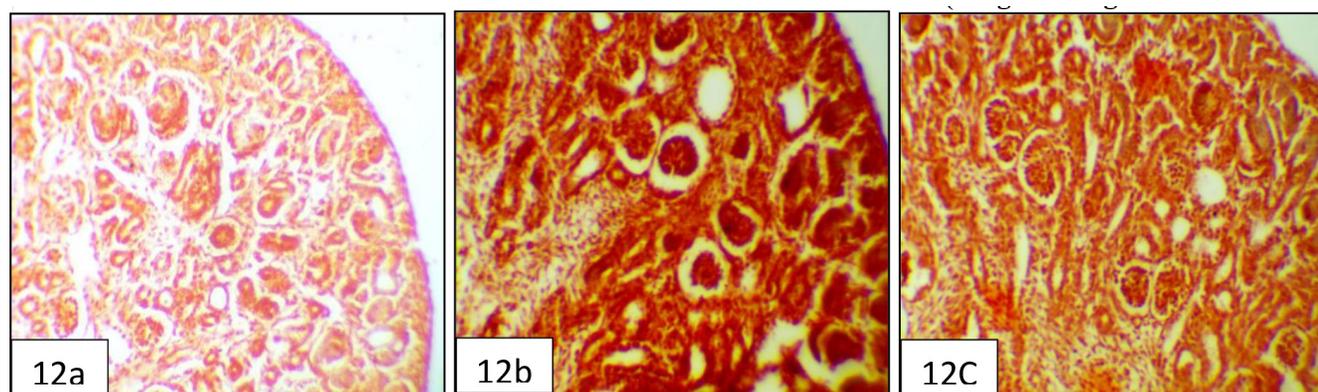
**Fig.9(a-c):** Photomicrographs of sections in fetal renal cortex stained with mercuric bromophenol blue showing:(a) the control group -deeply stained glomeruli and cells of the distal and proximal convoluted tubules of fetal kidney cortex ,(b) S1 group- decreased stain affinity of total protein in distal and proximal convoluted tubules and glomeruli, (c) S2 group -decreased staining affinity of total protein in the necrotic and fibrotic areas. (Original magnification X200)



**Fig.10(a-c):** Photomicrographs of sections in fetal renal cortex stained with periodic acid Schiff technique showing: (a) the control group - deeply stained glomeruli, Bowman's capsules, proximal convoluted tubules, but distal convoluted tubules are less stained ,(b)S1group - poorly stained polysaccharides ,(c) S2 group - faintly stained PAS +ve materials in the glomeruli and some epithelial cells of the convoluted tubules. (Original magnification X200)



**Fig.11(a-c):** Photomicrographs of sections in fetal renal cortex stained with Feulgen reaction showing: (a) control group - normal distribution of DNA in glomerular capillaries and convoluted tubules, (b) S1 group - decreased total DNA content with faintly stain affinity in the glomeruli and in the epithelial cells of proximal and distal convoluted tubules, (c) S2 group - poorly to moderately stained DNA materials. (Original magnification X 400)



**Fig.12(a-c):** Photomicrographs of sections in the fetal renal cortex stained with Congo red showing: ( a ) the control group - faintly stained amyloid-β protein, (b) S1 group - densely stained amyloid β –protein in the glomerular capillaries, especially in the basement membranes of some convoluted tubules,(c) S2 group -deeply stained amyloid deposits. (Original magnification X 200)

**Table 1:** Revealing mean optical density values of total protein, PAS + ve materials, DNA content and amyloid β protein in the kidney cortex of pregnant rats

Groups	C	S1	S2
Parameters	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)
Total protein	0.96±0.11	0.60±0.16*	0.56±0.16*
PAS+ve materials	0.31±0.06	0.22±0.01*	0.19±0.02*
Total DNA conten	0.40±0.04	0.30±0.02*	0.29±0.02*
Amyloid – β protein	0.26±0.03	0.89±0.13*	1.00±0.13*

SD means standard deviation, \* Significant from the control group ( $P \leq 0.05$ ).

**Table 2:** Revealing mean optical density values of total protein, PAS + ve materials, DNA content and amyloid β protein in fetal renal cortex

Groups	C	S1	S2
Parameters	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)
Total protein	0.46±0.03	0.41±0.02*	0.23±0.01*
PAS+ve materials	0.38±0.04	0.25±0.02*	0.23±0.02*
Total DNA content	0.23±0.05	0.15±0.02*	0.12±0.03*
Amyloid – β protein	0.21±0.02	1.12±0.16*	0.85±0.11*

SD means standard deviation, \* Significant from the control group ( $P \leq 0.05$ ).

## DISCUSSION

Renal is a main probable path for imbibing of risky substances occurred in the environment<sup>[21]</sup>. It was chosen in this study because it is a very vital organ for healthy life of mammals. Soma is processed to meprobamate, a schedule II drug that has been correlated with misuse, dependence and central nervous system side effects<sup>[22]</sup>. Harms of carisoprodol poses a danger of high dosage which may cause respiratory depression, hypotension, seizures and death. Carisoprodol intoxication may lead to Serotonin syndrome. Patients at huge hazards of carisoprodol effect may be due to lengthened use of it or those who use carisoprodol in blend with other injured drugs<sup>[23]</sup>.

In the present study, histopathological observation of renal cortex tissue of pregnant rats treated with

carisoprodol showed many deleterious changes in the renal cortex tissue of the pregnant rats of groups S1 and S2 such as highly distorted and degenerated cells of the distal and proximal convoluted tubules, hemorrhagic areas, thickening of the arterial walls, edema between the renal tubules, many of the glomerular tufts were lobulated or atrophied. Signs of pyknosis, karyorrhexis and karyolysis were realized in nuclei of cells of the convoluted tubules. Also, examination of the fetal renal cortex tissue of groups S1 and S2 showed many hurtful changes such as atrophied, lobulated, degenerated or elongated glomeruli with numerous pyknotic nuclei. Highly distorted and degenerated cells of the distal and proximal convoluted tubules were demonstrated and tubular epithelial cytoplasm showed brown-colored pigment. *In vitro* study established that carisoprodol owns barbiturate like properties are

analogous other Schedule IV drugs such as barbital, meprobamate and demonstrated that extreme carisoprodol use makes toxicity and withdrawal symptoms alike to other Schedule IV drug<sup>[12]</sup>. Results of the present study are supported by results of Chan<sup>[8]</sup> who declared that treatment with carisoprodol at doses of 400 mg/kg or greater in male rats caused a slight exacerbation of chronic nephropathy demonstrated by increased lesion rates and severities; in four males in the 1,600 mg/kg group, minimal tubule epithelial necrosis was found in the renal cortex and was proved by necrotic cells that were desquamated into lumens of the cortical tubules. The same author added that the average severity of nephropathy was minimally increased compared to the vehicle controls in males administered 200 mg/kg or greater while, in the 800 mg/kg group of females increased nephropathy was observed. The same author stated that the renal of male rat was more susceptible to the effects of carisoprodol than the renal of female. The hazards related to carisoprodol abuse may lead to seizures and death<sup>[24]</sup>. The true mechanism of action of carisoprodol is unidentified; however, past experiment recommended that carisoprodol might act via the  $\gamma$ -aminobutyric acid (GABA)-ergic system<sup>[25]</sup>. The capability to reabsorb, secrete and/or renally eject carisoprodol may affect both therapeutic and pharmacokinetic results; such as rise in plasma concentrations and a reduction in renal elimination of famotidine, a histamine H<sub>2</sub>-receptor antagonist in elderly and renal impaired patients<sup>[26]</sup>. Also, reduction in creatinine clearance was detected in both elderly and renal dysfunction patients because of weakening in glomerular filtration, the ratio of famotidine clearance to creatinine clearance also declined. This was suggestive of possible decaying of renal secretory devices that may be present in both elderly and renal defective patients<sup>[27]</sup>.

Congenital defects during the first trimester of pregnancy may be occurred by carisoprodol<sup>[28]</sup>. A animal study showed that carisoprodol crosses the placenta and causes opposing effects such as decreased fetal weights, postnatal weight gain and postnatal endurance at maternal doses equivalent to 1 to 1.5 times the human dose<sup>[23]</sup>. These results agree with our results and results of Abouel-Magd<sup>[29]</sup> since fetal tissues maternally treated with carisoprodol were highly affected, but these results don't agree with those of Briggs *et al.*<sup>[30]</sup> who stated that carisoprodol did not cause developmental injuries, but the long-term follow-up of this drug appeared later in life.

In this study, highly increased collagen fibres were detected in the renal tissue of pregnant rats of group S1 and S2; this increase was observed in the fetal renal cortex of group S1 especially in the brush borders and in the basement membranes of the convoluted tubules and in Bowman's capsules. Intensely stained collagen fibers were observed in the fetal renal cortex of group S2 compared to the control group. Hassan *et al.*<sup>[31]</sup> reported that increased collagen fibres may lead to protection from toxicant substance. Increased collagen deposition may be lead to oxidative stress that stimulates the expression of genes involved in collagen biosynthesis<sup>[32]</sup>. Increased superoxide

anion formation by inhibition of superoxide dismutase (antioxidant enzyme) stimulates collagen production and this indicated important role of superoxide dismutase and the generated reactive oxygen species in collagen accumulation<sup>[33]</sup>. These results agree with those of Abouel-Magd<sup>[29]</sup> who found increased collagen fibres in the liver tissue of pregnant rats and their fetuses maternally treated with carisoprodol.

Results of the present study showed decreased staining affinity of total protein in the glomeruli and walls of the convoluted tubules of groups S1 and S2 in the renal of pregnant rats and their fetuses compared to the control group. Proteins are chiefly involved in the architecture of cells<sup>[34]</sup>. It is related to insulin regulation, glucose control, muscle building and regulation or increased metabolism<sup>[35]</sup>. The accumulation and degeneration led to reduced protein content in the altered tissues<sup>[36]</sup>. Abu Elnaga and Abd Rabou<sup>[37]</sup> mentioned that decreased protein content due to broken cellular organoids or to reduced polyribosomes. The reduction in the protein content under the effect of many toxicants may be due to the disruption of lysosomal membranes which cause release of their hydrolytic enzymes in the cytoplasm and cause lysis and dissolution of the target material within the cytoplasm<sup>[38]</sup>.

Also, Abouel-Magd<sup>[29]</sup> found decreased in protein content in liver tissue of pregnant rats and their fetuses treated with carisoprodol. Reduced staining affinity of total protein in the different layers of fetal esophagus maternally treated with carisoprodol was recorded by Abd Rabou<sup>[39]</sup>.

In the present study, renal cortex tissue of pregnant rats and their fetuses of S1 and S2 groups showed a significant reduction in the polysaccharides. This reduction due to the stress on organs and that lead to use great energy in attempt to balance the pressure, or may be due to release of hydrolytic enzymes from broken lysosomes under the belongings of toxic agents<sup>[40, 41]</sup>. These results agree with the study of Abouel-Magd<sup>[29]</sup> who found the reduction in contents of carbohydrate in the liver tissue of pregnant rats treated with carisoprodol.

The current results showed highly reduced DNA materials in the glomeruli and in the epithelial cells of the proximal and distal convoluted tubules of renal cortex of pregnant rats and their fetuses compared to the control. The present results agree with results of Chan<sup>[8]</sup> who stated that carisoprodol induced chromosomal aberrations in the ovary hamster cells of cultured Chinese at the maximum concentration (1,000 or 1,250  $\mu\text{g/mL}$ ). The reduction in total protein content and DNA may be due to the arrest of metabolism or to use them to set up the new cells or enzymes to reduce the stress and also break of lysosomal membranes under the effect of many toxicants cause releasing the marked lysis and dissolution of destination materials<sup>[42]</sup>.

A significant increase was recorded in the amyloid protein of renal cortex of pregnant rats and their fetuses of S1 and S2 groups compared to the control group. Amyloids

are insoluble fibrous proteins and they arise from at least 18 unsuitably folded forms of proteins and polypeptides, which present naturally in the body<sup>[43]</sup>. These misfolded constructions alter their good configuration such that they erroneously interact with one another or other cell components forming insoluble fibrils. They have been related to the pathology of more than 20 serious human illnesses since abnormal accretion of amyloid fibrils in organs may lead to amyloidosis and have a role in numerous neurodegenerative diseases<sup>[44]</sup>. Kadowaki *et al.*<sup>[45]</sup> showed that amyloid deposition was related to mitochondrial dysfunction and caused generation of reactive oxygen species (ROS), which caused automatic cell death.

Fetal liver tissue took from mothers treated with carisoprodol showed increased amyloid protein in most liver cells, necrotic regions and blood cells<sup>[29]</sup>. Also, results of the present study are supported by results of Abd Rabou<sup>[39]</sup> who realized increased amyloid protein in the different layers of fetal esophagus maternally treated with carisoprodol. The histological changes that occurred in the renal cortex of both pregnant rats and their fetuses are due to carisoprodol alone because the rest of the components of Somadril as paracetamol, which is safe for pregnant women<sup>[46]</sup> and caffeine present in a small amount, which is ineffective<sup>[47]</sup>. Other studies are needed to assess the skeletal muscle relaxants medicines and their effect on the pregnant female and their fetuses.

#### **CONCLUSION**

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According to previous observations, we can be concluded that the using carisoprodol during pregnancy caused many hazards changes in the renal cortex tissue of pregnant rats and their fetuses. Crossing of carisoprodol from placenta of gravid rats to the fetuses, that a sign to avoid administration of carisoprodol during pregnancy period.

#### **CONFLICTS OF INTEREST**

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There are no conflicts of interest.

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## الملخص العربي

# التغيرات النسيجية والكيميائية التي يسببها الكاريزوبرودول (سومادريل) في قشرة كلى الجرذان الحوامل وأجنحتها

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**المقدمة:** يعتبر عقار كاريزوبرودول من قائمة العقاقير التي تساعد على إرخاء العضلات، وهو أحد أنواع الأدوية المسكنة التي تعمل على تخفيف الشعور بالألم في مختلف الأماكن في الجسم فهو يقوم بإرخاء العضلات من خلال السيطرة على بعض الإشارات أو حصر النبضات العصبية التي يتم إرسالها إلى المخ ويحدث تخفيف لأعراض الشد العضلي والتشنجات التي تسبب الألم وبالتالي لا يشعر المريض بأي ألم. وإساءة استخدام الكاريزوبرودول وآثاره الضارة على الصحة قد زاد بشكل كبير خلال السنوات القليلة الماضية بسبب آثاره المهدئة.

**الهدف من العمل:** تهدف هذه الدراسة إلى إيضاح التأثير الضار للجرعات العالية من عقار كاريزوبرودول على التركيب النسيجي والكيميائي لنسيج قشرة الكلى للجرذان الحوامل وأجنحتها.

**المواد والطرق:** تم تقسيم الجرذان إلى ثلاث مجموعات (عشرة جرذان حوامل في كل مجموعة). المجموعة الأولى استخدمت كمجموعة ضابطة، أما المجموعة الثانية والثالثة فتم إعطائهم جرعات فمية من عقار الكاريزوبرودول (سومادريل) تعادل 10,8 مجم و 21,6 مجم/100 جم من وزن الجسم يوميا لمدة 15 يوما من اليوم السادس إلى اليوم العشرين من الحمل. وأجريت دراسات نسيجية وكيميائية على خلايا قشرة الكلى للأمهات وأجنحتها.

**النتائج:** وقد أوضحت النتائج ظهور تغيرات مرضية عديدة بنسيج قشرة الكلى للأمهات والأجنة. وكانت التغيرات أكثر وضوحا في نسيج قشرة الكلى الجنيني لكلا المجموعتين وتشمل هذه الأضرار ما يلي: إحتقان وتفصص وضمور في الكبيبات البولية مع اتساع في محفظة بومان، معظم الأنابيب فقدت شكلها الطبيعي حيث حدث تمدد في الأنابيب البولية وضعف الكشف عن حدود تلك الأنابيب. وترسيب لحطام الخلايا وفقد اتصال الخلايا ببعضها وارتشاح لكريات الدم البيضاء داخل الأنابيب، إستسقاء بين الأنابيب الكلوية، كما لوحظ أيضا تمدد في الأوعية الدموية، بالإضافة إلى اتساع في الأنابيب ونزف داخلي كما أدى استخدام عقار الكاريزوبرودول أيضا إلى زيادة كبيرة في الألياف الكولا جينية في نسيج الكلى خصوصا في الغشاء القاعدي للأنابيب المتوتية. كما أظهرت المجموعات المعاملة بالكاريزوبرودول للأمهات وأجنحتها انخفاضا ملحوظا في المواد عديدة التسكر، المحتوى الكلى للبروتين وال DNA في قشرة الكلى وذلك مقارنة بالمجموعة الضابطة، ولكن لوحظت زيادة واضحة في بروتين الأميلويد في قشرة الكلى في المجموعات المعاملة بالكاريزوبرودول مقارنة بالمجموعة الضابطة.

**الخلاصة:** إن استخدام عقار الكاريزوبرودول أثناء الحمل يؤدي إلى مخاطر وإلى تغييرات عديدة في نسيج الكلى للأمهات والأجنة.