

# Histological and Histochemical Studies on Kidneys of the Pregnant Rats and Their Foetuses under the Effect of Buspirone Hydrochloride

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## ABSTRACT

**Background:** anxiety is a powerful central nervous system depression that can slow normal brain function. Buspirone is often prescribed to reduce the feelings of tension; this drug is also known as sedative drug.

**Aim of the work:** this study aimed to detect the effect of buspirone hydrochloride on kidney of the pregnant rats and their foetuses (histological and histochemical studies).

**Material and methods:** the current study was applied on thirty pregnant female rats that were categorized into three groups (ten pregnant rats in each group): Group I (the control pregnant rats that were orally administrated with distilled water, group II (pregnant rats were given oral dosage of buspirone hydrochloride at dose level of 0.27 mg /100 g body weight /day daily for 15 days from the 6<sup>th</sup> day to the 20<sup>th</sup> day of gestation and group III (pregnant rats were treated with buspirone hydrochloride at a dose level of 0.41 mg/100 gm body weight/day for 15 days from the 6<sup>th</sup> to the 20<sup>th</sup> day of gestation. Kidney tissues were taken from pregnant rats and picked out from their foetuses of all groups which killed on the 20<sup>th</sup> day of gestation for the histological and histochemical studies.

**Results:** maternal and foetal kidney tissues of both treated groups showed numerous changes post-treatment with buspirone that well-marked at the high concentration dose.

**Conclusion:** the present study showed that administration of Buspirone drug resulted in several histological and histochemical alterations in the kidney tissues.

**Keywords:** Pregnant, Foetuses, Buspirone, Histological and Histochemical Changes, Kidney.

## INTRODUCTION

Anxiety is a powerful central nervous system depression that can slow normal brain function. Buspirone is often prescribed to reduce the feelings of tension; this drug is also known as sedative drug <sup>(1)</sup>. Anxiety becomes a disorder when the individual experienced regularly and refers to specific psychiatric disorders that involve extreme fear or worry and includes generalized anxiety disorder, panic disorder and panic attacks, agoraphobia, social anxiety disorder, selective mutism, separation anxiety and specific phobias <sup>(2)</sup>.

A number of psychoactive agents are currently available for treatment of anxiety. The source of anxious behaviour is usually undefined or unknown <sup>(1)</sup>. In contrast to normal adaptive anxiety, anxiety disorders affect the individual performance of daily life tasks<sup>(3)</sup> representing a high cost for public health care all over the world <sup>(4-5)</sup>.

Buspirone is a good novel anti-anxiety drug which has neither a benzodiazepine structure nor any other benzodiazepine-like properties. Since buspirone is known to block dopamine auto receptors and increase dopamine metabolism, it was compared to classical antipsychotic drugs for sub chronic and chronic effects on dopaminergic function <sup>(6)</sup>.

This study aimed to detect the effect of buspirone on kidney of the pregnant female rats and their foetuses (histologically and histochemically).

## MATERIAL and METHODS

### Drug:

Buspirone hydrochloride was taken as tablets from Beecham, Haram, Giza, Egypt. The drug was dissolved in the distilled water and given orally by a

gastric tube. The daily single oral dose was 0.27 mg and 0.41 mg/100 g body weight/day, respectively. The dose for rats was calculated according to the method **Paget and Barnes** formula on the basis of the human dose <sup>(7)</sup>.

### Animals:

Adult Albino rats (Sprague Dawely strain) were used in this experiment with average weight from 150 to 200 g. They were taken from the animal house of EL-Nasr pharmaceutical chemicals company. Rats were caged separately, males in cages and females in another cages and fed on an ordinary diet. Adult females and males were mated in the proportion of two females for one male overnight. Each morning a vaginal smear was taken to check for the presence of vaginal plug. Zero day of pregnancy was considered to be the day on which sperms or plugs were found in the vagina.

### The experimental design:

Thirty pregnant female rats were categorized into 3 groups (Ten pregnant female rats in each group):

**Group 1 (control):** pregnant healthy normal rats administrated with distilled water.

**Group 2 (low dose B1):** pregnant rats were treated oral dose of buspirone hydrochloride equivalent to 0.27 mg /100gm body weight/day for 15 days from the 6<sup>th</sup> day to the 20<sup>th</sup> day of gestation.

**Group 3 (high dose B2):** pregnant female rats were treated with oral dose of buspirone hydrochloride equivalent to 0.41 mg/100g body weight/day for 15 days from the 6<sup>th</sup> day to the 20<sup>th</sup> day of gestation.

### The histopathological and histochemical studies:

All the pregnant rats were sacrificed on the 20<sup>th</sup> day of gestation after 4 hours from the last dose administration and small samples of kidney tissues from mothers and their foetuses were picked out for the histological and histochemical studies.

Sections of kidney tissues from mother and their foetuses placed in 10% neutral buffer formol and Bouin's solution then, washed, dehydrated in ascending concentrations of alcohol, embedded in melted paraffin wax, sectioned by microtome at 4  $\mu$ m and mounted by DPX. The slides were stained by haematoxylin and eosin (Hx and E) according to method of **Bancroft and Gamble** <sup>(8)</sup>.

Collagen fibres were detected by Mallory trichrome stain <sup>(9)</sup>. Total protein was detected by using mercury bromophenol blue method <sup>(10)</sup>; polysaccharides were detected by using PAS method <sup>(11)</sup> and amyloid  $\beta$  protein was detected by Congo red technique <sup>(12)</sup>.

#### **Ethical approval:**

**The study was approved by the Ethics Board of Al-Azhar University. This study was conducted in accordance with ethical procedures and policies approved by Animal Care and Use Committee of Faculty of science, Al-Azhar University, Cairo, Egypt.**

#### **Statistical analysis**

The current results were expressed as mean  $\pm$ SE. Data were analyzed by using the statistical package (SPSS) program. Significant differences between the treatment means were determined by student T-test. The data were presented as mean  $\pm$  SE and  $P \leq 0.05$  was considered statistically significant.

Image analysis: the optical density (pexil) of the kidney tissue in the current study was calculated by using image pro. Program.

## **RESULTS**

### **Kidney of pregnant rats:**

#### **The histopathological observations:**

Normal structure of kidney cortex of a pregnant rat was detected in figs. 1A&1B. Most glomeruli were

normal; both parietal and visceral epithelium appeared clearly with normal proximal and distal convoluted tubules.

The pregnant rats of group B1 showed distension of glomerular capsular space, degeneration glomerular tuft, some congested, lobulated or atrophied glomeruli with pyknotic nuclei (Figs. 1: C&D), while the pregnant rats of group B2 group showed degenerated proximal and distal convoluted tubules, congested blood vessels, bleeding, edema and atrophoid glomeruli(Figs. 1 E&F).

Mallory's trichrome stained sections of pregnant female kidney cortex of the control group showed well distribution of collagen fibres (Fig. 2A&B); they were distributed in walls of the blood vessels , proximal convoluted tubules and in between them while, treatment with the low dose (B1) and high dose (B2) of buspirone hydrochloride showed increased collagen fibres in the brush borders of the proximal convoluted tubules and basement membranes of the distal convoluted tubules with congested blood vessels(haemorrhagic area ) and fibrosis of arterial wall (Figs. 2 C&D).

#### **The histochemical observations:**

In pregnant rats of the control group cubic epithelial cells of the proximal tubules showed positive reaction of PAS stain (Fig.3A) while, in B1 and B2 slightly decreased polysaccharides was realized ( Fig. 3 B&C, table 1, histogram 1)

Kidney tissue of rats of the control group showed a moderate distribution of total protein content in the glomeruli, proximal and distal convoluted tubules (Fig. 4A, table 1, histogram1).

On other hand, examination of the kidney cortex of pregnant rats of groups B1 and B2 showed a decreased total protein (Figs. 4 B&C, table 1, histogram 2).Kidney cortex of pregnant rats of the control group showed a slight deposition of amyloid  $\beta$  in kidney tissue (Fig. 5A, table 1, histogram1). However, examination of the kidney cortex of the pregnant rats of group B1 and B2 showed increased deposition of amyloid  $\beta$  protein (Figs.5 B& C, table 1, histogram 1).

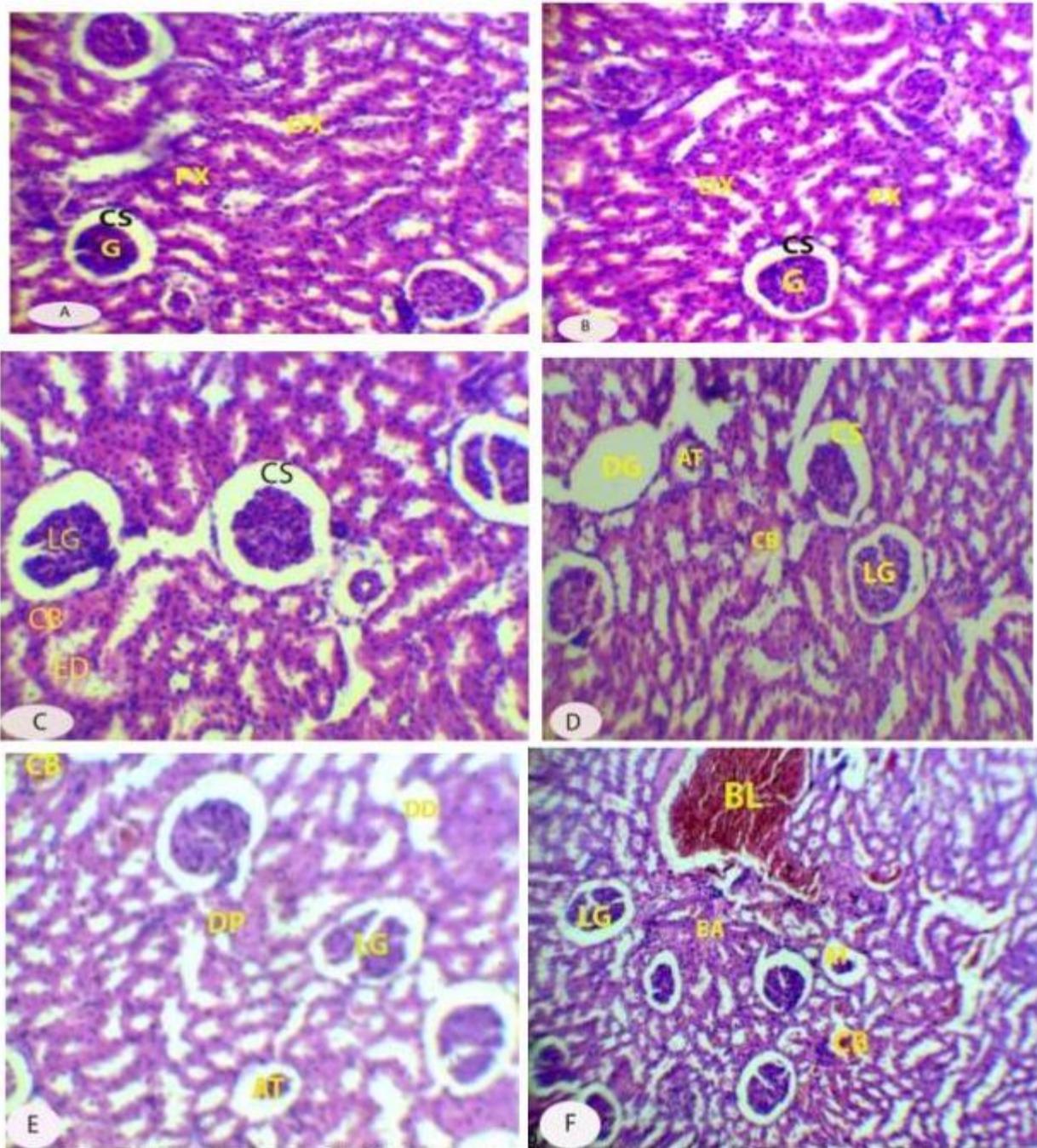


Fig 1(A-F): photomicrographs of sections of kidney cortex of pregnant control and treated rats stained with haematoxylin and eosin –

A-B: control pregnant rats (group1) showing the normal structure of glomerulus (G), proximal convoluted tubules (PX) , glomerular capsular space (CS) and distal convoluted tubules (DX). (X250)

C-D: kidney cortex of rats of B1 group showing many alterations such as dilation of glomerular capsular spaces (CS), congested blood vessels (CB), lobulation of glomerulus (LG), edema (ED), atrophoid glomeruli (AT) and degenerated glomeruli (DG). (X250)

E- F: kidney cortex of rats of B2 group, showing bleeding (BL), lobulation of glomerulus (LG), degenerated of proximal (DP) and distal convoluted tubules (DD), atrophoid glomeruli (AT),congested blood vessels (CB) and edema (EA) (X250) .

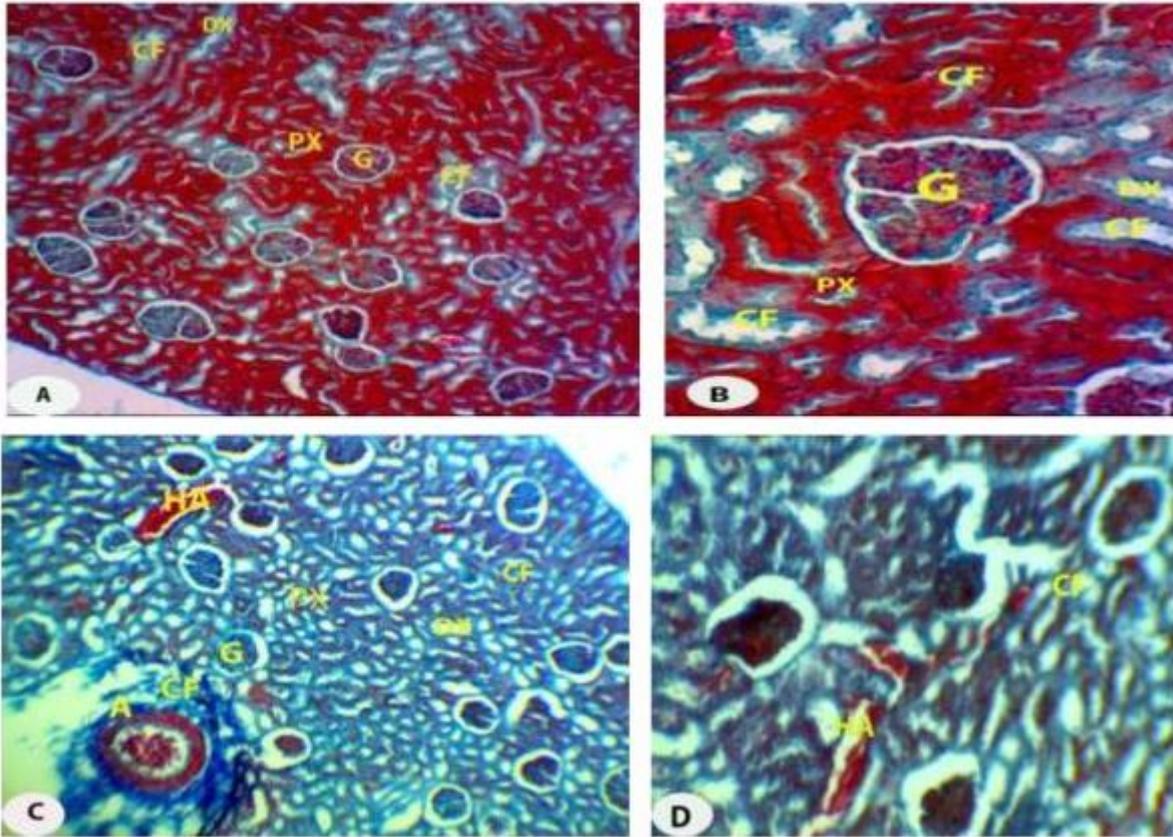


Fig.2 (A-D): photomicrographs of sections of kidney cortex of the control pregnant and treated rats stained with Mallory's trichrome stain

A:-B control rat (group1) showing normal distribution of collagen fibres in Bowman's capsule (CF) in the glomerulus (G) , brush borders of the proximal convoluted tubules(PX) and basement membranes of the distal convoluted tubules(DX) . (X100 &400).

C: treated group B1 showing increased collagen fibres (CF) in brush border of proximal convoluted tubules(PX) and basement membranes of the distal convoluted tubules(DX) with brightly red stained haemorrhagic areas (HA).The arterial wall shows fibrosis(A). (X200).

D: group B2 showing highly increased collagen fibres (CF) with brightly red stained haemorrhagic areas (HA). (X250)

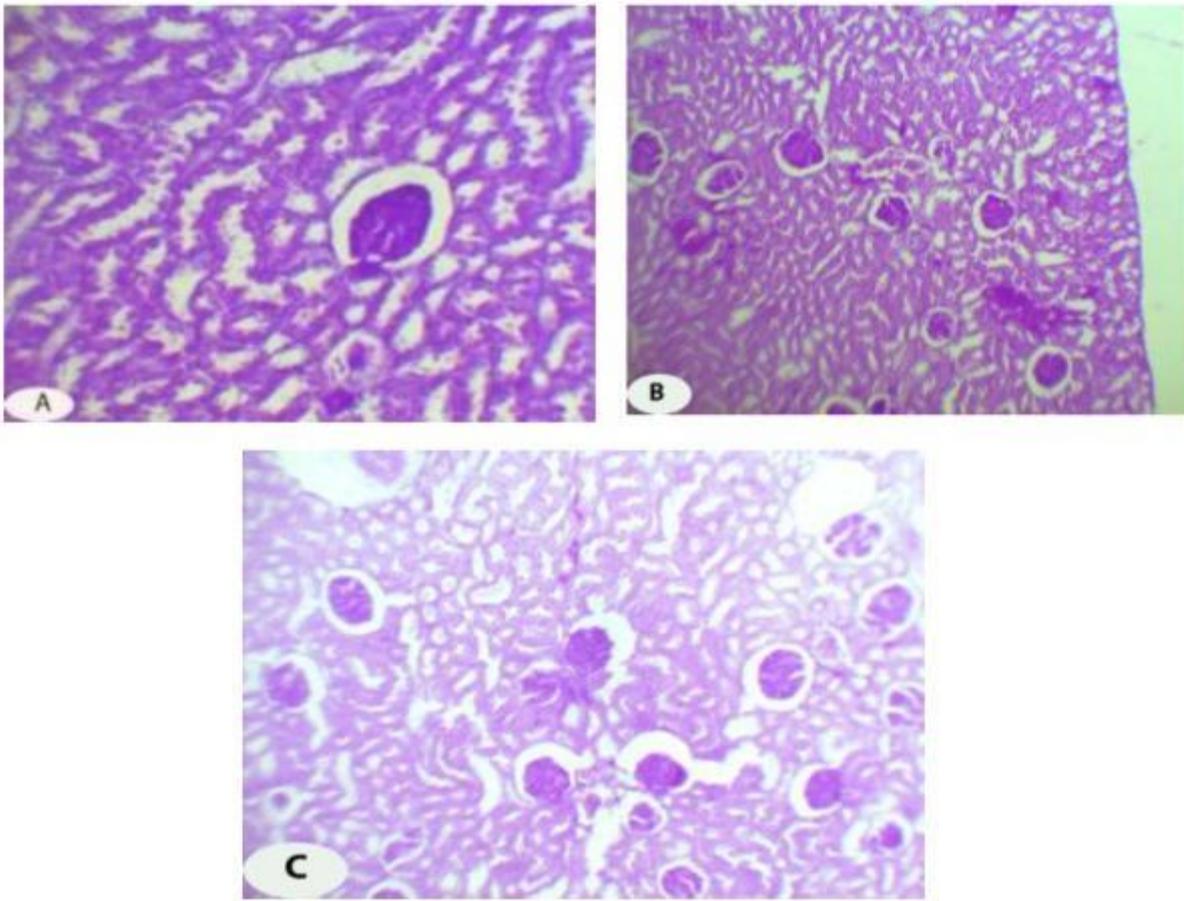


Fig.3 (A-C): photomicrographs of sections of kidney cortex of pregnant control and treated rats stained with PAS for detecting polysaccharides

A: Control rat (group1) showing a normal distribution of polysaccharides. (X250)

B- C: Low dose (B1) and high dose (B2) of buspirone showing decreased carbohydrates. (X200).

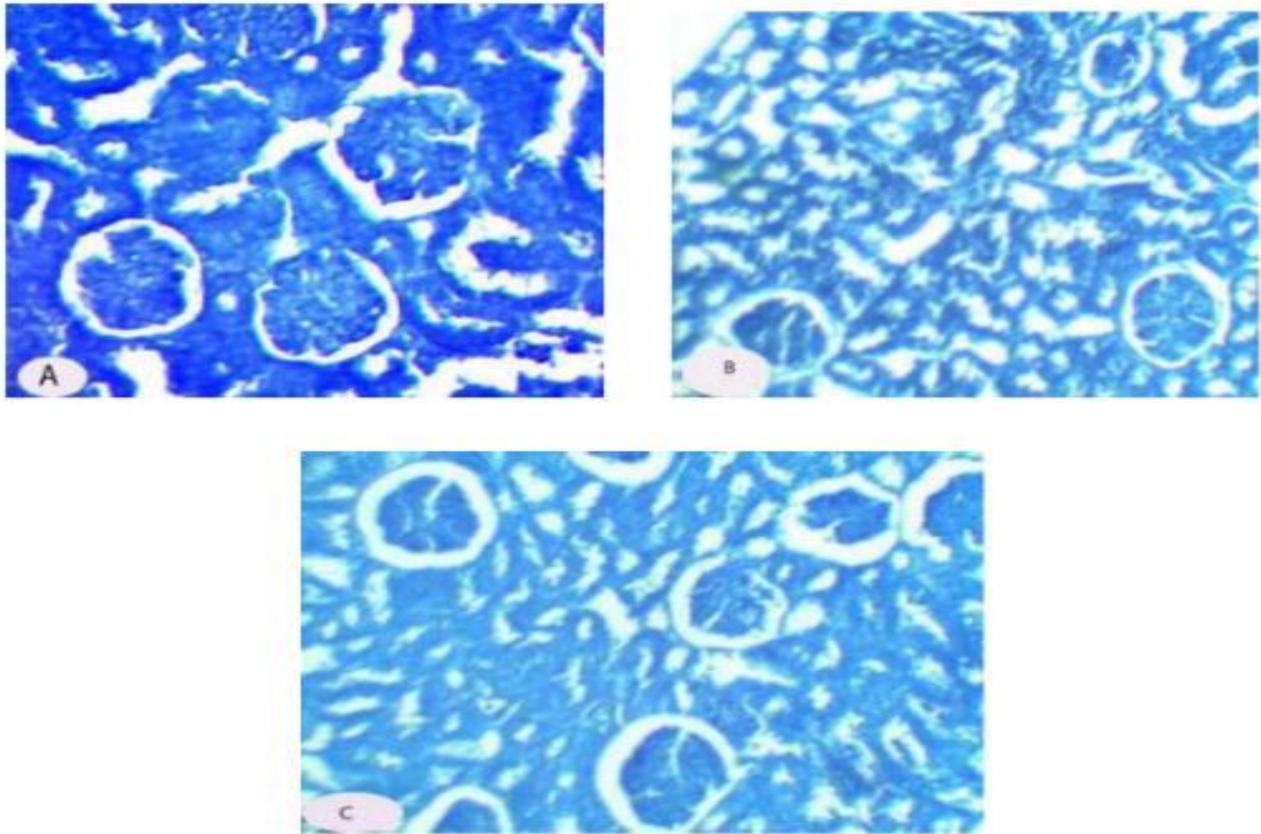


Fig.4(A- C): photomicrographs of sections of kidney cortex of the control and treated pregnant rats stained with mercury bromophenol blue for detecting total proteins

A: Control rats (group1) showing normal distribution of protein in the cortical region. (X250)

B- C: Low dose B1 (group2) and high dose B2 (group 3) showing decreased distribution of total protein in the cortical region. (X250)

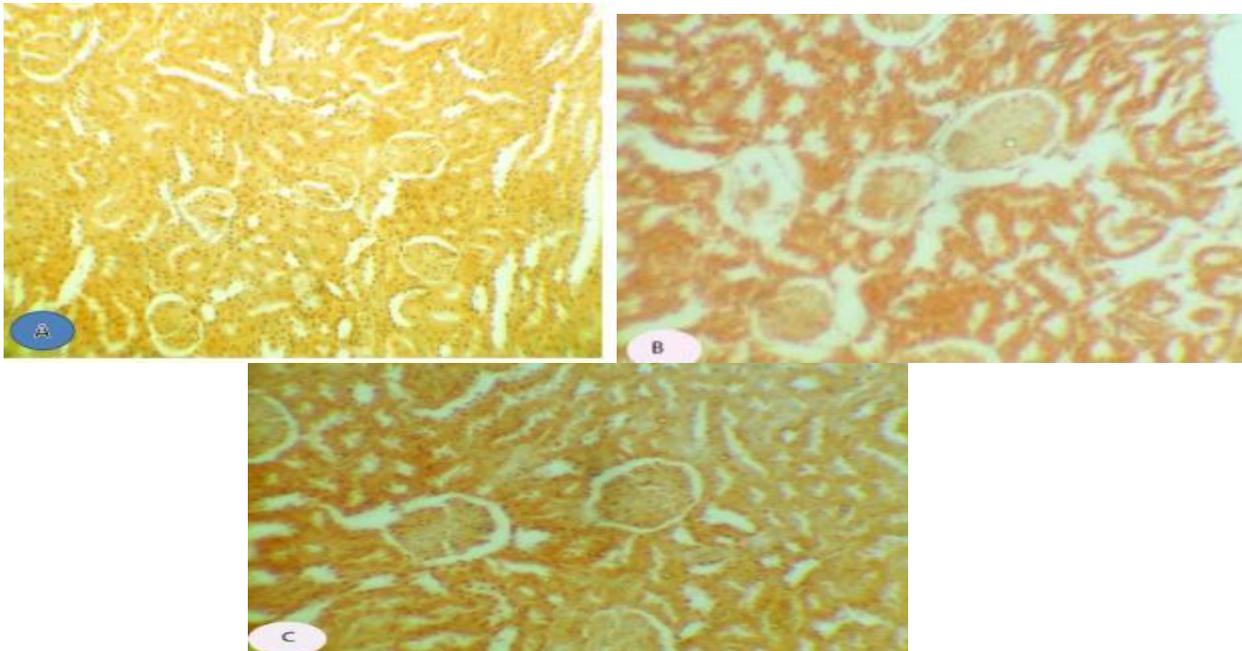


Fig.5 (A-C): photomicrographs of sections of kidney cortex of pregnant rats stained by Congo red for detecting amyloid  $\beta$  protein

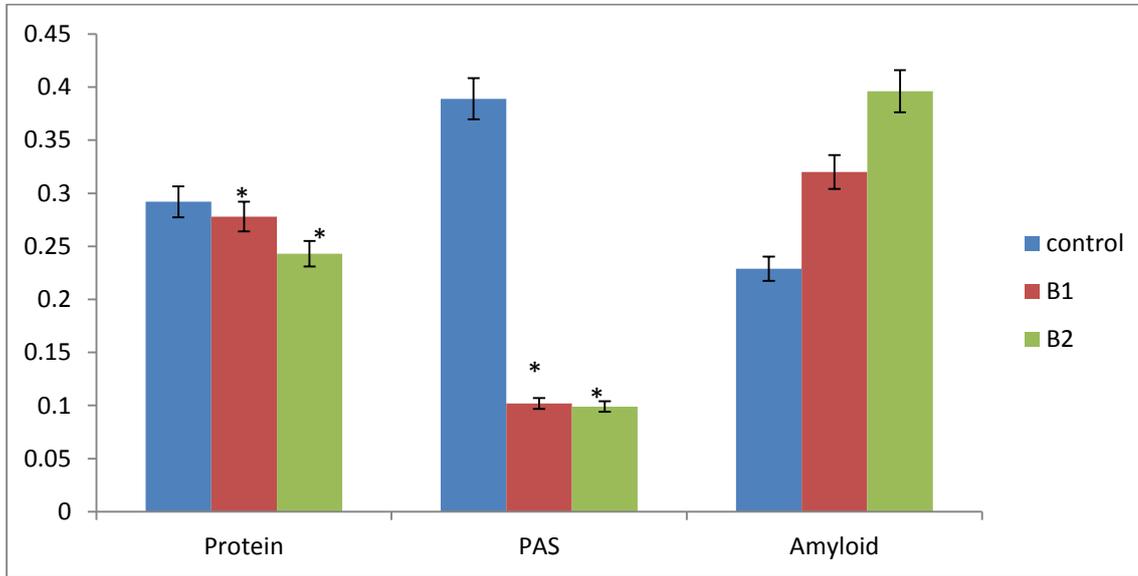
A: control rats (group1) showing a slight deposition of amyloid  $\beta$  protein in cortex region (X 250).

B- C: low dose B1 (group2) and high dose B2 (group3) showing increased deposition of amyloid  $\beta$  protein in the kidney cortex (X250).

**Table 1: showing the optical density of total proteins, polysaccharides (PAS) and amyloid  $\beta$  protein in the kidney cortex of female rats between control pregnant rats and treated groups**

Groups	Total proteins Mean $\pm$ Standard error	PAS (polysaccharides) Mean $\pm$ Standard error	Amyloid $\beta$ protein Mean $\pm$ Standard error
Control	0.2925 $\pm$ 0.0045	0.3847 $\pm$ 0.00713	0.2297 $\pm$ 0.00542
B1	0.2784 $\pm$ 0.00249	0.1029 $\pm$ 0.0038	0.3203 $\pm$ 0.0124
B2	0.2433 $\pm$ 0.00408	0.0994 $\pm$ 0.030	0.396 $\pm$ 0.0127

The values are considered significant at  $p \leq 0.05$ , data are presented as means  $\pm$  Standard error



**Histogram 1:** showing the optical density of total protein, polysaccharides (PAS) and amyloid  $\beta$  protein in the kidney cortex of the control and treated pregnant rats

**Kidney of foetuses:**

**The histopathological observations:**

Fig. 6A showed histological structure of foetal kidney cortex of a control foetus with normal glomeruli, proximal and distal convoluted tubules.

The kidney cortex of foetal rats of group B1 showed degeneration of the distal convoluted tubules, reduced number of proximal convoluted tubules, necrotic area and atrophy of glomeruli (Figs. 6 B-C). On other hand, the foetal kidney of group B2 showed congested blood vessels, hemorrhagic areas, degeneration and atrophy of some glomeruli, necrotic area and degeneration of distal convoluted tubules (Figs 6D - E). Mallory's trichrome stained sections of foetal rat kidney cortex of the control group showed normal distribution of collagen fibres (Fig. 7A), while in low dose (B1) and high dose (B2) of buspirone hydrochloride showed increased collagen fibres in the cortex region (Figs.7 B&C).

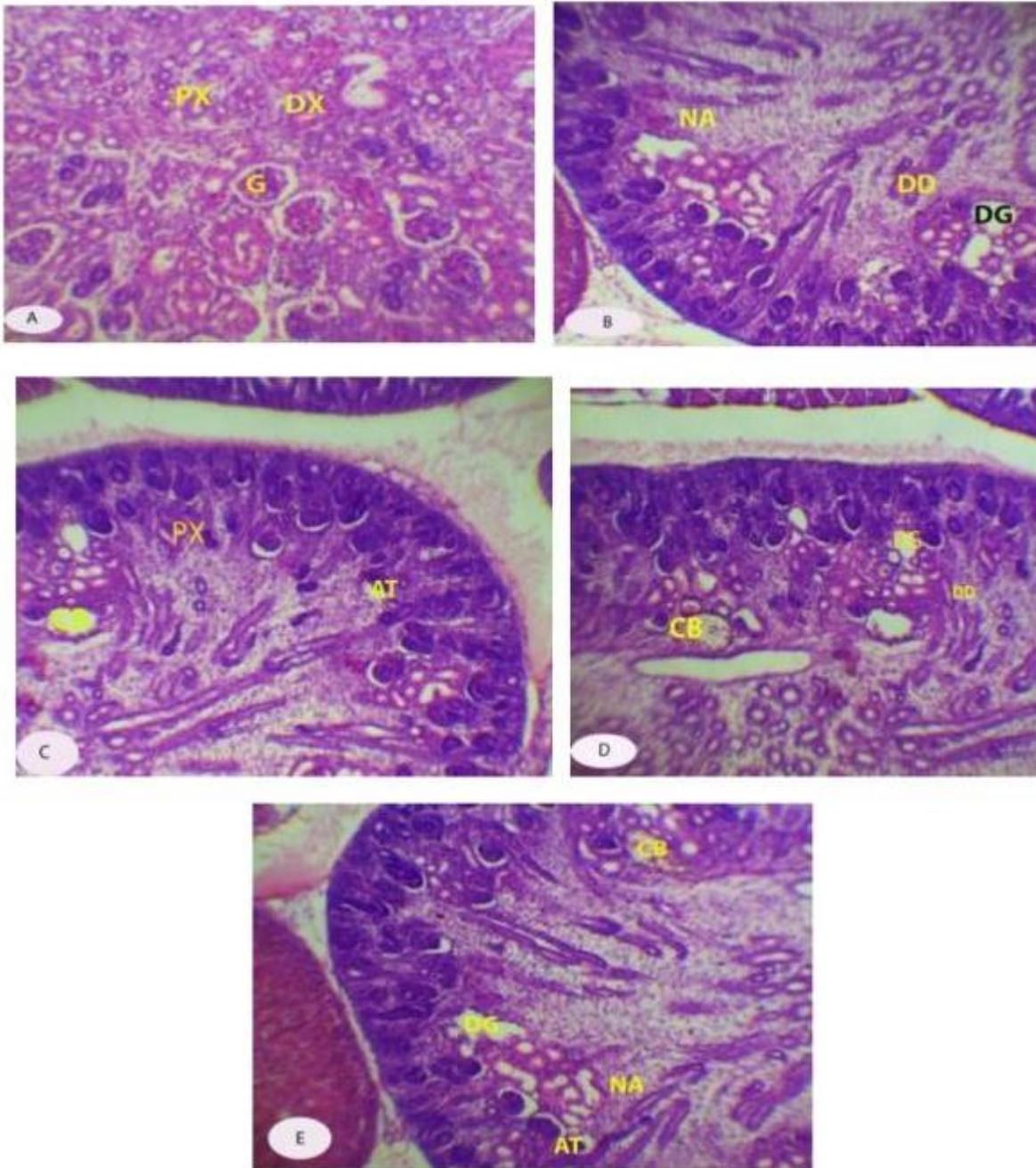


Fig. 6 (A-E): photomicrographs of sections of foetal kidney haematoxylin and eosin

A: control rat showing normal structure of glomerulus(G), proximal(PX) and distal convoluted tubules(DX). (X200)

B - C: low dose B1 showing degeneration of the distal convoluted tubules (DD), reduced number of the proximal convoluted tubules (PX), necrotic area (NA) , degenerated glomeruli (DG), atrophy of glomeruli(AT) and congested blood vessels (CB) . (X200).

D - E: high dose B2 showing congested blood areas (CB), , necrotic area (NA),degeneration of distal convoluted tubules (DD) degeneration of glomeruli (DG) and atrophy glomeruli (AT) . (X200)

**The histochemical observations:**

In foetal kidney tissue of the control group stained with PAS showed polysaccharides with normal magenta colour (Fig.8A,table 2&hist. 2).

On the other hand , examination of foetal kidney cortex of groups B1 and B2 indicated slightly decreased polysaccharides (Fig. 8 B- C, table 2, histogram 2).Kidney tissue of rats of the control group showed moderate distribution of total protein content in glomeruli ,proximal and distal convoluted tubules (Fig. 9A; table 2, histogram 2). On other hand, examination of foetal kidney cortex of groups B1 and B2 showed decreased density of total proteins (Fig. 9 B- C, table 2, histogram 2).

Foetal kidney cortex of the control group showed a slight deposition of amyloid beta protein (Fig. 10A table2&hist2). On other hand, examination of foetal kidney cortex of groups B1 and B2 showed increased amyloid beta protein (Fig.10 B& C, table 2, histogram 2).

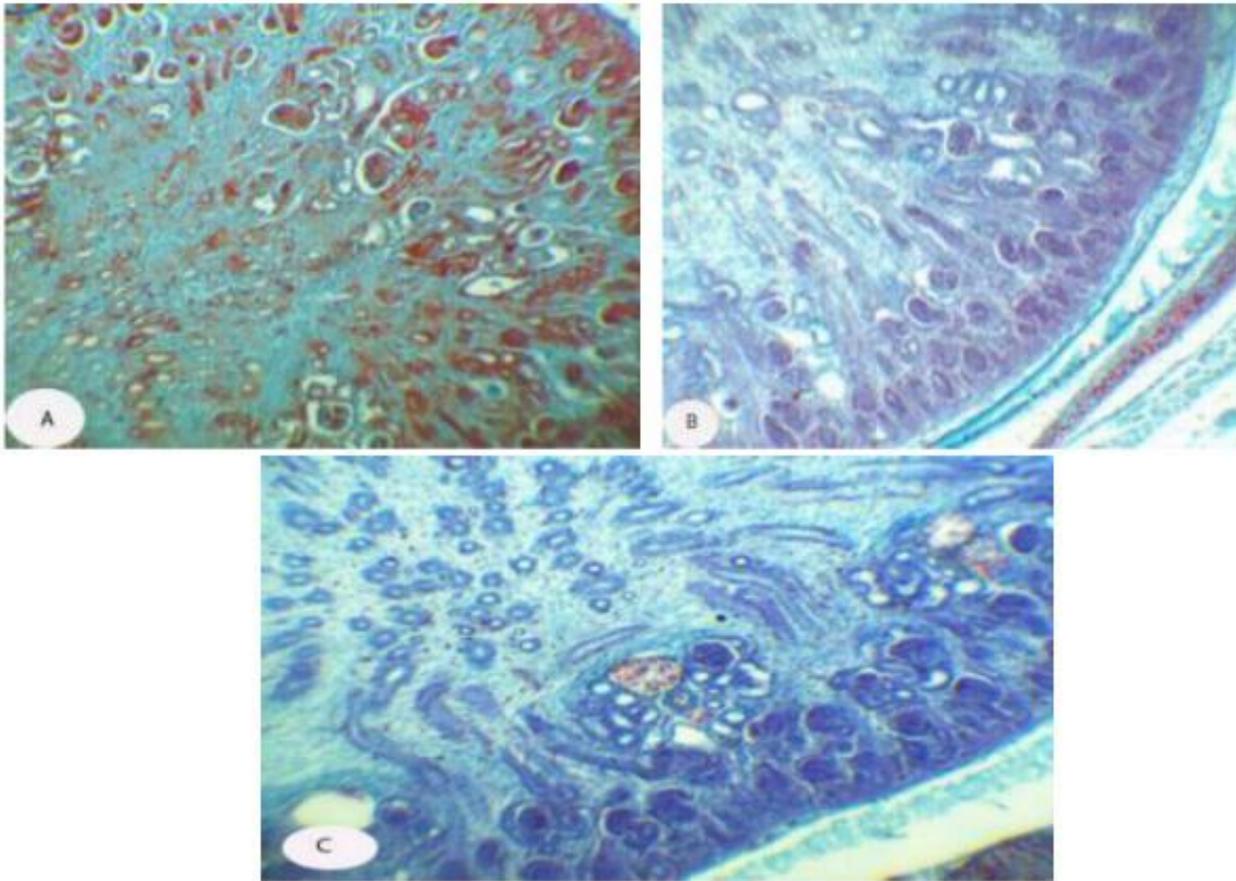


Fig.7( A-C) : photomicrographs of foetal kidney of the control and treated groups stained with Mallory's trichome stain.

A: control rats showing normal distribution of collagen fibres in the proximal and distal convoluted tubules . (X200)

B& C: low dose (B1) and high dose ( B2 ) showing moderate distribution of collagen fibres in the cortex region. (X200)

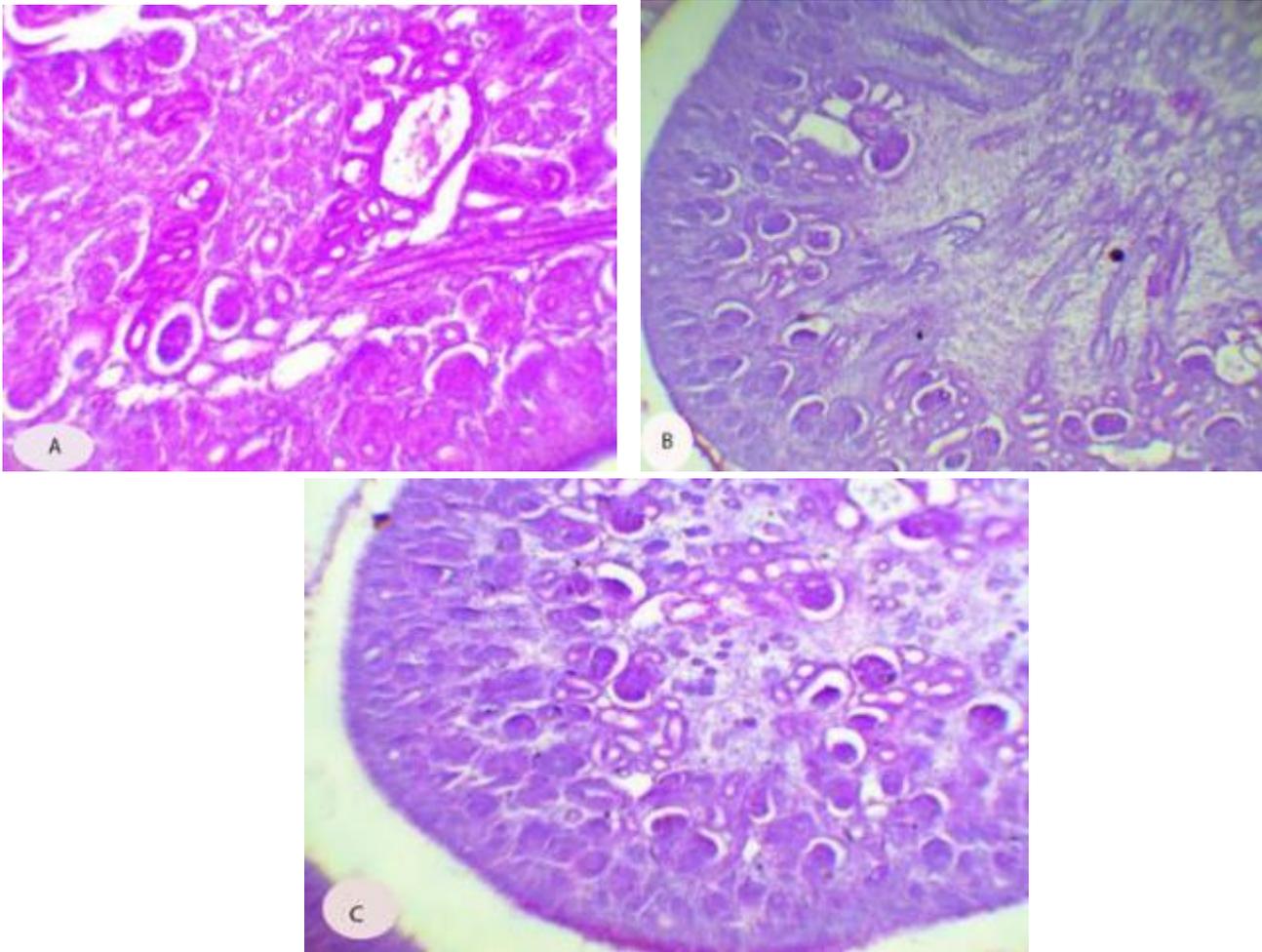


Fig. 8 (A-C): photomicrographs of sections of foetal kidney of the control and treated groups stained with PAS for detecting polysaccharides

A: control rat showing normal distribution of polysaccharides in the cortex region. (X200)

B- C: low dose B1 and high dose B2 showing reduced polysaccharides in the cortex region. (X200)

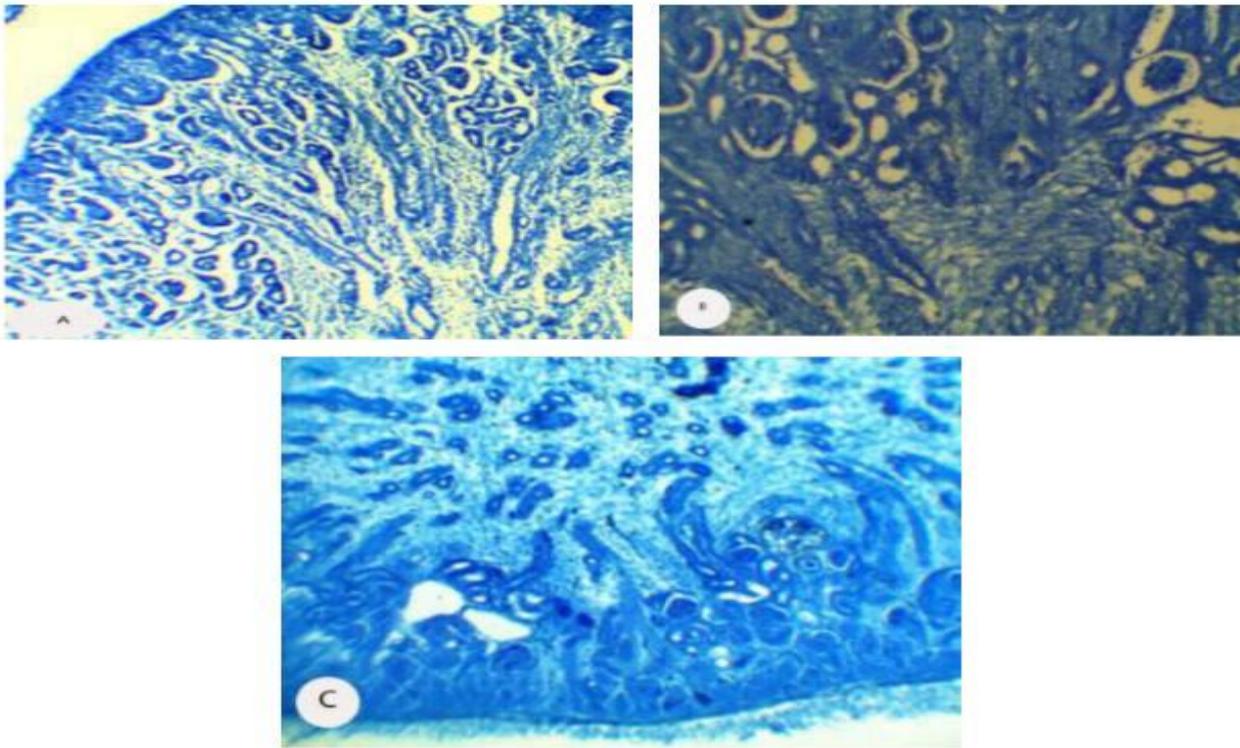


Fig. 9 (A-C): photomicrographs of sections of foetal kidney cortex of the control and treated groups stained with mercury bromophenol blue for detecting total proteins.

A: control foetus rat (group1) showing normal distribution of total protein in the cortex region. (X200)

B & C: low dose B1 and high dose B2 showing decreased total protein in the cortex region. (X200)

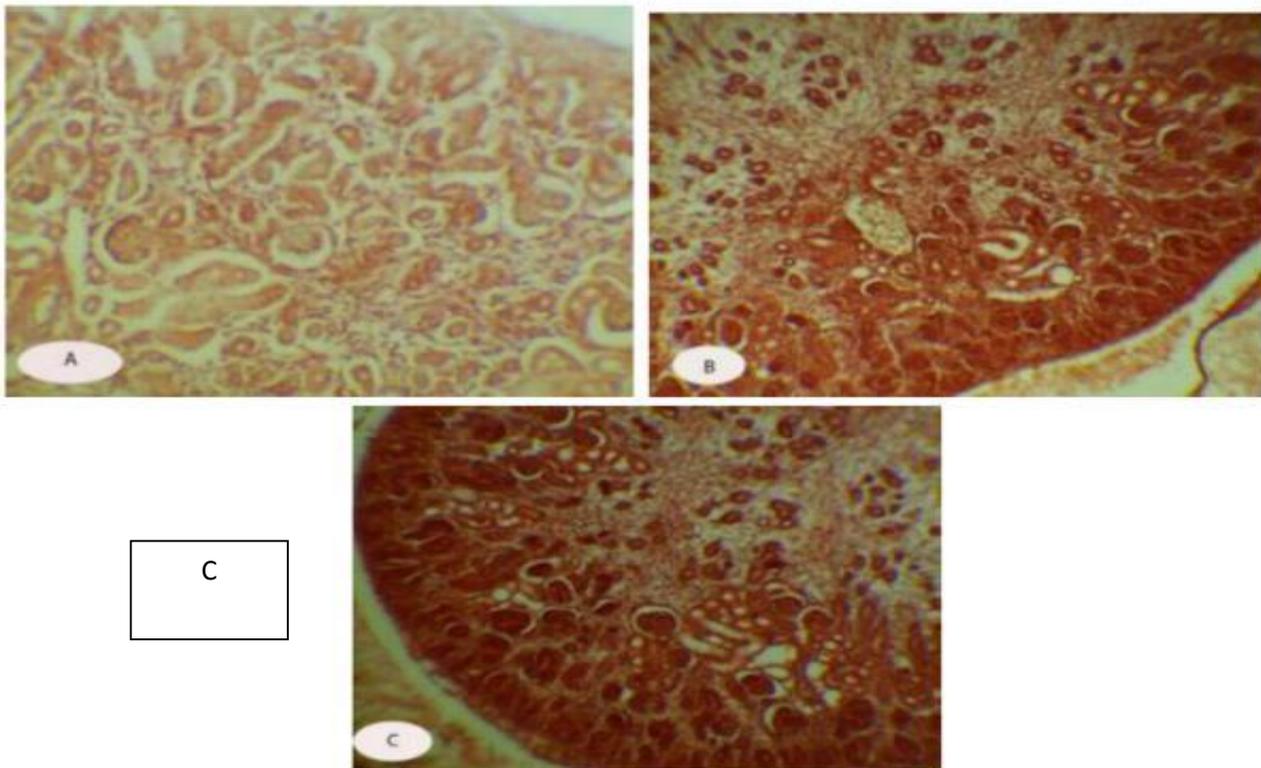


Fig. 10(A-C): photomicrographs of sections of foetal kidney of the control and treated groups stained with Congo red for detecting amyloid  $\beta$  protein

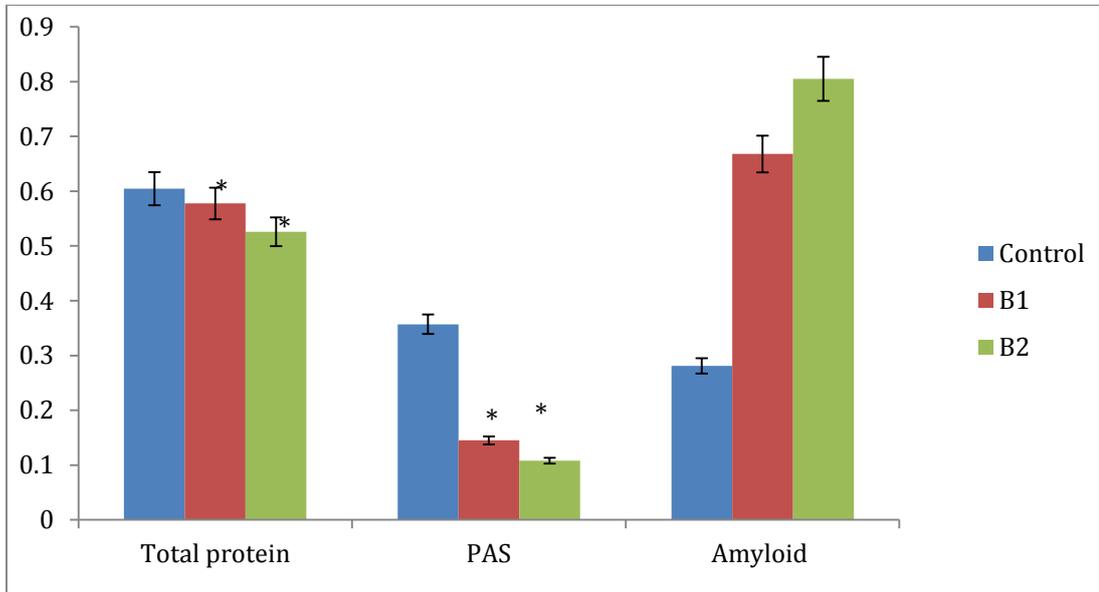
A: control rat showing faintly stained amyloid  $\beta$  protein in the cortex region. (X200)

B&C: Low dose B1 and high dose B2 showing increased deposits of amyloid  $\beta$  protein in the cortex region. ( X200 )

**Table 2: the optical density of total proteins, PAS + ve materials and amyloid β protein in the foetal kidney cortex of the control and the different treated experimental groups**

Groups	Total proteins Mean ±Standard error	PAS +ve materials Mean ±Standard error	Amyloid β proteins Mean ±Standard error
Control	0.6047±0.01078	0.3538±0.00439	0.281±0.004
B1	0.5777±0.00447	0.1450±0.00208	0.668±0.0154
B2	0.5265±0.0164	0.108±0.00206	0.805±0.0113

The values are considered significant at p ≤ 0.05, data are presented as means ± Standard error



**Histogram 2:** the optical density of total protein, PAS +ve materials and amyloid β protein in the foetal kidney cortex of the control and different treated experimental groups

**DISCUSSION**

Anxiety and fear are normal emotions with a great adaptive value that have been selected along the evolutionary process; in addition fear occurs in response to specific threats. These disorders which are associated with anxiety are mostly increased in women during pregnancy due to changes in the levels of progesterone and oestrogen in the blood level (13).

In the present study, treatment of pregnant rats and their foetuses with buspirone hydrochloride in a dose of 0.27 mg /100g and 0.41 mg/100 g body weight /day respectively for 15 days from the 6<sup>th</sup> day to the 20<sup>th</sup> day of gestation showed some histological changes in the cortical region in the mothers and their foetuses which were represented by dilation of urinary spaces, congested blood vessels, degeneration of some of glomeruli, necrotic area, lobulation of some glomeruli and atrophied glomeruli. Histochemical observation showed decreased PAS +ve materials in low and high treated groups in mothers and their foetuse; such decrease could be attributed to glucose uptake (14). These results are supported by the present study which showed a slight decrease in the PAS+ve materials as compared to the control ones. The decreased of total protein and increased deposition of amyloid β protein reflected the dangerous effects of buspirone (14).The decreased total protein and increased

amyloid β protein was also noticed by **Mello et al.** (15) and **Bari et al.** (16).

Such decrease may be due to the histopathological changes observed in the different regions of the cortical region in the maternal and foetal kidney cortex.

**Zaki and Abouel-Magd** (17), showed that exposure of pregnant rats to buspirone hydrochloride in the low and high doses led to some dystrophic changes in the Purkinje cells layer represented by decreased number of degenerated and accumulated granular layer cells with numerous oedematous areas in the cerebellum.

**Khana et al.** (18) declared that the decreased collagen lytic enzymes synthesis by the impaired cells could be contributed to accumulation of collagen fibres and to the distributed histochemical pattern of polysaccharides. **El-shaer and abd El-Aziz** (19) showed partial failure of development of some acini of pancreas beside degenerative and necrotic changes in some cells after administration of a low and high dose of buspirone in pregnant female rats. **Kadota et al.** (20) showed a destination of the stomach in rats treated with buspirone hydrochloride orally and hyper-secretion of gastric juice and numerous alterations.

## CONCLUSION

Administration of buspirone hydrochloride for long duration resulted in histological and histochemical alterations as evident by deformities of maternal kidney and their fetuses and remarkably increased amyloid  $\beta$  protein.

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