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EFFECTS OF UROGRAFIN ON THE MORPHOLOGY OF KIDNEY CELLS OF ADULT WISTAR RATS

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This study aimed at assessing the effects of urografinon the morphology of kidney cells of the Wistar rat. Thirty (30) Wistar rats weighing between 182-212Kg body weights were grouped into three groups of ten animals per group. Group A was used as the control, while group B and C were experimental groups. The experimental groups were administered low and high dosage of urografin (diatrizoate) being the Radiographic Contrast Medium (RCM) used for the experiment. The collection of tissue samples from all three groups was carried out simultaneously at intervals of 30 minutes, 60 minutes, 2 hours, 12 hours and 24 hours after administration of calculated doses of the RCM to the experimental groups. The result obtained showed marked alteration of kidney cells morphology from 30 minutes to 24 hours post administration of both low and high dosages of urografin. The effects of urografin (ionic RCM) on kidney cells morphology is persistent compared to results obtained for ultravist (non-ionic RCM).

Keywords: Hypercellular mesangium, Kidney, Urografin, Wistar rats.

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INTRODUCTION

Urografin (diatrizoate) is a water-soluble, organic, ionic Radiographic Contrast Medium (RCM) suitable for intravenous administration. It is quickly distributed in the extracellular space following intravascular administration, but will not cross an intact blood-brain barrier. It is used as a RCM for Intravenous Urography (IVU), Hysterosalpingography (HSG), Retrograde Urography, Angiography, Arthrography, Intraoperative Cholangiography, and Endoscopic Retrograde Cholangio Pancreatography (ERCP) among other investigations (Armstrong & Wastie, 2001). A RCM is a substance introduced into the body in order to make an organ, surface of an organ, or the lumen of an organ visible on the radiograph. Studies on the structure, development, osmolality, risk and safety of RCM are well documented (Rigler, 1967, Spect, 1993, Thomsen& Marcos, 2000; Esplugas*et al*, 2002; Morcos, 2003; Marshall, 2006_a; Caca *et al*, 2007; Becker, 2007, Cutroneo *et al*, 2007; Ikamaise*et al*, 2015).The kidneys are bean-shaped organs located at the retroperitoneal space. The kidneys perform homeostatic functions by regulating electrolytes, maintaining acid-base balance and regulating blood pressure (via maintaining salt and water balance). Kidneys are body's natural filter of the blood by removing water soluble wastes which are diverted to the bladder. In producing urine, the kidneys excrete waste such as urea and ammonium, and they are also responsible for the re-absorption of water, glucose and amino acids. Kidneys in addition produce hormones, namely; calatriol, erythropoeitin and enzyme renin that perform important hormonal and enzymatic regulatory roles in the body (Cotran*et al*, 2005). Injected RCM is eliminated by glomerular filtration and is concentrated in the urine by tubular reabsorption of water. About 83% of the injected dose of RCM is eliminated from the body by six hours without any metabolism of the molecules. Despite the fact that there is no metabolism and marked protein binding, RCM has a certain degree of nephrotoxicity (Berg *et al*, 1958; Junqueira et al, 1986; Katayama et al, 1990; Shellock&Kunal, 1999).Marshall (2006_b) reported that ideally, RCM should only alter the linear attenuation coefficient in the area to be imaged while remaining biologically inert. However, in reality because it differs in osmolality and ionic charge, a variety of physiological concerns result, which affects the cardiovascular and renal systems. Acute renal failure (ARF) occurs in less than 72 hours following injection of RCM. This is as a result of increase in creatinine levels which comes to its peak within the 48 hours and then starts to decrease daily with a complete recovery in 10-14 days. As the use of RCM increases with higher volumes and concentrations in critically ill patients, RCM induced nephrotoxicity is a growing and disturbing crisis. Medical Radiographers are by role extension performing what were previously designated medical tasks such as intravenous injections of RCM. Consequently, there

is need for Radiographers to receive more information regarding these agents to fully understand the characteristics of the agent they inject and possible effects of these substances. The study was accordingly designed to undertake a direct assessment of the effect of RCM at the cellular level by establishing the histological patterns of post RCM injection on kidney of the Wistar rats. The collection of tissue samples was carried out at intervals of 30 minutes, 60 minutes, 2 hours, 12 hours and 24 hours after administration of calculated doses of the RCM (urografin)

METHODOLOGY

The experiment was carried out using Thirty (30) Wistar rats and two brands of RCM namely; ultravist (iopromide) and urografin (diatrizoate). The grouping, identification and treatment of the rats, the collection of tissue samples at intervals of 30 minutes, 60 minutes, 2 hours, 12 hours and 24 hours after administration of calculated doses of the RCM (urografin) was reported in Ikamaise *et al*, (2015).
The dose of radiographic contrast medium for each rat was calculated using this equation:

$$\textbf{Dosage} = \frac{Vc \times Wr}{Wman}$$

where V_c= the volume of contrast
W_r =Weight of rat
W_{man} =weight of standard physiological man (70kg)

The doses of 60ml and 100ml on a physiological 70kg man are considered low and high doses in conventional radiographic contrast medium administration. Therefore, volumes of 60ml and 100ml respectively was used for the calculation of lo dose and high dose for the rats (table 1)

The rats were sacrificed by cervical dislocation and placed on a dissecting board in a supine position for laparotomy in accordance with the Helsinki declaration regarding the use of laboratory animals. The kidneys were removed immediately and fixed in Bouin's fluid after extraction and histological procedures completed as reported in Ikamaise *et al* (2015).The glomerulus, bowman capsule, nucleus, mesangium and the cytoplasm of the kidney cells were observed. Histological changes between the experimental and control groups and between two different brands of RCM on the photomicrographs were noted. The result obtained for utravist was reported in Ikamaise *et al* (2015) while the result for urografin is reported in this article.

RESULTS

Plate 1 showed a photomicrograph of the renal cortex of a control Wistar rat with prominent glomeruli, distinct bowman capsule, prominent cellular mesangium and consists of distinct basophilic nuclei and moderately eosinophilic cytoplasm. The cells are closely packed with sparse interstitium consisting of blood vessels.

Plate 2 showed a photomicrograph of the kidney section at 30 minutes after injection of 0.25 ml (high dose) of urografin. The renal cortical section showed swollen glomerulus with obliterated Bowman's space and hypercellular mesangium.

Plate 3 showed a photomicrograph of the kidney section at 30 minutes after injection of 0.15 ml (low dose) of urografin. The cortical section has swollen glomeruli with loss of Bowman's space and hypercellular mesangium. The renal tubules are lined by mildly swollen epithelial cells with prominent nuclei.

Plate 4 showed a photomicrograph of the kidney section at 60 minutes after injection of 0.30 ml (high dose) ofurografin. The renal cortical section showed swollen glomeruli, loss of Bowman's space, diffused glomerulosclerosis and degenerated cortical renal tubules with distinct cellular outline. The lining cells are swollen with sparse interstitium.

Plate 5 showed a photomicrograph of the kidney section at 60 minutes after injection of 0.15 ml (low dose) of urografin. The section showed mainly cortical tubules lined with epithelium with prominent nuclei. Some cells are moderately swollen. There is a single deeply stained glomerulus with poorly defined Bowman's space which may be due to poor imaging.

Plate 6 showed a photomicrograph of the kidney section at 2hours after injection of 0.30 ml (high dose) of urografin. There are prominent glomeruli with hepercellular mesangium with distinct Bowman's capsule. The glomeruli are swollen. The cortical tubules are moderately swollen. There are prominent epithelial cells which are also swollen.

Plate 7 showed a photomicrograph of the kidney section at 2 hours after injection of 0.18 ml (low dose) of urografin. The section demonstrated atrophic renal tubules and glomeruli which are deeply stained. The renal tubules show distinct outline and also deeply stained nuclei. The mesangium has sparse cells.

Plate 8 showed a photomicrograph of the kidney section at 24 hours after injection of 0.27 ml (high dose) of urografin. The section showed swollen glomeruli with loss of Bowman's capsule and hypercellular mesangium. The tubules are closely packed with distinct cellular outline.

Plate 9 showed a photomicrograph of the kidney section at 24 hours after injection of 0.16 ml (low dose) of urografin. The section showed shrunken glomeruli with increased Bowman's space, hypercellular mesangium and cortical tubules lined by moderately swollen epithelium.

Result obtained at 12 hours interval yielded a diffused image with poor resolution and was omitted from the presentation.

DISCUSSION

The result obtained for urografin (ionic monomer – diatrizoate) 30 minutes to two hours following it administration presented in plate 2 to plate 7 demonstrate severe alterations in the cellular morphology of the kidney. The alteration ranged from complete obliteration of the Bowman capsule, oedematous glomeruli, swollen epithelial cells, loss of Bowman capsule to hypercellular mesangium (proliferation of cells in the mesangium). The implication of this result is that cells with these morphological alterations may not be capable of performing effectively the homeostatic functions of regulating electrolytes, maintaining acid-base balance, regulating blood pressure as well as perform important hormonal and enzymatic regulatory roles in the body (Cotran *et al*, 2005; Ikamaise *et al*, 2015). The consequence of the above may lead to acute renal failure (Marshall, 2006_a). Ueda *et al* (1992) in a study on the influence of contrast media on single nephron glomerular filtration rate in rat kidney reported a decreased in single nephron glomerular filtration.

When this results are compared with results obtained for ultravist at the time intervals of 30 minutes and 60 minutes after contrast administration similar features were observed for high dose of ultravist, while signs of cell recovery were observed at 60 minutes interval for low dose of ultravist (Ikamaise *et al,* 2015).

The results presented in plate 8 and 9 for 24 hours after administration of urografin showed persistent swollen glomeruli and loss of bowman space for high dose and atrophic glomeruli with expanded Bowman's capsule. This shows that the elimination of urografin is not as rapid as that of ultravist (Ikamaise *et al*, 2015). Atrophic glomeruli and expanded Bowman's capsule implies that urografin is capable of permanent damage of the kidney cells. Person and Tepel (2006) reported a direct cytotoxic effect on the kidney as one of the existing mechanism underlying contrast media induced nephropathy. He described this as impairment in renal function occurring within 3 days following the intravascular administration of contrast media in the absence of an alternative aetiology. Hardiek *et al* (2001) reported injury to proximal tubule cells by iopamidolwhich increases extracellular adenosine, an indicator of cellular stress.

CONCLUSION

A study on the effect of urografin on the histological patterns of the Kidney was successfully carried and the following conclusion can be drawn from the findings.The kidney of the Wistar rat is affected by urografin by alterations of the normal histological pattern of cells. The alterations of the normal morphology of cells occasioned by effects of high dose of urografin compared to low dose were not directly proportional. The effects of urografin an ionic monomer, compared to that of ultravist a non-ionic monomer (Ikamaise *et al*, 2015) is persistent and may induce permanent damage to the kidney cells. Generally, the awareness of the impact of radiographic contrast medium on cells serves as a guide for accurate choice of contrast medium for a particular radiographic procedure in the clinical setting.

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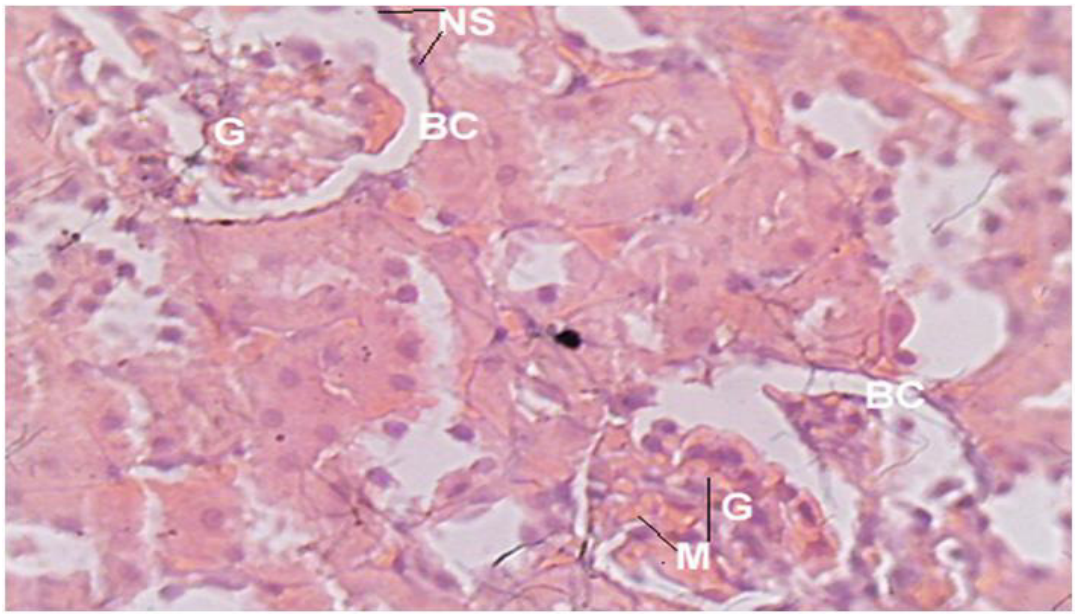

Plate 1: Photomicrograph of the renal cortex of a control Wistar rat. H&E stain, X400
BC- Bowman capsule **G**- Glomerulus **M-** Mensangia lcells **NS**- Nuclei of squamous cells

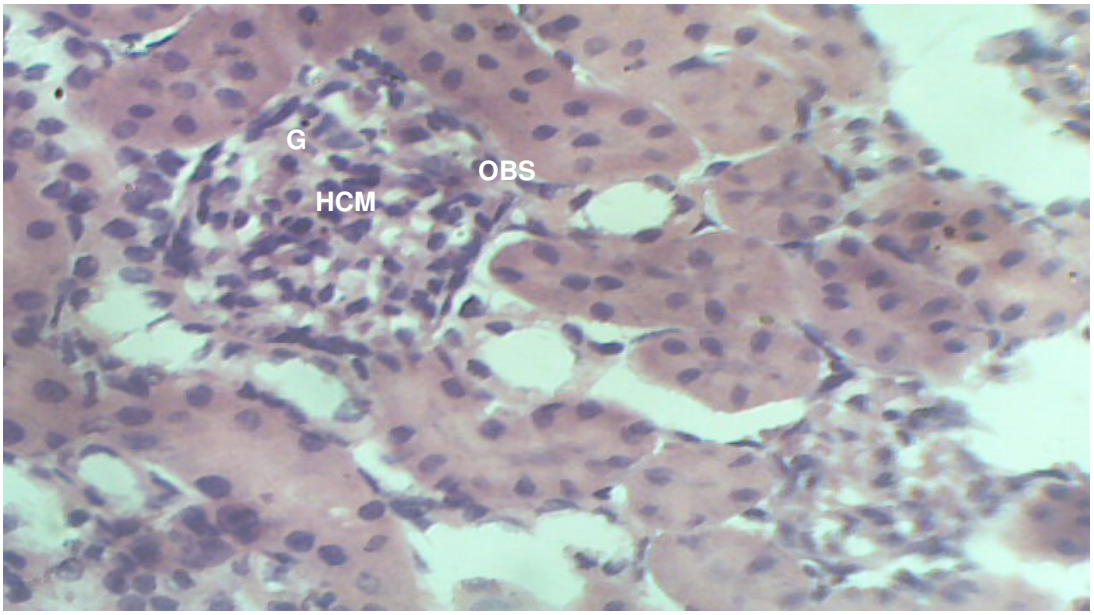

Plate2: Photomicrograph of the renal cortex of Wistar rat 30 minutes after injection of 0.25ml of urografin. H&E stain, X400.
G – Glomerulus **OBS** – Obliterated Bowman space **HCM** – Hypercellularmesangium

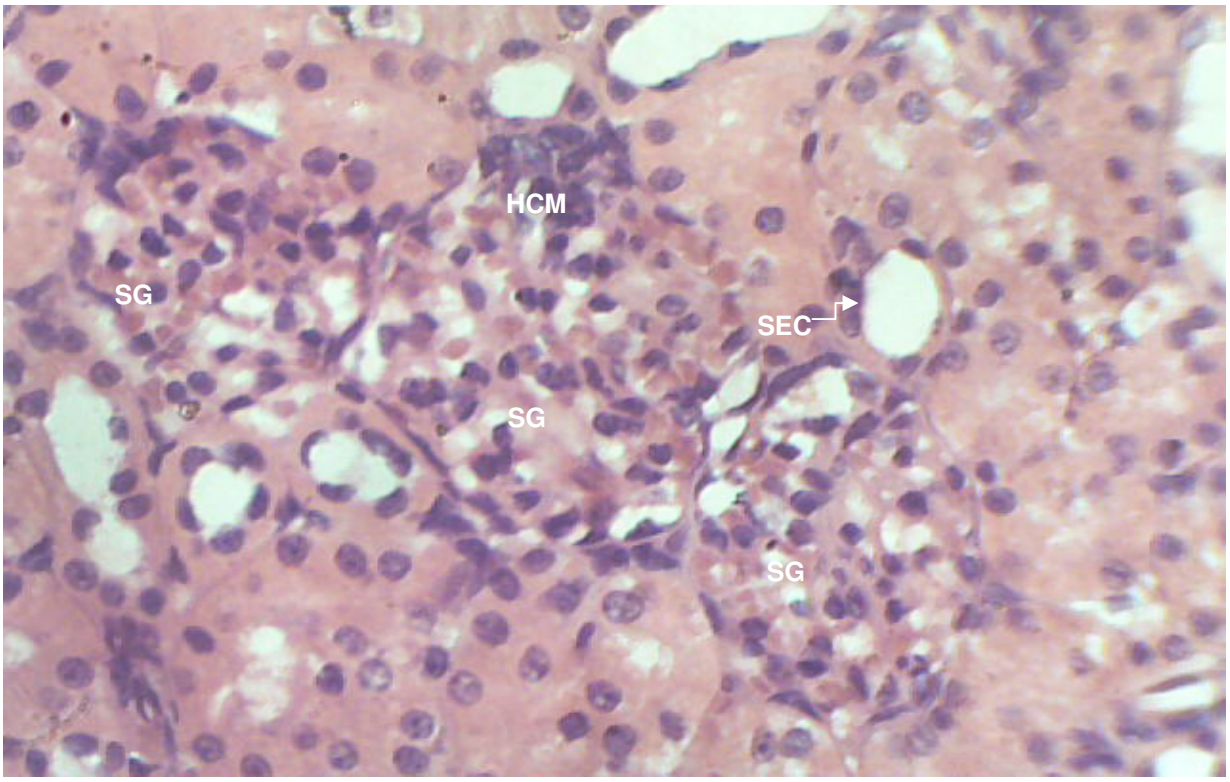

Plate 3: Photomicrogragh of the renal cortex 30 minutes after injection of 0.15ml of urografin. H&E stain, X400.
HCM- Hypercellularmesangium, **SG** – Swollen glomerulus, **SEC** – Swollen epithelial cells

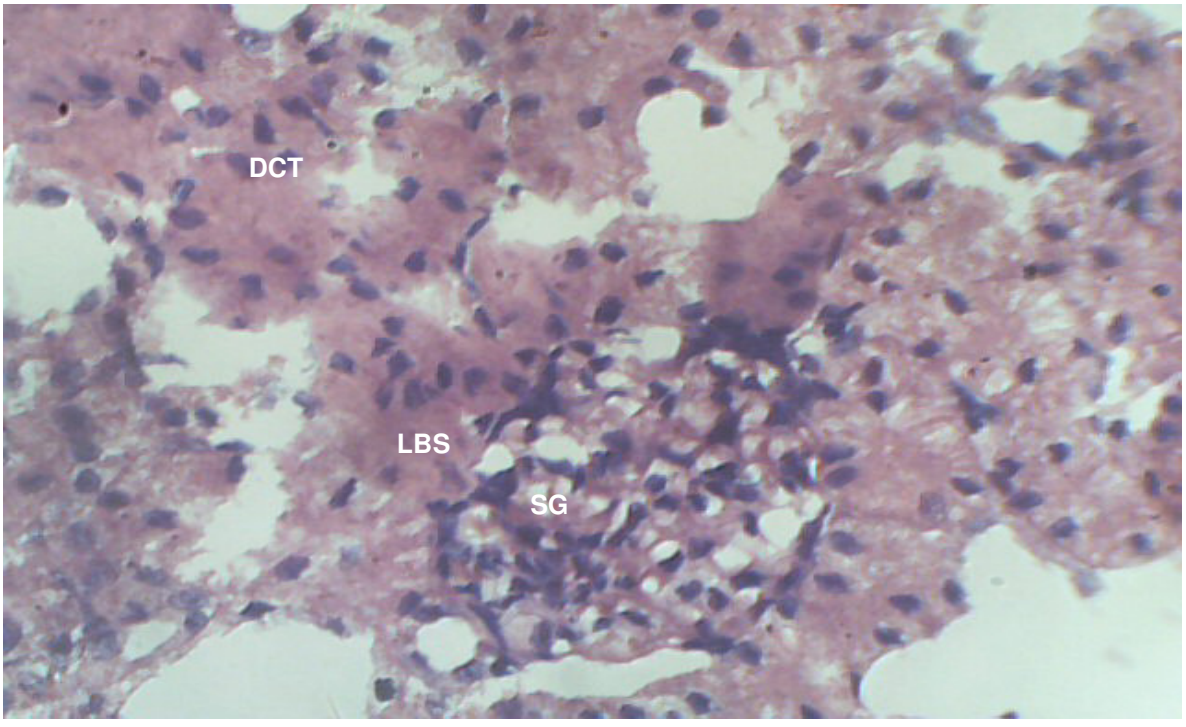

Plate 4: Photomicrogragh of the renal cortex 60 minutes after injection of 0.30ml of urografinH&E stain, X400.*
DCT– Degenerated convoluted tubule **SG** – Swollen glomerulus **LBS**– Lost Bowman's space

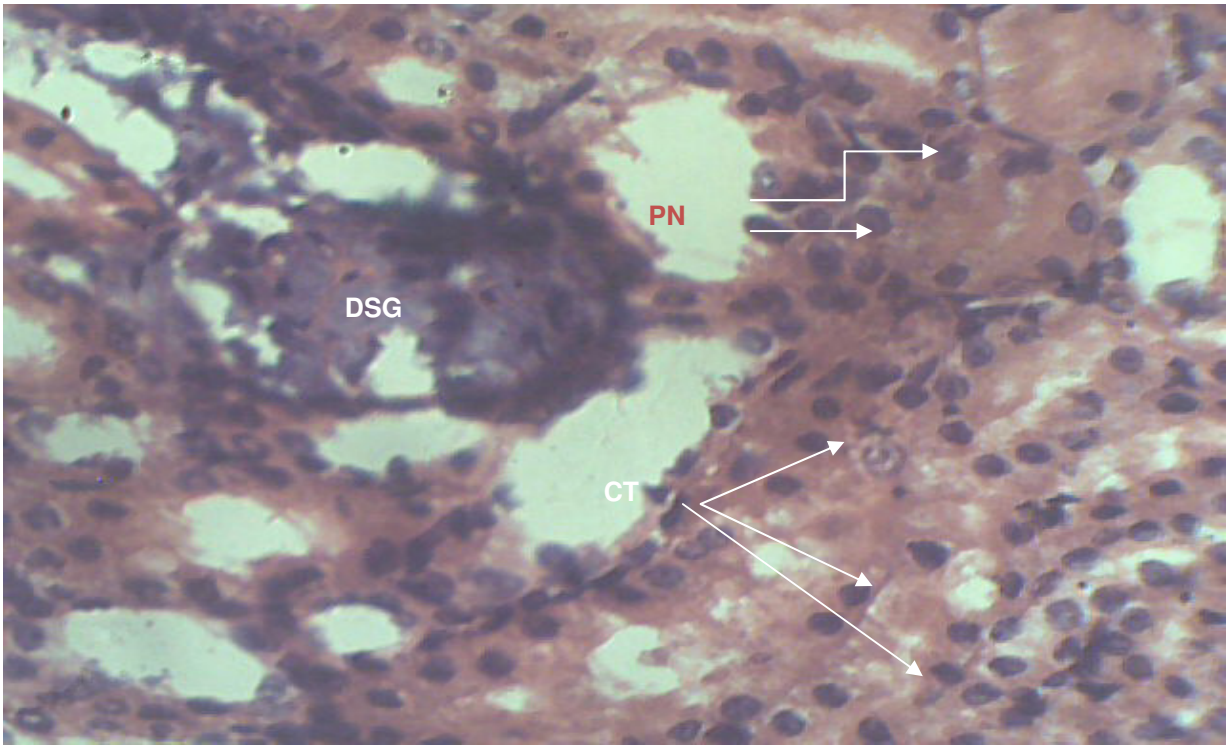

Plate 5: Photomicrogragh of the renal cortex 60 minutes after injection of 0.15 of urografin. H&E stain, X400.
PN – Prominent nuclei **DSG** – Deeply stained glomerulus [poor imaging] **CT** – cortical tubules

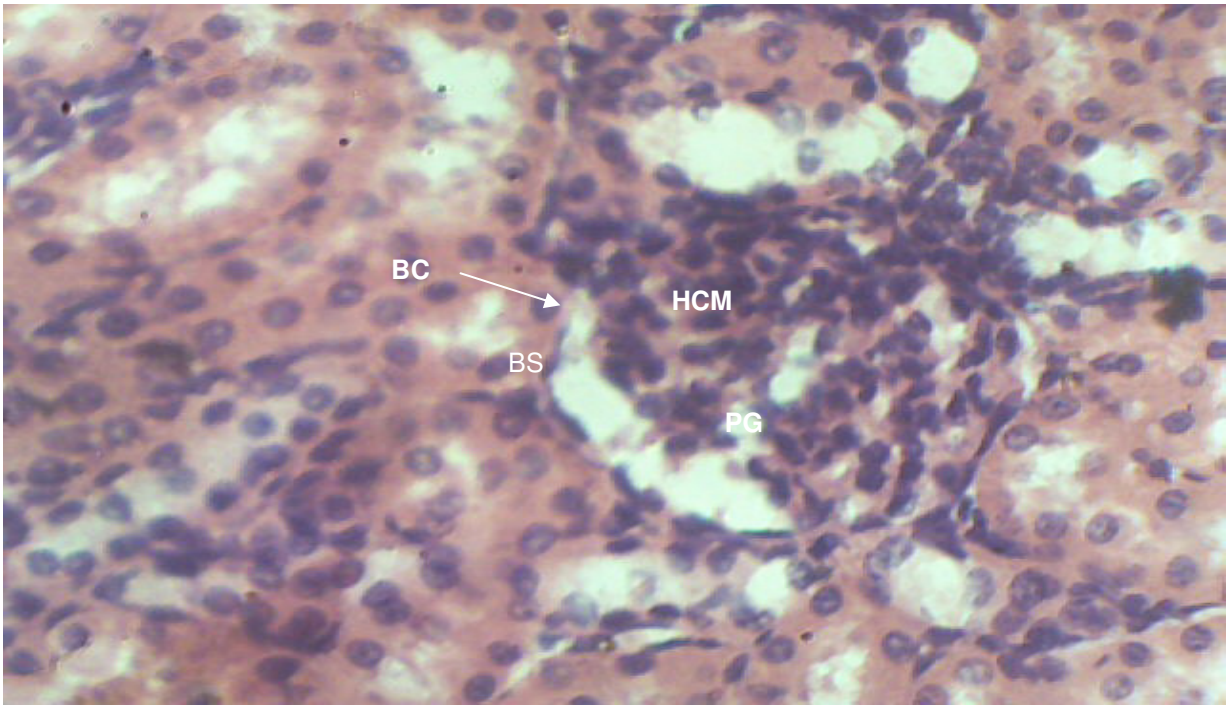

Plate 6: Photomicrogragh of renal cortex of Wistar rat 2 hours after injection of 0.30ml of urografin.H&E stain, X400.
HCM – Herpercellularmesangium **BC** – Bowman's capsule **PG** – Prominent glomerulus **BS** – Bowman's space

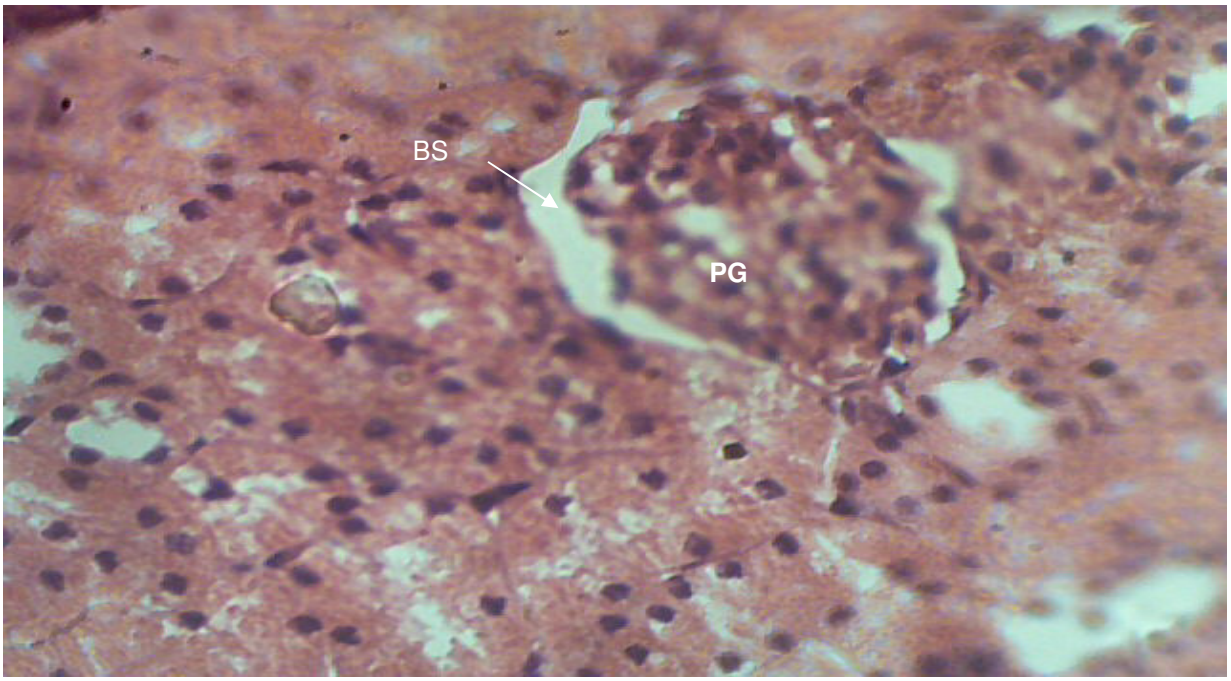

Plate 7: Photomicrogragh of the renal cortex of Wistar rat 2 hours after injection of 0.18ml of urografin. H&E stain, X400.
BS – Bowman's space **PG** – Prominent glomerulus

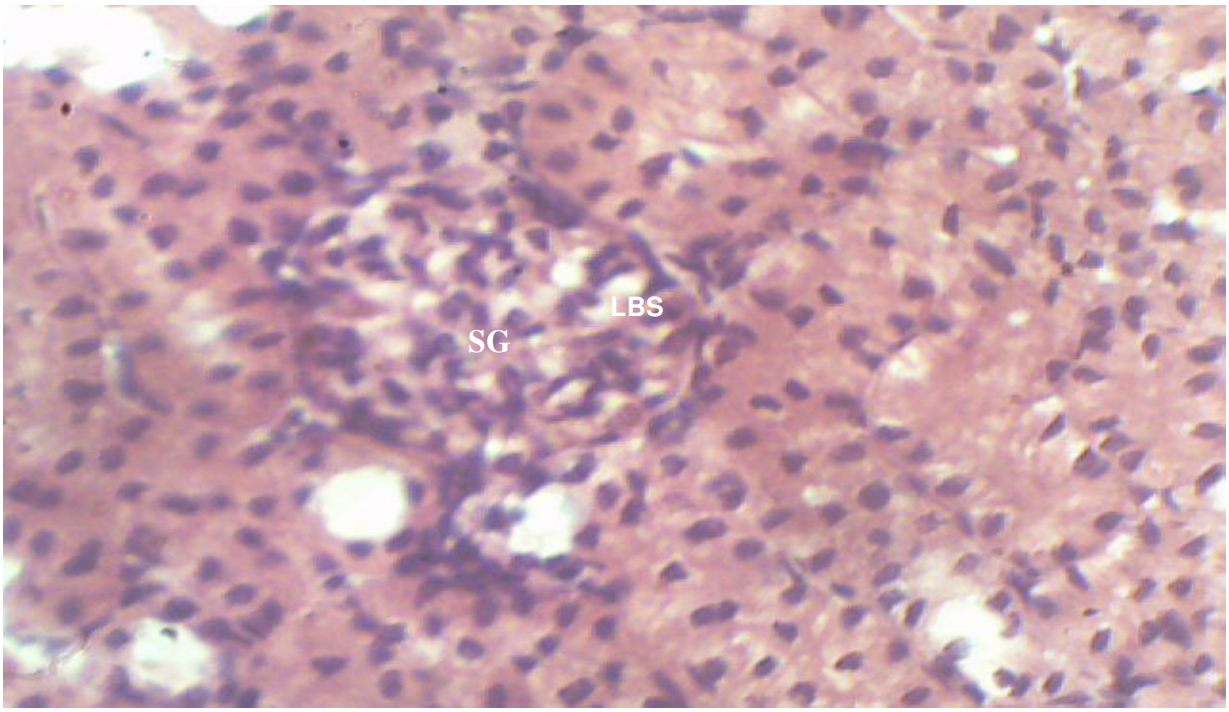

Plate 8: Photomicrogragh of the renal cortex of Wistar rat 24 hours after injection of 0.27ml of urografin. H&E stain, X400.
SG – Swollen glomerulus **LBS**– Lost Bowman's space

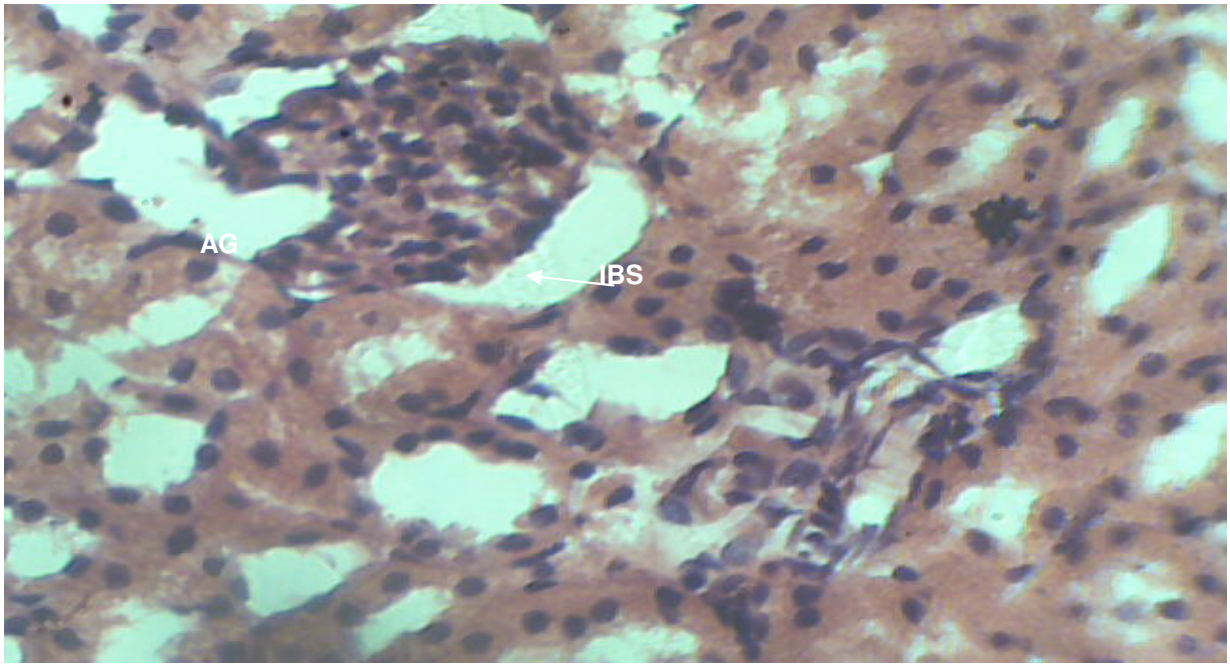

Plate 9: Photomicrogragh of the renal cortex of Wistar rat 24 hours after injection of 0.16ml of urografin. H&E stain, X400. **AG** – Shrunken glomerulus **IBS** – Increased Bowman's space

TABLE 1 :Body weight of rats and the dosage of contrast medium administered

Coded No.	Body weight of Rats (g)	Concentration/ Type of Contrast Medium	Duration after Contrast Administration (Hours)	Dosage of Contrast Medium (mL)	Coded marks for identification
1	202	ULT – HD	.5	0.29	Tail black
2	182	ULT – HD	1	0.26	Head black
3	190	ULT – HD	2	0.27	L hind limb black
4	210	ULT – HD	24	0.30	L fore limb black
17	169.5	ULT – HD	12	0.24	Neck black
5	222	ULT – LD	.5	0.19	R hind limb red
6	186	ULT – LD	1	0.16	Head red
7	181	ULT – LD	2	0.16	Tail red
8	193	ULT – LD	24	0.17	L hind limb red
18	211	ULT – LD	12	0.18	Neck red
9	176	URO – HD	.5	0.25	L hind limb blue
10	207	URO – HD	1	0.30	Back blue
11	212	URO – HD	2	0.30	Tail blue back blue
12	188	URO – HD	24	0.27	R hind limb blue
19	189.5	URO – HD	12	0.27	Neck blue
13	177	URO – LD	.5	0.15	Head brown
14	179	URO – LD	1	0.15	L forelimb brown
15	209	URO – LD	2	0.18	Back brown
16	183	URO – LD	24	0.16	R forelimb brown
20	209	URO – LD	12	0.15	Neck brown
21	201	-	.5	None	No mark
22	200	-	1	None	No mark
23	192	-	2	None	No mark
24	186	-	24	None	No mark
25	190.2	-	12	None	No mark
26	177	-	.5	None	No mark
27	198	-	1	None	No mark
28	206	-	2	None	No mark
29	211	-	24	None	No mark
30	204	-	12	none	No mark

URO = Urografin . ULT = Ultavist. LD = Low dose. HD =Haigh dose.