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Assessment of Hepato-Renal Functions and Markers of Oxidative Stress in Animals Exposed to Ionic Contrast Media

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Abstract

The increase in imaging technologies as well as many disease conditions requiring contrast media for diagnosis makes it important to investigate the effect of contrast media on hepato-renal function and its relation to oxidant and antioxidant status. To achieve this, twenty-five male albino rats were divided into 5 groups and administered with different doses of contrast agents (urografin and iohexol). After 48 hours of administration, 5 ml of blood samples was collected for the assessment of renal function, hepatic function and oxidative stress. The liver and kidneys were also excised for histological analysis. The result showed a significant increase (p=0.003) in the mean serum creatinine and urea in animals treated with either iohexol or urografin at different doses compared to the control. There was a significant increase (p=0.003) in serum malondialdehyde (MDA) level in contrast media group when compared with the control but serum total antioxidant status (TAS) level decreased in the groups administered with contrast media though the difference was not significant (p=0.003). Groups injected with iohexol showed no obvious histopathological alteration in both renal and hepatic tissues. In the groups treated with urografin, especially in higher dose, inflammatory cellular infiltration was observed at the peri-glomerular region and within the medulla in the kidney, while in the liver the portal tract appeared enlarged with infiltration of inflammatory cells. The result suggests that both iohexol and urografin are risk factors of kidney and liver damage as a result of increase in the pro-oxidant MDA and marked histomorphological alteration.

Keywords: Hepatic Function, Iohexol, Oxidative Stress, Renal Function, Serum Creatinine, Urografin.

1. Introduction

Technological advancements have led to the development of sophisticated imaging techniques used in the diagnosis of various human diseases. Such imaging techniques include computed tomography (CT), magnetic resonance imaging (MRI), digital subtraction angiography (DSA) and others which make use of contrast media/materials/agents that are introduced into the patient to aid image resolution by contrasting the selected areas of the body from surrounding tissue.[1] Contrast material can be administered orally, rectally or intravenously. Iodine-based contrast media are classified as high osmolality contrast media and low osmolality contrast media and are intravenously administered.[2]

Humans are exposed to many endogenous and xenobiotic substances that could have detrimental pathological outcomes if left un-modified and excreted.[3]When contrast materials are introduced intravenously, they move into the extracellular space through the vascular compartment and get eliminated by glomerular filtration. This may lead to dysfunction of the kidney, most especially in individuals with pre-existing health conditions like diabetes mellitus and renal impairment.[4] Apart from the kidney, the liver also plays an important role in the metabolism and biotransformation of these contrast materials. The detoxification of xenobiotics in the body is an important process in the maintenance of homeostasis, and any change in the homeostatic state could result to an imbalance in the dynamic equilibrium of metabolism leading to oxidative stress because of generation of reactive oxygen species (ROS) and liver dysfunction.[5-8]

To counter the damaging effects of reactive oxygen species, the body is endowed with antioxidant defence system that is made up of enzymes like glutathioneperoxidase (GPX), superoxide dismutase (SOD) and catalase, vitamins like vitamin E, C and A, and minerals like selenium, zinc, copper, and manganese which are produced either endogenously or received from exogenous sources. All of these play crucial roles in maintaining homeostasis.[9-12]

Creatinine, urea, serum electrolytes and uric acid are makers of renal function test[13], while markers of hepatic function test involve serum measurements of liver-derived enzymes like alanine aminotransferase (ALT), aspartate aminotransferase(AST), alkaline phosphatase and other non-enzymatic proteins.[14]Adverse reactions to intravenously administered contrast agents have been extensively investigated in humans and in animal models using mainly serum creatinine as a biomarker.[15-17] In

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humans, the use of contrast media may lead to contrast-induced nephrotoxicity (CIN) which is characterized by an increase in serum creatinine level within 24–72 hours following administration.[18-19]In addition to co-morbidities such as peripheral vascular disease, hypertension and anaemia[20], advanced congestive heart failure, diabetes mellitus and pre-existing renal impairment are some of the risk factors for contrast-induced nephrotoxicity.[21-22]Oxidative stress is one of the several mechanisms underlying CIN which result in the hypoxia of medulla and subsequent tubular damage.[23] Hypoxia may lead to the formation of reactive oxygen species which have been implicated in the toxicity of contrast agents.[24-25]

With the increase in disease conditions requiring contrast media for diagnosis, there is need to investigate the impact of contrast media using other markers of renal function apart from creatinine and markers of hepatic function among the exposed patients and also look at the level and effect of ROS generated in the course of using such contrast agents.

2. Materials and Methods

2.1. Experimental animals and groupings

Twenty-five (25) adult male albino rats weighing between 180-250g were procured for this study. The animals were acclimatized for 7 days under standard environmental conditions (temperature and humidity) and were given free access to rat chow and clean water *ad libitum*. The rats were randomly divided into 5 groups of 5 animals each. Group 1 (the control group) received l ml/kg body weight (bw) of physiological saline, group 2 and 3 received 1 and 3 ml/kg body weight of urografin, respectively, while group 4 and 5 received 1 and 3ml/kg body weight of iohexol, respectively. Animals were placed in individual metabolic cages and were deprived of water 24 hours before the administration of contrast media.

2.2. Collection of blood samples and processing

Exactly 48 hours after urografin and iohexol administration, 5 ml of blood samples was collected from the animals by retro-orbital bleeding under mild anesthesia (diethylether). Blood samples were collected using plain and EDTA tubes. Samples in the plain tubes were allowed to clot and centrifuged to get the serum which was stored at 4 °C until used. The liver and the kidney of all the rats were excised for histological investigations.

2.3. Biochemical analysis

Serum creatinine and urea levels were measured for the evaluation of renal function.

Creatinine level was determined as described by Mitchell (1973)[26], while the enzymatic method as described by Machado and Horizonte (1958)[27] was used for urea determination.

Levels of aminotransferase (ALT) and aspartate aminotransferase (AST) were measured for the evaluation of hepatic function. Alanine aminotransferase and aspartate aminotransferase levels were determined according to the method described by Reitman and Frankel (1975)[28].

Total antioxidant status (TAS) and malondialdehyde (MDA) were also measured to evaluate the level of oxidative stress. Based on the trolox equivalent antioxidant capacity method of Miller et al. (1993)[29] for quantitative assessment of *in vivo* antioxidant status, serum total antioxidant status (TAS) was determined using commercial Randox kit. The method of Ohkawa et al.(1979)[30] was used in the determination of malondialdehyde level. Using this method, 0.5 ml of plasma and 2.5 ml of 10% trichloroacetic acid were mixed and incubated for 15 min at 90°C. After cooling, the mixture was centrifuged at 3000 rpm for 10 min, 2 ml of the supernatant was added to 1 ml of 0.675% TBA solution in a test tube, sealed and incubated for 15 min at 90°C and then allowed to cool to room temperature. MDA level was measured spectrophotometrically at 532 nm wavelength.

2.4. Histological analyses

Liver and kidney of each rat were removed and, after histological processing, stained with ematoxylin and eosin. Stained sections were examined under light microscopy with different magnification, and the photograph of each of the slides was taken.

2.5. Statistical analysis

IBM SPSS 20.0 for windows was adopted for statistical analysis. Data were expressed as the mean ± SD. One-way analysis of variance (ANOVA) was used for comparisons of group data. Ducan multiple range test was used as a post-hoc test to determine where the exact difference lies, and p value $\leq$ 0.05 were considered as statistically significant.

2.6. Ethical Considerations

This study was carried out in accordance with the ethical standards of the University of Nigeria ethics committee on animal experimentation.

3. Results

3.1. Assessment of the effect of urografin and iohexol on renal function

The result of effect of urografin and iohexol on renal function is shown in table 1. It is shown that the mean creatinine and urea levels was significantly lower in the control groups compared to groups administered with contrast media, but there were no significant differences between groups with different doses of contrast media.

Table 1. Serum creatinine and urea values following administration of graded doses of urografin and iohexol

Groups	Creatinine (mg/dL)	Urea (mg/dL)
Group 1 (control)	2.46±0.36^{a}	67.27±8.56^{a}
Group 2 (1ml/kg bw of Urografin)	3.26±0.35^{b}	83.94±2.75^{b}
Group 3(3ml/kg bw of Urografin)	3.21±0.41^{b}	84.72±2.23^{b}
Group 4(1ml/kg bw of Iohexol)	3.20±0.18^{b}	81.24±4.49^{b}
Group 5(3ml/kg bw of Iohexol)	3.50±0.43^{b}	82.99±6.78^{b}

No significant difference exists between any groups with similar superscript in each row, and there is a significant difference if there is no similar superscript (p=0.003).

3.2. Assessment of the effect of urografin and iohexol on hepatic function

The serum ALT level of the animals in group 5 and group 3 that were treated with 3ml/kg of iohexol and 3ml/kg of urografin, respectively, were significantly higher (p=0.003) than the serum ALT levels of animals in group 2 and the control group, but there was no significant difference between the ALT level of animals in group 4 (iohexol 1ml/kg) and the other groups (Table 2).

Table 2.Serum ALT and AST values following administration of graded doses of urografin and iohexol

Groups	ALT (IU/L)	AST (IU/L)
Group 1 (control)	4.48±0.44[a]	13.80±5.29[a]
Group 2 (1ml/kg bw of Urografin)	4.60±0.60[a]	15.60±3.13[a]
Group 3(3ml/kg bw of Urografin)	6.28±1.38[b]	16.50±1.91[a]
Group 4(1ml/kg bw of Iohexol)	5.47±1.09[ab]	14.00±2.55[a]
Group 5(3ml/kg bw of Iohexol)	6.21±1.12[b]	13.75±1.80[a]

No significant difference exists between any groups with similar superscript in each row, and there is a significant difference if there is no similar superscript (p=0.003).

3.3. Assessment of effect of urografin and iohexol on oxidative stress

No significant difference was observed in the mean TAS level between the groups though the mean TAS level were found to be higher in the control group when compared with groups administered with contrast media (Table 3).

Table 3. Serum TAS and MDA values following administration of graded doses of urografin and iohexol

Groups	TAS (mg/dL)	MDA (mg/dL)
Group 1 (control)	2.43±0.28[a]	4.26±0.72[a]
Group 2 (1ml/kg bw of Urografin)	2.43±0.28[a]	4.98±0.80[a]
Group 3 (3ml/kg bw of Urografin)	1.99±0.35[a]	5.31±0.58[a]
Group 4 (1ml/kg bw of Iohexol)	1.80±0.31[a]	5.42±0.87[ab]
Group 5 (3ml/kg bw of Iohexol)	1.98±0.59[a]	6.50±0.99[b]

No significant difference exists between any groups with similar superscript in each row, and there is a significant difference if there is no similar superscript (p=0.003).

3.4. Histopathological examination of liver tissues

Histopathological examination of liver tissues of the control group showed normal histo-architecture of the hepatic tissue. The central vein (Cv), portal tract (Pt), and sinusoidal spaces (S) flanked by plates of hepatocytes (H) appear normal (figure 1). Those treated with iohexol (groups 4 and 5) showed no obvious histoarchitectural disruption with intact portal tract (Pt), hepatocytes (H), sinusoidal spaces (S) and central veins (Cv) (figure 2).

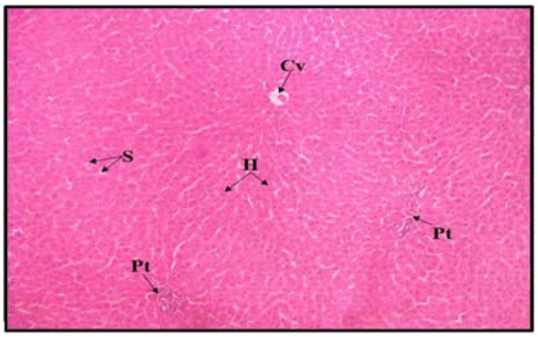

Figure 1: Photomicrograph of liver tissue section from control group 1 showing normal histo-architecture of the hepatic tissue.

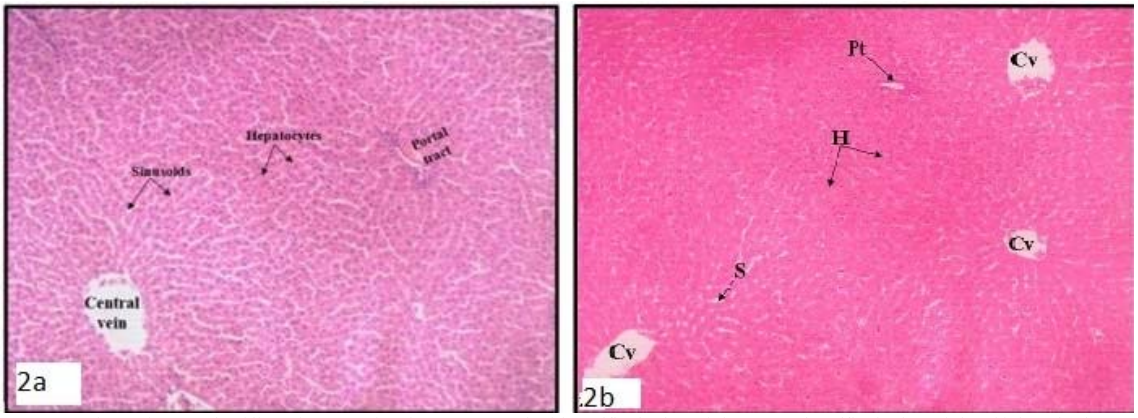

Figure 2: Photomicrograph of liver tissue section from rat treated with 1ml/kg body weight (2a) and 3ml/kg body weight of iohexol (2b) showing no obvious histo-architectural disruption with intact portal tract (Pt), hepatocytes (H), sinusoidal spaces (S) and central veins (Cv).

However, the photomicrograph of liver tissue of rats treated with 1ml/kg body weight of urografin (Group 2) showed intact tissue parenchyma, but the portal tracts (Pt) appeared mildly enlarged with infiltration of inflammatory cells (figure 3a) while the photomicrograph of liver tissue of rat treated with 3ml/kg body weight of urografin (Group 3) showed marked inflammatory cellular infiltration (black arrows) along the interlobular septa and portal tracts with damage of limiting plate. However, most hepatocytes at midzonal and centrilobular regions appeared intact (figure 3b).

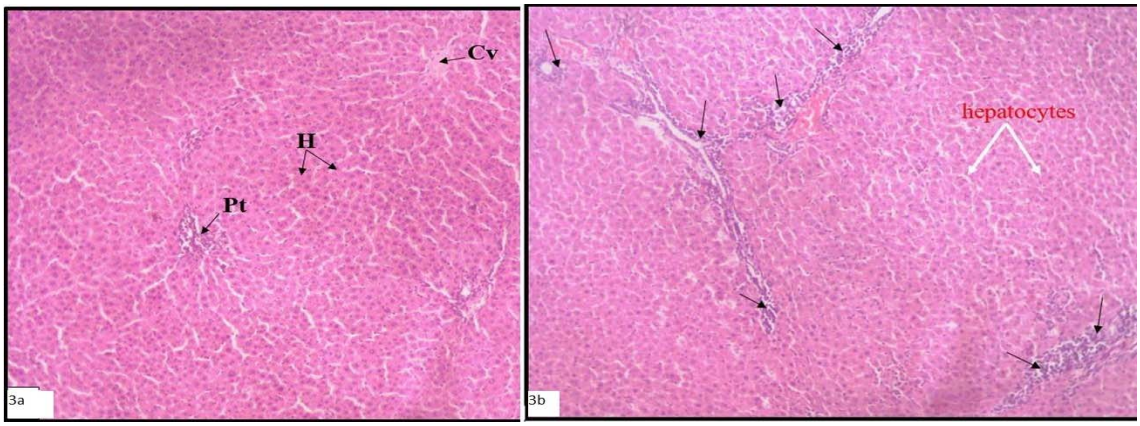

Figure 3: Photomicrograph of liver tissue section from rats treated with 1ml/kg body weight (3a) showing intact tissue parenchyma, but the portal tracts (Pt) appearing mildly enlarged with infiltration of inflammatory cells and 3ml/kg body weight of urografin (3b) showing marked inflammatory cellular infiltration (black arrows) along the interlobular septa.

3.5. Histopathological examination of kidney tissue

The histological examination of kidney tissues of the control group (figure 4), the group treated with 1ml/kg body weight of urografin and the groups treated 1ml/kg and 3ml/kg body weight of iohexol (figure 5), respectively, showed normal histoarchitecture of the cortical and medullary portions of the renal tissue with no obvious histopathological change.

However, the glomeruli of those treated with 3ml/kg body weight of urografin appeared to be shrunken with corresponding increase in bowman's capsular space. Inflammatory cellular infiltration was also observed at the peri-glomerular regions and within the medulla (figure 6).

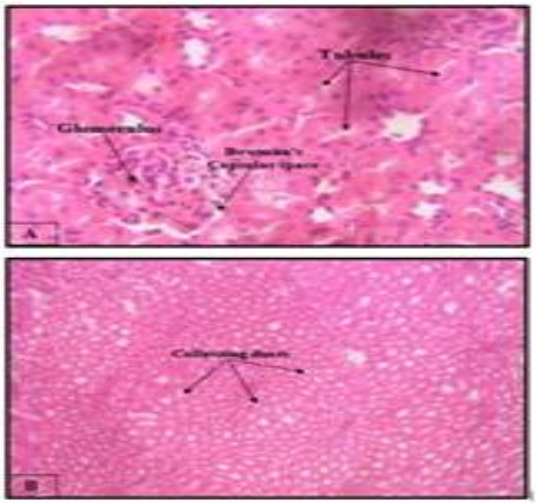

Figure 4: Photomicrograph showing the cortical (A) and medullary (B) portions of kidney tissue from control group 1 showing normal histoarchitecture of the cortical and medullary portions of the renal tissue.

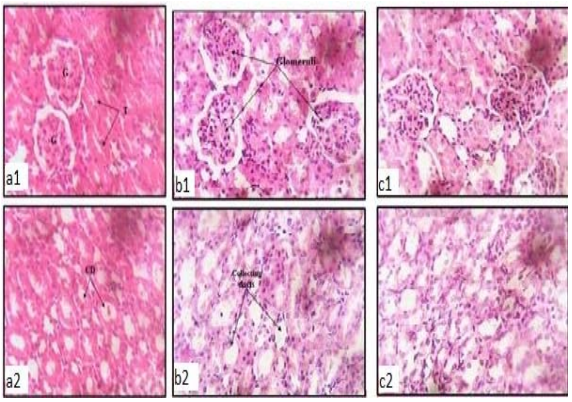

Figure 5: Photomicrograph showing the cortical (a1) and medullary (a2) portions of kidney tissue of rat treated with 1ml/kg body weight of urografin; cortical (b1) and medullary (b2) portions of kidney tissue of rat treated with 1ml/kg body weight of Iohexol; cortical (c1) and medullary (c2) portions of kidney tissue of rat treated with 3ml/kg body weight of Iohexol showing normal histoarchitecture of the cortical and medullary portions of the renal tissue.

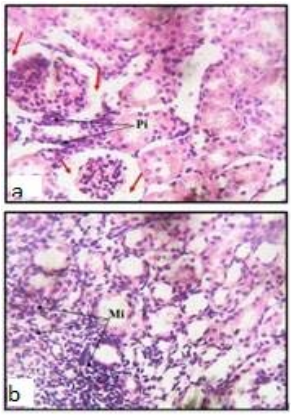

Figure 6: Photomicrograph showing the cortical (a) and medullary (b) portions of kidney tissue of rat treated with 3ml/kg body weight of urografin showing shrunken glomeruli with corresponding increase in bowman's capsular space.

4. Discussion

In the assessment of effect of urografin and iohexol on renal function, the mean serum creatinine level was highest in group 5 (3.50±0.43) that was treated with 3ml/kg of iohexol, followed by group 2 (3.26±0.35) that was treated with 1ml/kg of urografin, while the lowestvalue was observed in group 4 (3.20±0.18) that was exposed to 1ml/kg of urografin. The mean serum urea level was highest in group 3 (84.72±2.23) that was treated with 3ml/kg of urografin, followed by group 2 (83.94±2.75) that was treated with 1ml/kg of urografin while the least value was observed in group 4 (81.24±4.49) that was exposed to 1ml/kg of iohexol.

All rats in the contrast media groups treated with either urografin or iohexol at different doses exhibited a significant increase in serum creatinine and urea when compared with the control group. Variation in serum creatinine urea levels after contrast media administration has been interpreted as an indication of nephrotoxicity.[31] A study done by Choi et al. (2001)[32] reported an increased serum urea levels after administration of contrast agents in dogs with normal renal function.

In the assessment of effect of urografin and iohexol on hepatic function, the animals administered with 3ml/kg urografin (group 3) exhibited an increase in serum AST when compared with control group and other contrast media groups. However, no significant difference in the mean AST level was observed between the groups (Table 2). Elevations in serum enzyme levels (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase) are indicators of hepatotoxicity; a dysfunction, damage or injury of the liver that is associated with an overload of xenobiotics or drugs.[33-34] Hepatic function test may not be able to reveal detectable toxicity of contrast materials unless the liver is severely affected. Billström et al. (1987)[35] observed a slight increase in serum values of liver enzymes following administration of contrast agents particularly in patients with impaired hepatic function.

Total antioxidant status (TAS) measures the overall antioxidant status of the body.[36] Oxidative stress occurs in response to the oxidative damage caused when the body's scavenging and antioxidant activities cannot withstand the oxidants produced by a harmful stimulant[37] (in this case the contrast agents). Malondialdehyde (MDA) is a stable end product of lipid peroxidation, and its level is used as a marker of oxidative stress and of antioxidant status.[38]Table 3 also showed an increase in serum malondialdehyde (MDA) in all the groups injected with contrast media when compared with those of the animals in the control group. However, only the MDA level of animals in group 5 (treated with 3ml/kg of iohexol) were significantly increased when compared to those of the animals in the control group and in other groups. The increase in MDA and decrease in TAS values following administration of graded doses of contrast agents suggest that contrast agents play an important role in oxidative stress.

The liver and the kidney are the organs that play an important role in the metabolism, biotransformation and excretion of foreign compound which makes them highly susceptible to their adverse and toxic effects leading to hepatotoxicity and nephrotoxicity which refers to liver or kidney dysfunction, injury or damage that is associated with an overload of drugs or other foreign compounds. The histo-architectural disruption observed mainly in the liver and kidney of animals administered with urografin (especially high dose) could be attributed to the toxic effect of urografin on the organs. Iodine-based contrast media are classified as high osmolality contrast media (HOCM) and low osmolality contrast media (LOCM). Low osmolality contrast media (iohexol) are less nephrotoxic and hepatotoxic than high osmolality contrast media (urografin).[39]

5. Conclusions

Both iohexol and urografin are risk factors of kidney and liver damage as a result of increase in the pro-oxidant MDA and marked histomorphological alteration. However, the histopathological analsysis showed that it is safer and more reliable to use iohexol (a low osmolality contrast media) especially a lower dose.

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None.

Conflict of interest

We have no conflict of interests to declare.

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