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Pathophysiology of iron overload-induced renal injury and dysfunction: Roles of renal oxidative stress and systemic inflammatory mediators

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ABSTRACT

Iron-overload has been recognized as a risk factor for organ dysfunction and damage resulting in diseases such as liver and heart disease, diabetes mellitus, and neurodegenerative diseases. This study investigated renal function and some systemic inflammatory indices in iron-overloaded male Wistar rats.

Thirty animals were equally distributed into 3groups and treated daily i.p. with either normal saline (0.2 ml; control), iron (as ferrous sulphate) (15 mg/kg) or iron (30 mg/kg) for 21days respectively. Post-treatment, blood samples were obtained from each animal by cardiac puncture after light anaesthesia into plain sample bottles. Iron, ferritin, transferrin, creatinine, urea, albumin, total protein, interleukin-6 (IL-6), prostaglandins-E2 and tumor necrosis factor-α (TNF-α) were analysed in serum. Kidney homogenates were obtained per group and analysed for superoxide dismutase (SOD), total antioxidant capacity (TAC), reduced glutathione (GSH), lipid peroxidation (MDA) and nitric oxide (NO). Kidney histology was evaluated per group using both Haematoxylin and Eosin and periodic acid Schiff stains.

Iron-overload caused a graded increase ($p < 0.05$) in serum iron, ferritin, transferrin, creatinine, urea, IL-6, TNF-α, TAC, MDA and NO levels as well as a reduction in albumin levels, renal SOD and GSH in groups 2 (iron 15 mg/kg) and 3 (iron 30 mg/kg) respectively compared to control. Histological evaluation of the kidney showed structural and tubular aberrations consistent with renal damage via inflammatory processes in iron overloaded rats.

Our present study suggests that iron-overloading causes renal dysfunction by triggering the evolution of several inflammatory mediators which lead to a cascade of systemic and renal inflammatory processes that alter renal structure and function.

1. Introduction

Iron overload is considered one of the most common metal toxicities [1] and has been recognized to be a risk factor for numerous acute and chronic illnesses [2]. It has been reported that humans have no mechanism for elimination of excess iron [3]; hence, it is likely that apart from genetic predisposition to iron overload in some individuals (genetic hemochromatosis); multiple transfusion of red blood cells [4], parenteral iron administration in transfusion dependent anaemias [5], random prescription of iron supplements during pregnancy [6], consumption of home brewed beer made locally in iron containing vessels [7] and consumption of iron-rich food substances may inevitably result in iron overload.

Several studies have associated iron overload with organ dysfunction and damage resulting in diseases such as liver and heart disease, diabetes mellitus, dysfunctional immune system, hormonal abnormalities, Alzheimer's disease and Parkinson's diseases [8–10].

There is however gap in knowledge as to whether iron overload predisposes to renal disease as well as the likely mechanism associated with iron toxicity in the kidney. Hence, this study was designed to investigate renal function and histology in iron overloaded male Wistar rats.

2. Materials and methods

2.1. Animal grouping and experimental protocol

Thirty (30) male Wistar rats were housed in well aerated standard laboratory cages and maintained at room temperature with

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Table 1
Serum iron indices in control and experimental groups.

Groups	Iron (μg/dL)	Ferritin (μg/L)	Transferrin (mg/dL)
Group 1 (Control)	58.84 ± 2.10	11.37 ± 1.60	64.78 ± 6.59
Group 2 (Iron 15 mg/kg Treated)	84.64 ± 14.44*	27.46 ± 5.83*	149.37 ± 14.6*
Group 3 (Iron 30 mg/kg Treated)	90.46 ± 5.52#	58.60 ± 5.7#	163.32 ± 4.93#

* Indicates significant differences between group 2 and control.
# indicates significant differences between group 3 and control.

alternate day and night time cycles. They were fed on standard rat chow and allowed access to drinking water ad libitum in accordance with guidelines and protocol approved by the Animal Care and Use Research Ethics Committee (ACUREC) of the University of Ibadan, Nigeria (Approval no.: UIACUREC/18/0017). The animals were randomly divided into 3 equal groups as follows: group 1 was control and received daily intraperitoneal administration of 0.2 ml normal saline. Animals in group 2 and 3 received iron as ferrous sulphate at doses of 15 mg/kg and 30 mg/kg intraperitonealy [11] respectively. Treatments were done for 21 days.

2.2. Sample collection, biochemical and histological analysis

Blood samples were obtained by cardiac puncture after light anaesthesia (thiopentone sodium, 30 mg/kg) from all animals in each group into plain specimen bottles. The samples were allowed to coagulate and centrifuged at 3000 *g* for 10 min at 4 °C to obtain serum. Aliquot of the clear serum obtained was analysed for iron [12] and urea [13]. Aliquots of the serum were also analysed for ferritin, transferrin, creatinine and total protein using Fortress Diagnostics kits, United Kingdom; albumin was analysed using kits obtained from Pointe Scientific, Inc. United States of America while interleukin-6 (IL-6), prostaglandins E_2 (PGE2) and tumor necrosis factor-α (TNF-α) were all assayed using kits obtained from Elabscience Biotechnology Inc. United States. Globulin level was derived mathematically from the total protein and albumin levels obtained.

Kidney samples were also obtained from five (5) animals in each group, weighed and homogenized on ice in 1.15% KCl buffer (pH = 7.4). The kidney homogenates were centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant obtained was analysed for superoxide dismutase (SOD), total antioxidant capacity (TAC), reduced glutathione (GSH), lipid peroxidation and nitric oxide (NO) levels using commercially available kits obtained from Sigma-Aldrich Inc., Germany. Kidney samples were obtained from the remaining five (5) animals in each group and analysed for structural changes using haematoxylin and eosin (H and E) stains while tubular changes were evaluated using Periodic Acid Schiff (PAS) reaction techniques respectively.

2.3. Statistical analysis

Data is presented as mean ± SEM of five samples from 3 independent groups, and analysed using one-way analysis of variance while Tukey's honest significant difference post-hoc test was used to establish the statistical significance between experimental groups and control at $p < 0.05$.

3. Results

3.1. Serum Iron indices

Serum iron (μg/dL) level was significantly increased ($p < 0.05$) in the 15 mg/kg iron treated (43.9%) and 30 mg/kg iron treated (53.7%) group respectively compared to control. Ferritin (μg/L) level was also significantly increased in the 15 mg/kg (27.46 ± 5.83 vs. 11.37 ± 1.60) and 30 mg/kg iron treated (58.60 ± 5.70 vs. 11.37 ± 1.60) group compared to control. Transferrin levels (mg/dL) in the 15 mg/kg (149.37 ± 14.6) and 30 mg/kg (163.32 ± 4.93) iron treated groups were also significantly increased compared to control (64.78 ± 6.59) (Table 1).

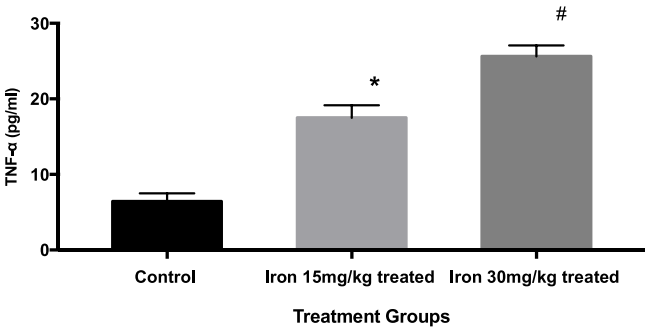

Fig. 1. Tumor necrosis factor - α levels in control and experimental groups * Indicates significant differences between group 2 and control, # indicates significant differences between group 3 and control.

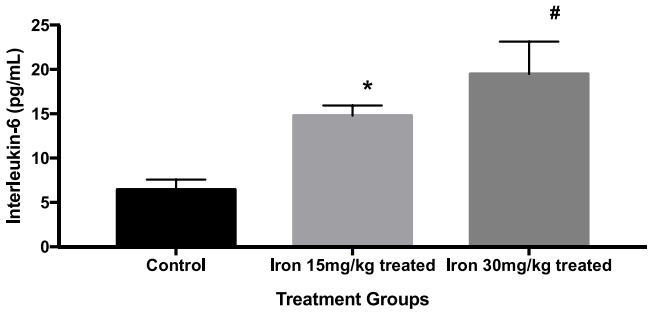

Fig. 2. Interleukin-6 levels in control and experimental groups * Indicates significant differences between group 2 and control, # indicates significant differences between group 3 and control.

3.2. Serum inflammatory studies

Serum tumour necrosis factor-α (mg/dL) (Fig. 1) and interleukin-6 levels (pg/mL) (Fig. 2) were significantly increased ($p < 0.05$) in the 15 mg/kg (14.77 ± 1.16; 17.50 ± 1.66) and 30 mg/kg (19.49 ± 3.65; 25.64 ± 1.44) iron treatment groups respectively compared to control (6.44 ± 1.14; 6.43 ± 1.09). Prostaglandin E_2 level did not however show any difference between the control and experimental groups (Fig. 3).

3.3. Renal tissue antioxidant studies

The values obtained show a reduction ($p < 0.05$) in renal superoxide dismutase (SOD) and reduced glutathione (GSH) levels in the 15 mg/kg and 30 mg/kg iron treatment groups compared to control (Table 2). Total antioxidant capacity (TAC) in the control group (0.23 ± 0.12) was reduced ($p < 0.05$) compared to the 15 mg/kg (0.81 ± 0.25) and 30 mg/kg (1.86 ± 0.51) iron treatment groups respectively. Renal malondialdehyde (MDA), a marker of lipid peroxidation, was elevated in the 15 mg/kg (0.53 ± 0.03) and

Table 2
Renal tissue oxidative stress status in control and experimental groups.

Groups	SOD (U/mL)	GSH (mM)	TAC (mM)	MDA (μM)	NO (μM)
Group 1 (Control)	0.34 ± 0.02	0.25 ± 0.03	0.23 ± 0.12	0.26 ± 0.03	10.21 ± 1.79
Group 2 (Iron 15 mg/kg Treated)	0.16 ± 0.01*	0.11 ± 0.04*	0.81 ± 0.25*	0.53 ± 0.03*	30.70 ± 3.14*
Group 3 (Iron 30 mg/kg Treated)	0.12 ± 0.01#	0.17 ± 0.02#	1.86 ± 0.51#	0.60 ± 0.01#	31.38 ± 2.64#

* Indicates significant differences between group 2 and control.
# indicates significant differences between group 3 and control.

Table 3
Renal function and serum protein levels in control and experimental groups.

Groups	Creatinine (μg/L)	Urea (mg/dL)	Albumin (g/dL)	Globulin (g/dL)	Total Protein (g/dL)
Group 1 (Control)	120.09 ± 18.51	0.81 ± 0.19	3.10 ± 0.03	3.62 ± 0.14	6.71 ± 0.14
Group 2 (Iron 15 mg/kg Treated)	243.44 ± 33.44*	1.04 ± 0.01*	2.09 ± 0.02	2.41 ± 0.23	4.51 ± 0.24*
Group 3 (Iron 30 mg/kg Treated)	163.60 ± 14.30#	1.38 ± 0.17#	1.64 ± 0.16#	2.37 ± 0.14	4.02 ± 0.32#

* Indicates significant differences between group 2 and control.
# indicates significant differences between group 3 and control.

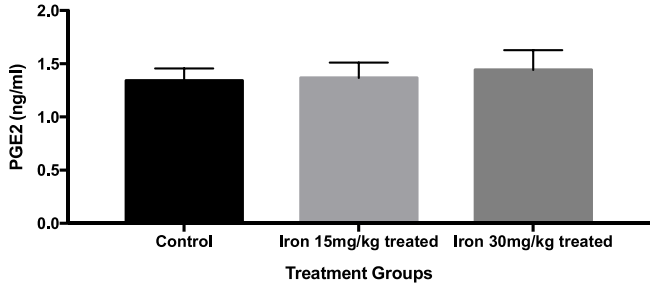

Fig. 3. Prostaglandin E_2 levels in control and experimental groups.

30 mg/kg (0.60 ± 0.01) treatment groups respectively compared to control (0.26 ± 0.03). Renal nitric oxide (NO) values in the 15 mg/kg and 30 mg/kg iron treated groups were also significantly increased respectively compared to control (Table 2).

3.4. Renal function and serum total protein evaluation

Urea (mg/dL) levels were significantly increased ($p < 0.05$) in the experimental groups (15 mg/kg and 30 mg/kg iron treated) compared to control (Table 3). Creatinine (μg/L) values show an increase in the 15 mg/kg (243.44 ± 33.44) and 30 mg/kg (163.60 ± 14.30) compared to control (120.09 ± 18.51) (Table 3). Serum albumin, globulin and total protein (g/dL) were reduced in the 15 mg/kg iron treated group (32.6%, 33.4%, 32.8%) and 30 mg/kg iron treated group (47.1%, 34.5%, 40.1%) compared to control (Table 3).

3.5. Histological assessment

Histological evaluation of renal tissue using H and E stains for gross structural changes, show control group with normal glomeruli, bowman capsule, tubules and no significant lesion. Animals in group 2 (15 mg/kg iron treated) displayed renal tissue with mild peritubular inflammation and haemorrhagic lesion (pointed arrow). Some tubules exhibited eosinophilic substance within them while some had atresia (thick white arrow). Group 3 (30 mg/kg iron treated) showed renal tissue with moderate perivascular inflammation (pointed arrow), mild haemorrhagic lesion (blue arrow) and the presence of eosinophilic material within the interstitial space (thick white arrow) (Fig. 4: A–C).

Using PAS stains for evaluation of tubular changes, renal tissue in the control group showed normal basement membrane with glomerular capillary loops and tubular epithelium. The capillary loops of the glomerulus are well defined and thin. The endothelial cells are seen in capillary loops and the messangial regions are of normal size. In group 2 (15 mg/kg iron treated), renal tissues showed mild peritubular inflammation, periglomerular inflammation (green arrow) and mild haemorrhagic lesion. Some tubules are dilated with eosinophilic substance within them. Some tubules show hydrophobic degeneration (pointed arrow). There is strong PAS positive reaction of the apical brush borders of the proximal convoluted tubules (PCTs) (black arrow). In group 3 (30 mg/kg iron treated), renal tissue showed pericapsular fibrosis with chronic inflammation, mild peritubular inflammation and mild haemorrhagic lesion. There is disseminated atresia and these tubules displayed strong PAS positive reaction of the apical brush borders (thick white arrow). Some tubules exhibited hydrophobic degeneration with dilated lumen (pointed arrow) (Fig. 5: A–C).

4. Discussions and conclusion

The kidneys are essential organs that perform several functions that include the regulation of electrolyte balance, red blood cell production, pH balance and excretion of water-soluble wastes from the body. These functions are necessary to preserve life and hence, any damage to the kidneys can result in a series of complications that are deleterious and life threatening. Iron, a vital metal essential for life, when taken in excess (iron overload) has been reported to cause hyperglycaemia, insulin resistance, and pancreatic beta cell dysfunction thus predisposing to type-2-diabetes mellitus [14] and other organ pathologies [8–10]. These effects of iron overloading are likely mediated via increased free radicals production that impairs the oxidative balance in the body resulting in oxidative stress and inflammation [14].

In this study, serum iron, ferritin and transferrin levels were increased dose dependently by iron supplementation (Table 1) thus suggesting iron overload in the experimental groups and is consistent with the observations of Pari et al., [11] who also reported iron overload in laboratory animals administered 30 mg/kg ferrous sulphate. Iron overload as observed in the experimental groups may likely be as a result of oversaturation of iron storage (as ferritin) and transport (iron bound to transferrin) thus leading to the presence in circulation of non–transferrin-bound iron (NTBI) and redox active labile plasma iron [15]. Certain cell types (hepatocytes and cardiomyocytes) readily take up these NTBIs, resulting in iron oxidant-mediated cellular injury in these cells [15]. In this study, renal tissues showed a depletion of renal antioxidants such as reduced glutathione (GSH) and superoxide dismutase (SOD) with increased malondialdehyde (MDA), a marker of tissue lipid peroxidation, and nitric oxide (NO) levels (Table 2) suggesting cellular

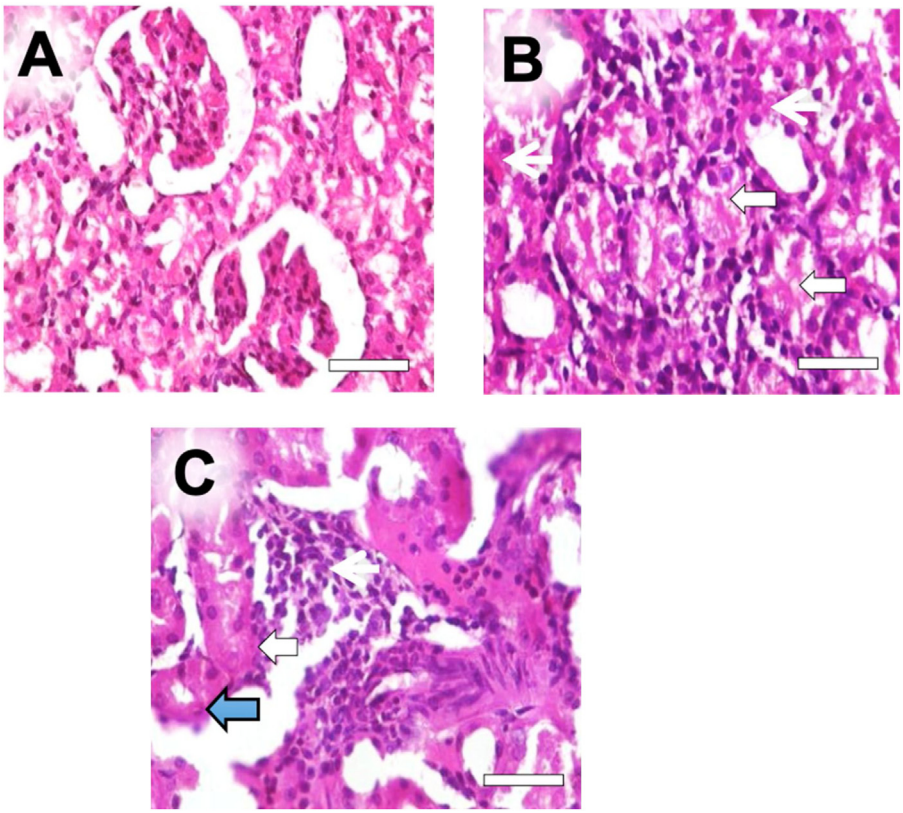

Fig. 4. (A–C) Photomicrograph of kidney samples in control and experimental groups using Haematoxylin and Eosin stains. Scale bar = 0.15 μm. Renal tissue of the control group shows normal glomeruli, Bowman capsule, tubules and no significant lesion were observed. Animals in group 2 (15 mg/kg iron treated) show renal tissue with mild peritubular, inflammation, mild haemorrhagic lesion (pointed arrow). Some tubules exhibited eosinophilic substance, within them while some have atresia (thick white arrow). Group 3 (30 mg/kg iron treated) had renal tissue with moderate perivascular inflammation (pointed arrow), mild haemorrhagic lesion (blue arrow) and showed presence of eosinophilic material within the interstitial. Some tubules and glomerulus seen were also unremarkable (thick white arrow) (Fig. 4: A–C) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

damage within the kidney. It is likely that the excess iron in circulation as NTBI in the experimental groups may have entered the kidneys and induced cellular damage by catalysing the production of reactive oxygen species in excess of the capacity of renal cellular antioxidant systems and thus result in a consequent decrease in antioxidants, such as reduced glutathione (GSH), superoxide dismutase (SOD) and catalase [16]. Histological evaluation for gross morphological changes (H and E Stain) and renal tubule changes (PAS) also support these observations as the iron treated groups displayed renal structural (Fig. 4A–C) and tubular (Fig. 5 A–C) pathologies consistent with NTBI induced oxidative stress and lesions [17]. This hypothesis however may need to be further investigated, as this study did not investigate iron deposition within the renal tissues and thus therefore may be regarded as a limitation of this study.

This study also shows an increase in the total antioxidant capacity (TAC) in iron-overloaded rats compared to control and this may be a reflection of increased urate production due to renal impairment as previously reported by Jackson et al. [18] and Young [19] and not associated with NTBI's which should cause a depletion of both serum and tissue antioxidant capacity.

Increased inflammation as a result of iron-induced oxidative damage has been associated with elevated serum tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) concentrations [20]. In this study, the elevated serum TNF-α (Fig. 1) and IL-6 (Fig. 2) observed may be due to iron-induced oxidative stress and activation of inflammatory cascade as has been associated with iron overload [20]. However, prostaglandin E_2 (PGE2) level observed in this study was not significantly different from controls (Fig. 3).

Amongst several clinical markers used routinely to predict renal function, creatinine and urea levels appear to be the most prominent [21], with urea being useful in the differential diagnosis of pre-renal and renal causes of Acute Renal Failure (ARF) [22] and creatinine giving a measure of the progression of renal disease [21]. In this study, serum urea and creatinine levels were increased suggesting impairment in renal function. Furthermore, the reduction in serum albumin levels observed in this study may also be indicative of a decline in kidney function [23]. This further corroborates earlier observations in this study that, iron overload may likely cause an increase in free radical production, inflammatory cascade, deplete serum and renal antioxidant capacity resulting in an increase in renal lipid peroxidation which can ultimately lead to renal injury and dysfunction.

In summary, the body has poor active iron excretion mechanisms. Hence, iron overloading impairs iron homeostasis resulting in an increase in non-transferrin bound iron levels that can activate an increase in systemic inflammatory cascade via oxidative stress. The increased oxidative stress and systemic inflammation may likely cause renal tissue damage and deplete renal antioxidants leading to impairment in renal structure and function.

While care should be taken in extrapolating results from laboratory studies to human subjects, this study suggests that in patients receiving blood transfusion, parenteral iron administration in transfusion-dependent anaemia's and in patients receiving supplemental iron to augment the effects of erythropoiesis-stimulating agents (ESAs), there may be a need to monitor iron status and renal function in order to prevent iron related toxicities and chronic renal disease in such patients.

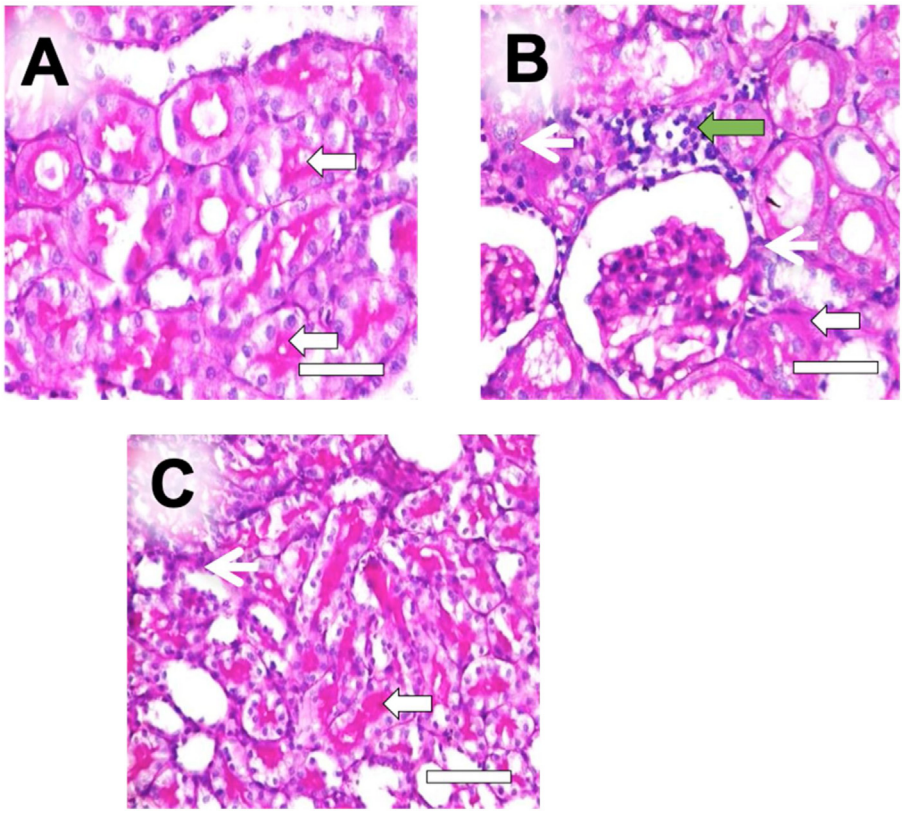

Fig. 5. (A–C) Photomicrograph of kidney samples in control and experimental groups using Periodic Acid Schiff stains. Scale bar = 0.15 μm. Renal tubules in control group show normal basement membrane of glomerular capillary loops and tubular epithelium. The capillary loops of the glomerulus are well defined, thin and endothelia cells therein are seen. The messengial regions are of normal size and there is strong PAS positive reaction of the apical brush borders of the PCTs (thick white arrow). Animals in group 2 (15 mg/kg iron treated) show renal tubules with mild peritubular inflammation, periglomerular inflammation (green arrow) and haemorrhagic lesion. Some tubules are dilated with eosinophilic substance within them while some tubules show hydrophobic degeneration (pointed arrow). There is also strong PAS positive reaction of the apical brush borders of the PCTs (thick white arrow). Group 3 (30 mg/kg iron treated) exhibited renal tubules with pericapsular fibrosis with chronic inflammation, mild peritubular inflammation and mild haemorrhagic lesion. There is disseminated atresia and these tubules have strong PAS positive reaction of the apical brush borders (thick white arrow). Some tubules seen also exhibit hydrophobic degeneration with dilated lumen (pointed arrow) (Fig. 5: A–C) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

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