



# Acute and chronic effects of broiler chicken enriched diet on kidneys morphology of male albino wistar rats

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## ABSTRACT:

**Objective:** To study the effect of broiler chicken enriched diet on acute and chronic morphological changes on kidneys of male albino Wistar rats.

**Materials and methods:** A total of 20 male albino Wistar rats were divided into two groups. Group A consisted of 10 animals fed on normal rat chow and group B consisted of 10 animals that were fed on commercially available broiler chicken meat. H & E, PAS and Mallory trichrome stains were used to detect any histopathological changes in the kidneys.

**Results:** The rats in group B which were fed on commercially available broiler chicken meat showed on H & E stained section acute tubular injury and necrosis with marked epithelial vacuolization and renal artery lesions in group B rats. Whereas PAS staining showed significant loss of brush border fluff in group B animals as compared to group A animals. Trichrome stain revealed normal renal parenchyma with unnoticeable glomeruli and tubules in both groups.

**Conclusion:** Present study concludes that consumption of broiler chicken meat causes acute toxic effects on histopathological parameters on the kidneys of male albino Wistar rats.

**Keywords:** Broiler Chicken Enriched Diet; Kidney; Albino Wistar Rats.

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## Introduction:

Broiler chickens are the chickens reared for meat purpose in short time reaching the weight of 2.5kg in 42 days.<sup>1</sup> The major negative impact on the quality of meat is contributed by the constituents of chicken

feed.<sup>2,3</sup> Previous studies indicated that harmful substances such as Aflatoxins B1, crude carbohydrates, crude proteins, and crude fibre, premixes of vitamins and toxicities plus pesticides along with heavy metals such as lead (Pb), Manganese (Mn),



Cadmium (Cd), nickel (Ni), mercury (Hg) and zinc (Zn) are present in the chicken feed.<sup>4-6</sup>

The concentration of Pb, Hg and Cd was found to be above the maximum permissible level in the chicken feed and accumulation of these metals was observed in the liver, breast and thigh muscles of chicken along with significant amount of Mn in liver, gizzard, breast and thigh muscles of broiler chicken making it unsafe for human consumption.<sup>5,7</sup>

In a study, it was observed that heavy metal accumulation was seen in tumours of renal cell carcinoma compared to its adjacent tissues which lacked any particle accumulation indicating the role played by heavy metals in renal cell carcinogenesis.<sup>8</sup> Previous studies reported that heavy metals lead to formation of free radicals causing inflammation, apoptosis and denaturation being probable cause of various carcinomas and other diseases.<sup>9,10</sup>

Kidney being the most common organ for reabsorbing and accumulating divalent metals become one of the highly targeted organ for heavy metal toxicity. These heavy metals are toxic at very low doses and one of the potential harmful environmental hazards by being non-biodegradable with long half-life.<sup>11</sup> In a study, male rats were used to determine nephrotoxicity of Cd using kidney injury molecule-1 (Kim-1) as a biomarker which is excreted in urine due to injury. It was observed that significant amount of Kim-1 was detected in urine at six weeks.<sup>12</sup> In another study, female rats were exposed to commercially available chicken feed and commercial chicken meat. Hormonal profile was found to be disrupted in these rats along with excessive weight gain, obesity and polycystic ovaries.<sup>13</sup> Hence forming a relation with other studies indicating that high cholesterol/high density lipoprotein(HDL-C) ratio have negative impact on renal function causing elevated creatinine level ( $\geq 1.5$  mg/dl) and decreased glomerular filtration rate( $\leq 55$ ml/min).<sup>14,15</sup>

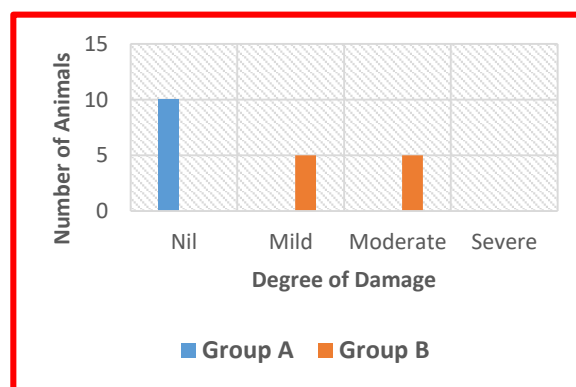
There is tremendous increase in the chronic kidney disorder raising the suspicion that broiler chicken may be one of the contributing factor but data in regard to effect

of broiler chicken consumption on morphology of kidney is limited and lacks reliability.<sup>16</sup> Therefore, present study was designed to observe the acute and chronic morphological changes in kidneys using various stains such as hematoxylin and eosin, Periodic Schiff reagent and mallory's trichrome reagent in response to broiler chicken enriched diet.

#### Material and methods:

The experimental study was conducted in Baqai Medical University after the research approval was taken from the Ethical Committee of Baqai Medical University. This study was performed at the Department of Anatomy, Baqai Medical University, Karachi. Histo-pathological investigation was conducted on rat's kidney in Javed I. Kazi Histopathology Department, SIUT Karachi. The duration of study was of six weeks. The Sample size of 10 in each group was estimated using Cohen's d table<sup>17</sup> and by considering the power of test as 80%, detectable effect size of 1.16 and 5% level of significance. Total 20 male albino Wistar rats were included in the study.

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Total 20 male albino Wistar rats of weight 120 gm to 180 gm were included. These rats were randomly divided into two group's i.e.

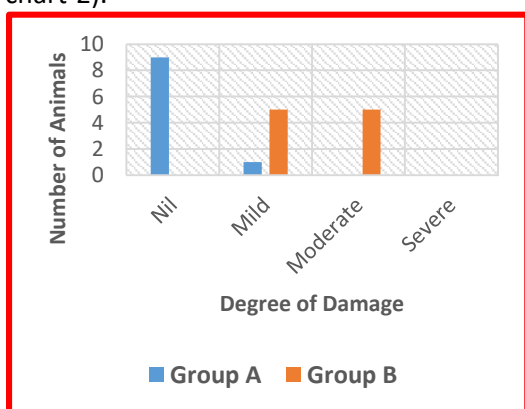
- **Group A** was control group comprising of 10 male albino wistar rats and were fed on normal rat chow.
- **Group B** was test group comprising of 10 male albino wistar rats which were fed on commercially available broiler chicken meat.

The rats were kept in the animal house of Baqai Medical University under normal room temperature. After completion of 6 weeks, the rats in group A and B were sacrificed by using ether for anesthesia. After achieving complete unconsciousness of the male albino Wistar rats, they were placed on the dissection board and their limbs were fixed with the help of fixing pins. Midline incision was made in order to expose the abdominal region, fat and intestine were removed to collect the kidney.

Kidney samples were collected in specimen bottles containing 10% formalin immediately, to preserve the tissue and to assess the morphological changes using various stains.

Data was analyzed using SPSS version 23. Mean and SD were computed for

When compared, the mean acute tubular necrosis between group A with B animals, statistically significant difference was found ( $p = 0.001$ ). Only one (10%) animal showed mild acute tubular necrosis in group A animals whereas in group B animals, 5 (50%) animals showed mild acute tubular necrosis and 5 (50%) animals showed moderate acute tubular necrosis (Table-1, Figure-1 and Bar chart-2).



**Bar Chart-2: Comparison of Acute Tubular Necrosis (ATN) between groups.**

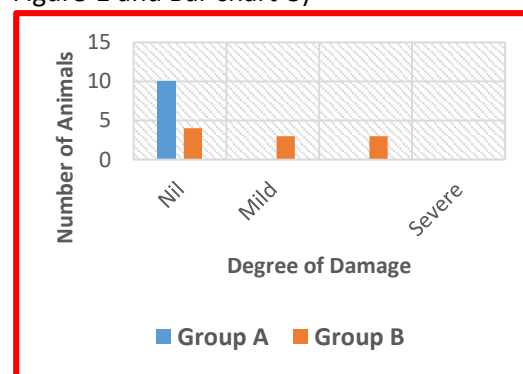
continuous variables. Fisher's exact test was applied to compare the morphological and molecular changes between two groups at 6<sup>th</sup> week.  $P \leq 0.05$  was taken as statistically significant.

**Results:**

There was statistically significant differences found in proportion of mean acute tubular injury between the group A with B animals ( $p = 0.0001$ ). There was no acute tubular injury in group A animals whereas in group B animals, 50% animals showed mild acute tubular injury and 50% animals showed moderate tubular injury.

**Bar Chart-1: Comparison of Acute Tubular Injury (ATI) between groups.**

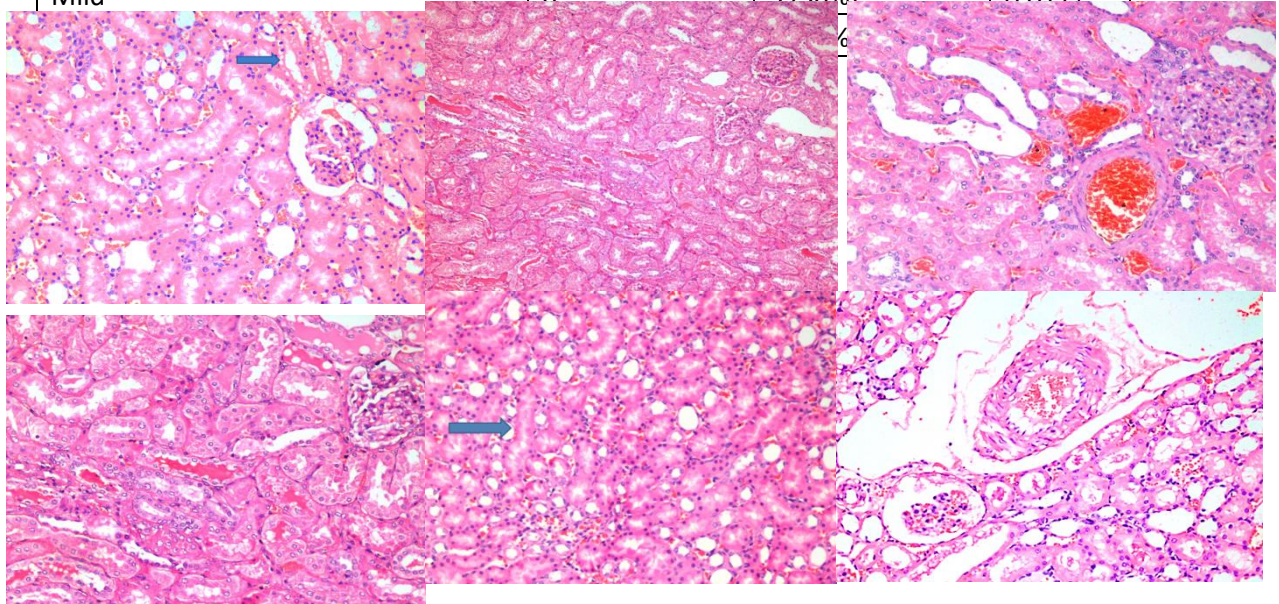
There was statistically significant differences found in proportion of mean arterial lesions between the group A with B animals ( $p = 0.011$ ). In group A, all the animals had negative findings for arterial lesions, whereas in group B, 30% had mild vacuolization and 30% had moderate arterial lesions with very prominent epithelial vacuolization. (Table 1, Figure-1 and Bar chart-3)



**Bar Chart-3: Comparison of Arterial lesions between groups.**

Hematoxylin and Eosin Staining	Group A (n=10)	Group B (n=10)	p-value
<b>Acute tubular injury</b>			
Nil	10 (100%)	0	0.0001*

Mild	0	5 (50%)	
Moderate	0	5 (50%)	
<b>Acute tubular necrosis</b>			
Nil	9 (90%)	0	
Mild	1 (10%)	5 (50%)	0.001*
Moderate	0	5 (50%)	
<b>Arterial lesions</b>			
Negative	10 (100%)	4 (40%)	
Mild	0	3 (30%)	0.011*



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**Table No 1: Hematoxylin and Eosin Staining Results**

**A**

**C**

**E**

**Figure-1:** H & E stained, 5µm thick section of kidney at 40X showing renal parenchyma in control (A) and chicken feed treated animals (B). Section B is showing epithelial vacuolization and acute tubular injury in renal tubules. 5µm thick section of kidney at 20X showing renal parenchyma in control (C) and chicken feed treated animals (D). Section D is showing renal tubules exhibiting moderate Acute Tubular Necrosis (blue arrow). Renal artery in control (E) and chicken feed treated animals (F). Section F showing an appreciated vessel with medial wall thickening (blue arrow).

**B**

**D**

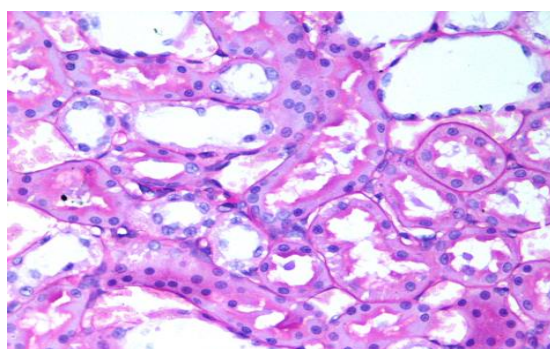
**F**

**PERIODIC SCHIFF REAGENT RESULTS**  
 Statistically significant difference in proportion of mean brush border fluff loss was found with p-value=0.003. In group A, 30% of the rats had mild (Focal loss) brush border fluff. Whereas in group B, 60% of the

rats had mild (Focal loss) brush border fluff, 30% had moderate (Patchy loss) brush border fluff and 10% had severe (Marked loss) brush border fluff. All rats in group A and B had negative findings for tubular atrophy. (Table 2, Figure-2)

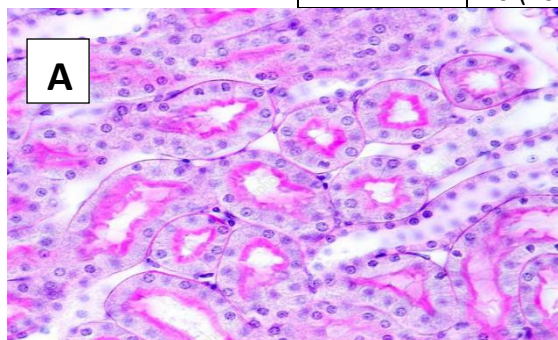
**Table No 2: Comparison of Periodic Schiff Reagent Analysis between Group A and Group B Animals.**

Periodic Schiff Reagent Analysis	Group A (n=10)	Group B (n=10)	p-value
<b>Brush border fluff</b>			
Nil	7 (70%)	0	
Mild-Focal loss	3 (30%)	6 (60%)	
Moderate-Patchy loss	0	3 (30%)	



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Severe-Marked loss	0	1 (10%)	0.003*
<b>Tubular atrophy</b>			
Nil	10 (100%)	10 (100%)	-



0.003\*

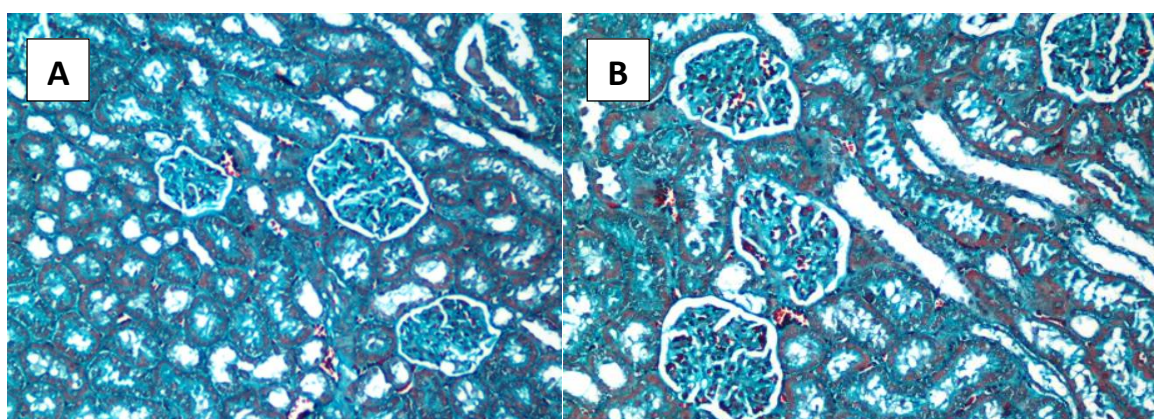
**Figure-2:** PAS stain, 5µm thick section of kidney at 40X showing brush border fluff in control (A) and chicken feed treated animals (B). Section B showing the distorted brush border fluff in tubules with focal, patchy and marked loss (black arrows).

**MALLORY TRICHROME RESULTS:**

Mallory trichrome stain was used to determine the presence of fibrosis in the collagen fibers making up the renal parenchyma. All rats in group A and B had negative findings for fibrosis. (Table-10, Figure-23)

**Table No 10:**  
**Comparison of Mallory's trichrome staining (%) between groups**

Semi-quantitative analysis of fibrosis with Mallory's trichrome staining	Group A (n=10)	Group B (n=10)	p-value
Mallory's trichrome staining (%)			
Negative	10 (100%)	10 (100%)	-



**Figure-23:** Mallory Trichrome stain, 5µm thick sections of kidney at 40X showing kidney parenchyma with unremarkable glomeruli and tubules. No glomerular or interstitial fibrosis is seen in section A and B

**DISCUSSION:**

The global inflation in kidney diseases have raised to such an extent that world health organisation declared public health emergency. Kidney disorders were found to have prevalence of 14.3% in general population rising to 36.1% in high risk groups. Amidst this situation, the condition is worsened by environmental risk factors and diet.<sup>18</sup>Therefore, this study was designed to find the negative effects of commercially available broiler chicken meat on the

morphology of kidneys in male albino Wistar rats.

The consistency characteristics of chicken meat include health and toxicology hygienic factors (presence of harmful microorganisms and pollutants such as pesticides, hormones and environmental contaminants), nutritional value, and technical and sensory qualities. Various overlapping influences, including genotype, nutrition, accommodation, pre-slaughter care, slaughtering, and storage, affect meat complex properties.<sup>19,20</sup>

The growing demand for chicken poultry meat has produced, throughout the years, in an extraordinary push to improve the growth rate of birds for breeders, nutritionists and farmers. Implementation of procedures that have led to histological and biochemical changes in tissue through the damage to certain meat quality characteristics, such as vulnerability to stress-induced myopathies that could have important repercussions on meat quality and unusual conditions, such as dark, tender and exudatory meat. This illness is linked to increased paleness of meat, reduced water supply and a decreased consistency in meals and fresh meat.<sup>21-23,</sup>

Different studies have shown that harmful substances such as aflatoxins B1, raw sugars, crude starch, nutrient and toxicity premixes and pesticides along with heavy metals are present in the chicken feed which ultimately leads to a major negative impact on the quality of meat.<sup>24</sup>

Rehman R et al., 2018 conducted an experimental study at the Pakistan Council of Scientific and Industrial Research laboratory, Karachi to assess the additives present in broiler chicken feed and meat and their consequences for human health. Samples of commercial broiler chicken feed and meat were obtained in 2015. In an animal house, another collection of organic chickens were born. The feed, meat and falling samples were then tested in the laboratory to determine the essential constituents and additives. In the commercial chicken meat, additive constituents were present. For organic chicken meat and droppings these components were absent, which means they were missing for their feeding material. Hence, commercial chickens were found to be unsafe for consumption.<sup>25</sup>

In this study, hematoxylin and eosin (H &E) stain showed significant acute tubular injury and necrosis and marked significant arterial lesions in chicken fed animals. Whereas, the interstitial edema and inflammation remained non-significant. It was also observed that 50% of rats in chicken fed group had mild and 50% of rats had moderate acute tubular injury and necrosis. There was statistically significant difference between the

mean arterial lesions within the groups. The chicken fed animals showed 30% of rats with mild arterial lesions and 30% of rat kidneys showed moderate arterial lesions with prominent epithelial vacuolization.

Significant acute tubular injury and necrosis in chicken fed animals could be because of presence of heavy metals such as cadmium, lead, nickel and mercury in chicken's meat.<sup>26</sup> These heavy metals are responsible for marked damage to the proximal convoluted tubules by its accumulation and producing reactive oxygen species. This search comes in line with this study findings, as excess of reactive oxygen species could be the cause for significant acute tubular injury and necrosis along with damage to the arteries.<sup>27</sup>

On periodic Schiff reagent (PAS) stain, significant loss of brush border fluff was found in chicken fed rats as 60% had mild focal loss, 30% showing moderate and 10% showed marked loss of brush border fluff. This is the first study showing the relation between the brush border fluff loss due to chicken meat intake in male albino wistar rats. This brush border fluff damage could be because of free oxygen radicals increased in the body due to defected functioning of cyclooxygenase and phospholipases leading to oxidative stress damage.<sup>28</sup>

#### CONCLUSION:

This study concludes that consumption of broiler chicken meat causes acute toxic effects on histopathological parameters on the kidneys of male albino Wistar rats. Further studies are warranted to find out the exact mechanism of broiler chicken meat induced toxicity.

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