ORIGINAL ARTICLE

Glomerular Changes in Kidneys of Albino Rats after using Copper diet – a biological study

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ABSTRACT

Aim: To evaluate the effects of copper metal on renal glomeruli.
Study design: AnExperimental study.
Duration and place of study: This study was conducted from May to November 2013 in Pathology Department of University of Health Sciences Lahore.
Methods: 20 albino rats were selected and divided into two groups. The control group 1 was fed on standard pellet rodent diet and tap water. The experimental group 2 was fed 0.5mg/kg of body weight for 18 weeks on alternate days; the dose was calculated according to the body weight. Rats were sacrificed at the end of experiment and the kidneys were removed for processing and H&E staining for routine histological study.Glomerular changes observed by microscope and statistically analyzed using Chi-square test with $p<0.05$.
Results: Results showed that glomerular changes in terms of mesangial cell proliferation byusing copper in the diet ofrats. When the control group 1 on normal diet was compared with the experimental group 2, statistically significant difference ($p<0.05$) was noticed.
Conclusion: It is therefore concluded that copper in diet effect the glomeruli in terms of mesangial cell proliferation,adhesion and crescent formation in kidneys of albino rats.
Keywords:Copper, mesangial cell, proliferation, adhesion, crescent formation

INTRODUCTION

Copper (Cu) is a heavy metal and essential trace element which is vital for the physical and mental health. But due to the wide spread occurrence of copper in daily routine increases the chances of the toxicity of it.Tubular epithelium, Glomerularbasement membrane and capillary blood vessels are appeared to be injured by the compounds of copper metals when used in prolonged times. These Compounds causing harm to the kidneys by degeneration of the tubules and vacuolization of the epithelial cells.

Capillaries of glomeruli are also affected and Bowman capsuleexpansions are also reported, these changes increase over the period of time[1]. Therefore, Cu compounds are lethal to the cells of tubules and the glomeruli and appreciably changed the functions of kidneys (Jarupet al., 1998). However, these metal compounds themselves behave like substances foreign to the body and trapped in the kidneys Bowman's capsule, a target for immune mechanisms. Compoundof copper can also cause proliferation of hyaline thickening in glomeruli[4,11].

Kidney damage after the use of copper compounds may occur through manypossibilities. For example they act as haptens, that could intervene in the body's defense mechanism and at the result behaving like foreign toxic substance just like other heavy metals[3].·Many factors prove their role in causing damaging effects by the use of these compounds of heavy metals such as the lethal dose of metal, total duration of exposure with metals, the total dose absorbed by the body, physical condition of the exposed person such as age gender and chronic illness as well as the route of drug administration[10].

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Functional unit ofkidney is nephrons as they filtered the whole blood so when these compounds of copper have been used, they are filtered through the nephrons and tubules of kidneys and then trapped in them, causing damage to the kidneys. Sometimes, it happens to irreversible damage of kidneys and can lead to death. Damage to the kidneys in terms of Glomerulonephritis would be occurred by the use of these toxic metal compounds.

After the use of these heavy metals compounds physical gaps (also called rent or holes) in the Glomerular Capillary Wall, the Glomerular basement membrane and in the Bowman capsule are developed initially. These gaps allow the clotting factors to be filled in the space of Bowman, and led to the formation of fibrin (due to the conversion of Fibrinogen into fibrin and polymers of delayed fibrinolysis) and elements (such as monocytes and lymphocytes) these events promote the formation of crescent as the Glomerularcrescent formation seems to represent a non-specific response to serious injury to the glomerular capillary wall. Glomerular crescents formation is defined as two or more layers of epithelial cells proliferate in the Bowman space due to inflammation causing by toxic substances. It is a histological marker of Glomerular injury. Similarly mesangial cell proliferation as well as adhesions also caused by these metallic compounds toxicity.

MATERIAL AND METHODS

It was an experimental intervention, randomized controlled study in adult rats. The study was conducted at University of Health Sciences, Lahore. The animals were kept in the animal house and experimental work was further processed in the pathology department of University of Health Sciences, Lahore. The total duration of this study was 18 weeks. Twenty male and female albino rats of Wister strain, 6-8 weeks of age; weighing 200-250gms were procured from University of Health Sciences Lahore. Albino rats were separated in different cages and maintained in the animal house of the University of Health

Sciences Lahore under controlled environment (temperature 22-25 c, humidity 65%±5) and light and dark cycle of 12 hours each. Each rat was to receive the prescribed dose on alternate days. Cage cards were used to indicate the group of albino rats.

Group 1: is the control group. It included 10 healthy rats receiving normal diet and tap water for 18 weeks on alternate days.

Group2: It included 10 rats and Cu compounds of 0.5 mg/kg body weight, mixed and homogenized with wheat flour, and dispensed as pellets, was given orally to the rats of this group on alternate days for 18 weeks.

Experimental schedule: Allrats were weighed before the commencement of the experiment. No sex differences were made and all rats were kept in separate cages. They received nutritionally standard diet and water. After acclimatization, rats were divided into two groups as mentioned above. Each rat was marked for its identification by giving a number on its back.

Copper Compounds in Diet: Copper compounds used in this study were analyzed chemically from Biochemistry Department of University of Health Sciences Lahore, for exact quantification of the heavy metal. According to the body weight (0.5mg/kg body weight) doses were calculated, mixed and homogenized with wheat flour and dispensed as pallets. This dose has been used in various studies (Parbu et al 2008). Oral LD_{50} of rats for Copper compound has been reported as 2mg/kg body weight (7).Doses have been translated from human dose to rat dose by normalization of the body surface area (Animal dose mg/kg=Human Dose mg/kg multiplied by Animal Km/Human Km); where Km is 5.9 for rat and 37 for human [(6)].The doses were adjusted daily according to the weight of each rat.

Dissection: At the end of experiment, animals were sacrificed under anesthesia and dissected according to proper procedure and ethics. All the rats were kept in jar containing cotton soaked in ether alcohol till death one by one and sacrificed.Sterile Instruments were used for the dissection of rats and an inscion was made vertically from xiphoid process to the pubic symphsis, the skin and the abdominal muscles were incised and retracted laterally. The kidneys were removed from the connective tissue coverings and examined both macroscopically and microscopically.

Gross examination of the kidney: Gross examination of the kidneys was normal. No grossly visible anomaly was seen.

Microscopic examination: Both kidneys were removed and fixed in 10% formalin solution for 72hours,3-5mm thick pieces were excised from the organ and each piece was placed separately in a single tissue cassette after labeling its identification .Automatic tissue processor was used for tissue processing and specimens dehydrated in 70%,95%.100%alcohol and cleared in Xylene for 18 hours. Paraffin blocks were prepared by placing tissue piece in a metal trough with its desired surface flat on the trays then infiltrated with molten paraffin (56-58 ºC) from the heated wax dispenser,. The blocks were labeled with the group of animal and kept for fifteen minutes in freezer before sectioningand then placing front of the block to expose the tissue, fixed in the Chuck of rotary microtome(Leica RM 2125).The microtome knife was fixed at an inclination of 3º towards block,. Ribbons comprising of 5µ thick sections were transferred to preheated water bath at temperature of 45 ºC.Individual sections were separated using a pointed probe ,then transferred to labeled albumenized surface of glass slides and slides were prepared for Hematoxylin& Eosin stain.

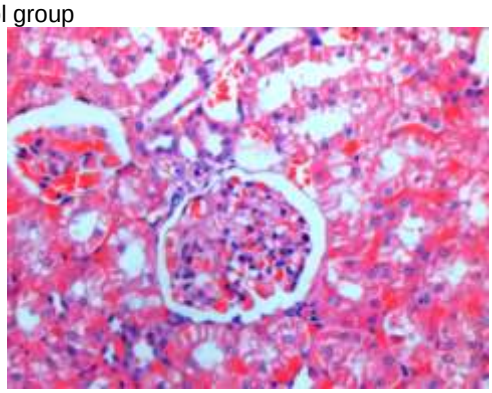
Microscopic observations: Microscopic examinations of slides prepared from kidneys of rats were performed to observe the changes in various components of the kidneys. These findings were documented in the relevant Performa (attached).

Glomeruli: Glomeruli were evaluated for Mesangial widening, Cellularity, Adhesions and Crescent formation.

RESULTS

Glomerular changes:Rats in all groups were sacrificed after completion of the experiment and their kidneys were removed and processed for routine histopathology examination. Histopathological examination in terms of Glomeruli shows that in Group-1 rat's mesangium and capillary wall were normal while in Group-2 mesangium was expanded and capillary wall was thickened in all rats. Necrosis, thrombosis, deposit of fibrin, crescent formation and sclerosis were absent in Group-1 rats while these features were present in rats of Group-2. Hyaline deposit was also significantly different in all groups. Mesangial proliferation was focal in some rats of group 2 and diffuses in other rats in group 2 while none of the rats in Group-1 had mesangial proliferation. Similarly mesangial cellularity was increased in Group-2 while none of the rats in Group-1 had increased cellularity.(Table-1)

Control group



Mesangial proliferation

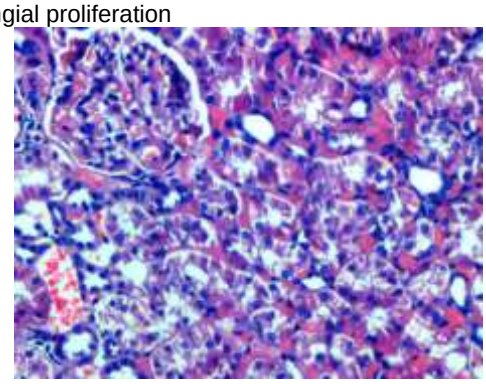
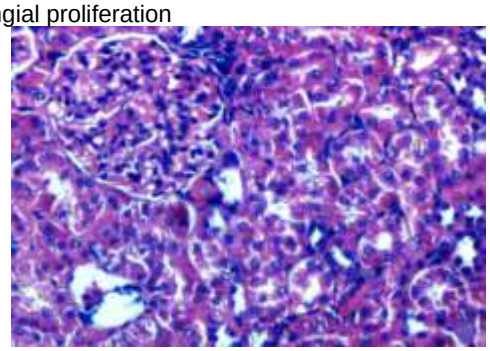


Table 1:

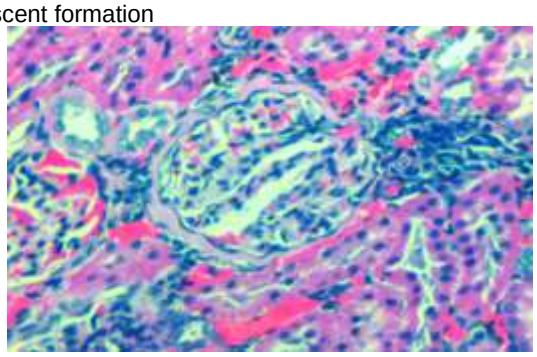
		Groups		
		Group 1	Group-2	P value
Mesangium	Expanded	0	10	0.000
	Normal	10	0	
Capillary Wall	Thickened	0	10	0.000
	Normal	10	0	
Necrosis	Present	0	10	0.000
	Absent	10	0	
Thrombosis	Present	0	10	0.000
	Absent	10	0	
Deposits (Fibrin)	Present	0	5	0.000
	Absent	10	5	
Crescent Formation	Present	0	5	0.000
	Absent	10	5	
Sclerosis	Present	0	8	0.000
	Absent	10	2	
Hyaline Deposits	Focal	10	0	0.000
	Diffuse	0	10	

Note**:** Significance Level*: p-value≤0.05

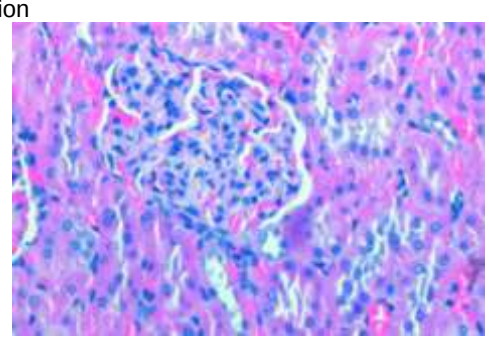
Mesangial proliferation



Crescent formation



Adhesion



DISCUSSION

The present study elaborates the clear association between toxicity of these metals and renal damageof various severities.25-50% glomerular adhesions were found in group 2. This is why we can consider a clear association between the intake of copper compounds and kidney damage.Albino rats which received diet of copper compound developed focal and diffusemesangial widening hence it explained the direct relationship of the toxicity of these heavy metals.Effects of mesangial cellularity were also likewise, the cellularity was remarkably increased in ten rats out of total twenty excluding ten rats of control group.Similarly thickening of capillary walls of glomeruli was shown more in group 2; it was of diffuse and focal thickness.

These finding are in contrast to the finding byRizkalla et al., 1993 in that study glomerular basement membrane were not affected by toxicity of some metals like copper cadmium etc.While the above described changes of glomeruli just like glomerular adhesions, , mesangial widening and increased mesangial cellularity irregular thickness of capillary walls are consistent with the results of other studies by various other researchers who have explored the renal damage after the exposure of copper metal as shown by El-Masry (2012) and Abu Zindah (2011)

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