



**Effect of Experimentally Induced Chronic
Kidney disease on Skeletal, Cardiac and
Smooth Muscle fibers of albino rat.
A light and transmission electron
microscopic study.**

Thesis

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Histology

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List of Abbreviations

Akt	: Family of protein kinase B enzymes
ATII	: Angiotensin II
ATP	: Adenosine tri-phosphate
BUN	: Blood urea nitrogen
CKD	: Chronic kidney disease
CRF	: Chronic renal failure
CVD	: Cardiovascular diseases
DALYs	: Disability-adjusted life-years
DCTs	: Distal convoluted tubules
DM	: Diabetes mellitus
DOCA	: Deoxycorticosterone acetate
ESRD	: End stage renal disease
FGF23	: Fibroblast growth factor 23
GBD	: Global Burden of Disease
GBM	: Glomerular basement membrane
GERD	: Gastroesophageal reflux disease
GFR	: Glomerular filtration rate
GM	: Gentamicin
GN	: Glomerulonephritis
HIV	: Human immunodeficiency virus
HT	: Hypertension
ICD	: International Classification of Diseases
IGF-1	: Insulin growth factor 1
IL-6	: Interleukin-6
IM	: Intramuscular

LES	: Lower esophageal sphincter
LV	: Left ventricle
LVH	: Left ventricular hypertrophy
MMP-2	: Matrix metalloprotease 2
NADPH	: Nicotinamide adenine dinucleotide phosphate
NFAT	: Nuclear factor of activated T-cells
NFκB	: Nuclear factor Kappa
PCTs	: Proximal convoluted tubules
PDGF:	Platelet-derived growth factor
PI3K	: Phosphoinositide 3-kinase
ROS	: Reactive oxygen species
SD	: Standard deviation
Smad 2/3	: Mothers against decapentaplegic homolog 2/3
TEM	: Transmission electron microscope
TGF-β	: Transforming growth factor-beta
THY-1	: THYmocyte differentiation antigen 1
TNF-α	: Tumour necrosis factor-alpha
UPS	: Ubiquitin proteasome system
VSMC	: Vascular smooth muscle cell

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Abstract

Background: Chronic kidney disease (CKD) is a serious disease affecting many organs but mainly the cardiovascular system.

Aim of the work: The aim of this study was to investigate the effect of experimentally induced chronic kidney disease by gentamicin intramuscular injection on the histological structure of gastrocnemius skeletal muscle, left ventricular cardiac muscle and smooth muscle fibers of lower esophagus.

Materials and methods: Twenty male adult Wistar albino rats were randomly and equally divided into two groups. Group I (control group) received physiological saline intramuscular injection, once daily for 28 consecutive days, in a dose equivalent to that taken in group II. Group II (Gentamicin-treated group) were given Gentamicin intramuscular injection for induction of CKD. Gentamicin was given as Gentamycin sulfate, 40 mg/ml (Sandoz, Switzerland), once daily, in a dose of 80 mg/kg/day for 28 days to induce CKD. After 28 days of the first injection of gentamicin, rats were anaesthetized and blood samples were collected to measure the level of serum urea and creatinine. The left kidneys, the middle third of left gastrocnemius muscle, the lateral wall of left ventricle (LV) and the gastroesophageal junction of all rats of both groups (I and II) were processed for light microscopic study. The middle third of left gastrocnemius muscle, the lateral wall of left ventricle (LV) were further processed for transmission electron microscopic study. Histomorphometrical and statistical analysis were carried out for mean cross sectional area of the transversely cut left gastrocnemius skeletal muscle fibers (40x), mean thickness of left ventricular (LV) wall (20x), mean area percentage of collagen fibers in-between the renal tubules (40x), mean area percentage of collagen fibers in the extracellular matrix of the left gastrocnemius skeletal muscle fibers (40x), mean area percentage of collagen fibers in the extracellular matrix of the cardiac myocytes (40x) and mean area percentage of collagen fibers in the extracellular matrix of the smooth muscle fibers, in the muscularis mucosa and the muscularis externa, of the gastroesophageal junction (40x) and all parameters were measured by using an image analyzer Leica Q win V.3 program installed on a computer in the Histology and Cell Biology Department, Faculty of Medicine, Ain Shams University. The computer was connected to a Leica DM2500 microscope (Wetzlar, Germany). The standard deviation (SD) was calculated and statistical analysis was carried out using SPSS program version 21. The data were evaluated using the independent samples T test. Values were presented as mean $\pm$

standard deviation (SD). With regard to the probability, the least significant level used was at P-value less than 0.05.

Results: The light microscopic examination of the kidney in group II (CKD) revealed moderate obliteration of glomeruli, dilatation in some renal tubules and collapse in others, mainly in distal convoluted tubules, with epithelial and granular casts and increased interstitial collagen deposition ($P < 0.05$) in renal parenchyma. Serum urea and creatinine levels were increased, $P < 0.05$ for both, compared with that of the control group. The skeletal muscle fibers of the rats in group II (CKD) showed fibers thinning and splitting with decreased cross sectional areas ($P < 0.05$), internalization of nuclei with clumping of heterochromatin, focal loss of myofilaments, increased sarcoplasmic glycogen granules, mitochondrial swelling, increased interstitial collagen deposition ($P < 0.05$) and mild mononuclear cellular infiltration. The cardiac muscle fibers of the rats in the group II (CKD) showed increased thickness of the wall of LV ($P < 0.05$), cardiomyocytes showed cytoplasmic vacuolations and pyknotic irregular nuclei with clumping and margination of the heterochromatin and irregular nuclear envelope, myofilamentous loss, disrupted intercalated disc, irregularly arranged mitochondria, disrupted sarcolemma with bleb formation and apparent increased interstitial collagen deposition ($P < 0.05$). The smooth muscle fibers of the lower esophageal sphincter of the rats in group II (CKD) showed no significant structural changes compared with the control group but the myenteric plexus of nerves was seen to have multiple pyknotic and karyolytic nuclei with vacuolated cytoplasm in addition to increase in the amount of collagen fibers ($P > 0.05$) in the lamina propria, inbetween smooth muscle bundles and in the submucosa and also inbetween the individual cells of muscularis mucosa and externa.

Conclusion: CKD in the early stage produced moderate atrophy of skeletal muscle fibers, significant increase in the cardiomyocyte size and no significant structural effect of smooth muscle fibers of the lower esophageal sphincter.

Keywords: gentamicin, sarcopenia, cardiomyopathy, chronic kidney disease, gastroesophageal reflux disease

Introduction

Chronic kidney disease (CKD) or chronic renal failure (CRF) as it was historically termed. This term includes all degrees of decreased renal function, starting from mild, moderate, to severe chronic kidney failure. It is a worldwide public health problem, with a rising incidence and prevalence within areas of poor outcomes (**Lameire and Van-Biesen, 2010**).

In Egypt, the estimated annual incidence of end stage renal disease (ESRD) is around 74 per million and the total prevalence of patients on dialysis is 264 per million. The prevalence of dialysis patients is presumed to be increasing. The main causes of ESRD in Egypt include diabetic nephropathy, hypertensive kidney disease, chronic glomerulonephritis (GN), chronic pyelonephritis, schistosomal obstructive uropathy, and schistosomal nephropathy (**El-Arbagy et al., 2016**).

This disease is more prevalent in the elderly population. However, the course of the disease is variable. While younger patients with CKD typically experience progressive loss of kidney functions, 30% of patients over 65 years of age with CKD have stable disease. International guidelines stated that regardless the underlying etiology, once the loss of nephrons and reduction of functional renal mass reached a certain point, the remaining nephrons soon begin a process of irreversible sclerosis that might lead to a

progressive decline in the glomerular filtration rate (O'Hare et al., 2007).

Skeletal muscle atrophy frequently complicates the course of CKD and is associated with excess morbidity and mortality (Wang et al., 2006). This was supported by Cano et al., 2007, who reported that several catabolic conditions, including CKD, might result in loss of muscle mass, which has no readily available treatments. Uremia was demonstrated to activate a mechanism of cellular protein catabolism which led to loss of muscle mass, and that loss would not be replenished by increasing dietary protein unless the catabolic mechanism had been blocked (Carrero et al., 2013).

Likewise, the incidence of cardiovascular complications, including hypertension, left ventricular hypertrophy, both systolic and diastolic dysfunctions and arrhythmia, have been reported to be about 20 times higher in CKD (Kundhal and Lok, 2005). Cardiovascular diseases have been reported to be the leading causes of death in CKD patients (Svíglerová et al., 2012).

Gastrointestinal disorders were also reported to occur frequently in children and adults with chronic renal failure. Many dialysis patients consider these symptoms to cause major impairment of daily life as nausea and vomiting (Punkkinen et al., 2001). Gastroesophageal reflux is often associated with esophageal and lower

esophageal sphincter motor dysfunction; the causes of which are not well understood (**Cekin et al., 2002**). Chronic Kidney Disease was also reported to be associated with an increased incidence of acid-related gastrointestinal disorders (**Stompor et al., 2002**).

Aim of the work

The aim of this study was to investigate the effect of experimentally induced chronic kidney disease by gentamicin intramuscular injection on the histological structure of gastrocnemius skeletal muscle, cardiac muscle and smooth muscle fibers of lower esophageal sphincter.

Review of literature

The kidney structure (Ross and Pawlina, 2016):

Histologically, the kidney is covered by a connective tissue capsule, and the renal tissue is divided into two distinct regions: **Cortex**, the outer dark part and **Medulla**, the much lighter-colored inner part. The cortex consists of renal corpuscles along with the convoluted tubules and straight tubules of the nephron, the collecting tubules, collecting ducts, and an extensive vascular supply. Vertical striations emanate from the medulla, representing the medullary rays of Ferrein. Each medullary ray contains straight tubules of the nephrons and collecting ducts. The regions between medullary rays contain the renal corpuscles, the convoluted tubules of the nephrons, and the collecting tubules.

The straight tubules of the nephrons and the collecting ducts continue from the cortex into the medulla. They are accompanied by a capillary network, the vasa recta, that runs in parallel with the various tubules. About 8 to 12 conical medullary pyramids, consisting of medullary tubules, are present with their bases facing the cortex, and the apices facing the renal sinus. Each medullary pyramid and the associated cortical tissue at its base and sides (one half of each adjacent renal column) constitutes a lobe of the kidney. The lobes of the kidney are further subdivided into lobules consisting of a central medullary ray and surrounding cortical tissue.