Exogenous melatonin induces histological changes in collecting tubules and ducts of male rat's kidney.

↔*Mustafa M. Ibraheem*

↔Assistant Lecturer
Department of Anatomy,
Histology & Embryology
College of Medicine
Al-Mustansiriyah University
Baghdad

Abstract:

Selective re-absorption and secretion are the functions of the collecting tubules and ducts, in addition to concentrate urine through ADH-regulated and ADH- independent water channels.

In this study, twenty four male rats were used; they were divided into two groups of animals: Group (A) included twelve rats of five weeks old age (before puberty) that were divided into three subgroups, four rats in each subgroup. Subgroup I was control one, subgroups II and III were treated orally with melatonin in a dose of 250 & 500 µg/kg body weights subsequently. Group (B) included twelve rats of seventeen weeks old age (after puberty) that were divided into the same subgroups and treated with the doses of melatonin as in the rats of group (A). In this experiment, the exogenous melatonin induced structural changes on the collecting tubules and ducts, in dose dependent manner as well as onset-dependent manner whether used in pre- or post-puberty of male albino rats.

Key Words: Kidney, collecting tubules and ducts, melatonin.

Correspondence to:
Mustafa M. Ibraheem

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Introduction:

Generally, the kidney can be divided into an outer cortex and an inner medulla. In human, the medulla comprises (10-18) renal pyramids and from the base of medullary pyramid which arise the medullary rays which penetrate into the cortex. Each ray consists of one or more collecting tubules with the descending and ascending limbs of the loop of Henle. [1]

Surrounding each medullary ray is the cortical labyrinth which consists of renal corpuscles and convoluted portions of the nephron. [2] The kidney of man contains many lobes where as the kidney of rat contains only one lobe, so it has a single medullary pyramid with a cap of cortical tissue [3].

The distal convoluted tubule which is the last part of the renal tubule of the nephron that joins the second component of the uriniferous tubule which is the collecting system that include collecting tubules and ducts, minor and major calyces, renal pelvis and ureter. [2]

The collecting duct is subdivided, with respect to localization, into cortical, outer medullary and inner medullary collecting duct. Collecting tubule and collecting duct are lined by a total of three different cell types: the collecting tubule by the collecting tubule cell and the intercalated cell; the collecting duct by the principal cell and the intercalated cell present only in the cortical and outer medullary segment. The intercalated cell can have different manifestations ("gray", "black", and "light").

The principal cell undergoes gradual alterations from a very complicatedly organized prismatic cell (approximately 7.5 micron cellular height) in the cortical collecting duct to a very simply organized, high prismatic cell (approximately 75 micron cellular height) in the inner medullary collecting duct. [8]

In this research, dealing with these previous different cellular types of the collecting tubule and duct as a single entity for simplification in histological and statistical analysis of the results. Song *et al* (1993) has identified iodomelatonin binding sites in the kidneys of mammals which supports the possibility of the direct action of melatonin on the renal system. Melatonin affects tubular uptake of sodium in mammals [5], it inhibits anti diuretic hormone [6] and decreases the blood urea and creatinine [7].

The aim of this study is to study the effects of different oral doses of exogenous melatonin on collecting tubules and ducts on different age groups regarding sexual maturation of rats.

Materials & Methods:

Male Wister albino rats, (24) in number were used; they were of two age groups. The first half of rats aged five weeks (before puberty) (**group A**), their average body weight was (64) gram at the beginning of the experiment, the second half aged seventeen weeks (after puberty) (**group B**), their average body weight was (312) gram at the beginning of the experiment.

The drug used in the experiments was; N-acetyl-5-methoxytryptamine (melatonin) tablets, from Nature's Bounty INC, Bohemia, NY11716, and U.S.A. The temperature was ranging between 20-24°C; they were housed individually in wire-meshed stainless steel cages. The light-dark cycle was 12:12, with free access to food, except for one and half hour prior to meal containing melatonin supply. Water was provided *ad libitum*.

Twelve rats of each age group (A&B) were divided into three subgroups, each consisting of four rats. Subgroup **I** was control one, subgroups **II** and **III** were treated orally with melatonin in a dose of 250 & 500 µg/kg body weight subsequently as a single daily dose for (14) days, the drug was given two hours prior to sunset. The animals were sacrificed by dissection under effect of diethyl ether; left kidney was removed when the heart was still beating, and it was separated from the surrounding connective tissues, fat and suprarenal gland, then bisected longitudinally through the pelvis of the kidney and used for paraffin sections. Transverse serial sections of kidneys of 3µm in thickness were stained with H. &E.

Results:

Although, collecting tubules and ducts are composed of cells that stain weakly with the H. & E. stains, the intercellular limits of their cells are clearly visible in the light microscope, figures (1 and 4). The medulla has a striated appearance due to the presence of collecting tubules.

In the cortical region, collecting tubules are regularly shaped in cross section as compared with the often irregularly shaped section of the nephron. The lining epithelium of the collecting system gradually increases in height from a simple cuboidal epithelium in the arched (collecting) tubules to a tall columnar epithelium in the papillary ducts (collecting ducts) which have large, more regular diameters and are comprised of uniform, lightly stained columnar epithelial cells. The collecting tubule and duct cells have spherical nucleus with prominent nucleolus, figure (1).

In transverse sections of the kidney of both groups that are stained with H&E, the measurement of the diameter of the lumen of the collecting tubules and ducts with the measurement of the height of their lining epithelium were studied.

The measurement of the diameter and the height of their lining epithelium were showing no significant difference between control rats and those treated with exogenous melatonin in a dose of 250 µg/kg body weight and 500 µg/kg body weight ($P>0.05$) (table 1 and 2) . Except, significant increase was seen in the diameter of five weeks old male rats treated with 500 µg/kg body weight ($P<0.002$) (table 1), figures (2 and 3). However, cellular debris or cast was seen in some of the lumen of collecting tubules and ducts of treated rats of both groups, but more in those of five weeks old male rats treated with 500 µg/kg body weights.

Table (1): *The effect of dietary exogenous melatonin on the diameter of the lumen of collecting tubules and collecting ducts in the renal cortex and the height of their epithelial cell lining in five weeks old male rats.*

Daily dose of melatonin(µg/kg)body weight	**Diameter of the lumen of the collecting tubules (in µm) (F1a)**	**Height of the epithelial cell lining of the collecting tubules (in µm) (F1b)**	**Diameter of the lumen of the collecting ducts (in µm) (F2a)**	**Height of the epithelial cell lining of the collecting ducts (in µm) (F2b)**
Subgroup I (control)	28 ± 2.05	11.7 ± 2.8	44.3 ± 2.7	14.2 ± 7.15
Subgroup II (250µg/kg)	28.3 ± 1.4	11.3 ± 3.75	44.7 ± 2.26	14.3 ± 6.4
SubgroupIII (500µg/kg)	29.6 ± 1.8 *	12 ± 2.5	45 ± 2	13.8 ± 7.7

☼ Values were presented as mean ± SD of 4 rats.

☼ Multiple groups comparisons were performed by analysis of variance (ANOVA).F1a= 6.7, *P<0.002.

F2a=0.42, NSP>0.85.

F1b=0.75, NSP>0.42.

F2b=1.594, NSP>0.215.

☼ Inter group's difference was assessed by the Student's t- test. Significance of difference in comparison with the control subgroup: NS= not significant (P>0.05), *P<0.002.

Table (2): *The effect of dietary exogenous melatonin on the diameter of the lumen of collecting tubules and collecting ducts in the renal cortex and the height of their epithelial cell lining in seventeen weeks old male rats.*

Daily dose of melatonin(µg/kg)body weight	**Diameter of the lumen of the collecting tubules (in µm) (F1a)**	**Height of the epithelial cell lining of the collecting tubules (in µm) (F1b)**	**Diameter of the lumen of the collecting ducts (in µm) (F2a)**	**Height of the epithelial cell lining of the collecting ducts (in µm) (F2b)**
Subgroup I (control)	30 ± 5.3	12 ± 1.1	45.2 ± 4.6	15.5 ± 4.2
Subgroup II (250µg/kg)	30.2 ± 6.2	11.8 ± 2.5	45.8 ± 4.1	15.35 ± 4.4
SubgroupIII (500µg/kg)	30.5 ± 6.7	11.6 ± 2.9	45.9 ± 4.8	15.23 ± 4.5

☼ Values were presented as mean ± SD of 4 rats.

☼ Multiple groups comparisons were performed by analysis of variance (ANOVA).F1a= 0.7, NSP>0.56.

F2a=0.093, NSP>0.912.

F1b=0.95, NSP>0.393.

F2b=0.28, NSP>0.87.

☼ Inter group's difference was assessed by the Student's t- test. Significance of difference in comparison with the control subgroup: NS= not significant (P>0.05).

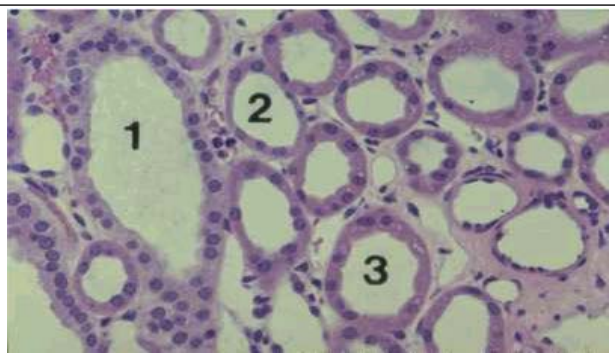

Figure 1: Transverse section of kidney's rat (five weeks old rat, control) in the cortical region demonstrates collecting duct (1), distal tubule (2) and proximal tubule (3). H&E stain, x400.

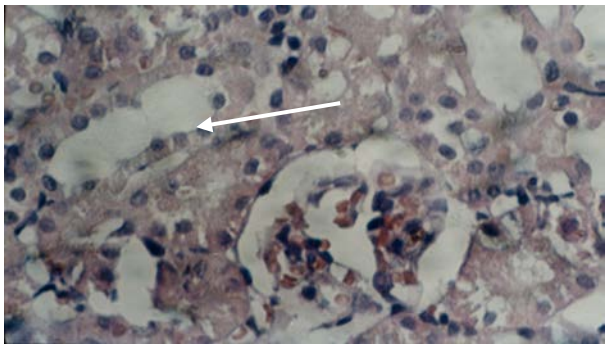

Figure 2: Transverse section of kidney's rat (five weeks old rat, treated with a dose of 500 µg/kg) in the cortical region demonstrates dilated collecting tubule (arrow).H&E stain, x400.

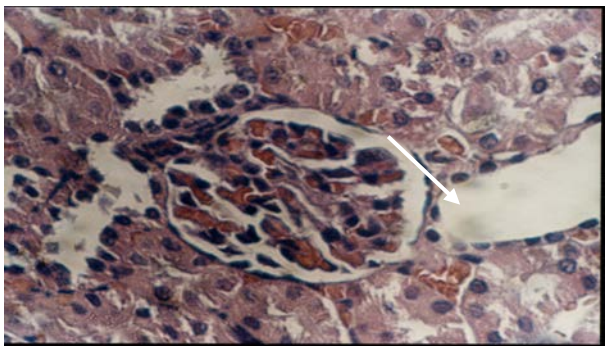

Figure 3: Transverse section of kidney's rat (five weeks old rat, treated with a dose of 500 µg/kg) in the cortical region demonstrates dilated collecting tubule (arrow).H&E stain, x400.

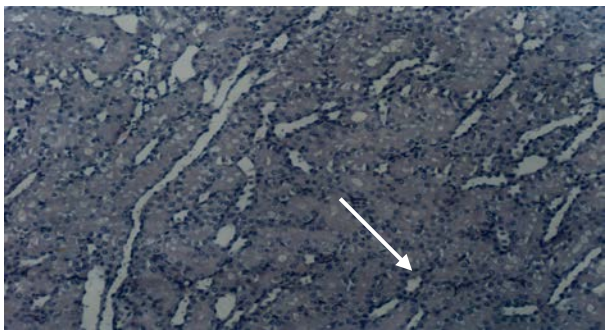

Figure 4: Transverse section of kidney's rat (seventeen weeks old rat, control) in the cortical region demonstrates collecting tubule (arrow). H&E stain, x100.

Discussion:

Study the effect of exogenous melatonin on the collecting tubules and ducts which represent the hypertonic region of the kidney where water is reabsorbed by the action of the hypophyseal antidiuretic hormone, thus the collecting tubules and ducts controlling the water balance of the body and are the major component of the urine-concentrating mechanism [2].

In this research which is concentrated on the study of histological changes in the kidney that associated with the supplement of exogenous melatonin in two different doses for relatively long period, of two weeks, melatonin being the only variable.

Generally, this study demonstrated that the kidneys of rats of group-A- (before puberty) were more affected by melatonin than that of group-B- (after puberty) in that the number of renal melatonin receptors is more before puberty [9].

In both groups, the kidneys of rats treated with the dose of 500 µg/kg body weight were more affected than those treated with the dose of 250 µg/kg body weight, in which melatonin effects are dose dependent [10 & 11]. The lumens of the collecting tubules and ducts contained cellular debris and cast. Walter and Talbot (1996) found that this material is consisting of glycoprotein substance which probably was shedding from the tubular cells, with blood cells, hemoglobin, myoglobin, plasma proteins and cell debris in urine to form the eosinophilic hyaline casts [13]. This observation is in agreement with the finding of Bolton and Rahman (2001), they recorded that the melatonin can be considered as nephrotoxic agent, in a dose-dependent manner.

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