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RESEARCH ARTICLE

STEREOLOGICAL INVESTIGATION OF CURCUMIN EFFECTS ON RENAL INJURY INDUCED BY DIETHYLNITROSAMINE EXPERIMENTALLY*

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*Ismail DENIZ's Master Thesis

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ABSTRACT

This investigation has been conducted to study the effect of Curcumin on renal injury induced diethylnitrosamine experimentally in rats. The rats were allocated into four groups each group contain seven animals; Control, Curcumin, Diethylnitrosamine, Curcumin+Diethylnitrosamine. Control group was injected by intra-peritoneal of saline (0.5 ml/kg) for 15 days, curcumin group given by gavage (200 mg/kg/day) for 15 days, diethylnitrosamine group was injected a single dose of diethylnitrosamine at the 5th day (intra-peritoneal, 150 mg/kg), curcumin+ diethylnitrosamine group was given by gavage curcumin (200 mg/kg/day) for 15 days and was injected a single dose of diethylnitrosamine at the 5th day (intra-peritoneal, 150 mg/kg). At end of the experiment, the rats were anesthetized then right kidney was taken for process was followed for light microscopic research. The kidney volume and glomerular number were calculated for each group using physical disector. A reduction of the kidney volume in diethylnitrosamine group and a significant decrease in the number of glomeruli has occurred in diethylnitrosamine group.

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INTRODUCTION

Curcumin is a crystalline powder substance that gives orange–yellow color to turmeric and it is responsible for turmeric's phytochemical effect. Curcumin is accounts for 3-5% of turmeric. Curcumin is a very powerful antioxidant and anti-inflammatory substance. Curcumin shows antioxidant efficacy by inhibiting reactive oxygene species (ROS) production (Sreeajavan and Rao, 1996). Curcumin's anti-inflammatory efficacy occurs by inhibiting cyclooxygenase (COX) pathways of arachidonic acid production. Curcumin's protective effects have been demostrated to against many nephrotoxic agents (Trujillo *et al*., 2013). Diethylnitrosamine that given to rats a single caused an increase in tumor incidence and renal carcinoma count (Mohr and Hilfrich, 1972). Both intragastric feeding and intratracheal instillation of diethylnitrosamine (DENA) induced multiple squamous-cell papillomas of the trachea and bronchi and carcinomas of the ethmoid region of the nasal cavity in the Syrian hamster (Mesocricetus auratus). Feeding DENA also induced proliferative lesions of the kidney and carcinomas of the liver (Herrold and Dunham, 1963).

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MATERIALS AND METHODS

Animals

We obtained ethical approval for our study from Yüzüncü Yıl University Animal Research Local Ethic Committee. 28 healthy wistar albino rats, weighing 250-300 gr and averaging 20 weeks old were utilized in this study. The rats were allocated into four experimental groups each group contain 7 animals: Control, Curcumin (CC), Diethylnitrosamine (DENA), Curcumin+Diethylnitrosamine. Control group was injected by intra-peritoneal (IP) of saline (0.5 ml/kg) for 15 days, CC group was given by gavage (200 mg/kg/day) for 15 days, DENA group was injected a single dose of DENA at the 5th day (I.P, 150 mg/kg) (Sigma). CC+DENA group was given CCby gavage (200 mg/kg/day) for 15 days and was injected a single dose of DENA at the 5th day (IP, 150 mg/kg). The rats were perfused after being anesthetized with ketamine 50mg/kg and xylazine 4mg/kg.

Histology

Following perfusion, right kidneys were removed and fixed in 10% neutral buffered formalin and washing the kidney were immersed in Bouin's solutions for repeat fixation. Right kidneys were dehydrated through a graded alcohol series,

cleared in xylene and infiltrated with paraffin for embedding. Serial sections of each kidney were cut in a vertical plane at 5µm using a rotary microtome. Selected sections were mounted on slides, hydrated through descending concentrations of alcohol series and stained with haematoxylin and eosin (H&E). Photos were taken by using a light microscope with a digital color camera attachment.

Stereology

We used the physical disector counting method for the total number of glomerulus. The physical disector consists of two paralel cross-sectional planes separated by a certain distance "t". One counts the particles that are visible on the reference section, but not the look-up section by unbiased counting frame. To achieve systematic random sampling for stereology, the first cross-section was chosen randomly while the next section was taken after cutting every 30.section. About nine to 11 sections were taken. The values obtained were multiplied by the sampling rate (Sterio, 1984).

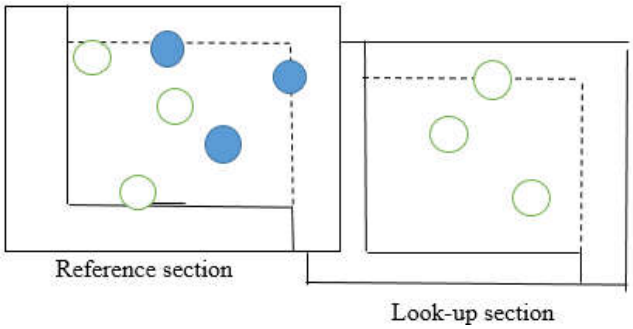

Figure 1. Unbiased counting frame. The blue particle seen in the reference section but not seen in the look-up section was counted. Thus, a disector particle (Q^-)=3

The total number of glomerulus in the kidney estimated using the formula:

$$N = N_v \, x \, V_{ref}$$

N_v is the numerical density of the particle of interest (glomerulus/unit volume) and V_{ref} is the total (reference) volume of the object. We used Cavalieri's principle for total kidney volume calculation. With the Cavalieri's principle, the kidney was divided into parallel slices at equal intervals with systematic random sampling from beginning to end.

Some random slices were taken and divided into spaced sections equally (t). The total areas of the received sections were calculated using the point counting grade (Fig.2). The total surface area obtained from all the slices was multiplied by the slice thickness to calculate the volume. The values obtained were multiplied by the sampling rate. We can formulate it like this:

$$V_{ref} = t \, x \, \Sigma a$$

''t'' is section thickness and ''Σa'' is total surface area in sections (Howard and Reed, 1998).

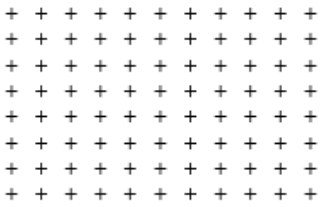

Figure 2. The point counting grid

Statistical analysis

Statistical Analyses were performed using Microsoft SPSS Version 13.0 for statistical analyses. Kruskal-Wallis test was performed to compare groups.

RESULTS

Total number of glomerulus

The total glomerular number were decreased in the DENA group compared to control group ($p<0.05$) while no significant change in the other group. The results suggest that Curcumin may prevent DENA induced nephrotoxity.

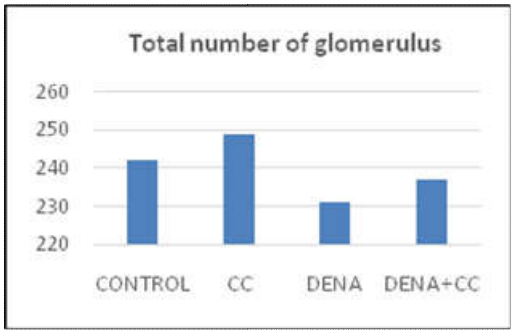

Figure 3. Total number of glomerulus

Table 1. Total number of the glomerulus

	Groups	Median	Mean	St. Dev.	Min.	Max.	p.*
Total number of the glomerulus.	Control	80421	81901a	13961	65251	106583	0.044
	CC	84100	85909a	16031	60121	101790	
	DENA	67509	66103b	7415	55240	76658	
	CC+DENA	74584	77461ab	13535	64892	106188	

Table 2. The total kidney volume

Volume (mm^3)	Groups	Median	Mean	St. Dev.	Min.	Max.	p.
	Control	224	242	54	198	356	0.560
	CC	263	249	42	173	304	
	DENA	223	231	25	206	271	
	CC+DENA	241	237	16	213	257	

Total kidney volume

No significant change in the o groups. However, there was a slight decline in the DENA group.

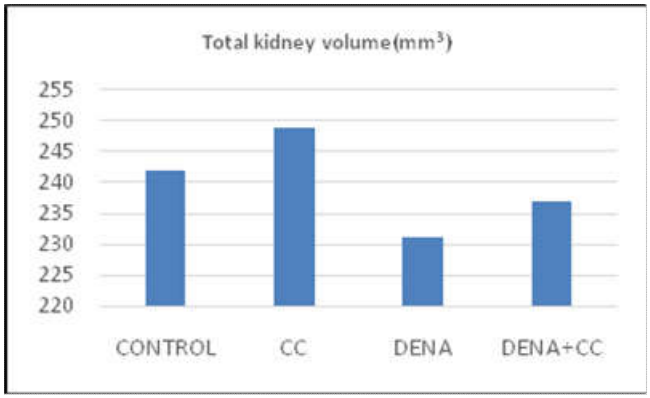

Figure 4. The total kidney volume

The coefficient variation (CV) and coefficient error (CE) values were calculated. CV values of groups; Control 0.1, CC 0.1, DENA 0.1, CC+DENA 0.1 and CE value of all groups<0.05.

by using different therapeutic strategies (Guyton and Hall, 2011; Trujillo *et al*., 2013). Curcumin has been used widely as an agent in foods. Recently, curcumin was found to possess chemopreventive effects against skin cancer, forestomach cancer, colon cancer and oral cancer in mice (Chuang *et al*., 2000). According to Chuang work, the curcumin group compared with the diethylnitrosamine group, had a 62% reduction in incidence of development of hepatocellular carcinoma (Chuang *et al*., 2000).It has been reported that curcumin prevents renal lesions in streptozotocin-induced diabetic rats (Babu, P.S. and Srinivasan, 1997). Curcumin has been show to protect against oxidative stress in a renal cell (Cohlya *et al*., 1998). Blood cholesterol was lowered significantly by curcumin streptozotocin induced diabetic rats (Babu, P.S. and Srinivasan, 1997).The Curcuma shows a high anti-inflammatory effect after parenteral application in standard animal models of inflammation according to Ammon (Ammon and Wahl, 1991).

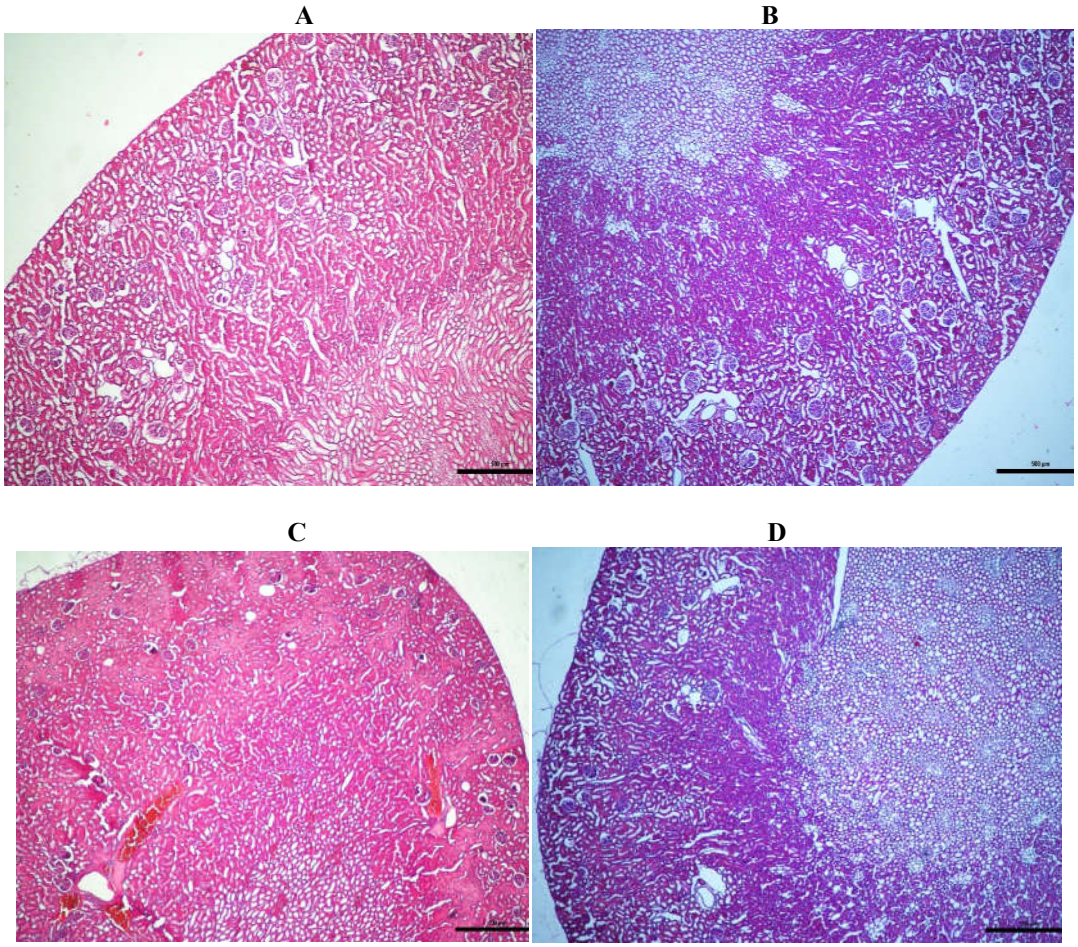

Figure 5. Microscopical section of kidney, A: Control group, B: CC group, C: DENA group, D: CC+DENA group (scale bar= 500µm)H&E x4

DISCUSSION AND CONCLUSION

The Kidney plays an important role in extracellular electrolyte, fluid balance and blood pressure. Patients have chronic kidney disease are at an increased risk of acute kidney injury (AKI). AKI might occur with the use of several drugs, such as non-steroidal anti-inflammatory drugs, antineoplastic drugs, antibiotics and angiotensin-converting-enzyme inhibitors. Acute and chronic renal failures can be treated and prevented

CC pre- and post-treatment resulted in a significant reduction in serum urea and creatinine levels in the contrast-induced nephropathy (Buyuklu *et al*., 2014).According to our study, in the DENA group the total glomerular number were decreased when compared to control group. But our result don't compared to another studies, because there is no any study about effect of CC on the glomerulus number by stereology method. CC group indicated an increase in glomerular number. Moreover, CC inhibited the renotoxic effect of DENA because total glomerular number increased in CC+DENA

group comparison to DENA group. Finally, DENA causeto reduce kidney volume and glomerular number. CC can be a protective agent against DENA in kidney injury.

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