

Full Length Research Paper

## Effect of fresh *Citrus limon* juice on the kidney histomorphology of growing rabbits (*Oryctolagus cuniculus*)

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The effect of *Citrus limon* juice on the kidney histomorphology of growing rabbits was investigated. A total of 24 rabbits, male and female (10-16 weeks), were weighed and distributed into 4 treatment groups. Control, which is group 1, was treated with normal diets without *C. limon* juice. 25% *C. limon* juice was administered to treatment group II, 50% *C. limon* juice was administered to treatment group III, and 75% *C. limon* was administered to treatment group IV. After 5 weeks of administration of the juice, animals were weighed and sacrificed. Kidney tissues were extracted, weighed and processed for histopathological analysis. Kidney weight of the experimental groups III and IV was significant ( $P < 0.01$ ), whereas that of treatment group II was not significant ( $P > 0.05$ ) and the weight gain in all the treatment groups was not significant when compared to that of the control group. The histopathological findings from the experimental groups II, III and IV revealed preserved cyto-architecture without any area of cellular abnormality when compared to the control group. In conclusion, *C. limon* juice does not pose deleterious or abnormal effects on the kidney of growing rabbits, as such, it can be included in rabbit feed or water.

**Key words:** Rabbit, kidney, *Citrus limon*, histomorphology.

### INTRODUCTION

For a long period in history, plants have been valuable and indispensable sources of natural products for the health of human beings as well as animals, thus having great potential for production of new drugs (Pellegrini et al., 2003).

The fruit of *Citrus limon* is used for culinary and non culinary purposes throughout the world. It is an excellent source of ascorbic acid which helps in preventing scurvy, developing resistance against infectious agents and scavenges harmful, pro-inflammatory free radicals from the blood (Olukanni, 2013). *C. limon* like oranges contains a variety of phytochemicals namely: hesperetin, naringin and naringenin flavonoid glycosides. Naringenin is found to have bio-active effect on animal health as antioxidant, free radical scavenger, anti-inflammatory, and immune system modulator (Monforte et al., 2005). It is a scientifically established fact that citrus fruits, especially *C. limon* by virtue of their richness

in vitamins and minerals, have many proven health benefits. Moreover, it is now beginning to be appreciated that the other biologically active, non-nutrient compounds found in citrus fruits such as phyto-chemical antioxidants, and soluble as well as insoluble dietary fibres are helpful in reducing the risk for cancers, many chronic diseases like arthritis, obesity, coronary heart diseases and kidney failure (Peterson et al., 2006). Limonins found in *C. limon* could also be anti-carcinogens. Citrus flavonoids improve the permeability of vascular vessels; they show anti-phlogistic effects and diuretic properties. Citrus flavonoids inhibit bacterial mutagenesis (Calomme et al., 2006).

Rabbits are herbivores that feed on forages and

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concentrates. Their diets contain large amounts of cellulose, which are hard to digest. Apart from being a good source of white meat which is “pearly white” and low in fat and cholesterol, they provide useful wool (fur), skins, manure, toys and novelties (Banerjee, 2005). The rabbit is the only farm animal which produces meat at 10-15 times or more its own weight in a year through progenies.

The primary function of the kidney is the formation of urine. The kidney performs a number of functions which help maintain physiological integrity of the extracellular fluid volume. These processes are: conservation of water, fixed cations, glucose, and amino acids; conservation being used in the broad sense to imply the return to the body fluids of the amount of the substance required by the body. Malfunctioning of the kidney can lead to renal failure and chronic kidney diseases which may be caused by severe dehydration, sudden loss of blood, obstruction due to stones or cancerous growth and injury.

Abnormalities arising from the inability of the kidney to adequately regulate fluid electrolyte, acid-base balance and to adequately excrete metabolic waste products can pose a great challenge to rabbit production. The farmer's ability to breed his animals successfully has a high dependence on the health status of the animals used. The needed improvement of rabbit performance may be achieved by investigating the effect of medicinal plants like *C. limon* on the kidney of rabbits. Considering the use of rabbits in research, food and drug industries, this work was carried out to study the effect of *C. limon* juice on the kidney histomorphology of growing rabbits and to determine the possible cyto-architectural effects.

## MATERIALS AND METHODS

### Materials and reagents

The following materials were used in this study: rabbits (24), hutches, concentrate feed (grower mash), syringes and hypodermic needles, universal treated bottles, latex hand gloves, weighing balance, centrifuge, graduated vials, measuring tape, *C. limon* juice, microscope, microtome, oven, paraffin wax, embedding moulds, staining racks, slides, cover slide, chloroform, feeders and drinkers. Reagents used were: normal saline solution, sodium metabisulfite, metaheamoglobin, sodium chloride, potassium chloride, mountant, neutral buffered formalin, absolute alcohol, eosin, xylene, iron allum, mountant (DPX) and paraffin wax.

### Experimental animals and management

Twenty four rabbits, aged 10-16 weeks old, were purchased from reputable farms in Uyo metropolis for the experiment. They were acclimatized for two weeks before the administration of the treatment (*Citrus limon* juice).

The rabbits were divided into four groups; each group comprised 6 animals, 3 males and 3 females. Two mild strains of rabbits were used: the chinchilla and New Zealand white. The experiment was carried out at the Rabbitry unit of the Teaching and Research Farm of Animal Science Department, University of Uyo, Akwa Ibom State, situated on latitude 5°02'32 N and longitude 7°54'06E and lies at an altitude of 120 m above sea level with average rainfall of 1500 mm. The state is in the south-south geopolitical zone of Nigeria.

### Experimental plan and fresh *Citrus limon* juice administration

The experiment was designed to have four treatments:

- Treatment 1 - Distilled water for 5 weeks;
- Treatment 2 - 25% *Citrus limon* juice concentration + 75 ml of water for 5 weeks;
- Treatment 3 - 50% *Citrus limon* concentration + 50 ml of water for 5 weeks;
- Treatment 4 - 75% *Citrus limon* concentration + 25 ml of water for 5 weeks.

The variety of *C. limon* used was the rough lemon bought from a reliable source at Itam Market, Uyo, Akwa Ibom state, Nigeria. The rabbits were weighed before the administration, progressive administration was given with time. *C. limon* were peeled and the juice squeezed out into a clean container manually. The extract was filtered using a clean sieve and the filtrate collected into clean bottles.

### Sample collection and histopathological analysis

The rabbits were anesthetized with chloroform vapour soaked in cotton wool in a dessicator for 24 h after the last juice administration. The animals were dissected and the kidney of each rabbit was carefully cut out, trimmed of all fat and connective tissue and weighed immediately. The tissues were immediately fixed in neutral buffered formalin in well labelled Petri-dish. The extracted kidney tissues were placed in 10% neutral buffered formalin (fixation), transferred to a graded series of ethanol (dehydration). On day 1, they were placed in 70% alcohol for 7 h, then transferred to 90% alcohol and left in the latter overnight. On day 2, the tissues were passed through three changes of absolute alcohol for an hour each then cleared in xylene (clearing). Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Three changes of molten paraffin wax (impregnation) at one-hour interval were made, after which the tissues were embedded (embedding) in wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientated perpendicularly to the long axis of the tissues. The sections were designated “vertical sections”.

**Table 1.** Weight gain and kidney weight of rabbits as affected by *Citrus limon*.

Group	Weight gain (g)	Kidney weight (g)
Group 1 (Control at 0%)	456.7 ± 33.09	9.30 ± 0.134
Group 2 (25%)	386.0 ± 29.11 <sup>ns</sup>	8.35 ± 0.961 <sup>ns</sup>
Group 3 (50%)	390.3 ± 58.84 <sup>ns</sup>	12.15 ± 0.469 <sup>n**</sup>
Group 4 (75%)	335.2 ± 57.27 <sup>ns</sup>	11.90 ± 0.357*

Means ± SEM (n=6); ns = Not-significant (P> 0.05), \*\* = very significant (P< 0.01), \* = significant (P< 0.05).

Serial sections of 5 µm thick were obtained from a solid block of tissue (microtomy), fixed on clean albuminized slides to prevent sections coming off the slides and later stained with haematoxylin and eosin staining techniques, after which they were passed through ascending grade of alcohol, cleared in xylene and mounted in DPX mountant, allowed to dry at room temperature and observed histopathologically under digital light microscope.

### Gross morphological and statistical analysis

The initial and final weight of each rabbit and the weight of each kidney in each group were taken using sensitive weighing balance graduated at 0.000 g. Analysis was carried out using Graphpad prism 6 version 2; all data were expressed as mean ± SEM. One way analysis of variance (ANOVA) was used to test for difference among the groups. Dunnet's multiple range tests was used to test for significant differences among treatment means. P<0.001 was considered extremely significant, P<0.01 was very significant and P>0.05 was non-significant.

### Photomicrography

Histological results were obtained by photomicrography using digital photomicrographic microscope at the Gross Anatomy Research Laboratory, Department of Human Anatomy, College of Health Sciences, University of Uyo, Uyo, Akwa-Ibom State, Nigeria as illustrated in Plates 1 to 4.

## RESULTS

### Histological finding

**Plate A:** Control Kidney without treatment at magnification revealed normal cellular profile of proximal and distal convoluted tubules, collecting ducts, renal corpuscle containing glomerulus and lined with squamous epithelial within normal cellular architecture.

**Plate B:** kidney treated 25% concentration of Citrus limon juice revealed normal cellular profile, no abnormality seen as compared to control group

**Plate C:** kidney treated 50% concentration of Citrus limon juice revealed normal cellular profile, no abnormality seen

as compared to control group

**Plate D:** kidney treated 75% concentration of Citrus limon juice revealed normal cellular profile, no abnormality seen as compared to control group

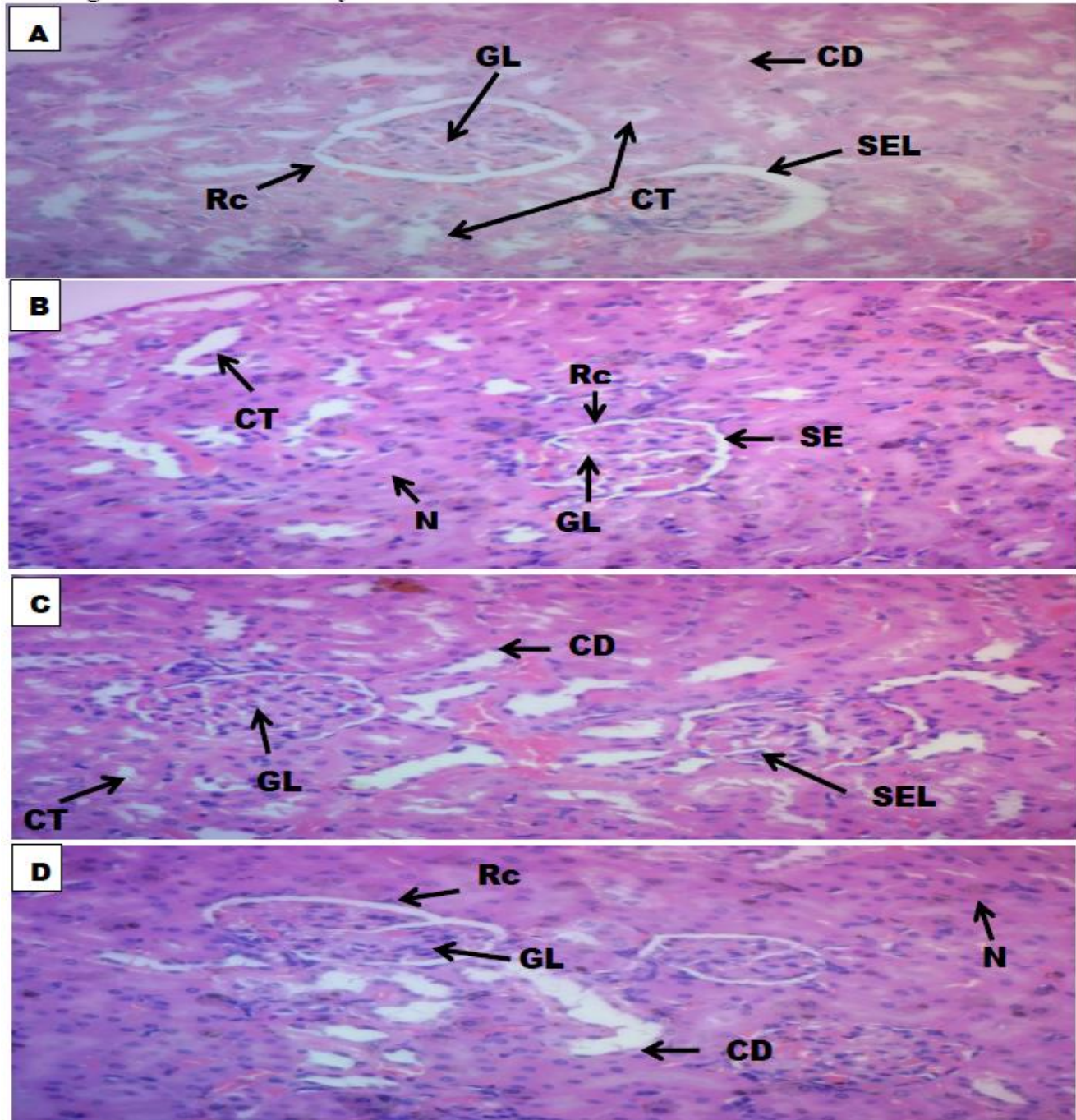
### Body weight and Organ weights

It was revealed that there was a slight increase in the Kidney weight of the treated groups, the group treated with 25% was slightly reduce in weight while the 50% and 75 % treated groups gained weight exponentially. Meanwhile the total body weight of the animal among the treatment group was decreased in a normal proportion.

## DISCUSSION

The histopathology of the effect of the different concentrations of *C. limon* juice revealed no changes in the kidney structure in all the treated groups as illustrated in Plates B to H when compared to the control group (Plates A and B). The histological features of kidney has been known to reveal detail information on abnormality or disorder whenever there is danger or assault on the kidney tissues, ranging from the cortex to pyramid. It was observed that *C. limon* preserved and enhanced kidney cyto-architecture in this experiment. The functional unit of the kidney (nephron) revealed the well preserved cellular architecture of *C. limon* containing high content of flavonoid which enhances cellular structure with its molecular compounds. The presence could be an enhancing factor which prevent the tissues from been damaged, thereby increasing the cellular profiles. This is very important as it enhances the effects of Vitamin C and provides a powerful defense mechanism against oxidative stress. They have powerful anti-inflammatory benefits, though they may also act as chemical messengers, physiological regulators and cell cycle inhibitors.

The kidney plays a central role in the excretion of waste products and maintenance of acid base balance. It aids selective re-absorption and ultra filtration thereby enhancing production of hormone regulating factors and water retention. Meanwhile kidney weight was very significant in the treatment groups when compared to the control group and the weight gain was not significant in all the treated groups as illustrated in Table 1 and Figure



**Figure 1. Plate A.** Control Kidney without treatment stained with H&E method, Mag. X400. **Plate B.** Kidney treated with 25% *C. Limon* juice stained with H&E method, Mag. X400. **Plate C.** Kidney treated with 50% *C. Limon* juice stained with H&E method, Mag. X400. **Plate D.** Kidney treated with 75% *C. Limon* juice stained with H&E method, Mag. X400. **Keys:** Renal corpuscle (RC) Convoluted tubules (CT), Collecting ducts (CD), Squamous epithelial lining (SEL), Glomerulus (GL) and Nucleus (N).

1. This indicated that administration of *C. limon* to rabbits had a positive effect on the kidney in accordance with the studies of Manners (2007) who reported that citric acid present in *C. limon* helps in dissolving kidney stones, and Benavente-Garcia et al. (2007) who reported that ascorbic acid present in *C. limon* is a natural antioxidant.

## CONCLUSION

From this study, it was observed that administration of *C. limon* juice to rabbits has a positive effect on the kidney cellular properties. It can be suggested that the breeder should supplement animal feed with this beneficial fruit as

it will help improve animal health and prevent any incidence of kidney malfunctioning.

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