

Full Length Research Paper

Changes in serum electrolyte, creatinine and urea of fresh *Citrus limon* juice administered to growing rabbits (*Oryctolagus cuniculus*)

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Changes in serum electrolyte, creatinine and urea of fresh *Citrus limon* juice administered to growing rabbits were investigated. A total of 24 rabbits, male and female (10-16 weeks), were weighed and distributed into 4 groups. Control, group 1, was administered with normal diets without *C. limon* juice. 25% *C. limon* juice was administered to treatment group 2; 50% *C. limon* juice was administered to treatment group 3; and 75% *C. limon* juice was administered to treatment group 4. After 5 weeks of administration of juice, animals were weighed and sacrificed. Blood samples were taken into plain bottles and centrifuged, after which serum was collected and sent to laboratory for kidney function assay. Analysis was carried out on electrolyte such as sodium, potassium, creatinine and urea. Result of the sodium and potassium electrolyte was not significant in all the *C. limon* treatment groups as compared to the control group, while the serum creatinine and urea were extremely significant when compared to the control group at $P < 0.001$. In conclusion, it was revealed that *C. limon* juice did not pose deleterious or abnormal effects on kidney function parameters of growing rabbits, rather it encouraged adequate and functional clearance.

Key words: Rabbit, kidney, *Citrus limon*, creatinine, urea, body weight.

INTRODUCTION

Among several beneficial plants, citrus fruit is very popular in many parts of the world due to its distinctive flavour, taste, aroma and also multiple health benefits associated with it. The consumption of citrus fruits or their products is believed to have beneficial effects against different diseases, the main reason being the presence of important bioactive compounds (Pellegrini et al., 2003; Peterson et al., 2006). It is now widely known that vitamin C (ascorbic acid) and carotenoids phenolic compounds play an integral role in total antioxidant capacity of citrus fruits (Gorienstein et al., 2004). The major phenolic compounds detected in different citrus fruits are categorized as flavonoids and phenolic acids (Balasundram et al., 2006).

According to International Journal of Science and Research (2013), 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). Lemons like oranges contain a variety of phytochemicals. Hesperetin, naringin and naringenin are flavonoid glycosides. Naringenin is found to have bio-active effect on human health as antioxidant, free radical scavenger, anti-inflammatory, and immune system modulator (Monforte et al., 2005). It is scientifically established fact that citrus fruits, especially lemons by virtue of their richness in vitamins and minerals, have

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many proven health benefits.

Rabbits are herbivores that feed on forages and concentrate, apart from being a good source of white meat which is “pearly white” and low in fat and cholesterol. They can be used for patients and they provide useful wool (fur), skins, manure, toys and novelties (Banerjee, 2005). Rabbit is the only farm animal which produces meat at 10-15 times or more its own weight in a year through progenies. Being such a prolific multiplier, it is expected to ease the demand of pressure on chicken and mutton. Rabbits are efficient converters of feed to meat. They also produce skins of pelts and fur which are very important raw materials in the leather industry. They produce high class protein characterised as lean meat and excellent fur.

The primary function of the kidney is the formation of urine. In this, the kidney performs a number of function 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. The excess being excreted into urine, elimination of nitrogenous end products of protein metabolism, primary urine, creatinine, and ammonia, elimination of excess hydrogen ions and the maintenance of physiological pH of the body fluids, and elimination of complex organic compounds both endogenous and exogenous (Kluwe, 2001).

From literature search, although the effects of *C. limon* on urinary compositions and its laxative effects have not been reported, no study has clearly demonstrated its influence on plasma electrolytes, urea, and creatinine in association with urinary composition changes. This study thus sought to investigate the effect of *C. limon* juice on serum electrolytes, urea, and creatinine as indicators of renal functions. Such 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.

MATERIALS AND METHODS

Materials and reagents

In this study, the following materials were used: 24 rabbits, hutches, concentrate feed (grower mash), syringes and hypodermic needles, universal treated bottles, latex hand gloves, weighing balance, centrifuge, graduated vials, measuring tape, and *C. lemon* juice. Reagents used were: Turll solution, sodium metabolite, metaheamoglobin, sodium chloride, potassium chloride, mountant, formalin, absolute alcohol, creatinine, urea, and electrolyte kits for rabbit from Biochain Inc. USA.

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 (*C. 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°54'06 E and lies at altitude of 120 m above sea level with average rainfall of 1500 mm. The state is in the south-south geopolitical zone, 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% *C. limon* juice concentration + 75 ml of water for 5 weeks.

Treatment 3 - 50% *C. limon* concentration + 50 ml of water for 5 weeks.

Treatment 4 - 75% *C. 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 determination of electrolyte, urea and creatinine

Blood was collected from the heart of the dissected animal using syringes and needles. The collected blood samples were put into well labelled sterile plain bottles. The blood samples were centrifuged using the centrifuge machine and serum was collected into well labelled plain bottles, while the blood cells were discarded. The serum was taken to the laboratory for kidney function tests. Serum sodium and potassium levels were determined using the Flame photometry method (410 flame photometer, Chiron Diagnostics) following the manufacturer's guidelines (Akhigbe et al., 2008). Serum bicarbonate, urea, and creatinine were determined using the standard assay kit following back titration, diacetyl monoxime, and alkaline picrate methods, respectively.

Statistical analysis

Analysis was carried out using Graphpad prism 6 version 2; all data were expressed as mean \pm SEM. One way analysis of variance was used to test for difference

Table 1. Rabbit serum electrolyte, urea and creatinine indices as affected by fresh *Citrus limon*.

Parameter	Control	25%	50%	75%
Sodium	136.2 ± 3.198	136.8 ± 2.651 ^{ns}	144.7 ± 1.382 ^{ns}	148.3 ± 1.229*
Potassium	4.167 ± 0.307	4.683 ± 0.245 ^{ns}	4.867 ± 0.224 ^{ns}	5.367 ± 0.140 ^{ns}
Bicarbonate	19.85 ± 1.400	22.170 ± 0.792 ^{ns}	22.50 ± 0.176 ^{ns}	23.83 ± 1.332 ^{ns}
Creatinine	95.000 ± 0.7071	82.000 ± 1.140***	96.000 ± 0.7071***	76.000 ± 1.140***
Urea	9.200 ± 0.1414	7.400 ± 0.1414***	6.900 ± 0.1000***	6.500 ± 0.1000***

Means ± SEM (n=6); *** = extremely significant (P<0.001); * = significantly (P<0.05); ns = not-significant (P>0.05).

among the groups. Dunnet's multiple range test was used to compare the significant differences among the means. P<0.001 was considered extremely significant, P<0.01 was very significant, P<0.05 was significant and P>0.05 was not significant.

RESULTS AND DISCUSSION

This study aimed at investigating the effect of *C. limon* on renal function by evaluating the serum concentrations of electrolytes, urea, and creatinine. This appears to be the first study to investigate and document changes in serum electrolytes as indices of renal function following *C. limon* treatment in association with changes in histomorphological studies composition previously reported. The result from this study demonstrates that *C. limon* treatment does not induce electrolyte imbalance in experimental animals. Sodium is the most abundant extracellular ion, and it plays an important role in muscle contraction. Similarly, potassium, an abundant intracellular ion, plays a vital role in muscle contraction. The intact electrolyte levels within normal range in rabbits resulting from the serum level of sodium seen in this study thus provides evidence that the use of *citrus limon* does not present risk for arrhythmia, abdominal pain and cramping, and muscle weakness as revealed in the experimental groups that the sodium and potassium levels were not significant when compared to the control groups. The creatinine and urea concentration were extremely significant in all the treated groups as compared to the control group at P<0.0001 as represented in Table 1. Creatinine clearance calculated from creatinine concentrations plasma sample is used to determine the glomerular filtration rate of the kidneys. Although not commonly done anymore, they remain useful tests for renal function. Thus, plasma concentrations of creatinine and urea could be used as indicators of nephrotoxicity. Low clearance of creatinine or/and urea indicates a diminished impaired ability of the kidneys to filter these waste products from the blood and excrete them in urine. As their clearance values decrease, their blood levels increase. Hence, an abnormally elevated blood creatinine is diagnostic of impaired renal function (Pagana et al., 1998; Wallech,

2009; Henry, 2001).

The result indicated that administration of *C. limon* to rabbits had a positive effect on the kidney in accordance with 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. It also showed that lemon juice has an effect on the level of uric acid in the kidney. Kang et al. (2007) reported that when lemon has been fully metabolized in the body, the pH of the body is raised. This implies that reduced level of uric acid improves kidney function.

Conclusion

From this study, it was observed that administration of *C. limon* juice to rabbits has a positive effect on the kidney. 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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