

Effect of Citric Acid as Food Additive on the Renal Function of Experimental Rats

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Abstract: This study was aimed to investigate the effect of oral administration of citric acid on the renal functions of white American rats. Thirty two rats were divided into four groups (8 rats each). One group serves as a control. The rest of the three groups were differently treated (at rates of 100, 500 and 1250 mg kg⁻¹ body weight were applied) with citric acid. Compared to the control, sudden ($p \leq 0.05$) increase in the serum creatinine and urea nitrogen levels in the rats with increasing the dose of citric acid (100 - 1250 mg kg⁻¹ body weight) was observed. Results also revealed an insignificant difference in serum creatinine and urea nitrogen on administering citric acid. Kidney changes in the rats received different doses of citric acid was observed.

Key words: Renal function, Citric acid, Food additive, Creatinine and Urea nitrogen

INTRODUCTION

Citric acid is a weak organic acid found in citrus fruits. It is a natural preservative and is also used to add an acidic (sour) taste to foods and soft drinks. Citric acid is used along with sodium bicarbonate in a range of effervescent formulae, both for ingestion and for personal care. Citric acid exists in a variety of fruits and vegetables, but is most concentrated in lemons and limes where it comprises as much as 8% of the dry weight of the fruit (Garden *et al.*, 2003). The major industrial route for citric acid production is by feeding cultures of *Aspergillus niger* on sucrose. Sometimes a high concentration of citric acid can damage hair (Assimos *et al.*, 2008).

Contact with dry citric acid or with concentrated solutions can result in skin and eye irritations. Excessive consumption is capable of eroding the tooth enamel (Hossner, 2005). It is an important intermediate in the citric acid cycle and therefore occurs in the metabolism of almost all metabolically living organisms. Mutations in enzymes that catalyze steps in the citric acid cycle result in human diseases with various clinical presentations. It has been found that a protein (GPR91) functions as a receptor for the citric acid intermediate succinate. This protein has been shown to play a possible role in renovascular hypertension and renal failure (He *et al.*, 2004).

MATERIALS AND METHODS

Biological Experiment:

Thirty two of white American rats of different sex, weight (0.8-1.4 g) and age (2-4 weeks) were provided the basal diet for a week as an adaptation period, as shown in (Table 1).

Table 1: Shown the Composition of Basal Diet of Rats During Experiment

Ingredients	Beef meat	Sesame oil	Corn flour	Table salt
Quantity (g)	250	1110	1000	7

The rats were divided into four groups, each with 8 rats. Rats were allowed free access to feed and water during the whole period of the experiment that lasted on the 28th day. Oral administration of citric acid was provided to 3 groups. Oral doses of citric acid at rates of 100, 500 and 1250 mg kg⁻¹ body weight were applied to 3 groups (100, 500 and 1250 mg kg⁻¹ body weight). One non-treated group served as the control. Clinical symptoms were observed. Blood was collected from blood capillaries of rats' eyes and stored at 5°C until analysis. Before chemical analysis the clotted blood was centrifuged at 30000 rpm to obtain serum.

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Measurement of Creatinine Concentration:

Serum creatinine level was determined by the method of Fabiny and Etlingshausen (1971). One hundred microliter of blood serum was transferred into a spectrophotometer cuvette. One milliliter of creatinine kit reagent [equal volumes of reagent 1 (picric acid, 8.73 mmol L⁻¹) and reagent 2 (sodium hydroxide, 312.5 mmol L⁻¹ and disodium phosphate, 12.5 mmol L⁻¹)] was added, mixed well and then left for 10 sec at room temperature. The absorbance was read at 500 nm using a spectrophotometer (Model No. 1904 plus, serial No. 1904-5252). A blank was used to calibrate the spectrophotometer. Creatinine was used as reference standard.

Measurement of Urea Concentration:

Urea kits were used to determine urea N concentration in blood serum samples according to the method of Chaney and Marbach (1962). One hundred µL of blood serum were transferred into a test tube. One milliliter of reagent 1 [(Phosphate buffer, 120 mmol L⁻¹, Sodium salicylate, 60 mmol L⁻¹, Sodium nitroproside, 5 mmol L⁻¹, EDTA 1 mmol L⁻¹ and Urease 5 KU L⁻¹)] was added, mixed and left to stand for 5 min at room temperature. Thereafter, 1 mL of reagent 2 [(Phosphate buffer, 120 mmol L⁻¹, Sodium hydroxide, 400 mmol L⁻¹ and Sodium hypochlorite, 10 mmol L⁻¹)] was added and left for 10 min before the absorbance was read at 600 nm using a spectrophotometer (Model No. 1904 plus, serial No. 1904-5252). A Blank and urea standards were prepared and read as before.

Statistical Analysis:

Mean values of each parameter for differs serum sample were computed. Data were subjected to the ANOVA under a randomized design. Duncan's multiple test was applied for each multiple mean comparison, using the SPSS (version 14.0). The level of significance was ≤ 0.05.

RESULTS AND DISCUSSION

Toxicological Study:

In this study, oral administration of citric acids at rate of 100, 500, and 1250 mg Kg⁻¹ body weight resulted in significant (p≤0.05) gradual increase in the level of serum creatinine in white American rats from 0.9 ml dl⁻¹ for the control to 1.7 ml dl⁻¹ for the rats received 1250 Kg⁻¹ body weight(Table2). Similar significant (p≤ 0.05) gradual increase in the level of serum urea nitrogen in white American rats from 1.3 ml dl⁻¹ for the control to 38.9 ml dl⁻¹ for the rats received 1250 Kg⁻¹ body weight (Table 2).

Table 2: Effect of citric acid on serum creatinine and urea N levels of rats.

	Oral dose treatment(Citric acid) mg Kg ⁻¹ body weight			
Metabolite test	Control	100	500	1250
Serum creatinine (mldl ⁻¹)	0.9±0.10	1.1±0.15	1.4±0.15	1.7±0.15
Serum urea N, (mldl ⁻¹)	1.3±0.23	29.8±0.1	30.3±1.7	38.9±3.

An increase in plasma urea nitrogen indicated impairment of renal function (Marshall and Bangert, 2004). Typical reference ranges for the serum creatinine are 0.5-1.0 mg dL⁻¹ for the women and 0.7-1.2 mg dL⁻¹ for men (Gross *et al.*, 2005).

Clinical Observations:

Clinical observations on the experimental rats revealed symptoms of quick movement and convulsions within the first week after oral administration of citric acid. After that The rats became quiet and sleepy for a while and then they showed some nervous movement that led to injuries of some rats. By the end of the experimental period almost all the rats died.

Kidney Dissection:

The kidney cross sectional area of rats non- treated with citric acid showed no inflammatory changes and hydrobic degeneration of white American rats (Fig1a), rats orally treated with citric acid at rate 100 mg kg⁻¹ indicated lobulated tufts, distinct Bowman's spaces, some tubules show desquamated cells and also some tubules show moderate dilatation of white American rats (Fig 1b), the kidney of rats received citric acid at rate of 500 mg kg⁻¹ characterized by lobulated tufts and desquamation of some tubules of white American (Fig1c) and the kidney section of rats received citric acid at rate of 1250 mg kg⁻¹ characterized by contracted tufts and desquamated cells in kidney of rats.

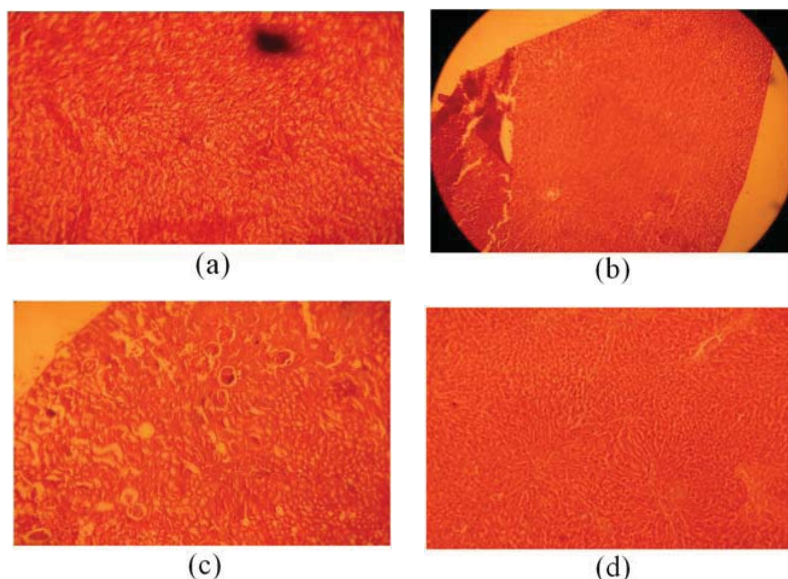


Fig. 1: Plates of kidney section of experimental white American rats orally treated with citric acids, (a) None treated (control); (b) Dose of 100 mg kg⁻¹ body weight; (c) Dose of 500 mg kg⁻¹ body weight; (d) Dose of 1250 mg kg⁻¹ body weight.

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