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Effect of Benzoic Acid and Combination of Benzoic with Citric Acid as Food Additives on the Renal Function of Experimental Rats

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Abstract: This study was aimed to investigate the effect of oral administration of benzoic acid and combination of benzoic with citric acid (1:1 v/v) on the renal functions of white American rats. Twenty one rats were divided in to seven groups (3 rats each). One group serves as a control. The rest of the 6 groups were differently treated (at rates of 100, 500 and 1250 mg kg^{-1} body weight were applied) with benzoic acid and benzoic plus citric acid. Compared to the control, significant ($p \leq 0.05$) gradual increase in the serum creatinine and urea nitrogen levels in the rats with increasing the dose of benzoic acid and benzoic with citric acid was observed. Results also revealed an insignificant difference in serum creatinine and urea nitrogen on administering benzoic acid or its combination with citric acid. Kidney changes in the rats received different doses of benzoic acid and combination of benzoic with citric acid was observed.

Key words: Benzoic acid, citric acid, rats, renal function, glomerular changes

INTRODUCTION

Benzoates are food additives that are used for different food categories, including dairy based deserts, margarine and emulsions. Benzoic acid inhibits the growth of mold, yeast and some bacteria by lowering the intracellular pH to less than 5. This decreases the anaerobic fermentation of glucose by almost 95%. Accordingly, benzoic acid is used as antimicrobial agent (at level 0.05-0.1%) in acidic foods and beverages (Wilson *et al*., 2004).

Chemical food additives may affect normal human body metabolism, organ function or may be precipitated in body tissues, leading to different types of adverse reactions. Benzoic acid has no adverse effects in humans at doses of 650-800 mg kg^{-1} body weight per day. In combination with ascorbic acid, sodium benzoate and potassium benzoate may form benzene, a known carcinogen. Heat, light and storage can affect the rate at which benzene is formed. Benzoic acid and its biphenol derivatives can be absorbed into a lipid bilayer and thus can modify the gel to liquid crystal phase transition to the lipid vesicles. For this, some foods, particularly fruits and vegetables, are believed to have protective effects against cardiovascular disease and cancer due to the presence of antioxidant hydroxyaromatic compounds. Lipoic acid inhibits glycine conjugation of benzoic acid in the liver, resulting in reduced clearance of benzoic acid from blood, which leads to decreased excretion of benzoylglycine in urine.

High level of plasma creatinine may indicate impaired kidney function due to nutritional disorder or a disease. In spite of that the deviation of blood urea nitrogen from normal is often used as an index of changes in renal glomerular function (Marshall and Bangert, 2004). However, an increase in blood urea nitrogen concentration occurs only after at least 65-75% of the functional kidney mass has been lost (Gregory *et al*., 2003).

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In the recent years the kidney failure appeared among wide sectors of the population in Sudan. Benzoate salts as food additives implied in food industries may have responsibility to this problem. This study was conducted to investigate on the effect of benzoic acid and benzoic plus citric acid on the renal function of the white American rats.

MATERIALS AND METHODS

Biological Experiment

Twenty one of white American rats of different sex, weight (0.8-1.4 kg) and age (2-4 weeks) were provided the basal diet (Composed of 250 g of beef meat, 1110 g of seasame oil, 1000 g of corn flour and 7 g of table salt) for a week as an adaptation period. The rats were divided in to 7 groups, each with 3 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 benzoic acid and combination of benzoic plus citric acid (1:1 v/v) was provided to 6 groups. Oral doses of benzoic acid at rates of 100, 500 and 1250 mg kg^{-1} body weight were applied to 3 groups. The same dose rates of benzoic plus citric acid were orally administered to another 3 groups. 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.

Measurement of Creatinine Concentration

Serum creatinine level was determined by the method of Fabiny and Etlingshausen (1971). One hundred microliter of blood serum were 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 nitroprusside, 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 various serum samples were computed. Data were subjected to the ANOVA under a randomized design. Duncan's multiple range test was applied for multiple mean comparisons, using the SPSS (version 12.0). The level of significance was $p \leq 0.05$.

RESULTS AND DISCUSSION

Toxicological Study

Creatinine and Urea N

In this study, oral administration of benzoic acid at rates of 100, 500 and 1250 mg kg^{-1} body weight resulted in significant ($p \leq 0.05$) gradual increase in the level of serum creatinine of the white

Table 1: Effect of benzoic acid and benzoic plus citric acid on serum creatinine and urea N levels of American white rats

		Oral dose treatment (mg Kg^{-1} body weight)					
	Non treated	Benzoic acid			Benzoic plus citric acid		
Metabolite test	(control)	100	500	1250	100	500	1250
Serum creatinine	0.73 ± 0.15^{e}	1.03 ± 0.23^{de}	1.57 ± 0.15^{bc}	2.37 ± 0.32^{a}	1.23 ± 0.14^{cd}	1.73 ± 0.32^{b}	2.47 ± 0.15^{a}
Serum urea N	28.00 ± 2.00^{d}	36.33 ± 2.31^{c}	37.33 ± 4.04^{c}	61.67 ± 2.52^{a}	42.66 ± 2.52^{b}	42.30 ± 2.08^{b}	65.70 ± 3.06^{a}

*Means of triplicate samples. Data are expressed as Mean±SD. Means having different superscripts within a row are significantly different at $p\leq0.05$

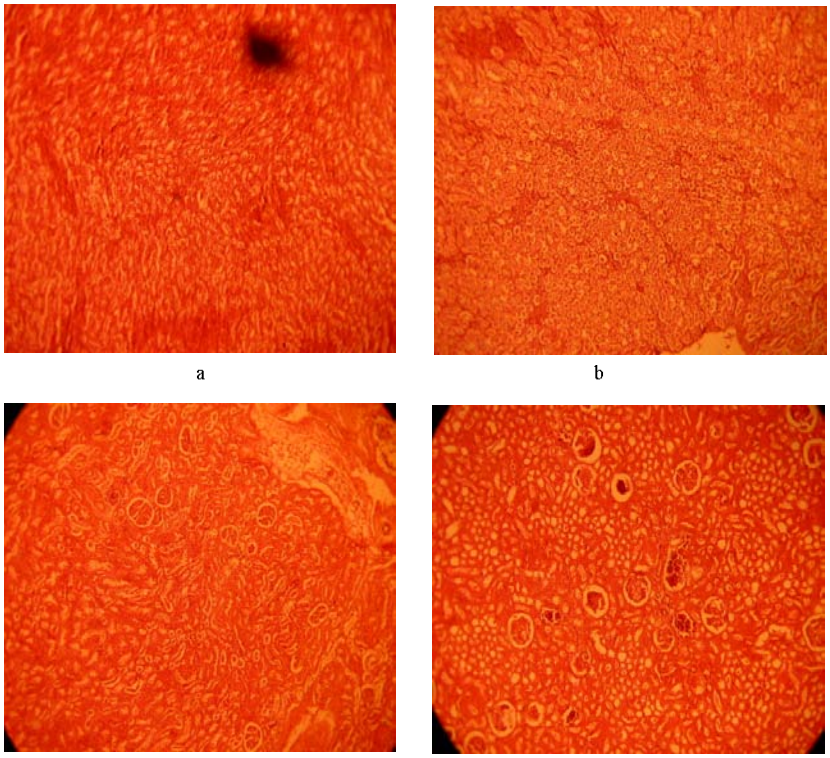

Fig. 1: Plate of kidney section of experimental rats only orally treated with benzoic acid, (a) Non-treated (Control); (b), Dose of 100 mg kg^{-1} body weight; (c) Dose of 500 mg kg^{-1} body weight; (d) Dose of 1250 mg kg^{-1} body weight

American rats from 0.73 mg dL^{-1} for the control to 2.37 mg dL^{-1} for the rats received the dose of 1250 mg kg^{-1} body weight. Similar significant ($p\leq0.05$) gradual increase in serum urea N level was found on managing benzoic acid with a maximum concentration observed at a dose of 1250 mg kg^{-1} body weight (Table 1). Substitution of 50% benzoic acid with citric acid in the oral dose resulted in almost similar significant ($p\leq0.05$) increase in both creatinine and urea N as benzoic acid alone did (Table 1). On the other hand, results revealed stepwise increase in creatinine and urea N with time after oral dosing of benzoic and benzoic plus citric acids at the different rates (Table 1). An increase in plasma creatinine and urea N indicated impairment of renal function (Marshall and Bangert, 2004). Long term toxicity studies demonstrated that exposure to benzoic acid in the diet at a rate of 500 mg kg^{-1} body weight did not cause observable toxic effects (Fanelli and Halliday, 1963). 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).

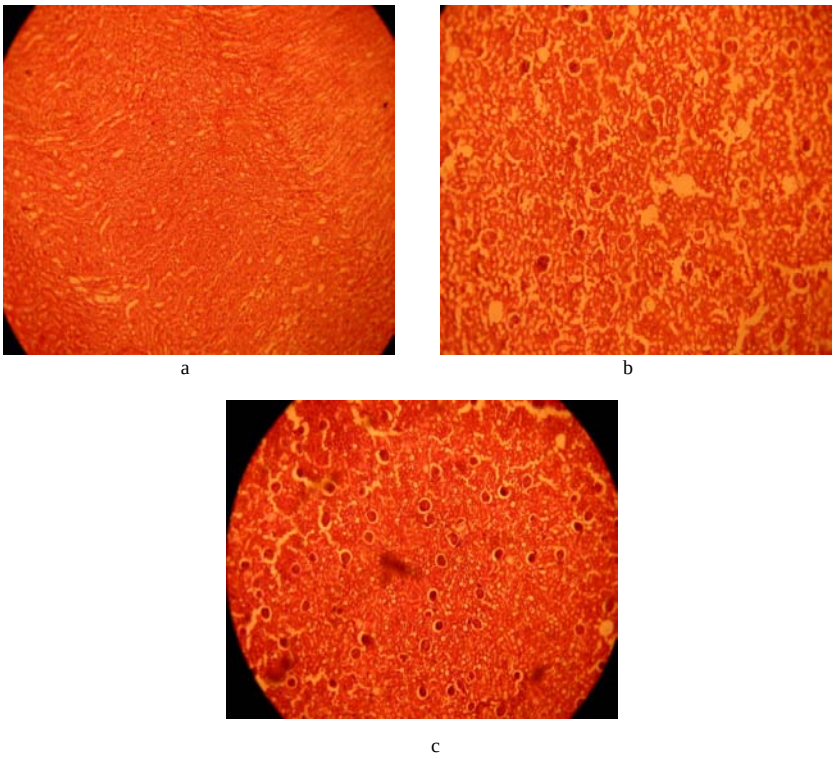

Fig. 2: Plates of kidney sections of white American rats orally treated with benzonic plus citric acid (1:1 v/v). (a) Non-treated (Control); (b) Dose of 100 mg kg^{-1} body weight and (c) Dose of 1250 mg kg^{-1} body weight

Clinical Observations

Clinical observations on the experimental rats revealed symptoms of quick movement and convulsions within the first 3 h after oral administration of benzoic acid and benzoic plus 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 orally treated with benzoic acid at a rate of 100 mg kg^{-1} body weight showed lobuloted tufts, tubules with desquamated cells and no inflammatory cells (Fig. 1b). Kidneys of rats received 500 mg kg^{-1} body weight of benzoic acid characterized by lobuloted tufts, desquamated cells and opponents RBCs (Fig. 1c). The most glomeruli appeared as hypercellular, dark staining and bluffing of tubular epithelium, with no inflammatory cells and no necrosis in kidney sections of rats that received 1250 mg kg^{-1} body weight of Benzoic acid (Fig. 1d). Similar toxicological characteristics was found on kidney sections of the rats received benzoic plus citric acids at rates comparable to those administered for benzoic acid alone (Fig. 2a-c). These dissection results suggest no effect of substituting citric acid on the potentiality of the toxicity of benzoic acid, which might be proved by the obtained serum tests (Table 1).

In conclusion both benzoic acid and combination of benzoic with citric acid, increases serum creatinine and urea nitrogen levels, that lead to inflammation and damage to kidneys indicating impaired renal function.

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