



# ADVERSE EFFECTS OF NON-BRANDED SOFT DRINK;

## ADVERSE EFFECTS OF NON-BRANDED SOFT DRINK ON THE RENAL HISTOLOGY - AN EXPERIMENTAL STUDY IN AN ALBINO RAT MODEL

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**ABSTRACT... Objectives:** Investigating the Adverse effects of non-branded soft drink on the renal histology in an albino rat model. **Study Design:** Experimental study. **Place and Duration:** Department of Anatomy, Isra University, in collaboration with Animal House, Department of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tando Jam over six months duration 2014. **Methodology:** A sample of 30 adult Wistar albino rats of was selected according to criteria. Animals were randomly divided into 3 groups. Group A (n=10) control rats, Group B (n=10) control rats given normal diet (12 hours fasting) and Group C (n=10) experimental rats. 3- 5  $\mu$  thick renal tissue sections were prepared, stained with H & E and examined by light microscopy. Data was analyzed on statistical software SPSS 21.0 ver (IBM, incorporation, USA) at 95% ( $\alpha$ )-level significance was taken at P-value  $\leq$  0.05. **Results:** Experimental group C showed an increase in kidney size and weight observed even after 12 hours of fasting (P=0.0001). Histological examination of experimental kidneys show gaping Bowman`s capsules, interstitial edema, glomerular distortion, calcifications, acidophilic hyalinization of renal tubules, vacuolization of epithelial cell, pyknotic nuclei and necrosis. Epithelial cells revealed fragmentation and sloughing in experimental rat kidney. **Conclusion:** The present study reports deleterious adverse effects of non-branded soft drink on the renal histology in an experimental albino rat model.

**Key words:** Soft Drink, Adverse Effects, Kidney Histology, Rats.

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## INTRODUCTION

Alarming situation of use of non- branded soda containing soft drink has inclined to dangerous levels in the World. This use is particular to the young and adolescents.<sup>1</sup> Research has reported harmful effects of soft cola drinks on various body systems. Specific problem of soft cola drinks induced are the obesity and subsequently the metabolic syndrome, both of these contribute to the atherosclerotic vascular, diabetes mellitus and coronary artery diseases. Despite of proven health hazardous effects of soft drinks, there use is on rise and this is posing a serious public health problem. Childhood use of soft drinks has resulted in the obesity at an early age. Use of soft drinks in the schools has been banned legally in the developed countries to prevent the childhood obesity and metabolic syndrome.<sup>2</sup> This shows the seriousness of the health issues which have been practically addressed in the developed

countries. Soft drinks contain many ingredients along with sweeteners such as the glucose and fructose. Additionally, the soft drinks contain the artificial sweeteners, caffeine, phosphates, etc. True recipe of soft cola drinks is a mystery that is known only by the manufacturer. However, the sugars, caffeine and phosphoric acids are known ingredients of soft cola drinks.<sup>3</sup> A previous research<sup>4</sup> was of opinion of fructose being the major cause of metabolic syndrome. Fructose exaggerates the glomerular filtration. Glomerular hypertension and renal microvasculature alterations have been noted. Fructose increases the serum triglycerides, uric acid and arterial blood pressure. High triglycerides and hyperuricemia injure vital organs such as the kidneys.<sup>4</sup> A previous study<sup>5</sup> noted the deleterious effects of fructose F60 type in rat kidney. This was given to them orally and renal changes were noted. Histology showed harmful effects on rat

kidney, noticeable changes noted were; tissue edema, cortical vascular injury, arteriopathy and glomerular hypertension.<sup>5</sup> It is described that the fructose intake increases the risk of albumin and protein loss in urine and induces diabetes mellitus. As the albuminuria is a surrogate marker of glomerular injury, this needs action against the use of soft cola drink.<sup>6,7</sup> The fructose containing carbonated soft drinks is a risk factor for the systemic hypertension, diabetes mellitus, kidney stone and chronic kidney disease (CKD). As the use of soft cola drinks is prevalent not only in large cities but also in villages where non-branded low cost drinks are available, but its adverse health issues are never reported. There is a knowledge gap to elucidate the different histological changes. The present study was conducted to analyze the deleterious and adverse effects of soft drinks in an albino rat model. The study provides in-vivo findings which are of clinical importance and highlights the emerging public health problem.

## MATERIALS AND METHODS

The present experimental study was conducted at the Department of Anatomy, Isra University, in collaboration with Animal House, Department of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tando Jam over six months duration during 2014. A sample of 30 adult Wistar albino rats were selected according to inclusion criteria of body weight 200 – 250 grams, age 8- 12 weeks, healthy active and moving albino rats of same breed. Sick, inactive, of different body weight and not feeding well were excluded. Animals were randomly divided into 3 groups. Group A (n=10) comprised control rats given normal diet, fresh water and without fasting, Group B (n=10) comprised control rats given normal diet, fresh water but 12 hours fasting and Group C (n=10) experimental rats were given non-branded soft drinks after a fasting of 12 hours time duration. Animal housing protocol was followed strictly. Animals were housed in stainless steel cages, provided with feed containers, plastic drinkers with stainless nozzles. All 3 groups were housed separately for maintaining the fasting of 12 hour time duration. Chow diet and fresh water was available during acclimatization period. Housing

environment provided was; room temperature (23- 25 °C), cross ventilation, and 12/12 hours dark and light cycle was ensured. Bedding saw dust was changed on daily basis. Hygiene and animal handling was in accordance to the standard protocol for animal research as mentioned by Animal Research Protocol (National Institutes of Health Guidelines for the Care and Use of Laboratory Animals). After 30 days of experiment duration, the animals were euthanized by cervical dislocation after Ketamine and Xylazine anesthesia. Laparotomy was performed in the presence of a veterinary surgeon. Kidneys were dissected out and put in normal saline. Gross morphological examination of kidneys (size & weight) was performed. Kidneys were fixed in 10 % formalin (48 - 60 hrs). Kidney tissues were sectioned on microtome with routine paraffin method. 3-5 $\mu$  thick tissue sections were prepared. Tissue sections were stained with Hematoxylin & Eosin (H & E). Tissue slides were examined by light microscopy for histological changes. Data was analyzed on statistical software SPSS 21.0 ver (IBM, incorporation, USA) using parametric tests, the analysis of variance and post Hoc Bonferonni test. Results of continuous variables were presented as mean  $\pm$ SD. Alpha ( $\alpha$ )-level significance was taken at P-value  $\leq$  0.05.

## RESULTS

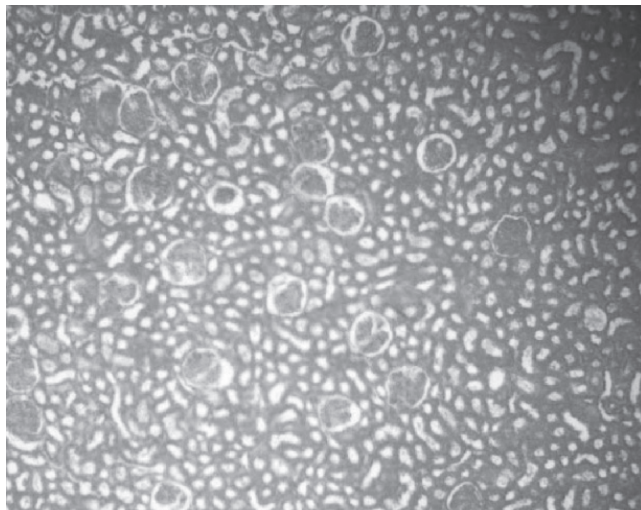
Significant differences in renal size and weight was observed in control groups A and B and experimental group C. Experimental group C showed a significant gain in kidney size and its weight observed even after 12 hours of fasting (P=0.0001). Table-I and II shows the mean  $\pm$  SD of kidney size and weight in controls and experimental group. Photomicrograph-1 and 2 shows the normal renal histological architecture of control group. Abnormal histological changes of experimental group C are shown in Photomicrograph-3 and 4. Histological examination of experimental kidneys show gaping Bowman's capsules, interstitial edema, glomerular distortion, calcifications, acidophilic hyalinization of renal tubules, vacuolization of epithelial cell, pyknotic nuclei and necrosis. Epithelial cells revealed fragmentation and sloughing in experimental rat kidney.

	Mean	SD	P-value
Control Group A.	1.430	0.018	0.0001
Control Group B.	1.394	0.181	
Group C. (Experimental group)	1.701	0.108	

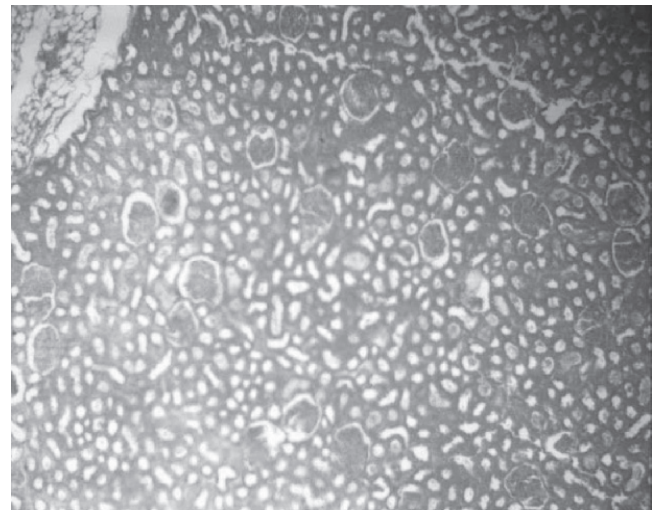
**Table- 1. Kidney size of study rats (measured as centimeters)**

Group	Mean	SD	p-value
Control Group A.	0.601	0.036	0.0001
Control Group B.	0.561	0.033	
Group C. (Experimental group)	0.768	0.072	

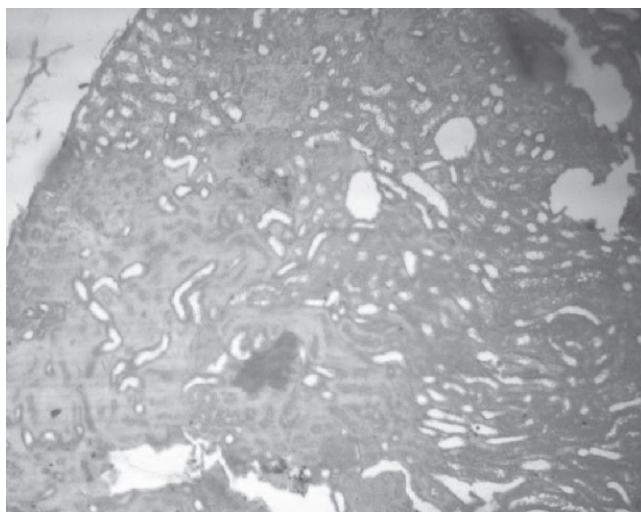
**Table-II. Kidney weight of study rats (measured as grams)**



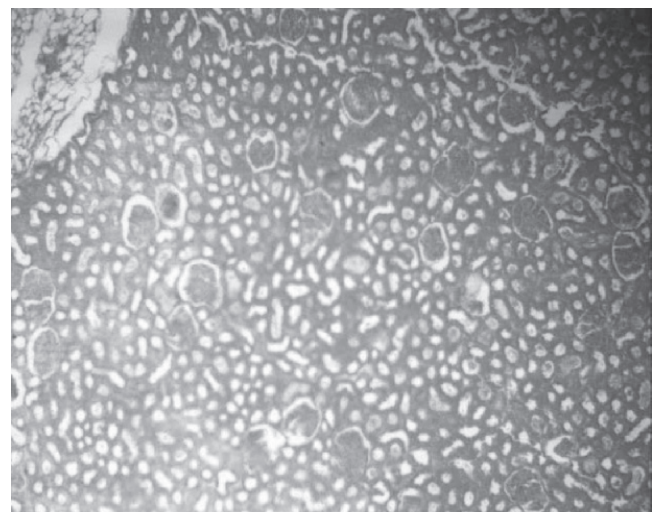
**Photomicrograph-1. Control group A showing normal renal corpuscles with normal DCT and PCT. (H & E x 100)**



**Photomicrograph-2. Control group B showing normal histological section. (H & E x 100)**



**Photomicrograph 3. Group C showing tissue section of kidney showing severely distorted glomeruli, fade looking renal tubules, edematous interstices, hyalinization, micro-calcification, and vacuolation. (H&E x100)**



**Photomicrograph 4. Group C showing tissue section of kidney showing severely distorted renal tubular architecture, cells reveals hydropic changes, edematous interstices and vacuolation (H & E x 400)**

## DISCUSSION

The present is the first in-vivo research study on the deleterious adverse effects of the non-branded soft drink in fasting rat model. Many studies<sup>8-10</sup> had reported on the use of soft drinks and kidney stone formation, but the present study is the first which reports on the renal soft tissue injury caused by non-branded soft drinks in a rat model. We noted significant differences in renal size and weight was observed in control groups A and B and experimental group C.

Experimental group C showed a significant gain in kidney size and its weight observed even after 12 hours of fasting ( $P=0.0001$ ). As the use of non-branded soft drinks is very common in developing countries because of lack of check and balance, hence this type of public health issue is emerging one which needs close supervision by government agencies. This will protect the public morbidity and mortality because of renal injury. Soft drinks contain many chemicals such as the fructose, caffeine, phosphoric acid and artificial sweeteners. Exact composition of non-branded soft drinks is not known and a culprit practice. The sugars and phosphoric acids are present in all available branded and non-branded soft drinks.<sup>3</sup> Phosphoric acid is lithogenic in nature and predisposes to the renal stone formation.<sup>8</sup> Significant differences in renal size and weight was observed in control groups A and B and experimental group C.

Experimental group C showed a significant gain in kidney size and its weight observed even after 12 hours of fasting ( $P=0.0001$ ). Our findings are consistent with previous studies.<sup>11-13</sup> Eluwa et al<sup>13</sup> conducted study on the histology of cerebellum after intake of soda carbonated soft drinks to female albino rats and found deleterious effects similar to the present study. Ferraro et al<sup>9</sup> concluded that the sugar containing soft drinks are associated with renal injury with predisposition of renal stone formation. They compared the soda drinks with the other common natural drinks such as the bear, tea, coffee, wine and orange juices. A previous study<sup>1</sup> reported renal histological injury of rats given different brands of cola drinks. These findings are consistent with the present study.

Adjene et al<sup>1</sup> reported renal tissue distortion, renal cortical injury, glomerulonephritis, capillary congestion and renal tubular necrosis. Evidence based findings of present study are in full agreement with the above study.

In present study, the histological examination of experimental kidneys show gaping Bowman`s capsules, interstitial edema, glomerular distortion, calcifications, acidophilic hyalinization of renal tubules, vacuolization of epithelial cell, pyknotic nuclei and necrosis. Epithelial cells revealed fragmentation and sloughing in experimental rat kidney. Renal micro calcifications are consistent with a previous studies.<sup>14-16</sup> Ukoha<sup>17</sup> reported on the histological and biochemical effects on kidneys. They reported that the frequency of albuminuria was very high. They concluded that the soft drink is a risk factor for the chronic kidney disease (CKD). As the albuminuria is a marker of glomerular injury, hence above study supports our present study. Glomerular injury was observed in the present experimental study. Akanede et al<sup>18</sup> has reported deleterious effects of soft drinks on the brain, liver and kidney histology in Sprague Dawley Rats.

Also the biochemical markers of organ injury were reported. The present study suggests adverse effects of non-branded soft drinks on kidney tissue. However, further studies are warranted in animals and human beings to determine the true deleterious effects of non-branded soft drinks on the kidney.

## CONCLUSIONS

The present study reports deleterious adverse effects of non-branded soft drink on the renal histology in an experimental albino rat model. In-vivo abnormal histological findings indicate the non-branded soft are a risk of kidney injury. The present study concludes that the intake of non-branded soft drinks is a modifiable risk factor of kidney damage and its use should be legally banned.



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Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Sajjad Ali Almani	Literature review, materials handling, collection of compilation of results, statistical analysis, Manuscript write up, Proof reading, Biochemical analysis, Correspondence.	
2	Shoukat Ali Memon	Statistical analysis, Manuscript write up, Proof reading, Biochemical analysis, Manuscript write up, Proof reading.	
3	Aftab Ahmed Shaikh	Concept, Materials handling, manuscript write up, Biochemical analysis and laboratory testing, compilation of results, Proof reading.	