Impact of Co-Administration of Vitamin C and Calcium Chloride on Kidneys Function of Adult Male Rats Treated with Sodium Fluoride

Sumayah Faruq Kasim[(1)] & Jassim M. A. Alkalby[(2)]

[(1)]Anesthesia Department, College of health and Medical Technology,

Middle Technical University, Baghdad, Iraq

[(2)]Department of Physiology, Pharmacology and Chemistry, College of Veterinary Medicine, University of Basrah, Basrah, Iraq.

Abstract

This study was carried out to investigate the role of both Vitamin C and calcium chloride in protection against the deterioration effect of sodium fluoride (NaF) exposure on kidney function .Fifty adult male rats were used, which divided randomly into five equal groups, the first group: The animals of this group served as control group administrated distilled water orally by gavage. Second group: administrated NaF (5.2mg/kg.bw/day) orally by gavage. Third group: administrated NaF (5.2mg/kg.bw/day) + Calcium Chloride (20mg/kg.bw/day) orally by gavage. Fourth group: administrated NaF (5.2mg/kg.bw/day) + Vitamin C (100mg/kg.bw/day) was given orally by gavage. Fifth group: administrated NaF (5.2mg/kg.bw/day) + Calcium Chloride (20mg/kg.bw/day) + Vitamin C (100mg/kg.bw/day) orally by gavage. The treatment continued for 45 days. At the end of the experiment, animals were sacrificed under anesthesia. Blood samples were taken and the serum was separated for the study of the urea, creatinine, and total protein and tissue samples of the kidney were taken for histological changes. The study showed a significant increase in the concentration of creatinine and urea in the fluoride treated group compared to the control group, while a significant improvements in these parameters were observed in all treated groups and in different degrees compared to the non-treated fluoride group. However, histological examination of the kidney in NaF treated group shows enlarged glomerulus, destruction of cuboidal epithelium of renal tubules. .

Keywords*: sodium fluoride, calcium chloride, vitamin c, kidney function*

تأثير الاعطاء المتزامن لفيتامين C وكلوريد الكالسيوم في وظيفة كلى الجرذان البالغة المعاملة بفلوريد الصوديوم

*سمية فاروق قاسم **جاسم محمد أحمد الكلبي

*الجامعة التقنية الوسطى/كلية التقنيات الصحية والطبية/ بغداد

**جامعة البصرة/كلية الطب البيطري/فرع الفسلجة والأدوية والكيمياء

الخلاصة

أجريت الدراسة الحالية في البيت الحيواني العائد لكلية الطب البيطري / جامعة البصرة لتقييم دور فيتامين C وكلوريد الكالسيوم كل واحد منهما لوحده أو كليهما معا في التغيرات الوظيفية والنسجية لكلى ذكور الجرذان المعرضة لفلوريد الصوديوم (NaF). أستخدم خمسون من ذكور الجرذان البالغة في هذه الدراسة قسمت عشوائيا إلى خمس مجموعات متساوية على النحو الأتي: المجموعة الأولى (مجموعة السيطرة): جرعت حيوانات هذه المجموعة ماء مقطر عن طريق الفم . المجموعة الثانية: أعطيت حيوانات هذه المجموعة فلوريد الصوديوم (5.2mg / kg.bw/day) عن طريق الفم بوساطة مجرعة. المجموعة الثالثة: أعطيت حيوانات هذه المجموعة فلوريد الصوديوم (5.2mg / kg.bw/day) + كلوريد الكالسيوم (20 mg / kg.bw/day) عن طريق الفم. المجموعة الرابعة: أعطيت حيوانات هذه المجموعة فلوريد الصوديوم (5.2mg / kg.bw/day) + Vitamin C (100mg / kg.bw/) عن طريق الفم. المجموعة الخامسة: أعطيت حيوانات هذه المجموعة فلوريد الصوديوم (5.2mg/kg.bw/day) + كلوريد الكالسيوم (20mg/kg.bw/day + فيتامين C (100mg/kg.bw/day) عن طريق الفم. استمرت التجربة لمدة 45 يوما وفي نهاية التجربة، تم التضحية بحيوانات الدراسة تحت التخدير. أخذت عينات الدم وتم فصل المصل لدراسة اليوريا، الكرياتنين والبروتين الكلي, وأخذت عينات من أنسجة الكلى لدراسة التغيرات النسجية وأظهرت الدراسة النتائج الآتية: أظهرت الدراسة زيادة معنوية في تركيز الكرياتينين واليوريا في المجموعة المعاملة بالفلوريد مقارنة مع المجموعة السيطرة، في حين لوحظ تحسن معنوي في هذه المعاير في جميع المجموعات المعاملة وبدرجات مختلفة مقارنة مع مجموعة الفلوريد غير المعاملة. وعلاوة على ذلك، فقد أظهر الفحص النسجي لكلية الحيوانات التي تم معاملتها بالفلوريد تضخم الكبيبات وتحطم الخلايا الظهارية المكعبة للنبيبات الكلوية.

1. Introduction

Humans are exposed to sodium fluoride (NaF) from many sources including water, pharmaceuticals, insecticides, pesticides, fertilizers and a few dental products such as toothpaste and some beverages [1]. Fluoride is a naturally occurring element in the environment. Fluoride has been added to drinking water in order to reducing tooth caries, but at present, fluoride can be observed in many sources such as food, beverages and dental products [2]. Fluoride aggregation was observed in soft tissue, causing oxidative stress by inhibiting different enzymatic systems and increasing the generation of free radicals [3]. Imanishi *et al*. [4] observed that oral administration of fluoride leads to the formation of hydrofluoric acid with gastric acid. Fluoride disrupts oxidative phosphorylation, glycolysis, coagulation, and transfer of neurotransmission (calcium binding during signal transfer). So the kidneys are more susceptible to fluoride poisoning than other soft tissues [5]. In addition to skeletal and dental tissues, the high fluoride permeability allows the fluoride ion to penetrate cell membranes and to accumulate

in many soft tissues such as the stomach, small intestine, liver and brain [6]. Heard *et al*. [7] showed that calcium chloride overcome the toxic effects of fluoride on various organ pathologies.

Several studies have been carried out to protect mammals from sodium fluoride poisoning but little research has been used vitamin C for protection [8], [9]. Early studies have noted that vitamin C has a strong antioxidant force and has an ability to neutralize the free radicals and is spread throughout the body. It also plays a protective role against oxidative stress, cell division and reproduction and improves fertility [10], [11], [12]. This study aimed to investigate whether the administration of calcium chloride and vitamin C each alone or both together can ameliorate renal function in adult male rats treated with sodium fluoride.

Materials and methods

Animals

The experiment was done at the animal house of the Faculty of Veterinary Medicine/ University of Basrah, Iraq. At the beginning of the study, the rats were housed in plastic cages with metal covers measuring (15×35×50), containing bedding of fine wood which was changed twice per week. They were kept three weeks without any treatment for an adaptation period before the onset of the experiment. The animals were maintained under controlled environment, light dark cycle (12/12) hours, at a temperature (21±3) C^{o}. Food and water were supplied *ad libitium* with tap water and fed with standard commercial rat chow.

Experimental Design

Fifty male rats were divided randomly into five equal groups as the following: First group: The animals of this group were given distilled water orally by gavage and served as control. Second group: The animals of this group were given Sodium Fluoride (1/10 of LD50) (5.2mg/kg.bw/day) orally by gavage. Third group: The animals of this group were given Sodium Fluoride (5.2mg/kg.bw/day) + Calcium Chloride in a dose of 20mg/kg.bw/day orally by gavage. Fourth group: The animals of this group were given Sodium Fluoride (5.2mg/kg.bw/day) + Vitamin C in a dose of 100mg/kg.bw/day orally by gavage. Fifth group: The animals of this group were given Sodium Fluoride (5.2mg/kg.bw/day) + Calcium Chloride in a dose of 20mg/kg.bw/day + Vitamin C in a dose of 100mg/kg.bw/day orally by gavage. The experiment was continued for 45 days.

The animals at the end of the experiment were anaesthetized by putting them in a closed container containing cotton soaked with chloroform. Once the rats were anaesthetized, blood samples were collected directly by heart puncture using disposable syringes of 5 ml capacity. The collected blood was poured into test tubes free from anticoagulant and centrifuged at 3000 rpm for 15 minutes.

Serum samples were collected after centrifugation and kept in Eppendorf tubes and stored at -20 C^{o} until using for the measurement of total proteins, creatinine and urea concentration . Moreover kidneys have been removed, and kept in 10% formal saline for histological examination.

Biochemical assay:

Creatinine and urea concentrations were determined by using a special creatinine Kit [13] and urea Kits (bioSystems, Spain) [14]. Total protein concentration also was determined by using a special total protein Kit (Agappe Diagnostic LTD., India) [15].

Histopathological study:

Samples of the kidney were taken and fixed in 10% formalin and then embedded in paraffin and sectioned at thickness of 6 micrometers and then carried on glass slides. It was stained with hematoxylene-eosin and was examined under a light microscope [16].

Results

The data in table (1) showed no significant differences in serum concentration of total protein in all treatments compared with control group with the exception of group that treated with NaF + $CaCl_2$, which showed significant ($P \leq 0.05$) decrease in serum total protein concentration compared with NaF + vitamin C treated group.

On the other hand, a significant ($P \leq 0.05$) increase in serum concentration of creatinine was observed in NaF treated group as compared to the control group. However, significant ($P \leq 0.05$) decrease in serum creatinine concentration was recorded in NaF + vitamin C and NaF + $CaCl_2$ + vitamin C treated groups compared with NaF treated group. While the group which treated with NaF + $CaCl_2$ shows a significant ($P \leq 0.05$) increase in concentration of serum creatinine as compared with control and the other treated groups.

Moreover, a significant ($P \leq 0.05$) increase in serum urea concentration was recorded in the NaF treated group as compared to the control group. While, significant ($P \leq 0.05$) decreases in serum urea concentration was observed in NaF + $CaCl_2$ and NaF + vitamin C compared with NaF treated group, but they were not significantly ($P \leq 0.05$) different from the control group.

Whereas, the serum urea concentration still significantly (P≤0.05) higher in NaF group that treated with both $CaCl_2$ and vitamin C compared with control group.

Table (1): Effect of $CaCl_2$, Vitamin C, and their Combination on Serum Total Protein, Creatinine and Urea Concentrations of Adult Male Rats Treated with NaF (M±SD) (n=10).

Parameters / Groups	Total Protein (g / dl)	Creatinine mg/dl	Urea mg/dl
Control	**6.23 ± 0.17 ab**	**0.33 ± 0.01 e**	**37.33 ± 1.55 b**
NaF	**5.98 ± 0.59 ab**	**0.47 ± 0.02 b**	**43.50 ± 2.65 a**
NaF + $CaCl_2$	**5.79 ± 0.40 b**	**0.59 ± 0.02 a**	**34.44 ± 3.05 b**
NaF + Vit. C	**6.30 ± 0.27 a**	**0.40 ± 0.02 d**	**36.60 ± 3.71 b**
NaF + $CaCl_2$ + Vit. C	**6.05 ± 0.46 ab**	**0.45 ± 0.03 c**	**40.62 ± 2.51 a**
LSD	**0.44**	**0.01**	**3.04**

The different letters refer to significant differences among groups at level of (p≤0.05).

Kidney histopathological examination

The section of kidney of control rats shows normal glomeruli found in kidney cortex. The different types of renal tubules are clear and their epithelium is normally composed of cuboidal cells with clear dense nuclei as shown in Figure (1). While the kidney section of rats treated with NaF shows enlarged glomerulus with clear destruction of cuboidal epithelium of the renal tubules indicated by enlargement and pyknosis of their nuclei with disappearance of cellular boundaries as shown in figure (2).

Microscopic examination of the kidney of animals treated with NaF + $CaCl_2$ shows that there is distortion of glomerular content with clear destruction of renal tubular cuboidal epithelium with several vacuolations as shown in Figure (3). However the kidney section of the animals treated with NaF + Vitamin C showing normal architecture of renal parenchyma, with mild vacuolation of some renal tubules as shown in Figure (4).

Finally, the kidney section of rats group treated with NaF + $CaCl_2$ + Vitamin C shows clear glomerular cells of glomerular epithelium which have round to oval nuclei, the renal tubules are clearly formed and lined by large cuboidal cells of large nuclei as shown in Figure (5).

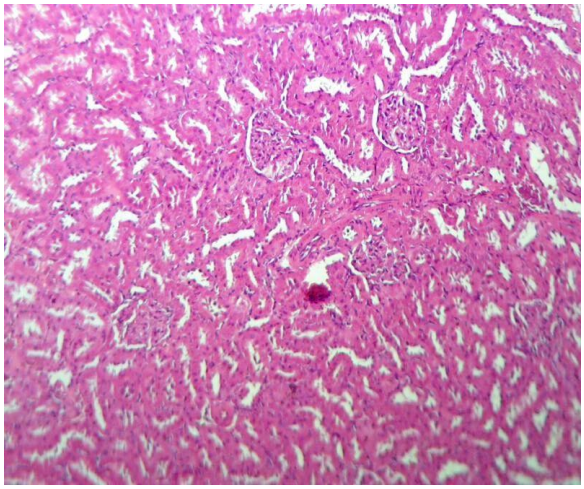

Fig. 1: Kidney of control rat. Showing normal glomerulus (G) and clear different types of renal tubules (RT) and their normal cuboidal cells with dense nuclei. (Hematoxylin-Eosin stain) 10X.

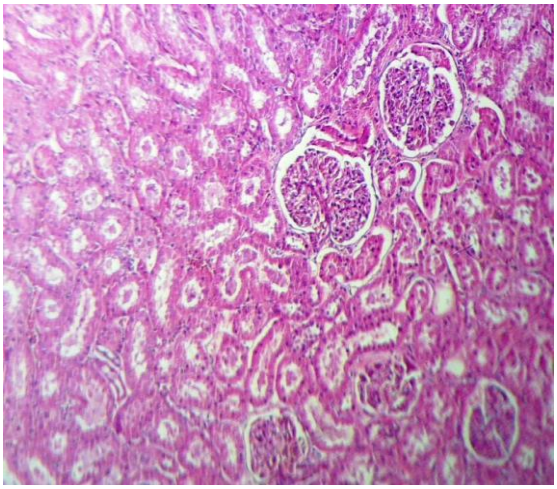

Fig. 2: Kidney of rat treated with NaF. Showing enlarged glomerulus (G) with clear destruction of cuboidal epithelium of renal tubules (RT). (Hematoxylin-Eosin stain) 10X.

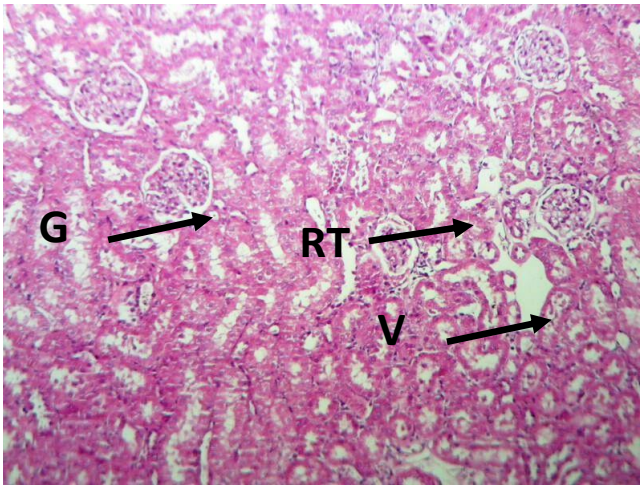

Fig. 3: Kidney of rat treated with NaF + $CaCl_2$. Showing distortion of glomerulus (G) content with clear destruction of cuboidal epithelium of renal tubules (RT) with several vacuolation (V). (H&E) stain 100X.

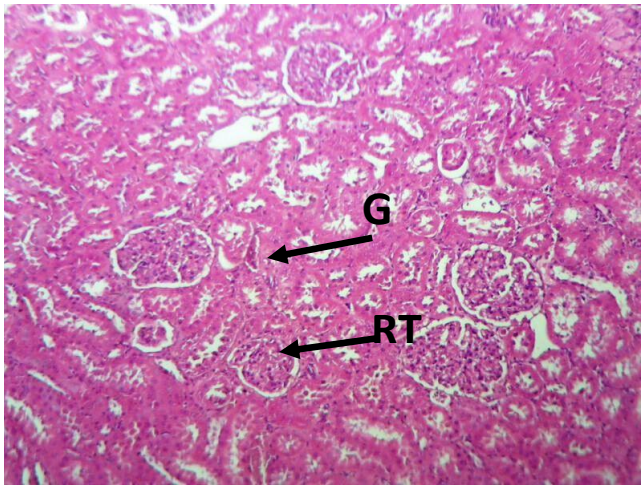

Fig. 4: Kidney of rat treated with NaF + Vitamin C. Magnification showing dilatation of proximal convoluted tubules (D). (H&E.) stain 100X.

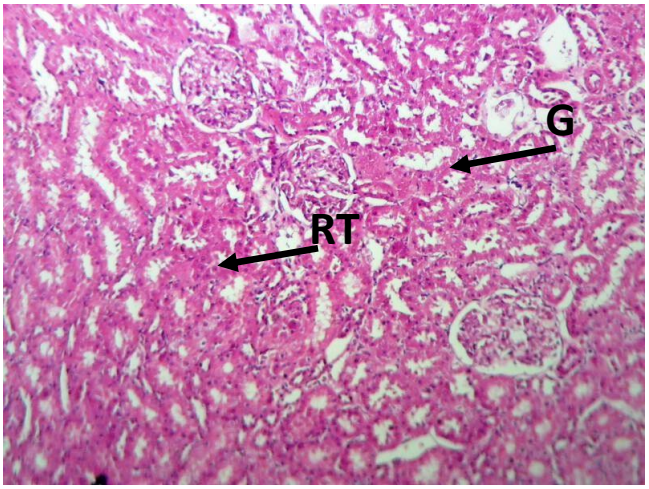

Fig. 5: Kidney of rat treated with NaF + CaCl2 + Vitamin C.

Showing clear glomerular cells, the renal tubules (RT) are clearl Formed and lined by large cuboidal cells of large nuclei. (H&E) stain. 100X.

Discussion

The data of present study in table (1) revealed that there are no significant differences in concentration of serum total protein in NaF treated group as compared with control group. This result is in agreement with the findings of [17]; and [18]. In contrast of the current result [19]; [20]; [21]; and [22] showed a significant decrease of serum total protein concentration in NaF group compared with control group. On the other hand Alharbi *et al*. [23] revealed an increase in total protein in NaF group compared with control.

Further more significant increase in serum concentrations of urea and creatinine were observed in NaF treated group as compared with the control group. The present results come in harmony with [24]; [3]; [21]; [25]. Emejulu *et al* [18] indicated a significant increase in urea concentration. While Birkner *et al* [17] reported that the concentrations of urea and creatinine in the serum of adult male rats did not undergo statistically significant changes.

Elevation of urea in serum could result from a defective excretory function of the kidneys, especially since increased urea concentration in blood serum is as a general rule caused by renal insufficiency, with or without obstruction of urinary tracts [26].

The results also indicated that serum urea levels were increased in NaF group treated along with $CaCl_2$ (20 mg/kg/day) by gavage for 6 weeks as compared to the NaF group. Similar result was observed by Mokhtar, [27] who showed that administration of calcium with NaF causes more great elevation in creatinine and urea concentration compared with NaF and control groups. This

result indicated that calcium not only failed to restore the renal deterioration resulted due to NaF exposure but also enhance the NaF toxicity.

Significant degrees of improvement were recorded in serum concentrations of creatinine and urea in NaF + Vitamin C and NaF + $CaCl_2$ + Vitamin C compared with NaF non treated group. Similarly found by Abdel Aziz and Masad, [28] who investigate signs of improvements in the previous biochemical parameters after the administration of vitamin C prior to sodium fluoride administration. This is due to the role of vitamin C in the removal of fluoride side effects due to its antioxidant and detoxification properties.

The kidney section of rats treated with NaF in the present study showed enlarged glomerulus with clear destruction of cuboidal epithelium of the renal tubules indicated by enlargement and pyknosis of their nuclei with disappearance of cellular boundaries as shown in figure (2). Our results are agree with [21]; [29]; [30]; [31] whom detected histological changes in kidney of animals treated with NaF such as necrosis, degeneration, hemorrhage. Calcium chloride co-administration (20 mg/kg/day) with NaF for 6 weeks not only failed to restore renal damage induced by NaF exposure but also enhanced the flouride-induced toxicity. These results are in consistent with those of Mokhtar [27]. In contrast to the present study' results it was found that rats that treated with calcium chloride and sodium fluoride (30 ppm F + 50 ppm calcium chloride) revealed amelioration from the toxic effects of F in terms of the histological changes in kidney [32]. However the kidney section of the animals treated with NaF + Vitamin C showing normal architecture of renal parenchyma, with mild vacuolation of some renal tubules as shown in Figure (4). Similarly Khan and Ranga [33] mentioned that mice treated with NaF for 28 days causes significant changes in renal function while treatment with Vitamin C for 28 days during with drawl resulted in a significant recovery in all parameters.

But, the combination treatment of NaF + $CaCl_2$ + Vitamin C showed some improvement near to normal represented by clear glomerular epithelial cells which have round to oval nuclei, the renal tubules are clearly formed and lined by large cuboidal cells of large nuclei as shown in Figure (5).

Conclusion

Exposure of adult male rats to NaF resulted in significant changes in urea and creatinine concentrations accompanied with histopathological changes in kidney. On the other hand co-administration of calcium, Vitamin C, each alone or both together reduce to some extent of these changes.

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