April 30, 2019

PharmacologyOnLine

Archives • 2019 • vol.1 • 87-95

# EFFECTS OF SOME CALCIUM CHANNEL BLOCKERS AGAINSTS $CCL_4$-INDUCED NEPHROTOXICITY IN ALBINO RATS

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## Abstract

Acute kidney injury remains associated with high morbidity and mortality despite progress in medical care. Carbon tetrachloride ($CCl_4$)-toxicity causes free radical generation and increases calcium influx, leading to pathogenesis in target tissues which includes the kidney. The present study reports the protective effects of some calcium channel blockers (nifedipine and amlodipine) against $CCl_4$-induced acute kidney injury. Twenty four (24) albino rats were randomly grouped into four groups: A, B, C and D; with six animals per group. Groups A-C received $CCl_4$ (0.4ml/kg b.wt, i.p) for 3 days. Group A served as toxic (negative) control. Group B received nifedipine (1mg/100g of rat, i.p), while Group C received amlodipine (1mg/100g of rat, i.p) for 3 days. No treatment was administered to group D (Normal control). Renal injury was assessed through biochemical and histopathological analyses. The $CCl_4$ administration alone caused a marked alteration in the levels of: $Na^+$ (120.83±2.14 mmol/l), $K^+$ (7.02±0.61 mmol/l), Creatinine (1.52±0.19 mg/dl) and Urea (34.02±4.46 mg/dl) ($p<0.01$). Treatment with the calcium channel blockers (nifedipine and amlodipine) separately, significantly attenuated the renal dysfunction with: $Na^+$ (129.54±1.68; 130.13±1.75 mmol/l), $K^+$ (5.10±0.72; 5.08±0.60 mmol/l), Creatinine (0.72±0.16; 0.71±0.12 mg/dl) and Urea (21.37±2.09; 20.08±1.83 mg/dl) respectively ($p<0.05$). Histopathological results showed a concomitant association with the biochemical findings. Calcium channel blockers are nephroprotective, and may be used to prevent drug/chemical-induced acute renal injury.

**Keywords**: *Calcium channel blockers, nephroprotection, $CCl_4$, nephrotoxicity*

## Introduction

Drugs such as aminoglycoside antibiotics, NSAIDS (non-steroidal anti-inflammatory drugs), amphotericin B, and ACE (angiotensin-converting enzyme) inhibitors, have been implicated as causes of nephrotoxicity (1). Factors such as age, presence of comorbidities, and exposure to multiple drugs and other therapeutic agents could trigger and culminate into drug-induced nephrotoxicity (2). The primary function of the renal system is the elimination of metabolic waste products (3, 4). The kidney is a common site of chemical toxicity as a result of its constant exposure to chemicals and toxicant xenobiotics, which it needs to eliminate from the host's body.

The production of toxic or active phase 1 metabolites during the biotransformation of drugs/chemicals is a key feature of nephrotoxicity and one known toxicant xenobiotic, among others, which can be activated during biotransformation, is carbon tetrachloride ($CCl_4$). $CCl_4$ is a well-established xenobiotic and both the liver and the kidneys are its target organs (5). It is known for its lytic injury on target organs. Studies have also shown that exposure to $CCl_4$ is metabolized by cytochrome P450 isoenzyme, CYP2E1 to the highly reactive trichloromethyl (CCl3 and CCl3OO) free radical which causes target tissue cell alteration through lipid peroxidation (6).

Drug-induced nephrotoxicity is fast becoming a common complication, with the ever-increasing plethora of drugs and the ease of availability of OTC (over-the-counter) drugs with detrimental effect on the renal function (7). Drugs cause approximately one-fifth of community- and hospital-acquired episodes of acute renal failure (7-9), Although renal impairment is often reversible if the offending drug is discontinued, the condition can be costly and may require multiple interventions including hospitalization (10). Inhospital drug use may contribute to 35% of all cases of acute tubular necrosis, most cases of allergic interstitial nephritis, as well as nephrotoxicity due to alterations in renal hemodynamics and postrenal obstruction (7, 11). Several biochemical mechanisms have been reported to be involved in the occurrence of renal cell injury. These mechanisms include cell death through apoptosis, disruption of cell volume/ion homeostasis, changes in cell membrane integrity, mitochondrial ATP (adenosine triphosphate) dysfunction, and disruption in cellular calcium ($Ca^{2+}$) homeostasis (8).

$Ca^{2+}$ is a second messenger that plays a critical role in a variety of cellular functions. Its distribution within renal cells is known to be complex and involves binding to anionic sites on macromolecules and compartmentalization within subcellular organelles (8). However, because the proximal renal tubular cells reabsorb approximately over 50% of the filtered load of $Ca^{2+}$, they must maintain low cytosolic $Ca^{2+}$ concentrations during a large $Ca^{2+}$ flux (7, 8). Sustained elevations or abnormally large increase in free cytosolic $Ca^{2+}$ can exert a number of harmful effects on the renal cells. While the precise role of $Ca^{2+}$ in toxicant-induced injury remains unclear, release of endoplasmic reticulum (ER) $Ca^{2+}$ stores may be a key step in initiating the injury process and increasing cytosolic-free $Ca^{2+}$ concentrations (8, 12, 13). However, prior depletion of ER $Ca^{2+}$ stores protect renal proximal tubules from extracellular $Ca^{2+}$ influx and cell death produced by mitochondrial inhibition and hypoxia (14). $Ca^{2+}$ channel blockers (CCBs) are known pharmacologically to inhibit the influx of $Ca^{2+}$ into cells thereby depleting the $Ca^{2+}$ stores in the renal cells, thus potentially exerting nephroprotective effect.

This study was designed to investigate the ameliorative effect of CCBs (nifedipine and amlodipine) against $CCl_4$-induced acute renal injury in rats. This study is important because, CCBs are commonly used for the treatment of hypertention in many parts of the world, but have not been clinically known to be used for treatment of acute kidney injuries. Recently, Uchendu *et al* (15) reported that combination of aqueous extracts of *curcuma longa* (turmeric) and some calcium channel blockers synergistically improves $CCl_4$-induced hepatotoxicity in albino rats. This suggests the protective potentials of CCBs against target-tissue injury by $CCl_4$. Furthermore, there is scarcity of literatures on the antinephotoxic effect of CCBs against drug/chemical-induced kidney injury. Again only one study, by Akindele *et al*. (7), has been carried out on the protective effects of CCBs against $CCl_4$-induced nephrotoxicity. Thus, further studies and more literatures are therefore needed to

validate their finding, either in support or in contradiction for justification of results and/or to affirm CCBs as potential nephroprotective drugs.

## Materials and Methods

*Chemical and drugs*

The chemical used in the study was of analytical grade and only includes $CCl_4$ solution (Alpha Pharmaceuticals, Enugu, Nigeria). Drugs used include Nifedipine and Amlodipine (Salutas Pharma GmBH for Lek Pharmaceutical and Chemical Company, Veroskova, Slovenia).

*Preparation of calcium channel blocker solutions.*

Thirty-two (32) tablets of 10 mg (i.e. 320 mg) amlodipine and 20 tablets of 20 mg nifedipine (400 mg) were grinded to powder, dissolved in 100ml distilled water to give a stock solution of 3.2mg/ml and 4mg/ml respectively.

*Induction of acute kidney injury.*

Renal injury was induced in each animal by intraperitoneal injection with $CCl_4$ (0.4ml/kg), daily for 3 days.

*Animals and maintenance*

A total of twenty-four (24) adult albino rats, weighing (120±20g), were obtained from the animal house of the College of Veterinary Medicine, University of Nigeria. The animals were housed in metallic cages in the animal house under ambient temperature (25±3°C) and 12-hour light and dark periodicity. They were adequately fed with commercial rat pellets (Neimeth Livestock Feeds Ltd., Ikeja) and water *ad libitum*. The animals were kept under observation for about 14 days before the onset of the experiment for acclimatization. Ethical approval for the use of animals for experimental research was obtained from the Institutional Ethics Committee at Department of Animal Science, University of Nigeria, Nsukka, Enugu State, Nigeria.

*Experimental Design*

The 24 albino rats were grouped into (A-D) and received the following treatments on a daily basis for three days:
Group A: (Negative Control): received intraperitoneal administration of carbon tetrachloride $CCl_4$ (0.4ml/kg body weight) only for 3 days.
Group B: received $CCl_4$ and nifedipine (1mg/100g of rat, i.p) for 3 days.
Group C: received $CCl_4$ and amlodipine (1mg/100g of rat, i.p) for 3 days.
Group D: (normal Control): No treatment was administered to this group.

*Sacrificing of animals and sample collection*

Blood samples for the determination of kidney biochemical markers were taken by cardiac puncture of the left ventricle of the heart under chloroform anaesthesia and the kidney harvested for histopathological analyses.

*Biochemical analysis*

The levels of Serum Electrolyte, Urea and Creatinine were estimated using the following methods: $K^+$ and $Na^+$ was determined using Perlong Medical PL1000A Electrolyte Analyser; Serum urea concentration was determined using the diacetyl monoxime method with protein precipitation according to Natelson *et al*. (16) Serum creatinine concentration was determined using the Jaffe Reaction according to Fabing and Ertingshausen (17).

*Histopathological analysis*

The excised kidneys were processed using the paraffin wax embedding technique, sectioned at 5 microns and stained using the Haematoxylin and Eosin [H and E] staining procedure (18). The histological sections were examined using an Olympus™ light microscope.

*Statistical analysis*

Data analysis was done using GraphPad prism version 6.0 (GraphPad, San Diego, CA, USA). The results of the biochemical assays were reported as mean±SEM (standard error of mean). The level of significance was tested using one way analysis of variance (ANOVA), followed by the Tukey post hoc analysis. Probability levels less than 0.05 ($p<0.05$) were considered significant.

## Results

*Biochemical results*

Table 1 shows the results of kidney biochemical parameters: urea, creatinine, $K^+$, $Na^+$ levels in four (4) groups of six (6) animals, which received intraperitoneal administration of carbon tetrachloride $CCl_4$ (0.4ml/kg body weight) and/or some calcium channel blockers (nifedipine and amlodipine; separately) for 3 days. From the results, The $CCl_4$ administration caused a marked alteration in the levels of: $Na^+$ (120.83±2.14 mmol/l), $K^+$ (7.02±0.61 mmol/l), Creatinine (1.52±0.19 mg/dl) and Urea (34.02±4.46 mg/dl) ($p<0.01$) in the negative control animals when compared to normal control animals. Treatment with the calcium channel blockers (Nifedipine and Amlodipine) separately, significantly attenuated the renal dysfunction with: $Na^+$ (129.54±1.68; 130.13±1.75 mmol/l), $K^+$ (5.10±0.72; 5.08±0.60 mmol/l), Creatinine (0.72 ± 0.16; 0.71 ± 0.12 mg/dl) and Urea (21.37±2.09; 20.08±1.83 mg/dl) respectively ($p<0.05$), when compared to the negative control animals.

*Histopathological results*

The results are represented in figures 1 (A-D). Microscopic examination of the kidney, revealed no histopathological alteration, in the normal control rats (Figure 1D). Presence of significant distortion the glomeruli was observed in the kidney of rats treated with $CCl_4$ only (Figure 1A); however non-significant degenerations were observed in rats administered with nifedipine or amlodipine separately, in the presence of $CCl_4$ challenge (Figure 1B and 1C respectively). The kidneys of rats in group B and C showed no significant histological alterations when compared with the normal control group.

## Discussion

In the present study, the aim was to evaluate the protective effects of two selected calcium channel blockers (nifedipine and amlodipine) against $CCl_4$-induced acute renal injury. Findings from our study showed that CCB effectively protected rat kidneys against nephrotoxic injury by $CCl_4$. This agrees with or strengthens similar findings by Akindele et al (7). It further scientifically supports the potential use of calcium channel blockers for the prevention or treatment of drug/chemical-induced renal injury in hospital patients.

Drugs are chemical substance, and their therapeutic use, usually alters the physiological homeostasis and affects organs during treatment process. Effective use of most drugs is limited because of their toxicity to various organs including the kidney (19, 20). Acute renal failure has remained associated with high mortality and morbidity in societies, considering major advances made in the field of medicine and medical care (21-26). There is increasing evidence that free radicals and reactive steps initiate and regulate the progression of kidney disease (7, 22).

$CCl_4$-induced-target tissue injury is one of the most characterized models of xenobiotic-induced tissue toxicity, and is employed for evaluating antihepatotoxicant or nephroprotective activities of drugs or bioactive substances (27). The metabolism of $CCl_4$, primarily through the activity of CYP2E1, generates highly reactive trichloromethyl free radicals CCl3 and CCl3OO which result in target-tissue damage. These free radicals initiate lipid peroxidation by abstracting a hydrogen atom from the polyunsaturated fatty acid of a phospholipid (28). The $CCl_4$-induced lipid peroxidation in turn increases the permeability of plasma membrane to calcium ion, resulting in severe disruption of calcium homeostasis and necrotic cell death (28, 29). The extent of kidney damage was assessed by the alterations in serum levels of Urea, Creatinine, potassium, sodium, and by histopathological examination. The aim of the study was to evaluate, the effects of selected calcium channel blockers (nifedipine and amlodipine) against carbon tetrachloride ($CCl_4$)-induced acute renal injury in rats.

Except for serum $Na^+$, other renal biochemical parameters, which include serum creatinine, urea, and $K^+$, were elevated in the group (negative control) administered with $CCl_4$ alone when compared to the normal group. Factors like gender, age, and the amount of the muscle mass can influence serum creatinine level (11). These factors were kept constant, as the rats were of the same weight range and were restricted from movement. The elevated urea and creatinine levels could be attributed to renal damage, since kidney function, with increased blood urea and creatinine (22).

Urea, creatinine and $K^+$ were seen to be significantly decreased, whereas $Na^+$ was increased in the group co-treated with a calcium channel blocker, which indicates that calcium channel blockers may afford protection against kidney injury. Hiroki *et al*. (29) report that hypocalcaemia has an ameliorating effect on $CCl_4$-induced hepatotoxicity. This again suggests that calcium influx is involved in $CCl_4$-induced tissue-toxicity. In this study, the effects of calcium channel blockers (nifedipine and amlodipine) on $CCl_4$-induced kidney damage were determined and the kidney showed a significant reestablishment of several of the renal biochemical parameters: a considerable decrease in Urea, Creatinine and $K^+$ levels was observed.

## Conclusion

The present study shows that carbon tetrachloride induced kidney injury; however the treatment with calcium channel blockers (nifedipine and amlodipine) ameliorated the effects in test groups. Thus this data suggests that calcium channel blockers may be of health benefit to patient suffering from acute kidney injury induced by nephrotoxic agents. Furthermore, this study supports discovery of the potential use of calcium channel blockers as complementary and alternative drugs for the prevention or treatment of drug/chemical-induced renal injury in hospital patients. Therefore, this study would serve as insight into further researches to harness more pharmacologic potentials of calcium channel blockers, aside their common use as antihypertensive agents.

## Acknowledgments

The authors express deep sense of gratitude to Mr. Chris Ireoba, The head of department of the Laboratory Division at Eastern Nigeria Medical Centre, Enugu, and all the technical staff for their kind cooperation.

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**Table 1:** Statistical comparison of kidney biochemical concentrations in different experimental animal groups.

| Groups | Urea (mg/dl) | Creatinine (mg/dl) | $K^+$ (mmol/l) | $Na^+$ (mmol/l) |
|---|---|---|---|---|
| **$CCl_4$ Alone** | 34.02 ± 4.46 | 1.52 ± 0.19 | 7.02 ± 0.61 | 120.83 ± 2.14 |
| **$CCl_4$ + Nifedipine** | 21.37 ± 2.09* | 0.72 ± 0.16* | 5.10 ± 0.72 | 129.54 ± 1.68* |
| **$CCl_4$ + Amlodipine** | 20.08 ± 1.83* | 0.71 ± 0.12* | 5.08 ±0.60 | 130.13 ± 1.75* |
| **Normal Control** | 17.85 ± 5.38** | 0.54 ± 0.06** | 4.02 ± 0.51* | 132.21 ± 1.61* |

Values given as Mean ± SEM. **P<0.01 or *P<0.05 is significant when $CCl_4$ alone (negative control) is compared with all other groups.

**Figure 1A:** Photomicrograph of kidney from (A) $CCl_4$ alone-treated rats. Presence of constricted glomerulus (CG), eroded glomerulus (EG), stromal hyperaemia (arrow head) and eroded tubules (arrows). [Stain: H and E; ×400].

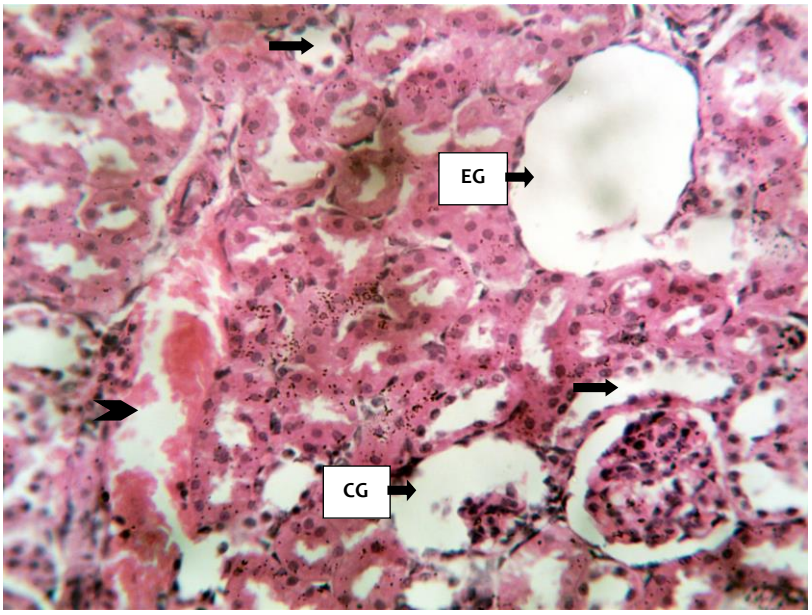

**Figure 1B:** Photomicrograph of kidney from (B) $CCl_4$ +nifedipine-treated rats. Presence of some normal glomeruli (G), the tubules (T) appear normal. A hyperaemic portion of the stroma is seen (arrow head). [Stain: H and E; ×400].

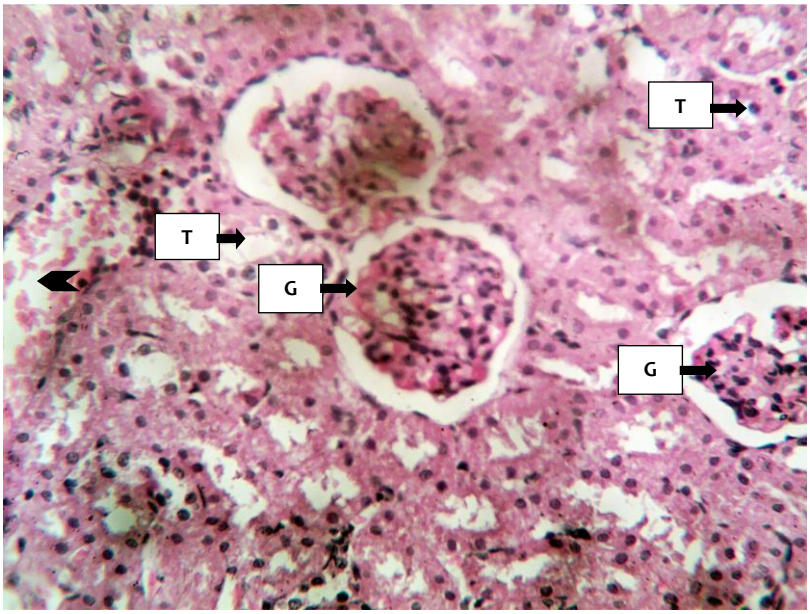


**Figure 1C:** Photomicrograph of kidney from (C) $CCl_4$+amlodipine-treated rats. Presence of some normal glomeruli (G), and the tubules (T) appear normal. [Stain: H and E; ×400].

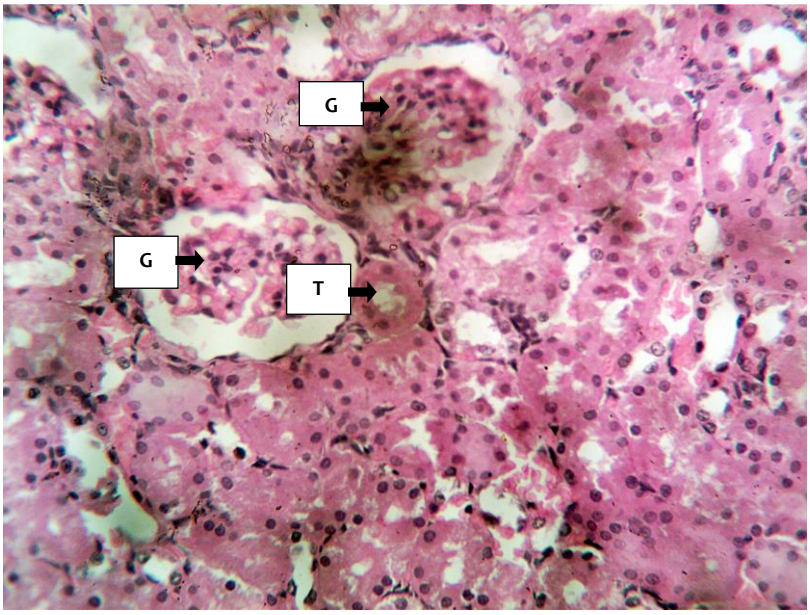

**Figure 1D:** Histopathology and photomicrograph of kidney from (D) normal control rats**.** Presence of normal Glomeruli (G) and renal tubules (T).[Stain: H and E; ×400].