doi.org/10.36721/PJPS.2020.33.5.REG.2059-2065.1

Combination of aqueous extracts of *Curcuma longa* (turmeric) and some calcium channel blockers synergistically improves CCl_4-induced nephrotoxicity in albino rats

Ikenna Kingsley Uchendu[1]*, Chidozie Elochukwu Agu[2], Ebuka Bitrus Nnedu[1] and Ikechukwu JohnPaul Chukwu[1]

[1]Department of Medical Laboratory Science, University of Nigeria, Enugu Campus, Nigeria
[2]Prime Health Response Initiative (PHRI), Kwara State, Nigeria

Abstract: In the present study, special attention was drawn to CCl_4-induced acute kidney injury (AKI) and how the nephrotoxicity could be treated or prevented by administration of aqueous extracts of *Curcuma longa* (AECL) alone or in combination with some calcium channel blockers. Thirty (30) male albino wister rats were grouped according to their weight into 6 groups (A-F) of 5 rats per group. Rats in groups A-D received CCl_4 (0.4ml/kg b.wt, i.p) for 3 days. Group B received AECL (200mg/kg, oral), Group C received AECL and nifedipine (1mg/100g of rat, i.p), Group D received AECL and amlodipine (1mg/100g of rat, i.p), and group E received AECL alone with no CCl_4 challenge for 3 days. No treatment was administered to group F (Normal control). Serum renal biochemical parameters; MDA level and SOD activity in the kidney homogenates were measured. CCl_4 administration to the rats resulted to acute kidney injury with significantly increased Urea, Creatinine, K^+ and MDA levels and decreased SOD activity ($p<0.05$, $p<0.01$ or $p<0.001$). The 3 days daily administration of AECL alone or plus nifedipine or amlodipine resulted in the attenuation of the CCl_4-induced kidney injury with significantly decreased Urea, Creatinine, K^+ and MDA levels and increased SOD activity ($p<0.05$. $p<0.01$). Histopathological results showed a concomitant association with the biochemical findings. This study shows that the combination of the extract and some calcium channel blockers is synergistically nephroprotective and can be used to prevent acute renal injury.

Keywords: Ethnopharmacology, CCl_4, nephroprotection, *Curcuma longa* extracts, nifedipine, amlodipine

INTRODUCTION

Turmeric has been used in folklore to treat numerous diseases, including renal diseases. Despite its numerous uses, turmeric is known to have some negative side effects (Hewlings and Kalman, 2017). Despite treatment advances, the mortality rate for Acute Kidney Injury (AKI) remains high-about 40% in critically ill patients (Uchendu *et al*., 2017). Acute Kidney Injury (AKI) is another term for Acute Renal Failure (ARF), and it affects kidney histo-architecture and function. AKI is characterized by a sudden decrease in kidney function (Kingsley, 2018). CCl_4 is well established as xenobiotics. Both the liver and the kidneys are the target organs of CCl_4 (Singh *et al*., 2008).

Carbon tetrachloride (CCl_4) is one of the many chemical substances implicated in liver or kidney damage in recent times. 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 (Manautou and Alekunes, 2010).

*Corresponding author: e-mail: ikenna.uchendu@unn.edu.ng

Studies have shown that several chemical compounds such as cystamine, 2-diethylaminoethyl 2, 2-diphenylvalerate (SKF 525-A), are protective against CCl_4-induced organ damage, however these compounds are expensive and may have severe side effects. This work was therefore necessary to provide a natural and cheap therapy for CCl_4-induced kidney damage. The aims of this research were to evaluate the phytochemicals in *Curcuma longa* and evaluate the combined effects of aqueous extract of *Curcuma longa* and some calcium channel blockers on function and histological structure of the kidney in albino rats treated with carbon tetrachloride (CCl_4).

MATERIALS AND METHODS

Collection and authentication of Tumeric

Fresh samples of tumeric (*Curcuma longa*) were obtained from Akwatta, a local market in Enugu, Nigeria. The plant material was authenticated by a consultant taxonomist at the herbarium section of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Preparation of aqueous extract of Tumeric

Preparation as described by Al-Taee *et al*. (2011) was done, with slight modification. Briefly, water extraction of turmeric was prepared by boiling 100gm in 1000ml distilled water over low flame for 15 minutes, using a heat-stable flask. The content of the flask was allowed to cool for 20 minutes. After cooling, the content of the flask was sieved using clean muslin cloth and filtered with Whatman No.1 filter paper (Whatman Clifton, NJ, USA). The filtrate was used to prepare the required concentration.

Acute toxicity test (LD50)

This was performed on mice and the Lorke procedure of LD50 determination was used (Lorke, 1983).

Phytochemical analysis of tumeric

Preliminary phytochemical screening of tumeric (*Curcuma longa*) for the presence of glycosides, flavonoids, saponins, steroids, tannins, carbohydrates, proteins and terpenoids was carried out at Department of Pharmacognosy, Faculty of Pharmaceutical Science, University of Nigeria Nsukka. Methods according to Trease and Evans (2002) were used for the analyses.

Reagent

The CCl_4 was of analytical grade and was purchased from Ogbete main market, Enugu.

Preparation of calcium channel blocker solutions

Thirty-two (32) tablets of 10mg (i.e. 320mg) Amlodipine and 20 tablets of 20mg Nifedipine (400mg) obtained from a licensed Pharmacist at Ogbete main market, Enugu, 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 experimental animal by intraperitoneal injection with CCl_4 (0.4ml/kg), daily for 3 days.

Animals and maintenance

A total of thirty (30) adult male albino wistar 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. All the animals used in this study were handled according to Institutional guidelines describing the use of rats and in accordance with the American Physiological Society guiding principles for research involving animals and human beings (APS, 2002). In addition, proper care was taken as per the ethical rule and regulation of the concerned committee of the University of Nigeria, Nsukka, Enugu State, Nigeria. An 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 30 experimental rats were grouped into (A-F) and received the following treatments on a daily basis for three days:

- *Group A:* (Negative Control): received intraperitoneal administration of CCl_4 (0.4ml/kg body weight) only for 3 days.
- *Group B*: received CCl_4 and AECL (200mg/kg, oral) for 3 days.
- *Group C*: received CCl_4; AECL and Nifedipine (1mg/100g of rat, i.p) for 3 days.
- *Group D*: received CCl_4, AECL and Amlodipine (1mg/100g of rat, i.p) for 3 days.
- *Group E*: received AECL only for 3 days.
- *Group F*: (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 kidneys harvested for histological and oxidative stress analyses.

Biochemical analysis

The levels of Serum Electrolyte, Urea and Creatinine were estimated using the following methods: K^+ and Na^+ determined using Perlong Medical PL1000A Electrolyte Analyser; Serum urea concentration was determined using the diacetylmonoxime method with protein precipitation according to Natelson *et al*. (1951), Serum creatinine concentration determined using the Jaffe Reaction according to Fabiny and Ertingshausen (1971)

Oxidative stress analysis

Rat kidney homogenates were prepared using the method described by Eldi *et al*., (2015). Lipid peroxidation (MDA) level in the kidney homogenate was determined according to the method described by Mihara and Uchiyama (1978). Super oxide dismutase (SOD) activity in kidney homogenate supernatant was determined spectrophotometrically according to the method by Roth and Gilbert (1984).

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 (Baker *et al*., 1998). The histological sections were examined using an Olympus ™ light microscope.

STATISTICAL ANALYSIS

Data analysis was done using Graph pad prism version 7.0 (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). Probability levels of less than 0.05 were considered significant.

RESULTS

Acute toxicity studies

LD50 value of the extract was 7500mg/kg which indicates that AECL is safe and is not toxic to mice.

Table 1: Qualitative phytochemical analysis of aqueous extract of *Curcuma longa*

Constituent	Indication
Carbohydrate	−
Reducing Sugar	−
Alkaloids	+
Glycosides	+
Saponins	+
Tannins	+
Flavonoids	++
Resins	−
Proteins	−
Oils	−
Phenolic Compounds	++
Terpenoids	++
Phytosterols	+

Key: ++ = present; + = present (in trace amount); − = absent

Phytochemical results

Phytochemical analysis indicated the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, tannins, phenolic compounds, terpenoids and phytosterols in the plant extract (table 1).

Combined effects of aqueous extract of Curcuma longa (Turmeric) and some calcium channel blockers on body weight

Combined effects of aqueous extract of *Curcuma longa* (Turmeric) and some calcium channel blockers on body weight of rats treated with carbon tetrachloride (CCl_4) is represented in fig. 1. It was observed that rats in the CCl_4 group were sluggish, lost appetite and response to stimulus was slow. The mean decrease in body weight was highest in the CCl_4-alone group (negative control) in comparison with other groups.

Biochemical results

Table 2 shows the results of kidney biochemical parameters: urea, creatinine, K^+, Na^+ levels in six (6) groups of five (5) animals which received intraperitoneal administration of carbon tetrachloride CCl_4 (0.4ml/kg body weight) and/or aqueous extracts of *curcuma longa* (AECL**)** or in combination with some calcium channel blockers (nifedipine and amlodipine) for 3 days. From the results, AECL showed significant renal protection (*P<0.05) in comparison with negative control (CCl_4 alone). Furthemore, in combination with the calcium channel blockers, nifedipine or amlodipine, there was enhanced renal protection against the nephrotoxicant, CCl_4 (*P<0.05).

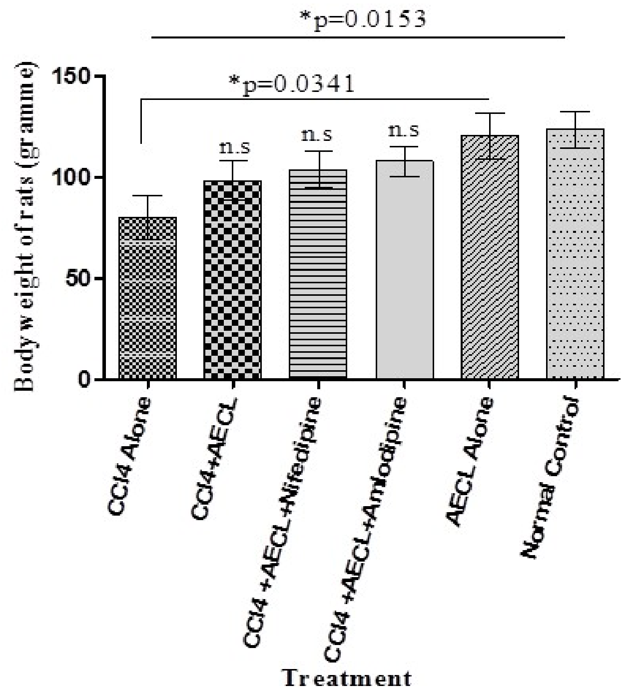

Fig. 1: Combined effects of aqueous extract of *curcuma longa* (Turmeric) and some calcium channel blockers on body weight of the rats. Histogram show the body weight of rats in the experimental groups. The preliminary data show intraperitoneal administration of CCl_4 induced a significant weight loss. However, oral administration of *Curcuma longa* (Turmeric) alone or in combination of Nifedipine and Amlodipine separately non-significantly induced higher body weight when compared with CCl_4 group (negative control). The data are presented as mean±SEM of body weight (gramme) for individual treatment. See *Materials and Methods* for experimental details. Statistical analyses were performed using ANOVA (*p<0.05).

In fig. 2, albino Wister rats which received CCl_4 alone (negative control) for 3 days, showed the highest MDA level and lowest SOD activity (fig 2A and 2B; respectively). Data show that intraperitoneal administration of CCl_4 for 3 days induced a significant increase and decrease in the tissue levels of MDA and SOD activity respectively (p<0.001) in the rat kidneys in comparison with normal control group. However, the treatment with AECL alone or in combination of a

Table 2: 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	40.33 ± 4.91	1.43 ± 0.09	6.93 ± 0.52	123.71 ± 2.03
CCl_4 + AECL	24.34 ± 2.96*	0.90 ± 0.17	5.30 ± 0.47	132.82 ± 1.14*
CCl_4 + AECL + Nifedipine	23.87 ± 2.33*	0.69 ± 0.18*	5.10 ± 0.55	130.68 ± 1.45*
CCl_4 + AECL + Amlodipine	22.69 ± 1.45*	0.70 ± 0.06*	5.14 ±0.54	131.02 ± 1.81*
AECL Alone	20.68 ± 1.72**	0.65 ± 0.13**	4.70 ± 0.25*	131.00 ± 2.65*
Normal Control	20.65 ± 2.73**	0.60 ± 0.06**	4.73 ± 0.43*	133.42 ± 1.76*

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.

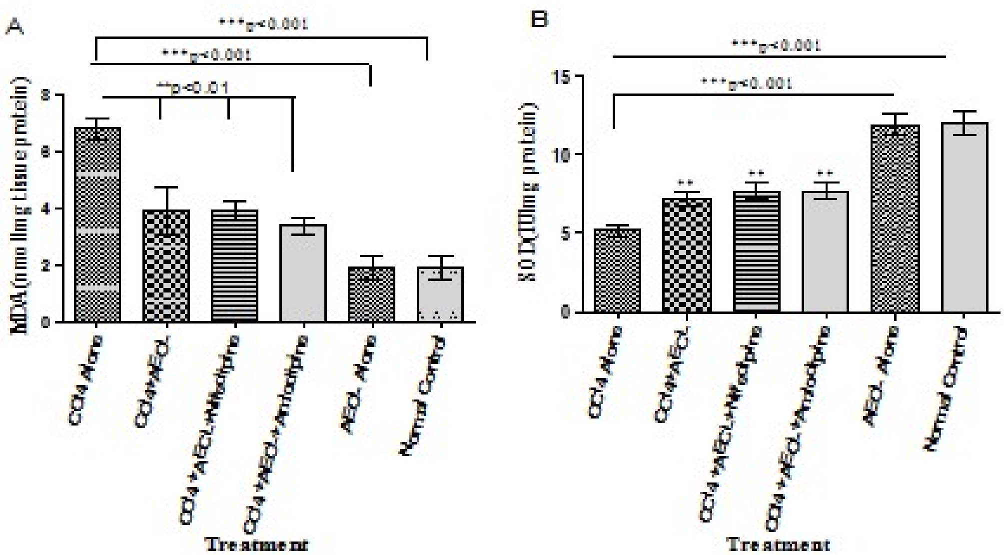

Fig. 2: Effect of oral administration of AECL at dose (200mg/kg b.w) plus nifedipine or amlodipine on MDA levels and SOD activity in the kidney of CCl_4-treated rats. The histograms A and B show kidney tissue MDA levels and SOD activity respectively following treatment with CCl_4 alone or in combination with AECL alone or AECL and nifedipine or amlodipine. The preliminary data show AECL alone or in combination with nifedipine or amlodipine induced lower MDA levels and higher SOD activity when compared with CCl_4 (negative control). Each column represents mean±S.E.M. of data from five rats. Normal control group was administered distilled water as a vehicle. The symbol (***) and (**) represent p<0.001 and p<0.01 respectively when CCl_4 group is compared with other groups. Statistical analyses were performed using ANOVA.

calcium channel blocker was shown to induce a significant decrease and increase in level of MDA and SOD activity respectively (p<0.01) in the rat kidney compared with CCl_4 alone. Interestingly, AECL combination with either nifedipine or amlodipine showed synergistic nephroprotection, much better than AECL treatment alone, when MDA level and SOD activity are compared with CCl_4 alone (p<0.01).

Histopathological results

In fig. 3, microscopic examination of the kidneys isolated from the rats at sacrifice revealed no histopathological alteration in the normal control rats (fig. 3F). Also AECL alone-treated rats showed no significant alteration (fig. 3E). Presence of significant distortion and erosion of the glomeruli were observed in the kidney of rats treated with CCl_4 only (fig. 3A); however non-significant degenerations were observed in rats administered with AECL alone or in combination with nifedipine or amlodipine separately, in the presence of CCl_4 challenge (fig. 3B, 3C and 3D respectively). The kidneys of rats in group B, C and D showed no significant histological alterations when compared with the normal control group.

DISCUSSION

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 (Brautbar and Williams, 2002). 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

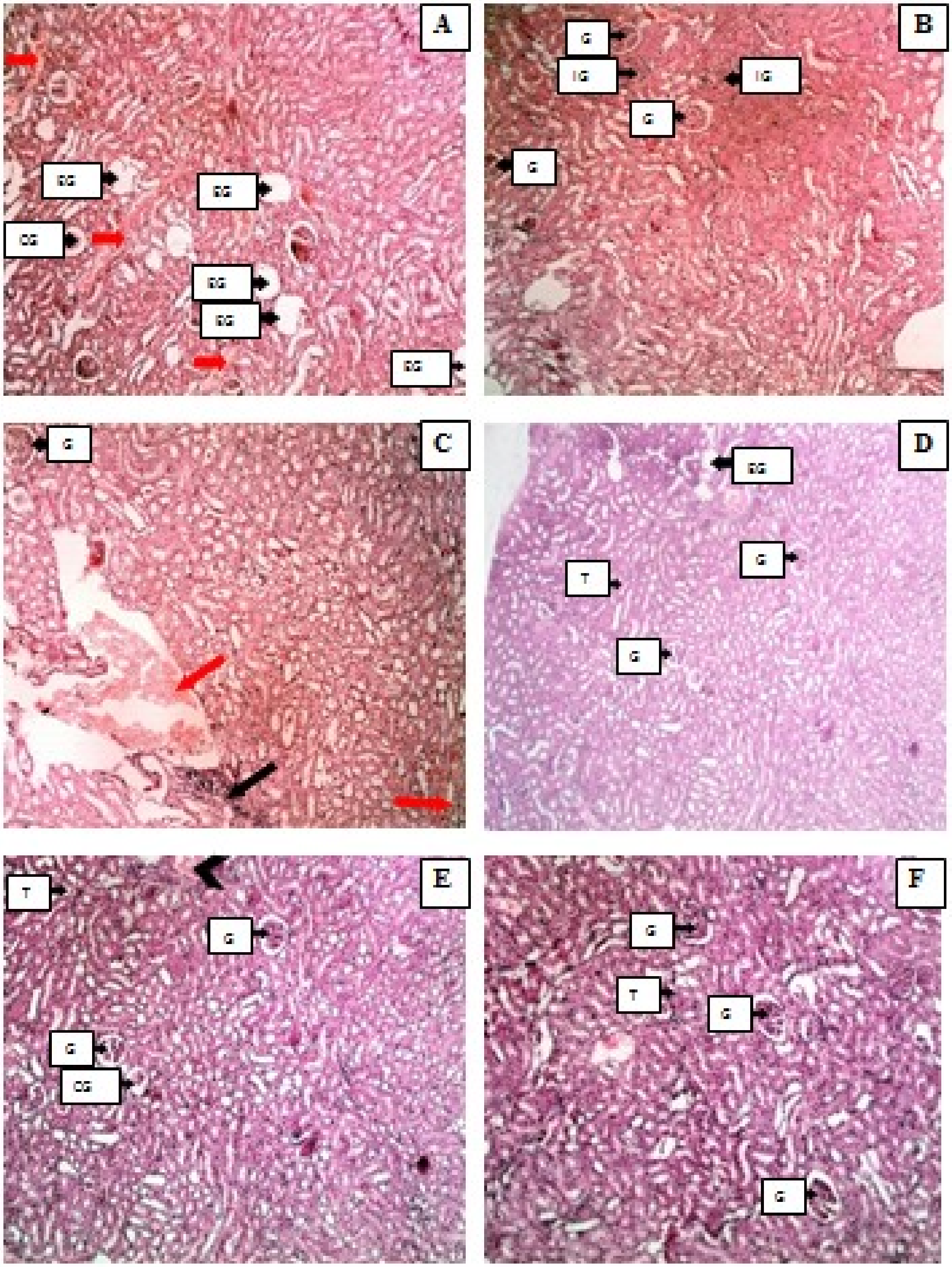

Fig. 3: Histopathology and photomicrograph of kidney. (A) CCl_4 alone- treated rats. Most of the glomeruli are eroded (EG) while others are constricted (CG). The tubules (T) appear normal and some areas of congestion are seen (red arrow). (B) CCl_4+AECL-treated rats: Some glomeruli (G) are inflamed with increased cellularity (IG). (C) CCl_4+AECL+Nifedipine-treated rats: Normal glomeruli seen. A zone of congested stroma (red arrow) and leucocyte infiltration (black arrow) is seen. (D) CCl_4+AECL+Amlodipine-treated rats: Normal glomeruli seen, a zone of eroded glomerulus seen. (E) AECL only-treated rats: Normal glomeruli seen, the tubules (T) appear normal. A hyperaemic portion of the stroma is seen (black arrow head). A constricted glomerulus (CG) is also seen (F) Normal control rats. There is normal appearance of glomeruli (G) and renal tubules (T). [Stain: H and E; ×100].

fatty acid of a phospholipid (Weber *et al*., 2003). 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 (Weber *et al*., 2003). The extent of kidney damage is assessed by the increase in serum levels of Urea, Creatinine, potassium, and by histopathological examination.

Hiroki *et al*. (2016) 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) in combination with aqueous extract of turmeric 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. These calcium channel blockers act by preventing the influx of calcium ions into kidney-tissue cells (Triggle, 2007).

The histological and biochemical changes indicate that combination of turmeric extract with nifedipine or amlodipine synergistically protects kidney from $CC1_4$-induced damage. This may be attributed to the bioactive antioxidant phytochemicals present in the extract; or alteration in extracellular .and intracellular Ca^{2+} concentration, general vasodilator action or to antilipoperoxidative properties of calcium channel blockers. Nifedipine and amlodipine are well known selective Ca^{2+} influx blockers in the myocardium, vascular smooth muscles and various parts of the brain (Singh, 1995).

It is possible that nifedipine and amlodipine inhibit Ca^{2+} influx and modulate intracellular calcium which helps in preventing Ca^{2+} accumulation in tissue cells, since it was demonstrated that cytosolic Ca^{2+} is elevated 100 folds in rat hepatocytes exposed to $CC1_4$ which is capable in initiating irreversible liver cell injury *(*Weber *et al.,* 2003). General vasodilator effect of these calcium antagonists improves tissue blood flow which may be useful in preventing $CC1_4$-induced hypoxia, since reduced tissue blood flow and associated hypoxia account for the tissue necrosis in $CC1_4$-poisoning. Mak and Weglicki (1990) have reported antiperoxidant effect of calcium antagonists in sarcolemmal membrane. Nayler and Britnell (1991) have suggested that Ca^{2+} antagonists provide cellular protection by an unknown mechanism.

In the present study, biochemical results show that AECL alone, in the presence of CCl_4 challenge, showed a significant nephroprotection. Interestingly, when combined with nifedipine or amlodipine separately, a positively significant synergy in nephroprotection was observed.

In the CCl_4 group, animals were sluggish, lost appetite and response to stimulus was slow. In this group, there was significant elevation in serum Urea, Creatinine and K^+ levels when compared to the normal control rats (table 2). The above results show that CCl_4-nephrototoxicity was effectively produced in the negative control group. The results of ours study show that CCl_4-nephrototoxicity was effectively produced in all cases. Earlier, Ozturk *et al*. (2003) and Khan *et al*. (2010) have independently reported similar observations.

The rats that were administered plant aqueous extract alone or in combination with a calcium channel blocker, serum Urea, Creatinine and K^+ values were significantly decreased. These results show that the extent of the kidney damage is reduced as the renal biomarker values are lower than CCl_4 (negative control) group. On histological examinations, there were minimal lesions on the nephrons and erosion of glomeruli was almost absent, as supported by the kidney oxidative stress analysis (MDA and SOD results), which reveals that turmeric extract contain the active ingredient(s) which protects the kidney. Although there was decrease in body weight but mean decrease of absolute body weight was lower than negative control group (fig. 1). It seemed likely that nephroprotective phytochemicals are substantially present in the aqueous extract to produce their protective action against the injury. Arguably, it is also possible that bioactive phytochemicals are soluble in aqueous extract. In this present study, aqueous extract of *C. longa* caused a significant decrease in the levels of serum urea, creatinine and K^+ ($P<0.05$ or $P<0.01$) when compared with negative control group. Increase in rat body weight could be due to recovery of the rats and thus better feeding and general well being.

CONCLUSION

This study showed that aqueous extract of *C. longa* has nephroprotective effects against CCl_4-induced kidney damage. In addition, the combination of the aqueous extracts of *C. longa* and calcium channel blockers synergistically improves recovery against nephrotoxicity in albino rats.

ACKNOWLEDGEMENTS

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.

REFERENCES

Al-Taee MF, Al-Ahmed HI and Abdul Malek HW (2011). Studying the Effect of Aqueous Extract from Curcuma Longa on Some Parameters of Cytogenetic, Immunity and Fertility in Female Mice. *Baghdad Sci. J.*, **8**(1): 73-80.

American Physiological Society (2002). Guiding principles for research involving animals and human beings. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **283**(2): R281-R283.

Baker FJ, Silverton RE and Pallister CJ (1998). *Baker and Silverton's Introduction to Laboratory Technology*. 7th Editon, Butterworth-Heinemann, Wobrun, MA, USA, p.448.

Brautbar N and Williams J (2002). Industrial solvents and liver toxicity: Risk assessment, risk factors and mechanisms. *Int. J. Hyg. Environ Health*, **205**(6): 479-491.

Eldi A, Pejman M, Jalal ZM and Parisa MM (2015). Hepatoprotective effects of Portulaca oleracea extract against CCl4-induced damage in rats. *Pharm Biol*., **53**(7): 1042-1051.

Fabiny DL and Ertingshausen G (1971). Automated reaction-rate method for determination of creatinine with the centrifichem. *J. Clin. Chem.*, **17**(8): 696-700.

Hewlings SJ and Kalman DS (2017). Curcumin: A review of its' effects on human health. *Food*, **6**(10): 92-96.

Hiroki Y, Nonogaki T, Fukuishi N and Onosoka S (2016). Carbon deficient diet attenuates carbon tetrachloride-induced hepatotoxicity in mice through suppression of lipid per oxidation and inflammatory responds. *Heliyon*, **2**(6); e00126.

Khan RA, Khan MR, Sahreen S and Bokhari J (2010). Prevention of CCl4-induced nephrotoxicity with Sonchus asper in rat. *Food Chem. Toxicol.,* **48**(8-9): 2469- 2476.

Kingsley UI (2018). Effects of tomato extract (*lycopersicon esculentum*) on carbimazole-induced alterations in kidney of albino rats. *Int J Res Rev*, **5**(1): 72-79.

Lorke D (1983). A new approach to practical acute toxicity testing. *Archives in Toxicology*, **54**(4): 275-287.

Mak IT and Weglicki BW (1990). Comparative antioxidant activity of propranolol, nifedipine, verapamil and diltiazem against sarcolemmal membrane lipid peroxidation. *Circul. Res.,* **66**(5): 1449-1452.

Manautou JE and Alekunes LM (2010). Hepatic toxicology. *Compr. Toxicol.*, **9**(8): 2-5.

Mihara M and Uchiyama M (1978). Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal. Biochem*, **86**(1): 271-278.

Natelson S, Scott ML and Beffa C (1951). A rapid method for the estimation of urea in biologic fluids. *Am J. Clin. Pathol.*, **21**(3): 275-281.

Nayler WG and Britnell S (1991). Calcium antagonist and tissue protection. *J. Cardiovas. Pharmac.,* **18**(SuppI): 1-5.

Ozturk F, Ucar M, Ozturk IC, Vardi N and Batcioglu K (2003). Carbon tetra chloride-induced nephrotoxicity and protective effects of betaine in Sprague-Dawley rats. *Urology*, **62**(2): 353-356.

Roth EF and Gilbert HS (1984). The pyrogallol assay for superoxide dismutase: Absence of a glutathione artifact. *Anal Biochem*, **137**(1): 50-53.

Singh J, Kaur and AH and Mathur SK (1995). Protection of CCl_4-induced liver damage in rats by some calcium channel blockers. *Indian J. Physiol. Pharmacol.,* **39**(3): 275-278.

Singh N, Kamath V, Narasimhamurthy K and Rajini PS (2008). Protective effect of potato peel extract against carbon tetrachloride induced liver injury in rats. *Environ. Toxicol. and Pharmacol*, **26**(2): 241-246.

Trease G and Evans SM (2002). Pharmacognosy: (15^{th} Edition). English Language Book Society. *Bailliere Tindall*, London, pp.23-67.

Triggle DJ (2007). Calcium channel antagonists: Clinical uses-past, present and future. *Biochem. Pharmacol.*, **74**(1): 1-9.

Uchendu IK, Orji OO and Agu CE (2017). Attenuation of glycerol-induced acute renal failure in albino rats by soy beans (*Glycine max*). *Int J Chem. Tech. Res*, **10**(12): 165-172

Weber LW, Boll M and Stampfl A (2003). Hepatotoxicity and mechanism of action of haloalkanes: Carbon tetrachloride as a toxicological model. *Crit. Rev. Toxicol.*, **33**(2): 105-36.