

Nephroprotective effect of *Cissampelos owariensis* extract on renal histomorphology of Wistar rats during exposure to Carbon tetrachloride-induced nephropathy

ABSTRACT

To assess nephroprotective potency of methanolic extract of *C. owariensis* on renal histomorphology of Wistar rats during exposure to nephropathic activity of CCl₄.

Twenty eight (28) albino Wistar rats divided into four groups which include normal control group administered with vehicles -distilled water (1 ml/kg b.w.) and olive oil (3 ml/kg b.w.), experimental control group administered with CCl₄ (3 ml/kg b.w.) twice a week, first treatment group administered with CCl₄ (3 ml/kg b.w.) twice a week + methanolic extract of *C. owariensis* (100 mg/kg b.w.) daily and second treatment group administered with CCl₄ (3 ml/kg b.w.) twice a week + methanolic extract of *C. owariensis* (300 mg/kg b.w.) daily for twenty eight (28) days. Phytochemical analysis of methanolic extract of *C. owariensis* was carried out using GC-MS. The body weight of study animals was measure at days 0, 7, 14, 21 and 28 of study. Then, kidney tissue of study animals was collected, weighed and processed for histopathological study. Tissue sections were stained using H & E, examined under microscope, photomicrographs were generated and observable histopathological changes were quantified using image-J software

Phytochemical analysis of methanolic extract of *C. owariensis* showed abundance of phenolic compounds which may in turn confer antioxidant property on the extract. Results of this study also showed that treatment with extract helped to reduce body and tissue weight loss that follows exposure to CCl₄. Also, treatment with the extract helped to reduce significantly ($p < 0.05$) renal histopathological changes following exposure to CCl₄.

The methanolic extract of *C. owariensis* contains abundant phenolic compounds which confer antioxidant property that in turn mediate the nephroprotective potency of the extract against nephropathic effect of CCl₄.

Keywords: *Cissampelos owariensis*; Carbon Tetrachloride; Nephroprotection; Wistar rats

1. INTRODUCTION

Nephropathy often results from exposure to chemical substances that can structurally distort or functionally impair the renal tissue. One of such chemical substances is Carbon tetrachloride (CCl₄) which is a potent cytotoxin with wide ranging toxic effects that affect several body tissues including the kidney. It is commonly employed as cytotoxin to produce experimental models of tissue pathology including nephropathy or nephrotoxicity [1,2]. The nephropathic effect of CCl₄ is usually due to the resulting oxidative damage of renal tissue through activity of reactive oxygen species (ROS) as well as the impairment of antioxidant defence system following exposure to the cytotoxin [3,4]. Invariably, therapeutic agent will exhibit nephroprotective effect during CCl₄ exposure via suppression of oxidative stress in renal tissue as well as improvement of antioxidant defence system of the body [5]. Currently, there is a significant increase in the application of phytotherapy in combating various tissue pathologies (including nephropathy) especially in developing countries [6,7]. Moreso, there exist an avalanche of medicinal plants with known and in many cases unknown pharmacological uses. One of such is *Cissampelos owariensis* P. Beauvalis ex D.C. which is a medicinal plant commonly found in the tropical region where it is widely applied for various ethnopharmacological uses [8]. Basically, the characteristic medicinal value of these plants or their extracts is a function of the properties or

biological activity of their constituent active compounds or phytochemicals [9,10]. According to findings in previous studies, *C. owariensis* extracts contains phytochemicals that exhibit potent antioxidant and free radical scavenging properties which may in turn culminate into their therapeutic or medicinal effects [11-13]. In particular, some of the ethno-therapeutic or pharmacological applications of *C. owariensis* include treatment of wounds, snake bites, amnesia, dysentery, diarrhoea, enteritis and other disease conditions of circulatory, gastrointestinal and reproductive systems [14-18]. Still, there persist inadequate pharmacological studies either on the whole or different parts of *C. owariensis* plant. Therefore, this study was aimed at assessing nephroprotective potency of methanolic extract of *C. owariensis* (MECO) on renal histomorphology of Wistar rats during exposure to nephropathic activity of CCl₄.

2. MATERIALS AND METHODS

2.1 Chemical reagents and plant material used

The chemical reagents used in this study were procured from Bristol Scientific Co. Ltd. (Lagos, Nigeria) and Sigma-Aldrich (St Louis, MO, USA) while fresh whole *C. owariensis* plant was harvested from the suburb of Okada community, Ovia North-East Local Government Area, Edo State, Nigeria. The plant was identified at the Department of Pharmacognosy, Igbinedion University, Okada, Edo State, Nigeria.

2.1.1 Preparation of chemical reagents and plant extract

The CCl₄ was dissolved in olive oil (ratio 1:1) to obtain the experimental nephropathic-inducing agent. The leaves of *C. owariensis* were detached, dried and pulverized into powdered form. To obtain MECO used for this study, 1000 g of the powdered leaf material was infused in methanol for 72 hours, filtered, weighed and evaporated to dryness to produce 64 g (representing 6.4%) yield. MECO was phytochemically analyzed using gas chromatography-mass spectrometer (GC-MS).

2.2 GC-MS phytochemical analysis of plant extract

Phytochemical analysis of MECO was performed using gas chromatograph (7890A series) coupled to TSQ quantum XLS mass spectrometer with an injector device (7683B series). 0.1 g of extract was dissolved in methanol (1 ml) for 5 minutes, filtered to obtain a clear filtrate used for the GC-MS method of identification and quantification of phytochemical constituents of the extract. Phytochemical compounds present in plant extract were identified through comparison of their mass spectra with reference spectra in the database of National Institute of Standard and Technology (NIST) library.

2.3 Experimental animal care and handling

Twenty eight albino Wistar rats, weighing between 160–185 g, used for this study were sourced from the Central Animal House Facility, Igbinedion University, Okada, Edo State, Nigeria wherein the study was also carried out. Within the facility, the study animals were housed in animal cages under hygienic conditions, exposed to 12 hour light/dark cycle, fed on standard animal feed and granted free access to drinking water *ad libitum* throughout the period of study.

2.4 Experimental design

The study animals were randomly divided into four groups with each group comprising seven animals (n=7) and the treatment regimen used for this study is as follows: first group of animals represented normal control group (NCG) administered with vehicles- distilled water once daily orally and olive oil twice a week intraperitoneally (i.p.) at dosages of 1 ml/kg body weight (b.w.) and 3 ml/kg b.w. respectively. The second group of animals represented experimental control group (ECG) administered with CCl₄ (30% in olive oil) twice a week i.p. at dosage of 3 ml/kg b.w. The third group of animals represented first treatment group (FTG) administered with CCl₄ (30% in olive oil) twice a week i.p. and MECO once daily orally at dosages of 3 ml/kg b.w. and 100 mg/kg b.w. respectively. The fourth group of animals represented second treatment group (STG) administered with CCl₄ (30% in olive oil) twice a week i.p. and MECO once daily orally at dosages of 3 ml/kg b.w. and 300 mg/kg b.w. respectively. The dosages of CCl₄ and MECO used in this study were according to studies by Khan et al. [19], Ekeanyawu et al. [20] and Omotoso et al. [21]. The period administration in this study was 28 days while the body weight of study animals were measured and recorded during days 0, 7, 14, 21 and 28 of this study.

2.5 Study tissue collection and processing

The study animals were sacrificed 24 hours after the 28-day treatment period and their kidney tissue collected, weighed and processed. The average organ weight calculated as the average weight of right and left kidneys while the relative organ weight was calculated as percentage ratio of average organ weight to final body weight. Tissue processing for histopathological study involved fixation in 10% Neutral Buffered Formalin, dehydration in ascending grades of alcohol (70%, 90% and absolute alcohol), clearing in xylene and embedding in paraffin wax to form tissue blocks.

2.6 Study tissue sectioning and staining

Using kidney tissue blocks and with the aid of rotary microtome, 5 μ thick tissue sections were produced and mounted on microscope slides. Histological staining of tissue sections was done by Haematoxylin and Eosin (H & E) technique.

2.7 Histopathological study

Stained tissue sections for all study groups were examined under microscope to assess histopathological changes in the renal histomorphology of study animals. Photomicrographs of tissue sections were generated and image-J software (NIH, Bethesda, MA, USA) was used to quantify observable histopathological features which include interstitial inflammation, epithelial necrosis, glomerular congestion and tubular dilatation within the renal parenchyma of study animals.

2.8 Statistical analysis

Experimentally derived values during this study were statistically analyzed using IBM-SPSS (version 20) (IBM Corp, NY, USA). Statistical results were presented as mean \pm standard error of mean (SEM) and comparison of statistical results was done using *t*-test while multiple comparisons were done using one way analysis of variance (ANOVA). For all statistical comparisons, the significant probability level was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Phytochemical analysis of plant extract using GC-MS

The gas chromatogram of MECO (Figure 1) showed retention time which represents relative concentration of compounds being eluted and peak area which represents percentage concentration of phytochemical compounds present in the extract.

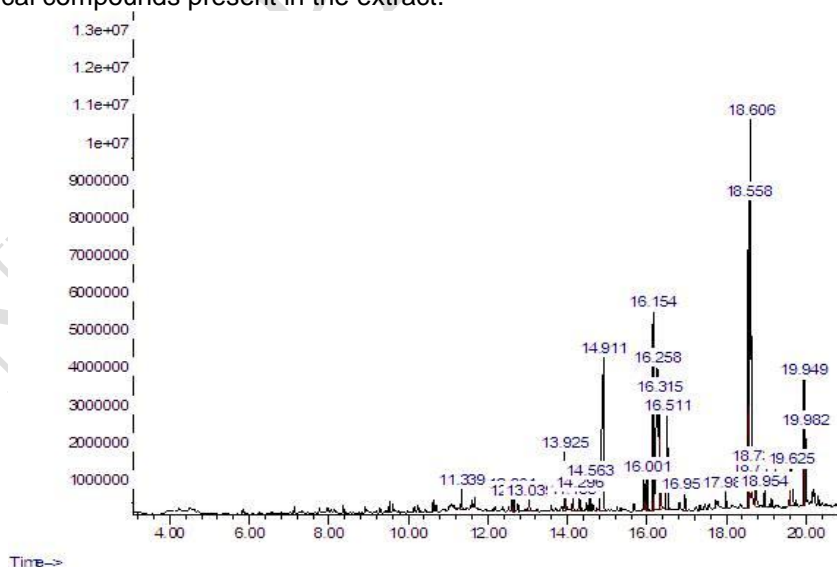


Figure 1: The gas chromatogram of MECO showing twenty six (26) peaks that indicate 26 phytochemical constituents of the extract.

The chromatogram of MECO showed twenty six (26) peaks indicating that the extract contains 26 phytochemical compounds. The retention time (in minutes), name and peak area (%) of phytochemical compounds identified in MECO were given in Table 1.

Table 1: Phytochemical compounds identified in the methanolic extract of *A. conyzoides* by GC-MS

| S/N | Retention Time (Minutes) | Name of Compound | Peak Area (%) |
|-----|--------------------------|---------------------------------------|---------------|
| 1 | 11.34 | 3-Tetradecen-5-yne | 0.86 |
| 2 | 12.60 | 11-Dodecenol | 0.60 |
| 3 | 12.65 | 2,6,10-trimethyl-Tetradecane | 0.56 |
| 4 | 13.04 | Tetradecanoic acid | 0.59 |
| 5 | 13.93 | 2,6,6-trimethyl-Bicyclo[3,1,1]heptane | 2.28 |
| 6 | 14.13 | 1-(2-methylpropyl)-Cyclohexene | 0.55 |
| 7 | 14.30 | Neophytadiene | 0.76 |
| 8 | 14.56 | Hexadecanoic acid | 1.07 |
| 9 | 14.91 | n-Hexadecanoic acid | 12.89 |
| 10 | 15.93 | 9,12-Octadecadienoic acid | 0.99 |
| 11 | 16.00 | cis-13-Octadecenoic acid | 1.21 |
| 12 | 16.15 | Phytol | 7.45 |
| 13 | 16.26 | 9-Hexadecyn-1-ol | 14.66 |
| 14 | 16.32 | 6-Octadecenoic acid | 4.32 |
| 15 | 16.51 | Octadecanoic acid | 4.27 |
| 16 | 16.95 | 11-Tetradecyn-1-ol | 0.71 |
| 17 | 17.98 | Eicosanoic acid | 0.59 |
| 18 | 18.56 | p-Cresol | 15.64 |
| 19 | 18.61 | 3-(pentadec-8-en-1-yl)phenol | 16.89 |
| 20 | 18.71 | 3-pentadecyl- Phenol | 0.91 |
| 21 | 18.74 | 15-Hydroxypentadecanoic acid | 1.51 |
| 22 | 18.95 | Bis(2-ethylhexyl) phthalate | 0.54 |
| 23 | 19.60 | 9,12-Tetradecadien-1-ol | 1.26 |
| 24 | 19.63 | 9,12,15-Octadecatrien-1-ol | 2.23 |
| 25 | 19.95 | 4-methyl-1,4-Benzenediol | 4.40 |
| 26 | 19.98 | Orcinol | 2.26 |

The mean values of body weight of study animals in NCG, ECG, FTG and STG measured on days 0, 7, 14, 21 and 28 of study were given in Figure 2. Comparatively, mean values of body weight of study animals showed significant ($p < 0.05$) reduction in ECG while non-significant decrease was observed among treatment groups (FTG and STG) relative to the NCG.

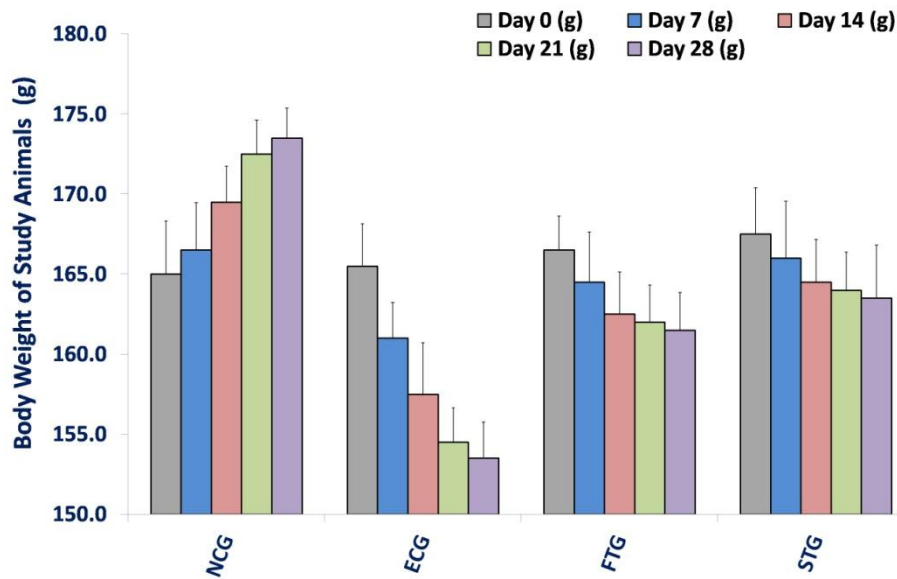


Figure 2: Mean values of body weight of study animals in NCG, ECG, FTG and STG. NCG = Normal control group; ECG = Experimental control group; FTG = First treatment group; STG = Second treatment group.

The mean values of average organ weight and relative organ weight of study animals in NCG, ECG, FTG and STG were given in Figure 3. Similarly, mean values of average organ weight and relative organ weight of ECG showed significant ($p < 0.05$) reduction while the treatment groups (FTG and STG) showed non-significant reduction relative to NCG.

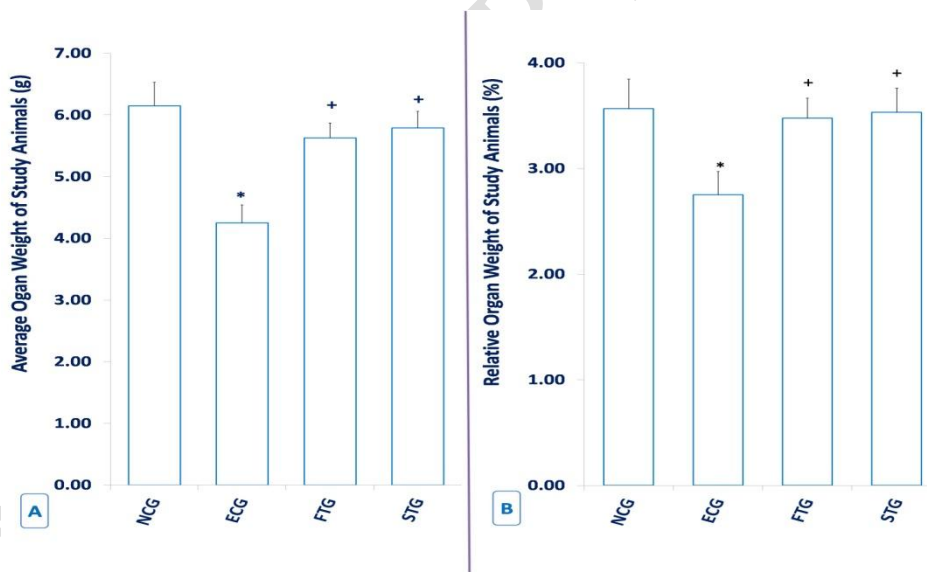


Figure 3: Mean values of average organ weight (A) and relative organ weight (B) of study animals in NCG, ECG, FTG and STG (*, + = significant from normal and experimental control respectively at $P < 0.05$). NCG = Normal control group; ECG = Experimental control group; FTG = First treatment group; STG = Second treatment group.

Microscopic examination of tissue sections revealed various histopathological features in renal tissue of study animals in NCG, ECG, FTG and STG (Figures 4). The evaluated renal histopathological features include interstitial inflammation, epithelial necrosis, glomerular congestion and tubular dilatation within renal parenchyma of study animals (Figure 5 and 6).

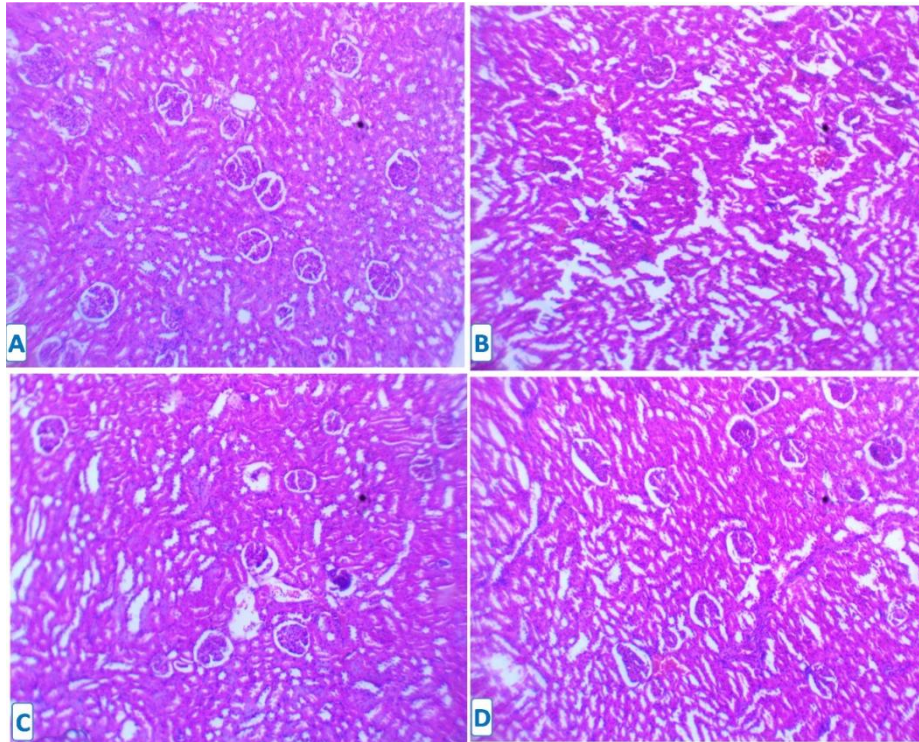


Figure 4: Representative photomicrograph of kidney tissue of study animals in NCG (A), ECG (B), FTG (C) and STG (D). NCG = Normal control group; ECG = Experimental control group; FTG = First treatment group; STG = Second treatment group.

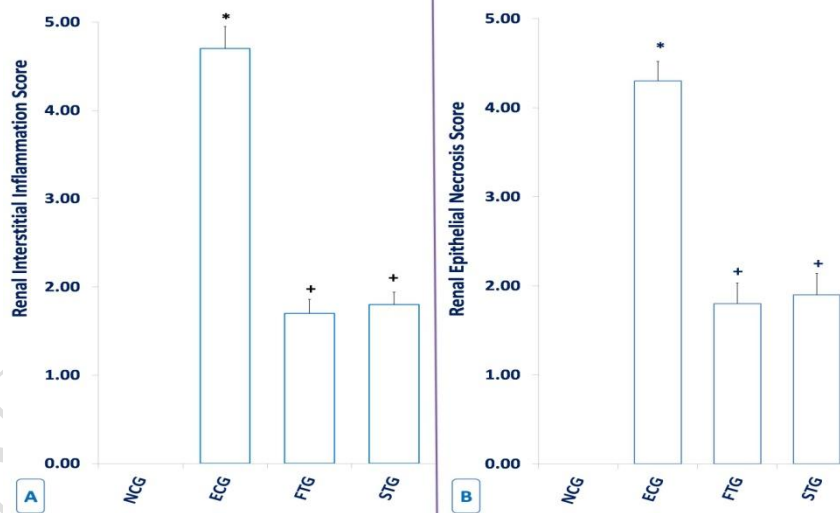


Figure 5: Evaluation of interstitial inflammation (A) and epithelial necrosis (B) within the renal parenchyma of study animals in NCG, ECG, FTG and STG. (*, + = significant from normal and experimental control respectively at $P < 0.05$). NCG = Normal control group; ECG = Experimental control group; FTG = First treatment group; STG = Second treatment group.

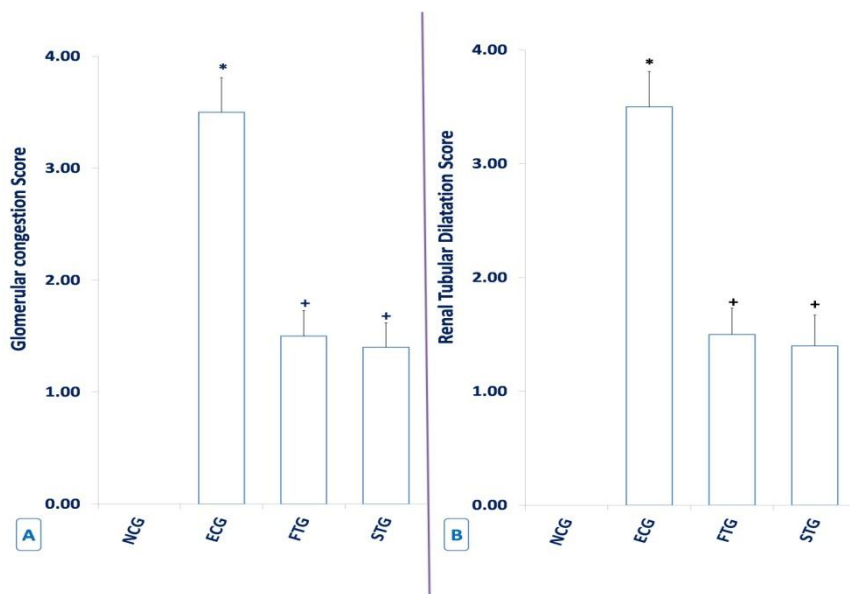


Figure 6: Evaluation of glomerular congestion (A) and tubular dilatation (B) within the renal parenchyma of study animals in NCG, ECG, FTG and STG. (*, + = significant from normal and experimental control respectively at $P < 0.05$). NCG = Normal control group; ECG = Experimental control group; FTG = First treatment group; STG = Second treatment group.

Medicinal plants have been described as plants containing phytochemicals in the whole or some of their parts that can be extracted and applied for therapeutic purposes [22,23]. These plants exhibit widespread distribution globally thereby constituting significant part of natural plant biodiversity and their therapeutic uses date back to antiquity [24,25]. As earlier noted, the therapeutic value of these medicinal plants is a function of their constituent active compounds or phytochemicals.

According to the study by Ekeanyanwu et al. [20], alcohol extract of *C. owariensis* is rich in flavonoids, saponins, tannins and alkaloids which are secondary metabolites that confer on the plant extract its medicinal value. From the result of this study, phytochemical analysis using GCMS revealed abundance of phenolic compounds in MECO (Table 1 and Figure 1). Typically, phenolic compounds in phytochemicals exhibit antioxidant effect especially through the inhibition of ROS activity [26]. The abundance of phenolic compounds in MECO may imply potency of the extract as an antioxidant that can neutralize and protect renal tissue from damaging effect of CCl_4 exposure.

Furthermore, widespread deleterious effects of CCl_4 on different organs of the body can impact negatively on individual organ weight and total body weight. From the result of this study (Figure 2 and 3), exposure to CCl_4 caused significant losses of body weight and kidney tissue weight. However, study animals treated with MECO showed non-significant body and kidney tissue weight loss relative to the normal. This may be linked to the possible cytoprotective potential of MECO in general and nephroprotective potential in particular. In addition, as a potent nephrotoxin, exposure of CCl_4 causes nephropathy with variable histopathological presentations affecting epithelial and interstitial cells, glomerulus, renal tubules and ducts within the renal parenchyma [27-29]. According to the findings in this study (Figure 4), exposure of CCl_4 caused prominent renal histopathological changes within the renal parenchyma. However, study animals treated with MECO showed mild renal histopathological changes.

Evaluation of some histopathological features within the renal tissue of study animals (Figures 5 and 6) showed similar result wherein animals treated with MECO showed non-significant increase in the features quantified. Previous studies by Adewole et al. [29], Sahareen et al. [31] and Dassarma et al. [32] on nephroprotective activity of different therapeutic agents against the CCl_4 -induced kidney damage reported similar qualitative and quantitative findings. According to the results of their studies therapeutic agents usually exhibit nephroprotective effect against damaging effect of CCl_4 exposure by suppression of oxidative damage of kidney tissue usually through the inactivation of free radicals generated during the cytotoxin exposure. Therefore, based on the results of this study, it can be opined that MECO possesses nephroprotective potential and its nephroprotective effect can be linked to the antioxidant properties of its constituent phytochemical compounds.

4. Conclusion

The methanolic extract of *Cissampelos owariensis* contain phytochemical compounds which possess antioxidant properties that in turn confer therapeutic potential on the plant extract including the nephroprotective potential against cytotoxins such as carbon tetrachloride.

ETHICAL APPROVAL

This study was approved by the Research and Ethics Committee, Igbinedion University, Okada, Edo State, Nigeria. **All experimental procedures complied with International guidelines for handling experimental animals.**

REFERENCES

1. Yoshioka H, Usuda H, Fukuishi N, Nonogaki T, Onosaka S. Carbon tetrachloride-induced nephrotoxicity in mice is prevented by pretreatment with Zinc sulfate. *Biol Pharmac Bull* 2016; 39(6): 1042–1046.
2. Safhi MM. Nephroprotective effect of Zingerone against CCl₄-induced renal toxicity in Swiss Albino mice. *Oxid Med Cell Longev* 2018; doi: 10.1155/2018/2474831
3. Khan MR, Siddique F. Antioxidant effects of *Citharexylum spinosum* in CCl₄ induced nephrotoxicity in rat. *Exp Toxicol Pathol* 2012; 64(4): 349-355. doi: 10.1016/j.etp.2010.09.009
4. Khan MR, Zehra H. Amelioration of CCl₄-induced nephrotoxicity by *Oxalis corniculata* in rat. *Exp Toxicol Pathol* 2013; 65(3): 327-334. doi: 10.1016/j.etp.2011.11.007
5. Khan RA, Khan MR, Sahreen S, Bokhari J. Prevention of CCl₄-induced nephrotoxicity with *Sonchus asper* in rat. *Food Chem Toxicol* 2010; 48(8-9): 2469-246. doi: 10.1016/j.fct.2010.06.016
6. Okigbo, R. N., Eme, U. E. and Ogbogu, S. (2008). Biodiversity and conservation of medicinal and aromatic plants in Africa. *Biotechnology and Molecular Biology Review*, 3(6): 127 – 134.
7. Adelanwa, E. B. and Tijjani A.A (2013). An ethno-medical survey of the flora of Kumbotso local government area of Kano State. *Nigerian Journal of Pharmaceutical Sciences*, 12(1): 1 – 9.
8. Akande, R., Okwute, S. K., Iliya, I. and Efiom O.O. (2013). Chemical constituents and anti-tuberculosis activity of the root extracts of *Cissampelos owariensis* (P. Beauv.) Menispermaceae. *African Journal of Pure and Applied Chemistry*, 7(1):21-30.
9. Krishnamoorthy, K., Subramaniam, P. Phytochemical profiling of leaf, stem and tuber parts of *Solena amplexicaulis* (Lam.) Gandhi using GC-MS. *International Scholarly Research Notices* 2014; DOI: 10.1155/2014/567409.
10. Omotoso DR, Akinola AO, Daramola O, Uwagboe V, Onyewotu C. Assessment of gastroprotective activity of leaf extracts of *Ageratum conyzoides* Linn. using pyloric-ligation method in animal model. *Int J Biomed Res* 2019; 10(7): e5214
11. Polterait O (1997). Antioxidants and Free radical Scavengers of Natural Origin, *Current Org. Chem.* 1:415-440.
12. Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, Atangbayila TO (2008). Phytochemical Screening and Anti-oxidant Activities of some selected Medicinal Plants Used for Malaria Therapy in South-Western Nigeria. *Trop. J. Pharm. Res.* 7(3):1019-1024.
13. Olajide O., Orishadipe A., Fatokun O., Akande T. and Iliya I. (2013). The chemical composition of essential oil from the root of *Cissampelos owariensis* (p.beauv) and free radical scavenging activities of its extracts. *African Journal of Pure and Applied Chemistry*, 7(6): 225 – 230.
14. Adjanohoun, E., Ahyi, M.R.A., Ake-Assi, L., Elewude, J.A., Dramane, K., Fadoju, S.O., Gbile, Z.O., Goudole, E., Johnson, C.L.A., Keita, A., Morakinyo, O., Ojewole, J.A.O., Olatunji, A.O., Sofowora, E.A., 1991. Traditional Medicine and Pharmacopoeia. Contribution to Ethnobotanical Floristic Studies in Western Nigeria. Pub. Organization of African Unity, Scientific Technical and Research Commission Lagos, Nigeria, p. 420.
15. Neuwinger H.D. 2000. African Traditional Medicine: A dictionary of plant use and applications. Medpharm Scientific Publishers, Stuttgart.
16. Schmelzer, G.H., 2008. Ressources végétales de l'Afrique tropicale 11(1). In: Plantes médicinales. PROTA, Wageningen, Netherlands.
17. Erhirhie, O. E., Moke, E. G. and Chinwuba P. (2015). *Cissampelos owariensis*: Experimental review. *The Pharma Innovation Journal*, 3(11): 75 – 77.

18. Omotoso DR, Uwagbor V, Lawal OS, Olatomide OD, Okojie IG. Gross and Histo-Morphological Study of Anti-Ulcerogenic Effects of *Cissampelos owariensis* (P. Beauv.) Methanolic Extract in Wistar Rats. *J Biomed Sci* 2019; 8(3): 15.
19. Khan RA, Khan MR, Sahreen S. CCl₄-induced hepatotoxicity: protective effect of rutin on p53, CYP2E1 and the antioxidative status in rat. *BMC Compl Alt Med* 2012; 12: 178 doi: 10.1186/1472-6882-12-178.
20. Ekeanyanwu, R. C., Udeme, A. A., Onuigbo, A. O. and Etienajirhevwe O. F. (2012). Anti-diabetic effect of ethanol leaf extract of *Cissampelos owariensis* (lungwort) on alloxan induced diabetic rats. *African Journal of Biotechnology*, 11(25): 6758 – 6762.
21. Omotoso DR, Okwuonu UC, Uwagbor V, Brown I, Oyaronbi OE. Immunohistochemical assessment of anti-proliferative potential of *Cissampelos owariensis* (P. Beauv.) methanolic extract in hepatic tissue of Wistar rats. *Am J Res Med Sci* 2020; 7(1): 1-7 doi: 10.5455/ajrms.20190828104719
22. Craig GM, David JN. Natural product drug discovery in the next millennium. *Pharm Biol* 2001; 39(supplementary 1): 8-17.
23. Omotoso DR, Akinola AO, Daramola O. Immunoexpression of cell proliferation (Ki-67) and tumor suppressor (p53) protein in hepatic tissue exposed to aqueous extract of *Ageratum conyzoides* Linn using rat model. *Ann Med Biomed Sci* 2019; 5(1): 8-12
24. Abe R, Ohtani K. An ethnobotanical study of medicinal plants and traditional therapies on Batan Island, the Philippines. *J Ethnopharmacol* 2013; 145(2): 554-565.
25. Abubakar A, Nazifi AB, Hassan FI, Duke KA, Edoh TD. Safety assessment of *Chlorophytum alismifolium* tuber extract (Liliaceae): Acute and sub-acute toxicity studies in Wistar rats. *J Acute Dis* 2019; 8(1): 21-27.
26. Aboonabi A, Rahmat A, Othman F. Effects of pomegranate on histopathology of liver and kidney on generated oxidative stress diabetic induced rats. *J Cytol Histol* 2014; 6: 1-5.
27. Ozturk F, Ucar M, Ozturk IC, Vardi N, Batcioglu K. Carbon tetrachloride-induced nephrotoxicity and protective effect of betaine in Sprague-Dawley rats. *Urology* 2003; 62(2): 353-356. doi: 10.1016/s0090-4295(03)00255-3
28. Es.Haghi M, Dehghan G, Banihabib N, Zare S, Mikaili P, Panahi F. Protective effects of *Conus mas* fruit extract on carbon tetrachloride induced nephrotoxicity in rats. *Ind J Nephrol* 2014; 24(5): 291-296. doi: 10.4103/0971-4065.133000
29. Awodele O, Adeneye AA, Aiyola SA, Benebo AS. Modulatory effect of *Mangifera indica* against carbon tetrachloride induced kidney damage in rats. *Intersdiscip Toxicol* 2015; 8(4): 175-183. doi: 10.1515/intox-2015-0027
30. Adewole SO, Salako AA, Doherty OW, Naicker T. Effect of Melatonin on carbon tetrachloride-induced kidney injury in Wistar rats. *Afr J Biomed Res* 2007; 10: 153-164
31. Sahareen S, Khan MR, Khan RA, Alkreathy HM. Protective effects of *Carissa opaca* fruits against CCl₄-induced oxidative kidney lipid peroxidation and trauma in rat. *Food Nutr Res* 2015; 59: 28438. doi: 10.3402/fnr.v59.28438
32. Dassarma B, Samanta S, Nandi DK, Gangopadhyay S, Protective effects of some Generally Recognized as Safe (GRAS) grade food preservatives against experimentally induced renal dysfunction. *J Drug Deliv Therap.* 2019; 9(3-s):412-419 doi: 10.22270/jddt.v9i3-s.2871