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Effects of Aqueous Extract of *Ocimum gratissimum* Leaf on Carbon Tetrachloride-induced Nephrotoxicity in Adult Wistar rats

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ABSTRACT: This study investigated the effects of aqueous leaf extract of *Ocimum gratissimum*on enzymatic antioxidants and renal function in carbon tetrachloride-induced nephrotoxicity. Twenty-five (25) adult Wistar rats were involved in this study. They were randomly assigned into five (5) groups of five (5) animals each, as follows: Group A received 1 ml of distilled water daily, Group B received 1.5 ml/kg of olive oiltwice a week for 15 days, Group C received 250 mg/kg of *Ocimum gratissimum* aqueous leaf extract daily, Group D received 1.5 ml/kg of carbon tetrachloride and olive oil (1:1 *v/v*), twice a week for 15 days, while Group E received 1.5 ml/kg ofcarbon tetrachloride and olive oil (1:1 *v/v*), twice a week for 15 days+ 250 mg/kg of *Ocimum gratissimum* aqueous leaf extract, daily. The extract was administered by orogastric tube, while olive oil and carbon tetrachloride were administered by intraperitoneal injection. Renal function markers (creatinine and urea), and malondialdehyde were assayed in plasma and tissue homogenate, respectively, using standard methods. Obtained data were subjected to statistical analysis using the IBM SPSS statistics software (Statistical Package for Social Science) (Version 25) and relevant statistical values were analyzed. Results showed that the intraperitoneal injection of carbon tetrachloride together with olive oil significantly ($p < 0.05$) increased the levels of urea and creatinine, and a similar outcome was observed in malondialdehyde levels. The administration of *Ocimum gratissimum* ensured a reversal of these levels to those comparable with the control. This demonstrates that aqueous leaf extract *Ocimum gratissimum* has the potential to manage carbon tetrachloride-induced nephrotoxicity.

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Carbon tetrachloride (CCl_4) is a lucid, colorless, fire-resistant liquid. It is a certified and well-known hepatotoxin and nephrotoxin that has been implicated in several medical conditions such as cirrhosis, necrosis and the classical steatosis (Abraham *et al*, 1999; Sherkatolabbasieh *et al.*, 2017). Intoxication with carbon tetrachloride leads to the generation of free radicals in several tissuessuch as the blood, brain, testes, lung, heart, liver and kidneys (Khan *et al.*, 2009; Kumar et al., 2005; Tlili *et al*., 2016). In the not so distant past, carbon tetrachloride was routinely used in dry cleaning and also as an anesthetic. In addition, it is said to be present in low quantities in foods in Britain (Timbrell, 2009). For example, milk contains about 0.2ug/kg; cheese, 5.0ug/kg; butter, 14.0ug/kg; pork liver, 9.0ug/kg; tomatoes, 4.5ug/kg; fresh bread, 5.0ug/kg (Klaassen and Amdur, 2013).Carbon tetrachloride is a very lipid-soluble compound. As a result, its distribution throughout the body is ensured (Timbrell, 2009).The cytochrome P450-mediated movement of a single electron to the C-Cl bond, which produces a radical anion as a transient intermediate that eliminates chlorine to form a carbon-centred radical - the trichloromethyl radical CCl3and chloride ion - is the first phase of nephrotoxicity caused by carbon tetrachloride (Sherkatolabbasieh *et al.*, 2017). In the presence of O_2, the formedCCl_3 is converted into trichloromethyl peroxyl radical (OOCCl3) (a form that is much more reactive). This then begins lipid peroxidation and results in formation of products such as malondialdehyde, which damage the membranes (Halliwell andGutteridge, 2007). The lipid peroxidation caused by the free radicals is a main trigger of cell membrane damage which results in

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several acute and chronic renal pathologies (Satyanarayana *et al*., 2001; Manna *et al*., 2006; Adewole *et al*., 2007).Medicinal plants which mostly possess a lot of phytochemicals with antioxidant properties have been recently in the focus of researchers and scientists for treatment and prevention of various oxidative stress-related complications (Rafieian-Kopaie and Baradaran, 2013; Nasri and Rafieian-Kopaei, 2013). Their antioxidant properties are as a result of the presence of their carotenoid and phenolic compounds which can decrease the intensity of degenerative and complications (Tavafi, 2013). Antioxidants from plants have been reported toprevent against the harmful effects of CCl_4 and other toxic substances (Amini *et al*., 2012; Kader *et al*., 2014).*O. gratissimum* is an herbaceous member of the Labiatae family. The plant is nativeto West Africaand India. It can be found in the coastal and savannah areas of Nigeria. Sri Lanka, Islands of Fiji and Nepal are some of the other locations where *O. gratissimum*is cultivated (Nadkarni, 1999). It goes by several local names which cut across different locations. For example, the Sanskrit and Hindi of India call it "Vriddhutulsi" and "Ram tulsi", respectively, while the Yorubas, Igbos and Hausas refer to it as "effinrin-nla", "Ahuji" and "Daidoya", respectively (Effraim *et al.*, 2003).It has several traditional, alternative and complementary medicinal uses (Akinmoladun *et al*., 2007; Rabelo *et al*., 2003; Orafidiya *et al*., 2001). In view of its glowing reviews, this study was carried out to investigate the effects of aqueous extract of *Ocimum gratissimum* leaf on carbon tetrachloride-induced nephrotoxicity in adult Wistar rats.

MATERIALS AND METHODS

Plant material: Ocimum gratissimum was obtained from Uselu, in Egor Local Government Area of Edo State, Nigeria. It was identified and authenticated with herbarium number UBH-0333in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.The leaves of *Ocimum gratissimum* were collected, washed, air dried under shade and grounded into powder form using a grinding machine and aqueous extraction was done using standard methods.

Experimental animals: Twenty-five (25) adult Wistar rats were involved in this study. They were purchased and then acclimatized for two weeks (14 days) at the animal house in the department of anatomy, University of Benin, Edo State before the commencement of the study. During this period, the animals were provided feed and water *ad libitum*. Handling of the animals was carried out in accordance with approved protocols and in compliance with the recommendations for the proper management and utilization of laboratory animals used for research (Buzek and Chastel, 2010).

Induction of nephrotoxicity: The 15-day intraperitoneal injection of 1.5ml/kg of carbon tetrachloride dissolved in olive oil at a ratio 1:1 will induce nephrotoxicity (Safhi, 2018).

Animal grouping and treatment: The animals were divied into five groups with varying treatment as shown below:

Group A: received 1 ml of distilled water

Group B: received1.5 ml/kg of olive oil, twice a week for 15 days

Group C: received250mg/kg body weight of*Ocimumgratissimum* aqueous leaf extract.

Group D: received 1.5 ml/kg ofcarbon tetrachloride and olive oil (1:1 *v/v*), twice a week for 15 days

Group E: received 1.5 ml/kg ofcarbon tetrachloride and olive oil (1:1 *v/v*), twice a week for 15 days+ 250mg/kg body weight of*Ocimumgratissimum* aqueous leaf extract.

Administration of *O. gratissimum* leaf extract was carried out by orogastric tube while those of olive oil and carbon tetrachloride was by intraperitoneal injection.

Sacrifice, tissue collection, processing and staining: After the period of administration, the animals were euthanized under chloroform anesthesia. Blood was collected by cardiac puncture using a syringe and needle into plain bottles and readied for biochemical assays. The kidneys were harvested and fixed in freshly prepared 10% neutral buffered formalin solution. Staining of tissues was done by established methods (Drury *et al.,* 1976).

Photomicrography: Stained slides were viewed using an optical photomicroscope (Leica MC170 HD, Leica Biosystems, Germany) and photomicrographs were taken at x100 magnification using an attached Eakins 14MP digital microscopic camera, model 2307su, manufactured by Eakins Microscope Store, UK.

Statistical analyses: All data were subjected to statistical analysis using the IBM SPSS statistics software (Statistical Package for Social Science) Version 25 (SPSS, Inc., Chicago, Illinois, USA) and relevant statistical values were obtained. The values of the treated groups were compared with those of nontreated group using the one-way analysis of variance (ANOVA) and the T-test method. Values of $P < 0.05$ were considered significant. LSD was used as the post-hoc test.

RESULTS AND DISCUSSION

Carbon tetrachloride (CCl_4) is a xenobiotic industrial solvent that is used to induce toxicity in experimental animals. A single exposure to carbon tetrachloridedirectly leads to severe tissue necrosis (Manibusan *et al*., 2007).In this study, carbon tetrachloride was responsible for the overt damage to the kidneys, as demonstrated by the elevation of creatinine and urea activities (Figures 1 and 2). An upsurge in the levels of malondialdehyde was also recorded (Figure 3). Creatinine is a nitrogenous end product of metabolism in the blood, distributed throughout the total body water and is normally removed from blood by the kidney. After the exposure to carbon tetrachloride, the kidney function slows down and increases the levels of creatinine in the blood (Safhi, 2018).

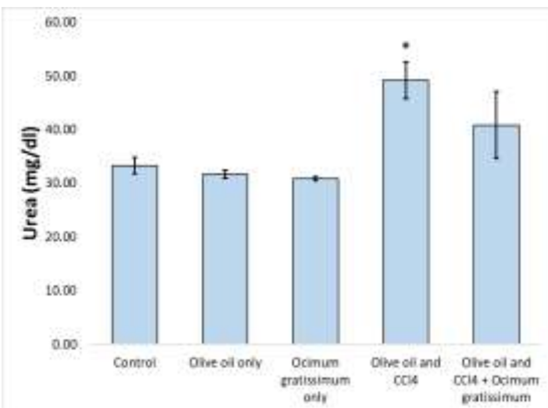

Fig 1: Chart showing the levels of urea (*significantly different from the control group. There was a significant increase ($P<0.05$) of urea in Group D, when compared to the control group)

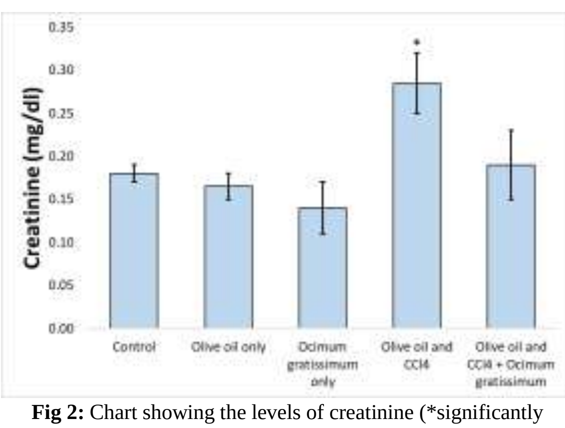

Fig 2: Chart showing the levels of creatinine (*significantly different from the control group. There was a significant increase ($P<0.05$) of creatinine in Group D, when compared to the control group).

The treatment with *O. gratissimum* significantly reduced the increased levels of creatinine to levels comparable with the control group. Urea, produced by the liver in the urea cycle as a waste product of metabolism of protein (either from the oxidation of amino acids or from ammonia), is dissolved into the blood and transported and excreted by the kidney as a component of urine. It is a sensitive biomarker used in the assessment of renal tissue damage.

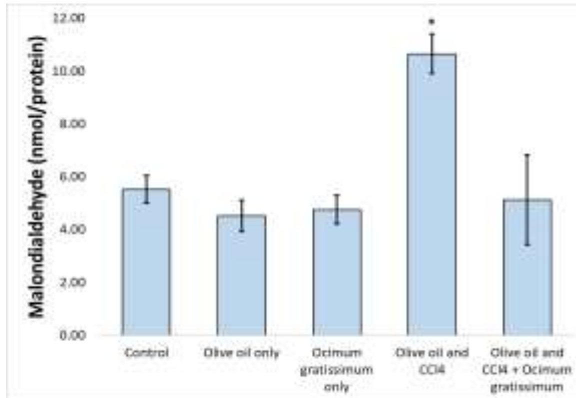

Fig 3: Chart showing the levels of malondialdehyde (MDA) (*significantly different from the control group (There was a significant increase ($P<0.05$) of malondialdehyde in Group D, when compared to the control group)

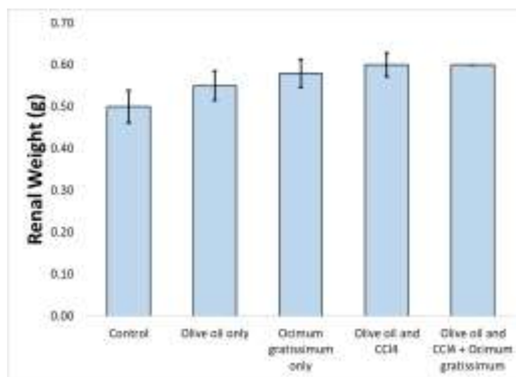

Fig 4: Chart showing renal weight. (There were no statistically significant differences ($P>0.05$) in renal weights across the groups).

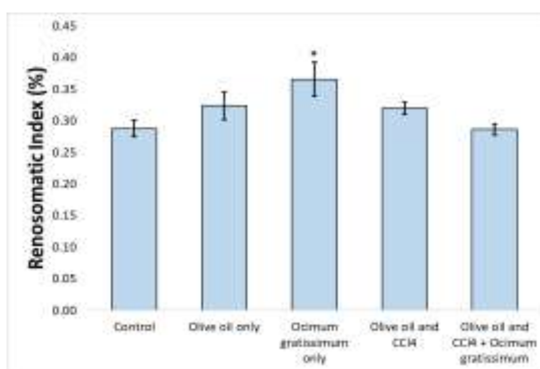

Fig 5: Chart showing the renosomatic index (*significantly different from the control group

(There was a significant increase (P<0.05) of renosomatic index in Group C, when compared to the control group)

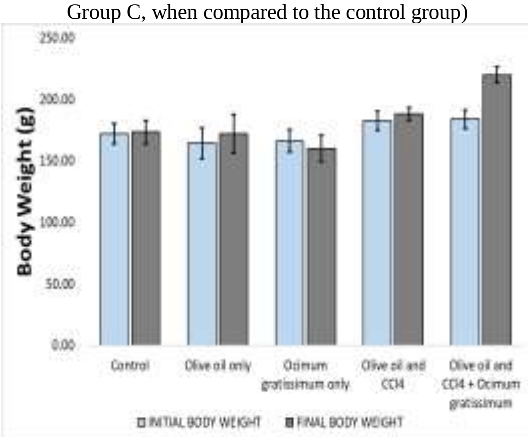

Fig 6: Chart showing the initial body weight in comparison to the final body weight.
(There were no statistically significant differences (P>0.05) when the initial body weight was compared to the final body weight in all the experimental groups)

Therefore, in renal tissue injury, there is retention of urea. Increase urea level is associated with nephritis, renal ischemia, urinary tract obstruction, and extrarenal diseases (Oyewole, 2011; Ogundipe *et al*., 2017).

The aqueous leaf extract of *Ocimum gratissimum*is rich in phenolic and flavonoids compounds (Olamilosoye *et al*., 2018). Flavonoids are a large group of naturally occurring phenolic compounds pervasively distributed in the plant kingdom (Das, 1994). The antioxidant properties of flavonoids are due to their ability to scavenge free radicals, to react with non-radicalic reactive oxygen species. As a result, there is a unanimity that the antioxidative properties of flavonoids aid in their anti-reactive oxygen species activities, which is corroborated by previous studies which have reported the protective action of flavonoids and phenols against oxidative stress induced cellular damage(Sharma and Rajani, 2011; Alabi *et al*., 2017). Studies have also revealed that administering carbon tetrachloride causes weight loss (Hemmings *et al*., 2002; Hemmings and Song, 2005). Contrastingly, this phenomenon was not recorded in our study (Figure 6).

Histologically, plate 1 which is the control group showed features of normal renal glomerulus, renal tubules and interstitial spaces. While the group that was given olive oil only (plate 2), showed normal glomeruli, tubules, normal vascular architecture and active interstitial congestion. Also, the rat group that was given extract only (plate 3) revealed normal glomeruli, active interstitial congestion and normal tubules. Our findings are supported by Ogundipe *et al*., 2017. They assessed the effect of aqueous extract of *Ocimum gratissimum* leaf on the kidney of rats with gentamicin-induced nephrotoxicity and concluded that aqueous extract of *Ocimum gratissimum* ameliorated gentamicin-induced kidney toxicity in rats.

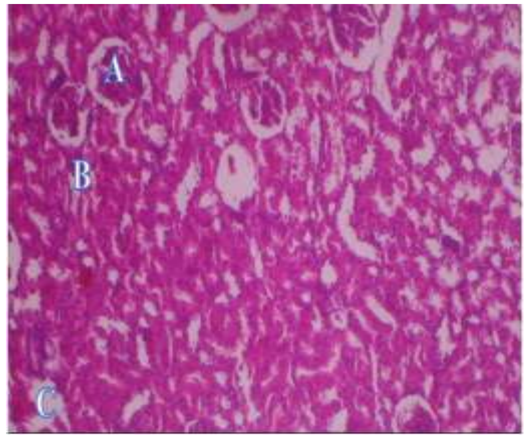

Plate 1: Kidney of control group composed of A: glomeruli, B: tubules and C: interstitial space (H&E x 40)

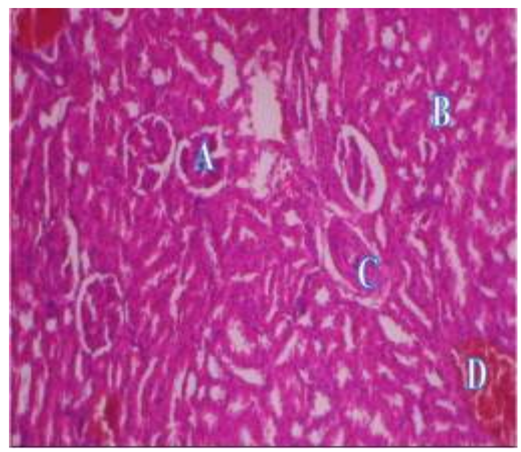

Plate 2: Rat given olive oil only showing A: normal glomeruli and B: tubules,C: normal vascular architecture and D: active interstitial congestion (H&E x 40)

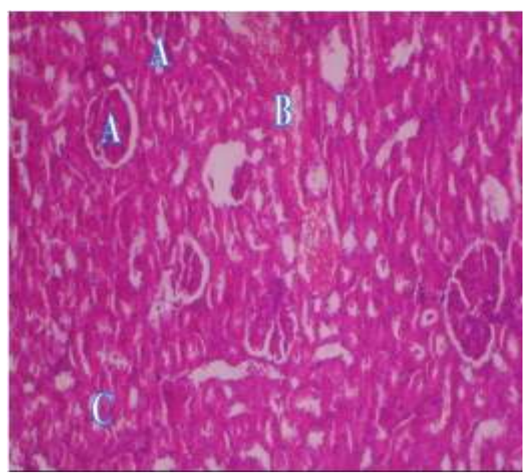

Plate 3: Rat given extract only showing A: normal glomeruli, B: activeinterstitial congestion and C: normal tubules (H&E x 40)

Ozturk, *et al*., 2003 studied the histological changes of kidney after carbon tetrachloride (CCl4) administration in rats and also the possible protective effects of betaine against CCl4-induced renal damage. They discovered that exposure to CCl4 resulted in glomerular and tubular changes in the renal cortex microscopically and that the renal changes were found to be prevented by betaine pretreatment. This result is in accordance with our research work (plate 4), where we discovered that this group that was given carbon tetrachloride and olive oil together showed focal tubular necrosis, vascular stenosis and heavy interstitial infiltrates of inflammatory cells.

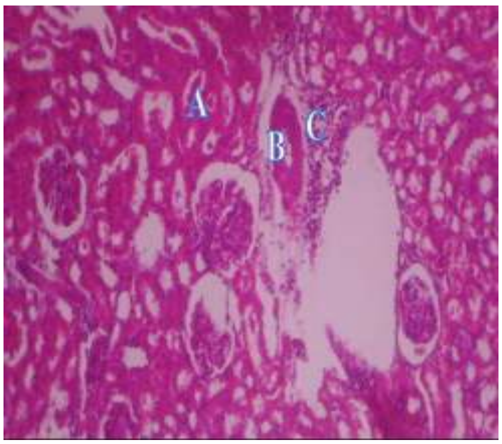

Plate 4: Rat given carbon tetrachloride and olive oil showing A: focal tubular necrosis, B: vascular stenosis and C: heavy interstitial infiltrates of inflammatory cells (H&E x 40)

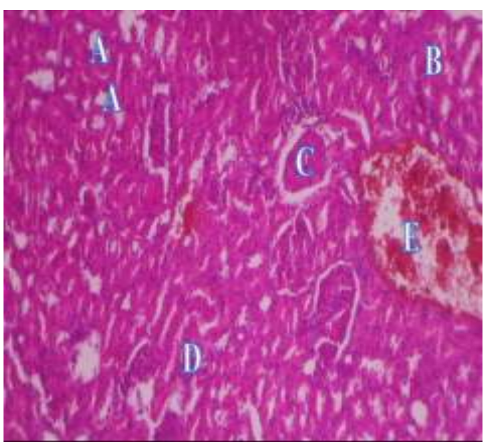

Plate 5: Rat given carbon tetrachloride and olive oil + extract showing A: normal tubules, B: focal tubular necrosis, C: vascular stenosis and D: mild interstitial infiltrates of inflammatory cells and E: congestion (H&E x 40)

Futhermore, in plate 5, rat group that was given carbon tetrachloride and olive oil and extract showed normal tubules, focal tubular necrosis, mild interstitial infiltrates of inflammatory cells and mild congestion. The result is in accordance to previous work done by Okerulu *et al*.,(2018). They studied the effect of aqueous extract of *Ocimum gratissimum* on acetaminophen induced renal toxicity in male. They discovered that aqueous extract of *O. gratissimum* exerted an ameliorative activity on acetaminophen induced renal injury in a dose dependent manner. They stated that consumption of *O. gratissimum* may protect the kidney from toxicity caused by some toxic agents.

Conclusion: From results of this study, it may be concluded that the aqueous leaf extract of *Ocimum gratissimum* moderated the toxic effects of carbon tetrachloride-induced nephrotoxicity. Its moderating effects are evident by the reversal of the increased levels of creatinine, urea and malondialdehyde, and the restoration of the renal architecture after the initial assault by carbon tetrachloride.

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