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Research Paper

Carbon Tetrachloride-Induced Testicular Toxicity And Histopathology In Male Wistar Rats: Sustained Effects Of Methanolic Extract Of Ocimum Gratissimum Leaves.

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Abstract
Background: *Recently there has been an increased association between environmental factors and male infertility. Ocimumgratissimum (Linn) (O. gratissimum) is one of the herbs commonly consumed in Africa and Asia and it has so many medicinal values.*
Materials and Methods: *This present study examined the ameliorative roles of O. gratissimum on carbon tetrachloride induced testicular toxicity in male wistar rats. Twenty (20) adults male albino wistar rats were randomly divided into five groups (A, B, C, D and E) with four rats in each group. Group A received distilled water, group B received a single oral dose of CCL_4 (2.5ml/kg) for two days, group C received 500mg/kg body weight methanolic extract of O. gratisimmum, group D received CCL_4 (2.5ml/kg) for two days followed by 250mg/kg body weight O. gratissimmum methanolic extract for 14 days, and group E received CCL_4 (2.5ml/kg) for two days followed by 500mg/kg body weight O. gratissimum methanolic extracts for 14 days.*
Results: *The exposure of experimental animals to CCL_4 showed deleterious effects on the testes. Treatment with methanolic extract of O.gratissimum revealed varying degrees of restoration after testicular toxicity by CCl_4.*
Conclusion: *This study suggests that the oral administration of methanolic extract of O. gratissimum can be used to manage testicular toxicity in rats.*
Keywords: *CCL_4, O. gratisssimum, testicular toxicity*

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I. INTRODUCTION

In recent years, human infertility has declined and this is as a result of toxic substances present in the environment as well as in drugs. The decline is not just due to choice but the high prevalence of toxins in both males and females (Pizzorno, 2018). The incidence of chemically- induced infertility has gradually increased over the years thereby causing a major clinical problem (Nawal *et al.,* 2015). It has been estimated that infertility affects 8-12% of married couples worldwide (WHO, 1991) and as high as 10-30% in Nigeria (Chimbatata and Malimba, 2016).

Carbon tetrachloride (CCL_4) is one of the most potent environmental toxins and humans are exposed to it via oral, inhalation or dermal routes (Hefnawy and Ramadan, 2013; Manibusan *et al.,* 2007). The long-term and short-term exposure of the chemical to humans can lead to its absorption by the gastrointestinal tract, respiratory tract and the skin, thereby affecting the testes, liver, brain, kidney and lungs (Ganie *et al*., 2011). The absorption can induce cell damage which results from covalent binding of the reactive intermediates to cellular components or from enhanced lipid peroxidation triggered by the interaction of free radical intermediates with oxygen which in turn attacks unsaturated fatty acids (Boll, 2001).

In males, CCL_4 diminishes the weight of testes, seminal vesicles and prostate glands, semen quality and quantity and also decreases the level of testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Abdel-Moniem, 2016). Testosterone is primarily produced and synthesized by the Leydig cells in the testes and LH and FSH through the action of testosterone are required for initiation and maintenance of spermatogenesis epithelium. And if there is disturbance in the hormone releasing process, the whole process leading to spermatogenesis is disrupted (Uadia and Emokpae 2015).

Synthetic drugs have been used to solve and maintain the problem but the effects of these drugs have almost exceeded their limits due to them being expensive and their provocation of serious adverse effects and so, traditional medicines have been suggested by various researches to improve fertility in both male and female individuals (Ekere*et al*., 2013). These plants are known to have active ingredients such as phenols, alkaloids, saponins and most especially flavonoids. The active ingredients make the plants to have both estrogenic and androgenic properties (Ekere*et al*., 2013). Plant of concern in this study is *Ocimumgratissimum L. (O. gratissimum)* which is an herbaceous plant of the Lamiacea family and commonly known as 'scent leaf' (Agarwal and Varma, 2014). It is indigenous to the tropical areas especially india and West Africa. The plant is found in the Savannah and coastal areas of Nigeria. In Nigeria, the plant is known with different names in various languages. It is known as 'Nchuanwu' in Igbo, 'Efirin' in Yoruba, 'Daidoyatagida' in Hausa and 'Ntonng' by the Ibibios and it can be cultivated or found wild in the tropics (Effraim *et al*., 2003). It is also known as 'wild basil, tree basil and clove basil' in English, 'menthe gabonaise' in French, 'horapha-chang' in Thai and so on (Orwa et al, 2009).

The extracts of different parts of the plant *O. gratissimum* have been used to relief and cure various diseases. It has been known to have antidiarrheal effect (Offiah and Chikwendu, 1999), antibacterial (Nwinyi, 2009), antihelmintic effect (Pessoa *et al*., 2002). The leaves of the plant are freshly used as a spice in cooking and it significantly reduce blood sugar level in hyperglycaemic patients (Olufisayo, 2008). With all the medicinal properties of *O. gratissimum*, the scientific data for the effect of the plant on chemically induced-infertility is inadequate and therefore, the study was aimed at determining the effect of methanolic extract of *O.gratissimum* leaves on carbon tetrachloride (CCl4) induced testicular toxicity in male wistar rats.

II. MATERIALS AND METHODS

Plant Collection and Extraction

*O. gratissimum*leaves were collected from Isuikwato Local Government area of Abia State Nigeria. The fresh O. gratissimum leaves were first separated from the stalk, rinsed with water to remove dirt and cut into pieces then air dried at room temperature for 7 days and then subjected to cold methanolic extraction. The dried leaves (148.26g) were soaked in methanol (1.5L) for 72 hours after which the filtrate was allowed to dry at room temperature.

Phytochemical Screening of Plant Extract.

Phytochemical tests were carried out on the plant extract using standard procedure as described by sofowora (1993) and Trease and Evans (2002).

Experimental animals

The experimental animals that were used for this study were locally bred male Wistar rats which were purchased from University of Nigeria, Enugu Campus laboratory (Animal House) and then brought to Gregory University Uturu for acclimatization and experiment. The animals were acclimatized for two weeks and maintained under the standard environmental conditions and were allowed to have free access to food and water *ad libitum*.

Experimental Design

Twenty (20) adult male Wistar rats were used and randomly divided into five groups (A–E) with four rats in each group. Group A served as the control group while Group B-E served as experimental group.

Group A: Each rat in this group received distilled water.

Group B: Each rat in this group received a single oral dose of CCl_4 diluted in equal volume of olive oil 1:1, 2.5 ml/kg body weight for two days.

Group C: Each rat in this group received 500 mg/kg body weight *O. gratissimum* methanolic extract for 14 days

Group D: Each rat in this group received 2.5 ml/kg body weight of CCl_4 diluted in equal volume of olive oil 1:1 for two days followed by 250 mg/kg body weight of *O. gratissimum*methanolic extract for 14 days.

Group E: Each rat in this group received 2.5 ml/kg body weight of CCl4 diluted in equal volume of olive oil 1:1 for two days followed by 500 mg/kg body weight of *O. gratissimum* methanolic extract for 14 days.

Sacrifice Of Animals

The rats were sacrificed 24 hours after the last day of administration. The rats were anesthetized by placing them in a closed jar containing cotton wool sucked with chloroform anaesthesia for 3–5 min. The abdominal cavity was opened up through a midline abdominal incision to expose the reproductive organs. The testes were

excised and trimmed of all fats. The testes of each rat were carefully exposed and removed. They were trimmed free of the epididymis and adjoining tissue.

Histopathological Studies

The testes samples collected from the animals were fixed in Bouin's fluid and freshly prepared formal saline respectively before tissue processing. The tissues were fixed so as to preserve them thereby preventing post mortem autolysis and decay. The tissues were then dehydrated using alcohol in increasing concentration (75%, 90%, and 100% respectively for 2 hours each). Dehydrated tissue was cleared at room temperature in two changes of xylene each lasting one hour. The tissue was then infiltrated in molten paraffin wax, at a room temperature of about 50-60C in an oven. The tissues were next embedded in trough containing molten wax. The tissues were transferred to moulds filled with molten paraffin wax. The tissues were inverted so as to face the section to be cut to free the surface of air bubbles. The paraffin blocked tissues were trimmed and mounted on wooden blocks for sectioning on a rotary microtome Sections of 15 microns thick were produced from the tissue blocks using a rotary microtome. Tissues were mounted on glass slides aided with albumin of egg. The section was put in the centre of the slide to enable the section to float. The section was immersed in water bath (at 50-55^{o}C) to prevent wrinkles. Water was drained off and the slide was put in an incubator so that the section is completely fixed on slide and becomes dry. Tissues were stained with haematoxylin and eosin. After tissue processing and staining procedures, the slide was mounted on the light leica microscope to be viewed and the photomicrographs were taken from each group and labelled using Microsoft power point.

III. RESULTS

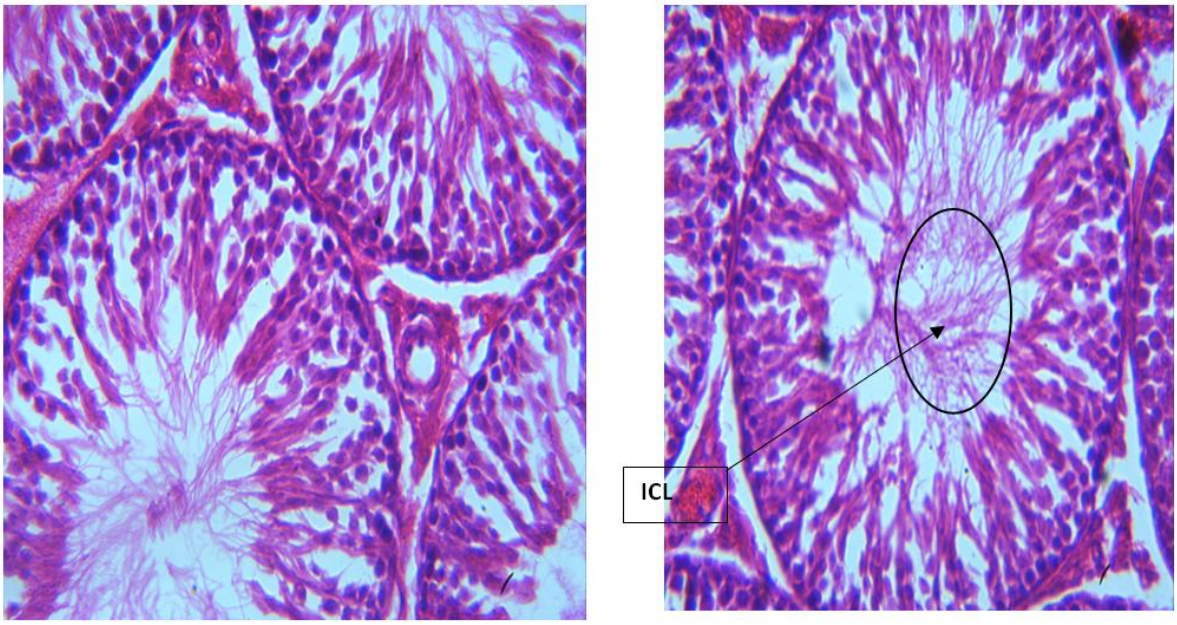
Table 1 shows the phytochemical constituents of the methanolic leaf extract of *O. gratissimum*

Phytochemicals	**Methanolic extract**
Alkaloids	+
Carbohydrates	+
Tannins	-
Saponins	+
Proteins	+
Flavonoids	+
Terpenoids	+
Steroids	+
Cardiac glycosides	+
Phenol	-

Table 1: Result of the qualitative phytochemical analysis of *O. gratissimum*

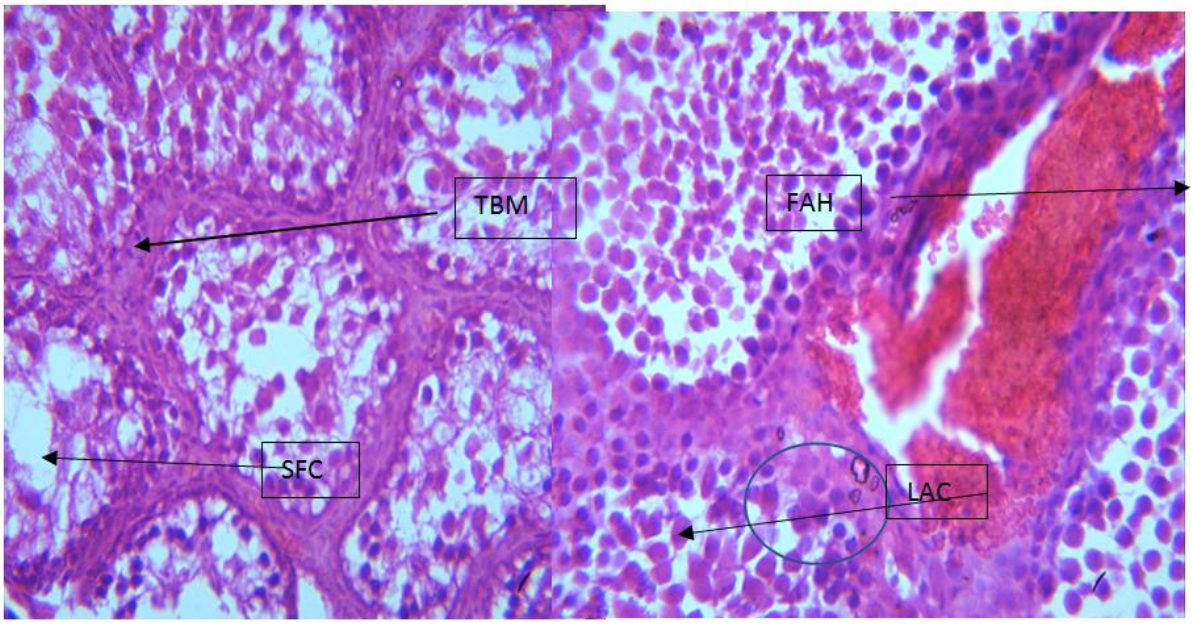
Results of histopathological studies

The testicular histology of the control rats showed well defined testicular architecture with seminiferous tubules that are lined with interstitial cells of the leydig and well enhanced spermatogenesis (figure 1). Animals treated with CCL_4 shows severe effect on the testicular tissue with seminiferous tubules that has thickened basemen membrane (figure 2). *O. gratissimum* treated rats' testicular histology shows defined lumen, moderately normal with well enhanced spermatogenesis and seminiferous tubules that are lined by interstitial cells of the leydig (figure 3). Administration of CCl_4 and low dose of *O. gratissimum* shows mild healing moderate normal with arrest of spermatogenesis (MAS) and mild apoptosis of the interstitial cells of the leydig (figure 4) while administration of CCl_4 and high dose of *O. gratissimum* shows moderate healing with mild arrest of spermatogenesis (figure 5).



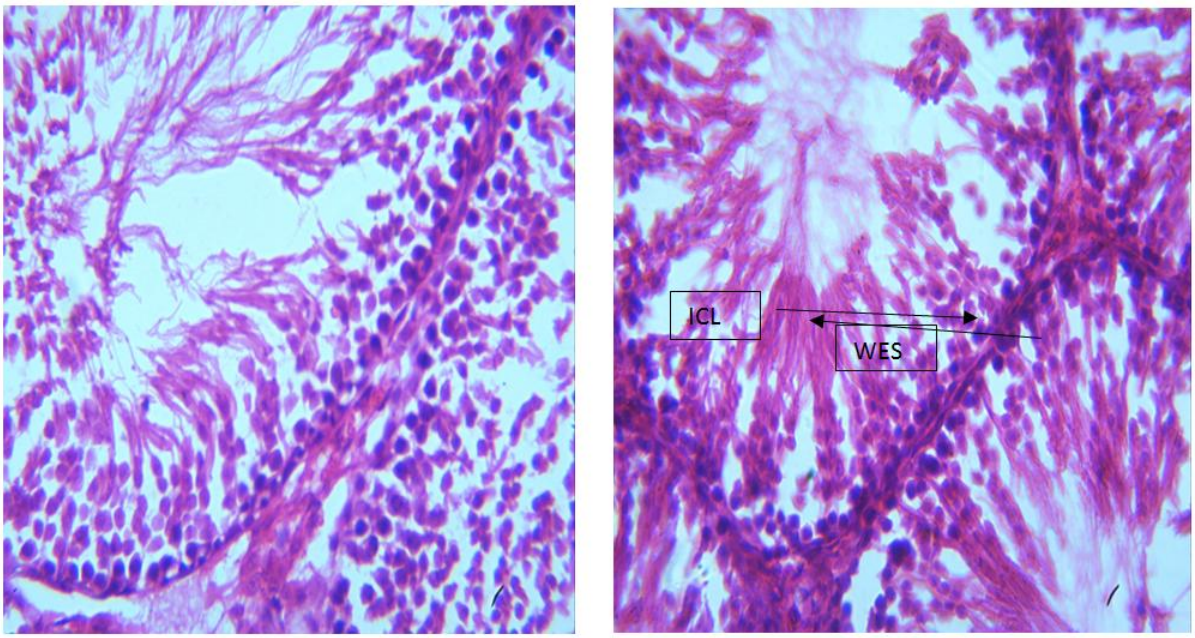
A B

Figure 1: Photomicrograph control section of testis (x400) (H/E) shows normal testicular architecture with seminiferous tubules that are lined with interstitial cells of the leydig (ICL) and well enhanced spermatogenesis (WES). The overall feature appears normal.



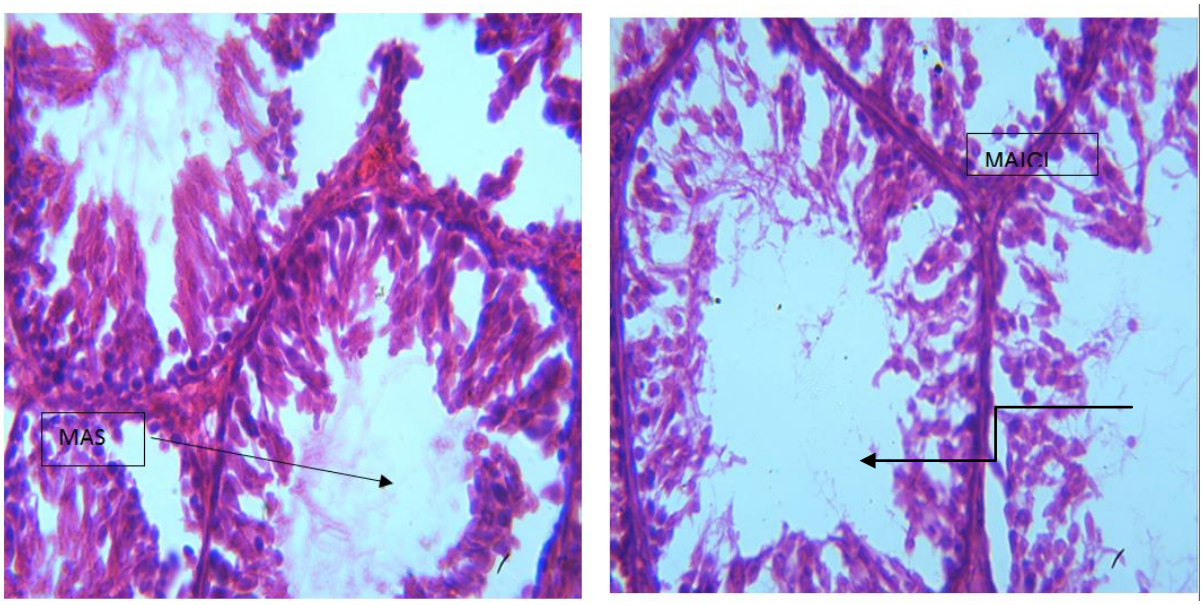
A B

Figure 2: Photomicrograph of Group B section of testes induced with 2.5mg/kg of CCL_4 for 2days (x400) (H/E) shows severe effect on the testicular tissue with seminiferous tubules that has thickened basemen membrane (TBM) and contain large atypical cell (LAC)with centrally located nuclei that have irregular outlines and prominent nucleoli without spermatogenesis ,severe fatty changes (SFC) within the lumen and focal area of hemorrhage (FAH).



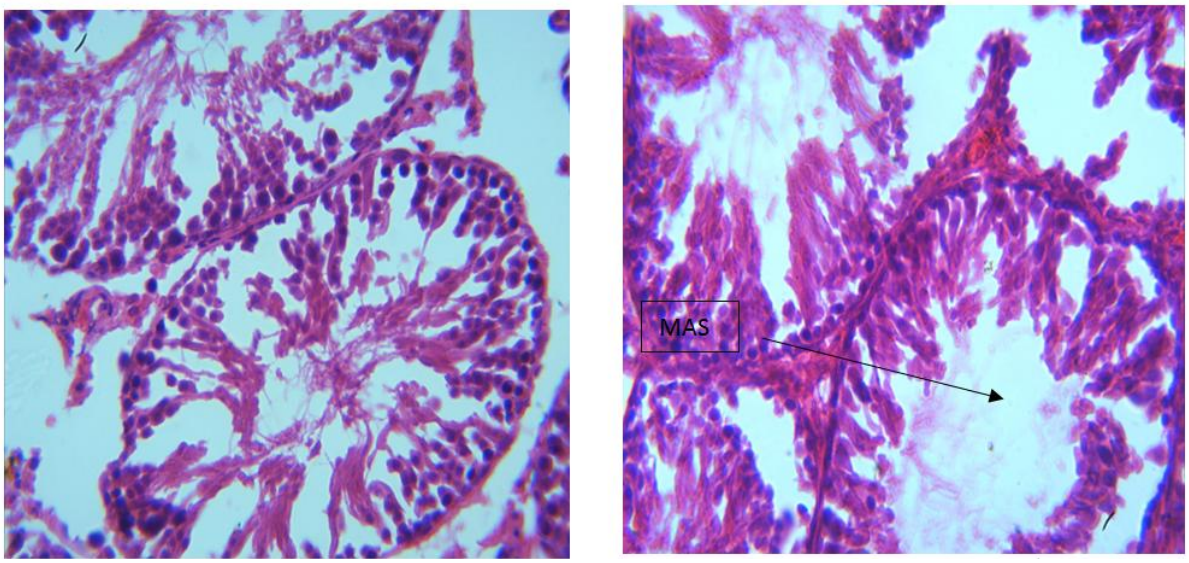
A B

Figure 3: Photomicrograph of Group C section of testes administered with 500mg/kg of *O. gratissimum* for 14days (x400) (H/E) shows testicular tissue that is moderately normal with well enhanced spermatogenesis (WES) and seminiferous tubules that are lined by interstitial cells of the leydig (ICL).



A B

Figure 4: Photomicrograph of Group D section of testes administered with 2.5mg/kg of CCl_4 and for 2 days followed by 300mg/kg of *O. gratissimum* for 14 days (x400) (H/E) shows mild healing moderate normal with arrest of spermatogenesis (MAS) and mild apoptosis of the interstitial cells of the leydig (MAICL).



A B

Figure 5: Photomicrograph of Group E section of testes administered with 2.5ml CCL_4 for 2 days followed by 500mg/kg of *O. gratissimum* for 14days (x400) (H/E) shows moderate healing with mild arrest of spermatogenesis (MAS)

IV. Discussion

Preliminary qualitative phytochemical analysis for the leaves of *O. gratissimum* revealed the presence of alkaloids, tannins, saponins, flavonoids, steroids, triterpenes, and cardiac glycosides. These secondary metabolites are reported to have many biological and therapeutic properties (Vishnu *et al.,* 2013; Narender *et al*., 2012). The phytochemical analysis showed that flavonoids are present in the methanolic extract of *O.gratissimum* leaves and according to Gil *et al.*, (2002), fruits showing high anti-oxidant capacity contains high amounts of phenolics which includes flavonoids, phenols and flavonols. Therefore, *O.gratissimum* is an anti-oxidant as it contains flavonoids. The presence of metabolites in plants account for their usefulness in folklore medicine.

Carbon tetrachloride is a toxic chemical and it has toxicology effect on the testes, liver and other visceral organs when exposed to it. The mechanism behind CCl_4 toxicity has been known to be due to oxidative stress (Sahreen*et al*., 2011; Khan *et al.*, 2011). From the present study, exposing the male rats to 2.5ml/kg body weight of CCL_4 for two days (Group B) affected the testicular morphology. The administration of CCl_4 led to severe destruction of the seminiferous tubules, interstial cells and spermatogonia and these histological changes seen in the testes of the animals exposed to CCl_4 are in agreement with Khan and Ahmed (2009) who reported alterations in the seminiferous tubules and reduction in spermatogenic cells. The disruption of the morphology of the testes is said to be due to the excess in production of free radicals which leads to oxidative stress thereby increasing the germ cell apoptosis and subsequent hypospermatogenesis (Yousef and Salama, 2009). Although reproductive hormonal assay was not done, a work done by Khan et al, 2011 showed that CCl_4 suppresses the level of testosterone, lutenizing hormone and follicle stimulating hormone.

It was also observed that the group treated with *O. gratissimum* alone (Group C) had morphology close to the control group. It showed a normal structure of seminiferous tubules that are lined by interstial cells of leydig with well enhanced spermatogenesis. These findings show that *O. gratissimum* is likely to improve the function of testes due to the fact that it is an antioxidant. Also, it was observed that the groups treated with CCl_4 plus *O. gratissimum* (group D and E) had significant changes. The changes however varied because of the high dose and low dose administration of *O. gratissimum*. The high dose administration moderately reversed the effect of CCl_4 on the testes compared to that of the low dose which still showed mild apoptosis. The high dose administration led to a moderate healing as well as a mild arrest of spermatogenesis in agreement with Khan and Ahmed, (2009), who studied the effect of *Digeramunicata* against CCl_4-induced toxicity on the rats' testes. There was no histological difference in the testes compared with the control (for the high dose administration of *O. gratissimum*). In accordance with these results, Asuquo *et al.*, (2010) found that *O. gratissimum* extract improved the testicular histopathological alterations in diabetic rats. Minimizing the hazard effects of CCl_4 by *O. gratissimum* treatment may be due to the flavoniods in *O. gratissimum*, which exert many health-promoting effects, including the ability to increase intercellular antioxidant levels, decrease capillary permeability and fragility and scavenge oxidants and free radicals (Singh and Jialal, 2006); Khan et al, 2011).

V. CONCLUSION

The exposure of experimental rats to CCl4 shows deleterious effects on the testes. The administration of methanolic leaf extracts of O.gratissimum may be used to manage testicular toxicity induced by CCl4.

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