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Research Article

Ethanol Extract of *Moringa oleifera* Leaves Modulates Ciprofloxacin-Induced Oxidant Stress in Testis and Semen of Rats

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Abstract

Infertility can primarily result from oxidative stress induced by the use of various drugs. Recently, many plant derived preparations have been used to treat various ailments due to their antioxidant potential. The modulatory effects of the ethanolic extract of *Moringa oleifera* leaves (MOE) were investigated in Ciprofloxacin (CIPX) induced oxidant stress in the testis and semen of rats. Antioxidant status of reproductive organs was assessed by estimating levels of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), reduced glutathione (GSH) and activities of glutathione-S-transferase (GST), glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT). Compromised tissue membrane integrity was estimated by lactate dehydrogenase (LDH) and gamma glutamyl transferase activities (GGT). Testicular sperm number (TSN), daily sperm production (DSP) and sperm morphology were also assessed. Ciprofloxacin induced oxidative stress was indicated by increased H_2O_2 and MDA levels with corresponding decrease in GSH, GST,GPX and SOD activities. CAT activity however, remained unchanged in testis but SOD activity increased significantly ($p < 0.05$) in semen. CIPX significantly ($p < 0.05$) induced elevation of GGT activities in both organs as well as LDH in testis only. TSN and DSP were also decreased. MOE significantly increased levels of GSH, GST, GPX and SOD in both organs and significantly restored their depleted levels in testis of CIPX-treated rats. MDA, H_2O_2, LDH and GGT levels were also modulated in organs as well as TSN and DSP. Histomophormetry showed that MOE attenuated CIPX-induced testicular damage. Our results suggest *Moringa oleifera* leaves possess antioxidant properties, and as such can mediate in free radical-induced testicular pathophysiologies.

Keywords: *Ciprofloxacin, Moringa oleifera, oxidative stress, antioxidant, testis*

Introduction

Approximately 15-20% of couples in the reproductive age are suffering from infertility in which male infertility is a contributory factor in half of all these couples (Kidd *et al*., 2001, Schoor, 2002). Pharmacologically mediated male infertility would be associated with misleading drug administration, invalid dietary supplementation or recreational drug use. Many antibiotics have been reported to exert adverse effects on male fertility (Schlegel *et al*., 1991), however; there are few data on the majority of these medications.

Ciprofloxacin (CIPX) is a 4-fluoro-quinolone antibiotic commonly used in treatment of many microbial infections. The antibacterial mechanism of CIPX is based on the inhibition of the bacterial type II topoisomerase/DNA gyrase enzyme (Koziel and Zablocki, 2006). The cross-reactivity of fluoroquinolones including CIPX with mammalian topoisomerase II has been reported previously (Bredberg *et al*, 1991, Curry *et al*, 1996). Studies related to the cytotoxic effects of CIPX revealed that this antibacterial agent inhibits the growth of various cultured mammalian cells (Aranha *et al*., 2003, Herold *et al*., 2002).

Although *in vivo* genotoxicity studies suggest ciprofloxacin as safe for therapeutic use (Herbold *et al*., 2001), others show that ciprofloxacin is phototoxic (Ferguson and Johnson, 1990), cytotoxic in primary cultures of rat astrocytes (Gurbay *et al*., 2006) and induces chondrotoxicity and myocardiotoxicity in juvenile rats (Adikwu and Brambaifa, 2012, Saracoqlu *et al*., 2009). Some other studies have demonstrated ciprofloxacin to significantly impair both testicular and epididymal function and structure (Abd-Allah *et al*., 2000, Demir *et al*., 2007, Nashwa *et al*., 2011), induce genotoxicity, chromatin and morphological abnormalities of sperm cells as well as biochemical and histochemical changes in testis of mice (Olajuyigbe *et al*., 2011, Zobeiri *et al*., 2013). Recently, a number of antibiotics, including ciprofloxacin, have been demonstrated to stimulate the production of reactive oxygen species (ROS) in bacterial cells as the mechanism of their antibacterial action (Albesa *et al*., 2004**,** Becerra and Albesa, 2002)**.** Inductions of these ROS have been found to underlie the toxicity observed with some of most fluoroquinolones**.** (Gurbay *et al*., 2001, Weyers *et al*., 2002)**.**

Moringa oleifera belongs to the Moringaceae family of perennial angiosperm plants (Olson, 2002). Though it's a native of the sub-Himalayan northern parts of India, it's cultivated throughout tropical and sub-tropical areas of the world. Common names are drumstick tree, horseradish tree, ben oil tree, malunggay, e.t.c. Phytochemical and nutritional screening of the leaves indicate they are particularly rich in potassium, calcium, phosphorous, iron,

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vitamins A and D, essential amino acids, as well as such known antioxidants such as β-carotene, vitamin C and E, and flavonoids (Aslam *et al*., 2005; Manguro and Lemmen, 2007; Amaglo *et al*., 2010; Gowrishankar *et al*., 2010).

It's an edible plant, consumed by humans throughout the world in diverse culinary ways (Iqbal and Bhanger, 2006). A variety of nutritional and medicinal virtues has been attributed to its roots, bark, leaves, seeds and flowers, (Anwar *et al*., 2007; Kumar *et al*., 2010) as these plant parts contain certain glycosides suggested to be responsible for the anti-diabetic, anti-dislipidaemic, hypolipidaemic, antiulcer and hepatoprotective property of the tree (Chumark *et al*., 2008, Kansara and Singhal 2013, Buraimoh *et al*., 2011, Majambu, 2012). Its antispasmodic (Gilani *et al*., 1994), antimicrobial (Moyo *et al*., 2012) and antitumor (Bharali *et al*., 2003) activities have also been reported.

Thus, with the array of data that supports the strong antioxidant potential of parts and especially leaves of the *Moringa oleifera* plant as a result of its high flavonoid content, this study was undertaken to justify claims of the potent antioxidant activity of *Moringa oleifera* leaves in a condition of ciprofloxacin induced oxidant stress in the testes and semen of rats.

Materials and Methods

Plant material and Preparation of extract: Moringa oleifera leaves obtained from and authenticated at the Department of Botany, University of Ibadan were air dried, powderized, and extracted (Soxhlet extraction) using 80% ethanol. The solvent was recovered by distillation. The extract (MOE) was concentrated with a rotary evaporator (50ºC) and stored at 4ºC until further use.

Chemicals: Ciprofloxacin (CIPX) manufactured by V.S. INTERNATIONAL PVT. LTD, India, was purchased from Danax Pharmaceuticals, Ibadan Nigeria. Thiobarbituric acid (TBA), Trichloroacetic acid (TCA), bovine serum albumin (BSA), 1- Chloro-2,4-dinitrobenzene (CDNB), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), and epinephrine were purchased from Sigma Chemical Co.(St. Louis, M.O.). Diagnostic kits for GGT and LDH were purchased from Randox Laboratories U.K. All other chemicals and solvents were of analytical grade and the purest quality available.

Animals and Treatment: Thirty male Wistar albino rats with an average weight of (174 ± 29)g were maintained in stainless steel cages in the departmental animal house at 25^0C and a natural photoperiod of 12h light and 12h dark cycle, fed commercial chow (Ladokun Feeds Ibadan, Nigeria) and given water ad-libitum. All animals received humane care according to criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institute of Health. Ethic regulations were followed in accordance with National and Institutional Guidelines for the Protection of Animal Welfare during the experiments (PHS, 1996). After acclimatization (2 weeks), rats were randomly distributed into 5 groups of 6 animals each.

Animals in group 1 served as the control and were given 2ml/kg normal saline. Group 2 received a therapeutic dose of 150mg/kg of CIPX. Group 3 received 100mg/kg of MOE, while those in groups 4 and 5 were pre-treated with MOE from day 1-5 and co-administered with ciprofloxacin from day 6-15. Rats in group 2 were also administered ciprofloxacin only from day 6-15 while rats in groups 1 and 3 received 0.9% normal saline and MOE from day 1-15 respectively. All administrations were done orally via gavage. At the end of administrations, rats were sacrificed by cervical dislocation, organs excised, rinsed in 1.15% ice-cold KCl solution, blotted dry and weighed. Cauda epididymis were homogenised in 8 volumes (w/v) of ice-cold 0.1M potassium phosphate buffer (pH 7.4) to release semen while testis was homogenised in 4 volumes (w/v) of same buffer and centrifuged at 4^0C for 10 minutes at 12,000*g*.

Biochemical Assays: The supernatant obtained after centrifugation was used for the estimation of total protein according to the Biuret method (Gornall *et al*., 1949) using BSA as a standard. Reduced glutathione was determined according to Beutler *et al*., (1963), GST was assayed according to Habig *et al*., (1974) using 1-Chloro-2, 4-dinitrobenzene (CDNB) as substrate and glutathione peroxidase activity (GPx) as described by Rotruck *et al*., (1973). Extent of lipid peroxidation was assessed by measuring the formation of thiobarbituric acid reacting substances, malondialdehyde (MDA) according to methods of Varshney and Kale (1990) and Hydrogen peroxide generation (H_20_2) by Wolff, (1994). Catalase (CAT) activity was determined according to the method of Sinha (1972), LDH and GGT activities were estimated using diagnostic kits obtained from Randox Laboratories U.K. Testicular Sperm Number (TSN) was carried out by the method of Yokoi *et al*., (2003)**.** Daily Sperm Production (DSP) was calculated from the TSN using the following formula – TSN x square factor (5) x haemocytometer factor (104) x dilution factor (50)/Testis (g) x 4.61.

Histopathological examination: For morphological studies, the left testes and epididymes were fixed in 10% formalin, embedded in paraffin and sectioned at 5µm. Sections were mounted on slides and stained with hematoxylin and eosin, examined under a light microscope and photomicrographs taken.

Statistical analysis: Statistical data are expressed as mean ± SD of six rats per group and analyzed with Microsoft Excel. Statistical analyses were done using Student's T-test. *p* value of less than 0.05 was considered significant

Results

Body and Reproductive organ weights: The effect of CIPX treatment on final body weight, relative weights of the testis and other accessory organs is shown in Table 1. There was no significant change ($p < 0.05$) in the relative weight of the reproductive organs of rats administered CIPX compared with the control group. However, a significant change was observed in the relative weight of the prostate. There was a slight increase in the relative

weights of the testis and seminal vesicles of rats pretreatment with MOE.

Biochemical Assays

Testis: In the group treated with only MOE, a significant increase was observed in the activities of GSH, GST and GPX ($p < 0.05$), and no significant changes in SOD and CAT activities in comparison with the control group. However, the activities of GST, GPX and SOD in CIPX-treated rats were significantly lower than that of the control group ($p < 0.05$). There was also no significant decrease in the level of GSH and no changes in CAT activity in the rats treated with CIPX in comparison with the control group (Table 2). Levels of MDA, H_2O_2, GGT as well as LDH markedly increased in CIPX-treated rats compared with the control. Pretreatment with MOE at both doses significantly decreased the elevated levels of these markers of lipid peroxidation and activities of these enzymes ($p < 0.05$) (Figure 1).

Semen: The effect of CIPX treatment on the level of GSH and activities of GST, GPX and SOD in semen of rats are summarized in Table 3. There was a no significant decrease in GSH and GPX levels, while a significant increase was observed in SOD activity in CIPX-treated rats in comparison with the control group ($p < 0.05$). No significant change was observed in GST activity. A significant increase was recorded in MDA and GGT levels but no significant increase in H_2O_2 and LDH levels respectively in semen of CIPX treated rats ($p <0.05$) (Figure 2). These changes were however modulated in the groups administered MOE before CIPX treatment (Table 3, Figure 2).

Table 1:
Effect of *Moringa oleifera* extract (MOE) on body weight (g) and relative organ weights of rats administered Ciprofloxacin

TREATMENTS	Control: 0.9% N.S	MOE (100mg/kg)	CIPX (150mg/kg)	MOE (50mg/kg)+CIPX	MOE (100mg/kg)+CIPX
Final Body Weight	223.33 ± 21.60	175.00 ± 17.32[a]	264.00 ± 15.17[a]	212.50 ± 5.00[a]	170.00 ± 28.28[a]
Testis	0.41 ± 0.11	0.48 ± 0.07	0.46 ± 0.08	0.48 ± 0.10	0.60 ± 0.18
Epididymes	0.12 ± 0.02	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.09 ± 0.02
Seminal Vesicles	0.34 ± 0.02	0.36 ± 0.08	0.39 ± 0.04	0.35 ± 0.05	0.42 ± 0.15
Prostate Gland	0.17 ± 0.02	0.15 ± 0.04	0.14 ± 0.01[a]	0.13 ± 0.03	0.16 ± 0.02

Values are mean ± SD, n = 5, [a]p < 0.05 compared with control, [b]p < 0.05 compared with CIPX, MOE = Moringa oleifera extract, CIPX = Ciprofloxacin

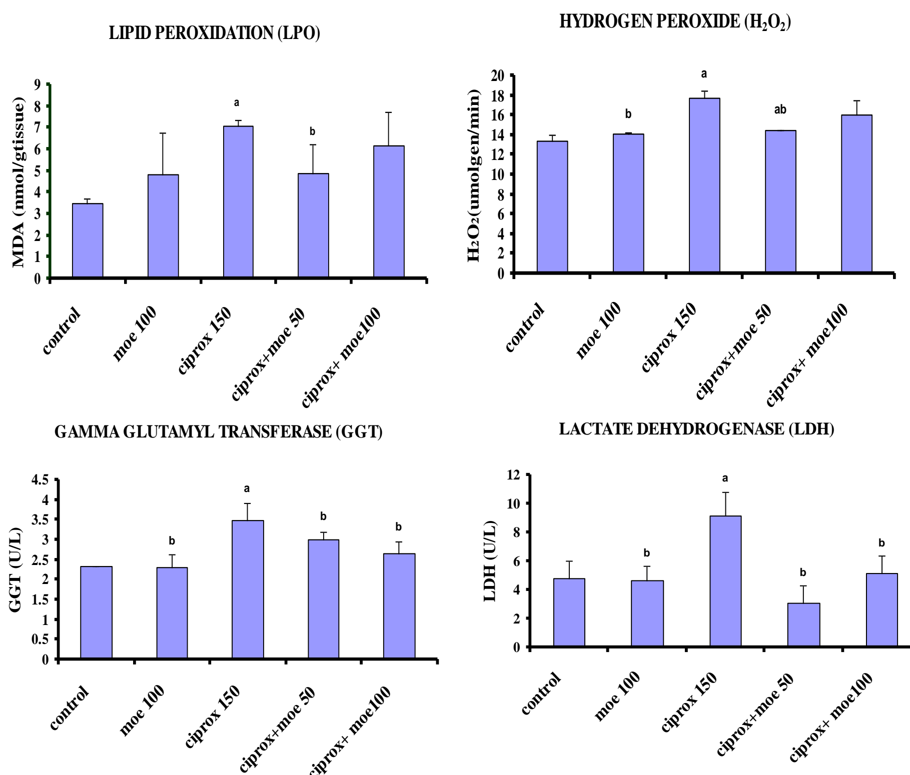

Figure 1:
Effect of *Moringa oleifera* extract (MOE) on Lipid peroxidation (LPO), Hydrogen peroxide generation (H_2O_2), Gamma glutamyl transferase (GGT), and Lactate dehydrogenase (LDH) levels in testis of rats administered Ciprofloxacin (CPX). Values are mean ± SD, n = 5, [a]p< 0.05 compared with control, [b]p < 0.05 compared with CIPX, MOE = *Moringa oleifera* extract, CIPX = Ciprofloxacin

Table 2:
Effect of *Moringa oleifera* extract (MOE) on Reduced glutathione (GSH), Glutahtione-S-Transferase (GST|), Glutathione peroxidase (GPX), Catalase (CAT) and Superoxide dismutase (SOD) levels in testis of rats administered Ciprofloxacin (CPX).

TREATMENTS	GSH (μg/gtissue)	GST (Unit/gtissue)	GPX (Unit/gtissue)	CATALASE (Unit/mgprotein)	SOD (Unit/gtissue)
Control:0.9% N.S	28.54 ± 2.93	0.02 ± 0.001	75.95 ± 6.62	22.99 ± 1.17	5.59 ± 1.51
MOE(100mg/kg)	39.55± 4.14^{ab}	0.05± 0.001^{ab}	126.53 ± 11.79^{ab}	23.13 ± 0.82	6.29 ± 1.01^{b}
CIPX(150mg/kg)	25.63 ± 0.95	0.01 ± 0.001^{a}	63.74 ± 5.21^{a}	22.55 ± 1.36	3.11± 1.12^{a}
MOE(50mg/kg) +CIPX	36.80± 3.86^{b}	0.04 ± 0.001^{b}	119.82 ± 19.51^{b}	29.20 ± 2.36^{b}	3.47 ± 0.1
MOE(100mg/kg) +CIPX	41.71± 4.11^{b}	0.03± 0.001^{b}	97.09 ± 11.37^{b}	22.05 ± 1.85	3.68 ± 0.72

Values are mean ± SD, n = 5, $^{a}p < 0.05$ compared with control, $^{b}p < 0.05$ compared with CIPX, MOE = Moringa oleifera extract, CIPX = Ciprofloxacin
GST: Unit = μmole/min; GPX: Unit = μmole/min; CAT: Unit = μmoles of H_2O_2 consumed/min; SOD: Unit = μmole of SOD consumed/min

Table 3:
Effect of *Moringa oleifera* extract (MOE) on reduced glutathione (GSH), Glutahtione-S-Transferase (GST|), Glutathione peroxidase (GPX), and Superoxide dismutase (SOD) levels in semen of rats administered Ciprofloxacin (CPX).

TREATMENTS	GSH (μg/mg protein)	GST (Unit/mg protein)	GPX (Unit/mgprotein)	SOD (Unit/mgprotein)
Control:0.9%N.S	1.54 ± 0.07	6.61 ± 1.55	3.35 ± 0.50	0.07 ± 0.004
MOE(100mg/kg)	1.51±0.22	9.14 ± 4.02	3.38 ± 0.79	0.10 ± 0.054^{b}
CIPX (150mg/kg)	1.39 ± 0.57	7.02 ± 0.74	2.86 ± 0.13	0.23± 0.026^{a}
MOE(50mg/kg)+CIPX	1.81 ± 0.02	9.90 ± 0.21^{b}	3.00 ± 0.32	0.26± 0.105
MOE(100mg/kg+CIPX	1.55 ± 0.16	8.03 ± 3.55	2.37 ± 0.62	0.085± 0.012^{b}

Values are mean ± SD, n = 5, $^{a}p < 0.05$ compared with control, $^{b}p < 0.05$ compared with CIPX

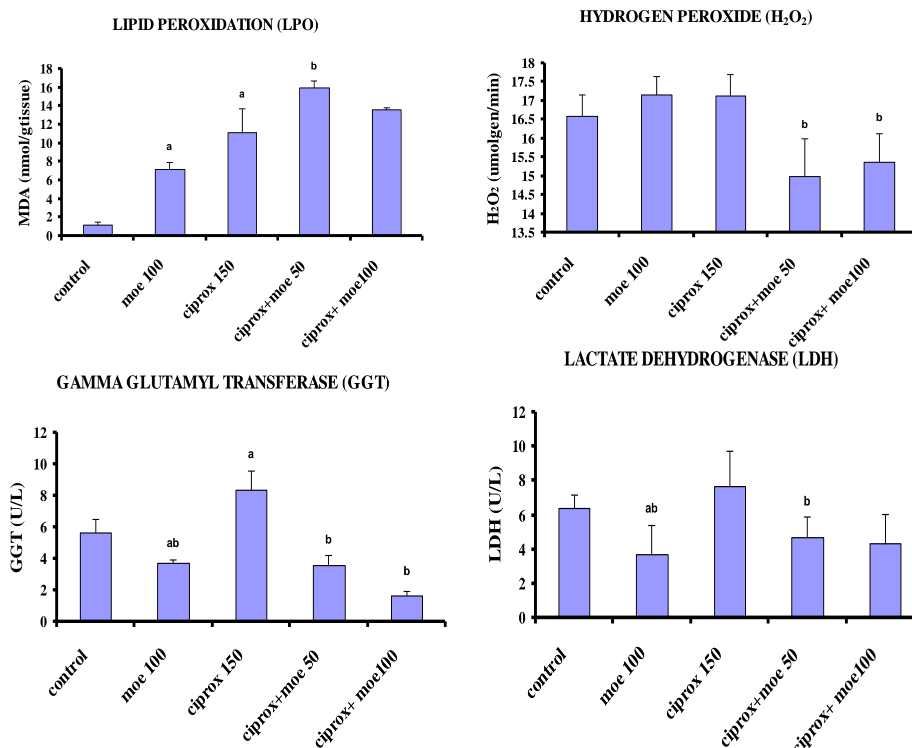

Figure 2:
Effect of *Moringa oleifera* extract (MOE) on Lipid peroxidation (LPO), Hydrogen peroxide generation (H_2O_2), Gamma glutamyl transferase (GGT), and Lactate dehydrogenase (LDH) levels in semen of rats administered Ciprofloxacin (CPX). Values are mean ± SD, n = 5, $^{a}p < 0.05$ compared with control, $^{b}p < 0.05$ compared with CIPX, MOE = *Moringa oleifera* extract, CIPRX = Ciprofloxacin

Histopathology of Testes and Epididymis: Photomicrographs of testes from rats treated with CIPX showed severe erosion (patchy) of the germ cells of seminiferous tubules and diffuse interstitial oedema. No histopathological changes were noticed in examined sections from control and MOE treated rats. Testes section from the 50mg/kg MOE pre-treated group showed slight oedema of the interstitial cells (Plate 1). No visible lesions were noticed in all sections from the epididymis of control, CIPX and MOE treated rats (Plate 2).

Testicular Sperm Number (TSN) and Daily Sperm Production (DSP): Table 4 showed significantly lower TSN and DSP in rats treated with CIPX than in the control group ($p < 0.05$). It was observed however, that TSN and DSP slightly increased on pretreatment with MOE. Increased morphological changes were also noticed in sperm cells of CIPX treated group (Table 4).

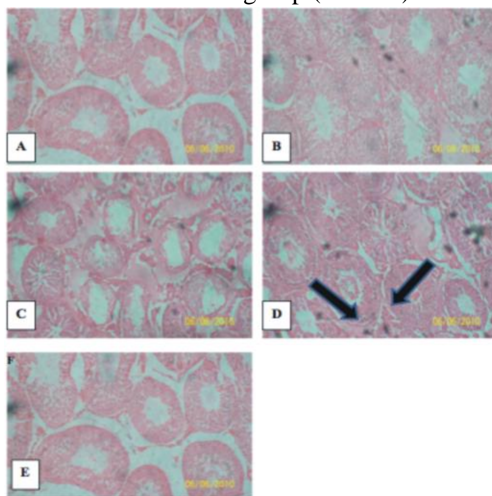

Plate 1
Histology section of the testis of rats from the various treatment groups. Mag. X100. (A) Section of testis from control, with no visible sign of lesion. (B) Section of testis from animals treated with 100mg/kg MOE with no visible sign of lesion. (C) Section of testis from animals treated with 150mg/kg CIPX showing severe germinal erosion (patchy) of the seminiferous tubules and diffuse interstitial oedema (arrows) (D) Section of testis from animals treated with 50mg/kg MOE and then CIPX with no visible sign of lesion

Discussion

Several reports abound with respect to the acclaimed toxicity exhibited by certain antibiotics including Flouroquinolones. Recently, few studies show that the widely used antibacterial Ciprofloxacin may pose a treat to normal testicular and sperm structure and function which are paramount determinants of male fertility (Abd-Allah *et al*., 2000 and Demir *et al*., 2007, Nashwa *et al*., 2011, Zobeiri *et al*., 2013). However, mechanisms underlying CIPX-induced damages in testicular tissue and sperm are still underway.

Our results reveal no significant changes in the relative weights of reproductive organs except in the prostrate of rats administered CIPX at 150mg/kg body weight. The prostrate plays an important role in male reproductive functioning. It secretes enzymes, amines, lipids and metal ions responsible for normal function of spermatozoa (Kumar and Majumder, 1995). Citric acid in prostatic fluid is used by sperm for ATP production via the Krebs cycle. Prostatic secretions make up about 25% of the volume of semen and contributes to sperm motility and viability. Thus a reduction in weight of prostatic tissue may reveal reduced seminal volume and may affect sperm motility if citric acid concentration is reduced.

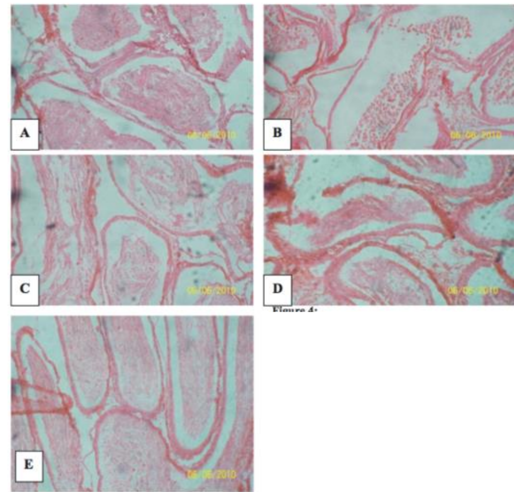

Plate 2
Histology section of the epididymis of rats from the various treatment groups, Mag. X100. (A) Section of epididymis from control, with no visible sign of lesion. (B) Section of epididymis from animals treated with 100mg/kg MOE with no visible sign of lesion. (C) Section of epididymis from animals treated with 150mg/kg CIPX showing no visible sign of lesion (D) Section of epididymis from animals treated with 50mg/kg MOE and then CIPX with no visible sign of lesion. (E) Section of epididymis from animals treated with 100mg/kg MOE and then CIPX with no visible sign of lesion

Testicular sperm number (TSN) and Daily sperm production (DSP) were significantly reduced in CIPX-treated rats. Determination of the number of spermatids per gram testicular tissue (TSN) and sperm produced per day in whole testicle (DSP) are commonly used for evaluation of male reproductive function in rodents (Takeda et al., 2009, Yoshida et al., 2010, Hemmingsen et al., 2009). Daily sperm production was calculated by dividing the total number of spermatids (TSN) per gram of testicular tissue (testis weight) by 6.1 days, the duration of step 19 spermatids in the seminiferous epithelial cycle (Robb *et al.*, 1978). Thus TSN and DSP specifically reflect the state of spermatogenesis which occurs in the testes and not the epididymis. However, Decreased TSN and DSP may or may not affect the number of sperm stored in epididymis (ESN) which wasn't reported in our study (Goyal et al., 2001). Thus epididymal morphology may not be affected. Abarikwu *et al*., (2010) reported a dose-dependent decrease in testicular (TSN) and epididymal sperm number (ESN) with no perceptible changes in epididymal morphology in Atrazine-treated rats.

The testis as one of the major organs of reproduction in the male, functions primarily in the production of spermatozoa which occurs in the seminiferous tubules (spermatogenesis). Testicular spermatogenesis comprises a precisely timed and synchronized development of several generations of germ cells into highly specialized motile sperm. Throughout this complex development, the Sertoli

cell structurally supports and moves germ cells from the base to the lumen of the tubule, regulating the progress of spermatogenesis through to completion. Newly formed cells are transported into the epididymis and on arrival undergo further important maturation processes. The epididymis stores mature sperm cells prior to ejaculation.

Interference with any of these processes has the potential to reduce fertility of the male, but in such cases histopathology is unlikely to show any adverse effects. However, it is usually possible to identify the probable target organ and or cell of toxicity by careful examination of the earliest morphological changes (Creasy, 2001). Thus organ morphology may be preserved in a case of toxicity but an effect noticed at the cellular levels as seen in the disruption of intracellular antioxidant enzymes in testis and semen of rats. In our study, severe erosion (patchy) of the germ cells of seminiferous tubules and diffuse interstitial oedema was observed from testicular histomorphometry but no histopathological changes were observed in the epididymis. It is thus likely that the testis and thus the process of spermatogenesis (the quantity or quality of sperm cells produced) were the target points of CIPX induced reproductive toxicity. This is reflected in disrupted changes observed in the seminiferous tubules. The erosion of germ cells in the tubules is reflected in reduced TSN and DSP subsequently observed in our results. The severe erosion of germ cells observed in the seminiferous tubules which can lead to decrease in the number of spermatogenic cells also agree with those of previous studies in a condition of CIPX-induced reproductive toxicity in male rats (Nashwa *et al*., 2011., Arash *et al*., 2008).

Fluid secretion in the testis serves an important function in the transport of oxygen and nutrients from blood to tubule via the interstitial fluid. The avascular nature of seminiferous tubule epithelium places it on the borderline of hypoxia and thus it is susceptible to reductions in blood flow. This results in a rapid and massive increase in the permeability of its endothelial cells. Resultant edema markedly reduces blood flow and results in death of germ cells (Creasy, 2001). The diffuse interstitial edema observed in testicular tissue in our study, may reflect the disturbance in the blood-testis barrier. With subsequent reduction in blood flow to the tubules and death of germ cells reflected in reduced TSN and DSP in CIPX-treated rats.

Intracellular antioxidant defenses were also significantly altered in our study particularly in the testes of rats administered CIPX. Previous studies show that reactive oxygen species such as superoxide radicals and hydrogen peroxide are produced during the microsomal metabolism of CIPX (Gurbay *et al.,* 2001), and have been implicated in its antibacterial action (Goswami *et al*., 2006). The Increased generation of H_2O_2 observed in the testes and semen could have resulted in the peroxidation of the lipid portions of tissue membranes especially in the sperm cells. Sperm plasma membrane has a high content of polyunsaturated fatty acids which is easily susceptible to lipid peroxidation caused by oxidative stress (Agarwal *et al*., 2005) because of the multiple unsaturation points found along their backbone. This leads to increased lipid hydroperoxide levels and consequently peroxidation products like MDA. In the study of Weyers *et al.,* 2002, CIPX increased levels of hepatic and renal lipid hydroperoxides which are oxidative mediators and intermediates of lipid peroxiadtion. Our results are thus in agreement with the findings of Nashwa *et al*., 2011 who reported an increase in testicular MDA levels in CIPX-treated rats relative to the control group.

The significant increase in levels of these reactive oxygen species also resulted in a concomitant decrease in the levels of the intracellular antioxidant defenses; GSH, GST, GPX and SOD, particularly in the testis leading to oxidative stress. SOD is the first intracellular enzymatic antioxidant of the body's antioxidant defenses. It catalyses the dismutation of the superoxide radical to oxygen and hydrogen peroxide which is readily degraded by Catalase and glutathione peroxidase using GSH. GSH concentrations may be very low in conditions of oxidative stress, and the major peroxide detoxification enzyme, GSH peroxidase, which functions very inefficiently under conditions of GSH depletion (Nakamura *et al.,* 1974), is expected to be inhibited. GSTs on the other hand catalyses the detoxification of xenobiotics by conjugation with GSH which acts as the electron donating cofactor in the reaction. The inactivation of these antioxidant enzymes may be caused by excess ROS generated in the system (Pigeolet *et al.*, 1990) leading to their reduced capacity to defend the cells from oxidative attack.

Compromised tissue membrane integrity resulting from membrane damage due to lipid peroxidation can cause the leakage of cellular components including proteins and enzymes into the surrounding milieu. This reason can account for the increased activities of GGT and LDH observed in the testis and semen of CIPX-treated rats in our study in comparison to the control group. The increased LDH activity in CPX treated rats denotes its cytotoxic effect on germ cells. It may be elevated in most types of testicular cancer (Stenman and Alfthan, 2002). Increase in GGT activity is also a characteristic of damage to testicular germ cells and sertoli cells by many xenobiotics (Pant *et al*., 1995; Pant and Srivastava, 2003). In agreement with our observations, increased GGT activity was also reported in the testes of tetracycline treated rats (Farombi *et al*., 2008).

However, it was observed in our study that treatment with MOE prevented the CIPX induced oxidative stress in the testis and epididymis of rats. MOE restored depleted levels of intracellular antioxidant enzymes while preventing increased generation of ROS and consequently H_2O_2 and MDA levels. *Moringa Oleifera* is a rich source of antioxidant (Chumark *et al*., 2008). During a study reporting antioxidant property of freeze dried *Moringa* leaves from different extraction procedures, it was found that methanol and ethanol extracts have the highest antioxidant activity with 65.1 and 66.8%, respectively (Lalas and Tsaknis, 2002; Siddhuraju and Becker, 2003). It was also reported that its major bioactive compounds of phenolics, such as quercetin and kaempferol are responsible for its antioxidant activity (Bajpai *et al*., 2005; Siddhuraju and Becker, 2003) and thus its treatment favorably modulates biochemical enzymatic parameters including, superoxide dismutase, catalase, glutathione peroxidase, lactate dehydrogenase, and creatine kinase-MB (Nandave *et al*., 2009). It is therefore suggested that the restoration of these depleted intracellular antioxidant enzymes observed in our study, may have reduced the

incident of oxidative attack in cells of the testis and semen, thereby preserving the GSH pool and the activity of GST. Our data agree with several others that reported elevation of a variety of antioxidant enzymes and testicular biomarkers as a result of treatment with *Moringa oleifera* or with phytochemicals isolated from *M. oleifera* (Sharma and Vaghela 2010, Saalu *et al*., 2011, Paliwal *et al*., 2011). Other studies have also shown the ameliorating effect of the treatment with MOE in conditions of drug-induced male reproductive toxicity in experimental animals as well as its ability to attenuate male sexual dysfunction induced by stress (Saalu *et al.,* 2011, Bassey *et al*., 2013, Prabsattroo *et al*., 2012).

Thus our study shows that the ethanol extract of *Moringa oleifera* leaves posses antioxidant properties that ameliorates oxidative stress induced by Ciprofloxacin in the testes and semen of rats.

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