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Reproductive and histological effects of garlic, antioxidant vitamins C and E on pefloxacin-induced toxicity in wistar rats

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ABSTRACT

The reproductive effect of pefloxacin (PF) and antioxidant vitamins C, E and garlic in wistar rats were evaluated. Fifty adult Albino wistar rats (120-180), of either sex were randomly selected into five study groups. Fifty rats were cohabited in matting ratio of 1:1 to give 5 study groups of 5 matting pairs per group. Reproductive parameters assessed were litter size, weight of offspring, morphological, organ weights. Control group was given placebo 0.5ml of normal saline while group II (pefloxacin 11.43mg/kg body weight), group III same dose of pefloxacin with 14.29mg/kg body weight of vitamin C supplement, group IV same dose of pefloxacin with 14.29mg/kg body weight of vitamin E supplement and group V pefloxacin with 4.28mg/kg body weight of garlic supplement in normal saline (vehicle) was administered orally by intubation to both male and female of groups 11 to V for 14 days. There was decrease in number of births (litter size) from 10 in the control to 2 in the PF treated and the PF treated and antioxidant supplemented group had higher litter size (8, 10 and 6) compared to PF treated only. Mean weight of off springs were significantly ($P<0.05$) higher in PF supplemented groups than PF treated only. The antioxidant properties of garlic, vitamins C and E particularly, their ability to ameliorate toxic effect of pefloxacin on affected reproductive organs were clearly depicted by the reversal of pefloxacin toxic effect on these organs and litter size.

Keyword: Pefloxacin, garlic, Vitamins C and E, reproductive function, histological tissues, hormonal and morphological

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INTRODUCTION

Reproductive toxicity adversely affects male and female sexual structures, function, reproductive lactation potentials and viability of the offspring. This concept may include organ toxicity which interferes with normal system functioning and teratogenicity which induced dysmorphogenesis in fetus development but behavioral teratogenicity affects mental development of the fectus, development toxicity embraces teratogenicity and postnatal development [14]. Adequate study design for evaluating the effects of compounds on fertility and general reproductive functions always yield information on the effect of chemicals on neonatal morbidity, mortality and teratogenesis [15]

Some therapeutic actions may adversely interfered with the host cell processes by affecting the membrane cells and tissues, blocking enzyme pathways denaturing proteins or disrupting their osmotic and ionic balances [1]. Evidence of toxemia especially diarrhea, heart failure, pneumonia and death are reported as adverse effects and complications of typhoid infection. Bacteria resistance to chloramphenicol has become an increasing menace associated with its use since 1972 [16]. The present studies was undertaken to evaluate the effect of pefloxacin interactions with garlic, antioxidant vitamin C and E on some reproductive functions of wistar rats, in order to confirm its safety with or other.

As far back as world war I and ii, soldiers were administered garlic to prevent gangrene and is traditionally used to prevent atherosclerosis [7]. Garlic have antioxidant properties and often prevent cell damage, aging and cancer [12].

MATERIALS AND METHODS

The drugs:
Pefloxacin injection (400mg/5ml), garlic supplement (300mg/5ml), Vitamins C and E supplement (1000mg/ml) respectively were obtained Rabana Pharmacy, Calabar and used for the study.

***Experimental animals, treatment protocol and animal mating*:**
Fifty mature albino wistar rats of both sexes, weighing between 120-180g obtained from the disease free stock of the animal facility of Biochemistry Department, University of Calabar, Calabar, Nigeria were used for the study. Prior to experimentation, permission for the use of animals and animal protocol was obtained from the facility of Basic Medical Science animal ethics Committee, University of Calabar.

Twenty five experimentally naive virgin female albino Wistar rats were cohabited with 25 experience males in the mating ratio of 1:1 to give 25 mating pairs. These were assigned into five experimental study groups. Group 1, consist of 5 mating pairs (males versus females) which were not treated with pefloxacin but were allowed to mate. Group 2 consist of 5 mating pairs (males versus females) and were treated with pefloxacin prior to matting. Group 3 to 5 consist of matting pairs treated with pefloxacin and either vitamin C, E or garlic co-administered respectively. Animals were after respective treatments allowed through successful mating, fertilization and gestation, until when pregnancy came to term when they littered.

The experimental virgin female of albino wistar rats were housed in the ratio of I:I with equal number of males overnight to achieved mating. Successful mating was detected by inspection of virginal smear obtained from females, for the presence of spermatozoa and this was designated as gestation day zero. Each male and female of the study group was housed differently in stainless cages of dimension 15m x 15m, with plastic bottom and wire screen top and were housed 2 animals per cage.

The animal room were adequately ventilated and kept at room temperature and relative humidity of 29 ± 2^0 C and 40-70% respectively with 12 hours natural light/dark cycle. Rat chow (Pfizer feeds Nigeria Ltd, Calabar, Nigeria), and water were given to the animals *ad-libutum*. Good hygiene maintained by constant cleansing and removal of waste products of metabolism and spilled from cages daily.

Assessment of pregnancy outcome and preparation of blood and tissue samples:
At the end of the gestation period , female animal parent animals gave to birth from the various mating groups. The litter size, weight and morphology were assessed.

Parents of pefloxacin with garlic, antioxidant vitamin C and E were subjected to overnight fast and the animals were anaesthesized in chloroform vapour and dissected. Blood samples were collected by cardiac puncture into plain sample tubes from where sera were prepared by centrifugation and used for hormonal assay. Ovary and testes tissues were excised and fatty tissues removed and weighed and preserved for histological.

Assay of progesterone and testosterone:
Enzyme linked immuno-absorbent assay (ELISA) (1971) was used for testosterone and progesterone concentrations. The ELISA kits were manufactured and supplied by Grilford, a corning Laboratory U.S.A.

RESULTS AND DISCUSSION

Table 1: Effect of pefloxacin administration and supplementation with garlic and antioxidant vitamins C and E on male rats' body weight and growth rate indices.

	Initial body weight	Final body weight	Weight increase (%)	% weight increase	Growth rate	% growth rate
Group 1 (control)	205.29	230.70	25.41	12.38	1.82	181.50
	2.18	0.93	0.03	1.34	0.09	0.09
Group 2 (PF-treated)	204.00	210.00	6.00	2.94	0.43	43.00
	5.40	0.01	0.02 ^{f}	0.03 ^{f}	0.04 ^{f}	0.04 ^{f}
Group 3 (PF+ vit. C)	208.25	235.20	26.95	12.94	1.93	192.50
	3.15	5.61	1.11^{g}	2.01^{g}	0.40^{g}	11.03^{g}
Group 4 (PF+ vit. E)	208.43	229.88	21.45	10.29	1.53	153.21
	2.33	5.95	2.40^{g}	1.15^{g}	0.45^{g}	28.30^{g}
Group 5 (PF+ galic)	207.10	215.21	8.11	3.92	0.58	57.93
	5.68	6.33	1.51^{f}	2.24^{f}	0.09^{f}	15.94^{f}

Values: Mean ± SEM, N =10, PF = Pefloxacin.

f = *Indicates significant difference in the result of pefloxacin exposed only compared with the control at (P < 0.05) level of confidence.*

g = *Indicates significant difference in the result of antioxidant supplemented rats compared with the pefloxacin exposed only group at (P<0.05) level of confidence.*

Table 2: Effect of pefloxacin administration and supplementation with garlic and antioxidant vitamins C and E on female rats' body weight and growth rate indices.

	Initial body weight	Final body weight	Weight increase (%)	% weight increase	Growth rate	% growth rate
Group 1 (control)	204.55	238.01	33.46	16.36	2.39	239.00
	2.66	2.13	1.21	0.03	0.01	0.25
Group 2 (PF-treated)	203.25	211.27	8.02	3.95	0.57	57.29
	0.41	0.33	0.45^{f}	0.07^{f}	0.03^{f}	0.11^{f}
Group 3 (PF+ vit. C)	201.30	236.76	35.46	17.62	2.53	253.29
	4.45	3.35	3.61^{g}	4.23^{g}	0.08^{g}	6.28^{g}
Group 4 (PF+ vit. E)	208.55	238.27	29.72	14.25	2.12	212.29
	5.21	5.12	8.20^{g}	2.71^{g}	0.34^{g}	15.02^{g}
Group 5 (PF+ galic)	205.50	217.83	12.33	6.00	0.88	88.07
	5.68	7.25	5.10^{f}	3.15^{f}	0.24^{f}	7.22^{f}

Values: Mean ± SEM, N=10, PF = Pefloxacin.

f = *Indicates significant difference in the result of pefloxacin exposed only compared with the control at (P < 0.05) level of confidence.*

g = *Indicates significant difference in the result of antioxidant supplemented rats compared with the pefloxacin exposed only group at (P<0.05) level of confidence.*

Table 3: Effect of pefloxacin administration and supplementation with garlic and antioxidant vitamins C and E on male testosterone level

Treatment group	Testosterone (ng/ml)
Group 1 (control)	1.27±0.04
Group 2 (PF-treated)	0.58 ± 0.01^{f}
Group 3 (PF+ vit. C)	0.95 ± 0.15^{g}
Group 4 (PF+ vit. E)	1.22 ± 0.03^{g}
Group 5 (PF+ galic)	1.05 ± 0.18^{g}

Value: Mean ± SEM, N =10, PF = Pefloxacin

f = *Indicates significant difference in the result of pefloxacin exposed group compared with the control at (P< 0.05) level of confidence.*

g = *Indicates significant difference in the result of pefloxacin exposed and antioxidant supplemented groups compared with the pefloxacin exposed group at (P < 0.05) level of confidence.*

Histological Analysis:

The method of Drunny and Wellington (1980) was used for the histological analysis.

Statistical Analysis

Data generated were analyzed for statistical significance by one way ANOVA and t-test of the SPSS (Statistical Package for Social Science) statistical programme using the Microsoft (MS) excel programme. All data were expressed as Mean ± SEM and the probability tested at 95% level of confidence so as to established research hypothesis.

Table 4: Effect of pefloxacin administration and supplementation with garlic and antioxidant vitamins C and E on female progesterone level.

Treatment group	Testosterone (ng/ml)
Group 1 (control)	1.66 ± 0.03
Group 2 (PF-treated)	0.74 ± 0.03[f]
Group 3 (PF+ vit. C)	1.24 ± 0.20[g]
Group 4 (PF+ vit. E)	1.37 ± 0.25[g]
Group 5 (PF+ galic)	1.30 ± 0.20[g]

Value: Mean ± SEM, N =10, PF = Pefloxacin

f = Indicates significant difference in the result of pefloxacin exposed group compared with the control at (P< 0.05) level of confidence.

g = Indicates significant difference in the result of pefloxacin exposed and antioxidant supplemented groups compared with the pefloxacin exposed group at (P < 0.05) level of confidence.

Table 5: Effect of pefloxacin administration and supplementation with garlic and antioxidant vitamins C and E on reproductive organs weight of offspring.

Treatment group	Reproductive organ weight	
	Testes:	Ovaries:
Group 1 Male control parents vs Female control parents	0.95 ± 0.01	0.03 ± 0.11
Group 2 Male Pf- Parents vs Female Pf- parents	0.42 ± 0.03[f]	0.02 ± 0.01[f]
Group 3 Male Pf+ Vit. C parents vs Female Pf + C parents	0.92±0.15[g]	0.04 ± 0.22[g]
Group 4 Male Pf+ Vit. E parents vs Female Pf+ Vit. E parents	1.10 ± 0.12[g]	0.04 ± 0.00[g]
Group 5 Male Pf+ Garlic parents vs Female Pf+Garlic parents	0.52 ± 0.05[f]	0.03 ± 0.00[f]

Value: Mean ± SEM, N =10, PF = Pefloxacin.

f = Indicates significant difference in the result of pefloxacin exposed group compared with the control at (P< 0.05) level of confidence.

g = Indicates significant difference in the result of pefloxacin exposed and antioxidant supplemented groups compared with the pefloxacin exposed group at (P < 0.05) level of confidence.

Table 6:Effect of pefloxacin administration and supplementation with garlic and antioxidant vitamins C and E on hormonal level of off-springs

Treatment group	Hormonal indices	
	Testosterone:	Progesterone:
Group 1 Male control parents vs Female control parents	1.03 ± 0.01	0.71± 0.04
Group 2 Male Pf-Treated Parents vs Female Pf-treated parents	0.48 ± 0.02[f]	0.25 ± 0.05[f]
Group 3 Male Pf+ Vit. C treated parent vs Female Pf + C treated	0.740±.05[g]	0.40 ±0.03[g]
Group 4 Male Pf+ Vit. E treated vs Female Pf+ Vit. E treated	0.82 ± 0.03[g]	0. 67 ± 0.01[g]
Group 5 Male Pf+ Garlic parents vs Female Pf+Garlic parents	0.60 ± 0.20[f]	0.31 ± 0.25[f]

Value: Mean ± SEM, N =10, PF = Pefloxacin.

f = Indicates significant difference in the result of pefloxacin exposed group compared with the control at (P< 0.05) level of confidence.

g = Indicates significant difference in the result of pefloxacin exposed and antioxidant supplemented groups compared with the pefloxacin exposed group at (P < 0.05) level of confidence.

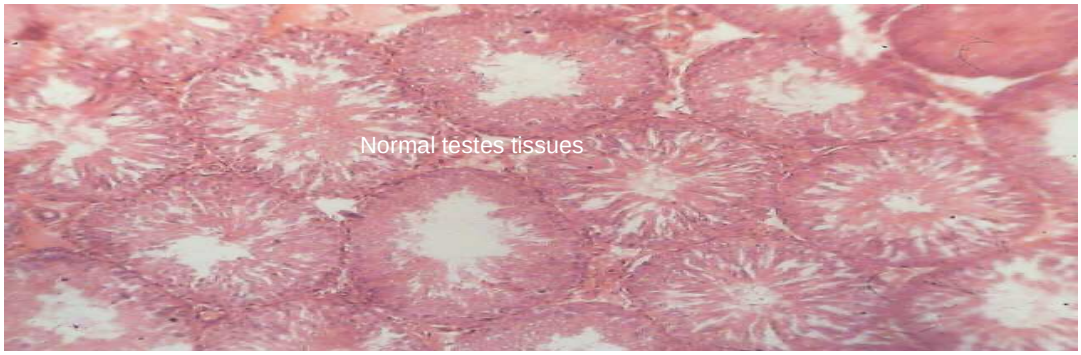
Table 7: Effect of pefloxacin administration and supplementation with garlicand antioxidant vitamins C and E on fecundity (reproductive outcome) number of births and appearance.

Treatment group	Fecundity Indices		
	Number of birth	Weight of offspring	Morphological appearance
Group 1 Male control parents vs Female control parents	10	6.71 ± 0.04	No physical abnormality
Group 2 Male Pf-Treated Parents vs Female Pf-treated parents	2[f]	2.11 ± 0.06[f]	No physical abnormality
Group 3 Male Pf+ Vit. C parents vs Female Pf + C parents	8[g]	5.18 ± 1.17[g]	No physical abnormality
Group 4 Male Pf+ Vit. E parents vs Female Pf+ Vit. E parents	10[g]	4.77 ± 0.00[g]	No physical abnormality
Group 5 Male Pf+ Garlic parents Vs Female Pf+Garlic parents	6[g]	2.89± 0.15[f]	No physical abnormality

Value: Mean ± SEM, N =10, PF = Pefloxacin

f = Indicates significant difference in the result of pefloxacin exposed groupcompared with the control at (P< 0.05) level of confidence.

g = Indicates significant difference in the result of pefloxacin exposed and antioxidant supplemented groups compared with the pefloxacin exposed group at (P < 0.05) level of confidence.



(plate1.1a) Control male testes ×40mag

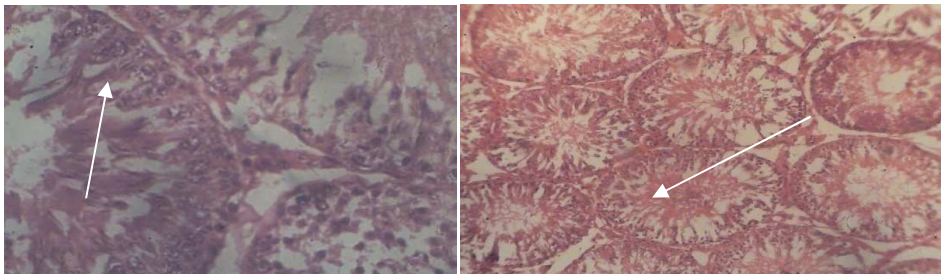
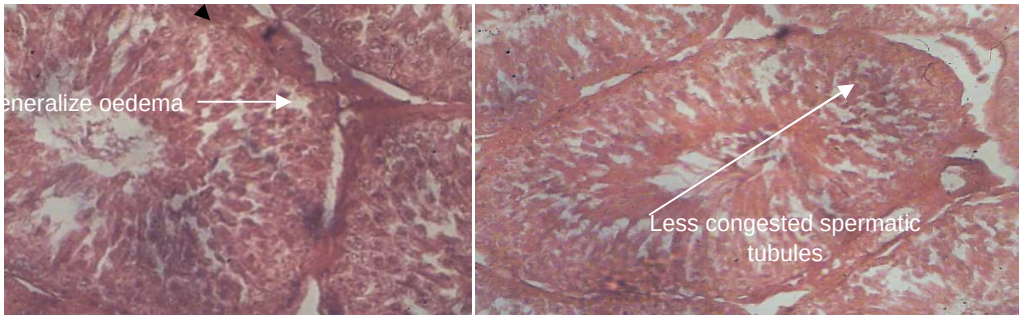
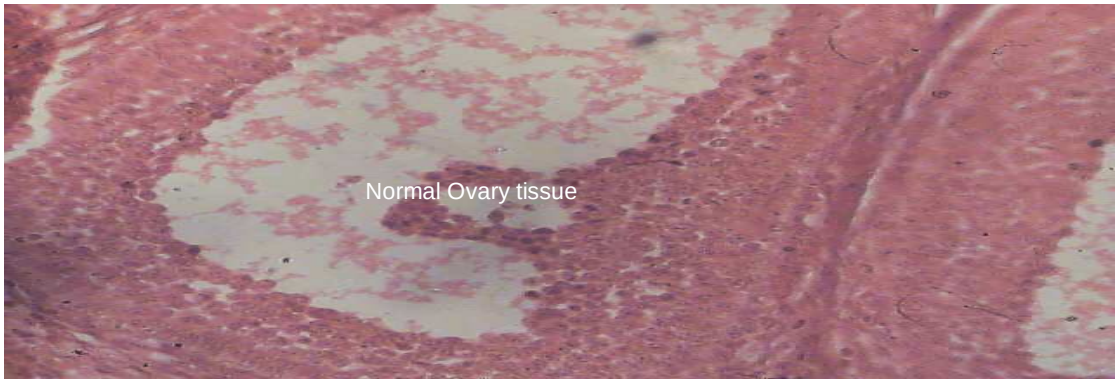


plate 1.1b) testes ×40mag **(plate 1.1c) testes × 40mag**

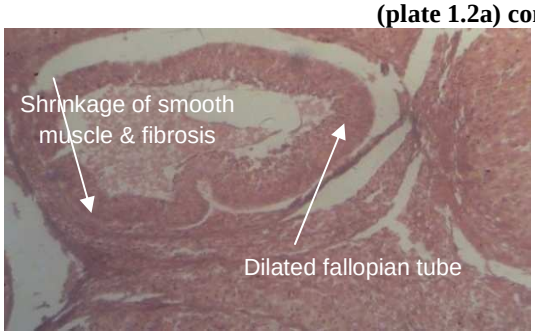


(plate 1.1d) testes × 40mag **(plate 1.1e) testes ×40mag**

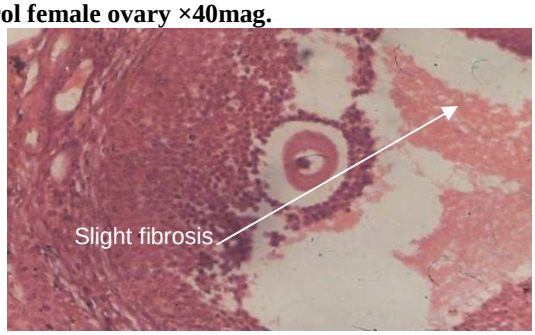
Plate 1.1: photographs of rats testes showing effect of pefloxacin at 11.43mg/kg body weight (a) control, normal testes cell architecture (b), pefloxacin only showing congested spermatic tubules and congested vascular channels (c), pefloxacin + vit. c with slight oedema (d) pefloxacin + garlic showing mild generalize oedema (e) pefloxacin + vit.e showing less congested spermatic tubules (magnification × 40).



(plate 1.2a) control female ovary ×40mag.



(plate 1.2b) female ovary × 40mag



(plate 1.2c) female ovary ×40mag

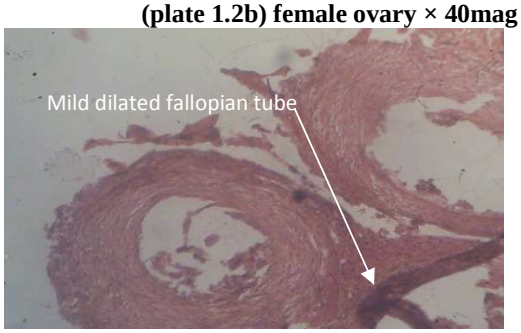
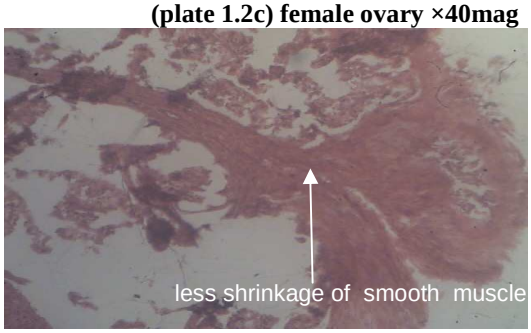


Plate 1.2d) female ovary × 40mag



(plate 1.2e) female ovary × 40mag

Plate 1.2: photographs of rats ovary showing effect of pefloxacin at 11.43mg/kg body weight (a) control, normal ovary cell architecture (b), pefloxacin only showing dilated fallopian tube and shrinkage of smooth muscle with fibrosis (c), pefloxacin + vitamin c with slight fibrosis(d) pefloxacin + garlic showing mild dilated fallopian tube (e) pefloxacin + vitamin e showing less shrinkage of smooth muscle (magnification × 40)

DISCUSSION

It has also been reported that exposure to xenobiotics markedly induces body weight decrease despite increase in food and water intakes [11].The percentage weight increase and the growth rate obtained from the final body weight of rats treated with pefloxacin only group II were observed to be significantly lower than those obtained for rats treated with pefloxacin and antioxidant vitamins and those in the control.

The decrease in body weight is one of the important diagnostic indices for anemia which was depicted in decrease in packed cell volume. It therefore means that pefloxacin treatment may cause weight loss, which may likely results in anemia. Treatment with antioxidants vitamins C, E and garlic ameliorated the conditions that are associated with anaemia by causing increased weight gain and increase in concentrations of haemoglobin and packed cell volume. The recovery process of percentage weight gain and growth rate in antioxidant treated groups were comparable to the control group. Reports by [2] and [9] have showed the importance of antioxidant vitamins in the process of

growth and development. Antioxidant vitamins trigger cell division, proliferation and replication thereby enhancing recovery from ailments and effectively reduce susceptibility to anemic condition [10] and [13]. Also the antioxidant vitamin protects the liver tissues against oxidative damage and may stimulate repair mechanism present in the liver [4]. The weight increase observed in this study may be attributed to stimulation of protein synthesis and stimulation of the repair mechanism present in the liver by the antioxidants.

The plasma hormonal levels of testosterone and progesterone were also determined in this study. There was a significant decrease in testosterone level of pefloxacin treated group compared with the control. This was expected since the damage testes caused by pefloxacin could affect its functions, one of which is the synthesis of the male hormone, hence the low testosterone levels obtained in this study. This study is supported by the report of [5] that Cd^{2+} and Pb^{2+} accumulated in male reproductive organs, causing alterations in hormone concentration, male fertility and sperm parameters. These metals act by poisoning the Ca^{2+} and K^{+} channels and are said to be involved in early events of acrosome reaction.

All the antioxidant supplemented groups showed a significant increase in their various testosterone levels, compared to pefloxacin treated rats. This showed that the supplements masked the testes from much damage by pefloxacin leading to no significant distortion in testosterone levels compared with the control probably by acting as free radical quenching devices.

Moreover, a significant decrease in progesterone level of pefloxacin treated female rats compared to the control was observed. This was confirmed by the distortion in the ovaries of the pefloxacin treated rats. The supplemented rats ameliorated this condition as there was a significant increase in the hormonal level when compared with pefloxacin treated rats.

The relatively low testes weight of the pefloxacin treated rats was a general effect of organ toxicity of pefloxacin and agreed with the report of [3] that testes weights of mice were markedly reduced after (11.43mg/kg) of benxaprofen treatment. This result equally collaborated with the low levels of testosterone obtained with the same group. The result also showed a significant increase in testicular weights of rats given supplementary diets (pefloxacin + vitamin C, pefloxacin + vitamin E and pefloxacin + garlic) compared to non supplemented rats. The significant decrease in ovarian weight of rats treated with pefloxacin in comparison with the control values showed that female sex organs was affected adversely by pefloxacin toxicity and the result collaborated with the low progesterone level obtained in the same group. Antioxidant treatment reversed the decreased ovarian weights.

Fecundity outcome, morphology and physical appearance of the offspring achieved through matting of different groups of parent rats showed that, control male versus control female produced 10 off springs. This was expected because there was no impediment to their reproductive systems by any external chemical or endodermic insult. Pefloxacin male and female produced 2 off-springs. This may have been caused by the damage on the reproductive organs (testes and ovaries) of the parent rats making, it impossible to achieve an impressive birth number. This result was a secondary effect of the low hormonal levels (progesterone and testosterone) observed in this group. The histopathological results of both testes and ovaries of rats in this group also collaborated with the findings of low number of births. Pefloxacin plus vitamin C male versus pefloxacin plus vitamin C female produced 8 off- springs. This was also expected because vitamin C in both parents has been able to remedy the damage effect of pefloxacin on the reproductive integrity. Pefloxacin plus vitamin E male versus pefloxacin plus vitamin female produced 10 off-springs. This observation showed that the acute dosage of pefloxacin (11.43mg/kg) to parents rats were reversed. Pefloxacin plus garlic male versus pefloxacin plus garlic female produced 6 off-springs. The result shows that the number of off-springs for rats given antioxidant supplements were significantly increased compared to the pefloxacin treated group without supplement. The results of this study showed with an exception of garlic supplemented group that there was a significant increased in the number of births in all pefloxacin and antioxidant vitamin supplemented groups compared with non supplemented group. The mean result of testes and ovaries weights of off springs exposed to pefloxacin only and pefloxacin plus garlic treated group showed a significant decreased when compared to the control group. This result could be attributed to the carried over of reproductive mal-formation traits of the parent rats caused by the toxic distortion of the parent female reproductive ability by pefloxacin. The results of hormonal assays (testosterone and progesterone) off-springs are also reported in this study for the group receiving pefloxacin only. Testosterone and progesterone levels in was significantly low compared to control group. This result is in agreement with testes and ovaries weights of the same group. The general picture showed that pefloxacin administration affected reproductive capability and outcome of wistar rats.

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