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Haematological studies of antioxidant vitamins C, E and garlic on Pefloxacin Induced Toxicity in Wistar Rats

Ukpanukpong, R .U[1], Eteng, M.U[2], Dasofunjo, K[1], Pekene, D.B.J[3], Utu-Baku, B. A[1] and Onyeama, H.P[2]
[1]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Cross River University of Technology, P. M. B 1123, Calabar, Cross River State, Nigeria.
[2]Department of Biochemistry, College of Medical Sciences, University of Calabar P. M. B 1115 Calabar, Cross River State, Nigeria.
[3]Department of Physics, Faculty of Science, Niger Delta University Lordberfree Island, Nigeira

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ABSTRACT

The Haematological studies of garlic, antioxidant vitamins C and E on pefloxacin-induced toxicity in wistar rat was evaluated. Method: One hundred adult wistar rats (120-180), of either sex were randomly selected into five study groups. Each group comprised of 10 pairs (ten males and ten females) were not allowed to mate, with group 1 as control. Group 11 were pefloxacin treated only while group 111 to 1V were pefloxacin treated with either garlic, vitamins C and E. Pefloxacin, garlic vitamin C and E in doses 11.43mg/kg, 4.28mg/kg, 14.29mg/kg body weight in normal saline (vehicle) was administered orally by intubation to both male and female of groups 11 to V for 14 days. Control animals received 0.5ml of normal saline. In life observation measurements were taken and at the end of drug, garlic, antioxidant vitamins C and E combined administration animals were sacrificed and tissues obtained for biochemical assessment. Result: Physical signs of toxicity and ameliorating effects of antioxidant vitamins and garlic were also expressed in rats, significant ($p<0.05$) decrease in Hb, PCV, RBC and Total WBC were observed. Conclusion: These results suggest adverse effect of pefloxacin and ameliorating role of garlic, vitamins C and E on wistar rats'

INTRODUCTION

It is estimated that about seventeen million cases of typhoid infection are recorded each year, among which six hundred thousand cases are reported to be fatal (Hakanen *et al.*, 2007). Evidence of toxemia especially diarrhea, heart failure, pneumonia and death are reported as adverse effects and complications of the disease (Woodruff and Wright, 1987). 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 (Agomo *et al*.,1992). Bacteria resistance to chloramphenicol has become an increasing menace associated with its use since 1972 (Woodruff and Wright, 1987). Pefloxacin, a broad spectrum antibiotic, was found to be more active than chloramphenicol in the treatment of typhoid infection (Cristiano *et al*.1995).

* *Corresponding Author*
Dr. Ukpanukpong, R. U, Department of Medical Biochemistry
Faculty of Basic Medical Sciences, Cross River University of Technology, P. M. B 1123, Calabar, Cross River State, Nigeria.

An antioxidant is a substance that prevents the oxidation of other molecule (Ternay and Sorokin, 2009). Although oxidative reactions are crucial to life, they can be destructive; hence plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamins C and E as well as enzymes such as catalase, superoxide dismutase and various peroxidases (Vardakas *et al.*, 2008).

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 and treatment protocol:

One hundred 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.

The animals were randomly selected based on average body weight into five study groups of 20 animals (10 males and 10 females) per group.

Each male and female of the study group was housed differently in a stainless cages of dimension 15m x 15m, with plastic bottom and wire screen top and were housed 10 animals per cage.

The animal room was 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.

Group 1 served as the control and groups 11 to V were pefloxacin, garlic, vitamin C and E supplemented groups.Pefloxacin, garlic, vitamins C and E supplements in dose 11.43mg/kg, 4.28kg/ml and 14.29mg/kg body weight in normal saline were co-administered via oral route by intubation to animals of the test groups 11-V while control received 0.5ml of normal saline for 14 days. Dose administration was done between the hours of 0.90am and 10am daily and the doses calculated corresponds with therapeutic dosage in humans of 800/70kg, 300/70kg and1000kg body weight respectively. In test group 11, male and female animal were treated with pefloxacin but not allowed to mate. In test group 111 to V, pefloxacin, garlic, vitamins C and E supplements were co-administered to male and female animals but not allowed to mate.

The animals were checked daily to ascertain for number of dead animals. Clinical signs of over poisoning such as hair coat, motor activity and state of feces were observed. Urine colour was also monitor daily. The animals were weighed at the commencement of the experiment and thereafter weekly to assess body weight gains and growth rate.

Assay of heamatological parameters

Haemoglobin level was determined by the method of Crosby *et al*, 1954 and as modified by Pla and Fritz, 1971.

Estimation of percentage packed cell volume

Values were read and converted from a micro-heamatocrit reading device (Dacies and Lewis, 1975).

Red blood cell count and total white blood cell count:

Red blood cell and total white blood cell counts were computed using the method of Dacies and Lewis 1975

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 $\pm$ SEM and the probability tested at 95% level of confidence so as to established research hypothesis.

RESULTS AND DISCUSSION

Abnormality in physical appearance and behaviours accompanying pefloxacin administration has been reported as physical signs of toxicity in rats (Bosque *et al*, 1995). It has also been reported that exposure to xenobiotics markedly induces body weight decrease despite increase in food and water intakes (Lovati *et al*, 1996). 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.

Table. 1: Effect of pefloxacin in diet supplemented with garlic and antioxidant vitamins C and E on male rat 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 $\pm$ SEM, N =10, PF = Pefloxacin.

f = Indicates significant difference in the effect of pefloxacin (only) in diet compared with the control at ($P < 0.05$) level of confidence.

g =Indicates significant difference in the effect of diet with pefloxacin supplemented with antioxidant vitamins when compared with diet with pefloxacin only at ($p<0.05$) level of confidence.

Table. 2: Effect of pefloxacin in diet supplemented with garlic and antioxidant vitamins C and E on female rat 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]

VALUE: MEAN ± SEM, N=10, PF =PEFLOXACIN.
f =Indicates significant difference in the effect of pefloxacin only in diet compared with the control at (P < 0.05) level of confidence.
g =Indicates significant difference in the effect of diet with pefloxacin supplemented with antioxidant vitamins when compared with diet with pefloxacin only at (p<0.05) level of confidence.

Table. 3: Effect of pefloxacin in diet supplemented with garlic and antioxidant vitamins C and E on male rat haematological indices.

	Haemoglobin (g/dl)	Packed cell Volume(%)	White blood cell count ($Nx10^3/mm^3$)	Red blood cell count ($Nx10^6/mm^3$)
Group 1 (control)	13.30+0.21	42±0.28	5.62±0.57	8.40±0.28
Group 2 (PF-treated)	5.71+0.34[f]	17±0.05[f]	12.11±0.20[f]	4.06±0.01[f]
Group 3 (PF+ vit. C)	10.22±1.50[g]	32±6.20[g]	6.28±1.33[g]	6.20±0.01[g]
Group 4 (PF+ vit. E)	11.45±1.80[g]	35±4.11[g]	8.07±1.91[g]	7.01±0.85[g]
Group 5 (PF+ galic)	12.05 1.20[g]	38±3.42[g]	7.22±1.41[g]	6.50±0.02[g]

VALUE: MEAN ± SEM, N=10, PF =PEFLOXACIN.
f =Indicates significant difference in the effect of pefloxacin (only) in diet compared with the control at (P < 0.05) level of confidence.
g =Indicates significant difference in the effect of diet with pefloxacin supplemented with antioxidant vitamins when compared with diet with pefloxacin only at (p<0.05) level of confidence.

Table. 4: Effect of pefloxacin in diet supplemented with garlic and antioxidant vitamins C and E on female rat haematological Indices.

	Haemoglobin(g/dl)	Packed cellVolume(%)	White blood cell count ($Nx10^3/mm^3$)	Red blood cell count ($Nx10^6/mm^3$)
Group1 (control)	13.27 ± 0.43	39 ±0.41	5.57 ±0.40	7.33 ±0.24
Group 2(PF-treated)	7.23 ± 0.77[f]	23 ± 2.45[f]	12.27 ±0.43[f]	4.18 ±0.30[f]
Group 3(PF+ vit. C)	12.05 ± 1.30 [g]	38 ± 1.21[g]	6.39 ±0.62[g]	6.75 ±1.08[g]
Group 4(PF+ vit. E)	10.75 ± 1.42[g]	33 ±4.07[g]	8.05 ±1.52[g]	6.00 ±0.62[g]
Group 5(PF+ galic)	11.22 ±1.58[g]	35 ±3.10[g]	7.44 ±0.81[g]	7.01 ±0.46[g]

VALUE: MEAN ± SEM, N=10, PF = PEFLOXACIN.
f = Indicates significant difference in the effect of pefloxacin (only) in diet compared with the control at (P < 0.05) level of confidence.
g = Indicates significant difference in the effect of diet with pefloxacin supplemented with antioxidant vitamins when compared with diet with pefloxacin only at (p<0.05) level of confidence.

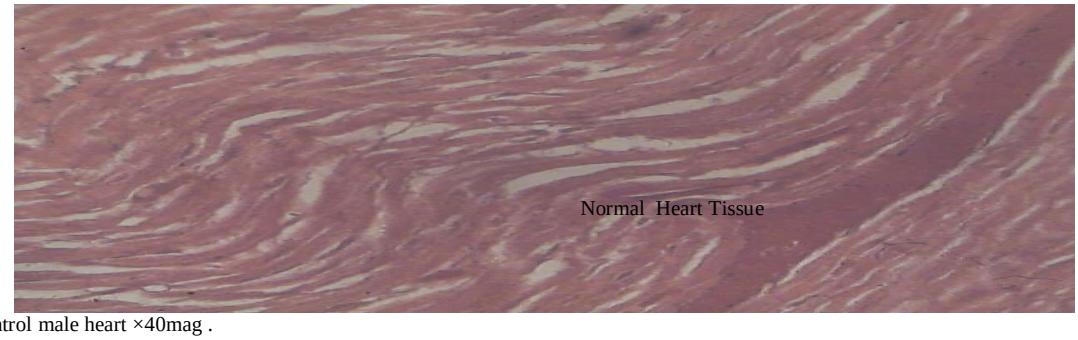

Plate 4.1a: control male heart ×40mag .

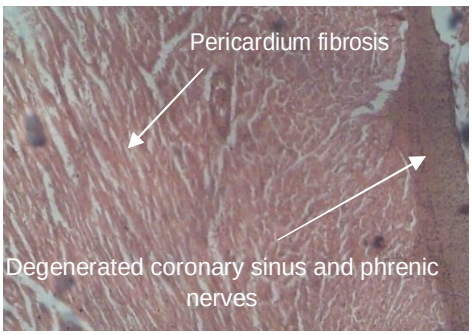

Plate 4.1b: Male Heart ×40mag .

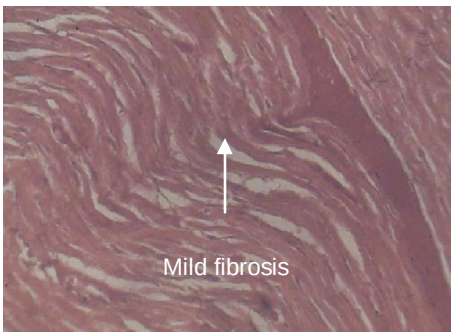

Plate . 4.1c: Male Heart ×40mag

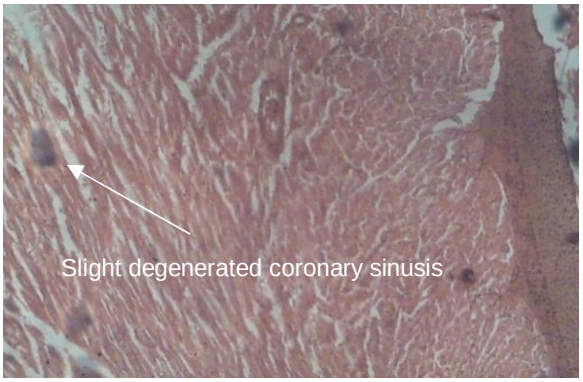

Plate 4.1d: Male Heart × 40mag

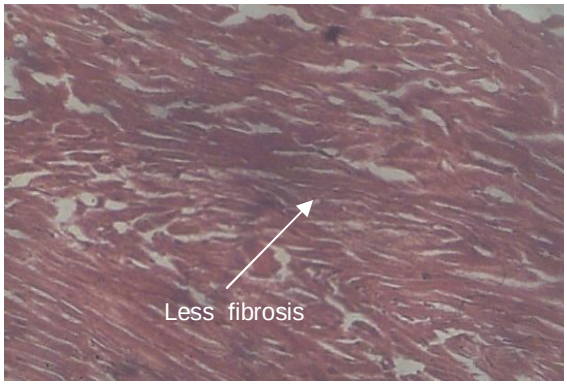

Plate 4.1e): Male Heart ×40mag

Plate 4.1: photographs of rat heart showing effect of pefloxacin at 11.43mg/kg body weight.. (a) Control:normal heart cell architecture (b), pefloxacin only: pericardium fibrosis, degenerated coronary sinusis and phrenic nerves (c), pefloxacin + vitamin C: mild fibrosis (d) pefloxacin + garlic: slight degenerated coronary sinusis (e) pefloxacin + vitamin E: showing less fibrosis (magnification × 40)

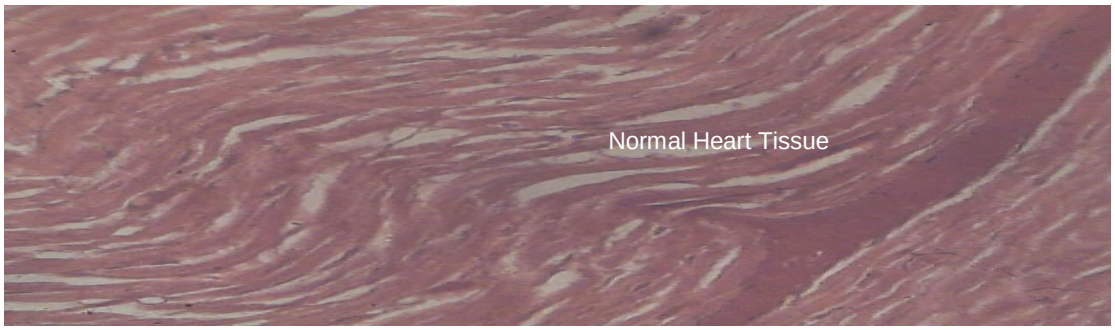

Plate 4.2a: Control Female Heart ×40mag

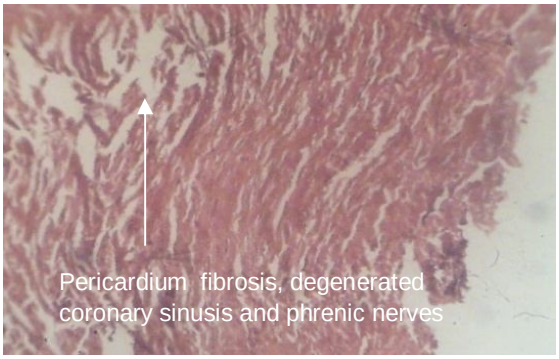

Plate4.2b: Heart×40mag

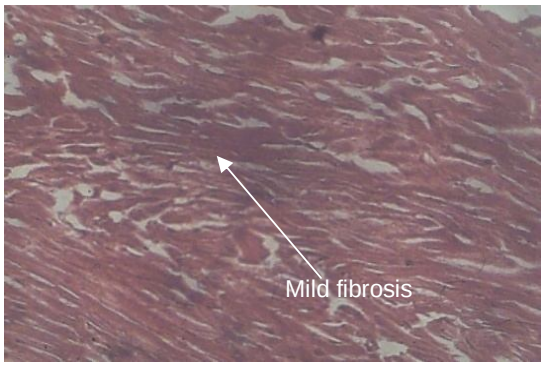

Plate4.2c: Heart×40mag

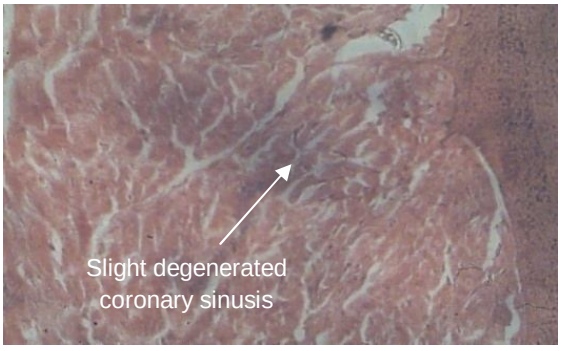

Plate 4.2d: Heart ×40mag

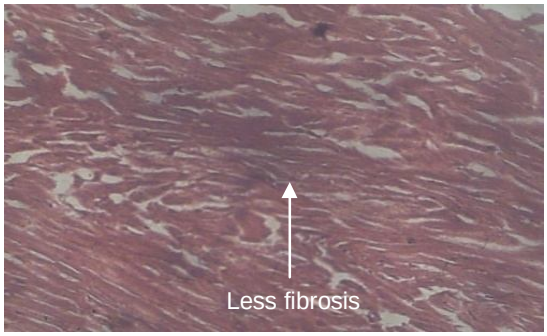

Plate 4.2e: Heart ×40mag

Plate 4.2 photographs of rats heart showing effect of pefloxacin at 11.43mg/kg body weight (a) Control: normal heart cell architecture (b), pefloxacin only: pericardium fibrosis, degenerated coronary sinus and phrenic nerves (c), pefloxacin + vitamin C: mild fibrosis (d) pefloxacin + garlic slight degenerated coronary sinusis (e) pefloxacin + vitamin E: less fibrosis (magnification ×40).

The recovery process of percentage weight gain and growth rate in antioxidant treated groups were comparable to the control group. Reports by Apori *et al* (2007) and Kassuya *et al* (2009) 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 (Krause and Mahan, 1984; Mayers, 1990). Also the antioxidant vitamin protects the liver tissues against oxidative damage and may stimulate repair mechanism present in the liver (Battacharjee and Sil, 2007). 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.

Pefloxacin caused a significant decrease in PCV, Hb, RBC, and increase in WBC. This effect may be due to decrease in myelosuppression and bone marrow cellularity as reported by kumar and kuttan (2005) and cyclophosphamatid exchanges in bone marrow cells and micronuclei in polychromatic erythrocytes (Moorthy and Murthy, 1994). The increase in PCV, Hb, RBC and decrease in WBC reported in this study to be associated with antioxidant supplements may be attributed the reversal of bone marrow depression with attendant improvement in erythrocyte membrane stability and these antioxidant properties reduced haemolysis (Krause and Mahan,1984; Naaz *et al,* 2007).

The histology in this study showed that garlic, vitamin C and E pretreatment considerably reduced the toxic effects of pefloxacin in rats heart compared to those of pefloxacin treatment.

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