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Antioxidant interactions of Pefloxacin, garlic, vitamins C and E on lipid profile level of Albino wistar rats

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ABSTRACT

The lipid profile 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 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, pefloxacin treatment induced significant ($P<0.05$) increase in total cholesterol, low density lipoprotein, very low density lipoprotein, triacylglycerol and decrease in high density lipoprotein relative to control but PF exposed and antioxidant vitamins C, E and garlic treated groups produced significant ($P<O.O5$) reduction in TC, LDL, VLDL, TG and with an increase in HDL levels relative to PF only treated groups were observed. Conclusion: These results suggest adverse effect of pefloxacin and ameliorating role of garlic, vitamins C and E on wistar rats'.

INTRODUCTION

Quinolones, synthetic chemotherapeutic agents, have a broad spectrum of antimicrobial activity as well as a unique mechanism of action, resulting in inhibition of bacterial DNA gyrase and topoisomerase IV. Quinolones inhibit the bacterial DNA gyrase or the topoisomerase IV enzyme, thereby inhibiting DNA replication and transcription (Bergan *et al*, 1985). Quinolones can enter cell easily via porins and, therefore, are often used to treat intracellular pathogens such as *Legionella pneumophila* and *Mycoplasma pneumoniae*. For many gram-negative bacteria, DNA gyrase is the target, whereas topoisomerase IV is the target for many gram-positive bacteria (Mcqueen and Williams, 1987). They are substances that protect other chemicals of the body from damaging oxidative reactions by reacting with free radicals and other reactive oxygen species within the body, hence, hindering oxidation. Although oxidative reactions are crucial to life, they can also be destructive; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C and E as well as enzymes such as catalase, superoxide dismutase and various peroxidases (Vardakas *et al.*, 2008).

As oxidative stress might initiate many human diseases and the use of antioxidants in pharmacology is presently gaining acceptance, particularly as potential treatments for atheriosclerosis, cancer and neurodegenerative diseases (Murray *et al.*, 2006). Antioxidants are now used as dietary supplements in maintaining health and preventing diseases such as cancer and coronary heart disease. A dietary antioxidant is a substance that significantly decreases the harmful effects of reactive oxygen species and nitrogen molecules, which disrupt normal physiological functions at the cellular level in animals and humans.

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Examples of dietary antioxidants include vitamins C and E, selenium and carotenoids.

A number of other nutrients, including minerals such as copper, manganese, and zinc, phytochemicals such as flavonoids in grape seed extract and phenols found in green tea, and coenzymes also possess antioxidant properties (Stacy and Childs, 2000). The primary function of vitamin C is for production of collagen, which forms the basis for connective tissues in bones, teeth and cartilege (Cann and Verhulst, 2007).

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 and 1000kg 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.

Estimation lipid profile

Triacylglycerol (CHOD-PAP) randox kits

The triacylglycerols are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-1-minopherazone and 4-chlorophenol under the catalytic influence of peroxides. See Appendix X for details.

Triacylglycerols + H_2O $\xrightarrow{\text{Lipase}}$ Glycerol + Fatty acids

Glycerol + ATP $\xrightarrow{\text{Gk}}$ Glycerol phosphate + ADP

Glycerol-3-phosphate + O_2 $\xrightarrow{\text{GPO}}$ Dihydroxyacetone phosphate + H_2O_2

$2H_2O_2$ + 4-aminophenazon 3 one + 4-chlorophenol $\xrightarrow{\text{POD}}$ Quinoneimine + HCl + $4H_2O$

Calculations

$$\text{Triacylglycerol concentration} = \frac{\text{A sample} \times 2.29}{\text{A standard}} = \text{mol/l}$$

or

$$\text{Triacylglylcerol concentration} = \frac{\text{A sample} \times 200}{\text{A standard}} = \text{mg/dl}$$

Estimation of total cholesterol (CHOD-PAD) Randox kits

Cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-amino antipyrine in the presence of phenol and peroxidase.

Cholesterol ester + H_20 cholesterol $\xrightarrow[\text{Esterase}]{\text{Cholesterol}}$ + Fatty acids

Cholesterol + 0_2 Cholesterol $\xrightarrow[\text{oxidase}]{\text{Cholestero}}$ l-3-one + H_20

$2H_20_2$ + 4-aminoantipyine $\xrightarrow{\text{peroxidase}}$ Quinoneimine + H_20

$$\text{Concentration of the cholesterol in sample} = \frac{\text{A sample} \times \text{Concentration of standard}}{\text{A Standard}}$$

Concentration of standard: 5.17mmol/l (200mg/dl). See Appendix XI for details.

Estimation of high density lipoprotein (CHOD-PAP) Randox kit

Low density lipoproteins (LDL), very low density lipoprotein (VLDL) and chylomicrons fractions were precipitated quantitatively by the addition of phosphotungstic acids in the presence of magnesium ions. After centrifugation, the cholesterol in the (HDL) fraction, which remains in the supernatant were determined.

Estimation of cholesterol (CHOD-PAP) Assay

Concentration of HDL cholesterol in supernatant =

$$\frac{\text{A sample X concentration of standard}}{\text{A standard}}$$

Concentration of standard: 5.17mmol/l

Estimation low density lipoprotein

Low density lipoprotein (LDL) Cholesterol concentration was calculated as follows:

$$\text{LDL–Cholesterol} = \text{Totalcholesterol} - \frac{\text{Triacylglycerol}}{2.2} - \text{HDL(mmol/l)}$$

$$\text{LDL – Cholesterol} = \text{Totalcholesterol} - \frac{\text{Triacylglycerol}}{5} - \text{HDL cholesterol}$$

Estimation very low density lipoprotein

The VLDL concentration was calculates as follows:

$$\text{VLDL} = \frac{\text{Triacylglycerol (mmol/l)}}{2.2}$$

$$\text{VLDL} = \frac{\text{Triacylglycerol (mg/d1)}}{5}$$

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.

Statistical analysis

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RESULTS AND DISCUSSION

Results

Table. 1: Effect of pefloxacin administration and supplementation with garlic and antioxidant vitamins C and E on male rats' lipid profile.

	TC mmol/l	HDL-c(mmol/l)	LDL mmol/l	VLDL mmol/l	TG mmol/l
Group 1 (control)	1.77± 0.05	1.23± 0.3	0.45± 0.03	0.54± 0.04	1.33± 0.06
Group 2 (PF-treated)	2.08± 0.04^{f}	0.91± 0.04^{f}	0.71± 0.03^{f}	0.73± 0.03^{f}	1.70± 0.01^{f}
Group 3 (PF+ vit. C)	1.79± 0.06^{g}	1.50± 0.18^{g}	0.46± 0.01^{g}	0.60± 0.01^{g}	1.40± 0.08^{g}
Group 4 (PF+ vit. E)	1.84± 0.07^{g}	1.20± 0.04^{g}	0.50± 0.04^{g}	0.57± 0.03^{g}	1.45± 0.04^{g}
Group 5 (PF+ galic)	1.82± 0.00^{g}	1.16± 0.05^{g}	0.48± 0.04^{g}	0.59± 0.00^{g}	1.44± 0.03^{g}

Value are expressed as 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. 2: Effect of pefloxacin administration and supplementation with garlic and antioxidant vitamins C and E on female rats'lipid profile**.**

	TC mmol/l	HDL-c(mmol/l)	LDL mmol/l	VLDL mmol/l	TG mmol/l
Group 1 (control)	1.08± 0.04	1.13± 0.00	0.40± 0.01	0.56± 0.04	1.28± 0.03
Group 2 (PF-treated)	2.26± 0.08^{f}	0.75± 0.01^{f}	0.51± 0.02^{f}	0.76± 0.01^{f}	1.70± 0.01^{f}
Group 3 (PF+ vit. C)	1.20± 0.05^{g}	1.01± 0.10^{g}	0.43± 0.03^{g}	0.60± 0.11^{g}	1.42± 0.11^{g}
Group 4 (PF+ vit. E)	1.31± 0.40^{g}	0.95± 0.09^{g}	0.41± 0.10^{g}	0.58± 0.02^{g}	1.31± 0.01^{g}
Group 5 (PF+ galic)	1.15+ 0.05^{g}	1.00+ 0.10^{g}	0.42+ 0.02^{g}	0.63+ 0.05^{g}	1.36+ 0.02^{g}

Value are expressed as 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.

DISCUSSION

The Total cholesterol, triacylglycerol, LDL–cholesterol and very low density lipoprotein concentrations have been proved to be indicative in the diagnosis of some clinical conditions such as hepatitis, chronic obstructive jaundice and coronary heart disease precipitated by atherosclerosis with attendant hyperglycemia (Lawn, 1992). Among the serum lipid fraction, cholesterol is the most implicated and predominant constituent of atherogenic plaque (Ganong, 1987). Increase in the serum level of these lipids leads to precipitation and accumulation of fatty deposit called atherosclerotic plaque in arterial wall, a condition referred to as atherosclerosis which often progresses to the development of coronary heart disease (Lawn, 1992).

In this research, total cholesterol, TG, LDL-C, and VLDL-c concentrations were increased significantly in the animals treated with pefloxacin only when compared the control.

Liver plays a pivotal role in the metabolism of lipids, hence increased level of serum lipids observed in this research as result of pefloxacin treatment may be due to distortions in the architectural and functional integrity of the liver, leading to liver damage. However, administration of pefloxacin with antioxidant vitamins C, E and garlic caused a significant decrease in total cholesterol, TG, LDL-C and VLDL-c when compared with pefloxacin treated only but no significant different when compared with the control.

Report by Yakubu *et al,* (2007) have shown the hypolidemic effect of plant extract which can be due to their antioxidant effect on the liver. Srividya *et al,* (2002) and Khanna *et al* (2006) have also shown the hypolipidemic effect of the antioxidant on the liver.

Antioxidant vitamins have been shown to have reductive effect on total serum cholesterol and can be explained due to their inhibitory effect on β-hydroxy–β-methylglutaryl (HMG) CoA reductase activity, thus inhibiting cholesterol biosynthesis. Anti-antherogenic role of the vitamins may therefore be due to drastic reduction in the filtration of fatty acids from the blood into the artery and consequent reduction in cholesterol and other fatty acids (Stephens *et al, 1*995).

Results of this study indicate significant increase in VLDL and TG in rats treated with pefloxacin only. A significant increase was also noted in total cholesterol and decrease HDL values in the group treated with normal dose of pefloxacin. Several epidemiological laboratory studies have demonstrated the relationship between the blood level of total cholesterol, triacylglycerol lipoproteins and the incidence of coronary heart disease, cholesterol remains the most fundamental (Ganong, 1987).

Increase cholesterol levels might be due to increase synthesis of cholesterol and cholesterol ester hydrolase following the increase levels of TG and VLDL. It has been reported that the cholesterol production is regulated intracellularly.

The rate limiting pathways being the comers ion HMG COA into mevalonate by the enzyme HMG COA reductase (Laningher *et al,* 1993). The decrease in HDL reported in this study to be associated with pefloxacin administration suggests that, there is risk of coronary heart disease, hence there was a positive correlation between HDL levels and arterial disease (Leningher *et al,* 1993). Atheriosclerosis is linked to high levels of cholesterol in the blood, and particularly to high levels of LDL - bound cholesterol, but findings from this work showed significant difference when the test groups were compared to the control, confirming that there was risk of arterial disease.

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