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Hepatocellular enzyme activities and protein level following administration of vitamins during Chloroquine induced hepatoxicity in wistar rats

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Abstract

***Objective**:* The effect of vitamins against chloroquine induced hepatoxicity was studied in wistar rats. Analysis of serum AST, ALT and ALP activities with those of total protein and albumin were carried out.
Method: Single oral administration of chloroquine (970mg/kg body weight) caused significant increase in AST, ALT and ALP while protein and albumin concentration were reduced. However, simultaneous treatment of chloroquine with 200mg/kg body weight and 4.3 mg/kg of vitamin C and E respectively were administered for 14days.
Results: The levels serum AST, ALT and ALP were significantly decreased following the treatment with vitamin C and E.
***Conclusion**:* These observations suggest that administration of high dose of chloroquine can cause increase in serum hepatocellular enzymes activities, which is suggestive of liver necrosis which can be ameliorated to varying degrees by vitamins C and E. Hence, fruits rich in vitamin C and E are highly recommended in liver disorders.

Key words: Hepato-cellular enzymes; Protein; Chloroquine; Hepatoxicity.

Introduction

Chloroquine (CQ) is a member of an important series of chemically related anti-malarial agents and quinolone derivatives (Fig.1). It is a synthetic drug used in the treatment of malaria. Being a 4-aminoquinoline, it is a rapidly acting blood schizontocide with some gametocytocidal activity (Ekanem *et al*., 1990).

Chloroquine is very cheap and until recently was very effective which made it the anti-malarial drug of choice for many years in most parts of the world. In malaria endemic regions like south eastern part of Nigeria, where *Plasmodium falciparum* is sensitive to chloroquine, the drug is still useful for the treatment and prophylaxis of the infection, in spite of increasing prevalence of resistance of the parasite to the drug (Sowunmi *et*

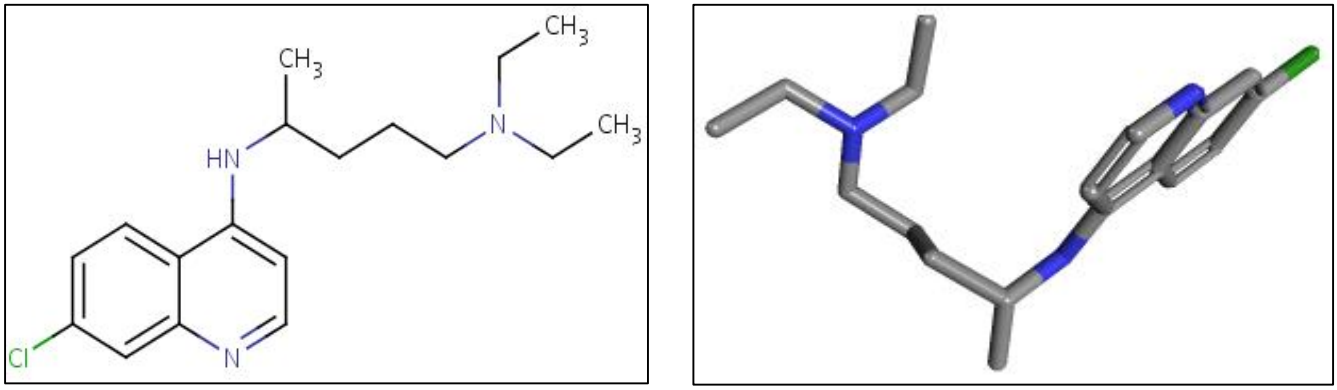

Fig.1. (a) Primary structure of Chloroquine, (b) Secondary structure of Chloroquine

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al., 2000). Chloroquine is a synthetic quinoline drug commonly used for the treatment of malaria (Pari and Amali, 2005). Augustijus and Verbeke (1993) reported that chloroquine is used for the treatment of a wide range of disease including inflammation and intestinal amobiasis. Chloroquine treatment is often accompanied by serious side effect such as headache and visual disturbances.

Chloroquine has been reported to cause hepatic damage (Pari and murugavel, 2004; Dass and Shah, 2000). The reactive oxygen species have been implicated in much disease including hepatotoxicity (Ali *et al*. 2001). Experimental, clinical and epidemiological studies have provided evidence in support of the role of ROS in the etiology of hepatotoxicity by anti malaria drugs (Nnodim *et al*., 2010). When produced in excess, ROS cause tissue injury. All aerobic organisms including human have anti oxidative damage. However, the natural antioxidants defense mechanisms can be inadequate and therefore dietary intake of antioxidant component is essential and recommended (Iniaghe *et al*., 2008).

Many minor components of food, such as mineral and antioxidant vitamins have been linked to alteration in biological processes which may reduce risk of chronic diseases in humans (Owu *et al*., 2006). For instance, vitamin C and E have been reported to improve glucose disposal in healthy subjects and in diabetic patients (Paolisso *et al*., 1994). Since vitamins C and E have been described to be beneficial, the present study investigates the protective role of vitamin C and E in chloroquine induced hepatotoxicity as there have been relatively few studies on this model.

Materials and methods

Drugs used

Chloroquine (Emzor) vitamin C (Emzor) and vitamin E (Ephynal) were purchased from a registered pharmacy shop in Owerri, Imo State of Nigeria. The tablets were dissolved in distilled water according to the required concentrations for administration to the wistar rats on the basis of their body weight.

Experimental Animals

The wistar albino rats weighing between 150-230g, ages 8-10 weeks were used in the study. These animals were obtained from the animal house of College of Medicine and Health Sciences, Imo State University, Owerri Nigeria. They were kept under standard laboratory conditions, fed with commercial growers mash, product of Tops Feeds Ltd, Sapele, Nigeria. Water and feed were provided *ad libitum.* The animals were left for two weeks to acclimatize and then divided into groups for experimentation.

Experimental Design

The animals were randomly assigned to four experimental groups (n = 6 x 4group). The first group of animals which served as control was given distilled water. Group II, III and 1V were given chloroquine (970mg/kilogram body weights), chloroquine and vitamin C (200mg/body weight), chloroquine and vitamin E (4.3mg/body weight) respectively for 14 days. In all groups the drug was administered through oral route using a feeding tube attached to a 5ml syringe. All animals were allowed free access to food and water throughout the experiment.

Blood Collection

Twenty four hours after the last doses were administered; the animals were anaesthetized with chloroform vapour, quickly brought out of the jar and sacrificed. Whole blood was collected by cardiac puncture from each animal into clean dry test tubes. The blood were allowed to stand for about 15 minutes to clot and further spun in a Westerfuge centrifuge (model 1384) at 10,000g for 5 minutes. Serum was separated from the clot with Pasteur pipette into sterile sample tubes for the measurement of the biochemical parameters.

Biochemical Analysis

Serum AST and ALT were assayed by the method of Reitman and Frankel (1957). ALP was determined by the method of king and king (King and King, 1954). Also, serum protein and albumin were determined by the method of Biuret (Tietz,

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Table 1. Hepatocellular enzymes activities, total protein and albumin levels in rats given Chloroquine with or without vitamin C and E

Group	Treatment	AST	ALT	ALP	Total protein	Albumin
1	Control	15.21±1.9	12.36±3.1	65.14±6.8	5.9±1.6±1.07	3.7±1.3
2	Chloroquine	34.61± 2.8*	25.17±3.0*	98.60±6.4*	3.7±0.92*	2.9±1.6*
3	Chloroquine+ vitamin C	24.92±2.1*	19.98±2.4*	81.14±6.6*	5.2±0.099*	3.2±0.9*
4	Chloroquine + vitamin E	26.11±2.3*	20.01±2.9*	80.82±5.2*	4.9±0.094*	3.0±0.7*

**Significantly different from control (P<0.05)*

1995) and Bromocresol Green method (Grant, 1987) respectively.

Statistical analysis

The results were expressed as mean ± standard deviation. The statistical evaluation of data was performed by using one-way anova (Analysis of variance) followed by Duncan's multiple range tests (Duncan, 1957).

Results and Discussions

Hepatocellular enzymes activities, a total protein and albumin level in rats given chloroquine with or without vitamin C and E has shown in Table 1. Chloroquine induced hepatotoxicity like many other disease conditions is believed to be linked with the generation of reactive oxygen species (Ita *et al*., 2007). Valko *et al*. (2007) reported that antioxidants play prominent roles in prevention of ROS generation. These antioxidants are readily available in vegetables.

The commonest enzymes regarded as indicators of liver damages are AST, ALT and ALP. The damage to the hepatocelluar cells results in increase in their enzymes activities. In this study, chloroquine administration in the dose of 970mg/kg body weight of rats results in increase serum AST, ALT and ALP activities (Ekam and Ebong, 2007). The increase are roughly proportional to the extent of liver enzymes in serum following anti malaria administration has been earlier reported (Nwanjo *et al*., 2007; Nwanjo and Oze, 2009). The liver cell damage may be associated with the generation of free radicals by chloroquine overdose which are also partly responsible for their anti-malarial effects. Hence the harmful effects were considered to be caused by free radical produced during peroxide formation. The level of hydroxyl and peroxide radical induced by chloroquine treatment may be responsible for the hepatic impairment in wistar rats (Nnodim *et al*., 2010). The observed higher increased in enzymes activities may be probably due to a leakage of cytoplasmic enzyme into circulation as a result of inflammation of the liver cells. However, the simultaneous administration of chloroquine, vitamin C and vitamin E significantly reduced the effect of chloroquine by lowering AST, ALT and ALP activities. As well as significantly increasing total serum protein and albumin concentration when compared with the control. This result suggests that vitamin C and E offer protection by preserving the structural integrity of hepatocelluar membrane against chloroquine.

Conclusion

In conclusion the present investigation indicates that vitamin C and E exerts significant protection against chloroquine induced hepatotoxicity.

Reference

1. Ali Y, Munir O and Vahit B (2001) The antioxidant activities of leaves of *cydonia vulgaris*. *Turk .J. Med. Sci.,* 31, 23-27.
2. Augustijus P and verbeke N (1993) Stereoselective pharmacokinetic properties of chloroquine de-ethyl-chloroquine in humans. *Clin. Pharmacokinetic.,* 24, 259-269.
3. Dass EE and Shah K (2000) Paracetamol and conventional anti-malarial drugs induced hapatoxicity and its protection by methionine in rats. *Ind. J. Exp biol*., 38, 1134-1142.

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4. Duncan BD (1957) Multiple range test for correlated and hetero sedastic means. *Biometric.,* 13, 359-364.

5. Ekam VS and Ebong PE (2007) Serum protein and enzymes levels in rats following administration of antioxidant vitamins during caffeinated and non caffeinated paracetamol induced hepatotoxicity. *Nigeria J. Physiol. Sci.,* 22(1), 65-68.

6. Ekanem DJ, Weisfeld JS and Salako LA (1990) Sensitivity of Plasmodium falciparum to chloroqine and Sulphadoxine in Nigerian children. *Bulletin of the World Health Organization.,* 68(1), 45-52.

7. Grant GH (1987) Amino acids and proteins: Fundamental Clinical Chemistry, Tietz, NW, Third edition. WB Saunders company: Philadephia 328-329.

8. Iniaghe MO, Malomo SO and Adebayo JO (2008) Hepatoprotective effect of the aqueous extract of leaves of *Acalypha racemosa* in carbon tetrachloride treated rats. *J Medicinal Plant Research,* 2(10), 301-305.

9. Ita SO, Etim OE, Ben EE and Ekpo OF (2007) Haemotopoietic properties of ethanolic leaf extract of Ageratum conyzoides (goat weed) in albino rats. *NIG J. Physiolog. Sci.,* 22, 83-87.

10. King EJ and King PR (1954) Estimation of plasma phosphatase by determination of hydrolysed phenol with amino antipyrene. *J Chem Path.,* 7, 322-326.

11. Nnodim JK, Emejulu A and Nwosunjoku EC (2010) Alterations in biochemical parameters of wistar rats administrated with sulfudoxine and pyrimethamine (*Fansidar*^{R}). *Almeen J. Med. Sci.,* 3(4), 317-321.

12. Nwanjo AU, Okolie NJ, Oze G, Okafor, MC, Nwosu D, Ajero C and Anyaehie B (2007) Halofantrine (antimalaria) toxicity in wistar rats, Biochemical evaluation of hepatic dysfunction. *Res. j. med. Sci*., 1(2), 102-105.

13. Nwanjo HU and Oze G (2009) Acute hepatoxicity following administration of artesunate in Guinea pigs. *Internal J Toxicol.,* 4, 1.

14. Owu DU, Antal AB Udofia KH, Obembe AO, Obasi KO and Eteng MU (2006) Vitamin C improves basal metabolic rate and lipid profile in alloxan–induced diabetes mellitus in rats *J.Bio sci.,* 31(5), 575-579.

15. Paolisso GD, Amore A, Balbic V, Volpe C, Galzerano D, Guigliano D, Sgambato S, Varricchio M and D`Onofrio F (1994) Plasma vitamin c affects glucose homeostasis In healthy subjects and in non–insulin dependent diabeteic. *Am. J. Physiol.,* 266, E261-E268.

16. Pari and Amali RD (2005) protective role of tetrahydrocurmin (THC) an active principle of turmeric on chloroquine induced hepatotoxicity in rats. *J.pharm pharmaceut sci*., 8(1), 115-123.

17. Pari and Murugavel P (2004) Protective effect of a lipoic acid against CQ induced hepatoxicity in rats. *J APP Toxicol.,* 24, 21-26.

18. Reitman, S and Frankel, S. (1957) Transaminases. *Am J Clin Pathol.,* 28, 56.

19. Sowunmi A, Fehintola FA, Falade AG, Akinyinka OO, Oduola AMJ (2000) Comparative efficacy of chloroquine plus chlorpheniramine alone or in sequential combination with sulfadoxine-pyrimethamine for treatment of acute uncomplicated falciparum malaria in Nigerian children. *Ann. Trop. Med. Para.,* 94(3), 209-217.

20. Tietz NW (1995) Clinical guide to laboratory tests Third edition. WB Saunders Company: Philadephia, 518-519.

21. Valko M, Leibfritz D, Monocol J, Cronin MT, Mazur M and Tester J (2007) Free radicals and anti oxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.,* 39, 44-84.