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Ameliorative effect of Vitamin E on the deleterious effect of Amodiaquine hydrochloride (AQ.HCl) on the reproductive function of the adult cyclic Sprague-Dawley (S-D) rats

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Abstract
This study determined whether exogenous supplementation with Vitamin E could confer ameliorative effect against the deleterious effect of AQ.HCl on ovarian function of matured S-D rats. Thirty S-D rats divided into two groups were used in this study: Group A – determined the effect of AQ.HCl + Vitamin E administered for 28 days on the oestrous cycle and antioxidant activities of superoxide dismutase (SOD) and catalase (CAT). Group B – determined the effect of a single dose of AQ.HCl + Vitamin E administered at 5 p.m. on proestrus on ovulation and serum concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), and prolactin (PRL). The exogenous supplementation with Vitamin E increased ($p < 0.05$) the length of the oestrous cycle and the diestrus phase, decreased the activities of CAT and SOD slightly, and did not hinder ovulation in adult S-D rats. Vitamin E proffered ameliorative effect against the deleterious effect of AQ.HCl through its antioxidant activity.

Keywords: Vitamin E; oestrous cycle; catalase; superoxide dismutase; AQ.HCl.

Introduction

It has been shown that the gonads are affected by a series of factors like exposure to physical agents, irradiation, hypoxia, and certain types of drugs (Heywood and Wardsworth, 1980). A number of antimalarial drugs have been implicated in infertility: halofantrine hydrochloride caused reduction in follicle size and cytoplasmic vacuolation in the ovary of the rats (Adjene and Agoreyo, 2003); chloroquine altered the oestrus cycle, lowered serum oestrogen, and LH levels, while serum FSH was unaltered and prevented the expected ovulation (Okanlawon and Ashiru, 1992); sperm motility as well as sperm counts were significantly decreased in pyrimethamine (PYR)-treated animals, and the fertility rate fell to zero (Kalla *et al.*, 1997). In our earlier study, we reported that the oral administration of AQ.HCl produced oxidative stress in the ovary by reducing the activities of CAT and SOD in the female adult cyclic S-D rats. This AQ-induced oxidative stress also led to increased follicular atresia which in turn led to reduced follicle numbers and reduced oocyte numbers at ovulation (Gbotolorun *et al.*, 2011a, b).

The mechanism of the physiological action of Vitamin E is not very clear but some of the biological activities are attributed to its antioxidant activity (Burton and Ingold, 1981; Burton *et al.*, 1983; Packer *et al.*, 2001). Vitamin E plays a major role in the prevention of lipid peroxidation in biological membranes and is an important intramembrane antioxidant, membrane stabilizer, and lipid-soluble antioxidant (Burton *et al.*, 1983; Burton and Ingold, 1986; Herrera and Barbas, 2001; Traber and Atkinson, 2007). Adequate intake of Vitamin E protects rats from free radical generation (Jordao *et al.*, 2004) and rats receiving Vitamin E supplementation exhibit decreased levels of urinary peroxidation products (Sodergren *et al.*, 2000). In adult women, consumption of antioxidant-rich fruit and vegetables is negatively associated with oxidative

stress (Djuric *et al.*, 1998). Furthermore, antioxidants have an important role in the female reproductive system (Ruder *et al.*, 2008).

The deleterious effect of the oral administration of AQ.HCl on the histoarchitecture of the ovary and its function may raise serious concerns as regards fertility particularly in women of reproductive age.

This study was carried out to determine whether exogenous supplementation with Vitamin E may ameliorate the deleterious effect of AQ.HCl on the reproductive function of adult cyclic S-D rats.

Materials and Methods

(i) Drugs

AQ.HCl (manufactured by Rhone Poulenc Pharmaceutical Company, France) was administered at both 6 mg/kg body weight (low dose) and 12 mg/kg body weight (high dose) orally. These doses were selected on the basis of our previous study (Gbotolorun *et al.*, 2011a).

Vitamin E (Pharco Pharmaceuticals, Egypt) was administered intramuscularly at the dose of 20 mg/kg body weight once a day 5 days in a week-Monday to Friday (Mishra and Acharya, 2004).

(ii) Animals

Thirty mature female S-D rats weighing 120 g supplied by the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria were used in this study. They were randomly divided into groups and were housed five animals per cage at the Animal Facility of the Department of Anatomy, College of Medicine, University of Lagos, Nigeria. The animals had free access to food (rat chow; Agric Farms Lagos) and water. They were maintained at 12-h light/12-h dark cycle and at temperatures between 25 to 28°C. The animals were allowed to acclimatize for 2 weeks before the commencement of the experiment. Throughout the duration of the experiment, the animals were observed for clinical signs of drug toxicity. All procedures involving animals in this study were approved by the Departmental Committee on the use and care of animals and tissue collection.

(iii) Determination of the oestrous cycle

Oestrous cycle was monitored for 28 days on 30 adult cyclic Sprague-Dawley rats. Oestrous cyclicity was determined daily between 8 a.m. and 10 a.m. using the vaginal smear method. (Nobunaga and Nakamura, 1968). Vaginal secretion was collected with a plastic pipette filled with 10 µL of normal saline (NaCl 0.9%). The vagina was flushed two or three times with the pipette and the vaginal fluid was placed on a glass slide. A different slide was used for each animal. The unstained secretion was observed under a light microscope. Only animals with a 4-day oestrous cycle were selected for this study.

(iv) Oestrous cyclicity study

Fifteen rats were divided into 3 groups of 5 rats in each and treated daily for 28 days with distilled water (DW), low dose AQ + Vitamin E and high dose AQ + Vitamin E. DW served as control. Animals were sacrificed by cervical dislocation. Laparatomy was performed; ovaries were dissected and removed, trimmed of fat and stored at −80°C for biochemical analysis.

(v) Ovulation study

A total of 15 rats were used for this experiment. They were divided into 3 groups of 5 rats in each. The animals received at 9 a.m. a single dose of DW, low dose AQ + Vitamin E, and high dose AQ + Vitamin E on proestrous. DW served as control. Animals were sacrificed by cervical dislocation at 10 a.m. in the morning of estrus. The lower abdominal wall was dissected and the oviducts were excised. The excised oviducts were placed on glass slides with a drop of saline, covered with cover-slips and squeezed with both sides being gently rocked (Kim *et al.*, 1994). Each ovum found in the distended ampulla was counted under a light microscope.

(vi) Hormonal assay

Blood was obtained from the angular vein of the S-D rats at 6 p.m. in the evening of proestrus and collected into a heparinised bottle. Each blood sample was spun at 2,500 rpm for 10 minutes in an angle-head desktop centrifuge at 25°C. Serum samples were assayed in batches with control sera at both physiological and pathological levels by Standard Quantitative Enzyme-Linked Immunosorbent Assay (ELISA) technique with Microwell kits from Syntro Bioresearch Inc., California, USA.

(vii) Determination of SOD

SOD was assayed utilizing the technique of (Kakkar *et al.*, 1984). A single unit of enzyme was

expressed as 50% inhibition of Nitroblue tetrazolium (NBT) reduction/min/mg/protein.

(viii) Determination of CAT
CAT was assayed colorimetrically at 620 nm and expressed as µmoles of H_2O_2 Consumed/min/mg/protein as described by Sinha (1972). The reaction mixture 1.5 mL contained 1.0 mL of 0.01 M pH 7.0 phosphate buffer, 0.1 mL of ovary homogenate, and 0.4 mL of 2 M H_2O_2. The reaction was stopped by the addition of 2.0 mL of dichromate-acetic acid reagent 5% potassium dichromate and glacial acetic acid was mixed in 1:3 ratios.

(ix) Statistical analysis
Results were expressed as mean ± standard deviation (Mean ± SD) and subjected to statistical analysis using Student's t-test. Statistical significance was considered at $p < 0.05$ or 5%.

Results

(i) Oestrus cyclicity
Co-administration of Vitamin E with AQ.HCl produced an increase ($p < 0.05$) in the length of the oestrus cycle at both dosages (Table 1). An irregularity in cycle pattern was observed with an increase ($p < 0.05$) in the diestrus phase and a decrease in the proestrus ($p < 0.05$), metestrus, and estrus phases of the cycle at the low dose compared with the control. At the high dose, the study recorded a reduction in the metestrus, proestrus ($p < 0.05$) and estrus phases, and an increase in the diestrus phase compared to the control (Table 2).

Table 1: Effect of the oral administration of AQ.HCl + Vitamin E for 28 days on the length of the oestrous cycle in S-D rats.

Treatment groups	Length of the oestrous cycle
Control	4.00 ± 0.00
6 mg/kg + Vitamin E	5.04 ± 0.43*
12 mg/kg + Vitamin E	4.70 ± 0.58*

*$p < 0.05$.

(ii) Ovulation study
Table 3 presents that the number of oocytes released in the oviduct following co-administration of AQ.HCl and Vitamin E were comparable to control at low dose (8.50 ± 0.58) and high dose (7.00 ± 2.16).

(iii) Serum concentrations of FSH, LH, and PRL
Serum concentrations of FSH, LH, and PRL were not significantly different from control values (Table 4).

(iv) Antioxidant status of CAT and SOD
Co-administration of AQ.HCl with Vitamin E resulted in a slight decrease in the activities of CAT and SOD compared to the control (Table 5).

Discussion

In our previous study, we had reported that AQ produced increase ($p < 0.05$) in the length of the oestrus cycle and also caused a disruption in the pattern of the cycle (Gbotolorun *et al.*, 2011a). In this present study, co-administration of AQ.HCl and Vitamin E produced a similar result compared with the control. The increase in cycle length occurred as a result of the animals spending more days in the diestrus phase. From this report, it can be deduced that Vitamin E conferred no ameliorative effect on the deleterious effect of AQ.HCl on the length of the oestrus cycle and cycle pattern. The prolonged diestrus ($p < 0.05$) resulted in a decrease in the frequency of the proestrus and estrus phases of the cycle which consequently may reduce ovulation and

Table 2: Effect of the oral administration of AQ.HCl + Vitamin E for 28 days on the pattern of the oestrous cycle in adult cyclic S-D rats (Mean ± SD).

Treatment groups	Metestrus	Diestrus	Proestrus	Estrus
Control	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
6 mg/kg + Vitamin E	6.00 ± 1.00	9.80 ± 2.59*	5.40 ± 5.89*	6.40 ± 1.14
12 mg/kg + Vitamin E	6.00 ±1.22	9.40 ± 4.34	5.80 ±1.10*	6.40 ± 2.51

*$p < 0.05$.

Table 3: Effect of the oral administration of a single combined dose of AQ.HCl + Vitamin E on the number of oocytes shed in the oviduct on the morning of estrus in S-D rats.

Treatment groups	Number of oocytes shed in oviduct on the morning of estrus
Control	8.40 ± 0.89
6 mg/kg + Vitamin E	8.50 ± 0.58
12 mg/kg + Vitamin E	7.00 ± 2.16

$p < 0.05$.

Table 4: Effect of the oral administration of AQ.HCl + Vitamin E on the serum concentrations of FSH, LH and PRL on proestrus in S-D rats.

Treatment groups	Serum concentrations of FSH, LH, and PRL at 6 p.m. on proestrus		
	FSH	LH	PRL
Control	0.34 ± 0.02	0.14 ± 0.04	0.30 ± 0.02
6 mg/kg + Vitamin E	0.33 ± 0.05	0.16 ± 0.15	0.31 ± 0.02
12 mg/kg + Vitamin E	0.41 ± 0.13	0.18 ± 0.02	0.38 ± 0.08

Table 5: Effect of the oral administration of combined dose of AQ.HCl + Vitamin E on the enzymatic antioxidant activities of CAT and SOD in the ovary of S-D rats.

Treatment groups	Antioxidant activities of CAT and SOD	
	CAT	SOD
Control	84.8 ± 49.1	1.40 ± 0.85
6 mg/kg + Vitamin E	74.7 ± 31.3	1.38 ± 0.81
12 mg/kg + Vitamin E	72.3 ± 31.7	1.43 ± 0.63

$p < 0.05$.

fertility. This report is in agreement with other investigators who have reported a prolonged diestrus phase with reduced proestrus and estrus phases following the administration of chloroquine, Garcinia kola, Neem, and amodiaquine (Okanlawon and Ashiru, 1992; Akpantah *et al.*, 2005; Gbotolorun *et al.*, 2004, 2008, 2011a, b).

The proestrus surge of LH is responsible for the process of and events following ovulation. This rapid surge of LH induces follicular rupture and ovulation (Freeman, 1988). The result of this study showed no difference in the serum concentrations of FSH, LH, and PRL of the animals treated with AQ.HCl + Vitamin E and the control so it can be inferred therefore that the expected surge in these hormonal levels occurred without interference.

An earlier study had reported that AQ.HCl caused widespread follicular atresia which subsequently disrupted the process of follicular maturation and selection and consequently resulted in a partial block in ovulation (Gbotolorun *et al.*, 2011a, b). Our study showed that the number of oocytes released in the oviduct in the groups that received AQ.HCl + Vitamin E were comparable to the control particularly at the low dose suggesting that Vitamin E had an ameliorative effect on the ovary by counteracting the deleterious effect of AQ.HCl on follicle maturation and selection as earlier reported.

Previous study had shown that AQ.HCl produced a decrease in the antioxidant status of SOD and CAT ($p < 0.05$) in the ovary of Sprague-Dawley rats (Gbotolorun *et al.*, 2011a). This present study however showed a slight decrease in the enzymatic antioxidant activities of CAT and SOD compared with the control. The result of this study suggests therefore that Vitamin E had an ameliorative effect on the adverse effect of AQ.HCl on ovarian function. Our finding is in

concert with the elegant studies of other investigators who have administered Vitamin E as supplements in their studies (Nugent *et al.*, 1998; Rao *et al.*, 2009).

A study on the protective effect of Vitamin E on ischaemia-reperfusion injury in ovarian grafts, total lipid peroxides, and malondialdehyde concentrations were investigated. The results showed that products of lipid peroxidation were higher in non-supplemented murine autografts compared with control ovaries ($p < 0.05$), and were significantly reduced on day 3 by Vitamin E administration ($p < 0.05$). Similarly, in human xenografts, there was a significant reduction in lipid peroxidation with Vitamin E administration. These results corresponded to a significantly greater total follicle survival in the murine grafts of the supplemented group (45 versus 72%; $p < 0.05$). The authors concluded that antioxidant treatment improved the survival of follicles in ovarian grafts by reducing ischaemia-reperfusion injury (Nugent *et al.*, 1998).

In another study, the in vivo effects of nickel chloride and/or potassium dichromate in the ovary of adult mice were determined. The protective role of Vitamin E (2 mg/kg body weight) along with their combination was also studied. Nickel and/or chromium to mice enhanced the levels of lipid peroxides in the ovary, which was accompanied by a significant decline in the levels of protein, glutathione, total ascorbic acid and activities of SOD and CAT. Supplementation of Vitamin E along with nickel chloride and potassium dichromate significantly lowered the levels of lipid peroxidation and enhanced the antioxidant status. In conclusion, these authors suggested that Vitamin E exerted its protective effect against nickel and/or chromium induced toxicity by preventing lipid peroxidation and protecting antioxidant system in the mouse ovary (Rao *et al.*, 2009).

Conclusion

The result of our present study showed that Vitamin E provided a protective environment against the oxidative stress induced by amodiaquine in the ovary through its antioxidant activity.

Ethical Approval

The study was approved by the animal ethics committee of the College of Medicine, University of Lagos.

Conflict of Interests

None declared.

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