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SUB-ACUTE CHLOROQUINE TOXICITY ON TESTIS OF SWISS ALBINO MICE AND ITS AMELIORATION BY CURCUMIN

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ABSTRACT: The objective of the present study is to investigate the adverse effect of chloroquine (CQ) toxicity on reproductive tissues and its attenuation by curcumin. Chloroquine (CQ) has been used widely as an antimalarial drug due to its quick action on blood schizontocide of different malarial parasite species for prophylaxis and treatment of malaria. Thirty-six male mice were divided into six experimental groups: control, curcumin (80 mg/kg b.w.), CQ low dose (100mg/kg b.w.), CQ mid dose (200mg/kg b.w.), CQ high dose (300mg/kg b.w.) and CQ high dose + curcumin treated group. An oral dose of artesunate and curcumin was administered for a period of 14 and 21 days to male Swiss albino mice. Results obtained indicated significant alterations in biochemical parameters as well as energy metabolism parameters in testicular tissue of animals after the treatment with chloroquine. Moreover, sperm count, sperm motility percent, sperm viability and sperm morphology of cauda epididymis of chloroquine treated mice revealed significant fluctuations as compared to the control group. Administration of curcumin along with the chloroquine revealed comparable values of biochemical as well as sperm parameters with the control group. Hence, obtained results clearly indicates the strong mitigative potential of curcumin against chloroquine induced toxicity in Swiss albino male mice.

INTRODUCTION: Globally the major public health issue in endemic regions [1] causing high mortality and morbidity, is the vector borne disease Malaria. The disease is a multifactorial burden - economically, medically, and a health calamity as the synergy of co-infections with the HIV and Tuberculosis is high [2]. About 90% cases of malaria occur in sub-Saharan Africa where the disease contributes substantially to underdevelopment and places a severe strain on limited health care facilities [3].

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The treatment of malaria has posed great challenge to medicine and development of efficient antimalarial drugs. This is due to the development of resistance of parasite to mainly antimalarial agents [4, 5] resulting in huge impact on the socio-economy [6]. The possibility of overdose and misappropriation in the usage of antimalarial agents are very common, all of which could lead to toxic effects of the drugs [7, 8].

Chloroquine (CQ) has been used for the treatment of diverse diseases. It is an aminoquinoline commonly used in the tropics to treat malaria [9]. When unprotonated, it can diffuse across cell membranes, get protonated and accumulate in acidic organelles such as the lysosomes. This lysosomotropic property results in the inhibition of lysosomal enzymes and makes it useful in the

treatment of the malaria parasite [10]. Given the lack of affordable alternatives, chloroquine remains the first-line antimalarial agent in most African countries. In recent decades chloroquine has been used so extensively that it has now lost its efficacy against malarial parasites. Indeed, resistance to chloroquine is so widespread that this drug has been rendered useless in some parts of the world, especially South East Asia [11].

Chloroquine is a lysomotropic agent which inhibits the degradation of internalized human chorionic gonadotropin (hCG) in Leydig cell [12] and luteal cells [13]. It has been shown to have varied effects on male reproductive functions including fertility reduction in male rats [14]. Chloroquine was shown to inhibit testosterone secretion in hCG-stimulated testis of pubertal rats [9]. The evaluation of antimalarial drugs for possible reproductive toxicity especially at doses higher than recommended doses becomes very important as both malaria and infertility are of global concern.

Although there have been few documentaries, review of literature reveals a paucity in the field of reproductive toxicity of the said drug. Antimalarial drugs have been implicated in male infertility and CQ has been found to have dose dependent reduction in fertility of male rats [15, 16].

The global use of this antimalarial drug thus made its detailed investigation on male fertility imperative. Further, the literature documents a couple of chloroquine induced reproductive and other toxicities, though, the awareness regarding the same is scarce. This necessitates the need of mitigation of toxicity.

Medicinal plants and natural herbal products have potential antioxidant activity and are therefore often administered along with chemotherapeutic agents to provide better protection against their toxic side effects [17]. Natural products have a wealth of applications. Some of them are used as drugs, while others possess important biological properties or are used as dietary supplements, as dyes, flavouring agents, or ingredients in the cosmetics industry [18].

One such medicinal plant is Turmeric (*Curcuma longa* Linn. Syn *C. domestica* Valeton) which is extensively used as a spice, food preservative and colouring material commonly used in the Indian subcontinent [19, 20]. Since the time of Ayurveda (1900 BC) numerous activities have been assigned to turmeric for a wide variety of diseases and conditions, including those of the skin, pulmonary, and gastrointestinal systems, aches, pains, wounds, and liver disorders [19].

Various phytochemicals are present in turmeric and one of them is 'Curcumin' also known as diferuloyl methane and exhibits antioxidant, anti-inflammatory, anti-mutagenic, anti-carcinogenic, membrane-stabilizing, and hypolipidemic properties which constitute an important part of its therapeutic effects [21 - 23]. Curcumin has also been shown to retard lipid peroxidation and ameliorate chemically induced oxidative stress [24, 25]. The high oxidative potential of curcumin has not been extensively explored as specially in curbing reproductive toxicity in mice. Thus the present investigation was focused on the mitigating potential of curcumin on reproductive toxicity in male Swiss mice.

The study was hence designed to explore the possible protective effect of curcumin on chloroquine induced reproductive damage in albino mice.

MATERIALS AND METHODS:

Animals and Chemicals: Healthy adult male albino mice, *Mus musculus* of a Swiss strain, weighing between 25 and 40 g, were obtained from Cadila Pharmaceuticals (Dholka, India). All the animals were acclimatized 7 days before commencement of treatment and were maintained under controlled conditions, with 12 h light-dark cycles at a temperature of 26 ± 2°C and relative humidity of 30 - 70%. Animals were randomized into control and experimental groups and were caged separately. Standard chow (obtained from Amrut Laboratory, Baroda, India) and water were given *ad libitum*. Experiments were conducted in accord with the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India, and experimental protocols were approved by the institutional animals' ethics committee, under registration no. 167/1999/ CPCSEA. Chloroquine phosphate (99.30% pure) was generously gifted from IPCA Laboratories Ltd. (Mumbai, India).

Curcumin and other chemicals were procured from Himedia Laboratories (Mumbai, India) and Sigma-Aldrich (Dorset, UK). All chemicals used in the experiment were of analytical grade.

Experimental Design: A stock solution of CQ was prepared in double-distilled water and orally given to mice *via* feeding canula with a hypodermic syringe. All doses for CQ were derived from its oral LD_{50} value (500 mg/kg) [26]. The dose for curcumin is based on an earlier work [27]. Animals were divided into the following six groups: group 1, control (given distilled water only); group 2 was given 80 mg/kg b.w. of curcumin; group 3, 100 mg of CQ/kg b.w.; group 4,200 mg CQ/kg b.w.; group 5,300 mg CQ/kg b.w.; group 6, high dosage of CQ+curcumin (300 mg CQ/kg b.w. + 80 mg/kg b.w.) and treatment for 14 and 21 days.

TABLE 1: EXPERIMENTAL DESIGN

Groups	Treatment and Dose	Duration(Days)	Day of Autopsy
I	Control (untreated) Control + distilled water	-	Sacrificed with treated
II	Control + curcumin (80mg/kg body weight)	14, 21	15th, 22nd
III	Control + CQ L.D (100mg/kg body weight)	14, 21	15th, 22nd
IV	Control + CQ M.D (200mg/kg body weight)	14, 21	15th, 22nd
V	Control + CQ H.D (300mg/kg body weight)	14, 21	15th, 22nd
VI	Control + CQ H.D + Curcumin (doses as in Group V and Group II)	14, 21	15th, 22nd

All the groups were treated for a 14 and 21 days period. At the end of each treatment, animals were weighed and sacrificed using light ether anaesthesia. Testes were taken for biochemical and histological evaluation. Animals were then euthanized *via* exsanguination.

Tissue Collection: After termination of the treatment period, animals were euthanized and dissected. Testes were dissected out carefully and weighed. Tissue was then processed and homogenate was prepared according to standard biochemical protocols.

Protein Estimation: Protein estimation was done by using the standard protocol of Lowry *et al*., (1951) [28]. The optical density (OD) of blue colour was read at 540 nm in systronics digital spectrophotometer 167 against blank.

Acid Phosphatase (ACPase) Activity: Activity of ACPase was determined by the method of Bessey *et al*., (1946) [29]. ACPase catalyzes hydrolysis of p-nitrophenol nitrate at pH 4.8, liberating paranitrophenol and inorganic phosphate. The liberated p-nitrophenol combines with NaOH to form a yellow colored complex which is measured at 420 nm and is directly proportional to the enzyme activity. Enzyme activity was expressed as µ moles of p-nitrophenol released/30 minutes/mg protein.

Alkaline Phosphatase (ALKPase) Activity: Alkaline Phosphatase (ALPase) activity was determined by the method of Bessey *et al.,* (1946) [29]. The enzyme ALPase hydrolyses the substrate p-nitrophenyl phosphate into inorganic phosphate and p-nitrophenol. The quantity of p-nitrophenol released under standardised condition was measured at 410 nm. Enzyme activity was expressed as µ moles p-nitrophenol released/30 minutes/mg protein.

Lipid peroxidation (LPO): LPO level in testis of control and treated mice were determined by the method of Ohkawa *et al*., (1979) [30]. This method is based on the formation of red chromophore that absorbs at 532nm following the reaction of thiobarbituric acid (TBA) with malonyldialdehyde (MDA) and other breakdown products of peroxidised lipids collectively called as thiobarbituric acid reactive substances (TBARS).

Energy Metabolism Estimation:

Succinate Dehydrogenase Activity (SDH): SDH activity was measured by the method of Beatty *et al*., (1966) [31]. The electrons released by the enzyme SDH from the substrate are taken up by an electron acceptor INT which is reduced to red coloured formazan. After extracting it in ethyl acetate the colour intensity was measured at 420 nm against blank. SDH activity was expressed as µg formazan formed/15 min/mg tissue weight.

Adenosine Triphosphatase (ATPase) Activity: The ATPase activity in testis of control and all treated groups of animals was assayed by the method of Quinn and White (1968) [32] while

inorganic phosphate liberated was estimated using the method of Fiske and Subbarow (1925) [33]. Readings were taken at 660 nm on a Systronics Digital Spectrophotometer 167.

Measurement of Antioxidant Enzyme Activity:

Superoxide Dismutase Activity (SOD): Activity of SOD in testis of control and treated mice was estimated by modified spectrophotometric method of Kakkar *et al*., (1984) [34]. The formazan formed at the end of reaction indicates presence of enzyme. One unit of enzyme activity is defined as the enzyme concentration required to inhibit 50% of the optical density of chromogen formed in one minute at 560nm under the assay condition.

Catalase activity (CAT): Catalase activity in testis of control and treated mice was assayed by the modified method of Sinha (1972) [35].

Sperm Parameters:

Sperm Count and Sperm Motility: Sperm count and motility in cauda epididymis of control and treated mice was determined using the Neubauer chamber of hemocytometer according to the method of Prasad *et al*., (1972) [36].

Sperm Viability:

Live: Dead ratio of cauda epididymal sperms was estimated by using the method of Talbot and Chacon (1981) [37].

Sperm Morphology Smears of the sperm suspension were made, allowed to dry in air, fraction and stained with (10:1) 1% Eosin Y (H_2O) and 60 min later slides mounted under a coverslip with permount mounting medium. For each suspension 1000 sperm were examined at 400-fold magnification with blue-green filters; a total of 2000 sperm were thus examined for each group by the method of Wyrobek and Bruce (1975) [38].

Statistics Analysis: All data are presented as mean ± standard error (SE). Statistical analysis was performed using the SPSS software package (version 16.0; SPSS, Inc., Chicago, Illinois, USA). Comparison between groups was made by one way analysis of variance (ANOVA), taking significance at $p<0.05$, followed by the Student's t test, taking significance at $p<0.01$, $p<0.005$, and $p<0.001$. Tukey's honestly significance difference (HSD) post-hoc test was used for comparison among different treatment groups ($p<0.05$).

RESULTS:

Gravimetric Study (Terminal Body Weight and Tissue Weight)

Body Weight: Mice treated with low dose and moderate dose of CQ for an interval of 14 and 21 days showed no significant reduction in body weight, whereas when high dose was administered, depletion in body weight was witnessed ($p<0.005$). When curcumin was given as an antidote to the control mice, the results showed negligible variation in body weight, whereas when curcumin was administered along with the higher dose of CQ, reduction observed in the body weight was not significant and the values were comparable to control group in both the durations.

Organ Weight: Low and moderate dose treatment of CQ showed non - significant decrease at 14 days, whereas, high dose ($p<0.01$) treatment showed significant decrease in the organ weight.

Low dose treatment of CQ showed non - significant decrease at 21 days, whereas moderate ($p<0.01$) and high dose ($p<0.005$) treatment showed significant decrease in testis weight. Supplementation of curcumin alone to control animals and along with the high dose of CQ did not show any significant decrease after 14 and 21 days.

Protein Content: Non-significant decrease in the protein content of testis was witnessed in low, moderate and high dose treatment of CQ after 14 days. Low dose and moderate dose of CQ treatment did not show any significant decrease in protein content of testis after 21 days, whereas high dose of CQ resulted in significant decline in protein level of testis ($p<0.005$). Curcumin when administered to control mice alone and when supplemented along with the high dose of CQ exhibited insignificant decrease in protein content of testis after 14 and 21 days treatment.

Enzymatic Parameters: ACPase activity was found to be non-significant in testis after the low dose and moderate dose treatment, whereas there was significant increase ($p<0.01$) after the administration of high dose during both the time intervals.

ACPase remained unaltered after administering curcumin alone to control mice during 14 and 21 days. Also, animals treated with CQ and supplemented with curcumin did not show significant change in ACPase activity in testis after 14 and 21 days. ALKpase activity showed no significant change as compared to control after 14 and 21 days treatment of low dose and moderate dose of CQ. However, when high dose of CQ was administered, the ALKpase activity was found to be significantly decreased ($p<0.01$), ($p<0.005$) after 14 and 21 days respectively. ALKpase remained unaltered after administering curcumin alone to control mice. Also, animals treated with CQ and supplemented with curcumin did not show significant change in ALKpase activity in testis after 14 and 21 days.

SDH activity did not show any significant change in testis during 14 and 21 days treatment of low dose. Testis SDH activity decreased significantly after the administration of moderate dose ($p<0.01$) during both the time intervals. High dose of CQ when administered resulted in significant decrease ($p<0.01$) after 14 days and moderately significant decrease ($p<0.005$) after 21 days. SDH remained unaltered after administering curcumin alone to control mice. Also, animals treated with CQ and supplemented with curcumin did not show significant change in SDH activity in testis during both the durations.

ATPase activity was found to be non-significant in testis after the low dose and moderate dose treatment during both the time intervals. High dose also when administered was found to be non-significant after 14 days, however, there was significant decrease ($p<0.01$) after 21 days. ATPase remained unaltered after administering curcumin alone to control mice. Also, animals treated with CQ and supplemented with curcumin did not show significant change in ATPase activity in testis after 14 and 21 days.

Oxidative Parameters: SOD activity was found to be unaltered in testis at low dose treatment of CQ during both the time intervals. When moderate dose of CQ was administered SOD activity was found to be non-significant after 14 days and exhibited significant decline ($p<0.005$) after 21 days. Also, there was moderately significant ($p<0.005$) decline at high dose after 14 days and highly significant decline ($p<0.001$) after 21 days. Curcumin when administered to control mice alone and when supplemented along with the high dose of CQ exhibited insignificant decrease in SOD activity of testis after 14 and 21 days treatment. During both the time intervals, CAT activity was found to be non-significant for each of the three treatments of CQ. Curcumin alone and in combination with the high dose of CQ also showed non – significant decrease in CAT activity of testis after 14 and 21 days treatment.

In both the time interval of 14 and 21 days, LPO levels were found to be non-significant in testis after the low dose. Testis exhibited significant elevation ($p<0.005$) in LPO levels at moderate dose and highly significant elevation ($p<0.001$) at high dose of CQ during both the durations. Levels of LPO remained unaltered and changes in values were non - significant when curcumin was administered alone. LPO levels were comparable to control in testis after 14 and 21 days duration of CQ and curcumin co-administration.

Sperm Parameters: Low dose treatment of CQ did not show any significant change in all the sperm parameters like sperm count, sperm motility, sperm viability and sperm morphology at 14 and 21 days.

Moderate dose treatment of CQ showed non-significant changes in all the sperm parameters after 14 days of treatment. However, for 21 days, significant decline only in sperm motility and viability parameters was witnessed, while sperm count and morphology were found to be non-significant. For 14 days, high dose treatment of CQ, sperm count was found to be non-significant, whereas sperm motility and viability was significantly decreased ($p<0.01$) and sperm morphology was significantly increased ($p<0.01$). High dose treatment for 21 days of CQ revealed significant decline ($p<0.01$) in sperm count and moderately significant decline in sperm motility and viability ($p<0.005$), whereas sperm morphology was found to be significantly increased ($p<0.01$). Curcumin given to control animals alone and in combination with the high dose of CQ showed non - significant changes in all the sperm parameters after 14 and 21 days treatment.

TABLE 2: BODY WEIGHT AND ORGAN WEIGHT (TESTIS) OF CONTROL AND TREATED ANIMALS AFTER 14 AND 21 DAYS TREATMENT

Groups	Treatment	Body weight (gm) (14days)	Body weight (gm) (21 days)	Testis (mg) (14 days)	Testis (mg) (21 days)
I	Control	41.21 ± 0.32	42.20 ± 0.44	120 ± 1.20	120 ± 1.20
II	Curcumin	41.13 ± 0.49^{NS}	41.12 ± 0.40^{NS}	118 ± 1.09 ^{NS}	121 ± 1.10^{NS}
III	CQ 100 mg/kg bw	39.28 ± 0.58 ^{NS}	38.25 ± 0.67^{NS}	116 ± 0.26 ^{NS}	112 ± 0.98^{NS}
IV	CQ 200 mg/kg bw	37.31 ± 0.27 ^{NS}	35.41 ± 0.31^{NS}	114 ± 0.91 ^{NS}	109 ± 0.98*
V	CQ 300 mg/kg bw	36.22 ± 0.53*	32.22 ± 0.67**	107 ± 1.67*	101 ± 1.34**
VI	CQ 300 mg/kg bw + Curcumin	40.22 ± 0.25 ^{NS}	38.43 ± 0.53^{NS}	117 ± 1.37 ^{NS}	116 ± 1.32^{NS}

Values are mean ± S.E.,*p<0.01, **p<0.005, NS = Not significant

TABLE 3: PROTEIN CONTENT IN TESTES OF CONTROL AND TREATED ANIMALS AFTER 14 AND 21 DAYS TREATMENT

Groups	Treatment	Testis (mg) (14 days)	Testis (mg) (21 days)
I	Control	12.75 ± 1.02	12.75 ± 1.02
II	Curcumin	11.71 ± 0.37 ^{NS}	12.64 ± 0.74^{NS}
III	CQ 100 mg/kg bw	12.66 ± 0.42 ^{NS}	12.62 ± 0.83^{NS}
IV	CQ 200 mg/kg bw	12.19 ± 0.53 ^{NS}	11.59 ± 0.74^{NS}
V	CQ 300 mg/kg bw	11.45 ± 0.67 ^{NS}	10.45 ± 0.46**
VI	CQ 300 mg/kg bw + Curcumin	12.59 ± 0.98 ^{NS}	12.45 ± 0.65^{NS}

Values are mean ± S.E., **p<0.005, NS = Not significant, Protein – mg/100mg tissue weight

TABLE 4: ACTIVITIES OF ACPase, ALKPase, SDH AND ATPase IN TESTES OF CONTROL AND TREATED ANIMALS AFTER 14 AND 21 DAYS TREATMENT

		14 days			
Groups	Treatment	ACPase	ALKpase	SDH	ATPase
I	Control	1.58 ± 0.09	0.80 ± 0.04	10.21 ± 0.89	1.07 ± 0.08
II	Curcumin	1.60 ± 0.02 ^{NS}	0.79 ± 0.05 ^{NS}	10.22 ± 1.22^{NS}	1.01 ± 0.02 ^{NS}
III	CQ 100 mg/kg bw	1.67 ± 0.03 ^{NS}	0.76 ± 0.07 ^{NS}	9.59 ± 0.78 ^{NS}	0.96 ± 0.04 ^{NS}
IV	CQ 200 mg/kg bw	1.83 ± 0.06 ^{NS}	0.70 ± 0.06 ^{NS}	8.76 ± 0.27*	0.82 ± 0.10 ^{NS}
V	CQ 300 mg/kg bw	3.01 ± 0.07*	0.54 ± 0.02*	8.03 ± 0.97*	0.74 ± 0.06 ^{NS}
VI	CQ 300 mg/kg bw + Curcumin	1.62 ± 0.04 ^{NS}	0.78 ± 0.05 ^{NS}	10.14 ± 1.35 ^{NS}	1.01 ± 0.06 ^{NS}
		21 days			
Groups	Treatment	ACPase	ALKpase	SDH	ATPase
I	Control	1.58 ± 0.09	0.80 ± 0.04	10.21 ± 0.89	1.07 ± 0.08
II	Curcumin	1.56 ± 0.08^{NS}	0.80 ± 0.09^{NS}	10.03 ± 1.31^{NS}	1.02 ± 0.05^{NS}
III	CQ 100 mg/kg bw	2.03 ± 0.08^{NS}	0.71 ± 0.08^{NS}	9.79 ± 0.82^{NS}	0.78 ± 0.38^{NS}
IV	CQ 200 mg/kg bw	1.93 ± 0.05^{NS}	0.68 ± 0.03^{NS}	8.69 ± 0.63*	0.74 ± 0.08^{NS}
V	CQ 300 mg/kg bw	3.11 ± 0.06*	0.48 ± 0.06**	7.89 ± 0.98**	0.63 ± 0.05*
VI	CQ 300 mg/kg bw + Curcumin	1.81 ± 0.06^{NS}	0.76 ± 0.03^{NS}	10.16 ± 0.74^{NS}	0.99 ± 0.09^{NS}

Values are mean ± S.E.,*p<0.01, **p<0.005, NS = Not significant
ACPase – µmoles of p-nitrophenol released/mg protein
ALKPase – µmoles of p-nitrophenol released/mg protein
SDH – µg formazan released/15 min/mg protein
ATPase – µmoles i.p. released/30 min/mg protein

TABLE 5: ACTIVITIES OF SOD AND CAT IN TESTES OF CONTROL AND TREATED ANIMALS AFTER 14 AND 21 DAYS TREATMENT

		14 days		21 days	
Groups	Treatment	SOD	CAT	SOD	CAT
I	Control	0.44 ± 0.03	10.45 ± 2.70	0.44 ± 0.03	10.45 ± 2.70
II	Curcumin	0.40 ± 0.05^{NS}	10.58 ± 2.09^{NS}	0.45 ± 0.07^{NS}	11.66 ± 1.24^{NS}
III	CQ 100 mg/kg bw	0.40 ± 0.03 ^{NS}	10.39 ± 1.48^{NS}	0.31 ± 0.04^{NS}	10.12 ± 1.58^{NS}
IV	CQ 200 mg/kg bw	0.31 ± 0.02 ^{NS}	10.34 ± 0.55^{NS}	0.29 ± 0.03 **	10.01 ± 0.88^{NS}
V	CQ 300 mg/kg bw	0.28 ± 0.04**	10.12 ± 0.75^{NS}	0.17 ± 0.06***	9.67 ± 0.90^{NS}
VI	CQ 300 mg/kg bw +Curcumin	0.38 ± 0.03 ^{NS}	10.40 ± 2.14 ^{NS}	0.39 ± 0.02^{NS}	10.36 ± 1.15^{NS}

Values are mean ± S.E., **p<0.005, ***p<0.001, NS = Not significant
SOD – units/mg protein
Catalase (CAT) – µmoles H_2O_2 consumed/mg protein

TABLE 6: LEVEL OF LPO IN TESTES OF CONTROL AND TREATED ANIMALS AFTER 14 AND 21 DAYS TREATMENT

Groups	Treatment	LPO (14 days)	LPO (21 days)
I	Control	61.27 ± 0.78	61.27 ± 0.78
II	Curcumin	61.35 ± 0.73^{NS}	63.39 ± 0.58^{NS}
III	CQ 100 mg/kg bw	66.75 ± 1.49^{NS}	71.67 ± 0.79^{NS}
IV	CQ 200 mg/kg bw	77.89 ± 1.06*	79.89 ± 1.23*
V	CQ 300 mg/kg bw	92.37 ± 1.34***	105.07 ± 1.04***
VI	CQ 300 mg/kg bw + Curcumin	68.55 ± 0.63^{NS}	67.32 ± 0.54^{NS}

Values are mean ± S.E.,*p<0.01, ***p<0.001, NS = Not significant
LPO - nanomoles of MDA/100 mg tissue weight

TABLE 6: SPERM PARAMETERS OF CONTROL AND TREATED ANIMALS AFTER 14 AND 21 DAYS TREATMENT

14 days					
Groups	**Treatment**	**Sperm count (10^6/ml)**	**Sperm motility (%)**	**Sperm viability (%)**	**Sperm morphology (%)**
I	Control	42.24 ± 0.09	80.92 ± 0.69	71.50 ± 0.57	68.24 ± 1.34
II	Curcumin	41.45 ± 0.09^{NS}	79.55 ± 0.47^{NS}	68.44 ± 0.34^{NS}	68.87 ± 1.07^{NS}
III	CQ 100 mg/kg bw	37.56 ± 0.48^{NS}	74.48 ± 0.73^{NS}	63.39 ± 0.69^{NS}	74.36 ± 0.87^{NS}
IV	CQ 200 mg/kg bw	34.94 ± 0.83^{NS}	71.30 ± 0.29^{NS}	66.41 ± 1.31^{NS}	74.28 ± 0.82^{NS}
V	CQ 300 mg/kg bw	29.47 ± 1.27^{NS}	69.64 ± 0.72*	50.54 ± 1.12*	83.47 ± 0.89*
VI	CQ 300 mg/kg bw + Curcumin	39.55 ± 0.03^{NS}	78.61 ± 0.69^{NS}	69.74 ± 0.83^{NS}	81.39 ± 0.73^{NS}
21 days					
Groups	**Treatment**	**Sperm count (10^6/ml)**	**Sperm motility (%)**	**Sperm viability (%)**	**Sperm morphology (%)**
I	Control	42.24 ± 0.09	80.92 ± 0.69	71.50 ± 0.57	68.24 ± 1.34
II	Curcumin	40.65 ± 0.06^{NS}	82.64 ± 0.68^{NS}	70.73 ± 0.84^{NS}	75.72 ± 1.12^{NS}
III	CQ 100 mg/kg bw	36.45 ± 0.68^{NS}	72.26 ± 0.59^{NS}	62.43 ± 1.87^{NS}	77.64 ± 0.46^{NS}
IV	CQ 200 mg/kg bw	30.94 ± 0.93^{NS}	69.30 ± 0.74*	59.41 ± 1.23*	79.39 ± 0.46^{NS}
V	CQ 300 mg/kg bw	23.47 ± 1.03*	62.64 ± 0.69**	48.37 ± 1.02**	88.56 ± 0.46*
VI	CQ 300 mg/kg bw + Curcumin	38.73 ± 1.67^{NS}	77.43 ± 0.58^{NS}	68.83 ± 1.33^{NS}	77.48 ± 0.85^{NS}

Values are mean ± S.E.,*p<0.01, **p<0.005, NS = Not significant

DISCUSSION: Chloroquine is still having a good position in malaria therapy as it is the cheapest antimalarial drug and readily available; particularly in the rural setting [39]. The results of the harmful effects of the drug on the reproductive functions are very scarce. There was an increased demand of this drug in African countries in the past, especially those which are closer to line of tropics where there is a diversity of vectors. Thus chloroquine has been supplied through governmental health organizations as well as international bodies like WHO from last decade. As a result the endemic populations of these areas are at greater risk of chloroquine toxicity which may affect the reproductive efficacy of the medicating individuals. Hence, the present study is to evaluate effects of CQ on reproductive tissues of male mammal and to mitigate toxicity using a potent antioxidant curcumin.

Literature abounds on the adverse effects of chloroquine on tissues [40 - 44] and several studies have been carried out on its antifertility potentials. There are reports suggesting that CQ can be added to the long list of drugs and chemicals causing damage to chromosomes, which is in turn associated with genetic damage [45, 46].

Decreased protein content found post-treatment could be attributed to altered physiology, or impairment in protein synthesis or loss of appetite. Studies have reported that that protein content was decreased when CQ was administered in higher dose (970 mg/kg b.w.), which also resulted in liver damage [27]. Magwere *et al*. (1997) [47] have shown that a therapeutic dose of CQ leads to a decrease in protein turnover in humans. Cells which undergo rapid multiplication and protein synthesis are more prone to damage by CQ, as it readily accumulates in such tissues [48]. Okanlawon and Dyn (1996) [49] had reported that CQ increases trans-epithelial resistance in immature Sertoli cells and exhibits anti-proteases activity.

Moreover, significant reduction in body weight as well as testis weight in CQ-treated animals was noted which is concomitant with decrease in protein synthesis. Similar such results have been

reported in rats exposed to CQ [50]. Weight loss was probably a consequence of loss of appetite, thereby reduced intake of food by animals after drug treatment, an evident observation during treatment period. Lysosomes of Sertoli cells incorporate ACPase, which possess heterogenous function [51]. CQ possesses lysomotropic property which induces its uptake in the lysosomes thereby changing its size, leading to increase in size and number of hepatic lysosomes [50]. Along with this, there is also an elevation in enzyme activities of non-target cells that have been exposed to CQ. Together, all this results in the elevation of ACPase activity in the testis of CQ treated mice. Fredman *et al*., (1987) [48] reported CQ reduces the activity of the lysosomal enzymes while increasing the liver acid phosphatase.

Adverse effects on phosphatase activities are caused by wide range of chemicals including drugs. One of the important groups of enzymes is ALKpases which are omni-present in all the tissues of body, especially cell membranes. Thaker *et al*., (1997) [52] reported that ALKpases carry out the crucial functioning of transport of metabolite(s) across the membranes and are highly vulnerable to damage caused by xenobiotics, which may alter its regular machinery. Thus, CQ which was administered in a dose dependent manner in the present study disturbed the proper functioning of these enzymes thereby causing its impairment and overall decline.

Succinate dehydrogenase (SDH) oxidises succinate to fumarate as a component of the tricarboxylic acid cycle and ubiquinone to ubiquinol in the mitochondrial electron transport chain [53]. Any alteration in the morphology and function of mitochondria would hamper the activity of this enzyme. Mitochondrial dysfunction and hampered oxidative metabolism may therefore, be one of the reasons for change in the activity of SDH after undergoing CQ treatment. ATPases are important metabolic regulatory enzymes associated with ATP metabolism. The activities of SDH and ATPase were decreased in a dose-dependent manner, which resulted in impaired energy metabolism. CQ adversely affects mitochondrial energy transduction in vivo by acting as uncouplers of oxidative phosphorylation and lowers the cytochrome a, a_3, and b content [54]. Liang *et al*., (2016) [55] also showed mitochondrial damage and altered cytochrome C oxidase activities after CQ treatment in rats, which were in support of our study. Superoxide dismutases (SOD) are ubiquitous enzymes in aerobic organisms that function to catalytically convert $O_2^{\bullet-}$ to oxygen (O_2) and hydrogen peroxide (H_2O_2), the latter is then reduced to water (H_2O) and oxygen (O_2) with the help of catalase [56]. After the CQ treatment the SOD activity in the testis of mice decreased significantly, whereas LPO increased significantly having a probable role in oxidative stress. SOD thus, acts as a primary line of enzymatic defense and prevents further generation of free radicals [57]. Magwere *et al*., (1997) [47] demonstrated that the decreased SOD activity in the testis may be due to the accumulation of superoxide radical which consequently increases LPO levels after CQ treatment. Several other studies have also reported that CQ administration leads to increase in LPO levels and lowered enzymatic and non-enzymatic antioxidants [47, 58]. The inhibitory effect of CQ on sperm metabolism and regulation of cell survival and motility (measured by production of lactic acid and CO_2) have been also reported [15, 59, 60].

CQ caused disruption of spermatogenesis due to insufficient production of androgens by Leydig cells [43]. Investigation by Ashiru *et al*., (1991) [61] had shown a reduction in tubular length and diameter of seminiferous tubules and also reported that the injection of rats with CQ for 16 weeks eliminated all Leydig cells. Asuquo *et al*., (2009) [62] also reported degenerative changes in the seminiferous tubules of chloroquine phosphate-treated rats. The elimination of Leydig cells by chloroquine may eliminate testosterone and other Leydig factors that may be required for spermatogenesis [63]. Okanlawon and Ashiru (1998) [64] showed that in CQ treated rats, there was disruption of spermatogenesis, which was accompanied by a decline in serum concentration of testosterone in the rats. There are reports suggesting CQ and hydroxychloroquine (HCQ) cross the placenta with no significant difference in the mean concentration in maternal and cord blood [65]. Also, chloroquine is almost completely absorbed into the bloodstream and is concentrated in the tissues, with concentrations being 200 to 700 fold those in plasma [66].

Thus, it is possible that spermatozoa in individuals taking long-term chloroquine may be subjected to tissue CQ concentrations approaching those that are capable of producing adverse effects on sperm function.

As there was generation of toxicity due to CQ observed in present investigation, a need to look for an ameliorative agent emerged. Curcumin was opted for as it is considered as the "Indian solid gold" or "spice for life" [19], and has captured the spotlight in the phytochemical research arena. Due to its incorporation in daily cooking, curcumin becomes an especially important antioxidant due to this ease of access and availability, allowing for maintenance of relatively constant levels in the body and numerous health benefits.

Hence, curcumin was administered along with CQ administration in the present study. Duvoix *et al*., (2005) [67] reported that curcumin, a phenolic compound acts as a chemo-protective agent and protects the cells against the damage caused due to oxidative stress.

Curcumin, when administrated alone and in supplementation with the high dose of CQ, did not show any alterations in the parameters studied, with the values being comparable to the control. However, that does not hinder its property of being a potential candidate for reviving the damage caused by the toxicant. Apart from scavenging the free radicals, curcumin protects against a wide array of degenerative diseases by modulating the biochemical marker enzymes, LPO, and augmenting the antioxidant defense system [68, 69].

Further, Halliwell and Gutteridge (2002) [70] highlighted the beneficial effects of curcumin, which included capacity to quickly and efficiently scavenge lipid peroxyl radicals before they attack membrane lipids, thereby lowering oxidative damage.

The protective effect of curcumin on the testis may be explained by the fact that it prevents cellular damage occurring as a result of oxidative stress in spermatogenic cells of seminiferous tubules and Leydig cells of the stroma [71]. Similarly Mohanty *et al.,* (2006) [72] found that the role of turmeric in testicular protection may be referred also to its anti-oxidant property. It was also found that curcumin supplementation had prevented chromium-induced decrease in weight of accessory sex organs due to normal serum testosterone level [73]. Moreover, curcumin administration to male Wistar rats was able to ameliorate lindane-induced reproductive toxicity in pretreatment, post treatment and combination groups [74]. Additionally, it was demonstrated that curcumin exerts its protective effect by modulating lipid peroxidation and augmenting antioxidant defense system [69]. Curcumin may stop peroxidative alteration in the sperm and the testicular membrane which leads to enhancement of sperm motility and decrease in spermatozoa defects [75].

Mathuria and Verma (2008) [76] investigated the influences of curcumin on aflatoxin-induced toxicity in mice spermatozoa. Aflatoxin noticeably reduced sperm count, viability, and motility; different morphologic defects were encountered; thus, treatment with curcumin improved aflatoxin-induced sperm decrease, immobilization, and viability, and enhanced the morphological characteristics of the sperm. Another study revealed that curcumin protects Leydig cells of mice from the damage caused by chronic alcohol consumption [77]. Moreover, hepatoprotective effect of tetrahydrocurcumin has been also demonstrated in chloroquine-induced toxicity in rats [27].

Thus, curcumin, due to its ability to affect a wide range of molecular targets and an excellent safety profile, was shown to be a potential candidate for the prevention and/or treatment of a number of diseases. Several reports have demonstrated the protective role of curcumin against many known toxicants which is in support of the ameliorative effects observed in the present study.

CONCLUSION: Based on the present investigation and available review of literature it can be clearly indicated that short term use of Chloroquine to treat malaria could lead to anti-spermatogenic and possible anti-fertility effects, which were noted in the present work as early as 14 and 21 days. Further, curcumin being a natural compound, isolated from the rhizome of turmeric plant, has all the properties of being a wonder drug, and causes no adverse effect even when used in high dose. Present data reveals that administration of curcumin along with chloroquine could prevent

the toxic influences of chloroquine and thus may prove to be an effective mitigating agent while treating malaria. As the malaria prone countries are in the developing phase, it will be greatly beneficial if they rely on this natural medicine, as it is cheap and cost-effective.

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