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The Protective Role of Extract of *Allium cepa* Linn. (Liliaceae) (Red Onion) Bulb in Artesunate-induced Testicular Damage in Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NCO designed the study, performed the statistical analysis. Authors NCO and IGO wrote the protocol and the first draft of the manuscript. Authors NFC and OJO managed the analyses of the study. Authors NCO, IGO and OJO managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: *Allium cepa* is consumed fresh or cooked in Nigeria and used as herb in ethno-medicinal practice against many ailments. The aqueous extract is used in the management of prostrate inflammation, alleviation of low sperm count and testicular damage related disorders in ethno medicine and folkloric medicine in the South-east regions of Nigeria. This study investigates the protective role of extract of *Allium cepa* bulb on spermatogenesis and testicular oxidative stress in male rats using Artesunate as testicular damage and oxidative stress inducing agent.
Study Design and Methodology: The design consisted of 5 groups of 10 male Wistar rats (140-180g) each. Groups 1, 2, 3 and 5 were given continuous oral challenge with 4.4mg/kgb.w Artesunate orally for two weeks to induce testicular damage. Animals were treated with 50, 150 and 300mg/kgb.w extract for 7 weeks. Groups 4 and 5 were not

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treated and served as normal and negative controls respectively. Animals were humanely euthanized, testes collected, homogenized and used for sperm count, motility and oxidative stress evaluation.
Results: Artesunate mediated testicular damage caused a significant ($p<0.01$) reduction in sperm count ($17.5\pm2.4x10^6$ml/l) compared to normal control ($140X10^6$ml/l). Extract treatment of groups 1, 2 and 3 caused a dose-dependent reversal of sperm count, motility and morphology after 7 weeks. Treatment with the highest dose (300 mg/kg) of the extract reversed testicular oxidative induced lesions as shown by levels of enzymatic antioxidants; CAT (10.112±1.73µg/mg), SOD (39.41±2.35µg/mg) and GPx (0.55±0.17nmol/mg) and non-enzymatic antioxidants; MDA (0.98±0.05nmol/mg) and GSH (2.17±0.05mg) relative to normal control, respectively.
Conclusion: These results demonstrated the potential beneficial effects of *Allium cepa* in the management of prostrate inflammation and testicular lesions.

Keywords: Allium cepa bulb; artesunate; testicular damage; spermatogenesis; oxidative stress.

1. INTRODUCTION

Artesunate is one of the numerous drugs for effectively treating malaria in Nigeria. Artesunate (AS) is a clinically versatile Artemisinin derivative utilized for the treatment of mild to severe malaria infection. It may be administered via intravenous (IV), intramuscular (IM), oral or rectal routes [1]. In Nigeria the oral route of administration is more pre-dorminant because it is an over the counter (OTC) drug. It is a semi-synthetic derivative of artemisinin, the active compound of the Chinese herb *Artemisia annua* which consists of sodium succinyl salt of dihydroartemisinin [2]. Artesunate and its active metabolite dihydroartemisinin are potent blood schizonticides and are highly effective against the multi-drug resistant strains of *Plasmodium falciparum*. This has led to its increased wide usage for the treatment and management of malaria [3]. The pharmacokinetics of AS and its active metabolite dihydroartemisinin (DHA) are yet to be fully established. Inappropriate dosing of AS may result in unpleasant side effects including male testicular lesions [1]. Artesunate is also used in combination therapy and is effective in cases of uncomplicated *P. falciparum* infection.

Serious concerns have been raised about uncontrolled use of artemisinin derivatives because at the moment they are the last resort in the combat against multi-drug resistant *P. falciparum* malaria [4] although it is associated with severe side effects at high doses and under prolonged usage. The effects range from neurotoxicity, damaged brain stem centers, cellular heterotrophy to damage to reproductive organs and functions [5]. The occurrence of male infertility long after treatment with this antimalarial and anticancer drug is in fact of a serious concern owing to increased abnormalities noticed at birth among children nowadays [6]. Perhaps, Artesunate and its most active metabolite DHA may elicit teratogenesis and hemolysis in humans.

Many herbal plants play very important roles in the process of tissue repairs. Plant secondary compounds may exhibit more potent tissue repairs because they promote the repair mechanisms in a natural manner and are safer.

Allium cepa Linn. is a member of the Liliaceae, which consists of over 250 genera and 3700 species. Because of their evergreen bulbs, tubers and rhizomes, these plants are able to

survive under harsh conditions of wet or dry seasons. Different parts of the plant have been proven to possess antidiabetic [7], antioxidant [8], antihypertensive [9,10], antithrombotic [10], hypoglycemic [11] and antihyperlipidemic [12] effects. The bulb contains Kampferol, β-sitosterol, ferulic acid, myritic acid, prostaglandins and several compounds of secondary metabolism [8].

In traditional medicine in Eastern Nigeria, the bulb extract had reportedly been used to alleviate and improve low sperm count in male human subjects. The extract has also been shown in traditional medicine to improve male erectile function and ameliorate groin and testicular pains associated with prostate inflammation.

Therefore, the aim of the present study is to investigate the protective role of aqueous extract of *Allium cepa* bulb on spermatogenesis and testicular oxidative stress using male Wistar rats as study models.

2. MATERIALS AND METHODS

2.1 Plant Collection and Extraction

Two kilogram of fresh *Allium cepa* bulb was bought from Ogige market in Nsukka Local Government Area of Enugu State, Nigeria. They were macerated and soaked in 2000ml distilled water for 48h after which the mixture was filtered. The filtrate was concentrated by lyophilization (freeze drying) to afford 325.2g dry weight of extract. This was stored in a refrigerator at 4^{o}C until required.

2.2 Animals

Adult male Wistar rats (140-180g) obtained from the Laboratory Animal Unit of the Department of Biochemistry, University of Calabar, Nigeria were used for the study. Animals were kept in stainless steel cages and had access to feed (Vital feed®, Nigeria Ltd.) and water *ad libitum* except in situations where fasting was required. The rats were allowed 14 days to acclimatize prior to commencement of experiments. The use of the animals conformed with international accepted principles for laboratory animal use and care as documented in the European Community guidelines, Council Directive, 1986 (86/609/EEC), revised in Directive 2010/63/EU. The experimental protocols were approved by the Ethical Committee for Animal Experimentation, University of Calabar, Nigeria in accordance with Nigerian Federal Government legislation on Animal care.

2.3 Induction of Testicular Damage

Testicular damage was induced in experimental rat groups 1, 2, 3 and 5 with a concurrent administration of 4.4mg/kg Artesunate (GlaxoSmithKline Pharmaceutical Company, Nigeria) orally for two weeks. Testicular lesions were confirmed in animals with swollen testes, observable pains and inflammation around the male organs.

Groups 1, 2 and 3 animals were then treated orally with 50, 150 and 300mg/kg b.w extracts of *Allium cepa* for 7 weeks respectively by intubation. Groups 4 (normal control) and 5 (negative control) were not treated with extract and were given instead 0.03ml/10g distilled water orally.

2.4 Animal Sacrifice and Collection of Samples

At the end of treatments and recovery period, the rats were sacrificed under 25% urethane anesthesia (0.6ml/100gb.w). Urethane has no known spermatogenic or antifertility effects on rat testes [13]. The abdominal cavities were opened through a ventral midline abdominal incision to expose the reproductive organs. The testes were excised and all fats trimmed off, blotted dried and weighed with a Metler top loading weighing balance. Testes volumes were measured by water displacement method. The mean value of the two testicles from each rat was regarded as one score.

2.5 Epididymal Sperm Concentration

Spermatozoa in the right epididymis were counted by a modified method of Yokoi and Mayi [14]. In this method, the epididymis was minced with anatomic scissors in 5ml physiologic saline, placed in a rocker for 10min. This was allowed to incubate at room temperature for 2 min, after which the supernatant fluid was diluted 1:100 with a solution containing 5g sodium bicarbonate ($NaHCO_3$) in 1ml formalin (35 %). Total sperm number was determined by using a haemocytometer. Approximately 10µl of the diluted sperm suspension was transferred to each counting chamber of the haemocytometer and was allowed to stand for 5min. This was viewed under a light microscope. The sperm concentration was then calculated.

2.6 Sperm Progressive Motility

This was evaluated by an earlier method by Sonmez et al. [15]. Motility estimates were performed from 3 different fields in each sample. The mean of the 3 estimations was used as the final motility score. Samples for motility evaluation were stored at 25ºC.

2.7 Sperm Morphology

Sperm Cells morphologies were examined and evaluated using a light microscope at x400 magnification. Caudal sperms were taken from the original dilution for motility and diluted 1:20 with 10% neutral buffered formalin (Sigma-Aldrich, USA). Five hundred randomly selected sperm cells from each sample were scored for morphological abnormalities. In wet preparations, spermatozoa were as follows: (1) normal head and tail, (2) isolated heads, and with possibility of misshapen heads, (3) head-only defects, i.e. misshapen head with normal tail, (4) tail defects, i.e. normal head with abnormal tail or misshapen head with abnormal tail and (5) fused sperms. Each evaluation was expressed as a percentage of the morphologically normal sperms.

2.8 Assay of Testicular Enzymatic Antioxidants

2.8.1 Assay of catalase (CAT) activity

Catalase activity was measured according to the method of Aebi [16]. A 0.1ml of the testicular homogenate (supernatant) was pipetted into glass tube containing 1.9ml of 50mM phosphate buffer, pH 7.0. Reaction was initiated by the addition of 1.0ml of freshly prepared 30% (v/v) hydrogen peroxide (H_2O_2). The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 340nm. Activity of enzyme was expressed as units/mg protein.

2.8.2 Assay of superoxide dismutase (SOD) activity

Superoxide dismutase activity was measured according to the method of Winterboum et al. [17]. The principle of the assay was based on the ability of SOD to inhibit the reduction of nitro-blue tetrazolium (NBT). In this method, the reaction mixture contained 2.7ml of 0.067M phosphate buffer, pH 7.8, 0.05ml of 0.12mM riboflavin, 0.1ml of 1.5mM NBT, 0.05ml of 0.01mM methionine and 0.1ml of enzyme sample. Uniform illumination of the tubes was ensured by placing it in air aluminum foil in a box with a 15W fluorescent lamp for 10min. A control without the enzyme source was included. The absorbance was measured at 560nm. One unit of SOD was defined as the amount of enzyme required to inhibit the reduction of NBT by 50% under the specified conditions and was expressed as µ/mg protein.

2.8.3 Assay of glutathione peroxidase (GPx) activity

Glutathione peroxidase activity was measured by the method described by Rotruck et al. [18]. The reaction mixture contained 2.0ml of 0.4M Tris-HCl buffer, pH 7.0, and 0.01ml of 10mM sodium azide, 0.2ml of enzyme, 0.2ml of 10mM glutathione and 0.5ml of 0.2mM H_2O_2. The contents were incubated at 37ºC for 10min followed by the termination of the reaction by the addition of 0.4 ml 10% (v/v) TCA, centrifuged at 3000rpm for 5min. The absorbance of the product was read at 430nm and expressed as nmol/mg protein.

2.9 Assay of Testicular Non-enzymatic Antioxidant

2.9.1 Estimation of lipid peroxidation (malondialdehyde)

This method is based on the principle that acetic acid detaches the lipid and protein of a tissue, thiobarbituric acid reacting with lipid peroxide, hydrogen peroxide, and oxygen-labile double bond species to form the colour adducts with maximal absorbance at 530nm.

Lipid peroxidation in the samples was estimated spectrophotometrically as thiobarbituric acid reactive substances (TBARS) using the method of Buege and Aust [19]. A principal component of TBARS is malondialdehyde (MDA), a product of lipid peroxidation. In this method, 0.1ml of tissue homogenate in Tris-HCl buffer, pH 7.5 was treated with 2ml (1:1:1 ratio) of TBA-TCA-HCl reagent (thiobarbituric acid 0.37%, 0.25NHCl and 15% TCA). The mixture was placed in a water bath for 15 min and then allowed to cool. The absorbance of clear supernatant was measured against reference blank at 535 nm. Concentration was expressed as nmol/ml.

2.9.2 Assay of testicular reduced glutathione (GSH) concentration

The GSH was determined by the method of Ellman [20]. A 1.0ml of supernatant was treated with 0.5ml of Ellman's reagent (19.8mg of 5, 5'-dithio-bis-nitro benzoic acid (DTNB) in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2M, pH 8.0). A 0.4ml of distilled water was added and the mixture was thoroughly mixed. The absorbance was read at 412 nm and expressed as nmol/mg protein.

2.10 Statistical Analysis

All data were expressed as Mean±SEM of number of replicates (n=10). The groups were compared using Analysis of Variance (ANOVA) in the SPSS version 16 software. Values at $p<0.05$ were considered significant between experimental groups.

3. RESULTS

3.1 Sperm Morphology

The results of the sperm count, sperm motility and sperm morphology is shown in (Table 1). The results showed that the extract exerted some measure of improvement in the fertility of rats with Artesunate-induced testicular damage. This was indicated by the increased values of the different sperm parameters (sperm count, motility and morphology) studied relative to the negative control. Artesunate-induced testicular damaged rats that were treated with the high dose (300mg/kg) of the extract produced 115.2±5.5 $x10^6$m/L sperms while normal rats had 140.3±7.2$x10^6$m/L sperms but untreated rats had a comparatively low mean sperm count (17.5±2.4$x10^6$m/L. The sperm count values in normal and extract-treated rats were elevated and highly significant ($p<0.01$) compared to values in untreated, negative control rats (Group 4).

Table 1. Sperm count, motility and morphology in artesunate-induced testicular damage in male wistar rats treated with aqueous extract of *Allium cepa* bulb

Groups/doses (mg/kg)	Sperm count ($x10^6$m/L)	% Sperm motility	Sperm morphology	
			Normal	Abnormal
50 *Allium cepa*	66.5±4.7	45.3±1.9	28.9±1.8	70.1±3.3
150 *Allium cepa*	88.7±6.6*	85.2±1.5*	60.4±2.0*	38.6±3.1*
300 *Allium cepa*	115.2±5.5*	99.0±1.3*	94.1±2.3*	4.77±1.8*
Normal	140.3±7.2*[b]	99.3±1.1*[b]	91.8±4.1*[b]	7.2±3.1*[b]
Negative control	17.5±2.4[a]	11.6±2.5[a]	12.7±3.2[a]	86.3±4.2[a]

*[*]Significant at p<0.01 compared to negative control but *[b] is comparable to normal control at p<0.05 (One-way ANOVA, LSD post hoc); n=10*

3.2 Testicular Enzymatic Antioxidants

Increases were observed in testicular enzymatic antioxidants in Artesunate-induced testicular damage in male Wistar rats treated with *Allium cepa* when compared to normal and negative control. (Table 2). Also, the animals treated with the highest dose of extract (300mg/kg body weight) showed faster testicular enzymes recovery when compared to the negative control (group 5).

Table 2. Testicular enzymatic antioxidants in artesunate-induced testicular damage in male wistar rats treated with aqueous extract of *Allium cepa* bulb

Groups/doses (mg/kg)	CAT (µg/mg protein)	SOD (µg/mg protein)	GPx (nmol/mg protein)
50 *Allium cepa*	5.120±2.4	11.77±2.36	0.19±0.15
150 *Allium cepa*	7.680±3.28*	19.32±4.77*	0.22±0.16*
300 *Allium cepa*	10.112±1.73*	39.41±2.35*	0.55±0.17*
Normal	16.691±1.68*	51.65±3.91*	0.82±0.06*
Negative control	3.210±0.77	9.67±2.21	0.13±0.14

*Values are mean±SEM; n=10 in each group; * significant at p<0.05 compared to negative control*

3.3 Testicular Non-enzymatic Antioxidants

The results showed a significant (p<0.05) decrease in MDA levels and were dose-dependent when compared to the negative control. There was a significant increase in reduced glutathione (GSH) levels (P<0.05) in a dose-dependent manner with the highest dose of the extract giving an activity of 2.17±0.05 as compared to 0.49±0.06 of the negative control (Table 3).

Table 3. Testicular non-enzymatic antioxidants in artesunate-induced testicular damage in male wistar rats treated with aqueous extract of *Allium cepa* bulb

Groups/doses (mg/kg)	MDA (nmol/mg protein)	GSH (mg/protein)
50 *Allium cepa*	2.01±0.04	0.65±0.07
150 *Allium cepa*	1.52±0.06*	1.88±0.04
300 *Allium cepa*	0.98±0.05*	2.17±0.05*
Normal	0.78±0.06*	2.44±0.06*
Negative control	4.01±0.05	0.49±0.06

*Values are mean±SEM; n=10 in each group; * represent significant differences at P<0.05 compared to negative control*

4. DISCUSSION

Mammalian spermatozoa is particularly vulnerable to lipid peroxidation because of the molecular anatomy of its plasma membrane. Unlike somatic cells, mammalian sperm cells present highly specific lipid composition with high content of polyunsaturated fatty acids (PUFA), plasmalogenes and sphingomyelins. This unusual structure of sperm membrane is responsible for its flexibility and the functional ability of sperm cells to migrate through the uterus to fertilize ovum in the fallopian tube. Unfortunately, spermatozoa lipids especially PUFA are the main substrates for peroxidation, that may provoke severe functional disorder of sperm [21], sperm membrane disruption, testicular lesions and potential infertility in susceptible males.

Quiles et al. [22], Chularojmontri et al. [23] and Prahalathan et al. [24] have attempted to study the effects of chemotherapeutic drugs on the reproductive system. It was established that anti-malarial drugs induce oxidative damage to spermatozoa by generating free radicals either by the enzymatic pathway of redox cycling or by the non-enzymatic process [25]. In both pathways, molecular oxygen is reduced to superoxide anion ($O_2^{\cdot}$), which is converted to other forms of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and the

more toxic hydroxyl radical (OH•). These free radicals then cause membrane and macromolecular damage by three basic mechanisms involving lipid peroxidation, DNA fragmentation and protein oxidation [26]. Therefore, Artesunate-induced testicular damage occurs at molecular level.

Under biological conditions, the anthracycline and semiquinone (which are metabolites in urine of Artesunate biotransformation) or reduced metal ions selectively cleaves H_2O_2 to produce the hydroxyl radical (OH•) which is the most reactive and destructive chemical species ever known [27]. This ultimately leads to lipid peroxidation, causing irreversible damage to membrane structure and function [28].

Phytochemical screening revealed the presence of glycosides, saponins and flavonoids which are potent antioxidants and free radical scavengers from *Allium cepa* bulb [29,30]. It is likely the extract improves spermatogenesis and produces testicular protection by scavenging free radicals produced by Artesunate and its metabolites. This is observed in the improved sperm count, sperm motility, sperm morphology and increased levels of enzymatic and non- enzymatic antioxidants in the testicular homogenates. This potential protection is further observed in the lowering of the MDA levels in groups treated with the different doses of the extract when compared with the negative control group. These results showed that the groups treated with the extract, especially at high doses (300mg/kg b.w) gave similar results with the normal control group and elicited recovery of all parameters tested.

5. CONCLUSION

Anti-malarial drugs seem to have major side effects on both male and female reproductive systems, as well as being teratogenic and hepatotoxic in human subjects. The ability of plant extracts rich in antioxidative principles to reverse testicular damage associated with Artesunate toxicity in endemic malaria zones in Nigeria has been demonstrated using aqueous extracts of *Allium cepa* bulbs. It is concluded in this study that populations living in endemic malaria zones be encouraged to regularly take water extracts of red onions, as well as eat them raw. Further researches are on-going to evaluate the effect of *Allium cepa* extract on microbial load in the cervix region in female reproductive area and also on sex hormone regulation.

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CONSENT

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Morris CA, Duparc S, Borghini-Fuhrer I, Jung D, Shin C and Fleckenstein L. Review of the clinical pharmacokinetics of Artesunate and its active metabolite dihydroartemisinin following intravenous, intramuscular, oral or rectal administration. Malaria Journal. 2011;10:263. doi: 10.1186/1475-2875-10-263.
2. Ittarat WR, Udomsangpeth KT, Chotivanich, Looareesuwan S. The effects of quinine and Artesunate treatment on plasma tumor necrosis factor levels in malaria infected patients. Southeast Asian J. Trop. Med. Public Health. 1999;30:7-10.
3. Van Agtmael MA, Cheng-Qi S, Qing JX, Mull R, Van Boxtel CJ. Multiple dose pharmacokinetics of artemether in Chinese patients with uncomplicated falciparum malaria. Int. J. Antimicrob. Agents. 1999;12:151-158.
4. White NJ. Clinical Pharmacokinetics and Pharmacodynamics of artesunate and derivatives. T. Roy. Soc. Trop. Med. H. 1994;88(suppI1):S41-S43.
5. Nwanjo H and Oze G. Acute hepatotocixity following administration of artesunate in guinea pigs. Internet J. Toxicol. 2007;4(1).
6. Suominen JS, Linderborg J, Nikula H, Hakovirta H, Parvinen M, Toppari J. The effects of mono-2-ethylhexyl phathalate, adriamycin and N -ethyl-nitrosouria on stage-specific apoptosis and DNA synthesis in the mouse spermatogenesis. Toxicol. Lett. 2003;143:163-173.
7. Galal EE, Gawad MA. Antidiabetic activity of Egyptian onion *Allium cepa* extract. J. Egypt Med. Assoc. 1965;48:14-45.
8. Dhanprakash BN, Garima U. Antioxidant and free radical scavenging activities of phenols from onion (*Allium cepa*). Food Chemistry. 2007;102:1389-1393.
9. Sakai Y, Murakami T, Yamamoto Y. Antihypertensive effect of onion on no synthase inhibitor induced hypertensive rats. Biosc. Biotecnol. Biochem. 2003;67:1305-11.
10. Yamada K, Namaura A, Sawashita N, Naguchi Y, Yamamoto J. An onion variety has Natural antithrombotic effect as assessed by thrombosis/thrombolysis models in rodents. Thromb. Res. 2004;114:213-20.
11. Compos KE, Diniz YS, Cataneo AC, Faine LA, Alves MJ, Nobeli EL. Hypoglycemic and antioxidant effect of onion, *Allium cepa*: dietary onion addition, antioxidant activity and hypoglycemic effect on diabetic rats. Int. J. Food Sci. Nutr. 2003;54:241-6.
12. Lata S, Saxena KK, Bhasin V, Saxena RS, Kumar A, Shrivastva VK. Beneficial effect of *Allium sativum*. *Allium cepa* and *Comiphora mukul* on experimental hyperlipidemia and arthrosclerosis- A comparative evaluation. Indian J. Pharmacol. 1991;37:132-5.
13. Meneguz A, Fortuna S, Lorenzini P, Volpe MT. Influence of urethane and ketamine on rat hepatic cytochrome P450 *In vivo*. Exp. Toxicol. Pathol. 1999;51(4-5):392-6.
14. Yokoi K, ZK Mayi. Organ apoptosis with cytotoxic drugs. Toxicology. 2004;290:78-85.
15. Sonmez M, Turk G, Yuce A. The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats. Theriogenology. 2005;63:2063-2072.
16. Aebi HE. Catalase. In: Methods of Enzymatic Analysis, Bergmeyer, H.U. (Ed.), Verlag Chemie, Weinhem. 1983;273-286.
17. Winterbourn CC, Hawkins RE, Brian M, Carrell RW. The estimation of red cell superoxide dismutase activity. J. Lab. Clin. Med. 1975;85:337-341.
18. Rotruck IT, AL Pope HE, Ganther AB, Swanson DG, Hafeman, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. Science. 1973;179:588-590.
19. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol. 1978;52:302-310.
20. Ellman GL. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 1959;82:70-77.

21. Sachdev S, Davies K. Molecular biology. Free Rad. Biol. Med. 2008;44:215-223.
22. Quiles JL, Huertas JR, Battino M, Mataix J, Ramirez-Tortosa CM. Antioxidant nutrients and adriamycin toxicity. Toxicology. 2002;180:79-95.
23. Chularojmontri L, Wattanapitayakul SK, Herunsalee A, Charuchongkolwongse S, Niumsakul N, Srichairat S. Antioxidative and cardioprotective effects of *Phyllanthus urinaria* L. on doxorubicin-induced cardiotoxicity. Biol. Pharm. Bull. 2005;28:1165-1171.
24. Prahalathan C, Selvekurna E, Varalakshmi P. Lipoic acid ameliorates adriamycin-induced testicular mitochondriopathy. Reprod. Toxicol. 2005;20:111-116.
25. Armstrong LL, Lipsy RJ. Doxorubicin. Drug Information Handbook, Lexi-Comp, Husdon; 1993.
26. Pacher P. FRs induce Chain reaction. Physiol. Rev. 2007;87:315-424.
27. Panjrath GS, V Patel, Valdiviezo CI. Potentiation of doxorubicin cardiotoxicity by iron loading in a rodent model. J. Am. Coll, Cardiol. 2007;49:2457-2464.
28. Kirsi T, J Timo. Toxic effects of doxorubicin. Cancer Res. 2001;24:634-644.
29. Devipriya S, Shyamladevi CS. Protective effect of quercetin in cisplatin-induced cell injury in the kidney. Indian J. Pharmacol. 1999;13:422.
30. Chatterjee TK. Medicinal plant with antifertility property. Herbal Option. 1997;77.

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