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Artesunate Induced Hepato-Toxicity and its Amelioration by *Allium sativum* in Swiss Albino Male Mice

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Abstract

The use of natural antioxidants as mitigating agents has become prevalent. Garlic (*Allium sativum*) is one such agent which has been proven to have antibacterial, antiseptic, antifungal, antiparasitic, anticoagulant and antitumor properties. The present investigation deals with the ameliorative effects of *Allium sativum* on the antimalarial drug induced hepato-toxicity. Artesunate is being used as an alternative anti-malarial drug against conventional drugs like chloroquine. Experimental animals were divided into six groups of six mice each. Group A served as the control group. Group B and Group C animals were given 150 mg/kg b.wt and 300 mg/kg b.wt of artesunate respectively while Group D mice were given 100 mg/kg b.wt of *Allium sativum*. Group E and F mice were given 150 mg/kg b.w of artesunate+100 mg/kg b.wt *Allium sativum* and 300 mg/kg b.wt of artesunate+100 mg/kg b.wt *Allium sativum* respectively. Results exhibited recovery and re-establishment of various altered indices as opposed to Artesunate treated groups. Thus, *Allium sativum* might be used as a potent mitigating agent against anti-malarial drug toxicity. However, further research is needed to completely elucidate the ameliorative efficacy of *Allium sativum*.

Keywords: Artesunate; Antimalarial drug; *Allium sativum;* Liver

Introduction

Malaria is an endemic parasitic disease globally wide-spread especially in tropical and sub-tropical countries and it is a major health problem in many parts of the world [1]. The annual worldwide burden exceeds 600 million clinical cases of *Plasmodium falciparum* and *Plasmodium vivax* [2,3], leading to 1.1-2.7 million deaths [4], as well as disappropriate impact on infants, children and pregnant women [5]. Alternative drug therapy has become necessary now a days due to the advent of various drug resistance cases.

Antimalarial drugs play an important role in treating malarial cases as well as controlling the spread of infection. Usage of drugs to treat the disease was documented as early as in the 17th century. The initial effective treatment for malaria was derived from the bark of the Cinchona tree, which comprises of quinine. Quinine was the predominant malarial medication until the 1920s, after which it was replaced by chloroquine [6]. The Chinese scientist Tu Youyou isolated artemisinins from the plant *Artemisia annua*, in the 1970s [7]. The two most widely used artemisinin derivatives are artemether and artesunate.

The present investigation focuses on artesunate induced hepatotoxicity. Artesunate belongs to the category of antiprotozoal agents. It is tetracyclic and consists of a trioxane and a lactone ring. An endoperoxide bridge is formed in the trioxane ring. This bridge is particularly essential for the antimalarial action of artesunate. Artesunate, a dihydroartemisinin -10-α hemisuccinate is a water soluble semi-synthetic derivative of artemisinin with a molecular weight of 384.4 [8,9]. Artesunate is the most widely available form of the artemisinin-related compounds; it may be administered parenterally, intravenously, intramuscularly, orally, or rectally. The single dose toxicity of artesunate includes effects like reduced activity, weakness, loss of appetite, tremors etc. [10]. Reports suggest that use of artesunate may cause hepatic dysfunction. It has been reported that high doses of artesunate could produce neurotoxicity such as selective damage to brainstem centres, gait disturbances [11,12] and loss of spinal cord and pain response mechanisms in mice and rats [13]. Artesunate has also been shown to cause a decrease in sperm motility in guinea pigs [14]. Animal studies have reported the hepatotoxic effect of artesunate. Artesunate causes hepatocyte damage by formation of free radicals. Further, it causes a rise in the liver transaminases [15,16].

To ameliorate such side effects, the mitigating properties of *Allium sativum* on artesunate treated albino mice were investigated. *Allium sativum*, commonly known as Garlic is a plant native to Africa and Central Asia. The detailed uses of *A. sativum* have been mentioned in quite a few ancient scriptures. The father of modern medicine, Hippocrates, used *Allium sativum* for treating cancerous tumors. According to Ayurveda, the Indian system of healing; *Allium sativum* is a very effective anti-microbial herb, with anti-fungal, anti-viral and anti-bacterial properties.

It helps prevent heart diseases including atherosclerosis, high cholesterol and high blood pressure. A study carried out by Czech researchers showed that *Allium sativum* supplementation reduced the accumulation of cholesterol on the vascular walls of animals [17]. It possesses anticoagulant effects [18]. Infection by *Helicobacter pylori* causes colon and stomach cancer. *A. sativum* might fight these cancers by attacking the bacteria [19]. It has also been found to be effective against the intestinal parasite Eimeria [20]. *Allium sativum* aids thiamin absorption and thus reduces the chances of developing beriberi. It was also found to be effective against scurvy [21]. It has been used quite successfully in AIDS patients for treating Cryptosporidium in a study carried out in China [22]. Furthermore, it is reported that aqueous extract of *Allium cepa* improves spermatogenesis and provides testicular protection by scavenging free radicals produced by Artesunate and its metabolites [23].

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Liver is the centre of all metabolic activities in the body. Drugs and other foreign substances are metabolized and inactivated in the liver and therefore liver is susceptible to the toxicity from these agents. Certain medicinal agents when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the liver. The present study was carried out to study the ameliorative potential of *A. sativum* on artesunate induced hepato-toxicity.

Materials and Method

Healthy, adult male albino mice (*Mus musculus*) of Swiss strain, weighing 25-30 gm were used for the experiment. The experimental animals were acclimatized 7 days prior to the commencement of treatment. They were maintained in an air-conditioned animal house at a temperature of 25 ± 2°C. Animals were exposed to approximately 10-12 hours of day light, with food chow and water *ad libitum*. The relative humidity was maintained at 30-70%. Different experimental animals were caged separately with a maximum of six animals per cage. They were treated daily before feeding so as to avoid interference with food intake. The oral treatments were done by a feeding tube which was attached to a hypodermic syringe.

The drug used for treatment was Artesunate. The dose of the antimalarial drug (Artesunate) was selected according to the LD_{50} value for rat and mouse and mean oral lethal doses for humans [24]. The animals were treated with low dose of artesunate at 150 mg/kg body weight and a high dose of 300 mg/kg body weight at different time intervals of 14 and 21 days. Along with the dose of artesunate, *Allium sativum* (100 mg/kg body weight) was also administered to certain groups to study its ameliorative effects (Table 1).

After each treatment, the experimental animals were weighed on an animal weighing balance and were sacrificed in accordance with the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Preparation of *Allium sativum*

Crude extract of *Allium sativum* of the single clove variety was prepared from the bulbs purchased in bulk from the market. The cloves were sliced into pieces, ground into a paste and then dissolved in deionized distilled water. The concentration of extract was 10 mg *Allium sativum* per 1 ml, corresponding to 100 mg of *Allium sativum* per kg body weight of the animal. This concentration is being calculated on the basis of a daily human intake of 6.00 grams *Allium sativum* by a 60 kg human individual [25].

Parameters Studied

Body and organ weight

The body weight of control and treated groups of mice were recorded on a daily basis to the nearest milligram on a digital balance (Reptech). The weights of the liver were recorded to the nearest milligram on digital balance (Citizen, Japan).

Total protein

Protein level in the liver of control and treated groups of animals was estimated by the method of Lowry et al. (1951) [26]. When the protein containing preparation is treated with phenol reagent of Folin Ciocalteu (FCR), a deep blue colouration develops due to two reactions- the reaction of alkaline copper sulfate solution with peptide bonds and the reduction of phosphomolybdic and phosphotungstic acids by aromatic amino acids present in the protein- occurring simultaneously. The blue colour developed is quantitatively proportional to the total protein, which is measured colorimetrically at 540 nm.

Cholesterol

The levels of cholesterol in the liver of control and all treated groups of mice were estimated by the method of Zlatkis et al. (1953) [27]. In the presence of concentrated sulphuric acid and glacial acetic acid, cholesterol forms a coloured complex with ferric chloride ($FeCl_3$) which can be measured on Systronics Digital Spectrophotometer 167 against blank at 540 nm.

Glycogen

Glycogen levels were estimated in liver of control and all treated groups of mice using the method of Seifter et al. (1950) [28]. The glycogen in the tissue is converted to glucose allowing the glucose to react with anthrone which gives a green colour. Depending upon the concentration of glycogen, the colour form intensifies. The percent transmittance was read at 620 nm on a Systronics Digital Spectrophotometer 167.

Total, dehydro and reduced ascorbic acid levels (TAA, DHA and RAA)

Levels of Total, Dehydro and Reduced Ascorbic Acid were estimated in the liver of control and all treated groups of mice by the method of Roe and Kuether (1943) [29]. Principle Total ascorbic acid (TAA) is oxidized to dehydroascorbic acid (DHA) by Norit reagent in the presence of trichloroacetic acid (TCA). This couple with 2, 4 dinitrophenyl hydrazine to yield a orange coloured complex by reacting with sulphuric acid which is measured colorimetrically. The dehydro form is estimated by using 6% trichloroacetic acid. The difference between total and dehydro-ascorbic acid gives the value of reduced ascorbic acid (RAA). The optical density was measured at 540 nm against blank on Systronics Digital Spectrophotometer 167**.**

Alkaline phoshphatase (ALKPase)

Alkaline phosphatase activity was estimated in the liver by the method of Bessey et al. (1946) [30]. The enzyme alkaline phosphatase hydrolyses the substrate p-nitrophenyl phosphate into inorganic phosphate and p-nitrophenol. The quantity of p-nitrophenol released under standardized condition is measured at 410 nm.

Acid phosphatase (ACPase)

Activity of Acid phosphatase was determined in the liver of all treated and control mice by the method of Bessey et al. (1946) [30]. Acid phosphatase, orthophosphoric monoester phosphorhydrolase catalyses the hydrolysis of p-nitorphenyl phosphate at pH 4.8, liberating

Groups	Treatment and Dose	Duration (Days)
A	Control (untreated)	_
B	Control+Artesunate (150 mg/kg b.wt)	14, 21
C	Control+Artesunate (300 mg/kg b.wt)	14, 21
D	Control+*Allium sativum* (100 mg/kg b.wt)	14, 21
E	Control+Artesunate(150 mg/kg b.wt)+*Allium sativum* (100 mg/kg b.wt)	14, 21
F	Control+Artesunate(300 mg/kg b.wt)+*Allium sativum* (100 mg/kg b.wt)	14, 21

Number of animals (n)=6 animals in each group

Table 1: Category of animals according to treatment and dose.

paranitrophenol and inorganic phosphate. The liberated p-nitrophenol combines with NaOH to form a yellow coloured complex, which is measured at 420 nm and is directly proportional to the enzyme activity.

Adenosine triphosphatase (ATPase)

The ATPase activity in the liver of control and all treated groups of animals was assayed by the method of Quinn and White, 1968; while inorganic phosphate liberated was estimated using the method of Fiske and Subbarow (1925) [31]. The enzyme adenosine triphosphatase (ATPase) hydrolyses the substrate ATP into adenosine diphosphate (ADP) and inorganic phosphate (ip). Readings were taken at 660 nm on a Systronics Digital Spectrophotometer 167.

Succinate dehydrogenase (SDH)

The activity of SDH was estimated in the liver of control and all treated groups of animals according to the method of Beatty et al., (1966) [32] using 2-(4- Iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) as an electron acceptor. The electrons released by the enzyme SDH from the substrate are taken up by an electron acceptor i.e. INT which is reduced to red colour formazan. After extracting it in ethyl acetate, the colour intensity is measured at 420 nm (Table 2-5).

Statistical analysis

All the data are shown as Mean ± SE. Statistical analysis was carried out using the trial version of SPSS software package version 16.0 (USA). Comparison between groups was done by one-way analysis of variance (ANOVA) taking significance at $P<0.05$, followed by Student's t-test taking significance at ***$p<0.001$, **$p<0.005$ and *$p<0.01$. Tukey's honestly significance difference (HSD) post hoc test was used for comparison among different treatment groups ($P<0.05$) (Table 6**).**

Results

Body weight and organ weight

Mice treated with low dose of artesunate (150 mg/kg B.wt.) for an interval of 14 and 21 days showed no significant reduction in body weight. When higher dosage of artesunate (300 mg/kg B.wt.) was administered, the reduction in body weight was significant after 21 days ($p<0.01$). The ameliorative studies showed that when *Allium sativum* (Garlic) administered to the control mice, negligible variation was observed in all the parameters studied at different time intervals and *Allium sativum* (100 mg/kg B.wt.) when administered along with low (150 mg/kg B.wt.) or higher dose of artesunate (300 mg/kg B.wt.) showed a trend towards recovery in body weight when compared to the control groups.

The gross weight of liver tissue was unaffected by the artesunate (150 mg/kg B.wt.) treatment up to 21 days. High dose of artesunate (300 mg/kg B.wt.) when administrated to male mice showed significant decrease in gross liver weight at 14 days of treatment ($p<0.01$) and significant decrease at $p<0.005$ level after 21 days treatment. When *Allium sativum* was supplemented along with artesunate low (150 mg/kg B.wt.) or high (300 mg/kg B.wt.) dose treated animals, the results were found to be comparable to the control group for gross liver weight after 14 and 21 days showing minimal toxic influence of the drug - artesunate.

Protein

Male mice administered with low dose artesunate treatment (150 mg/kg B.wt.) did not show any significant change in the protein content of liver up to 14 days, but 21 days treatment showed significant decrease ($p<0.01$) in the protein content of the liver. High dose of artesunate (300 mg/kg B.wt.) treated male mice had a significant decline in the

Groups → Parameters ↓	A	B	C	D	E	F
Body weight	39.28± 0.09	39.22± 0.02 NS	38.24± 0.02 NS	39.24± 0.02 NS	38.88± 0.02 NS	38.49± 0.01 NS
Tissue weight (mg)	2244 ± 1.12	2241 ± 0.26 NS	1985 ± 0.62 *	2247 ± 0.16 NS	2244 ± 0.14 NS	2241 ± 0.09 NS
Protein (mg/100 mg tissue weight)	17.20 ± 0.28	15.52 ± 0.15 NS	12.65 ± 0.16*	16.90 ± 0.13 NS	15.56 ± 0.21 NS	14.74 ± 0.15 NS
Cholesterol (mg/100 mg tissue weight)	1.38 ± 0.01	1.87 ± 0.18 NS	1.62 ± 0.02 *	1.21 ± 0.12 NS	1.21 ± 0.24 NS	1.09 ± 0.29 NS
Glycogen (µg/100 mg tissue weight)	613.8 ± 22.74	698.8 ± 29.16 NS	704.4 ± 24.82 *	694.6 ± 25.43 NS	625.1 ± 32.84 NS	623.8 ± 23.93 NS
TAA (mg/100 mg tissue weight)	5.89 ± 0.18	5.03 ± 0.18 NS	4.65 ± 0.16 *	5.07 ± 0.16 NS	5.12 ± 0.21 NS	4.93 ± 0.17 NS
DHA (mg/100 mg tissue weight)	4.63 ± 0.28	3.79 ± 0.19 NS	3.50 ± 0.25 *	4.04 ± 0.14 NS	4.07 ± 0.12 NS	3.83 ± 0.15 NS
RAA (mg/100 mg tissue weight)	1.25 ± 0.31	1.24 ± 0.36 NS	1.15 ± 0.28 *	1.03 ± 0.14 NS	1.04 ± 0.24 NS	1.09 ± 0.31 NS

Values are mean ± S.E., *p <0.01, **p <0.005, *** p<0.001, NS-Non Significant

Table 2: Showing gravimetric indices, biochemical and antioxidant parameters of liver for dose duration of 14 days.

Groups → Parameters ↓	A	B	C	D	E	F
ALKpase (µ moles of p-nitro phenol released / mg protein / minutes)	0.54 ± 0.13	0.41 ± 0.12 NS	0.31 ± 0.03 *	0.37 ± 0.02 NS	0.36 ± 0.02 NS	0.35 ± 0.03 NS
ACPase (µ moles of p-nitro phenol released / mg protein / minutes)	1.45 ± 0.03	1.56 ± 0.05 NS	2.35 ± 0.04 **	1.83 ± 0.16 NS	1.31 ± 0.17 NS	1.58 ± 0.17 NS
ATPase (µ moles inorganic phosphate released / mg protein / 30 minutes)	3.89 ± 0.18	3.03 ± 0.18 NS	2.65 ± 0.16 *	3.07 ± 0.16 NS	3.12 ± 0.21 NS	2.93 ± 0.17 NS
SDH (µg formazan / mg protein / minutes)	17.80 ± 0.34	15.51 ± 0.15 NS	11.65 ± 0.27 *	16.88 ± 0.13 NS	15.59 ± 0.21 NS	14.72 ± 0.16 NS

Values are mean ± S.E., *p <0.01, **p <0.005, *** p<0.001, NS-Non Significant

Table 3: Showing enzymatic parameters of liver for dose duration of 14 days.

Groups → Parameters ↓	A	B	C	D	E	F
Body weight	39.03± 0.13	39.05 ± 0.03 NS	35.36 ± 0.07 *	38.80 ± 0.02 NS	37.98 ± 0.10 NS	37.30 ± 0.01 NS
Tissue weight (mg)	2120 ± 0.92	2115 ± 0.63 NS	1846 ± 0.29 **	2248 ± 0.08 NS	2245 ± 0.09 NS	2242 ± 0.15 NS
Protein (mg/100 mg tissue weight)	17.34 ± 0.32	12.99 ± 0.14*	11.22 ± 0.21**	16.93 ± 0.19 NS	14.03 ± 0.18 NS	14.50 ± 0.24 NS
Cholesterol (mg/100 mg tissue weight)	1.41 ± 0.04	1.93 ± 0.13 *	2.56 ± 0.27 **	1.19 ± 0.13 NS	1.18 ± 0.22 NS	1.06 ± 0.30 NS
Glycogen (µg/100 mg tissue weight)	625.5 ± 23.90	707.3 ± 25.01 *	729.5 ± 23.41 **	690.3 ± 20.74 NS	675.7 ± 32.72 NS	634.6 ± 14.12 NS
TAA (mg/100 mg tissue weight)	5.85 ± 0.19	4.14 ± 0.11 *	3.75 ± 0.76 **	4.94 ± 0.20 NS	4.85 ± 0.16 NS	4.80 ± 0.15 NS
DHA (mg/100 mg tissue weight)	4.61 ± 0.25	2.79 ± 0.56 *	3.44 ± 0.26 **	3.87 ± 0.20 NS	3.89 ± 0.10 NS	3.75 ± 0.16 NS
RAA (mg/100 mg tissue weight)	1.24 ± 0.32	0.96 ± 0.33 *	0.69 ± 0.30 **	1.07 ± 0.36 NS	0.96 ± 0.24 NS	1.05 ± 0.30 NS

Values are mean ± S.E., *p <0.01, **p <0.005, *** p<0.001, NS-Non Significant

Table 4: Showing gravimetric indices, biochemical and antioxidant parameters of liver for dose duration of 21 days.

Groups → Parameters ↓	A	B	C	D	E	F
ALKpase (µ moles of p-nitro phenol released / mg protein / minutes)	0.55 ± 0.13	0.33 ± 0.08 *	0.25 ± 0.03 **	0.35 ± 0.01 NS	0.33 ± 0.02 NS	0.31 ± 0.01 NS
ACPase (µ moles of p-nitro phenol released / mg protein / minutes)	1.49 ± 0.03	2.02 ± 0.15 *	2.60 ± 0.09 **	1.81 ± 0.12 NS	1.23 ± 0.15 NS	1.53 ± 0.13 NS
ATPase (µ moles inorganic phosphate released / mg protein / 30 minutes)	3.85 ± 0.19	2.14 ± 0.11 *	1.55 ± 0.28 **	2.94 ± 0.20 NS	2.85 ± 0.16 NS	2.80 ± 0.15 NS
SDH (µg formazan / mg protein / minutes)	17.94 ± 0.34	12.09 ± 0.32 NS	11.22 ± 0.21 **	16.93 ± 0.18 NS	14.06 ± 0.17 NS	14.49 ± 0.24 NS

Values are mean ± S.E., *p <0.01, **p <0.005, *** p<0.001, NS-Non Significant

Table 5: Showing enzymatic parameters of liver for dose duration of 21 days.

Parameter	Source of Variation	SS	df	MS	F-value	P-value
Body Weight	Between the Groups (Dosage)	464.6	3	154.9	61.92	0.0001
	Within the Groups (Duration)	1040	416	2.501		
Tissue Weight	Between the Groups (Dosage)	800836	3	266945	22.56	0.0001
	Within the Groups (Duration)	4.921	416	11830		
Protein	Between the Groups (Dosage)	32.40	3	10.80	2.743	0.0429
	Within the Groups (Duration)	1638	416	3.937		
Cholesterol	Between the Groups (Dosage)	2.981	3	0.9938	2.678	0.0467
	Within the Groups (Duration)	154.3	416	0.3710		
Glycogen	Between the Groups (Dosage)	53066	3	17689	20552	0.0552
	Within the Groups (Duration)	2.883	416	6931		
TAA	Between the Groups (Dosage)	19.19	3	6.398	8.770	0.0001
	Within the Groups (Duration)	303.5	416	0.7295		
DHA	Between the Groups (Dosage)	9.275	3	3.092	6.389	0.0003
	Within the Groups (Duration)	201.3	416	0.4839		
RAA	Between the Groups (Dosage)	1.823	3	0.6075	0.8289	0.4785
	Within the Groups (Duration)	304.9	416	0.7330		
ALKPase	Between the Groups (Dosage)	0.4257	3	0.1419	3.713	0.0117
	Within the Groups (Duration)	15.90	416	0.03822		
ACPase	Between the Groups (Dosage)	5.106	3	1.702	5.630	0.0009
	Within the Groups (Duration)	125.7	416	0.3023		
ATPase	Between the Groups (Dosage)	26.18	3	8.728	12.75	0.0001
	Within the Groups (Duration)	284.7	416	0.6844		
SDH	Between the Groups (Dosage)	35.04	3	11.68	2.581	0.0531
	Within the Groups (Duration)	1883	416	4.527		

Table 6: Two-way ANOVA analysis.

protein values at 14 days (p<0.01) and 21 days (p<0.005). When *Allium sativum* was supplemented along with artesunate low (150 mg/kg B.wt.) and high (300 mg/kg B.wt.) dose, the animals exhibited recovery of artesunate induced toxicity and the values than were comparable to control values.

Cholesterol

When artesunate was administered at a low dose, the cholesterol levels were increased significantly at 21 days (p<0.01). Animals treated with high dose of Artesunate showed significant increase in cholesterol content of liver after 14 days (p<0.01) and 21 days (p<0.005). *Allium*

sativum administered along with artesunate did not induce toxic changes in the cholesterol levels.

Glycogen

Low dose (150 mg/kg B.wt.) treatment of artesunate did not show any significant change up to 14 days, but 21 days treatment showed significant ($p<0.01$) decrease in the glycogen content of the liver. Animals treated with high dose of Artesunate showed significant increase in glycogen levels after 14 days ($p<0.01$) and 21 days ($p<0.005$). Supplementation of *Allium sativum* did not reveal any significant alterations.

TAA, DHA and RAA

TAA values decreased significantly at 21 days ($p<0.01$) and 45 days ($p<0.005$) when Artesunate was administered at low dose. TAA activity significantly decreased at 14 days ($p<0.01$) and 21 days ($p<0.005$). *Allium sativum* administered along with low or high dose of Artesunate exhibited an insignificant decrease in the TAA values at all time-intervals studied.

DHA value estimated in Artesunate treated male mice at low dose for different time intervals did not show any significant decrease up to 14 days, but showed a significant decrease at 21 days ($p<0.01$). High dose of artesunate treatment showed a decline at 14 days ($p<0.01$) and 21 days ($p<0.005$). *Allium sativum* revealed no significant changes in the values of DHA at all time-intervals studied.

Male mice administered low dose (150 mg/kg B.wt.) artesunate treatment did not show any significant change up to 14 days, but showed decline in the RAA values at 21 days ($p<0.01$). High dose of Artesunate (300 mg/kg B.wt.) significantly decreased the RAA content of liver at 14 days ($p<0.01$) and 21 days ($p<0.005$). Administration of *Allium sativum* showed insignificant changes in the RAA values.

Alkaline phosphatase (ALKpase)

The akaline phosphatase activity estimated in artesunate treated mice (low dose) for different time intervals did not show any significant decrease up to 14 days, but revealed a significant decrease in 21 days ($p<0.01$). High dose treatment of artesunate showed significant decline in ALKpase activity at 14 days ($p<0.01$) and 21 days ($p<0.005$). *Allium sativum* supplemented with artesunate low or high dose treated animals showed non-significant decline in the ALKpase enzyme activity.

Acid phosphatase (ACPase)

When low dose of artesunate was administered, the ACPase activity was increased significantly ($p<0.01$) at 21 days. On administration of high dose of artesunate the activity of ACPase increased significantly by 14 days of treatment and continued to increase by 21 days ($p<0.005$). *Allium sativum* supplemented along with low or high dose of artesunate showed insignificant change in ACPase activity at all time-intervals.

Adenosine triphosphatase (ATPase)

Male mice treated with low dose (150 mg/kg B.wt.) of artesunate did not show any significant changes up to 14 days, but 21 days treatment showed a significant decrease ($p<0.01$) in the ATPase activity. High dose of artesunate (300 mg/kg B.wt.) showed significant decline in ATPase activity at 14 days ($p<0.01$) and 21 days ($p<0.005$). *Allium sativum,* when supplemented along with low (150 mg/kg B.wt.) and high (300 mg/kg B.wt.) dose of artesunate, showed non-significant decrease at all time-intervals indicating recovery in the disrupted enzyme activity.

Succinate dehydrogenase (SDH)

Low dose of artesunate treatment did not show any significant decrease in SDH enzyme activity up to 21 days. High dose of artesunate treatment exhibited significant decrease ($p<0.01$) by 14 days and 21 days ($p<0.005$). Supplementation of *Allium sativum* to control animals did not show any significant changes in SDH activity after all time intervals. *Allium sativum* when supplemented along with artesunate low dose or high dose showed non- significant decrease in SDH enzyme activity at all time-intervals, similar to ATPase activity levels.

Discussion

Artesunate is an alternative drug of choice to combat the resistant malarial infections. Literature shows lacunae in the toxicity implications of the drug as well as possible ameliorative means. The present investigation therefore has been to some extent do justice by finding a simple effective ameliorative agent that could minimize the artesunate drug toxicity when administration along with it.

The disease malaria was almost on the verge of disappearance but has pounced back with twice the vigour and what exacerbates the situation is the resistance of the plasmodium parasites to various antimalarial drugs [33].

A research program was set up by the Chinese army in the 1960s to find an efficient treatment for malaria. In the course of this research program, Tu Youyou discovered artemisinin in the leaves of *Artemisia annua* [34].

Artemisinin displays the most rapid action among the current drugs against *Plasmodium falciparum* malaria. It is also increasingly being used for the treatment of *Plasmodium vivax* malaria [35]. Artemisinin and other Artemisinin-based combination therapies are highly effective against malarial parasite [36-38] and hence are choice drugs for the treatment of uncomplicated and multidrug resistant malaria [39,40]. Artemisinin-based combination therapies have been introduced in virtually all countries in which malaria is endemic, thereby making such drugs the most essential class of antimalarial agents. The present study deals with artesunate which is a derivative of artemisinin. The tendency of artemisinin to accumulate in RBC and mechanism of action [41] has raised questions regarding the safety of artemisinin and its derivatives, especially artesunate, since it is rapidly gaining acceptance globally in most malaria endemic areas.

In the present investigation, artesunate toxicity on the liver was studied. The animals were treated with low artesunate dose of 150 mg/kg body weight and a high dose of 300mg/kg body weight at different time intervals of 14 days and 21days.

Considering the side effects of artesunate, such as weakness, tremors, increase in the cholesterol and the lipid levels and decrease in the protein levels, it is important to use an ameliorative agent to reduce the adverse effects. *Allium sativum* has been studied to have several ameliorative effects. It was administered to a group of artesunate-treated mice and the effects were studied.

The results of this investigation displayed reduction in the terminal body weight in animals treated with high dose of artesunate for a period of 21 days. When a dose of 300 mg/kg body weight of artesunate was administered, the body weight reduced within 14 days of treatment. Decrease in body and organ weight has been observed after anti-malarial drug treatment [42-44].

In the present investigation the total protein content decreased

when low or high dose of artesunate was administered to male mice. The decline was time and dose dependent. This could be due to the artesunate toxicity which stops the enzymatic synthesis of DNA and RNA. It forms complexes with DNA and thus does not allow DNA to act as a template for its own replication, or transcription of RNA [45].

The glycogen levels of the liver were observed to be significantly increased in dose and tissue dependent fashion. This suggests impairment of glucose metabolism. This may probably be due to the dysfunction of phosphorylase activity which results in the decreased utilization of glycogen.

Total Ascorbic Acid (TAA), Dehydro Ascorbic Acid (DHA) and Reduced Ascorbic Acid (RAA) markedly declined with low and high dose of artesunate treatment in liver. The reduction was significant with increase in time interval as well as the dose. This decline can be attributed to the concomitant decrease in the glutathione levels (GSH) noted in the investigation. Ascorbic acid and GSH are important chain breaking antioxidants that are responsible for scavenging free radicals [46]. Glutathione further converts DHA to its reduced form. Thus these findings indicate that artesunate though considered being a potentially safe drug has toxic implications causing disturbances in utilization as well as synthesis of ascorbic acid leading to alterations in metabolism as well as reduction in glutathione (GSH) observed in this study.

Results of the present investigation showed that alkaline phosphatase (ALKPase) activity declined in artesunate treated mice in a dose dependent manner, resulting in cellular toxicity and metabolic impairment in the liver.

Reduction in ATPase activity in the liver suggests reduced utilization of the ATP produced in the cell. The reduced aerobic oxidation and ATP generation might be responsible for the decrease in ATPase activity. Reductions in ATPase activity due to various toxicants have been documented [47].

At high dose administration of artesunate, the livers of all the experimental group animals were found to have significantly declined SDH activity. Similar alterations after metal toxicity have been observed by Pang *et al*. in 1996. This implies mitochondrial dysfunction and hampered oxidative metabolism due to artesunate induced toxicity.

Thus, *Allium sativum* was found to be a very potent antioxidant ameliorative agent when used along with low dose of artesunate where it could reduce the toxicity. *Allium sativum* contains at least 33 sulfur compounds, several enzymes, 17 amino acids, and minerals such as selenium [48]. It contains a higher concentration of sulfur compounds than any other *Allium* species. *Allium sativum* exhibits direct antioxidant effects and enhance the serum levels of two antioxidant enzymes, catalyses and glutathione peroxides [49]. The efficiency of *Allium sativum* was perhaps due to the presence of these sulfur containing amino acids and compounds having free carboxyl (C=0) and amino (NH_2) groups in their structures. At a higher dosage of the drug we found that to achieve nil to negligible drug toxicity one needs to increase the dosage of *Allium sativum* which was used in our study. Pharmacological research on *Allium sativum* has also shown the thiosulfinates free radical scavenging and inhibition of lipid peroxidation [50]. The antioxidant activity of *Allium sp*. has been attributed mainly to a variety of sulphur-containing compounds and their precursors [51,52].

Furthermore, the results of present investigation suggested that there was great similarity in the pattern of response to toxicant (artesunate) and ameliorative agent (*Allium sativum)* at lower dosage while at higher dose of artesunate the toxicity is severe and recovery to antioxidant administration is slow.

Thus this implies that dosage of ameliorative agent plays an important role with respect to the toxicity induced.

Conclusion

The present investigation focuses on the possible toxicity of the antimalarial drug, artesunate and shows that it is not a completely safe drug. Therefore it should be used adequately, especially in developing countries where self-medication is quite common and sometimes at dosages higher than the therapeutic doses. The toxic effects of artesunate have been successfully ameliorated by using *Allium sativum* due to its antioxidative properties. Thus, the investigation carried out is an important contribution to the field of drug toxicology.

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