IJPSR (2017), Vol. 8, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 September, 2016; received in revised form, 22 October, 2016; accepted, 17 November, 2016; published 01 March, 2017

MITIGATING POTENTIAL OF *ALLLIUM SATIVUM* ON ARTESUNATE INDUCED RENAL TOXICITY IN MALE MICE

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Keywords:

Artesunate, *Allium sativum*, Antimalarial drug, Antioxidant, Renal

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ABSTRACT: In recent times, the use of natural antioxidants as ameliorative agents has become the focus of basic research. *Allium sativum* (garlic) 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 induced renal toxicity of the antimalarial drug, artesunate, which has been used as an alternative antimalarial drug against conventional drugs like chloroquine. Thirty six male mice were divided into six experimental groups. In the present study, *Allium sativum* was given at 100 mg/kg body weight with low dose (150 mg/kg body weight) and high dose (300 mg/kg body weight) of artesunate for 14 days and 21 days duration. Obtained results showed significant alterations in gravimetric indices as well as biochemical parameters. Moreover, administration of *Allium sativum* exhibited recovery and reestablishment of various altered indices in renal tissue as opposed to artesunate treated groups. This study obviously demonstrated that treatment with *Allium sativum* significantly attenuated the renal toxicity induced by artesunate in *Mus musculus*. Thus, it can be concluded from the observed results that *Allium sativum* could be used as a potent mitigating agent against antimalarial drug toxicity.

INTRODUCTION: *Plasmodium* (*vivax* and *falciparum*) a protozoan vector is a great threat to human population for centuries in causing a disease like malaria. Malaria has now become endemic in 87 countries endangering 2.5 billion people at risk of contracting the disease. [1, 2] Basic manifestations of the disease are flu like symptoms [3] which include chills, headache, fever, hemolytic anemia and retinal damage. [4] Resistance to the classical treatment to this disease has lead to use of alternative therapies. In current scenario chloroquine is being replaced by a new drug artesunate which is a derivative of artemisinin.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(3).1459-68

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (3).1459-68

Artemisinin and its derivatives are a group of antimalarial drugs having the most rapid action among the current drugs used against *P. falciparum*. The two most widely used artemisinin derivatives are artemether and artesunate. Artesunate ($C_{19}H_{28}O_8$) is a water soluble, semisynthetic and hemisuccinate derivative of artemisinin. It is a type of plant sesquicentennial and belongs to the category of antiprotozoal agents.

The molecular weight of artesunate is 384. The antimalarial activity of artesunate depends on the endoperoxide bridge in its structure which is appeared to interact with haem in the parasite. The iron- mediated cleavage of the bridge releases a highly reactive free radical species that binds to membrane proteins, causes lipid peroxidation, damages the endoplasmic reticulum, inhibits protein synthesis and ultimately results in the lysis of the parasite. [5]

Randomized trials have supported the use of artesunate over quinine in falciparum malaria. [6] The single dose toxicity of artesunate is quite low and includes effects like reduced activity, weakness, loss of appetite, tremors etc. Other side effects include transient and reversible reticulocytopenia, drug fever, drug rash, bradycardia, first degree heart block, elevation of serum transaminases, anaemia and slow heart rate.

Various agents, natural or otherwise have been used since ancient times to induce ameliorative effects. Numerous herbs and spices used by people for seasoning of food are also known to yield useful medicinal compounds. [7]

Allium sativum (garlic) is an ameliorative agent possessing antibacterial, antiseptic, antifungal, antiparasitic, anti-coagulant, antifatulent and anti-tumor properties. [8] The immuno-modulatory properties of *A. sativum* are by increasing the production of interferon and white blood cells. Further, the herb protects cardiovascular system [9-11] and also the non-pharmacological treatment with *A. sativum* preparation is observed to reduce blood pressure in hypertensive individuals. [11] The nervous system and the brain function have been widely studied [12] as well as it possesses anticoagulant properties. [13] Some contrary reports are available where studies have shown a spermicidal effect of *Allium sativum* [14] on one hand, while others have reported its beneficial effects in the recovery of testicular functions. [15] Moreover, Allium sativum is a mood elevating and hepato-protective agent [16] which is also protective against protozoan infections [17-20] and reduces platelet aggregation. [13-21]

The active compounds of *Allium sativum* are volatile oil, sulphur, iodine, zinc, manganese, phosphorus, potassium, selenium, vitamin A, vitamins B_1, B_2 and B_6 and vitamin C. In addition to various antioxidants, it also contains more than eighty sulfur-containing compounds. Out of these substances, allicin is the most known.

Thus, the present study has been carried out with reference to the mitigating efficacy of *Allium sativum* against the toxic manifestations incurred upon by the artemisinin derivative, Artesunate on renal tissues of Swiss albino male mice.

MATERIALS AND METHODS: 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. They 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%. The experimental animals undergoing different treatments 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 an oral gavage which was attached to a hypodermic syringe.

The drug used for treatment was Artesunate obtained from Sigma-Aldrich. 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. [22] 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. Thus, the animals were categorized into six groups.

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).

TABLE 1: DOSE AND DURATION OF STUDY FOR DIFFERENT GROUPS

Groups	Treatment and Dose	Duration
A	Control (untreated) Control + distilled water	-
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 for each the durations

Extract 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. [23]

Parameters Studied:

Body and Organ weights: 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 kidneys were recorded to the nearest milligram on digital balance (Citizen, Japan) after carrying out the euthanizing.

Total Protein: Protein level in the kidneys of control and treated groups of animals was estimated by the method of Lowry et al. (1951). [24] When the protein containing preparation is treated with phenol reagent of FolinCiocalteu (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 kidney of control and all treated groups of mice were estimated by the method of Zlatkis et al. [25] 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 kidney of control and all treated groups of mice using the method of Seifter et al. [26] 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 kidney of control and all treated groups of mice by the method of Roe and Kuether. [27] Total ascorbic acid (TAA) is oxidized to dehydroascorbic acid (DHA) by Norit reagent in the presence of trichloroacetic acid (TCA). This couples with 2, 4 dinitrophenyl hydrazine to yield an 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 kidney by the method of Bessey et al. [28] 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 kidney of all treated and control mice by the method of Bessey et al. [28] Acid phosphatase, orthophosphoric monoester phosphorhydrolase catalyses the hydrolysis of p-nitorphenyl phosphate at pH 4.8, liberating 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 kidney of control and all treated groups of animals was assayed by the method of Quinn and White [29], while inorganic phosphate liberated was estimated using the method of Fiske and Subbarow. [30] 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 kidney of control and all treated groups of animals according to the method of Beatty et al [31] 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.

Statistical Analysis: All the data are shown as Mean ± SE of minimum six animals per group and maximum twelve animals per group. Statistical analysis was carried out using the SPSS software package version 16.0 (USA). Comparison between groups was done by analysis of variance (ANOVA) taking significance at $p<0.05$. The Student's t-test taking significance at ***$p<0.001$, **$p<0.005$ and *$p<0.01$ was also carried out. Tukey's honestly significance difference (HSD) post hoc test was used for comparison among different treatment groups ($p<0.05$) (**Table 5**).

RESULTS: Mice treated with low dose of artesunate (150 mg/kg B.wt.) for duration of 14 and 21 days showed no significant reduction in body weight. When a higher dose 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* were given as an antidote to the control mice, negligible variation in body weight at different time intervals was observed. *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.) exhibited negligible reduction in body weight (**Table 1, Table 3).** Low dose of artesunate (150 mg/kg B.wt.) treatment did not show any significant change up to 21 days in the weight of the kidney. High dose of artesunate showed significant decrease in kidney weight in 21 days ($p<0.005$). *Allium sativum* administered with artesunate low (150 mg/kg B.wt.) or high (300 mg/kg B.wt.) dose treated animals also showed non- significant alterations in the weight of kidney (**Table 3**).

High dose administration of artesunate significantly decreased the protein level of kidney after 14 days ($p<0.005$) and 21 days ($p<0.005$). Supplementation of *Allium sativum* to control animals did not show any significant changes in the protein level at any time intervals. *Allium sativum* supplemented along with low or high dose of artesunate exhibited insignificant decrease in protein level of kidney at all time intervals (**Table 1**, **Table 3**).

Mice receiving Artesunate treatment at low dose (150 mg/kg B.wt.) did not show any significant change up to 21 days. High dose of Artesunate treatment (300 mg/kg B.wt.) showed significant increase in Cholesterol at 21 days ($p<0.005$). *Allium sativum* when supplemented along with Artesunate low (150 mg/kg B.wt.) or high (300 mg/kg B.wt.) dose animals, showed non-significant decrease in cholesterol at all treatment durations (**Table 3**).

Glycogen content in Artesunate treated mice (low dose) for different time intervals did not show any significant increase up to 14 days, but revealed significant increase in Glycogen ($p<0.01$) at 21 days. High dose of Artesunate treatment for 14 and 21 days showed significant ($p<0.001$) and ($p<0.005$) increase in glycogen level. *Allium sativum* supplemented with Artesunate low or high dose also showed non-significant change in the Glycogen content (**Table 1**, **Table 3**).

Low dose Artesunate treatment did not show any significant change up to 14 days, but 21 days treatment showed significant decrease at ($p<0.005$) in TAA values. High dose of Artesunte when administered, showed significant decrease in TAA after 14 days ($p<0.001$) and 21 days ($p<0.005$). *Allium sativum* given with Artesunate low or high dose also showed a non significant decline in TAA values at all time intervals. Low dose of Artesunate treatment did not show any significant decrease up to 14 days, but 21 days treatment showed some significant ($p<0.005$) decrease in DHA content of kidney. High dose of Artesunate also exhibited a significant decrease in 14 days ($p<0.001$) and 21 days ($p<0.005$) treated groups. *Allium sativum* when supplemented along with Artesunate low dose and high dose treatment showed non-significant decrease in DHA activity at all time intervals. RAA values were decreased significantly

in 21 days treated groups when Artesunate was administered at low dose.

RAA activity significantly decreased at 14 days ($p<0.001$) and 21 days ($p<0.005$) on high dose administration of Artesunate. *Allium sativum* supplemented along with low or high dose of Artesunate showed insignificant change in RAA level at all time intervals (**Table 1**, **Table 3**).

Low dose of artesunate treatment did not show any significant decrease in alkaline phosphatase activity up to 21 days, whereas, high dose of artesunate treatment exhibited a significant decrease in the ALKpase activity in kidney after 14 and 21 days ($p<0.01$). *Allium sativum* when supplemented along with artesunate low dose or high dose treatment also showed non-significant decrease in ALKpase activity at all time intervals (**Table 2**, **Table 4**).

Low dose of artesunate did not show any significant change after 14 days, but 21 days treatment showed significant increase at ($p<0.01$) in ACPase enzyme activity levels. High dose of artesunte when administered, resulted in significant increase in ACPase activity of kidney after 14 days ($p<0.01$) and 21 days ($p<0.005$) time interval. *Allium sativum* given with artesunate low or high dose treated animals showed non-significant alterations in ACPase activity at all time intervals (**Table 2**, **Table 4**).

ATPase activity was decreased significantly at 21 days ($p<0.005$) when artesunate was administered at a low dose. It significantly decreased in artesunate treated group at 14 days ($p<0.001$) and 21 days ($p<0.005$). *Allium sativum* supplemented along with low or high dose of artesunate exhibited insignificant decrease in ATPase at all time intervals (**Table 2**, **Table 4**).

The activity of SDH was decreased significantly ($p<0.01$) at 21 days when artesunate was administered at low dose (150 mg/kg B.wt.) Animals treated with high dose of artesunate also showed significant decline in the enzyme activity after 14 days ($p<0.01$) and 21 days ($p<0.005$). *Allium sativum* supplemented along with artesunate low dose and high dose treated animals also showed non-significant decrease in SDH at all time intervals (**Table 2**, **Table 4**).

TABLE 2: SHOWING GRAVIMETRIC INDICES, BIOCHEMICAL AND ANTIOXIDANT PARAMETERS OF KIDNEY FOR DOSE DURATION OF 14 DAYS

Groups → Parameters ↓	A	B	C	D	E	F
Body weight	39.28±0.09	39.22±0.02NS	38.24±0.02 NS	39.24±0.02 NS	38.88±0.02 NS	38.49±0.01 NS
Tissue weight (mg)	370.8±1.47	365 ± 0.88NS	364.1±0.94 NS	358.6±1.15 NS	363.9±1.35 NS	366.5±1.14 NS
Protein (mg/100 mg tissue weight)	16.32±0.24	15.48±0.16 NS	11.63±0.23 *	16.93±0.12 NS	15.50±0.22 NS	14.57±0.17 NS
Cholesterol (mg/100 mg tissue weight)	0.51±0.01	0.54±0.02 NS	0.57±0.02 NS	0.33±0.02 NS	0.33±0.03 NS	0.32±0.03 NS
Glycogen (µg/100 mg tissue weight)	396.3±24.5	429.1±17.5 NS	602.9±12.5 *	407.8±10.8 NS	407.6±21.7 NS	401.9±15.9 NS
TAA (mg/100 mg tissue weight)	5.09±0.14	4.96±0.15 NS	3.32±0.83 *	5.11±0.23 NS	4.98±0.17 NS	4.89±0.19 NS
DHA (mg/100 mg tissue weight)	3.02±0.14	2.62±0.17 NS	2.05±0.50 *	3.07±0.23 NS	2.93±0.18 NS	2.89±0.19 NS
RAA (mg/100 mg tissue weight)	2.07±0.01	2.33±0.15 NS	1.27±0.33 *	2.04±0.009 NS	2.05±0.01 NS	2.05±0.02 NS

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

TABLE 3: SHOWING ENZYMATIC PARAMETERS OF KIDNEY 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)	0.36±0.03	0.32±0.02 NS	0.31±0.03 *	0.38±0.02 NS	0.36±0.02 NS	0.33±0.02 NS
ACPase (µ moles of p-	0.65±0.03	0.76±0.03 NS	0.85±0.05 *	0.71±0.04 NS	0.78±0.01 NS	0.76±0.01 NS

nitro phenol released / mg protein / minutes)						
ATPase (µ moles inorganic phosphate released / mg protein / 30 minutes)	3.09±0.14	2.03±0.15 NS	1.74±0.32 *	2.89±0.23 NS	2.64±0.29 NS	2.62±0.21 NS
SDH (µg formazan / mg protein / minutes)	14.25±0.27	13.31±0.41 NS	10.03±0.26 *	14.74±0.17 NS	13.41±0.26 NS	11.65±0.24 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 KIDNEY FOR DOSE DURATION OF 21 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)	364.6±0.90	355.4±1.04 NS	326.7±1.14 **	363.3±2.31 NS	362.4±1.30 NS	362.1±1.01 NS
Protein (mg/100 mg tissue weight)	16.41±0.25	13.02±0.15 NS	10.22±0.26 **	16.97±0.20 NS	14.10±0.18 NS	14.26±0.28 NS
Cholesterol (mg/100 mg tissue weight)	0.49±0.02	0.56±0.04 NS	0.61±0.01**	0.32±0.02 NS	0.32±0.03 NS	0.30±0.03 NS
Glycogen (µg/100 mg tissue weight)	398.3±21.9	586.4±28.3 *	623.7±29.1**	405.4±17.0 NS	420.6±16.1 NS	425.7±18.3 NS
TAA (mg/100 mg tissue weight)	5.05±0.13	3.74±0.74 **	3.14±0.63 **	5.09±0.17 NS	4.80±0.15 NS	4.83±0.15 NS
DHA (mg/100 mg tissue weight)	2.99±0.13	2.28±0.46 **	1.92±0.52 **	3.06±0.17 NS	2.72±0.15 NS	2.83±0.15 NS
RAA (mg/100 mg tissue weight)	2.06±0.01	1.45±0.30 *	1.22±1.05 **	2.03±0.005 NS	2.08±0.01 NS	2.04±0.008 NS

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

TABLE 5: SHOWING ENZYMATIC PARAMETERS OF KIDNEY 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)	0.38 ± 0.02	0.33 ± 0.08 NS	0.32 ± 0.05 *	0.36 ± 0.01 NS	0.34 ± 0.02 NS	0.31 ± 0.01 NS
ACPase (µ moles of p-nitro phenol released / mg protein / minutes)	0.66 ± 0.04	0.80 ± 0.03 *	0.98 ± 0.08 **	0.70 ± 0.03 NS	0.80 ± 0.01 NS	0.77 ± 0.01 NS
ATPase (µ moles inorganic phosphate released / mg protein / 30 minutes)	3.05 ± 0.13	1.54 ± 0.26 **	1.32 ± 0.28 **	2.86 ± 0.22 NS	2.60 ± 0.16 NS	2.43 ± 0.21 NS
SDH (µg formazan / mg protein / minutes)	14.46 ± 0.27	9.79 ± 0.36 *	9.67 ± 0.34 **	14.79 ± 0.19 NS	11.63 ± 0.24 NS	12.16 ± 0.23 NS

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

TABLE 6: ANOVA ANALYSIS

Parameters	**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)	5293	3	1764	13.73	0.0001
	Within the Groups (Duration)	53461	416	128.5		
Protein	Between the Groups (Dosage)	83.49	3	27.83	6.81	0.0002
	Within the Groups (Duration)	1700	416	4.087		
Cholesterol	Between the Groups (Dosage)	0.09629	3	0.0321	2.073	0.1032

	Within the Groups (Duration)	6.442	416	0.01548		
Glycogen	Between the Groups (Dosage)	195357	3	65119	8.463	0.0001
	Within the Groups (Duration)	3.201	416	7694		
TAA	Between the Groups (Dosage)	15.32	3	5.108	4.476	0.0042
	Within the Groups (Duration)	474.8	416	1.141		
DHA	Between the Groups (Dosage)	6.132	3	2.044	3.806	0.0103
	Within the Groups (Duration)	223.4	416	0.537		
RAA	Between the Groups (Dosage)	2.387	3	0.7957	1.144	0.3309
	Within the Groups (Duration)	289.2	416	0.6953		
ALKPase	Between the Groups (Dosage)	0.1297	3	0.04324	3.405	0.0177
	Within the Groups (Duration)	5.284	416	0.0127		
ACPase	Between the Groups (Dosage)	0.2063	3	0.06878	2.368	0.0702
	Within the Groups (Duration)	12.08	416	0.02904		
ATPase	Between the Groups (Dosage)	20.59	3	6.864	10.54	0.0001
	Within the Groups (Duration)	270.8	416	0.651		
SDH	Between the Groups (Dosage)	122.3	3	40.78	10.51	0.0001
	Within the Groups (Duration)	1613	416	3.878		

DISCUSSION: Recently developed resistance (in the last decade) of *Plasmodium* to the anti malarial drugs hampers effective treatment of malaria. [32-34] Artemisinin displays the most rapid action among the current drugs against *Plasmodium falciparum* as well as *Plasmodium vivax* induced malaria. [35]

Further, Artemisinin and its derivatives have been observed to exhibit potency against the asexual and erythrocytic forms of *P. falciparum* and *P. vivax*. [36] The present investigation dealt with the artesunate induced renal toxicity in male albino mice using low (150 mg/kg body weight) and high dose (300 mg/kg body weight) of artesunate at different time intervals (14 and 21 days). Further, amelioration of this toxicity was observed by using *Allium sativum* (100 mg/kg body weight) along with the anti malarial drug.

The results showed reduction in the body weight, organ weight as well as reduction in the total protein content of the tissue after artesunate treatment (low and high doses) at both the time intervals (14 and 21 days). This reduction can be attributed to reduced food consumption, appetite loss as well as disturbed protein metabolism. Moreover, Meyer et al., (1976) [37], have reported that there is a reduction which probably hinders the enzymatic synthesis of DNA as well as RNA; hence the changes in the gravimetric indices.

The decline in the body/organ weight post anti malarial drug treatment has been reported by Dattani et al. [38] Further, similar pattern of reduction in absolute or relative weight of an organ after administration of a chemical or drug indicates toxic influences. [39] To combat the toxicity caused by the anti malarial drug, *Allium sativum* was used as an ameliorative agent. It was administered along with the artesunate dose (low and high at both the time intervals) and it was noted that there were negligible changes in the gravimetric parameters (non-significant) indicating that *A. sativum* is an effective ameliorative agent.

The glycogen content of the renal tissue was elevated post-artesunate treatment and showed non-toxic influence when anti-malarial drug was administered with *Allium sativum* (ameliorative agent) especially at lower dose of artesunate. The higher dose still showed mild toxicity (non-significant). The kidneys are known to have capacity of glycogen storage in very less amounts. Thus, the observed storage/increase in glycogen is probably due to the disruption of glucose metabolism. Similar reports by Lei et al [40] show disruption of G6Pase and concomitant GSD (Glycogen Storage Disease). Further, post Artesunate treatment increase in hepatic glycogen has also been observed. [16] Total Ascorbic Acid, TAA, Dehydro Ascorbic Acid (DHA) as well as Reduced Ascorbic Acid (RAA), showed significant decline with artesunate treatment. The reduction was significant with increase in time duration as well as the dose in the renal tissue. Reduction in ascorbic acid metabolism could be due to the decrease in GSH as well as ascorbic acid as these are the vital chain breaking antioxidants responsible for scavenging free radicals. [41] Phosphatases are required in specific amounts in the tissues for proper functioning of the organs. [42]

The present investigation revealed that the alkaline phosphatase activity declined with artesunate toxicity while acid phosphatase activity increased in dose and time dependent manner in the kidneys. A similar increase in the acid phosphatase activity in the cardiac tissue of rats after administration of amodiaquine and its co-administration with artesunate has been reported by Malomo et al. [43] The observed reduction in ATPase activity levels probably indicates disturbance in the utilization of ATP or its production. The study also showed that the impairment was more pronounced with high dose of artesunate. Reduction in ATPase is also noted after metal toxicity. [44, 45] Moreover, alterations in the heme and mitochondrial activity due to artesunate treatment *in vitro* has been also reported by Mercer et al. [46]

The present study also showed decline in SDH activity at high dose artesunate treatment. This further supports the presumed mitochondrial dysfunction due to said drug toxicity and lowered oxidative metabolism. Such a decline could be due to structural disorganization of mitochondria and rupturing of the cristae. Disruptions in biochemical parameters post artesunate treatment have also supportive histological findings. [47] The changes in the histoarchitecture due to artesunate toxicity in renal tissue were prevented when the drug was given along with *Allium sativum*.

Taking into consideration to avoid toxicity on renal tissue of mice, the use of ameliorative options was mandatory. Thus, in the present study, *Allium sativum* was used as an antidote which has gained popularity as a powerful antidote to oxidative stress. [48] *Allium sativum* is also widely used in infectious disease treatments. [19] The results obtained in our laboratory showed that *Allium sativum* was able to prevent the Artesunate toxicity in majority of parameters. Further, it was noted that high dose Artesunate toxicity was not totally prevented by 100 mg/kg *Allium sativum* implicating that the ameliorative agent dose needs to be reevaluated by increasing the dosage to combat high dose toxicity of Artesunate.

CONCLUSION: Contrary to the available literature the outcome of the present investigation emphasizes on artesunate toxicity and raises a question to it's so called 'safe-drug' image. This is of great concern due to its use as alternative drug to chloroquine resistant malaria. The results of the study clearly points towards amelioration of renal toxicity by *Allium sativum*. The use of *Allium sativum* has hence proved to be mitigating and if it is used along with the antimalarial drug (Artesunate) treatment, one can probably lower or prevent the drug induced toxicity.

ACKNOWLEDGMENTS: We acknowledge the Departmental head and staff of Statistics for their help in the statistical analysis of the data.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect tothe research, authorship, and/or publication of this article.

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How to cite this article:
Desai KR, Rajput DK, Patel PB, Pandit JP and Highland HN: Mitigating potential of *Alllium sativum* on artesunate induced renal toxicity in male mice. Int J Pharm Sci Res 2017; 8(3): 1459-68.doi: 10.13040/IJPSR.0975-8232.8(3).1459-68.