International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 8 | Issue 2 | APR-JUN | 2018 | 693-704
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

LONG TERM TOXIC EFFECTS OF ARTESUNATE ON SEMEN PARAMETERS AND ITS MITIGATION IN SWISS ALBINO MALE MICE

[*1]K. R. Desai, D. K. Rajput, P. Patel, Z. Siddiqui and H. N. Highland

[*1]Professor (M.Sc. PhD), [2]Research Scholar (M.Sc. PhD), Dept. of Zoology, Gujarat University, Gujarat University, School of Sciences, Department of Zoology, Ahmedabad-380009, Gujarat, India
[3]Research Scholar (M.Sc. PhD (ongoing)), Dept. of Zoology, Gujarat University, Gujarat University, School of Sciences, Department of Zoology, Ahmedabad-380009, Gujarat, India
[4]Research Scholar (M.Sc. PhD (ongoing)), Dept. of Zoology, Gujarat University, Gujarat University, School of Sciences, Department of Zoology, Ahmedabad-380009, Gujarat, India
[5]Professor (M.Sc. PhD), Dept. of Zoology, Gujarat University, Gujarat University, School of Sciences, Department of Zoology, Ahmedabad-380009, Gujarat, India

[*]Corresponding Author Email: ketalak60@yahoo.co.in

ABSTRACT

The safety over the use of antimalarial drug is day by day rising and is extensively studied topic as the parasite has sternly become resistant to it. It has thus become imperative to find an effective solution which is safe to administer and is easily available for the target population, especially those living in the tropical countries. Allium sativum is one such native indigenous species and is an integral part of diet since ages. This work focuses upon Artesunate (150 and 300 mg/kg body weight) toxicity for a period of 21 and 45 days on cauda epididymis and seminal vesicle of mice and evaluating the effect of garlic (150mg/ kg body weight) as an effective antidote. Artesunate lowered the organ weight of both the test tissues, decreased fructose levels, impaired sperm parameters and also declined testosterone levels. The current findings provide a valuable insight in the antimalarial drug toxicity.

KEY WORDS

Allium sativum; Antimalarial drug; Artesunate toxicity; Indigenous

1. INTRODUCTION

From the time of chloroquine introduction to European medicine in the 1630s until its deployment in the 1950s, quinine was the mainstay of severe malaria treatment. Resistance to chloroquine emerged in Southeast Asia and then spread to Africa at the end of the 1970s[1].

Treatment failures have been linked majorly to the development of resistance of the malaria parasite to standard antimalarial agents [2,3,4]. This resistance created a need for new drugs. Discovery of artemisinin, earlier known as Qinghaosu, is a phytoconstituent obtained from the aerial part of Chinese medicinal herb *Artemisia annua,* commonly known as wormwood have given renewed hope for combating resistant malaria [5,6].

Since artemisinin itself has poor bioavailability limiting its effectiveness, several semisynthetic derivatives of artemisinin have been developed (Fig. 1). The best known among these derivatives are oil soluble artemether, water-soluble artesunate andartenimol (β-dihydroartemisinin) [7]. The most potent among them is water soluble artesunate, which can be administered parenterally as well as by mouth and per rectum.

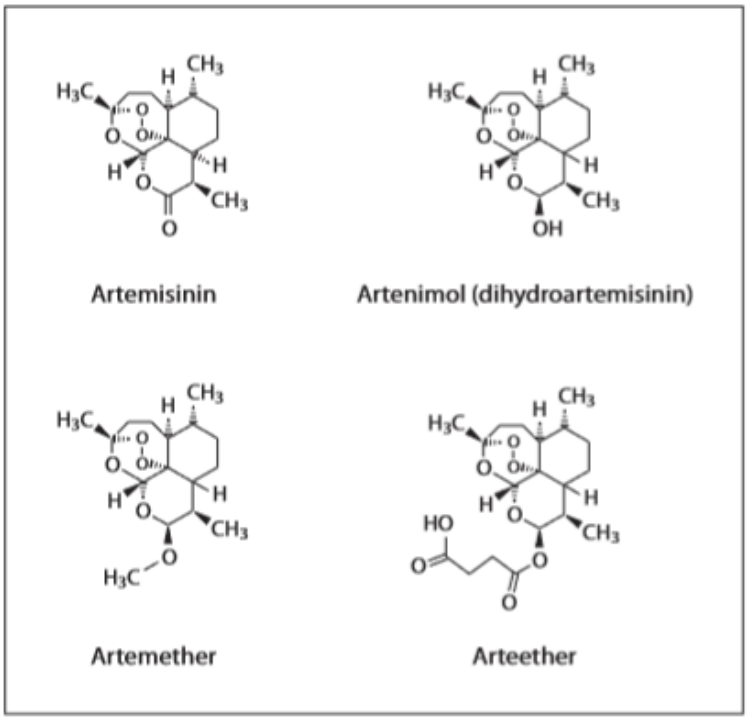

Fig. 1. Structural formulae for artemisinin and its derivatives [7]

Artesunate is an effective antimalarial drug, an alkylating agent which generates free radicals, thus, alkylating the parasite's membrane [8] and is used in combination therapies.

Since artemisinin's are potent and rapidly acting drugs to which there have been few cases of resistance [9,10,11], they have become one of the most important groups of drugs available to treat malaria. The World Health Organization (WHO) has recommended that artemisinin-containing combination therapies should be used to treat *P. falciparum* in areas of drug resistance [12]. Also, it has been reported that Artesunate reduces mortality by more than 34% when compared to quinine in adults with severe malaria [13].

So, by now it is an established fact that artemisinin-based drugs are way more efficient than the previously used conventional drugs like chloroquine.

Furthermore, previous reports have described artemisinin derivatives to be generally safe and well-tolerated [14,15]. However, there are concerns about their potentials for neuro- and reproductive toxicities [16-20]. In addition, previous toxicological studies on artemisinins evaluated their effects on specific systems, limiting such studies to make quantitative safety evaluations of these drugs [18,19].

Nevertheless, the recent approach on the development of new drugs from natural products for treatment of human diseases especially in developing countries still rely on traditional medicines for their primary health care based largely from various species of plants [21].

There are so many plants in the wild which have the calibre to revive or repair the damage caused by these antimalarial drugs. The potential candidates which we will be exploring here are *Allium sativum* and *Curcuma longa*, which have been used in folk medicine since ages.

Medical and pharmacological properties of *Allium sativum*, commonly known as Garlic belonging to family Lilliaceae are known to Hindus for centuries and documented in Ayurveda [22, 23]. Garlic has been proved to elicit antimicrobial [24], antihypertensive [25], hypolipidemic [26], hepatoprotective [27], antidiabetic[28], antitumor [29], antifungal[30] and insecticidal[31] properties.

Similarly, one cannot deny the innumerable health benefits which is obtained from Curcumin, which is isolated from the roots of the *Curcuma longa* plant and has been shown to regulate a number of biological responses [32]. In recent years, a wide biological and pharmacological property of curcumin has been extensively studied such as antioxidant, anti-inflammatory, antimicrobial, antimalarial, and anticarcinogenic activities [33-36].

The present study is therefore undertaken to gauge the effect of artesunate on semen parameters in rodent

model i.e. Swiss albino mice and also to determine the mitigating potential of garlic and curcumin on the same.

2. MATERIALS AND METHODS

2.1 Animals

Healthy adult male albino mice, Musmusculus of Swiss strain weighing between 30 and 35 gm, were obtained from Cadila Pharmaceuticals, Dholka, Gujarat, India. All the animals were acclimatized 7days prior to the commencement of treatment and were maintained under controlled condition with 12-hour light and 12-hour dark cycles at temperature of 26°C ± 2°C and relativehumidityof30%to70%. They were divided into 9 groups (A, B, C, D, E, F, G, H and I) of 5 mice each. The animals were fedona commercial pellet supplied by Amrut mice feed (Pranav Agro Industries, Vadodara, Gujarat, India) and water ad libitum. Experiments were conducted in accordance with the guidelines set by the CommitteeforthePurposeofControlandSupervisionofExperimentsonAnimals(CPCSEA), India, and experimental protocols were approved by the Institutional Animals' Ethics Committee (167/1999/CPCSEA).

2.2 Experimental design

The experimental design has been depicted in the table showing experimental protocol included:

A. Studies on the effect of artesunate at two dose concentrations were carried out and compared with control (untreated) mice.

B. Ameliorative studies were carried out using Curcumin and garlic as supplements with low and high dose of artesunate respectively and compared with artesunate treated and control mice.

2.3 Experimental protocol

The animals were divided into following groups:

GROUPS	TREATMENT AND DOSE	DURATION (DAYS)	DAY OF AUTOPSY
A	Control (untreated) Control +distilled water	-	Sacrificed along with scheduled treated animals
B	Artesunate (Low dose) (150 mg/kg body weight)	21, 45	22th, 46th Post treatment
C	Artesunate (High dose) (300 mg/kg body weight)	21, 45	22th, 46th Post treatment
D	Curcumin (80 mg/kg body weight)	21, 45	22th, 46th Post treatment
E	Garlic (100 mg/kg body weight)	21, 45	22th, 46th Post treatment
F	150 mg/kg Artesunate (L.D) + 80 mg/kg Curcumin	21, 45	22th, 46th Post treatment
G	300 mg/kg Artesunate (H.D) + 80 mg/kg Curcumin	21, 45	22th, 46th Post treatment
H	150 mg/kg Artesunate (L.D) + 100 mg/kg Garlic	21, 45	22th, 46th Post treatment
I	300 mg/kg Artesunate (H.D) + 100 mg/kg Garlic	21, 45	22th, 46th Post treatment

(n=6 animals/group)

2.4 Toxicant and dose selection

The increasing use of artesunate as antimalarial drug in recent years has demanded the necessity to evaluate its reproductive toxicity.

Artesunate was prepared in double-distilled water and orally given to mice via feeding canula with a hypodermic syringe. All the doses for artesunate were derived from its equivalent therapeutic dose for the treatment of uncomplicated malaria [37]. All the other chemicals used in different assays were procured from HI Media and Merck, Mumbai.

The dose for Curcumin is based on earlier work done in Annamalai University, Tamilnadu, India [38]. Crude extract of garlic (*Allium sativum*) of the single clove variety was prepared from bulbs purchased in bulk from the market. The cloves were sliced into pieces, ground into a paste and then dissolved in deionised distilled water. The concentration of the extract prepared was 10 mg garlic per 1 ml, corresponding to 100 mg of garlic

per kg body weight of the animal, this concentration being calculated on the basis of a daily human intake of 6.00 gm garlic by a 60 kg human individual [39].

2.5 Tissue collection

Artesunate was administered as per the experimental protocol. At the end of each treatment, animals were euthanized and necropsied according to the CPCSEA guidelines and caudaepididymis and seminal vesicle were carefully dissected out and weighed. Tissue was then processed for biochemical evaluation.

2.6 Parameters studied

2.6.1 Fructose

Fructose levels were assayed in seminal vesicle of control and treated groups of mice by the method of Foreman et al. (1973) [40].

A known amount of tissue was homogenized in 5 ml of 5% Perchloric acid. 0.2 ml of homogenate was followed by 1.8 ml of 5% Perchloric acid in sample tube while blank tube was run with 2 ml of 5% Perchloric acid. 3 ml of 30% HCL was added to all the tubes and heated in water bath at 80° C for one hour and cooled at room temperature.

The colour intensity was read on Systronics Digital Spectrophotometer 167 at 410 nm against blank tube.

2.6.2 Sperm Parameters

2.6.2.1 Sperm count:

Sperm count in cauda epididymis of control and all different groups of mice was determined using the Neubauer chamber of haemocytometer according to method of Prasad et al. (1972) [41].

2.6.2.2 Sperm motility:

The assessment of sperm motility (in percentage) in cauda epididymis of control and all different groups was carried out according to the method of Prasad et al. (1972) [41].

2.6.2.3 Sperm viability:

Sperm viability (Live: Dead ratio) of caudaepididymal sperms was estimated by the method of Talbot and Chacon (1981) [42].

2.6.3 Testosterone

The coated micro wells and test components were brought to room temperature before the assay. The coated wells were marked as recorded in data sheet. 50 µl of each reference standard, control and test samples were pipette in to the labelled wells using micropipette. 50 µl of enzyme conjugate was added to each well and gently shaken for 20 seconds, sealed with parafilm and incubated at 37°C for 60 minutes. After incubation these wells were decanted and rinsed with wash buffer three times each. The wells were dried by firm tapping to ensure removal of wash solution. 50 µl each of substrates hydrogen peroxide and 50 µl of TMB reagent were added to each well for the enzymatic- reaction. The wells were shaken for 20 seconds and incubated at 25° C for 15 minutes. Reactions were stopped by adding 50 µl of 1 N $H_2SO_4$to each well. The colour developed after mixing was read at 450 nm on a Merck Elisa Reader.

2.7 Statistics analysis

All the data are presented as mean + standard error. Statistical analysis was performed using the trial version of SPSS software package version 16.0. Comparison between the groups was made by 1-way analysis of variance (ANOVA) followed by Student't' test taking significance at $p< 0.05$.Tukey honestly significance difference post hoc test was used for comparison among different treatment groups ($p< 0.05$).

3. RESULTS

3.1 Organ Weight

The weight of both the organs i.e. Cauda epididymis and Seminal vesicle were found to be unaltered after 21 and 45 days treatment in control mice.

The weight of Cauda epididymis was significantly reduced ($p<0.005$) after the administration of low dose of artesunate in both the durations. Similarly, the weight was significantly reduced ($p<0.005$) after 21 days and highly significantly reduced ($p<0.001$) after 45 days, when high dose of artesunate was administered. However, when the antidotes i.e. Curcumin and Garlic were administered individually, the organ weight was found to be non-significant in both the durations. Also, when low and high dose of artesunate were administered with Curcumin and Garlic respectively, the results were found to be non-significant in both the durations (Table 1).

Table 1. Organ weight of Cauda and seminal vesicles (mg) of control and treated animals after 21 and 45 days treatment respectively.

Cauda epididymis			
Groups	**Treatment**	**21 days**	**45 days**
A	Control (untreated)	16.26 ± 0.02	16.23 ± 0.03
B	ART 150 mg	13.29 ± 0.03**	11.32 ± 0.03**
C	ART 300 mg	11.32 ± 0.03**	9.38 ± 0.02***
D	Curcumin	16.78 ± 0.03	17.03 ± 0.06
E	Garlic	16.52 ± 0.02	16.86 ± 0.13
F	ART 150 mg + Curcumin	14.62 ± 0.12	14.92 ± 0.13
G	ART 300 mg + Curcumin	14.85 ± 0.13	15.05 ± 0.21
H	ART 150 mg + Garlic	14.41 ± 0.03	15.39 ± 0.03
I	ART 300 mg + Garlic	14.43 ± 0.02	14.88 ± 0.09

Seminal Vesicles			
Groups	**Treatment**	**21 days**	**45 days**
A	Control (untreated)	379.26 ± 0.02	379.23 ± 0.03
B	ART 150 mg	370.34 ± 0.02	368.32 ± 0.03**
C	ART 300 mg	368.31 ± 0.03**	363.37 ± 0.02***
D	Curcumin	368.31 ± 0.03	363.37 ± 0.02
E	Garlic	379.54 ± 0.03	379.56 ± 0.04
F	ART 150 mg + Curcumin	376.54 ± 0.03	376.64 ± 0.13
G	ART 300 mg + Curcumin	373.43 ± 0.04	373.44 ± 0.04
H	ART 150 mg + Garlic	374.41 ± 0.03	374.46 ± 0.03
I	ART 300 mg + Garlic	377.44 ± 0.04	377.45 ± 0.02

Values are mean ± S.E., *p<0.01, **p<0.005, ***p<0.001

Table 2. Showing the Fructose level (µg/mg tissue weight) in seminal vesicle of control and treated mice after 21 and 45 days treatment.

Groups	**Treatment**	**21 days**	**45 days**
A	Control (untreated)	11.45 ± 0.03	11.33 ± 0.02
B	ART 150 mg	8.52 ± 0.12*	6.63 ± 0.10**
C	ART 300 mg	6.55 ± 0.06**	5.41 ± 0.03***
D	Curcumin	12.99 ± 0.16	13.28 ± 0.20
E	Garlic	10.97 ± 0.11	11.04 ± 0.08
F	ART 150 mg + Curcumin	10.76 ± 0.09	10.82 ± 0.07
G	ART 300 mg + Curcumin	10.26 ± 0.04	10.31 ± 0.04
H	ART 150 mg + Garlic	9.85 ± 0.16	9.76 ± 0.15
I	ART 300 mg + Garlic	10.90 ± 0.17	11.10 ± 0.21

Values are mean ± S.E., *p<0.01, **p<0.005, ***p<0.001

Table 3. Showing the Cauda epididymal Sperm Count (10^6/ml) in control and treated mice after 21 and 45 days treatment.

Groups	Treatment	21 days	45 days
A	Control (untreated)	35.20 ± 1.10	36.31 ± 0.67
B	ART 150 mg	28.86 ± 0.45*	26.95 ± 0.41**
C	ART 300 mg	25.72 ± 0.42 **	25.32 ± 0.30***
D	Curcumin	45.11 ± 0.69	46.24 ± 0.86
E	Garlic	39.67 ± 0.27	41.30 ± 0.89
F	ART 150 mg + Curcumin	33.26 ±0.46	31.91 ± 0.50
G	ART 300 mg + Curcumin	29.89 ± 0.47	28.65 ± 0.26
H	ART 150 mg + Garlic	36.02 ± 0.42	34.65 ± 0.82
I	ART 300 mg + Garlic	31.68 ± 0.41	28.99 ± 0.10

Values are mean ± S.E., *p<0.01, **p<0.005, ***p<0.001

Table 4. Showing the Cauda epididymal Sperm motility (%) in control and treated mice after 21 and 45 days treatment.

Groups	Treatment	21 days	45 days
A	Control (untreated)	73.84 ± 0.53	73.35 ± 0.24
B	ART 150 mg	62.82 ± 0.60 *	57.53 ± 0.40**
C	ART 300 mg	57.43 ± 0.41 **	56.32 ±0.74 ***
D	Curcumin	79.48 ± 0.28	79.99 ± 0.54
E	Garlic	70.50 ± 0.37	70.55 ± 0.37
F	ART 150 mg + Curcumin	67.61 ± 0.41	66.02 ± 0.40
G	ART 300 mg + Curcumin	62.43 ± 0.50	59.11 ± 0.92
H	ART 150 mg + Garlic	70.02 ± 0.16	68.82 ± 0.11
I	ART 300 mg + Garlic	68.35 ± 0.37	67.56 ± 0.42

Values are mean ± S.E., *p<0.01, **p<0.005, ***p<0.001

Table 5. Showing the Cauda epididymal Sperm Viability (%) in control and treated mice after 21 and 45 days treatment.

Groups	Treatment	21 days	45 days
A	Control (untreated)	65.94 ±0.13	62.79 ± 0.61
B	ART 150 mg	56.99 ± 0.95*	56.32 ± 0.74*
C	ART 300 mg	54.09 ± 0.10**	52.72 ± 0.20***
D	Curcumin	75.14 ± 0.34	75.94 ± 0.12
E	Garlic	70.49 ± 0.38	70.56 ± 0.37
F	ART 150 mg + Curcumin	62.58 ± 0.65	63.07 ± 0.68
G	ART 300 mg + Curcumin	59.03 ±0.20	58.08 ± 0.15
H	ART 150 mg + Garlic	70.01 ± 0.16	68.89 ± 0.13
I	ART 300 mg + Garlic	68.32 ± 0.37	67.53 ± 0.42

Values are mean ± S.E., *p<0.01, **p<0.005, ***p<0.001

Table 6. Showing alterations in testosterone level (ng/ml) in serum of control and treated mice after 21 and 45 days treatment.

Groups	Treatment	21 days	45 days
A	Control (untreated)	3.26 ± 0.02	3.23 ± 0.03
B	ART 150 mg	1.29 ± 0.03 *	0.82 ± 0.13 **
C	ART 300 mg	0.87 ± 0.19 **	0.41 ± 0.02 ***
D	Curcumin	3.79 ± 0.03	4.01 ± 0.18
E	Garlic	2.54 ± 0.03	2.56 ± 0.04
F	ART 150 mg + Curcumin	2.22 ± 0.04	2.06 ± 0.21
G	ART 300 mg + Curcumin	2.09 ± 0.19	2.04 ± 0.17
H	ART 150 mg + Garlic	1.99 ± 0.20	1.87 ± 0.18
I	ART 300 mg + Garlic	1.45 ± 0.04	1.42 ± 0.02

Values are mean ± S.E., *p<0.01, **p<0.005, ***p<0.001

When low dose of artesunate was administered the Seminal vesicle, weight was found to be non-significant after 21 days treatment. However, it was significantly reduced (p<0.005) after 45 days. The weight was significantly reduced (p<0.005) after 21 days and highly significantly reduced (p<0.001) after 45 days after the administration of high dose of artesunate. Curcumin and Garlic when administered alone and in addition with low and high dose of artesunate respectively gave non-significant results in both the durations (Table 1).

3.2 Fructose levels

As shown in Table 2, fructose levels were found to be significantly reduced (p<0.01) after 21 days and moderately significantly reduced (p<0.005) after 45 days when low dose of artesunate was administered. Reduction was moderately significant (p<0.005) after 21 days and highly significant (p<0.001) after 45 days when high dose was administered.

Curcumin and Garlic when administered alone and in addition with low and high dose of artesunate respectively gave non-significant results in both the durations.

3.3 Sperm Parameters

3.3.1 Sperm count:

When compared with control, sperm count was found to be significantly reduced (p<0.01) after 21 days and moderately significantly reduced (p<0.005) after 45 days when low dose of artesunate was administered. Reduction was moderately significant (p<0.005) after 21 days and highly significant (p<0.001) after 45 days when high dose was administered (Table 3).

Curcumin and Garlic when administered alone and in addition with low and high dose of artesunate respectively gave non-significant results in both the durations.

3.3.2 Sperm motility:

As shown in Table 4, when compared with control, sperm motility was found to be significantly reduced (p<0.01) after 21 days and moderately significantly reduced (p<0.005) after 45 days when low dose of artesunate was administered. Reduction was moderately significant (p<0.005) after 21 days and highly significant (p<0.001) after 45 days when high dose was administered.

Curcumin and Garlic when administered alone and in addition with low and high dose of artesunate respectively gave non-significant results in both the durations.

3.3.3 Sperm viability:

Sperm viability was found to be significantly reduced (p<0.01) in both the durations when low dose of artesunate was administered. Reduction was moderately significant (p<0.005) after 21 days and highly significant (p<0.001) after 45 days when high dose was administered (Table 5).

Curcumin and Garlic when administered alone and in addition with low and high dose of artesunate respectively gave non-significant results in both the durations.

3.4 Testosterone levels

Testosterone levels were found to be significantly reduced (p<0.01) after 21 days and moderately significantly reduced (p<0.005) after 45 days when low dose of artesunate was administered. Reduction was moderately significant (p<0.005) after 21 days and highly significant (p<0.001) after 45 days when high dose was administered (Table 6).

Curcumin and Garlic when administered alone and in addition with low and high dose of artesunate respectively gave non-significant results in both the durations.

4. DISCUSSION

The rediscovery of qinghaosu (artemisinin) in China in 1972 [43]and the subsequent synthesis of artemether and artesunate have provided highly effective alternatives to previously used conventional drug as quinine [44]. And among them, Artesunate, a semisynthetic derivative of artemisinin, is significantly more efficient in the treatment of malaria than other derivatives [45].

However, the effectiveness of artesunate is not worthy at the cost of generating toxicity, which is supported by vast number of literature [46-53].

The current study was designed to determine the effect of artesunate on reproductive tissues *viz*. caput epididymis and seminal vesicle and simultaneously to check the efficacy of chosen antidotes (Allium and Curcumin) on the same.

In the present study the weight of both the assessed organs i.e. Cauda epididymis and Seminal vesicle was significantly reduced in both the duration of 21 days and 45 days after the antimalarial drug (Artesunate) administration. Reduction in organ weight has also been recorded by other workers [54,55,56]and it may be due to loss of appetite or obstructive effect on hunger centre due to drug toxicity.

It is a well-established fact that during the process of spermatogenesis, fructose plays a vital role in providing energy to the developing sperm. In the present study, fructose levels in the seminal vesicle were declined to a remarkable extent when high dose of artesunate treatment was given during both the time intervals, which suggested that the metabolism was affected by the treatment of artesunate. The results can be correlated with decreased sperm count and motility from cauda epididymis, explaining damage induced by artesunate in a dose dependent manner. Mathur et al. (2010) [57] also showed decreased fructose levels relatively altered spermiogram after treatment of Tecomastans leaves ethanolic extract, which were in consistent with our results.

The present investigation elucidated that artesunate toxicity resulted in marked decline of caudaepididymal sperm motility, viability as well as count. Abnormality in spermatozoa with reduction in testicular weight was also noted by [18,19,56].

The testosterone levels were also found to be significantly reduced in mice treated with low and high dose artesunate at time interval of 45 days. Similar results were obtained by [58] where administration of $CdCl_2$ significantly lowered the enzyme activities of 3β-HSD and 17β-HSD which was associated with decreased serum testosterone level. This testosterone secretion in turn can be impaired as a result of oxidation insult (stress) and consequent Leydig cell degeneration, which was reported Khan et al., 2006 [59].

In 2011, Prasad et al. [60] has also documented similar findings where Imatinib affected testicular function and reduced testosterone levels probably due to increased ROS production and dysfunction of Leydig cells.

The problem of malaria is mostly associated with poor countries where there is little or no access to modern medicine due to lack of resources, and hence the only option for management of this situation is to rely on traditional medicine which is affordable and easily accessible.

Medicinal plants, since time immemorial, have been used in virtually all cultures as a source of medicine [61]. Both*Allium sativum* and Curcuminare used in the traditional system of medicine since ages and have large number of biological health responses. Both these folk medicines possess plethora of valuable phytochemicals which make them essential applicant to overcome the antimalarial toxicity. The literature [62-65] also validates them as having antimalarial, antiparasitic and antiprotozoal activity.

In the present study, when artesunate is given with either of the antidote i.e. Allium or Curcumin, the data implies that the use of antidotes Curcumin and Allium have been successfully able to combat the drug (artesunate) toxicity.

Literature has also provided enough documentation on the antioxidant potential of both Curcumin as well as Allium, to combat the damage caused by free radicals. The presence of an enol group with intramolecular hydrogen bonds enhances the radical scavenging activity of curcumin [66]. Moreover, curcumin enhances the activity of detoxifying enzymes like Glutathione-S-transferase [67]. Banerjee et al., (2003) [68] reported that garlic as an antioxidant has the potential to modulate the ROS.

Recent studies in the laboratory have shown that curcumin from turmeric has antimalarial activity [64]. Moreover, Curcumin loaded hydrogel nanoparticles synthesized by solvent emulsion-evaporation technique also exhibits anti-malarial efficiency [69]. Also, Curcumin

has been shown in various animal models and human studies to be safe even used at very high doses (12 g/day) [70,71,72].

Nandakumar and co-workers (2006) [65] reported that the combination therapy of curcumin and artemisinin was effective in removing the drug resistance caused by *P. falciparum* infected malaria. Also, Curcumin when used on cerebral malaria (CM) mice model, was able to prevent CM and delay death of animals by about 10 days [73].

Quercetin, genistein, eugenol and terpeneol can be considered as adjuvants to increase the bioavailability of curcumin as these compounds can modulate the activity/permeability of curcumin [74]. A study reported that Quercetin, a flavonoid present in garlic reduces inflammation, haemozoin effects on cytokine production from monocytes and reduces drug (quinine) toxicity in malaria patients [75,76,77].

Allicin, a naturally occurring compound generated when garlic cloves are crushed, can inhibit malaria infection. Previous studies with allicin have shown that it has inhibitory effects on a wide range of bacteria, as well as some fungi and a few protozoans [78-85]. Also, it was found that ajoene, an organosulphur compound found in garlic has an inhibitory effect on the erythrocytic stages of Plasmodium [86].

5. CONCLUSION

It can be concluded from the present work that since time immemorial traditional medicines have played an integral part in the lives of human beings, especially those colonizing in rural areas, and facing troubles due to lack of resources. As medicinal plants like curcumin and *Allium* are indigenous species, effortlessly reachable and one can easily afford these agents to get protection from the damage caused due to action of antimalarial drugs. Hence, it is advisable to include both the above-mentioned components to overcome the detrimental effects from the antimalarial therapy, though further validation of these studies would provide valuable insight.

Acknowledgement:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration statement:

The manuscript has been read and approved by all the authors, that the requirements for authorship as stated earlier in this document have been met, and that each author believes that the manuscript represents honest work.

References

[1] Dondorp A.M., Fanello C.I., Hendriksen I.C., Gomes E., Seni A., Chhaganlal K.D., Bojang K., Olaosebikan R., Anunobi N., Maitland K. and Kivaya E., Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial. *The Lancet*, *376*(9753), pp.1647-165, (2010).

[2] Ronn A.M., Msangeni H.A., Mhina J., Wernsdorfer W.H. and Bygbjerg I.C., High level of resistance of Plasmodium falciparum to sulfadoxine-pyrimethamine in children in Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *90*(2), pp.179-181, (1996).

[3] Ogutu B.R., Smoak B.L., Nduati R.W., Mbori-Ngacha D.A., Mwathe F. and Shanks G.D., The efficacy of pyrimethamine-sulfadoxine (Fansidar®) in the treatment of uncomplicated Plasmodium falciparum malaria in Kenyan children. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *94*(1), pp.83-84, (2000).

[4] Djimdé A., Doumbo O.K., Cortese J.F., Kayentao K., Doumbo S., Diourté Y., Coulibaly D., Dicko A., Su X.Z., Nomura T. and Fidock D.A., A molecular marker for chloroquine-resistant falciparum malaria. *New England journal of medicine*, *344*(4), pp.257-263, (2001).

[5] Harinasuta T. and Karbwang J., Qinghaosu: a promising antimalarial, (1994).

[6] Hien T.T., An overview of the clinical use of artemisinin and its derivatives in the treatment of falciparum malaria in Viet Nam. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *88*, pp.7-8, (1994).

[7] Medhi B., Patyar S., Rao R.S., Ds P.B. and Prakash A., Pharmacokinetic and toxicological profile of artemisinin compounds: an update. *Pharmacology*, *84*(6), pp.323-332, (2009).

[8] Heppner D.G. and Ballou W.R., Malaria in 1998: advances in diagnosis, drugs and vaccine development. *Current opinion in infectious diseases*, *11*(5), pp.519-530, (1998).

[9] Wongsrichanalai C. and MeshnickS.R., Declining artesunate-mefloquine efficacy against falciparum malaria on the Cambodia–Thailand border. *Emerging infectious diseases*, *14*(5), p.716, (2008).

[10] Lim P., Alker A.P., Khim N., Shah N.K., Incardona S., Doung S., Yi P., Bouth D.M., Bouchier C., Puijalon O.M. and Meshnick S.R., Pfmdr1 copy number and arteminisin derivatives combination therapy failure in falciparum malaria in Cambodia. *Malaria journal*, *8*(1), p.11, (2009).

[11] Rogers W.O., Sem R., Tero T., Chim P., Lim P., Muth S., Socheat D., Ariey F. and Wongsrichanalai C., Failure of artesunate-mefloquine combination therapy for uncomplicated Plasmodium falciparum malaria in southern Cambodia. *Malaria journal*, *8*(1), p.10, (2009).

[12] World health organization guidelines for the treatment of malaria. (2006, accessed 3 February 2012).

[13] Malaria, S.E.A.Q.A., Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. *The Lancet*, *366*(9487), pp.717-725, (2005).

[14] Nosten F. and White N.J., Artemisinin-based combination treatment of falciparum malaria. *The American journal of tropical medicine and hygiene*, *77*(6_Suppl), pp.181-192, (2007).

[15] Maiteki-Sebuguzi C., Jagannathan P., Yau V.M., Clark T.D., Njama-Meya D., Nzarubara B., Talisuna A.O., Kamya M.R., Rosenthal P.J., Dorsey G. and Staedke S.G., Safety and tolerability of combination antimalarial therapies for uncomplicated falciparum malaria in Ugandan children. *Malaria journal*, *7*(1), p.106, (2008).

[16] Nontprasert A., Pukrittayakamee S., Dondorp A.M., Clemens R., Looareesuwan S. and White N.J., Neuropathologic toxicity of artemisinin derivatives in a mouse model. *The American journal of tropical medicine and hygiene*, *67*(4), pp.423-429, (2002).

[17] World Health Organization, W., Assessment of the safety of artemisinin compounds in pregnancy. *Geneva: WHO*, (2003).

[18] Raji Y., Gonadotoxicity evaluation of oral artemisinin derivative in male rats. *J. Med. Sci.*, *5*, pp.303-306, (2005).

[19] Nwanjo H.U., Iroagba I.I., N natuanya I.N. and Eze N.A., Antifertility activity of dihydro artemisinin in male albino rats. *Internet J Endocrinol*, *4*(1), pp.15-20, (2007).

[20] Obianime A.W. and Aprioku J.S., Comparative testicular histopathological effects of artemisinin derivatives and some ACTS in the guinea-pig. *Journal of Experimental and Clinical Anatomy*, *8*(2), (2009).

[21] WHO Expert Committee, Prevention and control of schistosomiasis and soil-transmitted helminthiasis. *World Health Organization technical report series*, *912*, p.i, (2002).

[22] Rivlin R.S., Historical perspective on the use of garlic. *The Journal of nutrition*, *131*(3), pp.951S-954S, (2001).

[23] Mamtani R. and Mamtani R., Ayurveda and yoga in cardiovascular diseases. *Cardiology in review*, *13*(3), pp.155-162, (2005).

[24] Kumar M. and Berwal J.S., Sensitivity of food pathogens to garlic (*Allium sativum*). *Journal of Applied Microbiology*, *84*(2), pp.213-215, (1998).

[25] Suetsuna K., Isolation and characterization of angiotensin I-converting enzyme inhibitor dipeptides derived from *Allium sativum* L (garlic). *The Journal of Nutritional Biochemistry*, *9*(7), pp.415-419, (1998).

[26] Sumiyoshi H., New pharmacological activities of garlic and its constituents. *Nihon yakurigakuzasshi.Folia pharmacologica Japonica*, *110*, pp.93P-97P, (1997).

[27] Wang B.H., Zuzel K.A., Rahman K. and Billington D., Protective effects of aged garlic extract against bromobenzene toxicity to precision cut rat liver slices. *Toxicology*, *126*(3), pp.213-222, (1998).

[28] Ohaeri O.C. and Adoga G., Effects of garlic oil on body fluid acidity and electrolytes in experimental diabetic rats. *Medical science research*, *26*(7), pp.487-488, (1998).

[29] Kyo E., Uda N., Suzuki A., Kakimoto M., Ushijima M., Kasuga S. and Itakura Y., Immunomodulation and antitumor activities of aged garlic extract. *Phytomedicine*, *5*(4), pp.259-267, (1998).

[30] Fromtling R.A. and Bulmer G.S., *In vitro* effect of aqueous extract of garlic (*Alliumsativum*) on the growth and viability of *Cryptococcus neoformans*. *Mycologia*, pp.397-405, (1978).

[31] Singh R.N., Prithviraj B., Singh U.P., Singh P.K. and Wagner K.G., Effect of some pesticides and ajoene on biology of *tetranychusurticae* Koch. *J Plant Dis Prot*, 103-145, (1996).

[32] Araujo C.A.C. and Leon L.L., Biological activities of Curcuma longa L. *Memorias do InstitutoOswaldo Cruz*, *96*(5), pp.723-728, (2001).

[33] Anand P., Thomas S.G., Kunnumakkara A.B., Sundaram C., Harikumar K.B., Sung B., Tharakan S.T., Misra K., Priyadarsini I.K., Rajasekharan K.N. and Aggarwal B.B., Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. *Biochemical pharmacology*, *76*(11), pp.1590-1611, (2008).

[34] Goel A., Kunnumakkara A.B. and AggarwalB.B., Curcumin as "Curecumin": from kitchen to clinic. *Biochemical pharmacology*, *75*(4), pp.787-809, (2008).

[35] Aggarwal B.B. and Hari kumar K.B., Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. *The international journal of biochemistry & cell biology*, *41*(1), pp.40-59, (2009).

[36] Goel A., Jhurani S. and Aggarwal B.B., Multi-targeted therapy by curcumin: how spicy is it? *Molecular nutrition & food research*, *52*(9), pp.1010-1030, (2008).

[37] Angus B.J., Thaiaporn I., Chanthapadith K., Suputtamongkol Y. and White N.J., Oral artesunate dose-response relationship in acute falciparum malaria. *Antimicrobial agents and chemotherapy*, *46*(3), pp.778-782, (2002).

[38] Pari L. and Amali D.R., Protective role of tetrahydro curcumin (THC) an active principle of turmeric on chloroquine induced hepatotoxicity in rats. *J Pharm PharmSci*, *8*(1), pp.115-23, (2005).

[39] Roy Choudhury A., Das T., Sharma A. and Talukder G., Dietary garlic extract in modifying clastogenic effects of

inorganic arsenic in mice: two-generation studies. *Mutation Research/Environmental Mutagenesis and Related Subjects*, *359*(3), pp.165-170, (1996).

[40] Foreman D., Gaylor L., Evans E. and Trella C., A modification of the Roe procedure for determination of fructose in tissues with increased specificity. *Analytical biochemistry*, *56*(2), pp.584-590, (1973).

[41] Prasad M.R.N., Chinoy N.J. and Kadam K.M., Changes in succinic dehydrogenase levels in the rat epididymis under normal and altered physiologic conditions. *Fertility and Sterility*, *23*(3), pp.186-190, (1972).

[42] Talbot P. and Chacon R.S., A triple-stain technique for evaluating normal acrosome reactions of human sperm. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *215*(2), pp.201-208, (1981).

[43] China Cooperative Research Group on qinghaosu and its derivatives as antimalarials. Antimalarial studies on Qinghaosu. *Chin Med J*, 92: 811–16, (1979).

[44] Li G.Q., Guo X.B., Jin R., Wang Z.C., Jian H.X. and Li Z.Y., Clinical studies on treatment of cerebral malaria with qinghaosu and its derivatives. *J Tradit Chin Med*, 2: 125–30, (1982).

[45] Luo J., Zhu W., Tang Y., Cao H., Zhou Y., Ji R., Zhou X., Lu Z., Yang H., Zhang S. and Cao J., Artemisinin derivative artesunate induces radiosensitivity in cervical cancer cells in vitro and in vivo. *Radiation Oncology*, *9*(1), p.84, (2014).

[46] White T.E., Bushdid P.B., Ritter S., Laffan S.B. and Clark R.L.,Artesunate-induced depletion of embryonic erythroblasts precedes embryolethality and teratogenicity in vivo. *Birth Defects Research Part B: Developmental and Reproductive Toxicology*, *77*(5), pp.413-429, (2006).

[47] Clark R.L., White T.E., Clode S., Gaunt I., Winstanley P. and Ward S.A., Developmental toxicity of artesunate and an artesunate combination in the rat and rabbit. *Birth Defects Research Part B: Developmental and Reproductive Toxicology*, *71*(6), pp.380-394, (2004).

[48] White T.E. and Clark R.L., Sensitive periods for developmental toxicity of orally administered artesunate in the rat. *Birth Defects Research Part B: Developmental and Reproductive Toxicology*, *83*(4), pp.407-417, (2008).

[49] Konkimalla V.B., Blunder M., Korn B., Soomro S.A., Jansen H., Chang W., Posner G.H., Bauer R. and Efferth T., Effect of artemisinins and other endoperoxides on nitric oxide-related signaling pathway in RAW 264.7 mouse macrophage cells. *Nitric oxide*, *19*(2), pp.184-191, (2008).

[50] Li P.C., Lam E., Roos W.P., Zdzienicka M.Z., Kaina B. and Efferth T., Artesunate derived from traditional Chinese medicine induces DNA damage and repair. *Cancer Research*, *68*(11), pp.4347-4351, (2008).

[51] Clark R.L., Embryotoxicity of the artemisinin antimalarials and potential consequences for use in women in the first trimester. *Reproductive toxicology*, *28*(3), pp.285-296, (2009).

[52] Desai K.R., Dattani J.J., Rajput D.K., Moid N., Highland H.N. and George L.B., Role of curcumin on chloroquine phosphate-induced reproductive toxicity. *Drug and chemical toxicology*, *35*(2), pp.184-191, (2012).

[53] Desai K.R., Rajput D.K., Patel P.B. and Highland H.N., Ameliorative Effects of Curcumin on Artesunate-Induced Subchronic Toxicity in Testis of Swiss Albino Male Mice. *Dose-Response*, *13*(2), p.1559325815592393, (2015).

[54] Dattani J.J., Rajput D.K., Moid N., Highland H.N., George L.B. and Desai K.R., Ameliorative effect of curcumin on hepatotoxicity induced by chloroquine phosphate. *Environmental toxicology and pharmacology*, *30*(2), pp.103-109, (2010).

[55] Zahid A. and Abidi T.S., Effect of chloroquine on liver weight of developing albino rats. *Journal-Pakistan medical association*, *53*(1), pp.21-23, (2003).

[56] Salman T.M., Olayaki L.A., Shittu S.T.T. and Bamgboye S.O., Serum testosterone concentration in chloroquine treated rats: effects of ascorbic acid and alpha-tocopherol. *African Journal of Biotechnology*, *9*(27), pp.4278-4281, (2010).

[57] Mathur N., Iain G.C. and Pandey G., Effect of Tecomastans Leaves on the reproductive system of male albino rats. *International Journal of Pharmacology*, *6*(2), pp.152-156, (2010).

[58] Gupta R.S., Kim J., Gomes C., Oh S., Park J., Im W.B., Seong J.Y., Ahn R.S., Kwon H.B. and Soh J., Effect of ascorbic acid supplementation on testicular steroidogenesis and germ cell death in cadmium-treated male rats. *Molecular and cellular endocrinology*, *221*(1), pp.57-66, (2004).

[59] Khan M.R., Rizvi W., Khan G.N., Khan R.A. and Shaheen S., Carbon tetrachloride-induced nephrotoxicity in rats: Protective role of *Digeramuricata*. *Journal of ethnopharmacology*, *122*(1), pp.91-99, (2009).

[60] Prasad A.M., Ramnarayan K., Nalini K. and Bairy K.L., Effect of imatinib on the biochemical parameters of the reproductive function in male Swiss albino mice. *Indian journal of pharmacology*, *43*(4), p.389, (2011).

[61] Hoareau L. and DaSilva E.J., Medicinal plants: a re-emerging health aid. *Electronic Journal of biotechnology*, *2*(2), pp.3-4 (1999).

[62] Wills E.D., Enzyme inhibition by allicin, the active principle of garlic. *Biochemical journal*, *63*(3), p.514, (1956).

[63] Leuter H.D., Koch H.P. and Lawson L.D., Therapeutic effects and applications of garlic and its preparation. *Garlic.The science and therapeutic application of Allium sativum L. and related species.Edited by Koch HP, Lawson LD. Williams & Wilkins*, pp.135-212, (1996).

[64] Reddy R.C., Vatsala P.G., Keshamouni V.G., Padmanaban G. and Rangarajan P.N., Curcumin for malaria therapy. *Biochemical and biophysical research communications*, *326*(2), pp.472-474, (2005).

[65] Nandakumar D.N., Nagaraj V.A., Vathsala P.G., Rangarajan P. and Padmanaban G.,Curcumin-artemisinincombination therapy for malaria. *Antimicrobial Agents and Chemotherapy*, *50*(5), pp.1859-1860, (2006).

[66] Ohara K., Mizukami W., Tokunaga A., Nagaoka S.I., Uno H. and Mukai K., Kinetic study of the mechanism of free-radical scavenging action in curcumin: effects of solvent and pH. *Bulletin of the Chemical Society of Japan*, *78*(4), pp.615-621, (2005).

[67] Ray B. and Lahiri D.K., Neuroinflammation in Alzheimer's disease: different molecular targets and potential therapeutic agents including curcumin. *Current opinion in pharmacology*, *9*(4), pp.434-444, (2009).

[68] Banerjee S.K., Mukherjee P.K. and Maulik S.K., Garlic as an antioxidant: the good, the bad and the ugly. *Phytotherapy Research*, *17*(2), pp.97-106, (2003).

[69] Dandekar P., Dhumal R., Jain R., Tiwari D., Vanage G. and Patravale V., Toxicological evaluation of pH-sensitive nanoparticles of curcumin: acute, sub-acute and genotoxicity studies. *Food and Chemical Toxicology*, *48*(8), pp.2073-2089, (2010).

[70] Hsieh C.Y., Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer research*, *21*, pp.2895-2900, (2001).

[71] Sharma R.A., Euden S.A., Platton S.L., Cooke D.N., Shafayat A., Hewitt H.R., Marczylo T.H., Morgan B., Hemingway D., Plummer S.M., Pirmohamed M., Gescher A.J. and Steward W.P., Phase I clinical trial of oral curcumin: biomarkers of systemic activity and compliance. *Clin Cancer Res*, 10: 6847-6854, (2004).

[72] Itokawa H., Lee K.H., Shi Q., Morris-Natschke S.L. and Akiyama T., Recent advances in the investigation of curcuminoids. *Chinese Medicine*, *3*(1), p.11, (2008).

[73] Waknine-Grinberg J.H., McQuillan J.A., Hunt N., Ginsburg H. and Golenser J., Modulation of cerebral malaria by fasudil and other immune-modifying compounds. *Experimental parasitology*, *125*(2), pp.141-146, (2010).

[74] Fang J.Y., Hung C.F., Chiu H.C., Wang J.J. and Chan T.F., Efficacy and irritancy of enhancers on the in-vitro and in-vivo percutaneous absorption of curcumin. *Journal of pharmacy and pharmacology*, *55*(5), pp.593-601, (2003).

[75] Prato M., Gallo V., Giribaldi G., Aldieri E. and Arese P., Role of the NF-κB transcription pathway in the haemozoin-and 15-HETE-mediated activation of matrix metalloproteinase-9 in human adherent monocytes. *Cellular microbiology*, *12*(12), pp.1780-1791, (2010).

[76] Farombi E.O., Ekor M., Adedara I.A., Tonwe K.E., Ojujoh T.O. and Oyeyemi M.O., Quercetin protects against testicular toxicity induced by chronic administration of therapeutic dose of quinine sulfate in rats. *Journal of basic and clinical physiology and pharmacology*, *23*(1), pp.39-44, (2012).

[77] Polimeni M., Valente E., Aldieri E., Khadjavi A., Giribaldi G. and Prato M., Haemozoin induces early cytokine-mediated lysozyme release from human monocytes through p38 MAPK-and NF-kappaB-dependent mechanisms. *PLoS One*, *7*(6), p. e39497, (2012).

[78] Ankri S. and Mirelman D.,Antimicrobial properties of allicin from garlic. *Microbes and infection*, *1*(2), pp.125-129, (1999).

[79] Chowdhury A.K., Ahsan M., Islam S.N. and Ahmed Z.U., Efficacy of aqueous extract of garlic &allicin in experimental shigellosis in rabbits. *The Indian journal of medical research*, *93*, pp.33-36, (1991).

[80] Gupta K.C. and Viswanathan R., Combined Action of Streptomycin and Chloramphenicol with Plant Antibiotics against Tubercle Bacilli. Part I: Streptomycin and Chloramphenicol with Cepharanthine. Part II: Streptomycin and Allicin. *Antibiotics & Chemotherapy*, *5*(1), pp.24-7, (1955).

[81] Harris J., Plummer S. and Lloyd D., Antigiardial drugs. *Applied microbiology and biotechnology*, *57*(5-6), pp.614-619, (2001).

[82] JonkersD., Van den Broek E., Van Dooren I., Thijs C., Dorant E., Hageman G. and Stobberingh E., Antibacterial effect of garlic and omeprazole on Helicobacter pylori. *Journal of Antimicrobial Chemotherapy*, *43*(6), pp.837-839, (1999).

[83] Mirelman D., Monheit D. and Varon S., Inhibition of growth of *Entamoebahistolytica* by allicin, the active principle of garlic extract (*Allium sativum*). *Journal of Infectious Diseases*, *156*(1), pp.243-244, (1987).

[84] Shadkchan Y., Shemesh E., Mirelman D., Miron T., Rabinkov A., Wilchek M. and Osherov N., Efficacy of allicin, the reactive molecule of garlic, in inhibiting Aspergillus spp. in vitro, and in a murine model of disseminated aspergillosis. *Journal of Antimicrobial Chemotherapy*, *53*(5), pp.832-836, (2004).

[85] Uchida Y., Takahashi T. and Sato N., The characteristics of the antibacterial activity of garlic (author's transl). *The Japanese journal of antibiotics*, *28*(4), pp.638-642, (1975).

[86] Perez H.A., De la Rosa M. and Apitz R., *In vivo* activity of ajoene against rodent malaria. *Antimicrobial agents and chemotherapy*, *38*(2), pp.337-339, (1994).

***Corresponding Author:**
Ketaki R. Desai*
Email: ketalak60@yahoo.co.in