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Original Article

In vivo antiplasmodial potential of three herbal methanolic extracts in mice infected with *Plasmodium berghei* NK65

Pachiyappan Rajiv Gandhi[a], Chinnaperumal Kamaraj[b,*], Elangovan Vimalkumar[a], Selvaraj Mohana Roopan[c,*]

[a] *Division of Nanobiotechnology, Department of Zoology, Auxilium College (Autonomous) Affiliated to Thiruvalluvar University, Tamil Nadu 632006, India*
[b] *Department of Biotechnology, Periyar University, Tamil Nadu 636011, India*
[c] *Chemistry of Heterocycles & Natural Product Research Laboratory, Department of Chemistry, School of Advanced Sciences, Vellore Institute of Technology, Tamil Nadu 632014, India*

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ABSTRACT

Objective: The present study deals with the investigation of antiplasmodial potential of leaf methanolic extract of *Aegle marmelos, Aristolochia indica* and *Cassia auriculata* against *Plasmodium berghei* (NK65) infected mice.

Methods: The chloroquine-sensitive parasites *P. berghei* (1×10^6) were inoculated into Swiss albino mice intraperitoneally. The methanol extracts of three herbal plants were orally administered in *P. berghei* infected mice which were further assessed using the four-day suppressive test at different doses of 150, 300 and 600 mg/kg per day. Chloroquine (CQ) was used as the standard drug with of 1.25, 2.5 and 5 mg/kg concentrations and was orally administered.

Results: The leaves of *A. marmelos, A. indica*, and *C. auriculata* were found to suppress *P. berghei* parasitaemia in Swiss albino mice by (67.0 ± 4.02)%, (72.0 ± 8.44)% and (52.7 ± 2.06)% at 600 mg/kg/d with ED_{50} values of 284.73, 233.77 and 562.48 mg/kg, respectively. These herbal plants increased the mean survival time of infected mice and prevented body weight loss. GC-MS analysis revealed the presence of hentriacontan-16-one ($C_{31}H_{62}O$) in *A. indica* extract. The histopathology study showed non-toxic to kidney and liver at 600 mg/kg/body weight.

Conclusions: Overall results revealed that herbal plants may be active in the development of novel and cheap antimalarial compounds.

1. Introduction

Mosquito-borne diseases have an economic impact, including loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates; However, no part of the world is free from vector-borne diseases (Gandhi, Jayaseelan, Mary, Mathivanan, & Suseem, 2017). Malaria is caused by *Plasmodium* parasites, vectored to people through the bites of infected female *Anopheles* mosquitoes, which bite mainly between dusk and dawn (Gandhi, Jayaseelan, Kamaraj, Rajasree, & Mary, 2018; WHO, 2014). Malaria infects more than 500 million people each year, killing approximately 1.2 to 2.7 million per year (Gandhi, Jayaseelan, Vimalkumar, & Mary, 2016; WHO, 2012). In India, malaria is the most vital cause of morbidity and mortality with nearly 2 to 3 million new cases increasing every year (Jayaseelan, Gandhi, Rajasree, Suman, & Mary, 2018). Currently, malarial control is complicated task and challenging, due to insecticide resistance in vector populations, as well as to the development of *Plasmodium* strains resistant to a growing number of antimalarial drugs. The increasing problem of resistance to the classical drugs (Chloroquine, Atovaquone, Sulphadoxine and Pyrimethamine) and the problem of recrudescence of Artemisinin stress the need to look for new antimalarial agents. Recently, herbal plants have been used more widely than synthetic drugs due to its non-toxic, higher efficacy, no side effects and easy to proceed. Herbal plant extracts are noble source for a variety of drugs in many countries. Plant extracts are an important natural composite source for the validation of pharmacological properties and can act as new agent against pathogens.

Aegle marmelos L. (Rutaceae) is widely distributed in India and used as traditional medicines (Bailey, 1963). The aerial parts of *A. marmelos* were used to treat cardiac illnesses, fever, cough and

* Corresponding authors.
E-mail addresses: kamaraj84@hotmail.com (C. Kamaraj), mohanaroopan.s@vit.ac.in (S.M. Roopan).

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ulcer (Kakiuchi et al., 1991; Udupa, Udupa, & Kulkarni, 1994). *A. marmelos* leaves were used for hypoglycemic, inflammatory and wound healing effects (Sharma, Dwivedi, Varshney, & Swarup, 1996). The *A. marmelos* leaves showed antifungal properties (Khalid, Farouk, Geary, & Jensen, 1986; Rana, Singh, & Taneja, 1997; Renu, Dubey, & Dixit, 1986) and immature bark extracts of *A. marmelos* exhibited better antimalarial action against *Plasmodium falciparum*. The extract of *A. marmelos* displayed promising *in vitro* antimalarial potential against CQ-sensitive 3D7 and CQ-resistant *P. falciparum* INDO strains (Kamaraj et al., 2012a).

Aristolochia indica L. (Aristolochiaceae) is the most widely distributed species in India and has the greatest importance as a medicinal plant (Heinrich, Chan, Wanke, Neinhuis, & Simmonds, 2009; Mollik et al., 2010). The plant is used to treat cholera, fever, bowel troubles, ulcers, leprosy, and poisonous bites (Kanjilal, Kotoky, & Couladis, 2009; Krishnaraju et al., 2005). It is also used as antineoplastic, antiseptic, anti-inflammatory and antibacterial agents (Achari, Chakrabarty, & Pakrashi, 1981; Das, Kausik, & Pal, 2010). The methanol, ethyl acetate and hexane extracts from the leaves of *A. indica* were active against *Culex gelidus* and *Culex quinquefasciatus* (Kamaraj et al., 2010a). Our pervious study showed that the *A. indica* and *C. auriculata* extracts showed promising antimalarial activity to blood stage CQ-sensitive and CQ-resistant strains of *P. falciparum* (Kamaraj et al., 2012b).

Cassia auriculata L. (Cesalpinaceae) commonly known as “avaram” in Tamil is considered as a good traditionally used medicinal plant for diabetes. It establishes good control of sugar levels in the treatment of diabetes (Shrotri, Kelkar, Deshmukh, & Aiman, 1963). Dried leaves and flowers of *C. auriculata* are utilized to cure skin problems, asthma, conjunctivitis and renal disorders (Joshi, 1986; Sawhney, Khan, Ndaalio, Nkunya, & Wavers, 1978; Vedavathy, Mrudula, & Sudhakar, 1997). The *C. auriculata* leaves and flowers (methanol, acetone, ethyl acetate, chloroform, petroleum ether and hexane) extracts showed maximum activity against the larvae of *Anopheles subpictus* and *Culex tritaeniorhynchus* (Kamaraj et al., 2009). The *C. auriculata* leaf and flower (methanol, hexane, chloroform, ethyl acetate and acetone) extracts showed promising activity towards the 4th instar larvae of *A. stephensi* and *C. quinquefasciatus* (Kamaraj et al., 2010b).

Hence, in this study, we have carried out an antiplasmodial activity of leaf methanol extract from traditionally used herbal plants *A. marmelos, A. indica* and *C. auriculata* against *Plasmodium berghei* (NK65) infected mice. The histopathology study has also been performed. The volatile compounds were analysed by GC-MS analysis of methanol extract to identify the presence of the most active compounds. This is the first report on *in vivo* antiplasmodial study of *A. marmelos, A. indica* and *C. auriculata* from southern Indian herbal plants.

2. Materials and methods

2.1. Materials

The fresh leaves of *A. marmelos, A. indica* and *C. auriculata* were collected from Thanipadi, Tiruvannamalai district (12.1074° N, 78.8341° E, altitude 102 m), Tamil Nadu, India, and authenticated by Dr. S. Isabella Rosaline, Associate professor, Department of Botany, Auxilium College, Vellore, India. Methanol was obtained from sigma-Aldrich, India.

2.2. Extraction process of medicinal plants

Plant leaves were dried for 6–10 d under the shade and ground (28–32 °C), and the leaves (100 g) were powdered in an electric grinder and the methanol extract (500 mL, Qualigens) was obtained by using Soxhlet apparatus at 65–85 °C for 3 h. The solvent was removed by Rotatory evaporator at 60 °C and the extract was kept in refrigerator for further applications (10 °C). The extraction yield is a measure of solvent and extraction methods efficiency to extract the specific components from plant matrix. In the present study, *A. indica* leaves were extracted with methanol solvent and the yield of *A. indica* leaf extract was found to be the 17.6% in methanol solvent with Soxhlet extraction method.

2.3. GC-MS analysis

GC-MS analysis of *A. indica* leaf methanol extract was carried out on a Perkin Elmer Clarus 680 GC-MS instrument employing the following conditions: column elite-5MS (30.0 m, 0.25 mm ID, 250 µm), operating in electron impact mode; Helium was used as a carrier gas at a constant flow and split ratio was 10:1; Injector temperature was 250 °C, flow rate was 1 mL/min, oven temperature was initially 60 °C for 2 min, ramping 10 °C /min to 300 °C and holding for 6 min. The total run time was 32 min. The molecular weight and structure of the compounds were ascertained by interpretation using the database of National Institute Standard and Technology (Kamaraj et al., 2018; Mathivanan, Gandhi, Mary, & Suseem, 2018).

2.4. In vivo antiplasmodial activity

2.4.1. Collection of strains

A CQ-sensitive *P. berghei* (NK65 strain) was obtained from NIMR (National Institute of Malaria Research) New Delhi, India.

2.4.2. Animals

Male and female mice (Swiss albino mice) weighing 24–30 g, 6 weeks old, obtained from the Institute of Veterinary Preventive Medicine, Ranipet, Tamil Nadu, India were used for this study. The mice were grouped and housed in polyacrylic cages (38 × 23 × 10 cm) and acclimatized for a period of 30 d. Three animals per cage and maintained under standard laboratory condition [Temperature at (27 ± 2) °C with dark/light cycle 12/12 h]. They were allowed standard pellet diet (Hindustan Lever Limited, Mumbai, India) and clean drinking water *ad libitum*. The study was conducted in accordance with the permission and approval of Government of India, Ministry of Environment and Forest, New Delhi, India (Committee for the purpose of control and supervision of experiments on animals; Reg. No.: 1011/c/CPCSEA).

2.4.3. Inoculum

Parasitized erythrocytes were obtained from a donor-infected mouse by cardiac puncture in heparin and diluted with sterile blood from similar age group mice. Animals were inoculated intraperitoneally with infected blood suspension (0.2 mL) containing 10^6 parasitized erythrocytes lethal inoculum on day 0. Infected mice with parasitemia of 5%–7% were allocated to five groups with three mice in each group (David, Philip, Simon, Reto, & Solomon, 2004).

2.4.4. In vivo antiplasmodial activity

Experiments were performed using four-day curative standard test (David et al., 2004; Peter & Anatoli, 1998), and employing the chloroquine sensitive *P. berghei* (NK 65). For bioassay test, three mice were used for each plant test group, chloroquine (standard) and untreated control groups of mice were tested separately. Tween 80 (Qualigens) was used as an emulsifier at the concen-

tration of 0.3% in the final test solution. The plant extracts were orally administered to the test groups at different doses (150, 300 and 600 mg/kg body weight) from 0 to 4 d to *P. berghei* infected mice. Chloroquine (Sigma) was used as standard drug with normal saline (0.9%) at 1.25, 2.5 and 5 mg/kg. Saline (0.9%), Tween 80 (0.3%) with distilled water was used as a control for four experimental days. Treatments were performed daily for four consecutive days starting 24 h after infection receiving a total of three oral doses.

2.4.5. Estimation of parasitemia

Parasitemia was supervised from day 0 to day 4 using blood films, which were collected from the tail of the swiss albino mice (David et al., 2004; Ene, Atawodi, Ameh, Kwanshie, & Agomo, 2008). Blood smears were stained with 10% Giemsa (Sigma) at pH 7.2 for 15–20 min and parasitemia thicknesses were determined using the microscope at 100× to assess the level of parasitized red blood cells. The infected Swiss albino mice percentage was calculated according to following equation by Iwalewa, Oguntoye, Rai, and Iyaniwura (1997):

$$\text{Parasitemia}\ (\%) = \text{Treated parasites/Control parasites} \times 100\%$$

2.5. Determination of body weight

The body weights were measured to observe that leaf methanolic extract of *A. marmelos, A. indica* and *C. auriculata* treated mice controlled weight loss that was normally decreased with increasing parasitemia in *P. berghei* (NK 65) infected Swiss albino mice. The body weight of the animals was taken before infection day 0(D0) and on day 4 (D4).

2.6. Determination of mean survival time (MST)

Mortality was monitored daily and the number of days from the time of inoculation of the parasite up to death was recorded for each mouse in the treatment and control groups over a period of 30 d. The mean survival time (MST) for each group was calculated as:

$$\text{MST} = \frac{\text{Sum of survival time of all mice in a group (d)}}{\text{Total number of mice that in group}}$$

2.7. Histopathological studies

In the present study, liver and kidney was removed from the experimental mice through the dissection, washed in 0.9% sodium chloride solution and placed in 10% formalin for fixation. The organs were dehydrated by increasing concentrations of alcohol (0%–100%) and embedded in paraffin blocks which were sectioned in 4 μm thickness using Leica rotary microtome. The sections were stained in haematoxylin and eosin for parasite visualization and evaluation of tissue morphology using light microscopy with a camera (×40) (Labomed, India). Examination of liver and kidney sections was undertaken to determine morphological changes. Organs were collected from plant, chloroquine-treated, untreated (control infected) and noninfected (control) mice (Moore, Jago, & Batty, 2008).

2.8. Statistical analysis

The results were analyzed statistically using one-way and two-way ANOVA methods to identify the differences between treated group and control group. The data were considered significant at $P < 0.05$. The ED_{50} representing 50% suppression of parasites was estimated by a common statistical procedure when compared with untreated control group (Software DLSP Montpellier).

3. Result

3.1. In vivo *antiplasmodial activities*

The outcomes of the four consecutive days suppressive antiplasmodial activity of the plant leaf methanol extracts (*A. marmelos, A. indica,* and *C. auriculata*) with various doses in Swiss albino mice parasitized with *P. berghei* were showed in Table 1. The inhibition of parasitemia calculated in percentage (S.P.; %) and standard deviations ($n = 3$) were measured all extract/CQ. The values of ED_{50} were calculated and showed in Table 2. Three doses (from 150, 300 and 600 mg/kg) were screened for methanol extracts of *A. marmelos, A. indica,* and *C. auriculata*. The inhibition of *A. indica* at 150 mg/kg to parasites was low (32.4 ± 4.05)%; With the increase of dose to 300 mg/kg, the parasitemia inhibition was enhanced to (52.3 ± 6.26)% and maximum doses of *A. indica* extracts showed higher inhibition about (72.0 ± 8.44)% at 600 mg/kg. The percentage of parasitemia inhibition of *A. marmelos* and *C. auriculata* at 150 mg/kg was reduced to (47.4 ± 6.42)% and (22.3 ± 8.47)%, respectively. When the plant leaf methanol extract doses were increased to 300 mg/kg, which showed excellent percent of parasitemia inhibition of (58.6 ± 4.81)% (*A. marmelos*) and (38.0 ± 2.03)% (*C. auriculata)* and the maximum concentration of *A. marmelos* and *C. auriculata* methanol extracts showed (67.0 ± 4.02)% and (52.7 ± 2.06)%, respectively. Chloroquine showed

Table 1
Effect of leaf crude methanol extract of different plants on parasitemia suppression and mean survival time of *P. berghei* infected mice.

Groups	Doses/(mg·kg^{-1}·d^{-1})	Suppression of parasitemia (S.P. ± S.D.)/%				Mean survival time/(day) ±SD
		Day 1	Day 2	Day 3	Day 4	
A. marmelos	150	06.2 ± 0.83	14.8 ± 0.40	26.2 ± 2.80	47.4 ± 6.42	22.42 ± 0.29
	300	10.8 ± 1.62	21.6 ± 1.62	38.0 ± 0.64	58.6 ± 4.81	18.67 ± 0.82
	600	12.0 ± 2.45	35.4 ± 0.26	45.4 ± 1.63	67.0 ± 4.02*	18.17 ± 0.41
A. indica	150	10.0 ± 0.00	08.2 ± 1.82	13.8 ± 2.42	32.4 ± 4.05	27.67 ± 1.62
	300	14.3 ± 1.08	14.0 ± 2.60	28.6 ± 0.64	52.3 ± 6.26	24.83 ± 0.22
	600	22.9 ± 0.62	31.4 ± 1.63	47.2 ± 1.63	72.0 ± 8.44*	24.67 ± 1.24
C. auriculata	150	02.4 ± 2.08	08.0 ± 0.40	14.8 ± 0.64	22.3 ± 8.47	26.23 ± 1.62
	300	05.2 ± 0.42	12.8 ± 2.46	26.6 ± 1.82	38.0 ± 2.03	24.2 ± 0.08
	600	12.6 ± 1.08	20.4 ± 2.08	35.4 ± 2.03	52.7 ± 2.06*	18.5 ± 0.56
Chloroquine	1.25	16.3 ± 0.42	35.6 ± 0.22	40.5 ± 0.42	66.2 ± 0.82	30.20 ± 1.46
	2.5	20.0 ± 1.56	48.4 ± 0.35	68.3 ± 0.38	89.0 ± 2.06	26.45 ± 1.51
	5	28.4 ± 2.64	56.8 ± 2.64	82.6 ± 2.84	92.0 ± 0.04*	26.18 ± 2.34
Control	1mL	0.00	0.00	0.00	0.00	30.2 ± 0.44

Data were expressed as standard deviation (SD) ± for three mice per group when compared with control.
* Statistically significant ($P < 0.05$).

Table 2
Parasitaemia suppression and ED_{50} values of leaf methanol extract of different plants extract against *P. berghei* infected mice and control chloroquine drug (mg/kg/day) on day 4.

Groups	Dose ($mg \cdot kg^{-1} \cdot d^{-1}$)	Suppression of parasitemia (SP ± SD) (%) on day 4	Effective dose for 50% inhibition (ED_{50} ± SD)/ ($mg \cdot kg^{-1}$)
A. marmelos	150	47.4 ± 6.42	284.73 ± 2.78
	300	58.6 ± 4.81	
	600	67.0 ± 4.02*	
A. indica	150	32.4 ± 4.05	233.77 ± 4.32
	300	52.3 ± 6.26	
	600	72.0 ± 8.44*	
C. auriculata	150	22.3 ± 8.47	562.48 ± 8.46
	300	38.0 ± 2.03	
	600	52.7 ± 2.06*	
Chloroquine	1.25	66.2 ± 0.82	14.87 ± 2.27
	2.5	89.0 ± 2.06	
	5	92.0 ± 0.04	

Data are expressed as standard deviation (SD) ± for five mice per group when compared with control.
* Statistically significant ($P < 0.05$).

(92.0 ± 0.04)% percentage of parasitemia inhibition at 5 mg/kg quantity of high doses of the plant leaf extracts were required for parallel reports. Finally, our results revealed that *A. indica* plant leaf methanol extract had moderate antimalarial activity as compared to the standard drug CQ. The ED_{50} values of plant methanol extracts showed (14.87 ± 2.27), (284.73 ± 2.78), (233.77 ± 4.32) and (562.48 ± 8.46) mg/kg for chloroquine, *A. marmelos, A. indica*, and *C. auriculata*, respectively (Table 2).

The Giemsa stained blood smears of *A. marmelos, A. indica*, and *C. auriculata* leaf methanol extracts treated, chloroquine treated and untreated control group mice on day 4 at 100× magnification under light microscopy were shown in Fig. 1. The Photomicrograph of blood smears showed *A. marmelos* [(67.0 ± 4.02)%], *A. indica* [(72.0 ± 8.44)%] and *C. auriculata* [(52.7 ± 2.06)%] (Fig. 1A–C) parasitaemia suppression at 600 mg/kg body wt. and Fig. 1D showed CQ [(92.0 ± 0.04)%] treatment of parasitaemia suppression at 5 mg/kg body wt. and Fig. 1E showed untreated mice RBCs, Arrows indicated different stages of parasites that were seen at higher frequencies in the control group compared to the plant extract treated group.

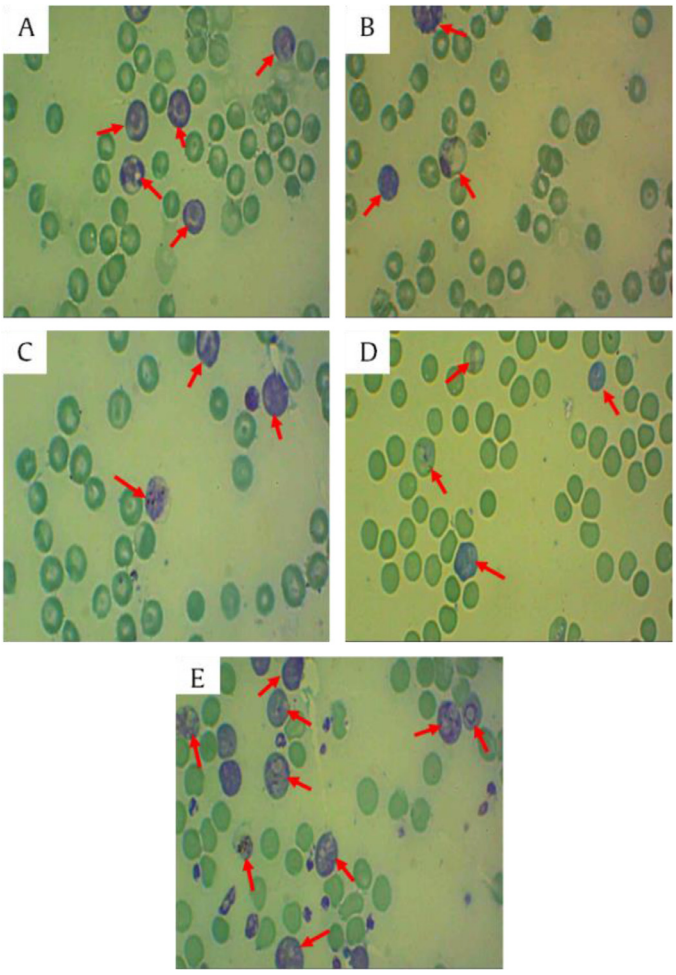


Fig. 1. Photomicrograph of blood smears of treatment and control groups on day 4 under microscopy (100×). (A) *A. marmelos* leaf methanol extract treatment [(67.0 ± 4.02)%]; (B) *A. indica* leaf methanol extract treatment [(72.0 ± 8.44)%]; (C) *C. auriculata* leaf methanol extract treatment [(52.7 ± 2.06)%] of parasitaemia suppression at 600 mg/kg body wt.; (D) Chloroquinine treatment [(92.0 ± 0.04)%] parasitaemia suppression at 5 mg/kg body wt. and (E) untreated mice RBCs; Arrows indicate different stages of parasites that were seen at higher frequencies in control group compared to plant extract treated group.

3.2. Body weight of animal

The test extracts of *A. marmelos, A. indica* and *C. auriculata* controlled loss of body weight with increasing parasitemia. From the results of loss of body weight, it can conclude that *A. indica* showed higher activity than other plant extracts at all concentrations in a dose-depended manner.

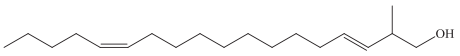
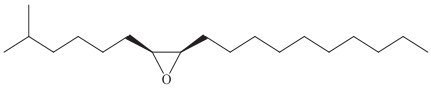
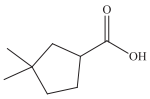
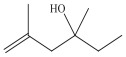
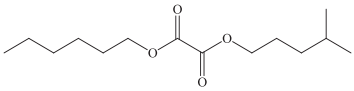
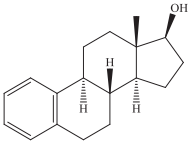
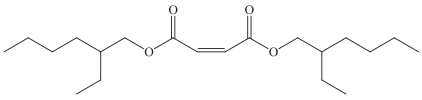
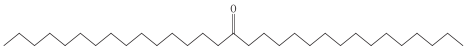
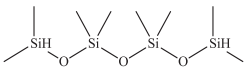
The Swiss albino mice body weights were observed at day 0 (D 0) and day 4 (D 4) in the leave extracts of *A. marmelos* [(29.10 ± 1.51)g] and [(30.10 ± 0.27)g], *A. indica* [(29.57 ± 0.24)g] and [(28.48 ± 2.04)g] and *C. auriculata* [(30.42 ± 0.42)g] and [(31.42 ± 1.60)g] were controlled weight loss of the body weight in infected mice at 600 mg/kg, respectively. Chloroquine treated mice at 5 mg/kg [(22.40 ± 0.26)g] and [(24.62 ± 0.73)g] and normal mice treated with control at 1 mL [(24.08 ± 3.01)g] and [(25.91 ± 2.8)g] were prevented the body weight. The loss of body weight was observed in untreated mice because parasitemia level was increased on day 4. The *in vivo* study showed that the plant leaf extracts significantly controlled weight loss at 150, 300 and 600 mg/kg dose compared to the controls (Table 3).

Table 3
Effect of crude methanol extract of different plants on body weight (B.wt.) of *P. berghei* infected mice after oral administration (mean±SD, $n = 3$).

Groups	Dose/($mg \cdot kg^{-1} \cdot d^{-1}$)	Body weight/g	
		Day 0	Day 4
A. marmelos	150	27.30 ± 0.38	28.64 ± 0.47*
	300	30.26 ± 0.33	32.90 ± 0.54*
	600	29.10 ± 1.51	30.10 ± 0.27*
A. indica	150	26.20 ± 0.46	25.24 ± 0.55*
	300	24.40 ± 1.80	27.03 ± 1.86
	600	29.57 ± 0.24	28.48 ± 2.04
C. auriculata	150	24.62 ± 0.30	26.20 ± 0.45
	300	28.92 ± 1.36	31.03 ± 0.24
	600	30.42 ± 0.42	31.42 ± 1.60*
Chloroquine	1.25	29.66 ± 0.25	30.10 ± 0.27
	2.5	25.34 ± 1.03	26.00 ± 0.16
	5	22.40 ± 0.26	24.62 ± 0.73
Control	1mL	24.08 ± 3.01	25.91 ± 2.8

Note: Day 0: day infection was initiated; day 4: 5th day of infection, each result was with a mean of three mice.
* $P < 0.05$ *vs* control group.

Table 4
Important compounds identified in GC-MS analysis of leaf methanol extract of *A. indica*.

Sample No.	Compound names	Rt/min	MW	Molecular formulas	Structures
1	(3*E*, 13*Z*)-2-methyloctadeca-3, 13-dien-1-ol	16.61	280	$C_{19}H_{36}O$	
2	(2*R*, 3*S*)-2-decyl-3-(5-methylhexyl)oxirane	17.54	282	$C_{19}H_{38}O$	
3	3,3-dimethylcyclopentanecarboxylic acid	17.84	142	$C_8H_{14}O_2$	
4	3,5-dimethylhex-5-en-3-ol	18.07	128	$C_8H_{16}O$	
5	Hexyl (4-methylpentyl) oxalate	19.39	258	$C_{14}H_{26}O_4$	
6	(8*R*, 9*S*, 13*S*, 14*S*, 17*S*)-13-methyl-7,8,9,11,12,13,14,15, 16, 17-decahydro-6*H*-cyclopenta[α]phenanthren-17-ol	19.42	256	$C_{18}H_{24}O$	
7	8-(2-octylcyclopropyl)octanal	21.01	280	$C_{19}H_{36}O$	
8	*Bis*(2-ethylhexyl) maleate	22.51	340	$C_{20}H_{36}O_4$	
9	Hentriacontan-16-one	28.91	450	$C_{31}H_{62}O$	
10	1,1,3,3,5,5,7,7-octamethyltetrasiloxane	29.46	282	$C_8H_{26}O_3Si_4$	

3.3. Observation of mean survival time

The methanol extract of *A. marmelos, A. indica* and *C. auriculata* prolonged mean survival time of the study mice at 600 mg/kg [(18.17 ± 0.41)d, (24.67 ± 1.24)d, (18.5 ± 0.56)d]. When we compared to the standard drug chloroquine treated mice at 5 mg/kg [(26.18 ± 2.34)d], our results were moderate mean survival time (Table 1).

3.4. GC-MS analysis

The GC-MS analysis of *A. indica* was carried out and database was matched with the National Institute of Standards and Technology (NIST) which were listed in Table 4. Major constitutes of methanol extracts of *A. indica* fraction were hentriacontan-16-one ($C_{31}H_{62}O$) with peak area of 34.58%, (3*E*, 13*Z*)-2-methyloctadeca-3,13-dien-1-ol ($C_{19}H_{36}O$) with peak area of 3.811%, (2*R*, 3*S*)-2-decyl-3-(5-methylhexyl)oxirane ($C_{19}H_{38}O$) with peak area of 6.74%, 3,3-dimethylcyclopentanecarboxylic acid ($C_8H_{14}O_2$) with peak area of 4.52%, 3,5-dimethylhex-5-en-3-ol ($C_8H_{16}O$) with peak area of 3.60%, hexyl (4-methylpentyl) oxalate ($C_{14}H_{26}O_4$) with peak area of 7.97%, (8*R*, 9*S*, 13*S*, 14*S*, 17*S*)-13-methyl-7,8,9,11,12,13,14,15, 16, 17-decahydro-6*H*-cyclopenta[α]phenanthren-17-ol ($C_{18}H_{24}O$) with peak area of 2.90%, 8-(2-octylcyclopropyl)octanal ($C_{19}H_{36}O$) with peak area of 27.79%, *bis*(2-ethylhexyl) maleate ($C_{20}H_{36}O_4$) with peak area of 1.78% and 1,1,3,3,5,5,7,7-octamethyltetrasiloxane ($C_8H_{26}O_3Si_4$) with peak area of 1.76% (Fig. 2).

3.5. Histopathological studies

The histopathological study of the kidney organ showed normal (uninfected) structure (Fig. 3a) of cells in untreated control mice. The kidney organ showed no notable damage or change treated with *A. marmelos, A. indica* and *C. auriculata* at 600 mg/kg/d over a period of four consecutive days (Fig. 3b, c & d respectively). Fig. 3e showed CQ treated Swiss albino mice at 100 mg/kg/d for four consecutive periods. Fig. 3f displayed the untreated (infected) kidney organ. The normal control Swiss albino mice hepatocytes in the liver was shown in Fig. 3g. Then the liver organ was treated with *A. marmelos, A. indica, C. auriculata* and CQ once daily for four consecutive days (Fig. 3h–k). The plant extract treated Swiss albino mice at 600 mg/kg/d for four consecutive days and liver section were observed the normal histological appearance and also usual intracellular break. The liver organ of untreated swiss albino mice, penetration of hepatocytes and deposition of malarial stain were observed (Fig. 3l). CQ treated swiss albino mice at 600 mg/kg/day for four consecutive days and there is no structure damage or change in the control mice.

4. Discussion

The *in vivo* antiplasmodial results were presented in Fig. 1, and methanol extract of *A. indica* exhibited higher inhibition of parasitemia suppressions ($P < 0.05$) on day 4 post infection, ranging from 32.4% to 72.0%, which strongly validated the tra-

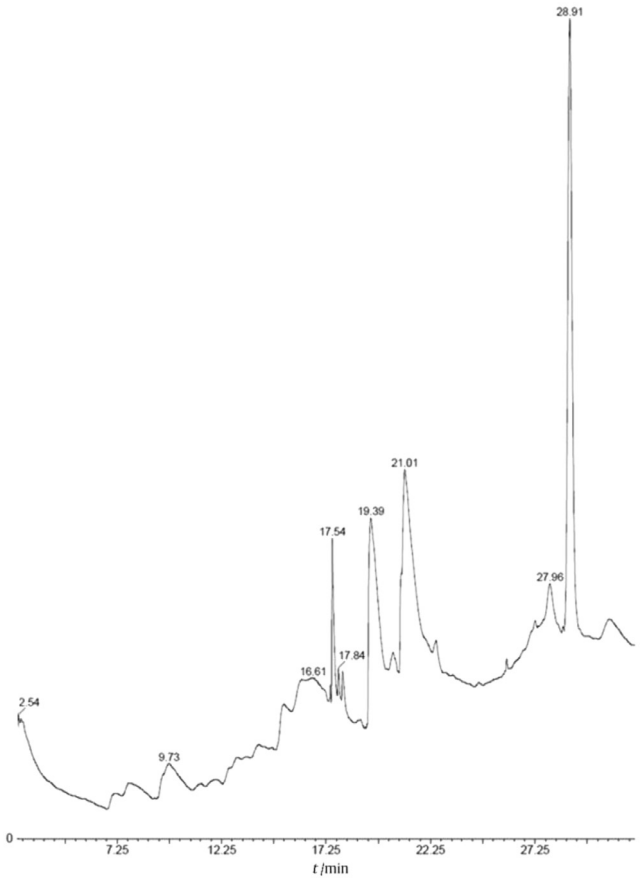


Fig. 2. GC-MS analysis of leaf methanol extract of *A. indica*.

ditionally use of south Indian medicinal plant leaf methanol extract as a malaria remedy. Our previous study reported that leaf methanol extract of *A. marmelos, A. indica* and *C. auriculata* showed good antimalarial property towards *P. falciparum* with inhibition of 50% (IC_{50}: 3D 7, 10, 10 and 14 µg/mL and parallel action with INDO IC_{50} of 6, 17 and 24 µg/mL) (Kamaraj et al., 2012a, 2012b); Khalid et al. (1986) have recorded *A. marmelos* leaf and bark extracts exhibited *in vitro* antimalarial activity (IC_{50} = 48.2 µg/mL) towards *P. falciparum*. The petroleum ether and chloroform (1:1) extract of *A. bracteolata* produced 100% inhibition at dosage of ≤50 µg/mL (Ahmed, Nour, Mohammed, & Khalid, 2010). Compared with the earlier researchers' report, the present *in vivo* observation leaf methanol extracts of *A. marmelos, A. indica* exhibited better parasitemia inhibition of (67.0 ± 4.02)% and (72.0 ± 8.44)% on day 4 against *P. berghei* (NK65) at 600 mg/kg/d, respectively. The plant leaf methanol extracts sustained the MST (mean survival time) of the study, Swiss albino mice treated with the leaf extracts suppressed *P. berghei* (NK65) and decreased the pathologic effect of the parasite in mice (de Andrade-Neto et al., 2007; Traore et al., 2008).

The present study, similar potency was also observed in leaf methanol extract of *C. auriculata* against *P. berghei* (NK65). Similarly, the root bark methanol extract of *C. singueana* exhibited inhibition against *P. berghei* with ED_{50} value of (847 ± 30) mg/kg (Adzu, Abbah, Vongtau, & Gamaniel, 2003). Tona et al. (2001) have reported the dichloromethane extract of *C. occidentalis* showed 60% growth suppression against *P. berghei* (ANKA) at 200 mg/kg of body wt. Kayembe, Taba, Ntumba, Tshiongo, and Kazadi (2010) have observed the four plants *C. occidentalis, C. alata, Ocimum basilicum* and *Garcinia kola* isolated from 20 quinones and studied the *in vitro* anti-malarial action towards *P. falciparum*. Six isolated quinones were showed the most activeness with an IC_{50} value of <1 µg/mL and other 14 quinines were bearing a moderate activity.

Previously, the *in vivo* antiplasmodial potential against *P. berghei* infected mice were evaluated in whole plant of *Eleucine indica* fractions and chloroform, ethyl acetate, n-hexane, butanol and aqueous extracts, extracts (600 mg/kg) and fractions (400 mg/kg) exhibited significant ($P < 0.05$, $P < 0.001$) activity in the 4-day consecutive test (Ettebong, Nwafor, & Okokon, 2012). *Bridelia ferruginea* stem bark aqueous extract showed significant ($P < 0.05$) antiplasmodial activity against *P. berghei* infected mice at 5000 mg/kg (Mbah, Akuodor, Anyalewechi, Iwuanyanwu, & Osunkwo, 2012). Our results showed that the methanol extract of *A. indica* showed the better *in vivo* parasitemia suppression of 72.0%. Similarly, the plant root bark extracts of *Vernonia lasiopus* showed the maximum *in vitro* antimalarial activity with IC_{50} values as low as 1.0 mg/mL, and significant *in vivo* parasitemia inhibition of 59.3% with mice mean survival rates of 60% on day 4 (Dua et al., 2004; Muregi, Ishih, Miyase, & Suzuki, 2007).

The antibacterial activity of hentriacontan-16-one (palmitone) against *Staphylococcus aureus, S. albus, S. viridans*, *Escherichia coli, Pseudomonas pyocyanea* and *Klebsiella* has been reported by Sharma (1993). Behari and Sharma (1986) have also reported the isolation of 16-hentriacontanone from methanol and petroleum ether extracts of *A. squamosa.* The GC-MS separation of hexane extract led to the removal of a $C_{31}H_{64}$ linear alkane ketone: 16-hentriacontanone (palmitone), reported to be a CNS depressant (Gonzalez-Trujano, Navarrete, Reyes, Cedillo-Portugal, & Hong, 2001). Phung, Casazza, Perego, Capranica, and Busca (2015) reported the 16-hentriacontanone compounds were the least noxious than fatty acids but very high boiling point (816 and 772 K for stearone and palmitone, respectively) and maximum melting point (362 and 357 K). Palmitone (hentriacontan-16-one) has remarkable antifungal and antibacterial activities (Shanker et al., 2007). Palmitone, an isolated active principle obtained in 0.2% yield from a crude extract of *A. diversifolia,* produces an anticonvulsant response to penicillin-induced seizures similar to that produced by the crude ethanol extract of this plant at a higher dose than the extract (Gonzalez-Trujano, Lopez-Meraz, Reyes-Ramirez, Aguillon, & Martinez, 2009). The palmitone reduces the glutamate-activated NMDA receptor and prevents the decrease in peptidase activity and hippocampal damage (Cano-Europa et al., 2010). Anticonvulsant properties and bio-guided isolation of palmitone from the leaves of *Annona diversifolia* indicate antiepileptic properties (Gonzalez-Trujano et al., 2001).

The histopathology studies of *A. marmelos, A. indica* and *C. auriculata* treated nephrotoxicity of kidney and hepatic damage of liver cells were performed. From histopathology of kidney and liver section, we observed the normal histological appearance compared with control Swiss albino mice kidney and liver section on day 4 at 600 mg/kg/d. Similarly, Kamaraj et al. (2014), have reported the isolation and characterization of active antiplasmodial compound β-caryophyllene with significant antiplasmodial activities and exhibited harmless to the body parts of *P. berghei* (NK65)-infected Swiss albino mice with the higher tested dose at 100 mg/kg/d. This study suggested that the south Indian medicinal plants *A. marmelos* and *A. indica* revealed effective antiplasmodial activities, and the histopathology study exposed that protection against kidney and liver of *P. berghei* (NK65) infected mice administered the maximum tested dose at 600 mg/kg/d.

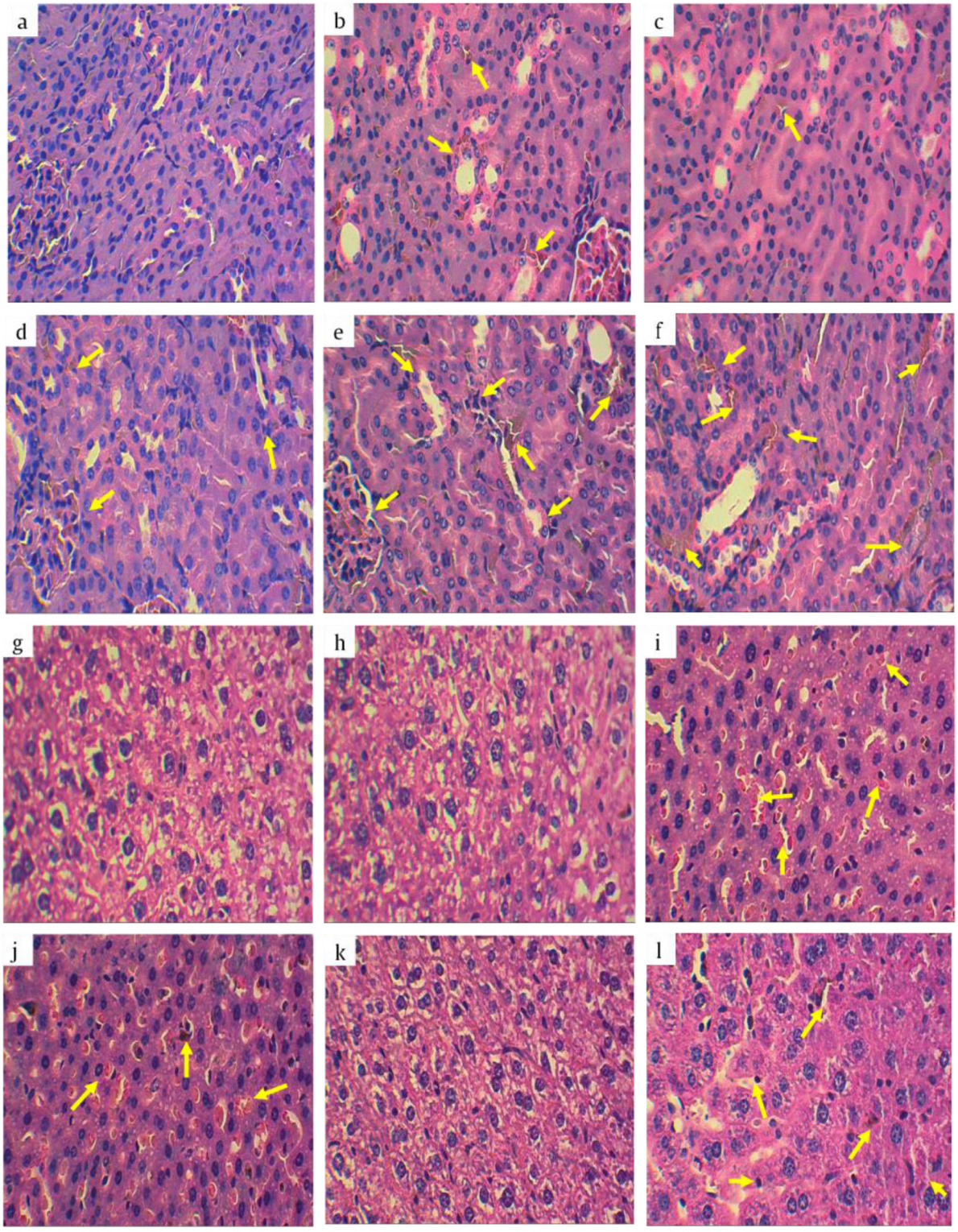


Fig. 3. Histopathology study of kidney cells in normal mice (a), *A. marmelos* (b), *A. indica* (c), *C. auriculata* (d) and chloroquine (e) treated mice kidney cells at 100 mg/kg once daily for 4 d. Deposition of malarial pigments in cells and intracellular gaps in the kidney section of untreated (infected) mice (f). Histopathology study of liver cells in normal mice (g), *A. marmelos* (h), *A. indica* (i), *C. auriculata* (j) and chloroquine (k) treated mice liver cells at 100 mg/kg once daily for 4 d. Infiltration of lymphocytes in liver section of untreated (infected) mice (l).

5. Conclusion

In the present study, the leaf methanol extracts of *A. marmelos*, *A. indica* and *C. auriculata* were tested against *Plasmodium berghei* (NK65) infected mice. The results obtained from methanol extract of *A. indica* active against *in vivo* antimalarial activity. The novel antimalarial compounds can also be isolated from the most active methanol extract of *A. indica* and tested against human malaria parasites in the future. The histopathology of kidney and liver microscopic examination clearly indicated no pathological changes in

the control and methanol extracts in treated mice. All these results would contribute to the development of potential antimalarial drug from the South Indian herbal plants.

Compliance with ethical standards

All applicable international and national guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Conflict of interest

We confirm that this manuscript was read by all authors before submission. The authors declare that they have no competing interests.

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