Antimalarial effect of the ethanolic extract of *Nigella sativa* seeds (black cumin) against *Plasmodium berghei* (NK65) infection in mice.

[1]Ibrahim A. Oreagba [2]Oluwagbenga O. Aina. [3]Peter U.Bassi [1]Olanrewaju A. Salako and [1]Ifeoluwa Ogunsola

[1]Department Of Pharmacology, College of Medicine, University of Lagos, Nigeria.
[2]Nigerian Institute of Medical Research Yaba, Lagos, Nigeria.
[3]Clinical Pharmacology Unit, Department of Medicine,
University of Maiduguri Teaching Hospital, Maiduguri, Nigeria.

Author for correspondence

Oluwagbenga O. Aina
Phone: 08033946735 Email: gbengaaina2003@yahoo.com

STRACT

ckground: *Nigella sativa* seeds have en used as a traditional medicine for e treatment of a variety of illnesses cluding parasitic diseases. The study ned to investigate the suppressive, rative and prophylactic effects of *gella sativa* seeds against *smodium berghei* infection in mice.

ethods: Oral doses of ethanolic seed tracts (100, 200 and 400 mg/kg) of *N. iva*, were screened using the four-y suppressive, curative and phylaxis assays, for their anti-alarial properties against *smodium berghei* (NK65) *infection* mice.

sults: Oral treatment of the seed ract showed suppressive activity in groups of mice with the highest pressive effect recorded at the 100 /kg dose of the extract and also for rative activity 100 mg/kg dose of the ract showed the highest curative ect which significantly ($p < 0.05$) creased the parasitaemia of the ected mice. On the other hand, for phylactic effect, the extract showed dose dependent protective effect h the highest effect at the dose of mg/kg.

nclusion: The results of this study firmed the usage of this plant in Northern Nigeria folklore medicine as an anti-malaria remedy.

Keywords: *Nigella sativa, Plasmodium berghei*, parasitaemia

INTRODUCTION

Malaria, one of the six most important parasitic diseases of man, is an infectious disease that is wide spread and endemic in tropical and subtropical regions of the world[1]. It is a major public health problem in sub-Saharan Africa, where over 85 - 90% of all global burden of malaria exists with up to 60% of all outpatient visits in areas with high malaria transmission and 30 -50% of all hospital admissions are attributed to malaria[2]. The disease kills 1.1 million people worldwide each year. Approximately 1 million of these deaths are in Africa[2].

The global strategy for malaria mainly focuses on case management through provision of drugs capable of reducing or eliminating the parasites, and consequently reducing the morbidity and mortality of malaria[3,4].

Drug resistant *Plasmodium falciparum* and the emergence of insecticide resistant *Anopheles mosquitoes* not only cause the spread of malaria to new areas but also its re-emergence in areas, where it had previously been eradicated[5]. This has prompted research towards the discovery and development of new, safe and affordable anti-malarial drugs.

In this respect, plant resources are potential targets for research and development of alternative malarials, with novel modes of action[6]. Although up to 80% of the African population uses traditional medicine especially plant remedies for the management of diseases including malaria, plants are not yet fully explored[7]. Furthermore, as anti-malarial drug resistance has become one of the greatest challenges against malaria control, drug-resistance to Chloroquine and quinine was responsible in the spread of malaria to new areas and recurrence of malaria in areas where the disease had been eradicated[8].

Drug resistance has also played an important role in the occurrence and severity of epidemics in some parts of the world. Population migration has introduced resistant parasites to areas previously free of drug resistance[9]. Moreover, reports also showed that more than 5% of 65 isolates of the parasite from South East Asia are resistant to artemisinin and artesunate[10].

Due to the increasing incidences of

resistance to anti-malarial agents there is a need to develop more effective new anti-malarial drugs[11] that are inexpensive, routinely available to people especially those in the developing countries and the curative course must be short[12].

The *Nigella sativa (Ranunculaceae)* commonly known as karayal, black cumin, and black seed is an annual flowering herbaceous plant native to southeast Asia but has been cultivated in other parts of the world including Africa. *N. sativa* seeds have a great medicinal importance and known to include many medicinal properties particularly in Greco-Arab/Unani-Tibb and Ayurveda system of medicine[13]. Black seed (*Nigella sativa*) is considered to be one of the greatest healing herbs of all times. This herb has been used for millennia to strengthen the immune system, cleanse the body, purify the blood, protect against irritants and support healthy longevity. Long forgotten this herb is now enjoying a positive and welcomed come-back. The seeds have been reported to exhibit many pharmacological effects including anti-parasitic anti-helminthes[14], anticestodal[15] and anti-schistosomal[16], anti-bacterial[17], anti-fungal[18], anti-viral[19], anti-oxidant[20], anti-inflammatory[21] activities and have been shown to enhance the T cell mediated immune response[22].

In spite of the large number of pharmacological studies carried out world wide on *N.sativa* seeds, there is a need to investigate the anti-malarial activity of *N. sativa* of Nigerian origin. The aims of using this plant extract in relation to malarial infections are:

To prevent and treat clinical attacks of malaria

To completely clear the parasite from the patient's circulation.

To reduce the human reservoir of infection that is, cut down transmission of parasites to mosquito.

These are achieved by attacking the parasite at its various stages of life cycle in the human host. A study was conducted in Malaysia to check the suppressive effect of *N. sativa* seed extract on *Plasmodium berghei* parasite[23].

This study aimed to ascertain the suppressive, curative and prophylactic effects of *Nigella sativa* seeds that are produced and used here in Northern Nigeria.

MATERIALS AND METHODS

Reagents and Chemicals:

Nigella sativa extract, tween 80, normal saline (Dana pharmaceuticals), chloroquine (Emzor pharmaceuticals), phosphate buffer saline (PBS) and distilled water.

Plant raw materials

Nigella sativa fresh/dry seeds were purchased from an herbal shop in Maiduguri, Nigeria and the seeds were authenticated by Mr. T.I. Adeleke of Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos

Preparation of extract

The seeds were dried and crushed in a mortar with a pestle into coarse powder. Four hundred grams of the powder was extracted by soaking it in ethanol for 48 hours (95% v/w).

The extract was filtered and the solvent was evaporated in vacuum with a rotatory evaporator that yielded a blackish-brown concentrate. This was kept at 4°C.

Malaria parasite

The malaria parasite used in this research work was the *Plasmodium berghei (NK65)* parasite which was obtained from Nigerian Institute of Medical Research, Yaba, Lagos. Blood was collected from a donor-infected mouse by cardiac puncture. 10 ml of PBS was diluted with 0.2 ml of the whole blood.

Experimental Animals

Eighty albino mice weighing between 18-20g, obtained from the Laboratory Animal Centre, College of Medicine University of Lagos, Idi-Araba, Lagos-Nigeria were used for this study.

The animals were fed with standard pelleted diet (Raf feeds, Akute, Lagos, Nigeria) and clean drinking water. The animals were caged in groups of five and kept in the animal house laboratory, following the guidelines for care and use of laboratory animals in biomedical research[24].

Models for antimalarial activity

The models employed included suppressive test[25], curative and prophylatic test.

Suppressive test

The Peter's 4-day suppressive test against chloroquine sensitive *P. berghei (NK65)* infection in mice was employed[25].Twenty five animals were used in this model, each consisting of five animals in each group treated orally as follows. Positive control given 25 mg/kg body weight chloroquine. Negative control distilled water 10 ml/kg. The other groups were given extract orally using the following doses respectively: 100, 200, 400 mg/kg.

The mice were inoculated with 0.1 ml of the diluted donor blood (containing approximately 1×10^6 *P. berghei* parasitised erythrocytes) intraperitoneally. After one hour, the mice were given distilled water, chloroquine and extract as stated above.

Treatment was continued for the next three days, (D0-D3) and on the fifth day (D4), thin blood smears were made, fixed and stained with 10% Giemsa's stain and rinsed off after 10 mins. The slides were viewed with a microscope using the oil immersion lens at the magnification of x100. The level of parasiteamia was determined by

counting the number of parasitized red blood cells over total red blood cells x100. The average percent suppression of parasiteamia was calculated. Data obtained from the experimental mice were analyzed statistically to indicate significant levels between groups.

Curative test

Evaluation of the curative potential of *Nigella sativa* extract was carried out according to the method described by Ryley and Peters[26]. Twenty five mice were used in this model, each consisting of five animals in each group treated orally as follows. The positive control which was treated with 25 mg/kg body weight of chloroquine.The negative control was given distilled water. The other groups were treated with the ethanolic extract of doses 100, 200, 400 mg/kg of the *Nigella sativa*. The mice were inoculated with *P. berghei (NK65)* parasite as earlier described intraperitoneally and 72 hours post inoculation, the mice were given chloroquine, ethanolic extract and distilled water accordingly for the next four days. The blood smears (thick and thin film) were collected daily, fixed with methanol and stained with 10% Giemsa stain. The level of parasitaemia was also determined.

Prophylatic test

Evaluation of the prophylactic potential of *N. sativa* ethanolic extract was carried out according to the method of Peters[25]. Twenty five animals were used in this model, each consisting of five animals in each group treated orally as follows. The positive control was treated with 25 mg/kg body weight chloroquine. Negative control with distilled water and the other groups with doses 100, 200, 400 mg/kg of the *Nigella sativa* ethanolic extract.

In this particular model, the drugs were given for four days and on the fifth day, the animals were inoculated with the *P. berghei (NK65)* parasite. Each animal was inoculated with 0.1 ml of the diluted donor blood intraperitoneally. On the third day, post infection (Day 8) blood smears were prepared to determine the level of parasiteamia in the mice. This was repeated two days later (Day 10).

Statistical analysis

The results were expressed as mean ± S.D; the data were analyzed using one way analysis of variance (ANOVA). Level of significance was taken as $P < 0.05$. All statistical calculations were performed using SPSS software (SPSS Inc., Chicago, IL).

RESULTS

Suppressive Test

The suppressive activity of different doses of the ethanolic extract of *Nigella sativa* administered orally are summarized in Table1. The ethanolic extract showed suppressive activities in all groups of mice with the highest value noted at 100 mg/kg dose of the *N. sativa* extract (69.62 % suppression), followed by 200 mg/kg and 400 mg/kg with 55.25% suppression and 53.39% respectively.

Table 1: Anti-malarial screening of the ethanolic extract of *N.sativa* seeds treated orally against *P. berghei (NK 65)* in mice for suppressive test.

Extracts	Dose (mg/kg)	Mean Parasitemia ± S.D.±	Average Suppression %
Nigella sativa	100	....	69.62
	200	1.50 ± 1.31*	55.25
	400	1.60 ± 1.50	53.39
- ve Control	0.2 ml	4.13 ± 1.70	0.00
+ ve Control	25 mg	0.00 ± 0.00	100

*Significantly different from the saline control group ($P < 0.05$)

†Results are expressed as mean count ± S.D. (n = 5)

urative Test

he ethanolic extract considerably decreased the level of parasitaemia in all groups of mice with the highest efficacy recorded t 100 mg/kg dose of the *N. sativa* extract, followed by 200 mg/kg and 400 mg/kg respectively.

Table 2: Anti-malarial screening of the ethanolic extracts of *N.sativa* seeds in *P. berghei (NK65)* infected mice treated orally (curative test).

Extracts	Dose (mg/kg)	DAY 3 Mean Parasitemia ± S.D.†	DAY 4 Mean Parasitemia ± S.D.†	DAY 5 Mean parasitemia± S.D.†	DAY 6 Mean parasitemia± S.D.†	DAY 7 Mean parasitemia± S.D.†
Nigella sativa	100	2.17 ± 2.48	1.46±1.67*	1.26±1.53	1.10±2.39*	0.90±2.44*
	200	2.74 ± 1.56*	2.38±2.49	2.22±1.78	2.14±1.66	1.40±1.85
	400	3.10± 1.51*	2.50±2.52*	2.43±2.48	2.20±2.76	2.04±2.58
-ve Control	0.2 ml	4.20 ± 1.37	3.90±1.43	3.98±1.35	3.70±1.21	4.12±1.18
+ve Control	25 mg	2.15 ± 2.26	1.38±0.86	1.22±0.72	0.00±0.00	0.00±0.00

*Significantly different from the saline control group ($P < 0.05$)
†Results are expressed as mean count ± S.D. (n = 5)

Prophylactic Test

The extract showed prophylactic activity in the three groups of mice with the highest value noted at the 400 mg/kg dose of the ***N. sativa*** extract, followed by 200 mg/kg and 100 mg/kg respectively.

Table 3: Anti-malarial screening of the ethanol extracts of *N.sativa* seeds treated orally against *Plasmodium beighei (NK65)*in mice for prophylaxis test.

		DAY 8		DAY 10	
Extracts	**Dose (mg/kg)**	**Mean Parasitemia ± S.D.†**	**Average prophylaxis %**	**Mean Parasitemia ± S.D.†**	**Average prophylaxis %**
Nigella sativa	100	3.12 ± 2.58	51.91	2.02 ± 2.38	58.91
	200	2.84 ± 1.46*	59.60	1.13 ± 0.88	66.60
	400	1.33± 1.31*	69.26	0.98± 1.49*	74.26
-ve Control	0.2 mL	4.29 ± 0.95	00.00	4.20 ± 1.17	00.00
+ve Control	25 mg	0.00 ± 0.00	100	0.00 ± 0.00	100

*Significantly different from the saline control group ($P < 0.05$)
†Results are expressed as mean count ± S.D. (n = 5)

DISCUSSION

The anti-malarial activities exhibited by the ethanolic extract of *N.sativa* were perhaps due to the possible presence of active compounds. The 4-day suppressive test is a standard test commonly used for anti-malarial screening and the determination of percentage inhibition of parasitaemia. A mean group parasitaemia level of less than or equal to 90% of the treated control animals usually indicate that the test material is active in standard screening studies[27]. The results obtained from this study showed significant decrease in parasitaemia of *P. berghei (NK65)*infected mice treated with the ethanolic seed extract of *N. sativa*. The results also showed that higher doses of the extracts not necessarily caused higher degree of suppression as 400 mg/kg dose gave considerable degree of suppression but not as high as 100 mg/kg when given orally.The lowest dose caused highest suppression in parasitaemia of *Plasmodium bergei (NK65)*infected mice while chloroquine a standard antimalaria drug exerted 100% suppression. A previous study showed a similar pattern of suppression *Nigella sativa* extract decreased the parasitaemias of the infected mice for

curative test but did not show if the plant extract will completely eliminate the parasite or not as the curative test was not carried out for more than eight days[8]

The limitation to this test result was that the curative test was not completely done. If the test was completely done, this would have shown if the plant extract showed complete curative results like the standard drug (chloroquine) which had already cleared the parasite from day 3.

When a standard antimalarial drug is used in mice infected with *NK65*, it suppresses parasitaemia to non-detectable levels[28], just like the effect of chloroquine in this study. The observed antimalarial activity is consistent with the traditional use of the plant as herbal medication against malaria in Nigeria. The extract exerted significant effect in mice treated with 100, 200 and 400 mg/kg body weight and also in prophylaxis. 100 mg/kg gave the highest value in both suppressive and curative tests while 400 mg/kg gave the highest value for prophylaxis. This may be due to the longer time it would take for the highest dose of extract to clear from the system compared to a lower dose.

The extract of *N.sativa* seeds contained different classes of alkaloids[29] that were believed to block protein synthesis in *Plasmodium falciparum*. *N.sativa* seeds also contain phenolic compounds[30]. These molecules are well known for their diverse physiological properties, including among others, anti-carcinogenic, anti-inflammatory and anti-parasitic[31]. Other works have shown that *N.sativa* seeds have possessed potential immunomodulatory effects[32], which as a consequence might give some impact on the host-parasite inter-relationship[32].

The anti-malarial activities of *N.sativa* seed extracts observed in this study could have resulted from a single or combined action of these mechanisms. However, the active responsible principles are yet to be identified, which need further studies to elucidate the anti-malarial mechanism of their action.

CONCLUSION

The results of this study justify and confirm the usage of this seed in the North Eastern Nigerian folk medicine as a suppressive antimalarial remedy. The seed possesses a significant suppressive, curative and prophylactic activity. However, more work is still to be done on the curative test and prophylaxis test to confirm its usefulness in anti-malaria remedy.

REFERENCES

1. World Health Organisation (1998). Malaria: Know the facts. World Health Organisation Newsletter 13(1): 6-7
2. World Health Organisation (2005). Malaria in Africa. Roll Back Malaria Infosheet WHO. Geneva, pp. 1-3
3. World Health Organisation (1993). Assessment of Therapeutic Efficacy of Anti-malarial Drugs of Uncomplicated Falciparum Malaria in Areas with Intense Transmission. Document WHO/MAL/96.1077 Geneva. P.22
4. Scaphira A, Beales PF, Halloran ME (1993). Malaria: Living with Drug. Resistance. Parasitol. Today. 9: 168-174.
5. CollinsWE, Jeffery GM (1996). Primaquine resistance in *Plasmodium vivax*. Am. J. Trop. Med. Hyg. 55: 243-249.
6. Muregi FW, Chhabra SC, Njagi ENM (2003). *In vitro* antiplasmodial activity of some plants used in Kisii Kenya against malaria and their chloroquine potentiation effects. J. Ethnopharmacol. 84: 235-239.
7. World Health Organisation (2002). Center for Health Development. Traditional Medicine: Planning for cost effective traditional health services in the new century- a discussion paper. http:/www.who.or.jp/tm/research . P. 19.
8. Cordell, G. A., C. K. Angerhofer & Jan Peggrito (1994). Recent studies on cytototic, anti-HIV & anti-malaria agents from plants. Am. J. Trop. Med. Hyg 58: 265-244
9. Foster S. D. (1991). Pricing, distribution & Use of antimalarial drugs. Bulletin of the World Health Organization, 69: 349-363.
10. Pickard, A.L., C. Wongsrichanalai, A. Purfield, D. Kamwendo, K. Emery and C. Zaewski (2003). Resistance to antimalarials in Southeast Asia and genetic polymorphisms in pfmdr1. Antimicrob Agents Chemother, 47: 2418-2423.
11. Ridley, R.G.(2002). Medical need, scientific opportunity and the drive for anti-malarial drugs. Nature, 415: 686-693.
12. Rosenthal, P.J.(2003). Antimalarial drug discovery: old and new approaches. The J. Expl. Biol., 206: 3735-3744.
13. Goreja, W. G, (2003) Black seed; nature's miracle remedy. Am.J. Pharm & Toxicol, 2(2): 46 – 50, 2007.
14. Agarwal, R., M.D. Kharya, R., Shrivastava, (1979). Antimicrobial and anthelmintic activities of the essential oil *Nigella sativa* Linn. Indian J. Expl. Biol., 17: 1264-1265.
15. Akhtar, M.S. and S. Rifaat (1991). Field trial of Saussurea lappa roots against nematodes and *Nigella sativa* seeds against

cestodes in children. J. Pak. Med. Assoc., 41: 185-187.
16. Azza, M.M., M.M. Nadia and S.M. Sohair (2005). Sativa seeds against *Schistosoma mansoni* different stages. Mem Inst Oswaldo Cruz, Rio dee Janeiro, 100: 205-211.
17. Hanafy MS, Hatem ME (1991). Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin). J. Ethnopharmacol., 34: 275-278.
18. Zeinab, E.M.E.B., Hala, A.F., Mohie, E.D.Z. E.F., Seham, Y.M.E.T. (2001).Inhibitory effect of gamma radiation and Nigella sativa seeds oil on growth, spore germination and toxin production of fungi radiation. Physics and Chemistry, 60: 181-189.
19. Salem, M.L. and M.S. Hossain, (2000). Protective effect from black seed oil from *Nigella sativa* against murine cytomegalovirus infection. Intl. J. Immunopharmacol., 22: 729-740.
20. Mahmood, M. S., Gilani, A. H., Khwaja, A., Rashid, A. and Ashfaq, M. K. (2003), The *in vitro* effect of aqueous extract of Nigella sativa seeds on nitric oxide production. Phytother. Res., 17: 921-924.
21. Al-Ghamdi MS (2001). The anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa*. J. Ethnopharmacol., 76: 45-48.
22. Haq A, Abdullatif M, Lobo PI, Khabar KS, Sheth KV, Al-Sedairy ST (1999). Immunomodulatory effect of Nigella sativa proteins fractionated by ion exchange chromatography. Int J Immunopharmacol; 21 (4): 283-5.
23. Abdulelah, H.A.A. and Zainal-Abidin, B.A.H. (2007). *In vivo* anti-malarial tests of *Nigella sativa* (black seed) different extracts. American Journal of Pharmacology and Toxicology 2(2): 46-50.
24. National Academy of Sciences. *Guide for the care and use of laboratory animals*, 8th Ed., National Academies Press, Washington, D.C.,USA, 2011.
25. Peters W (1965). Drug resistance in *Plasmodium berghei* I. Chloroquine Resistance. Exp. Parasitol. 17: 80-89.
26. Ryley JF, Peters W (1970). The antimalarial activity of some quinolone esters. Ann. Trop. Med. Parasitol. 84: 209-222.
27. Peter I.T., and Anatoli V.K. (1998). The current global malaria situation. Malaria parasite biology, pathogenesis and protection. ASM Press. WDC. pp. 11-22.
28. Kiseko K, Hiroyuki M, Syun-ichi F, Ryuiichi F, Tomotaka K, Seiji M 3480 Afr. J. Biotechnol. Arch. Toxicol. 57: 278-288
29. Morikawa, T., F. Xum, K. Ninomiya, H. Matsuda and M. Yoshikawa (2004). Nigellamines A3, A4, A5 and C, new dolabellane-type diterpene alkaloids, with lipid metabolism-promoting activities from the Egyptian medicinal food black cumin. Chem. Pharm. Bull. (Tokyo), 52: 494-497.
30. Nergiz, C. and S. Otles (1993). Chemical composition of *Nigella sativa* L. Seeds. Food Chem., 48: 259-261.
31. Ma, Q., K. Kinner.(2002). Chemoprevention by phenolic antioxidants. J. Biol. Chem., 277: 2477-2484.
32. Anthony, P.J., L. Fyfe and H. Smith (2005). Plant active components-a source for anti-parasitic agents. Trend in Parasitol., 21: 462-468.

NJP