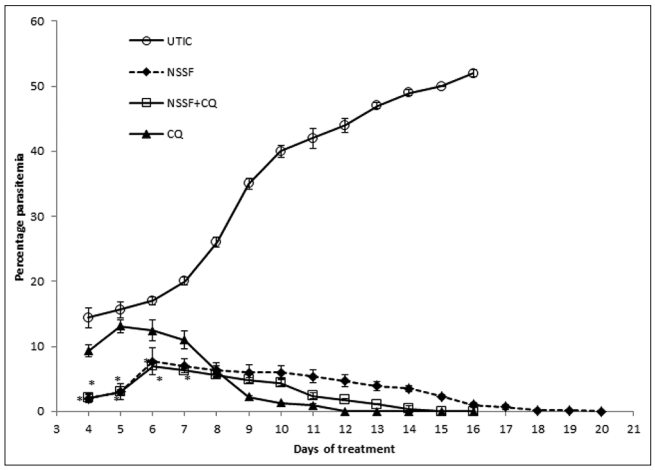

Figure 1: Comparison of Percentage (%) parasitaemia in mice treated with Nigella sativa seed in feed(NSSF) only, NSSF+ CQ (Chloroquine), CQ only and untreated infected control (UNTIC). Values are expressed as mean ± SD. *n*=3, * for =*P*< 0.001 is significant when compared with Chloroquine control (One-way ANOVA followed by Turkey HSD post-hoc test)

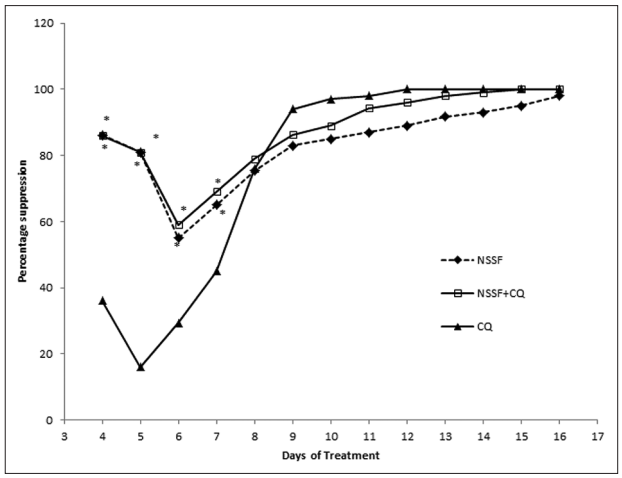

Figure 2: Percentage (%) Suppression of parasitaemia in mice treated with Nigella sativa seed in feed (NSSF) only, NSSF+ CQ (Chloroquine) and CQ only.Values are expressed as mean ± SD. n=3, *for =*P*< 0.001 is significant when compared with Chloroquine control (One-way ANOVA followed by Turkey HSD post-hoc test)

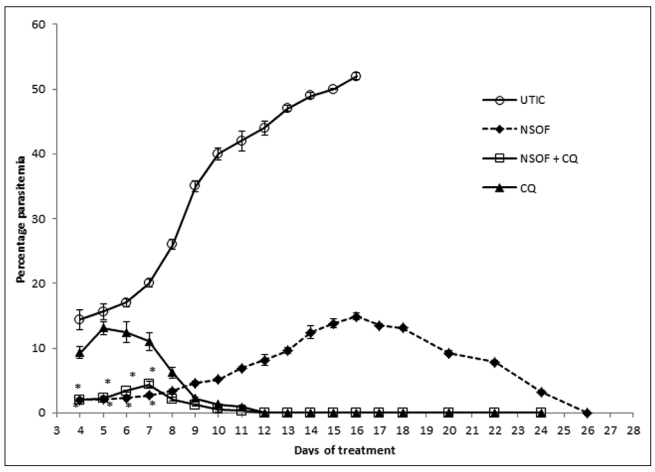

Figure 3: Comparing Percentage (%) of parasitaemia in mice treated with (NSOF) Nigella sativa oil in feed, NSOF+CQ (Nigella sativa oil in feed plus Chloroquine), CQ (Chloroquine) only and untreated infected control (UNTIC). Values are expressed as mean ± SD. *n*=3, *for =*P*< 0.001 is significant when compared with Chloroquine control (One-way ANOVA followed by Turkey HSD post-hoc test)

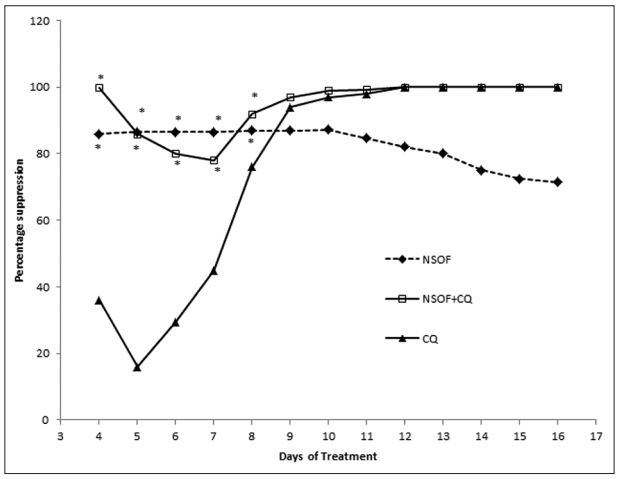

Figure 4: Percentage (%) Suppression of parasitaemia in mice treated with Nigella sativa oil in feed (NSOF), NSOF+CQ (Nigella sativa oil in feed plus Chloroquine) and CQ (Chloroquine) alone. Values are expressed as mean ± SD. *n*=3, *for =*P*< 0.001 is significant when compared with Chloroquine control (One-way ANOVA followed by Turkey HSD post-hoc test)

with conventional antimalarial drugs is not new [5,6] but has begun with traditional remedies in the light of ineffectiveness of existing mode of treatment. Traditional use of remedies such as *Nigella sativa* as adjuvant is becoming popular as some of them are common knowledge and have been employed for centuries in the treatment of different diseases.

The findings from the present study showed that there was an earlier parasite clearance with either NSSF or NSOF when combined with CQ than in CQ treatment alone. This shows that *N. sativa* seed or oil extracts as supplements enhanced parasite clearance when combine with CQ. Methanol and ethanol extracts of *N. sativa* have been demonstrated to have anti-plasmodia activity in mice infected with animal species of the plasmodium.[14,22,23] Reports indicate that *N. sativa* acts as an antioxidant by reducing the production of free radicals, hence by this action it augments the antioxidant status of the host, enabling the host to reduce the effects of reactive oxygen species and therefore decreases oxidative stress generated by malaria and in the cause of treatment with chloroquine.[23] This might explain the complete parasite clearance as seen in some of the groups during the present investigation. Treatment of malaria in Nigeria particularly in this era of drug resistance, is expensive for a majority of the sufferers who might barely afford the antimalarial drug designated as first line and will not see the need to buy any prescribed antioxidant as they are added expenses.

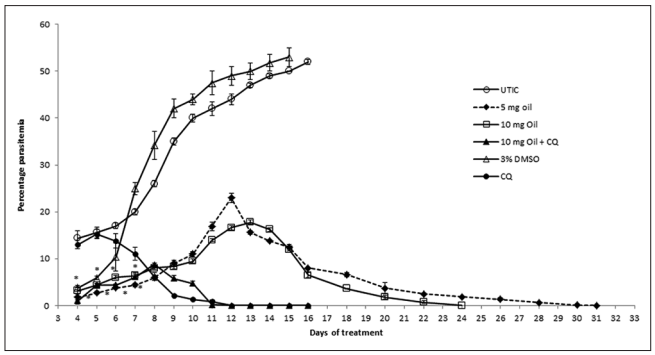

Figure 5: Comparison of Percentage (%) parasitaemia in mice treated with various concentrations of Nigella sativa oil extract (5 mg/kg and 10 mg/kg), 10 mg/kg + CQ (Chloroquine), CQ (Chloroquine) alone, UNTIC (untreated infected control) and 3% DMSO. Values are expressed as mean ± SD. *n*=3, * for = *P*< 0.001 is significant when compared with Chloroquine control (One-way ANOVA followed by Turkey HSD post-hoc test)

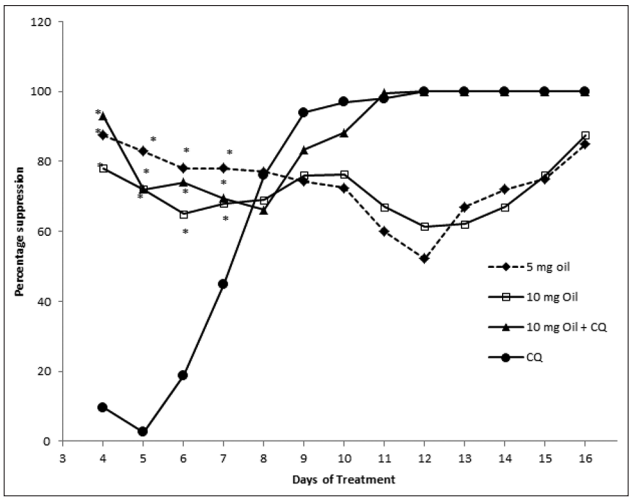

Figure 6: Percentage (%) suppression of parasitaemia in mice treated with various concentrations of Nigella sativa oil extract (5 mg/kg and 10 mg/kg), 10 mg/kg Nigella sativa oil extract plus CQ (Chloroquine) and CQ (Chloroquine) alone. Values are expressed as mean ± SD. *n*=3, *for =*P*< 0.001 is significant when compared with Chloroquine control (One-way ANOVA followed by Turkey HSD post-hoc test)

Ekeanyanwu *et al*.,[24] and Aghedo *et al*.,[25] showed from their studies that the antioxidant levels in plasmodium-parasitized children in the North-West of Nigeria were low and that the more severe the malarial infection, the lower the antioxidant level and the packed cell volume. They recommend that malaria-parasitized children, particularly those in the North-West of Nigeria, be placed routinely on antioxidant vitamins to manage the micronutrient deficiencies as was seen in the children. The non-usage of antioxidants to manage micronutrient deficiencies is not limited to one geographical region according to an earlier report by Ekeanyanwu *et al*.,[24] from studies carried out in Southeastern Nigeria. Therefore, there will be potential benefit of antioxidant supplementation with the use of CQ or other antimalarials.[7] We also observed that complementary use *N. sativa* with CQ produced enhanced parasite suppression which was very significant. In comparison, NSOF is seen to have produced earlier parasite suppression than NSS treatment group with this effect further improved with the addition of CQ. This potentially makes the *Nigella sativa* oil extract a more likely antimalarial agent as an adjuvant. The present finding is consistent with the work of Dwived *et al*.,[10] in which they reported that the co-administration of CQ and an immune stimulant Picrurhiza kurrua extract enhanced the efficacy of CQ in murine mice. Also, studies of co-administration of various antimalarial tested medicinal plant extracts in Madagascar and Kenya revealed a reversal in chloroquine resistance in animals.[26,27] In other studies, diet supplementation with genisten was reported to have suppressed liver infection with *P. berghei* thus reducing the blood parasite load.[28] It is important to note that the liver stage is the rate limiting step and critical for subsequent erythrocyte stage. The arrest of the hepatic parasitic stage is usually targeted for prophylaxis, since it will stop parasite multiplication. As studies have shown that *Nigella sativa* (seed and oil) will enhance antioxidant profile,[29,30] it will also assist the host to build up natural immunity needed to fight the clinical stage of the infection. In combination with antimalarial agent such as CQ, the effect will be the much needed cure as is shown by the results in the present study. Malaria is still claiming casualties because of difficulties in its eradication; it is not surprising that several treatment options have been advocated by both orthodox and traditional practitioners in this fight. The life expectancy of the average Nigeria has dropped considerably over the years with the emergence of chloroquine resistant Plasmodium parasites.[31] Due to multidrug resistance associated with the use of antimalarials, supplementation and/or combination with CQ have been tried.[32] *Nigella sativa* seed and oil as shown in this study have the potential to be used as both supplementation and as adjuvant. It is of great significance that the results from the present study showed that either the seed, oil or in combination with CQ produce no mortality and therefore safe.

CONCLUSION

This study has further highlighted the therapeutic potential of this plant seed extract as a medicinal supplement. In malarial endemic area, the use of *Nigella sativa* as an adjuvant will reduce the adverse effect of CQ and the cost of malarial treatment with CQ being the cheapest and most available.

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