Original Article

Effects of Short-Term Diet of *CucumisMeloand SesamumIndicum* Seeds on the Parasite Load, Immune Response, and Health of Mice Infected with *Plasmodium Berghei*

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Abstract

This study assessed the impact of Melon (Cucumis melo) seeds and Sesame (Sesamumindicum) seeds on the parasite load, immune response and health of mice infected with Plasmodium berghei. Fourty-five Albino mice used in this research were randomly distributed to five groups (1-5); Group 1 was fed a diet containing 2.6g of Melon seeds, Group 2 a diet containing 2.1g of Sesame seeds, Group 3 a diet containing 2.35g of Melon-sesame combination, while Groups 4 and 5 served as control groups. The percentage of parasitaemia in mice infected with Plasmodium berghei but not treated with the short-term diet was 6.78 ± 0.06%. However, feeding the mice a combination of melon and sesame in a short-term diet significantly reduced ($p<0.05$) the parasite load to 2.67 ± 0.56%. Similarly, using a single short-term diet of either melon (1.47 ± 0.31%) or sesame (2.33 ± 0.90%) also resulted in a significant decrease ($p<0.05$) in the percentage of parasitaemia compared to the negative control. A short-term diet combining melon and sesame reduced parasite load by 64%. When fed individually, the short-term diets of melon and sesame led to chemo-suppression rates of 66% and 78%, respectively, with the single melon diet demonstrating the greatest effectiveness among the three treatment options. However, there was no significant difference ($p>0.05$) in the chemosuppressive activity among the three types of short-term diets administered. The result also showed that the infection with P. berghei resulted in a significant decrease ($p < 0.05$) in the weight of the negative control group when compared to the normal group. Feeding the infected mice, a combination or single diet of melon and sesame seeds

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did not lead to a significant improvement ($p < 0.05$) in their weights compared to the negative control and the healthy uninfected group.

Keywords: Cucumismelo, Sesamumindicum, Albino mice, RBC, WBC, PCV, parasitaemia, malaria.

Introduction

According to[1], malaria is a major parasitic disease that is endemic in the tropics, particularly Sub-Saharan Africa and is transmitted through the bite of an infected female *Anopheles* mosquito. The transmission and control of this deadly disease involve a delicate interplay of factors, including human behaviour, mosquito populations, parasite dynamics, environmental conditions, healthcare infrastructure, and policy decisions. This complex web of interactions evolves, making malaria control a challenging and dynamic issue that requires sustained efforts and adaptive strategies to effectively mitigate its impact and protect vulnerable populations[2]. Also, the increasing prevalence of treatment-resistant infections highlights the critical imperative to pursue unconventional therapeutic options, such as nutritional modifications and other natural remedies. This shift is driven by the limitations of conventional pharmaceuticals in addressing evolving pathogens[3].

The persistence and growth of intracellular parasites like *Plasmodium* species rely heavily on the host's physiological state, especially the availability of vital nutrients[4]. These nutrients serve functions beyond simply supplying energy; they are key modulators of gene expression, metabolic pathways, and developmental processes[4]. Such regulation is mediated by intricate signalling networks that allow cells to adjust to constantly changing environmental conditions[5].

Melon seeds (*Cucumismelo*) display multiple medicinal properties, including anti-inflammatory and immunomodulatory effects[6]. These properties are attributed to diverse bioactive compounds found in both the peel and seeds, which may contribute to immune regulation, reduced inflammation, and overall support of immune health[7]. [8]noted that melon seeds are rich in protein (21-23%), crude fibre (33-39%), and contain significant amounts of minerals, including iron, zinc, and manganese. They also noted that melons contain bioactive compounds such as phenolic compounds, flavonoids, and antioxidants.

Similarly, *Sesamumindicum* showed dose-dependent antimalarial activity, enhancing survival rates and helping to maintain stable physiological measures like body weight and rectal temperature[3]. *Sesamumindicum*is rich in lignans such as sesamin and sesamolin, known for their dual antioxidant and anti-inflammatory roles[9]. These compounds could act synergistically with the host's immune defences, potentially enhancing resilience against Plasmodium infections[10].

Researchers frequently use *Plasmodium berghei*, a malaria parasite that infects rodents, as a model to study how these parasites interact with their hosts and to test new ways to combat the disease. This mouse model is a useful tool for investigating the malaria parasite's life cycle, immune responses, and potential treatments[11]. In this model, infected mosquitoes or sporozoites introduce the parasite, which then invades liver cells before entering the bloodstream to attack red blood cells in a cyclic manner that mimics human malaria infection[11], and in the search for effective and affordable treatment options, traditional plant-based remedies have gained renewed interest among researchers[12]. This study investigates the effect of these oily seeds on the parasite load, immune response and health of mice infected with *Plasmodium berghei.*

Methods

Experimental site: The study was carried out in the Zoology laboratory located at the Faculty of Biosciences, NnamdiAzikiwe University, Awka.

Duration of the study

The study was carried out for a period of 5 weeks. The short term treatment of experimental feed lasted from week two (immediately after acclimatization) to the final week (upon sample collection).

Ethical Considerations and Approval: The study adhered to ethical guidelines for the ethical treatment

of animals during capture, handling, and sample collection. Necessary permits were obtained from the Animal Research Committee of NnamdiAzikiwe University, Awkawith reference number NAU/AREC/2025/0062.

Procurement of food materials: *Cucumismelo* and *Sesamumindicum* seeds were purchased from Eke Awka market, Awka. The plants were subjected to the herbarium curator attached to the Department of Botany Laboratory, Faculty of Biosciences, NnamdiAzikiwe University, Awka, for identification.

Preparation of food material: The melon and sesame seeds were air-dried at room temperature for eleven (11) days to avoid the reduction of the phytochemical components[13]. The dried seeds were blended to a coarse texture using a Corona blender.

Procurement and Management of Experimental Animals: A total number of 45 Swiss albino mice (30.15g), 10 weeks old, were obtained from the Institute for Advanced Medical Research and Training (IAMRAT) College of Medicine, University of Ibadan. The mice were transported to the research area in a transportation box measuring 40 x 20 x 20 cm to ensure adequate ventilation.

The mice were acclimatised for a period of one week before the experiment began**.** They were fed with commercial food (Vital Feed Broiler Starter, 18.00 ± 0.50 g/100 g crude protein, and 2106.00 kcal/kg metabolizable energy, Vital Feed, Grand Cereals Limited, Jos, Plateau State, Nigeria) and water ad libitum daily[14].

Experimental Design: A cross-sectional descriptive survey was conducted amongst adults in both urban and rural areas to establish their consumption pattern of *C.melo* and *S.indicum* seeds. One hundred and five people participated, and the response was obtained using a Google questionnaire. Statistical analysis of the results gotten from the questionnaire was used to get the average amount of Melon and Sesame seeds consumed and the frequency of consumption. Based on the quantity consumed by humans, as obtained in the result of the survey; a standard conversion technique was applied to obtain an equivalent dose for mouse using the formula:

HED (mg/kg) = Animal does (mg/kg) × (Animal Km / Human Km)

Where;

HED - Human equivalent dose, Km factor for each species is constant; the Km ratio is used to simplify calculations

HED (mg/kg) = Animal does (mg/kg) × Km ratio. The Km ratio is obtained from the table below

Table 1. Animal Km Ratio

Species	Reference body weight (kg)	Working weight range (kg)	Body surface area (m^2)	To convert dose in mg/kg to dose in mg/m^2, multiply by K_m	To convert animal dose in mg/kg to HED in mg/kg, either	
					Divide animal dose by	Multiply animal dose by
Human	60	-	1.62	37	-	-
Mouse	0.02	0.011-0.034	0.007	3	12.3	0.081
Hamster	0.08	0.047-0.157	0.016	5	7.4	0.135
Rat	0.15	0.08-0.27	0.025	6	6.2	0.162
Ferret	0.30	0.16-0.54	0.043	7	5.3	0.189
Guinea pig	0.40	0.208-0.700	0.05	8	4.6	0.216
Rabbit	1.8	0.90-3.0	0.15	12	3.1	0.324
Dog	10	5-17	0.50	20	1.8	0.541
Monkeys (rhesus)	3	1.4-4.9	0.25	12	3.1	0.324
Marmoset	0.35	0.14-0.72	0.06	6	6.2	0.162
Squirrel monkey	0.60	0.29-0.97	0.09	7	5.3	0.189
Baboon	12	7-23	0.60	20	1.8	0.541
Micro pig	20	10-33	0.74	27	1.4	0.730
Mini pig	40	25-64	1.14	35	1.1	0.946

*Data obtained from FDA draft guidelines.[7] FDA: Food and Drug Administration, HED: Human equivalent dose

Source:[15].

The experimental animals were randomly housed in 15 stainless steel metabolic cages laid out in a complete randomised design (CRD) of three treatments, replicated thrice with each replicate having three mice. The experimental animals, which were of homogeneous sizes, were randomly stocked into five cages (24 by 24 cm) containing three mice each and labelled as follows: 1-5;

Group 1: were fed with 2.1g of Sesame and 10.5g of commercial feed. Before and after infection

Group 2 were fed with 2.6g of melon and 10.4g of commercial feed. Before and after infection

Group 3: were fed with 1.3g of Melon, 1.05g of sesame and 22.8g of commercial feed before and after infection.

Group 4: Normal Control (Uninfected, Untreated)

Group 5: Negative Control (Infected, Untreated)

Procurement of parasites: *Plasmodium berghei* parasites were obtained from the National Institute for Medical Research, Lagos (NIMR). The parasites were inoculated into four mice and transported down to NnamdiAzikiwe University, Awka, in a transportation box measuring 40 x 20 x 20cm to ensure adequate ventilation.

Inoculation: One millilitre (1ml) of *P. berghei-infected* blood was taken from a donor mouse and diluted with normal saline. The diluted blood containing approximately 1.25×10^6 parasites was used to inoculate the experimental animals from groups 1, 2, 3, and 5 via the intraperitoneal route. Inoculation took place one week into the experimental feeding, and infection has been confirmed in the donor mice. Parasitaemiain the experimental animals were determined on the third day and was estimated by making use of blood from the tail and checked microscopically with a thin film smear[16].

Determination of mean weight and temperature changes of the mice: The weights of the mice were measured daily using a sensitive weighing balance to monitor the change in weight of the mice. Rectal temperature was also measured with a digital thermometer before infection, and then daily. All the control groups and malaria-infected mice were observed visually throughout the experiment.

Determination of Parasitaemia and Inhibition: Parasitaemia was measured daily by collecting tail blood samples and staining with Giemsa stain[17]. Parasitaemia was calculated based on the number of infected erythrocytes in counted among the observed erythrocytes. The formula for parasitaemia is as follows:

Parasitemia (%) = Number of infected erythrocytes/ Counted erythrocytes x 100

The percentage of inhibition was calculated using the following formula:

Inhibition (%) = NC – TG x 100 NC

NC: mean of parasitaemia in negative control

TG: mean of parasitaemia in the treated group (G1, G2, G3)

Packed Cell Volume (PCV) Determination: The packed cell volume was determined by making use of the micro haematocrit centrifuge method as described by[18 19].

White Blood Cell (WBC): The WBCs were counted using the principle of calibrated capillary tube for blood sampling with a Haemocytometer[20]. The counted White Blood Cells were recorded and calculated with the formula:

Number of WBC in 1µL = $\frac{N \times 10 \times 20}{4}$. Source:[21].

Where N; is the number of cells counted,

10; is the depth of the counting chamber,

20; is the dilution ratio – 1:20, and

4; is the squares counted.

This is summarised into N × 50, which gives the value of WBC.

Red Blood Cell (RBC): The RBC count was determined using the principle of a calibrated tube for blood sampling with the hemocytometer method[20].

The counted RBCs were recorded and calculated with the formula;

Number of RBC in 1µL = $\frac{N \times 10 \times 200}{1/5}$. Source:[21].

Where N is the number of cells counted,

10 is the depth of the counting chamber,

200; is the dilution ratio – 1:200, and

5; are the squares counted?

This is summarised into N × 10000, which gives the value of RBC.

Statistical analysis: The data of haematological indices, weight, temperature and parasitaemia of mice were subjected to mean, standard deviation and One-Way Analysis of Variance (ANOVA) was carried out using SPSS (25)[22]. The Tukey's Multiple Comparison Test was used to separate mean significant differences between treatments at a 5% significant level. Results are expressed as Mean±SD; Mean values with different alphabets as superscripts are significantly different ($p<0.05$).

Results

Effects of short-term diets of melon and sesame seeds on parasite load of infected mice

The percentage of parasitaemia in mice infected with *P. berghei* and not treated with the short-term diet was 6.78 ± 0.06%. However, feeding the mice a combination of melon and sesame in a short-term diet significantly reduced ($p<0.05$) the parasite load to 2.67 ± 0.56%. Similarly, using a single short-term diet of either melon (1.47 ± 0.31%) or sesame (2.33 ± 0.90%) also resulted in a significant decrease ($p<0.05$) in the percentage of parasitaemia compared to the negative control (Table 2) short-term diet combining melon and sesame reduced parasite load by 64%. When fed individually, the short-term diets of melon and sesame led to chemo-suppression rates of 66% and 78%, respectively, with the single melon diet demonstrating the greatest effectiveness among the three treatment options. However, there was no significant difference ($p>0.05$) in the chemosuppressive activity among the three types of short-term diets administered (Table 2).

Table 2. Percentage Parasitaemia and Chemosuppression of mice infected with *P. berghei*and treated with a short-term diet of melon and sesame seeds.

Groups	Parasitaemia (%)	Chemosuppression (%)
Melon & Sesame	2.67 ± 0.56^{b}	63.73 ± 8.49^{a}
Melon	1.47 ± 0.31^{b}	78.43 ± 4.49^{a}
Sesame	2.33 ± 0.90^{b}	65.69 ± 13.26^{a}
Negative control	6.78 ± 0.06^{a}	-
Normal control	-	-

Immune Response of *Plasmodium berghei-infected* mice

Infection with *P. berghei* significantly increased ($p<0.05$) the immune response in the negative control group compared to the normal group. In contrast, treatment with both a combination of melon and sesame diets, as well as single diets, led to a significant reduction ($p<0.05$) in the immune response, as indicated by lower white blood cell (WBC) counts in the diettreatment groups(Figure 1).

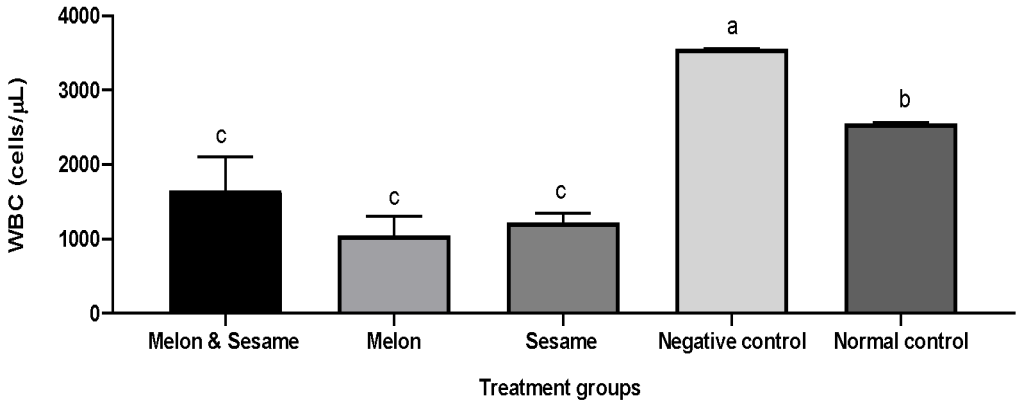

Figure 1: Effect of short-term diet feed on WBC of *P. berghei*-infected mice

Effects of short-term diets of melon and sesame seeds on RBC *P.berghei*-infected mice

The red blood cell (RBC) count in the negative control group significantly decreased after infection with *P. berghei*. Placing the infected mice on a short-term diet consisting of either a combination of melon and sesame or each individually did not improve their RBC counts. The RBC counts for the group fed the combination of melon and sesame, as well as the group on the single melon diet, decreased significantly compared to the negative control. However, there was no significant difference in the RBC counts for the group that received the sesame diet compared to the negative control (Figure 2).

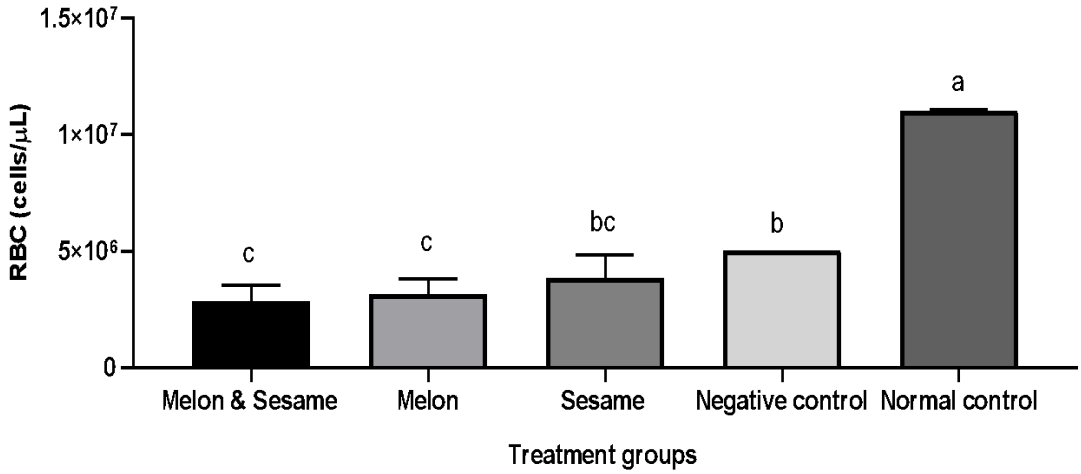

Figure 2: Effect of short-term diet feed on RBC of *P. berghei*-infected mice

Effects of short-term diets of melon and sesame seeds on PCV *P. berghei*-infected mice

Infection with *P. berghei* significantly reduced the packed cell volume (PCV) in the animals compared to the normal control group. However, treatment with either a combination or a single short-term diet of melon and sesame seeds did not lead to a significant improvement in PCV when compared to the negative control (Figure 3).

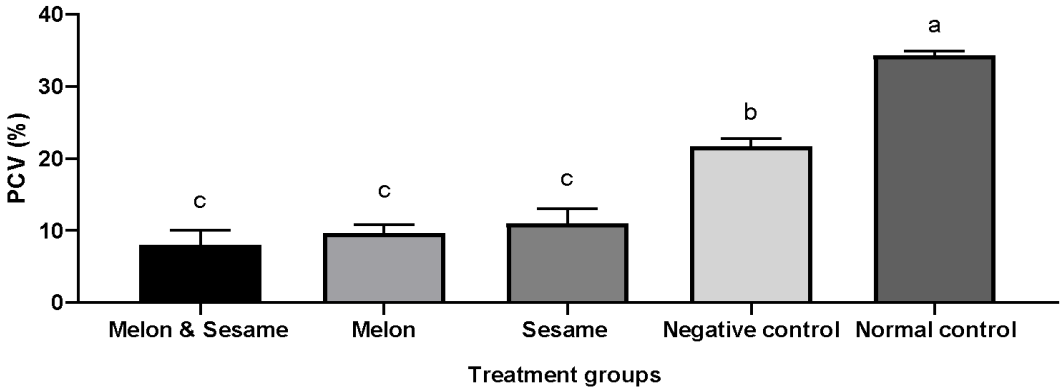

Figure 3: Effect of short-term diet feed on PCV of *P. berghei*-infected mice

Effects of short-term diets of melon and sesame seeds on body weight and temperature of *P. berghei*-infected mice

After acclimatisation, mice in the negative control group had a significantly higher mean weight (37.63 ± 0.06) compared to the other treatment groups. Infection with *P. berghei* resulted in a significant decrease ($p < 0.05$) in the weight of the negative control group when compared to the normal group. Feeding the infected mice, a combination or single diet of melon and sesame seeds did not lead to a significant improvement ($p < 0.05$) in their weights compared to the negative and normal controls. While mice in the normal group gained weight by the end of the study, those in the negative control group experienced a significant weight loss ($p < 0.05$) over the study period. However, the weight loss observed in the groups fed a combination of melon and sesame diets was significantly less ($p < 0.05$) than that of the negative control group. There was no significant difference ($p > 0.05$) in the weight loss between the group fed the sesame diet and the negative control group (Table 3).

After acclimatisation, the body weights of all treatment groups, except for the normal group, were not significantly different ($p > 0.05$) from those of the negative control group. Infection with *P. berghei* and subsequent treatment with the short-term diet did not significantly affect the body temperature of mice in both the negative control and diet groups ($p > 0.05$). Only the normal group experienced a notable decrease in temperature at the end of the study (Table 3).

Table 3. Effects of short-term diets of melon and sesame seeds on body weight and temperature of *P. berghei*-infected mice.

Groups	Weight of animals (g)			Temperature of animals (^{O}C)		
	Initial	Final	Change in weight	Initial	Final	Change in Temp.
Melon & Sesame	25.17 ± 1.05^{d}	23.70 ± 1.65^{c}	-1.90 ± 0.60^{b}	37.40 ± 0.10^{b}	36.40 ± 0.20^{b}	1.00 ± 0.30^{b}
Melon	28.60 ± 1.40^{c}	25.37 ± 1.45^{c}	-3.23 ± 2.85^{b}	37.87 ± 0.75^{b}	36.87 ± 0.06^{b}	1.00 ± 0.70^{ab}

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Sesame	27.57±0.45^{c}	22.90±1.45^{c}	-4.67±1.29^{bc}	38.33±0.55^{ab}	35.90±1.32^{b}	2.43±0.83^{a}
Negative Control	37.63±0.06^{a}	30.33±0.06^{b}	-7.30±0.10^{c}	37.43±0.06^{b}	36.88±0.06^{b}	0.60±0.10^{bc}
Normal Control	31.77±0.06^{b}	35.37±0.06^{a}	3.60±0.00^{a}	38.93±0.06^{a}	39.07±0.12^{a}	-0.13±0.06^{bc}

Discussion

The present study demonstrates that short-term dietary supplementation with a combination of melon and sesame seeds produced a significant reduction in parasite load in mice infected with *Plasmodium berghei*. Short-term supplementation with either melon or sesame alone also produced significant decreases in percentage parasitaemia compared with the negative control. These antiparasitic effects are plausibly related to the nutrient composition of the foods used: melon is a source of vitamins and proteins[23], and sesame is a rich source of calcium[24]. Micronutrients such as vitamin C and calcium have been shown previously to reduce parasitaemia in *P. berghei*-infected mice, supporting the present observations[25] which has medical implications in the treatment of malaria. More broadly, nutrient deficiencies are known to influence malaria progression and outcome[26], which further establishes a mechanistic link between diet and parasite control.

The combination and single-seed diets decreased parasitaemia, and they were associated with a reduction in measured immune response (lower total white blood cell counts). [27]in a similar study reported that the combined meal and single meals of coconut and cashew nuts significantly reduced the parasite load ($p<0.05$) compared to the negative control group. This finding may reflect diet-dependent modulation of immune parameters and/or the complex interaction between protein intake, parasite proliferation, and host immune activation. For example, feeding rodents low-protein diets has been reported to suppress parasitaemia while simultaneously depressing immune indices[28]. Because melon and sesame are protein contributors, the observed changes in WBC may reflect shifts in immune activation and nutrient allocation during infection, rather than a straightforward improvement in immune competence creating a new idea in the treatment of malaria.

The diets did not restore red blood cell (RBC) indices in infected mice. This agrees with prior work in which substitution diets based on processed melon seed in poultry did not change packed cell volume, RBC, or related haematological indices[29]. By contrast, other studies report that sesame supplementation can improve some haematological and biochemical parameters in rodents[30]. The discrepancy may reflect differences in species (mice versus rats or chickens), duration of feeding (short-term here vs. longer trials elsewhere), the processing or dose of the seed supplements, and the specific haematological parameters measured. In the present short-term feeding model, nutrient-driven changes in parasitaemia were detectable, but full hematologic recovery may require longer supplementation or different dosing regimens.

Infected, untreated mice lost weight compared with uninfected (normal) controls, consistent with the anorexia and catabolic state associated with malaria[31]. Short-term feeding with melon and/or) Sesame did not produce significant improvements in body weight relative to negative controls; mice in the normal group gained weight by the end of the study.

Conclusion

The study concludes that while certain seed-based diets can reduce peripheral parasitaemia in the short term, they may be insufficient to reverse infection-associated anorexia and cachexia over the same time frame. Taken together, the results indicate that short-term dietary supplementation with melon and sesame either singly or combined can lower parasitaemia in this mouse model. However, the

diets did not restore RBC or prevent weight loss within the short experimental period (4 weeks). Decreased parasitic presence suggests that these seeds may have beneficial properties that warrant further investigation.

Therefore, we suggest that longer or more comprehensive nutritional interventions might be required to achieve weight recovery and broader haematological benefits.

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