WEIGHT LOSSES IN *ANCYLOSTOMA CANINUM* INFECTED MICE RECEIVING *ALLIUM SATIVUM* AQUEOUS EXTRACT

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ABSTRACT

Globally, one billion people, mostly the poor bear hookworm burden. Quick fixes with A. sativum remediation are attractive but there is no information of host reactions when infected people consume this plant. The study set to ascertain whether A. sativum would modulate weight of Ancylostoma caninum-infected mice. Helminth free mice were randomly selected into one of three classes: uninfected group and unfed with A. sativum and two groups of mice infected with A. caninum. One of the infected group received extract of A. sativum while the other did not. All infected mice received 1000/0.2 ml of suspension of infective A. caninum larvae. Mice fed with A. sativum extract received 250 mg/kg weight of animal daily. All experimental animals were allowed mice chow and water ad libitum. Over a 10-day period, the weight of animals obtained were compared using analysis of variance (ANOVA) at 5 % α-level of significance and Tukey's test post hoc to evaluate data. Though weight instability was observed in all groups, uninfected healthy mice had a more consistent trend. Weight changes began to vary in infected animals from day 3. Infected garlic fed mice lost more weight significantly (p<0.05; range 0.3 – 4.6 g) than control mice (range 0.14 – 2.85 g). However, both infected groups at day 6 began showing sign of recovery with garlic unfed infected animals trending faster recovery. The use of A. sativum during hookworm infection is contra indicated because of the exacerbation of weight losses. The public health implication of A. sativum intake in concomitant infection with A. caninum was highlighted.

Keywords: *Ancylostoma caninum, Allium sativum*, Hookworm larvae, Weight changes, Host-parasite relationship

INTRODUCTION

Allium sativum Linnaeus (Amarylidaceae: Asparagales), commonly known as garlic is one of the oldest of all cultivated plants (Ali *et al.*, 2000; Lanzotti, 2006; Chanda *et al.*, 2011; Lopez-Bellido *et al.*, 2016). It is a monocotyledonous, white compound bulb covered by a few dry membranous scales which protect the plant and inner sheet (Collinc, 2006). The cloves, swollen, are the storage part of the plant leaves (Lopez-Bellido *et al.*, 2016). The major phytoconstituents of *A. sativum* are allicin and its decomposition products, volatile oils, peptides, enzymes, vitamins, terpenes, saponin, glycosides, carbohydrates, minerals and flavonoids (Romeilah *et al.*, 2010; Chanda *et al.*, 2011; Rehman *et al.*, 2017). Allicin is the major odorous compound in garlic (Lekshmi *et al.*, 2015; Rehman *et al.*, 2017). S-allylcysteine, an organosulphur compound is also isolated from *A. sativum* (Mostafa *et al.*, 2000; Eric *et al.*, 2021). Traditional use employed aqueous solution after crushing bulb (Arreola *et al.*, 2015; Orengo *et al.*, 2016). Lanzotti (2006) and Azzini *et al.* (2014) indicated that its phytonutrients from raw or cooked garlic are important elements of Mediterranean, Asian, European and American diets.

Allium sativum is also used in the treatment and prevention of a number of diseases, including cancer, coronary heart disease, obesity, hypercholesterolemia, diabetes type 2, hypertension, cataract and disturbances of the gastrointestinal tract like colic pain, flatulent colic and dyspepsia (Lanzotti, 2006). The plant had also shown antiviral, anti-bacterial, anti-fungal, anti-protozoan and anti-helminth activities (Romeilah *et al*., 2010; Orengo *et al*., 2016; Rehman *et al*., 2017; Khan *et al*., 2021). However, Faisal *et al.* (2019) listed irrigation of the skin and mucous membrane, allergic contact dermatitis and diarrhoea as some undesirable effects of large doses of *A. sativum*. It may also exacerbate liver damage (Eric *et al*., 2021). A protein known as sodium hydrogen exchanger isoform is essential for the absorption of sodium and water from the intestine. *A. sativum* suppress this protein in the terminal ileum and may therefore cause loss of sodium chloride and water during colitis (Khan and Ali, 1999).

Hookworms are haboured by about one billion people across the globe (Wang *et al*., 2010). *Ancylostoma caninum* Ercolani, 1859 (Rhabditida: Ancylostomatidae), is a Clade V nematode which serves as a model of hookworm infection in man (Wang *et al*., 2010). It is a definitive parasite of dog (Daba *et al*., 2021). *A. caninum* is a stout bodied nematode and may be coloured red or ash with sub globular bucal capsule containing three by two ventral teeth at its margin. The males are 11 – 13 mm long, 0.34 – 0.39 mm wide, spicules 730 – 960 µm long and the lateral lobes of the bursa 450 – 490 µm wide. The female is 14 – 20.5 mm long and 0.5 – 0.56 mm wide with posterior end tapering into a tail. This worm is responsible for asymptomatic to severe illness in dogs (Laatamna *et al*., 2021). It is a disease of children associated with poverty (Otranto *et al*., 2017; Harvey *et al*., 2020) and also associated with anaemia in infected animals (Dias *et al*., 2013). It is responsible for heterogeneous infections in animals and amphixenous anthropozoonosis in man manifesting as larva migrans apthous ulceration and eosinophilic enteritis.

Man and mice are generally known as paratenic hosts to *A. caninum* (Laatamna *et al*., 2021) and share anatomical and physiological similarities as well as dependence on the same basic food classes. Recently; it has also been indicated that adult *A. caninum* can be rarely found in man (Daba *et al*., 2021). Consequently, induced changes in host-parasite relationship in a muridan host may be transposable to man.

Owing to neglect, exposure, ignorance or prevailing diseases, all classes of people are readily open to helminthic infections, especially in a polity where the majority are poor (Harvey *et al*., 2020). Da Silva *et al.* (2019) showed that among those with acquired disease such as obesity, infection with this pathogen could exacerbate symptoms. Though mass drug therapy, water, sanitation and hygiene are employed to challenge hookworm infections (Haldeman *et al*., 2020), the pathogen is persisting and Castro *et al.* (2019) demonstrate its rising multiple drug resistance capability. Faced with hookworm burden, people could make rational alternative (Orengo *et al*., 2016) and resort to quick-fixes to alleviate perceived problems associated with this particularly invasive parasite. They will readily recourse to use of local herbs and vegetables to fill this gap. *A. sativum*, which is well known and widely used as a therapeutic that traditional healers depend on for their concoctions or decoctions, eminently presents the opportunity that this category of persons seek most. Its use is continually widened that processed forms are now available in patent medicine stores as capsules. Ordinary folks use or consume it as food seasoner. People may be using this plant and products in circumstances where it may be contraindicated. There is therefore the need to explore host reactions to this plant when faced with concomitant helminth infection using an animal model that share similarities with man. This study was designed to find out if consumption of *A. sativum* could protect against short term weight changes in groups of *A. caninum* infected mice receiving and not receiving *A. sativum*.

MATERIALS AND METHODS

***Allium sativum*:** Bulbs of *A. sativum* were bought from Ogige Main Market, Nsukka, Enugu State, Nigeria, identified and authenticated at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, where voucher specimen number (UNH/001 *A. sativum*) was deposited. An aqueous extract was prepared according to Arivazhagan *et al*. (1999). Scales on *A. sativum* bulbs were removed and appropriate cloves selected and weighed (100 g), crushed and blended with distilled water and made up to 100 ml (Gatsing *et al*., 2005). The blend was separated by centrifugation and supernatant placed in aliquots and frozen until required.

Behavioural Changes and Acute Toxicity Assay: Behavioural changes in mice exposed to concentrations of *A. sativum* extracts were observed. The method of Lorke (1983) was used to find the toxicity of *A. sativum*. Two stages was employed: Stage A involved the use of 9 mice (27.00 ± 1.00 g), where three mice formed another sub-group, that is 3 sub-groups given variable levels of *A. sativum* gavaged *per os* in the following concentration 10, 100 and 1000 mg/kg body weight (BW) respectively. The second stage (B) was applied after 24 hours when no death occurred to another set of mice as stage A. Stage B also involved the use of another set of sub-groups as the former that received respectively, 1,600, 2900 and 5000 mg/kg BW. Similarly the mice were checked for signs of toxicity or death up to 24 hours thereafter.

Phytochemical Assay: Phytoconstituents assay of *A. sativum* were adopted from the studies of Romeilah *et al.* (2010), Chanda *et al.* (2011), Lekshmi *et al.* (2015) and Rehman *et al.* (2017).

Culture of Infective Larvae: Local dogs were screened for hookworm infection by stool microscopy for ova. A dog that harboured *A. caninum* was given a mild laxative which enabled it to expel a few worms that were identified (Uppal *et al*., 2017) and confirmed as *A. caninum* by parasitologist in the Veterinary Department, University of Nigeria, Nsukka. The dog was thereafter kept in captivity to generate parasite ova. Infective larvae were raised in a plastic bowl containing 100 g of pooled dog faeces containing *A. caninum* ova. This was mixed with 300 g of sterile sand and cultured according to Sen *et al.* (1965). The culture solution with which the filter paper was kept wet consisted of 0.01 % hydrochloric acid and 0.2 % sodium chloride (Bhai and Pande, 1981).The larvae were harvested 12 days after inoculation at 25°C and were further cleaned in sucrose gradient or with 400 g sucrose in a litre of distilled water (Friedhoff, 1977). They were kept at 4°C until required. Finally active larvae were collected by using muslin impregnated with 1.5 % agar (Warren, 1965). Infective dose of 1000 larvae/0.2 ml suspension was prepared and delivered *per os* with a pipette.

Feeding of animals with *A. sativum* extract was according to Arivazhagan *et al.* (1999) who used 250 mg/kg of test animal. The weights of mice were obtained and respective volume to be fed each mouse calculated. This was delivered with a micropipette. Before infection with *A. caninum*, the extract of *A. sativum* was given 3 hours earlier.

Experimental Animal and Protocols: Experimental mice whose weight ranged from 22.2 – 29.2 g consisted of 9 randomly selected healthy helminth-free male Swiss Albino Wistar mice obtained from the Animal House of the Veterinary Department, University of Nigeria, Nsukka. The mice were transferred into new cages and allowed acclimatization for 7 days before any experimentation (Waheed, 2021). The experimental mice were kept according to the guideline for care and use of laboratory animals for scientific experiments (NRC, 2010). The floors of the cages were covered with wood shavings and were changed every 48 hours. The animals were given mice chow (Supreme Feeds Growers Mash, 15 % crude protein, 2700 kcal/kg metabolizable energy, Novum Agric Industries Limited, Nassarawa State, Nigeria) and water *ad libitum*. The mice were placed into groups. The first group was uninfected healthy mice (UHM, neither infected nor fed with *A. sativum*). The second group (G-mice) received both *A. sativum* and infective dose of 1000 larvae/0.2 ml suspension *per os*. The last group (C-mice) received the infective dose *per os* and corresponding amount of water in place of *A. sativum*.

Data Collection and Analysis: The weights of experimental mice were determined a day before infection and 24 hours consecutively for 10 days post infection during the experimental period using a weighing balance. Net weight gain or loss and mean for each day for all the groups were obtained. Microsoft Office Excel package was used for calculating mean and the standard error of means and plotting the graph of net weight gain or loss and SPSS version 16.0 was used to analyze the other data.

RESULTS

Behavioural Changes and Acute Toxicity of *Allium sativum* in Mice: In stage (a), there were no noticeable changes in behaviour such as aggressiveness, social interactions, feedback to noise, vitality or state of the tail, etc. Hours after and up to 48 hours after the first stage, their behaviours were not different from when they were being observed during acclimatization. However in stage (b), little changes in behavior were noticed such as diminished vitality and interactions amongst themselves within 24 hours in the group receiving the highest dose. These anomalies were normalized after 48 hours. No deaths were recorded in all the groups (Table 1), confirming the nontoxicity of *A. sativum* aqueous extract.

Phytochemicals in *Allium sativum* Aqueous Extract: From previous studies the major phytoconstituents of *A. sativum* include allicin, S-allylcysteine, volatile oils, peptides, enzymes, vitamins, terpenes, saponin, glycosides, carbohydrates, minerals and flavonoids. Other important compounds present in garlic aqueous extract are allyl methyl thiosulfonate, (E,Z)-4,5,9-trithiadodeca- l,6,11-triene 9- oxide (ajoene), 1 -propenyl allyl thiosulfonate, and y-L-glutamyl-S-alkyl- L-cysteine.

Table 1: Toxicity (acute) of aqueous extract of *Allium sativum* to Albino mice

Group	Amount of extract per kg body weight	Number of animals	Mortality
	Stage A		
I	10	3	0/3
Ii	100	3	0/3
Iii	1000	3	0/3
	Stage B		
I	1600	3	0/3
Ii	2900	3	0/3
Iii	5000	3	0/3

Body Weight Changes: The weight data obtained for infected animals are shown in Table 2. Within the short period of the experiment (10 days), significant ($p<0.05$) weight loss were recorded in the two groups of infected mice (both G-mice and C-mice), while uninfected healthy mice (UHM) had no weight loss. The UHM had a weight gain of 0.43 g within the period. G-mice lost more weight with a range of 0.3 – 4.6 g, while C-mice lost weight ranging from 0.14 – 2.85 g. In both groups, mice which appeared to be regaining weight could lose further weight. Net weight gain or loss is shown in Figure 1. It showed that there was no significant difference ($p>0.05$) in weight loss up till day three between the two infected groups. Significantly different ($p<0.05$) maximum weight loss (-2.6 g) occurred on the 5th day for G-mice and on the 6th day (-2.1 g) for C-mice. Weight recovery appears to be rapid in the control mice but less so in the group receiving *A. sativum*. Weight instability in all the groups were obvious but it was ascertained that uninfected healthy mice had a more consistent trend even though weights still undulated in this group, especially between days 1 – 5 of monitoring after which it began steady marginal gain.

One way analysis of variance was used to analyze the data in Table 2 by comparing the means. The F value was 3.2 confirming significant differences among the groups.

Table 2: Weight changes in mice fed/unfed with *Allium sativum* and infected *Ancylostoma caninum*

Days Post Infection	G-Mice		C-Mice		UHM	
	Mean weight (g)	Net weight (g) Gain /loss	Mean weight (g)	Net weight (g) Gain/loss	Mean weight (g) of mice	Net weight (g) Gain/loss
0	25.43 ± 1.129^{b4}	0.00	24.06± 1.198^{a}	0.00	26.83± 1.186^{c}	0.000
1	24.81± 1.295^{3}	-0.62	23.57± 1.041	-.49	27.06± 1.135	0.23
2	24.36± 1.382^{3}	-1.07	23.01± 1.158	-1.05	27.03± 0.811	0.20
3	23.5± 1.189^{2}	-1.93	22.21± 1.263	-1.85	26.66± 0.560	-0.17
4	23.23± 1.260^{2}	-2.20	22.16± 1.141	-1.90	27.03± 0.731	0.200
5	22.8± 1.167^{1}	-2.63	22.06± 1.235	-2.00	26.83± 0.578	0.00
6	22.9± 1.598^{1}	-2.53	21.96± 1.218	-2.10	27.06± 0.874	0.23
7	23.1± 1.898^{2}	-2.33	22.86± 1.217	-1.20	27.26± 0.970	0.43
8	23.03± 2.218^{2}	-2.40	22.9± 1.179	-1.16	27.16± 1.049	0.33
9	23.18± 1.736^{2}	-2.25	22.6± 1.217	-1.46	27.28± 0.977	0.45
10	23.83± 1.105^{2}	-1.60	23.8± 1.102	-0.26	27.23± 0.906	0.40

Keys: G-mice: Infected mice fed with Allium sativum and infected with A. caninum; C-mice: Infected control mice not fed with A. sativum; UMH: uninfected healthy mice were not fed with allium sativum or infected with A. caninum. †: Net weight gain/loss was obtained by subtracting the weight of animal before test (Day 0) from the weight recorded on that day. Means on the same row with different letter superscripts are significantly different (p<0.05), while means on the same column with different numeral superscripts are significantly different (p<0.05)

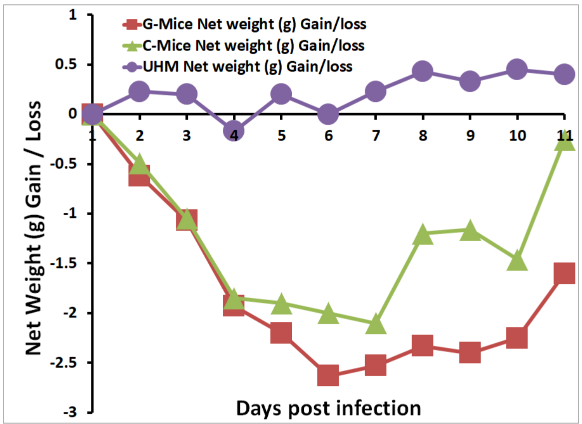

Figure 1: Net weight gain or loss of infected and uninfected mice compared over a ten day period. *Key: G-mice received both A sativum and A. caninum; C-mice received A. caninum and water; UHM, uninfected healthy mice received neither A. sativum nor A. caninum. Each point shown is derived from average of three mice*

Finally, Tukey's honestly significance test of the three categories of mice showed that difference ($p \geq 0.47$) occurred between UHM and G-mice; UHM and C-mice as well as between G-mice and C-mice. This therefore confirmed that weight loss in G-mice was different from C-mice and UHM mice were different from both G and C mice.

DISCUSSION

The study determined if *A. sativum* that is commonly used as food seasoner could have a role in host-parasite-relationship in hookworm infection. The extract of *A. sativum* was confirmed to be nontoxic because no adverse reaction or death was recorded in animals even when given 5,000 mg/Kg BW. This showed that the highest dose given was still less than the LD_{50} of *A. sativum* (Gatsing *et al.*, 2005; Waheed, 2021).

Among the phytochemical constituents of *A. sativum,* allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate) is the principal bioactive compound present in the aqueous extract of garlic or raw garlic homogenate. Allicin has been associated with anti-atherosclerotic effect (Yeh and Liu, 2001), inhibits proliferation of human mammary endometrial and colon cancer cells (Lin *et al.*, 2002), and reduces fasting blood sugar in diabetes mellitus patients (Padiya and Banerjee, 2013). Furthermore allicin has been implicated for its antiviral, antifungal, antibacterial, and antihelminths efficacy (Fenwick and Hanley, 1985; Yousuf *et al.*, 2011; Houshmand *et al.*, 2013; Metwally *et al.*, 2018). Thus, the weight losses in *A. caninum* infected mice receiving *A. sativum* may be attributed to the synergerical activities of allicin.

Weight changes existed in groups of mice receiving and not receiving *A. sativum.* Atuahene *et al.* (2013) also found it very safe in commercial production of broilers where its incorporation of up to 2.5 % in diet was advocated. Hasan *et al*. (2015) employed 10 % water solution of garlic to improve weight gain, reduce parasite load and promote health of goats. Similarly, safety related reason must have informed the dietary addition of garlic powder for improved growth performance of nematode infected lambs by reducing egg count while enhancing feed digestion (Zhong *et al*., 2019).

The results of this study were similar to that of Shetty and Shetty (1993), who used measurement of weight in their studies involving nutrition and infection in adult humans. When infection is incorporated into nutrition based study, it becomes more expedient in the study of host-parasite relationship because more information could be generated to unravel hidden details that phytoconstituents contribute to modulate outcomes of such relationship in diverse animal models. Moreover, such approach would encourage development of new strategic plans to engender prevalence reduction (Harvey *et al*., 2020) given that public health authorities neglect animal infections (Otranto *et al*., 2017).

Previous related studies revealed inconsistent results: at one end infections increased weight of the host (Shetty and Shetty, 1993) and in certain ambient temperatures (Franke *et al*., 2019). At the opposite end, infections lead to hosts weight loss and compromise of host fitness (Suman *et al*., 2013). Nematode infection may produce little interference in host output as shown by Mavrot *et al*. (2015) who found that wool production in sheep was only inhibited by 10 %. Infected and non-infected animals may grow with negligible differences. It seems that infection involving hookworms ineluctably lead to weight losses (Laatamna *et al*., 2021; Daba *et al*., 2021). Weight losses in infected animals may be due to other reasons such as poor nutrition, no protection of animals with vaccination, poor management practices among other factors. Vinayak *et al.* (1981) ascribed weight losses to irritation of the gastrointestinal tract (GIT) because his experimental animals developed diarrhoea and dysentery. The aggravated weight losses in mice given *A. sativum* was most probably due to its phytoconstituents as it had been implicated in irritation of the GIT (Khan and Ali, 1999). Weight gain in infected host could also be caused by worms which stimulate appetite by release of physiologically active substances such as hormone in host animals (Hite *et al*., 2020).

Crompton *et al*. (1981) noted two trends in experiments involving malnourished hosts: the first was that food intake was less in infected host, and secondly, the steady decline in body weight in infected host was usually greater than that of uninfected partner. They indicated that anorexia further deprived hosts of protein or lowered the value of protein because of coincidental reduction of energy intake. The deficit of protein may be due to impaired digestive function, the degeneration of the villus or plasma albumin instability. It appears that weight losses engendered in this study may after all have been provoked by two biogens provoking the same pathway. *A. sativum* has immunomodulatory potential which acts by stimulation of immune cells that respond by antibody production, macrophage activation/ phagocytosis, cytokine secretion among others (Arreola *et al*., 2015). Hookworm infection similarly provokes immune cell proliferation (Dias *et al*., 2013; Da Silva *et al*., 2019). Both *A. caninum* and *A. sativum* certainly have additive effects accounting for the increased weight losses in animals subjected to both conditions in this study. Therefore both entities most probably interfere with digestion, absorption or provoke anorexia. Da Silva *et al.* (2019) had reported that hookworm infection decrease glucose uptake. Overall, both organisms seem to have a temporary or short-lived interference on the gastro-intestinal system. As observed in this study, the experimental animals had begun to show signs of recovery after day 5 – 6 post infection, with control mice showing nearly full recovery at the end of the tests while test animals receiving garlic demonstrated far slower recovery.

This study has thrown more weight to other workers who indicate that *A. sativum* may be a GIT irritant; that is, hookworm infected animal by mouth could ordinarily lose weight which could be worsened further by garlic based nutritional supplements. The public health implication is that the duo of garlic and hookworm infection has undesirable consequence shown by weight losses. In persons who have other predisposing factors such as malaria, malnutrition or diarrhea, the weight loss that could be precipitated by both hookworm infection and garlic intake could subject them to greater, life threatening risk, so health care givers should be cognizant of this fact. This study also demonstrated that such weight losses could be short lived because despite the continual supplement intake, infected animals were inclined to weight recovery. It seems that host animals may have other biogenic pathways that either persisting parasitic infection and/or intake of garlic could provoke adaptation directed at mitigation of the adverse effects of each or a combination of the two GIT irritants. A corollary of this study is that fat persons could attempt weight reduction using either of the two organisms to reduce weight. Obese people who need drastic weight reduction can attempt shedding weight using both combinations. However, such exercise should be under strict medical supervision and only attenuated form of *A. caninum* in particular should be applied. It could be interesting to further explore the relationship by prolongation of supplement feeding and possible re-infection of host animals to further evaluate the effects of both on parasite host.

Conclusion: *A. sativum,* commonly known as garlic is a plant with many beneficial phytonutrients in its raw or cooked forms and used in the treatment and prevention of a number of diseases. However, this study showed that when applied to one who is already burdened with *A. caninum* infection; it could exacerbate weight losses in the short term. Furthermore, weight recovery follows as the plant extract is continued demonstrating that hosts resist abrupt change and strives to return to *status quo*. It is hereby recommended that further testing be done to provide more long term host-parasite relationship related information.

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