The efficacy of plant extracts on cecal amebiasis in rats

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Abstract

Amebiasis caused by *Entamoeba histolytica* is a major public health problem in tropical and subtropical countries. Treatment failure with specific chemotherapy has been reported suggesting the possibility of drug resistance. This study investigated the anti-amoebic effects of four plant extracts on cecal amebiasis in rats. The cecal amebiasis was induced by the injection of 3.0×10^5 troph/mL of *E. histolytica* parasite directly into the rat's caecum. A total of 137 rats were used for these studies; five rats in each group for both positive and negative control, 15 rats in each group to test the four plant extracts and metronidazole. The infected rats were treated for cecal amebiasis using each of the four plant extracts at graded doses of 100 mg/kg, 200 mg/kg and 400 mg/kg and with metronidzole at a dose of 62.5 mg/kg,100 mg/kg and 125 mg/kg for five consecutive days. The efficacy of the four plant extracts were evaluated based on Neal's, 1951 method. The plant extracts of Garlic, *Guava,* Pawpaw and Pumpkin at 400 mg/kg and 200 mg/kg bodyweight gave a cure rate of 80%, 100%, 60%, 40% and 40%, 80%, 40%, 0%, respectively. The mean parasite count in the cecal contents of the treated rats at a dose 400 mg/kg were 18.5±1.6, 0.0±0.0, 33.3±1.8 and 49.5±4.0, respectively. The difference was statistically significant ($P<0.05$). This study has revealed that Guava at a high dosing level (400 mg/kg body weight) is as good as the standard drug in reducing the both parasite load (probably with limited side effect).

Introduction

Amebiasis ranks third amongst lethal parasitic infection after malaria and schistosomiasis.[1] *Entamoeba histolytica* causes approximately 50 million cases of dysentery and 100,000 deaths annually.[2] Amoebic dysentery is more common in areas of low socio-economic status, poor sanitation and nutrition especially in the tropics thus majority of these infections, morbidity and mortality occur in Africa, Central and South America and the Indian subcontinent.[3,4] In Nigeria a prevalence of 22.3% has been reported in Calabar,[5] 21.6% in Enugu,[6] 14.3% in Kaduna,[7] and 13.7% in Illesa.[8] The current drug of choice for the treatment of amebiasis is metronidazole.[9] Drug resistance is rare but treatment failure has been reported by Hanna *et al.*,[10] and recently drug resistance clones have been generated in the laboratory suggesting a future natural development of drug resistance by *E. histolytica*.[11-13] Metronidazole has some side effects including metallic taste, nausea, vomiting, transient neutropenia, epigastric distress, abdominal cramps, interaction with warfarin and peripheral neuropathy.[14,15] This necessitates a search for a safe and effective alternative anti-amebic agent. Garlic (*Allium sativum*), Pumpkin (*Cucurbeta pepo*), Guava (*Psidium guajava*) and Pawpaw (*Carica papaya*) are edible plants which have generated lots of interests as medicinal panacea.[16] Antiprotozoan properties of Garlic have been established and recently,[17,18] its anti-amebic properties have been discovered.[19] Guava has also been shown to have anti-diarrheic properties.[20-23] The *in vitro* anti-amebic effect of Paw paw against *E. histolytica* has been confirmed.[24,25] Pumpkin is used as an anti-parasitic agent and its anti-amebic properties have been suggested due to its role in the treatment of intestinal parasite.[26,27] Rat has been recommended as an experimental animal model in the *in vivo* study of anti-amebic plants.[28,29] Intracecal inoculation of rat with trophozoites of *E. histolytica* parasite produces a lesion in the intestinal wall of the rat, similar to that seen in amebic colitis in human.[30] This study was an attempt to determine the efficacy of the four plant extracts on cecal amebiasis in rats, and to compare the effects of the four plants extracts with that of the standard drug metronidazole.

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Materials and Methods

Source/plant collection

The plants used for this study were Garlic (*Allium sativum*), Pawpaw (*Carica papaya*), Pumpkin (*Cucurbita pepo*) and Guava (*Psidum guajava*). Guava (*Psidium guajava*) leaves were collected from the botanical garden of Department of Botany, University of Calabar. The Paw paw seeds, Pumpkin seeds and bulbs of Garlic were purchased from the Local market in Calabar, Cross River State of Nigeria. The identification and authentication of these four plants was carried out by the Botany Department of the University of Calabar.

Plant extraction

The fresh leaves of Guava, seeds of Pawpaw, seeds of Pumpkin and bulbs of Garlic were air dried for three weeks and grounded using a manual blender to get a powdery form. One hundred grams (100 g) of powdery form for each of the four plants, Guava, Garlic, Pumpkin and Pawpaw were extracted by subjecting through maceration at 25°C for 48 hours using 80% methanol as solvent. The extracts obtained were then filtered using 1 filter paper (Whatman, Maidstone, UK). The filtrate was concentrated with a rotary evaporator at 45°C. The extracts were then stored at 4°C in sterile bottle for use when required.

Experimental animal

The 85 Wistar strain of albino rats of both sexes used for the study were obtained and also kept in cages in the animal house of Physiology Department of University of Calabar. The animals were healthy, with body

weight range of 150-200 g. They were kept at room temperature (28±2°C) and away from direct sunlight but with good ventilation. They were fed with food pellets and water *ad libitum*. The animal cages were cleaned twice a week to ensure good sanitary condition until required for use.

Parasite preparation

The Boeck and Drbohlav's diphasic and polyxenic medium was used for the culture of the *E. histolytica* parasites. The Boeck and Drbohlav's medium is made up of albumin slope and overlay solution. Albumin slope was prepared by mixing 270 mL of fresh egg albumin and 75 mL of sterilized Ringer's solution (0.8 g NaCl, 0.2 g $CaCl_2$, 0.2 g KCl in 100 mL of distilled water). Then, 2.5 mL of the mixture was dispensed aseptically after filtration through sterile gauze into sterile culture tubes and inspissated in slanted position at 100°C for 10 minutes. The overlay solution was obtained by mixing 100 mL of sterilized Locke's solution (0.8 g NaCl, 0.2 g Na_2HP0_4, 0.2 g KCl, 0.01 g $MgCl_2$, 0.4 g $NaHCO_3$ and 0.3 g kH_2PO_4) to 1 mL of calf serum. Then 5 mL of overlay solution was added to each tube containing the albumin to complete the medium.

The *E. histolytica* parasite was isolated from feces of confirmed cases of *E. histolytica* infections from parasitology laboratory of University of Calabar Teaching Hospital (UCTH), Calabar. The parasite was cultured on Boeck and Drbohlav's medium with some modification as described by Sawangjaroen *et al.*[31] Calf serum (10%) was used instead of horse serum and bijoux bottle was used as parasite culture tube. The culture was incubated at 37°C and *E. histolytica* trophozoites along with associated bacteria were sub cultured every 48 hours. Just before culture, a loopful of sterilized rice starch (1 mg) was added to the medium. Then a small quantity of the faces were inoculated in the culture medium and incubated at 37°C for 48 hours. After 48 hours incubation, the culture fluid in the tube was mixed and examined microscopically for amoebic growth. In order to renew the culture medium, culture tubes were chilled on ice for 5 minutes and the upper phase (around 4 mL) was discarded. The sedimented part containing the parasites was mixed and transferred to a fresh sterile culture tube containing the medium and rice starch. This procedure was repeated after every 48 hours to maintain the amoebic strain. Rawson and Hitchcock, method for counting of amoeba was use to determine the number of *E. histolytica* parasites.[32]

Experimental protocol

The Wistar rats used for the evaluation of the four plant extracts on cecal amebiasis were divided into 7 groups: two control groups (normal and infected-untreated) consisting of 5 rats each and 5 test groups consisting of 15 rats each.

The rats were inoculated intracecally with 3.0×10^5/mL trophozoites of *E. histolytica* parasite, and the methods used for maintaining *E. histolytica* and inoculations procedure in rats were according to Ray and Chatterjee, method.[33] After two days of inoculation, the rats were subjected to either one of the following treatments for 5 days through oral administration using feeding needle of plant extracts and drug (metronidazole).

Group 1: infected test subject treated with garlic extracts

Amebiasis was induced by intracecal inoculation of *E. histolytica* parasites to all the rats in the group + treatment with the garlic extract at 100 mg/kg to 5 rats, 200 mg/kg to another 5 rats and 400 mg/kg to remaining 5 rats.

Group 2: infected test subject treated with guava extracts

Amebiasis was induced by intracecal inoculation of *E. histolytica* parasites to all rats + treatment with the guava extract at 100 mg/kg to 5 rats, 200 mg/kg to another 5 rats and 400 mg/kg to the remaining 3 rats.

Group 3: infected test subject treated with pawpaw extracts

Amebiasis was induced by intracecal inoculation of *E. histolytica* parasites to all rats + treatment with the paw paw extract at 100 mg/kg to 5 rats, 200 mg/kg to another 5 rats and 400 mg/kg to the remaining 5 rats.

Group 4: infected test subject treated with pumpkin extracts

Amebiasis was induced by intracecal inoculation of *E. histolytica* parasites to all rats + treatment with the pumpkin extract at 100 mg/kg to 5 rats, 200 mg/kg to another 5 rats and 400 mg/kg to the remaining 5 rats.

Group 5: infected test subject treated with metronidazole

Amebiasis was induced by intracecal inoculation of *E. histolytica* parasites to all rats + 62.5 mg/kg to 5 rats, 100 mg/kg to another 5 rats and 125 mg/kg to the remaining 5 rats.

Group 6: normal control

The rats in this group were not infected and distilled water was given as placebo to the 5 rats in this group.

Group 7: infected-untreated control

Amebiasis was induced by intracecal inoculation of *E. histolytica* parasites to all the 5 rats in the group without any treatment given to them.

Evaluation of the effects of plant extracts on cecal amebiasis

Macroscopic examination of caecum

The effect of the four plant extracts and metronidazole on amebiasis induced by intracecal inoculation of *E. histolytica* in rats was evaluated after 5 days of treatment. The animals were sacrificed by cervical dislocation. The effects of the plant extracts and drugs were evaluated by macroscopic and gross examination of cecal walls for thickenings or ulcerations. Neal's, method was used for evaluation.[34]

Stool examination

Direct smear method: stool samples from each of the groups were examined by direct smear method according to the method described by Cheesbrough. A wet mount with saline and Lugol's iodine was prepared, microscopic examination for motile trophozoites and cysts of *E. histolytica* was carried out using the low and high power (×10) and (×40) objectives.

Concentration method: fecal samples were collected from each of the rats into clean petri dishes; the samples were examined microscopically using the formol ether concentration technique as reported by Cheesbrough.[35]

From each sample, about 1 g of the feces was taken, emulsified in tubes containing 4 mL of 10% formol saline and mixed properly. The emulsified feces were sieved, the sieved suspensions were transferred into centrifuge tubes and 3 mL of diethyl ether was added. The tubes were stoppered and mixed vigorously for 1 minute. The stoppers were removed and each tube centrifuged immediately at 1000 g for 1 minute. After centrifugation, 4 layers appeared in the tubes: ether layer on top, a plug of fecal debris, formol saline and sediments containing *E. histolytica* cysts. All layers were poured off except the bottom layer of the sediments. The sediments were mixed and a drop was put on a slide, covered with a cover slip and examined microscopically.

Data analysis

Data obtained from the study were statistically analyzed using ANOVA. Means were expressed as mean ± standard deviation. The Student's t-test was used to test the significant differences between treated groups and infected-untreated control; significant were considered at $P<0.05$.

Results

The effects of plant extracts of Garlic, Guava, Pawpaw and Pumpkin on cecal amebiasis in rats are shown in Table 1. The Garlic

extracts at concentrations of 100 mg/kg, 200 mg/kg and 400 mg/kg bodyweight per day given orally for five days in cecal amebiasis in rats gave a percentage cure rate of 0%, 40% and 80%, respectively. The average cecal score of cecal content and wall were 0.4, 0.2 and 0, respectively.

The Guava extracts at concentrations of 100 mg/kg, 200 mg/kg and 400 mg/kg bodyweights per day given orally for five days in cecal amebiasis in rats gave a percentage cure rate of 20%, 80% and 100%, respectively. The average cecal score of cecal contents and wall were 0.2, 0 and 0 respectively. The Pawpaw extracts at concentrations of 100 mg/kg, 200 mg/kg and 400 mg/kg given orally for five days gave a percentage cure of 0%, 40% and 60%, respectively. The average cecal score of cecal content and wall were 0.4, 0.2 and 0, respectively.

The pumpkin extracts at concentrations of 100 mg/kg, 200 mg/kg and 400 mg/kg given orally per day for five days gave a percentage cure of 0%, 0% and 40%, respectively. The average cecal score of cecal content and cecal wall were 2, 2 and 0.2, respectively.

The drug metronidazole at concentrations of 62.5 mg/kg, 100 mg/kg and 125 mg/kg given orally per day for 5 days gave a percentage cure of 60%, 80% and 100% respectively. The average cecal score of cecal content and wall were all 0. Two rats died in the infected-untreated control the average cecal score of cecal content and wall infected-untreated control were 3.3 and 3 respectively.

The mean parasitic count of *E. histolytica* parasite in the faeces of caecal amoebiasis in the four plant extracts treated rats are shown in Table 2. The effect of the four plant extracts on mean parasitic counts in feces in cecal amebiasis was discovered to be dose-dependents. There was a statistical significant difference between the effects of the four plant extracts on the mean parasitic count in the feces of rats in cecal amebiasis ($F=5.98$ df(3), $P<0.05$). There was no statistically significant difference ($P>0.05$) in the mean parasitic count of feces at a dose of 100 mg/kg in Garlic (38.2±3.1), Guava (37.5±2.5), Pawpaw (37.9±3.1) and Pumpkin (37.1±2.9) when compared with the infected-untreated control rats (38.9±3.4). There was statistical significant decrease ($P<0.05$) in the mean parasitic counts in feces of rat at a concentration of 200 mg/kg in the Guava (8.5±1.5), Garlic (23.0±2.8) and Pawpaw 0.2±2.2) extracts treated rat when compared with the infected-

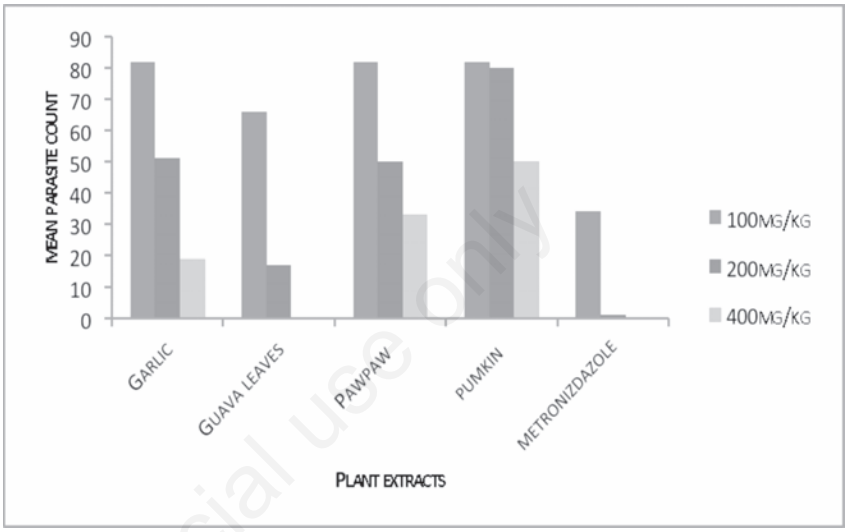

Figure 1. Mean parasite count in cecal content after treatment with the four plant extracts and metronidazole.

Table 1. The effects of the four plant extracts and metronidazole on cecal amebiasis in rats.

Test materials; dose (mg/kg per day for 5 days)	Rats cleared/treated (% cured)	Average cecal score (range)*	Content wall
Group 1: Garlic bulbs			
100	0/5 (0)	0.4 (0-1)	0.4 (0-1)
200	2/5 (40)	0.2 (0-1)	0.2 (0-1)
400	4/5 (80)	0.0 (0-0)	0.0 (0-0)
Group 2: Guava leaves			
100	1/5 (20)	0.2 (0-1)	0.2 (0-1)
200	4/5 (80)	0.0 (0-0)	0.0 (0-0)
400	5/5 (100)	0.0 (0-0)	0.0 (0-0)
Group 3: Pawpaw seeds			
100	0/5 (0)	0.4 (0-2)	0.4 (0-1)
200	2/5 (40)	0.2 (0-1)	0.2 (0-1)
400	3/5 (60)	0.0 (0-0)	0.0 (0-1)
Group 4: Pumpkin seeds			
100	0/5 (0)	2.0 (2-2)	2.0 (2-2)
200	0/5 (0)	2.0 (2-2)	2.0 (2-2)
400	2/5 (40)	0.2 (0-1)	0.2 (0-1)
Group 5: Metronidazole drug			
62.5	3/5 (60)	0.0 (0-0)	0.0 (0-0)
100	4/5 (80)	0.0 (0-0)	0.0 (0-0)
125	5/5 (100)	0.0 (0-0)	0.0 (0-0)
Group 6: Normal control	0/0/0	-	-
Group 7: Infected-untreated control	0/3°/0	3.3 (3-4)	3 (3-3)

*Cecal scores were graded upon the following criteria (Neal, 1951). Wall: normal, 0; slight thickening, 1; marked local thickening and contraction, 2; extensive thickening and contraction, 3; cecum shapeless (extensive ulceration with abscess formation), 4. Contents: normal, 0; slightly less solid than normal, 1; slightly mucoid, 2; mucoid (some solid matter present), 3; no solid matter (whiter or yellow mucus only), 4. °Two rats died due to the parasite infections, the infected-untreated control rat remaining 3.

Table 2. The mean parasitic count of *E. histolytica* (trophs/cyst) in the feces of the four plant and metronidazole treated rats.

Test materials; dose (mg/kg per day for 5 days)	Mean number of *E. histolytica* (trophs/cyst) g feces
Infected-untreated control	
0	38.9±3.4
Group 1: Garlic bulbs extracts	
100	38.2±3.1
200	23.0±2.8*
400	8.6±1.0*
Group 2: Guava leaves extracts	
100	37.5±3.2*
200	8.5±1.5*
400	0.0±0.0*
Group 3: Pawpaw seeds extracts	
100	37.9±3.1
200	23.2±2.2*
400	19.7±2.5*
Group 4: Pumpkin seeds extracts	
100	37.1±2.9
200	35.4±2.0
400	23.3±2.5
Group 5: Metronidazole	
62.5	11.0±1.2*
100	0.5±0.2*
125	0.0±0.0*

Values expressed as mean ± standard deviation, n=5. *P<0.05 compared to the infected-untreated control.

untreated (38.9±3.4) control rats. However, the decrease in mean parasitic count in feces of rat was not significantly different (P>0.05) in Pumpkin extract (35.4±2.0) treated rats. The mean parasitic counts of feces of rats at a concentration of 400mg/kg was significantly decreased (P<0.05) in Garlic (8.6±1.0), Guava (0.3±0.0), Pawpaw (19.7±2.5) and Pumpkin (23.3±2.5) when compared with the infected-untreated (38.9±3.4) control rat. The mean parasitic count in feces of rats treated with metronidazole at concentrations of 62.5 mg/kg,100 mg/kg and 125 mg/kg was significantly decreased (P<0.05). The mean parasitic count for rats treated with Guava (0.0±0.0) extracts at a concentration of 400mg/kg was comparable to metronidazole (0.0±0.0) at a dose 125 mg/kg.

Figure 1 shows the mean parasitic count of *E. histolytica* in the cecal contents of rats treated with the four plant extracts. The effect of the four plant extracts on mean parasite counts in cecal contents in cecal amebiasis is dose-dependent. There was a statistical significant difference between the effects of the four plant extracts on the mean parasitic count in the cecal contents of rats in cecal amebiasis (F=6.98 df(3), P<0.05).

The mean parasitic count of cecal contents of rat at a concentration of 100mg/kg in Garlic (82.2±5.0), pawpaw (81.99±3.7) and *pumpkin* (82.0±5.2) extracts treated rats were not statistical significantly difference (P>0.05) from the infected-untreated control rats (82.5±5.6). However, it was significantly different (P<0.05) in Guava (66.1±4.7) extract. There was a decrease (P<0.05) in the mean parasitic counts in caecal contents of rat at a concentration of 200 mg/kg in the Guava (17.4±3.2), Garlic (50.7±2.6) and Pawpaw (49.7±2.8) extracts treated rat when compared with the infected-untreated (82.5±5.6) control rats. These decrease in mean parasitic count in cecal contents were not statistically significant (P>0.05) in pumpkin (80.0±5.3) extract treated rats. The mean parasitic counts of cecal contents of rat at a concentration of 400 mg/kg showed a significantly reduction (P<0.05) in Garlic (18.5±1.6), Guava (0.5±0.0), Pawpaw (33.3±1.8) and *pumpkin* (49.5±4.0) when compared with the infected-untreated rat (82.5±5.6). Metronidazole at concentrations of 62.5 mg/kg, 100 mg/kg and 125 mg/kg, the mean parasitic count was significantly reduced (P<0.05). The mean parasitic count for rats treated with Guava (0.0±0.0) extracts at a concentration of 400 mg/kg was comparable to metronidazole (0.0±0.0) at a dose 125 mg/kg.

Discussion and Conclusions

Amebiasis is a common parasitic disease in developing countries and its eradication is very difficult due mainly to poverty, characterized by the absence of portable drinking water, proper sanitary habits, absence of good fecal disposal system and poor hygienic practices.[36] With reported cases of treatment failure of amebiasis with the commonly used drug metronidazole,[10] it has become imperative to study the effects our local medicinal plants of Garlic, Guava, Pawpaw *and* Pumpkin on cecal amebiasis in rats.

The results obtained in Table 1, shows the potential therapeutic effects of the four plant extracts of Garlic, Guava, Pawpaw and Pumpkin on cecal amebiasis in rats. However, these effects on cecal amebiasis are dose-dependent for each of the plant extracts. Two rats died as a result of *E. histolytica* parasite infection in the infected-untreated control rat. This is attributed to high virulence of the strain of *E. histolytica* parasite used in intracecal inoculation of the rats. This does not conform to other similar studies as no deaths have been reported on cecal amebiasis both in rats and mice.[37-39]

The three remaining rats in the infected-untreated control were all positive for amoebae at the time of sacrifice. This amoebic infection generally produces cecal score of content and wall ranging between 3 and 4 with the average of 3.3 and 3.0, respectively. This further re-confirms the virulence of the *E. histolytica* parasite used in this study.

The result of this study though with another plant disagrees with that of Sawangjaroen *et al.*,[37] on effects of *Piper longum* fruits, *Piper sarmentosum* roots and *Quercus infectoria* nut gall on cecal amebiasis in rats who reported cecal score of content and wall of rats ranging between 2 and 3 with average of 2.55 and 2.40, respectively.

Philips *et al*.[40,41] in their study observed that axenic strain of *E. histolytica* parasite becomes non-invasive after prolonged *in vitro* cultivation. From the result obtained in this study, the trophozoites of *E. histolytica* isolated from human bloody stool was still virulent in rats even after prolonged *in vitro* cultivation in Boeck and Drbohlav's medium. This is in agreement with a similar study by Sawangjaroen *et al.*,[37] who reported that amoebae isolated from control mice Infected with *E.*

histolytica parasite was still virulent in the study of the effects of *Piper longum* fruits, *Piper sarmentosum* roots and *Quercus infectoria* nut gall on cecal amebiasis in mice.

Guava extracts are medicinal plant used in tropical and subtropical countries to treat many disorders such as diarrhea, cough and gastrointestinal disorders. Jaiarj *et al.*[42] reported that leaf extracts of Guava has a wide spectrum of biological activities such as anti cough and antibacterial. From the results in this present study Guava extract appears to be most effective at a concentration of 400 mg/kg per day as this concentration cleared all *E. histolytica* from the caecum as seen in the percentage cure of 100%. The mean parasitic count in both the cecal contents were (0.0) and feces (0.0) on the day of examination. This can compared with metronidazole at a dose of 125mg/kg with percentage cure of 100% and mean parasitic counts of cecal content (0.0) and feces (0.0).

The concentrations 100 mg/kg and 200 mg/kg of Guava extracts gave a percentage cure of 20% and 80% in cecal amebiasis in rats. The mean parasitic count of *E. histolytica* parasite in the cecal contents showed a statistical significant difference ($P<0.05$) from the infected-untreated control rats in 100 mg/kg, 200 mg/kg and 400 mg/kg concentrations but was only significantly different ($P<0.05$) at concentrations of 200 mg/kg and 400 mg/kg in feces of rats.

The results obtained from this study is in disagreement with the observation of Moundipa *et al.*,[43] and Tona *et al.*,[44] who reported a less pronounced effects in the *in vitro* studies on amebecidal effects of Guava extracts. These further re-confirms previous studies of antidiarrheal properties of leaves extracts of Guava which quercetin, a chemical constituent of the extracts is thought to be responsible for its antidiarrheic properties.[21-23,45,46] Garlic extract have been reported to have antiparasitic properties in traditional medicine. Lun *et al.*[47] reported the *in vitro* antiparasitic activity of Garlic on pathogenic protozoa such as *E. histolytica, G. lamblia* and *Trypanosoma sp*.

The results of this study shows that Garlic at concentrations of 100 mg/kg, 200 mg/kg and 40 mg/kg gave a percentage cure of 0%, 40% and 80% of cecal amebiasis in rats, respectively (Table 1). In the present study the mean parasitic count of *E. histolytica* in Table 2 and Figure 1 reduce from 82.2 to 18.5 in cecal contents and 38.2 to 8.6 in feces of rats indicating the effectiveness of Garlic extract in reducing the severity of the parasite but not completely eradicating it. This study is in consistence with that of Behnia *et al.*,[19] who demonstrated that Garlic is effective against trophozoites of *E. histolytica* parasite *in vitro* and the essential oil exhibits the greatest anti-amebic activity at the lowest minimum inhibitory concentration. Ankri *et al.*,[48] and Mirelman *et al.*,[17] in their *in vitro* studies showed that allicin from freshly crushed garlic inhibited the activity of cysteine proteinases, an important contributor to amoebic virulence. Pawpaw seeds extracts is used in tropical and subtropical countries as remedy for parasitic infection. Okeniyi *et al*. established the effectiveness dried seed extract against human intestinal parasite. Pawpaw extracts at concentrations of 100 mg/kg, 200 mg/kg and 400 mg/kg gave a percentage cure of 0%, 40% and 60% of cecal amebiasis in rats, respectively. There was no statistical significant difference ($P>0.05$) in the mean parasitic count of *E. histolytica* parasite at concentration of 100 mg/kg when compared with the infected-untreated control rats. However, there was a statistical significant difference ($P<0.05$) at concentrations of 200 mg/kg and 400 mg/kg. These indicates reduction of the severity of the parasitic infections but not completely eradicating it. The findings is in agreement with Kumar *et al.*, who reported less effects in *in vitro* studies on the amebicidal effects methanol extract of Pawpaw seed extracts. Pumpkin is used all over the world both as vegetable and medicinal plant. Diaz Obregòn *et al*.[26] reported the use of Pumpkin as an antiparasitic agent. There was no statistical significant difference ($P>0.05$) in the mean parasitic count of *E. histolytica* parasite at concentrations of 100 mg/kg, 200 mg/kg when compared with the infected-untreated control however there was a significant difference ($P<0.05$) at concentration of 400 mg/kg. These indicate slight reduction of the severity of the parasitic infections but not completely eradicating it. Previous studies have reported on the anti-parasitic properties of pumpkin extracts, its anti-amebic activity is unknown.[26,49-53] From the results of this study, infected rats treated with metronidazole at concentrations of 62.5 mg/kg, 100 mg/kg and 125 mg/kg gave a percentage cure of 60%, 80% and 100% of cecal amebiasis in rats, confirming that this strain of *E. histolytica* was still sensitive to metronidazole. Our result on the effectiveness of metronidazole on cecal amebiasis in rats were similar to the studies of Bhopale *et al.,*[29] Sohni *et al.,*[38] Ghoshal *et al.,*[39] Sawangjaroen *et al.,*[31] who reported a 60%, 80% and 100% cure of cecal amebiasis both in rats and mice at concentrations of 62.5 mg/kg, 100 mg/kg and 12 mg/kg respectively.

This study has revealed that Guava at a high dosing level (400 mg/kg BW) is as good as the standard drug in reducing the both parasite load (probably with limited side effect). It is therefore recommended that a similar and more comprehensive work be done possibly on human subjects.

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