



EFFECT OF *Syzigium guineense* STEM BARK EXTRACT ON *Entamoeba histolytica* INDUCED AMOEBIC ABSCESS IN THE LIVER OF SWISS MICE

*Ahmed, H.O. and Agbo, V.K.

Department of Zoology, Federal University Lafia P.M.B 146.

*Corresponding Email: hussain.ahmed@fulafia.edu.ng

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ABSTRACT

The effects of ethanolic stem bark extracts of *Syzigium guineense* on trophozoites of *Entamoeba histolytica* in the liver of swiss mice was studied in an acute toxicological trial. Amoebiasis was induced by injection of *E.histolytica* trophozoites into the caecum and monitored for 7 days after inoculation. Successfully infected mice were divided into 5 groups and treated for 7 days. Group 1 was treated with 2mg/kg/day normal saline (negative control), groups 2, 3, 4 were treated with oral doses of *S. guineense* of 1000, 500 and 250 mg/kg/day respectively. Group 5 was treated with an oral dose of metronidazole (positive control) 15mg/kg/day. Mice were sacrificed on the 8th day and dissected to remove the liver for histological preparation and examination. Weight measurement revealed significant reduction ($p<0.05$) in all groups while the median lethal dose (LD50) of the extract was obtained to be 2828.43mg/kg. Histological investigations showed inflammation of hepatocyte cells and early signs of necrosis. The severity of cellular inflammation appeared more visible in clusters in all test treatment groups and negative control (normal saline) in contrast to metronidazole treatment (positive control). These findings indicate that *S. guineense* stem bark extract may be not be effective in the treatment of *E. histolytica* infections in the liver but recommend further investigation.

Keywords: *Syzigium guineense*, *Entamoeba histolytica*, Amoebic abscess, Liver.

INTRODUCTION

Amoebiasis is an infection caused by protozoan parasite *Entamoeba histolytica* of the class Sarcodina which occurs with or without the presence of clinical symptoms (Lübbert, *et al.*, 2014). It ranks third among parasitic diseases that result to death worldwide; being second to malaria as a protozoan cause of death (Nowak *et al.*, 2015). Although cosmopolitan in distribution, it mainly occurs in the tropics and sub tropics. It also occurs especially in areas where there is a low level of sanitation and very poor personal hygiene practices (Ibrahim, 2008). It parasitizes man causing amoebic dysentery, amoebic hepatitis and pulmonary amoebiasis. *E. histolytica* inhabits the large intestine of man, but can also establish itself in the liver, lungs, brain and other organs where secondary lesions are produced (Khairnar, 2007). Amoebic infections remain an important health problem in Nigeria due to inadequacies in sanitation infrastructure and health care facilities (Ibrahim, 2008).

E. histolytica is pathogenic in the caecum, colon and liver of humans and is the most unique among the Amoebas because of its ability to hydrolyse host tissue (Inabo, *et al.*, 2014). It can become a highly virulent and invasive organism causing diarrhoea. Amoebiasis may also give rise to amoebic liver abscess and intestinal pathologies (Aribodor *et al.*, 2012).

In Nigeria, amoebiasis is prevalent and widespread (Ajero *et al.*, 2008) and distribution of the infection was reported to relate more with inadequate environmental sanitation, poor personal hygiene and climate (Nowak *et al.*, 2015) leading to Amoebic dysentery cases. The high prevalence of *E. histolytica* infections is closely linked with poverty, poor personal hygiene, poor environmental hygiene, and poor health service providers having an inadequate supply of drugs and lack of adequate and proper awareness of the transmission mechanisms and life cycle patterns of these parasites (Adeyeba and Akinlabi, 2002; Mbanugo and Onyebuchi, 2002). Clinical features of amoebiasis range from asymptomatic colonization of amoebic colitis (dysentery or diarrhea) and invasive extra-intestinal amoebiasis, which is manifested most commonly in the form of liver abscesses (Fotadar *et al.*, 2007). The dependence on plants as a source of medicine is still relied on in many parts of the world and Tsakala *et al.* (1996) reported *S. guineense* has a potent antibacterial effect against diarrhea causing bacteria.

The aim of this study therefore is to assess the anti-hepatic effect of treatment with *S. guineense* stem bark extracts on the normal histology and physiology of the liver in mice under laboratory conditions. The

Specific Objectives of this study are to: Test for the phytochemical constituents of *S. guineense* stem bark, assess the effect of the extract on body weight in mice, determine the acute toxicity of the extract and thereby determine the median lethal dose (LD50) and study the effect of the extract of *S. guineense* on the histopathology of the Liver.

MATERIALS AND METHOD

S. guineense was collected from Tse-mo district of Makurdi, the capital of Benue State in February, 2016. The plant was taken to the department of Botany, Federal University Lafia for identification. The stem bark of *S. guineense* was cleaned with water and was air dried under shade. It was then pounded into coarse powder with a pestle and mortar. 100 g of the powder was measured using a digital weighing balance and soaked in 500ml ethanol and the process of shaking was performed for 7 days manually. The plant extract was filtered using filter paper a Buchner funnel. The filtrate was dried and concentrated by evaporating the solvent using a water bath at a temperature of 40° C to obtain a solvent-free paste-like deep brownish colour extract which was stored in a refrigerator at 4°C in well-closed container.

Animals were obtained from the National Veterinary Research Institute, Vom Plateau state. Both sexes of 4 – 7 weeks old Swiss mice weighing 18-30 grams were used. They were housed and maintained under specific pathogen free conditions at the Animal Facility Center of National Institute for Pharmaceutical Research and Development (NIPRD) Abuja. Mice were housed in plastic cages with saw dust as beddings at the Animal House Unit in NIPRD. Animals were given standard laboratory diet formulated by the Animal Facility Center, Department of Pharmacology and Toxicology of NIPRD, and water ad libitum and maintained under laboratory conditions with a temperature of 22 ± 1°C relative humidity and a non-reversed 12 hr light/12 hr dark cycle.

Seven days after inoculation, successfully infected mice were randomly divided into five groups of five mice each. Group 1 served as a control received 2 mg/kg oral dose of normal saline while groups 2, 3 and 4 were given different single oral doses of *S. guineense* (250, 500 and 1000 mg/kg) respectively. Group 5 was treated with a single dose of metronidazole (15mg/kg) once a day. All groups were given treatment at 24 hour intervals for seven consecutive days.

Phytochemical Screening

The *S. guineense* ethanolic stem bark extract was screened for phytochemical constituents using

standard procedures as described by Evans (2002) to test the presence of alkaloids, saponins, flavonoids, tannins, phlabatanins, polyphenol, anthraquinones and steroids.

Acute Toxicity Test and LD50 determination

The oral acute toxicity of the ethanolic extract of *S. guineense* was evaluated in albino mice using a modified Lorke's method (Lorke, 1983) in two phases. In the first phase, nine mice were randomly divided into three groups of three mice and were given 10, 100 and 1000 mg extract/kg body weight orally, respectively. Animals were observed for the first four critical hours after dosing and subsequently for 48 hours for signs of toxicity and mortality. In the second phase of the study three fresh mice, one per group, were each given doses of 1600, 2900 and 5000 mg/kg of the extract. They were also observed for signs of toxicity and mortality for the first critical 4 hr and subsequently for 72 hr. The median lethal dose (LD₅₀) was calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose, using the formula:

$$LD_{50} = \sqrt{\text{maximum dose for all survival} \times \text{minimum doses for all death}}$$

In vivo inoculation of *Entamoeba histolytica*

Mice were infected in accordance with Institutional Animal Care and Use Committee approved protocols (NIH, 1985). Animals were anesthetized with 50mg/kg of ketamine hydrochloride, their abdomens were shaved to incise the skin and each mouse was injected with 0.1 ml of 1×10^6 trophozoites into the apical site of the abdomen, blotted and the peritoneum and the skin were sutured. Mice were kept on warming blankets at 37°C throughout. After infection, diarrhoea, colitis, and weight loss were monitored in all infected mice from day 1 to 7. The mice were sacrificed on day 8 using chloroform and liver were dissected longitudinally and processed for tissue preparation.

Tissue preparation

Tissues were prepared in accordance to the method of Choji *et al.*, (2015). They were harvested and fixed in (10ml of formalin in 90ml of water) 10% formalin for 3 days, cut into thin slices of 5mm X 2mm X 1mm thick and then processed in the following order using The SPIN tissue processor, STP 120 (Thermo scientific) 10% buffered formalin for 72 hours, 70% Alcohol for 2 hours, 80% Alcohol for 2 hours, 90% Alcohol for 2 hours, 95% Alcohol for 2 hours, Absolute Alcohol I for 2 hours, Absolute Alcohol II for 2 hours,

Absolute Alcohol III for 2 hours, Xylene I for 2 hours, Xylene II for 2 hours, Paraffin Wax Oven I for 2 hours and Paraffin Wax Oven II for 2 hours. Tissues were embedded in molten paraffin wax using embedding moulds and were embedded using embedding cassettes on a tissue Tek Embedding Centre (SLEE MPS/P2), and cooled rapidly on the cooling component. Tissues were sectioned using a rotary microtome (MICROM HM340E Thermo-Scientific) set at 4 micromes, picked on slides and ready for staining. Sections were cleared and hydrated by passing through two changes of xylene and through descending grades of alcohol (100%, 80%, 70%) for three minutes each and then into water, stained in Harris' haematoxylin solution for 5 minutes and washed in running water. They were differentiated in 1% acid-alcohol and then washed well in water, blued in Scott's tap water substitute for 5 minutes and rinsed briefly in distilled water, counterstained in 1% aqueous eosin for 2 minutes, washed well in water, dehydrated in descending grades of alcohol, cleared in xylene and mounted in DPX (Destrene, Plasticiser and Xylene). Sections were then placed in slide carriers and placed in a 40°C oven to dry overnight. Prepared sections were then viewed under the microscope at different magnification.

Statistical Analyses

All the values in the test are presented as mean \pm SEM. Statistical differences between the means of various groups were evaluated by one-way analysis of variance (ANOVA) using the SPSS version 20 followed by student's t-test. P-values less than 0.05 were considered to be significant.

RESULTS AND DISCUSSION

Table 1: Phytochemical Tests for Ethanolic stem bark Extract of *S. guineense*.

TEST	<i>S. guineense</i>
FLAVONOIDS	+
ALKALOIDS	-
SAPONINS	+
TANNINS	+
PHLABATANINS	-
STEROID	+
POLYPHENOL	+

KEY: + indicates presence, - indicates absence

Phytochemical investigation showed the presence of flavonoids, saponins, tannins, steroid and polyphenol. This is in agreement with the findings of Nigatu (2004) who reported the presence of tannins, phytosteroids, flavonoids and saponins in some of the crude extracts and fractionates of *S. guineense*. However, the absence of alkaloid and phlabatanin was observed in this study. Eugenin which is reported to be an active

ingredient found at buds of *Syzygium* spp according to Takechi and Tanka (1981); Abebe, *et al.*(2003) was also not present in the stem bark extract. The combined presence of the phytochemicals may probably be responsible for the effect observed in this study and this is agreement to Tsakala *et al.*, (1996).

Observed Changes in weight in Mice

Table 2: Effect of treatments on the weight of swiss mice

Day	2mg/kg Normal Saline	1000 mg /kg	500 mg /kg	250 mg/kg	15 mg/kg Metronidazole
	(Group 1)	(Group 2)	(Group 3)	(Group 4)	(Group 5)
D0	22.80±4.66	23.00±4.15	22.40±4.47	24.20±5.07	20.00±2.96
D1	21.60±4.55	23.00±4.02	22.80±4.76	23.00±4.81	18.80±2.71
D2	21.60±4.31	22.40±3.60	22.20±4.59	22.60±4.69	19.00±2.58
D3	22.40±4.20	21.80±3.68	21.80±4.09	23.40±4.53	20.20±2.57
D4	22.60±3.89	23.00±3.21	22.80±3.89	22.40±4.88	20.60±2.80
D5	21.20±3.89	22.20±3.15	23.80±4.43	22.00±4.40	19.20±2.57
D6	21.60±3.80	21.40±3.37	21.60±4.06	21.20±4.35*	19.40±2.58
D7	20.60±4.15**	21.00±3.70	20.80±3.68	22.20±4.22	20.00±2.81
D8	20.80±3.65**	21.20±2.82	22.00±3.69	20.40±4.06**	19.80±2.92

Data are represented as mean ± SEM * $p < 0.05$, ** $p < 0.01$ significantly different as compared to D_0

Mice in all treatment groups showed similar trends with regards to differences between initial weight at D_0 and final weight at D_8 in this study. The final weight in all groups was less than the initial weight. This is in agreement with the findings of Palit *et al.* (1999). The reduction in body weight in all treatment groups can be attributed to the invasive nature of the trophozoites of *E. histolytica* which according to reports by Pritt and Clark (2008) and Haque *et al.*, (2009) can penetrate and invade the colonic mucosal barrier, leading to tissue destruction, secretory bloody diarrhea, and colitis resembling inflammatory bowel disease, which ultimately hamper the normal metabolic rate of absorption and assimilation in the intestine. This is in line with findings of Ghosh, *et al.*, (2010). Results in body weight changes showed statistically significant differences from the initial weight at D_0 in both group 1 at D_7 ($p < 0.01$) and D_8 ($p < 0.01$), and group 4 at D_6 ($p < 0.05$) and D_8 ($p < 0.01$) respectively.

Acute toxicity test and LD_{50} determination

Following the two phases of acute toxicity test, the LD_{50} was determined using the equation,

$$LD_{50} = \sqrt{ab}$$

Where a = maximum dose producing 0% mortality

b = minimum dose producing 100% mortality

$$= 1600 * 5000$$

$$LD_{50} = 2828.43 \text{ mg/kg}$$

Histological Investigation

The effect of the different treatments on the liver of mice brought about changes in morphology and photomicrographs of such tissues were taken.

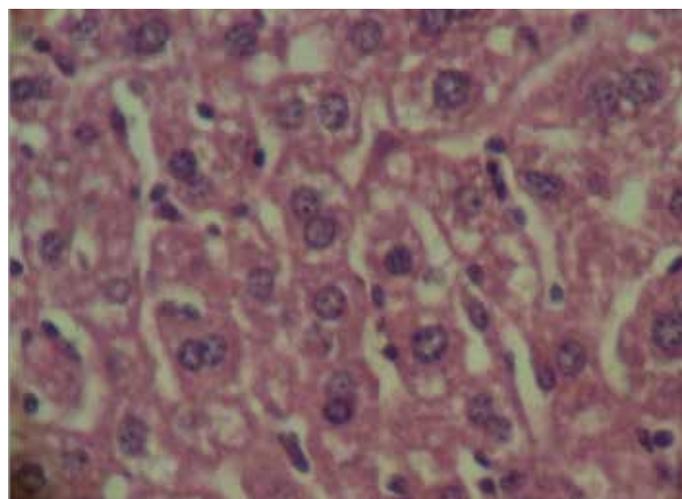


Plate I (GP5): Liver of a mouse infected with *Entamoeba histolytica* and treated with metronidazole (2mg/kg). The hepatocytes appear normal with nuclei (black arrows) within intact cytoplasm (white arrows). The sinusoids (black arrow heads) have few inflammatory cells (white arrowheads). H&E X400.

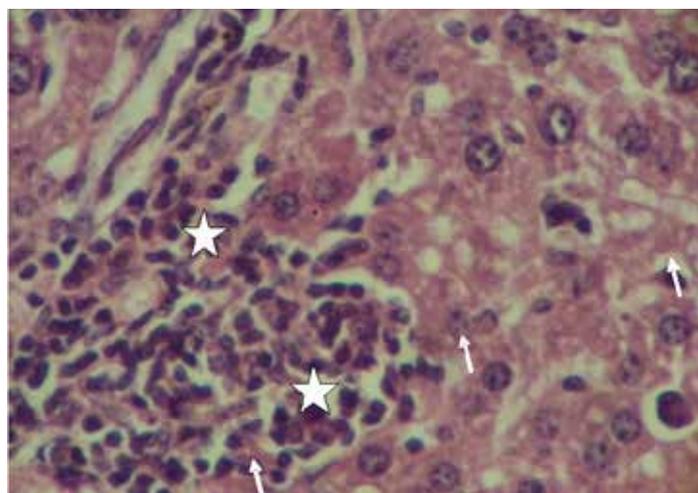


Plate II (GP4): Liver of a mouse infected with *Entamoeba histolytica* and treated with 1000mg/kg

kg/day per body weight aqueous extract. There is inflammation evident by the presence of inflammatory cells (white stars) and necrosis as noted by the complete destruction of hepatocytes and their replacement with inflammatory cells (white arrows). H&E X400.

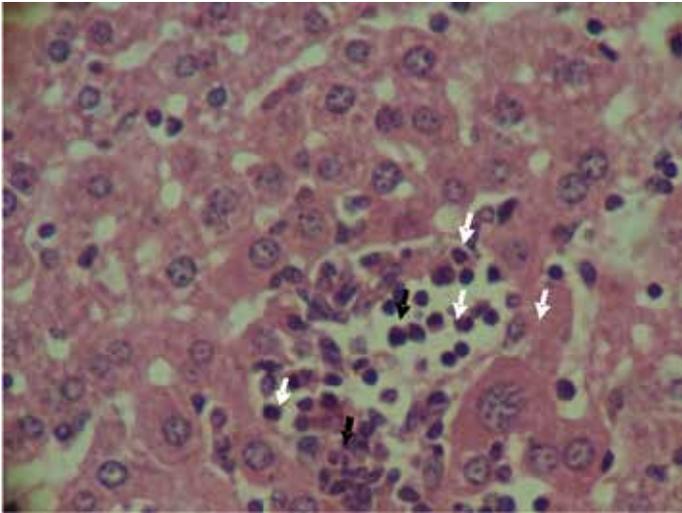


Plate III (GP3): Liver of a mouse infected with *Entamoeba histolytica* and treated with 500mg/kg body weight aqueous extract. Intercellular infiltration is clearly observed as noted with the presence of inflammatory cells (white arrows). Cellular degeneration (necrosis) is shown by the dissolution of cellular components (black arrows) and their replacement with inflammatory cells. H&E X400.

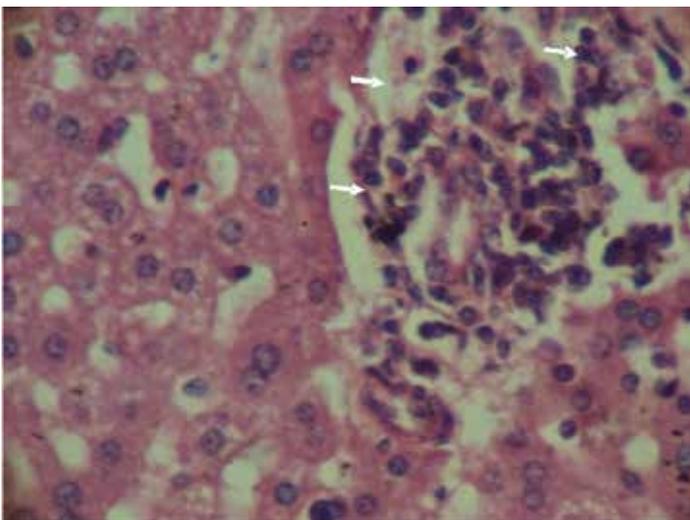


Plate IV (GP2): Liver of a mouse infected with *Entamoeba histolytica* and treated with 250mg/kg body weight aqueous extract. There is inflammation of the hepatocyte as well as necrosis. White arrows= inflammatory cells.

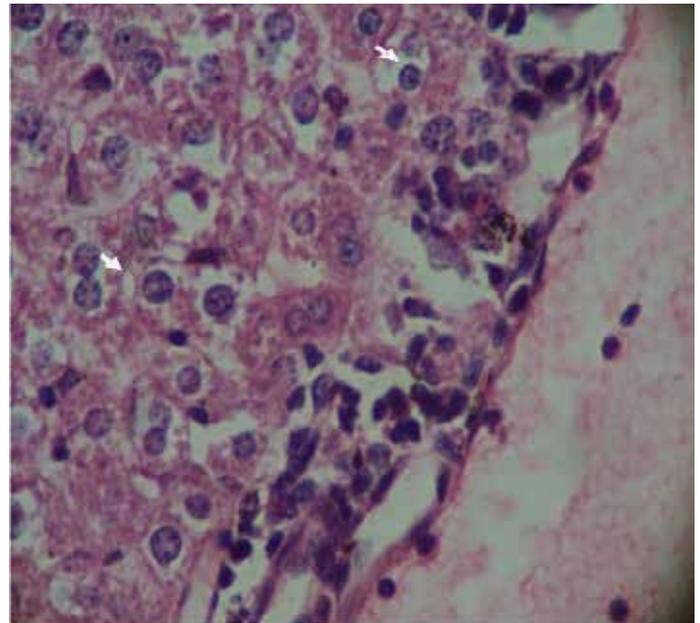


Plate V (GP1): Liver of a mouse infected with *Entamoeba histolytica* and treated with 2mg/kg body weight normal saline. There is inflammation and vacuolation of hepatocytes (evident by empty spaces within the cytoplasm) as well as necrosis evident by the presence of naked nuclei (white arrows). Black arrows= inflammatory cells. H&E X400.

CONCLUSION AND RECOMMENDATIONS

In this study, the effects of acute treatment with *S. guineense* ethanolic stem bark extract on the histology of the liver was investigated. Oral administrations of the extract in mice produced a positive result on the reduction of severity of caecal wall lesions in comparison to the untreated mice, as well as alterations in body weight. The antiamoebic effects of all extracts are clearly dose-dependent due to the response in the low treatment with 250mg/kg.

The recommendations are as follows;

1. Further investigations need to be carried out to elucidate the mechanism(s) of action of the extract on the metabolic process in the liver.
2. A chronic investigation should be done to evaluate the histological response of the treatment on the liver and other sensitive organs like the kidney, brain and heart.
3. People who use this plant as a source of medicine should be educated about the possible toxic effects of the plant when taken in large quantity due to the finding on the acute toxicity test carried out.

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