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Research Article

EFFICACY OF THE ETHANOLIC EXTRACTS OF *TALINUM TRIANGULARE* (JACQ) FOR CONTROL OF THE FRESH WATER SNAIL, *BULINUS GLOBOSUS*, THE VECTOR OF URINARY SCHISTOSOMIASIS

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ABSTRACT

Phyto- molluscides have received considerable attention in search of safe, environmentally friendly and cheaper alternative to existing synthetic molluscicides in the control of schistosomiasis. Bioassay was carried out using ethanolic extracts of leaf, root and a combination of leaf and root of *Talinum triangulare* on *Bulinus globosus* snails that are intermediate host of *Schistosoma haematobium* which causes urinary schistosomiasis. The experimental groups in triplicates were exposed to serial dilutions (160, 320, 480, 640 and 800 ppm) of ethanolic extracts of the different plants parts for three days while the control groups were exposed to water collected from the snails habitat and kept at the same laboratory conditions. The concentrations of all the extracts were mollluscicidal except the lower concentrations (160-480 ppm) of the leaf extracts. Ethanolic root extracts showed the highest potency (125.89 ppm), followed by the combination of leaf and root extracts (316.20 ppm) while the least potency was found in the leaf extracts (1000 ppm). Analysis of variance (ANOVA) showed significant variation between the treatments (F-ratio= 28.90 $P < 0.05$) in the ethanolic leaf extracts. Histopathological examination showed normal cells in the gastro intestinal tissue of *B. globosus* exposed to the control groups while there were tissue damages like necrosis, disruption of cytoplasm and nucleus, the appearance of dead cells and cytolysis of cells in the treated *B. globosus*. It is concluded that the plant extracts caused the histopathological damage which led to the death of these snails. Since organic compounds are more environmentally friendly than the synthetic chemicals, these plant extracts are recommended for further evaluation of field trials for the control of *B. globosus.*

Keywords: Ethanolic extracts, *Talinum triangulare*, Efficacy, Mollluscicidal**,** Histopathological damage *Bulinus globosus.*

INTRODUCTION

Schistosomiasis (Bilharziasis) also called snail fever is one of the parasitic trematode diseases infecting man in both the tropics and sub tropics (Hegan *et al.*, 1998). It is a major disease of public health in humans, occurring in over 72 countries of the world (WHO, 2010). It is one of the neglected tropical diseases due mainly to the fact that its effects are not dramatic like malaria and HIV/AIDS, but are hidden diseases and somewhat subtle (Jordan and Webbe 1993).

Depending on the species, different freshwater snails act as intermediate hosts for the parasite. These snails are found in the tropics where they breed in the wet season and become inactive during the dry season; however dry season farming encourages breeding of snails throughout the year (Chitsulo *et al.*, 2000).

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Although the snails do not play an active role in transmission of the parasites, the transmission of the infective stage of the parasite is accentuated through shedding of cercariae by the snail host (Imandeh and Echibe, 2001). The epidemiology of the disease schistosomiasis is influenced by major factors such as contamination of fresh water with excreta containing *Schistosoma* eggs, the presence of appropriate snail intermediate hosts and human contact with water infected with cercariae (Okon and Asor, 2012).

For the effective control of schistosomiasis, multifarious approaches are desirable, including control of the intermediate host snail. At present, Niclosamid (Baylucide, Bayer Germany) is the only commercially available synthetic molluscicides applied on a large scale (WHO, 2004). However, this synthetic molluscicide tends to be generally biocidal, toxic to fish and microscopic aquatic animals at lower concentrations than those required to kill the snails and affecting many of the plants in the snail habitat. Furthermore, the compound is often not affordable or available in many poor schistosomiasis endemic communities (Sher, 2001). The rising cost of proprietary mollusicides has stimulated a search for cheaper, safe and effective natural compounds derived from plants (Sher, 2001).

Studies have documented over 600 medicinal plant species from over 100 families used in the treatment of various illness (Odugbemi, 2006). *T. triangulare* (Portulaceae) is one of such important plants. *T. triangulare* belongs to the family Portulacaceae. It is a herbaceous, perennial and glabrous plant widely grown in tropical regions as a leaf vegetable (Ezekwe *et al.,* 2001). It serves as indispensable constituents of the human diet supplying the body with minerals, vitamins and certain hormone precursors, in addition to protein and energy (Philipson and Wright, 1991). Nutritionally, *T. triangulare* leaves have been shown to posses the essential nutrients like Beta carotene; minerals such as calcium, potassium and magnesium; Pectin protein and vitamins (Aletor and Adeogun, 1995). Traditionally, it is used as softener of other vegetables, and medically, *T. triangulare* has been implicated in the management of cardiovascular disease including stroke and obesity (Adewumi and Sofowora, 1980; Aja *et al*., 2010).

The objective of the present research is focused on the efficacy of the ethanolic extracts of *T. triangulare,* as a phyto-molluscicide in the control of the freshwater snail, *B. globosus.*

METHODOLOGY

Descriptions of experimental sites

The experiment was conducted at the University of Calabar, Calabar in Calabar Municipality Local Government Area of Cross River State, Nigeria in May 2013- August 2013. Cross River State covers a land area of 20,156km^2 and is located at 5^0 45^1 N, 80 3' E. It lies within the tropical climate and has three major vegetation zones namely: Mangrove, Rainforest, and Derived savannah zones. The Mangrove zones consist of creeks and swamps with annual rainfall of about 2000 mm. The rainforest zone with a mixture of tall and small trees and shrubs has a moderate total annual rain fall of 1500-2000mm while derived savannah in the Northern zone of the state has thorny bushes, scattered trees and low grasses with rainfall of 500-1000mm per annum. Humidity is about 65-90%, ambient temperature of 22.2^0-23.8^0C minimum and 27^0-40^0 C maximum (Mofinews, 2006).

Collection of snails (*B. globosus*)

Snails were collected from Ubam River which serves as a boundary between Adim and Abini community, all in Biase Local Government Area of Cross River State Nigeria. The information gathered revealed that the river has not been recently treated with molluscicides (Personal communication with the users of Ubam River). Trapping method as outlined by Azim and Ayad (1984) and employed by Hairston (1990) was used for snail collection. Freshly cut palm leaves and cassava leaves were placed along the bank of the snail- endemic River usually in the evening.

After 2 days, the leaves were recovered and inspected for the presence of snails. Collection of snails was done in the morning when water temperature was about 25^{o} C between 8 am and 12 noon. The collected snails were put in a sterile polythene bag along with water from the habitats and transported to Parasitology Research Laboratory, Department of Zoology and Environmental Biology, University of Calabar, Calabar.

Snail identification and culture in the laboratory

Snails were identified using keys outlined by Brown (1984) and Christensen and Frandsen (1985). Sand and earthenware pot were placed in 20 liters aquarium in an attempt to obtain a low temperature in the aquarium. Ten liters of the river water and the grass from the site of collection were then placed in the aquarium. A thermometer was placed in the aquarium to record the readings throughout the period of the study. The snails were acclimatized for a period of 7 days between 25^{o} C and 26^{o} C.

Plant collection and authentication

T. triangulare was bought from farmland within University of Calabar, Calabar-Nigeria. The plant specimens were put in sterile dark polythene bags and transported to the Herbarium Unit of Botany Department, University of Calabar, Calabar where they were identified by experts based on taxonomic keys available in the unit.

Preparation and preservation of plant powders

The selected plant parts were oven dried at a temperature of 65^{0} C for three days. The plant parts were thereafter separately pulverized using electric blender (Ken Wood). Each plant material/part well-labeled in the container was then stored in a refrigerator in the laboratory.

Preparation of *T. triangulare* extracts

Ethanolic extraction of plant substances was performed using the method of Harbone (2006) with a little modification. For each plant part, a stock solution was prepared by soaking 80 g of pulverized plant parts in 500 ml (160,000 ppm) ethanol sigma (Cat No. 1578) for 72 hours. The mixture was shaken vigorously for 2 hrs intervals to ensure proper soaking of the product. After which decanting of the extract was done and the liquid extract was filtered using Whatman No.1 filter paper and the excess water removed using evaporator to concentrate the volume of 50 ml. The extracts were put in the reagent bottles and stored at low temperature in the refrigerator. From known milliliters of each of the extracts, different concentration were later prepared (serial dilutions) using a known volumes of water taken from the snails habitat.

Extracts bioassay

The bioassay was performed as outlined by WHO (1995) and employed by Adenusi and Odaibo (2008, 2009), Singh and Yadav *et al.* (2010) and Philomena *et al.* (2013). The different volume of 0.0 (control), 0.5, 1.0, 1.5, 2.0 and 2.5 ml from the stock solution of the extracts were added to equal volume (500 ml) of dechlorinated water (collected from the snails habitat) in bioassay boxes of 6. 5 cm depth x 14 cm length 8.5 cm breadth. Ten (10) adult snails **(***B. globosus*) of weight range 6-12 g were immersed in different containers with varying serial dilution (Patole and Mahajan, 2010). There were triplicates for each concentration of each extract; three groups of ten snails each were kept in 500 ml of water collected from the snails' habitat as control groups. The concentration of each solution was calculated in ppm; 160, 320, 480, 620 and 800 ppm respectively. After 24 hrs of exposure to the plant extracts, the snails were first examined using irritability function and hydro sensitivity. Those snails that were motionless or do not react to needle probe by closing their opercula were transferred to fresh declorinated water for another 24 hrs after which mortality was ascertained. According to El-Sheerbini *et al* (2009), any plant extract that causes no mortality at 1000 ppm should be considered inactive and further investigation

should be discontinued. The total examination period was 72 hrs.

Histological examination of snails

The histological study involving collection of digestive tissues (gland) from the intestine of treated and untreated (control) snails was performed according to Kim *et al.* (2006), Mohamed and Saad (2009), Ming *et al*. (2011) and Philomena *et al.* (2013). The thick tissues were fixed in 2 ml of 10% neutral buffered formalin for 24 hrs then dehydrated in ascending grades of alcohol 70%, 90%, Absolute Alcohol 1, Absolute Alcohol 2, and Absolute Alcohol 3. Further procedures included clearing in xylol and paraffin as well as embedding. The snails were stained with Harris haematoxylin and eosin. The histological changes in tissue Sections of snails were mounted in Canada balsam and finally, examined with a light microscope using X10, X40 and X100 objectives.

Statistical analysis

Probit analysis of the raw data was carried out using Statistical Package for Social Science (SPSS) Software (Version 17.0) designed by Finney and Steven (1984) and employed by Philomena *et al.* (2013) to obtain the lethal concentration values. Analysis of variance (ANOVA) was used to test the significance differences in mean percentage mortality with different plant extract concentrations.

RESULTS

Percentage (%) mortality of *B. globosus* exposed to the different plant extracts

The results of mean percentage (%) mortality of *B. globosus* snails exposed to different concentrations of ethanolic extracts of *T.triangulare* are presented in Figure 1, 2 and 3. In ethanolic extract of *T. triangulare* leaves (Figure 1), there was no mortality in the lower concentrations (160-480 ppm) over the 3 days of exposure. Mortality was evident and increased as the concentration increases from 640-800 ppm which resulted in 100% mortality of all the *B. globosus* snails at the end of 72h exposure. Analysis of variance (ANOVA) showed significant difference between the treatments (F-ratio= 28.90 $P < 0.05$).

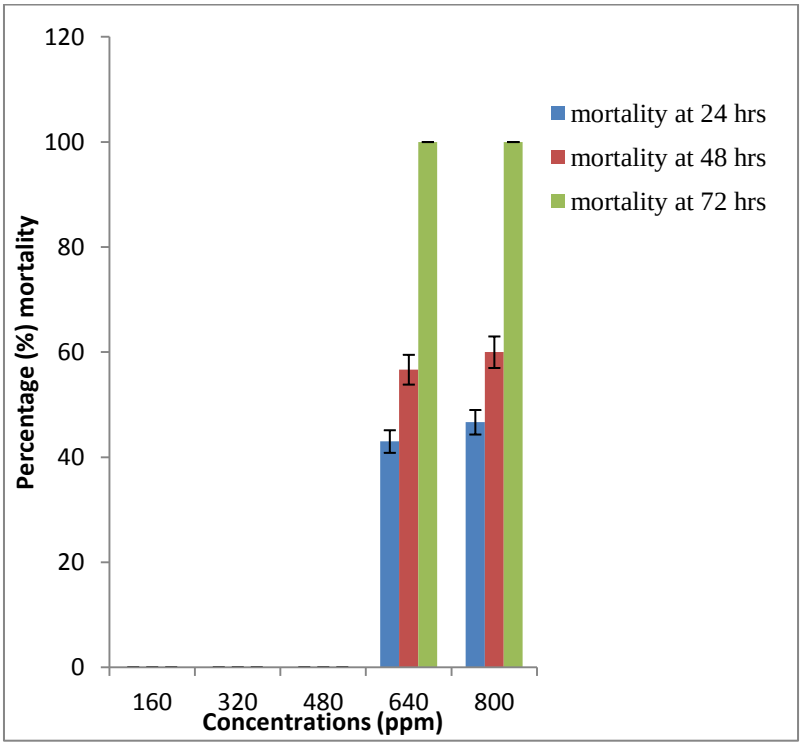
Mortality results in the ethanolic extracts of *T. triangulare* roots (Figure 2) showed greater mean percentage mortality when compared to the ethanolic extracts of the leaves. All the concentrations of the extracts from 160-800 ppm showed significant mortality effect and caused 100 % mortality of *B. globosus* snails. Result of analysis of variance (ANOVA) showed no significant variation between the treatments (F-ratio 0.28 $P>0.05$) in the mean percentage mortality with different concentrations of the extracts. Mortality results in a combination of ethanolic extracts of leaf and root (Figure 3) also showed increasing mortality from lowest concentration to the highest (160-800 ppm). Analysis of variance (ANOVA) of mortality results showed no significant difference (F- ratio 0.30 $P >0.05$) in the mean percentage mortality of the *B. globosus* exposed to the different concentration of the extracts.

Lethal concentration (LC_{50}) of ethanolic extracts of *T. triangulare* on *B. globoscus*

The result of lethal concentration (LC_{50}) of ethanolic extracts of *T. triangulare* is shown (Figure 4) The highest potency was found in ethanolic root extracts (125.89 ppm), followed by a combination of leaf and root (316.23 ppm) and the ethanolic leaf extracts (1000 ppm).

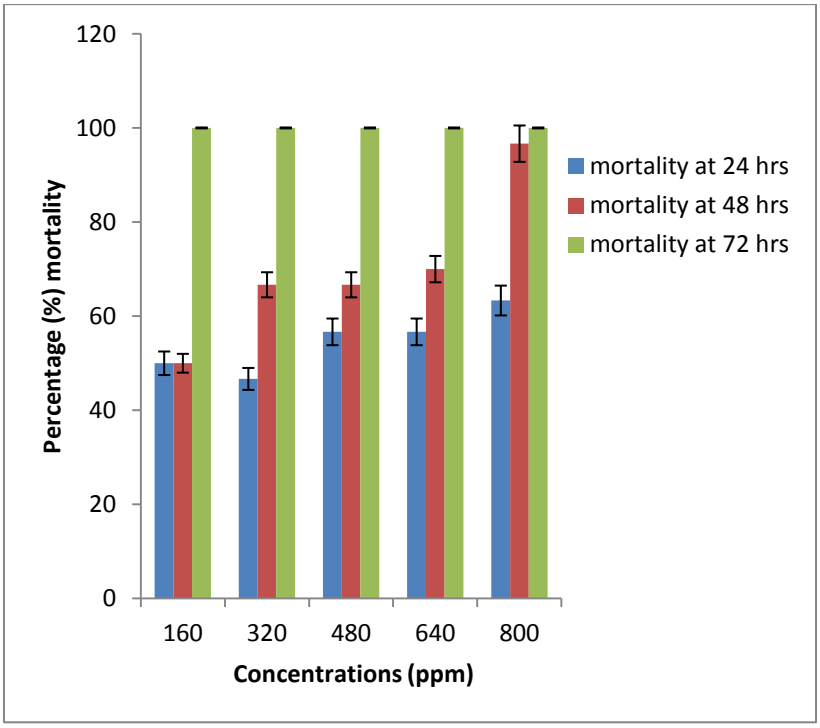
Histopathological analysis of *B. globosus* snails in treated and control experiment

The pathology of *B. globosus* exposed to different plant extracts (Plate 1-2) was manifested in the following conditions: disruption of cytoplasm (Plate 1b), disruption of cytoplasm and necrosis (Plate 2a), deformation of cytoplasm and dead cell (Plate 2b).



(a)

Figure 1. Mean percentage mortality of *B. globosus* exposed to ethanolic extracts of *T. triangulare* (leaves).



(b)

Figure 2. Mean percentage mortality of *B. globosus* exposed to ethanolic extracts of *T. triangulare* (root).

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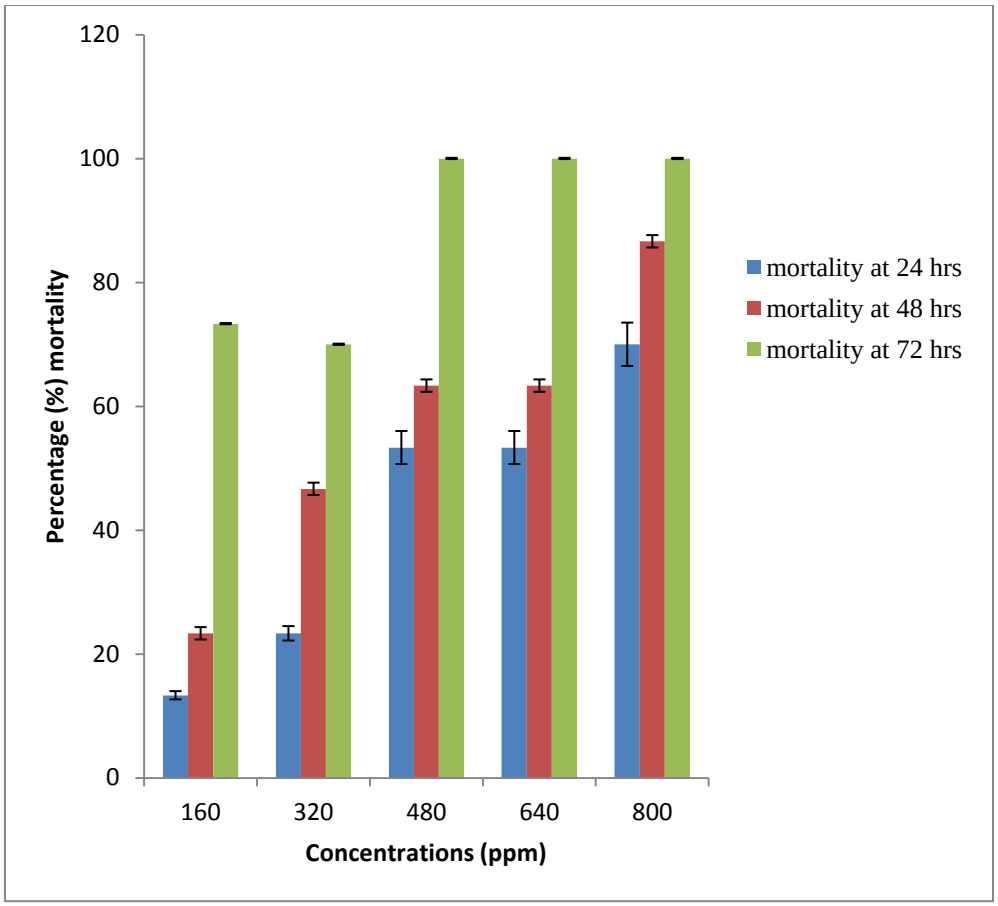


Figure 3. Mean percentage mortality of *B. globosus* exposed to a combination of ethanolic extracts of *T. triangulare* (leaves and roots).

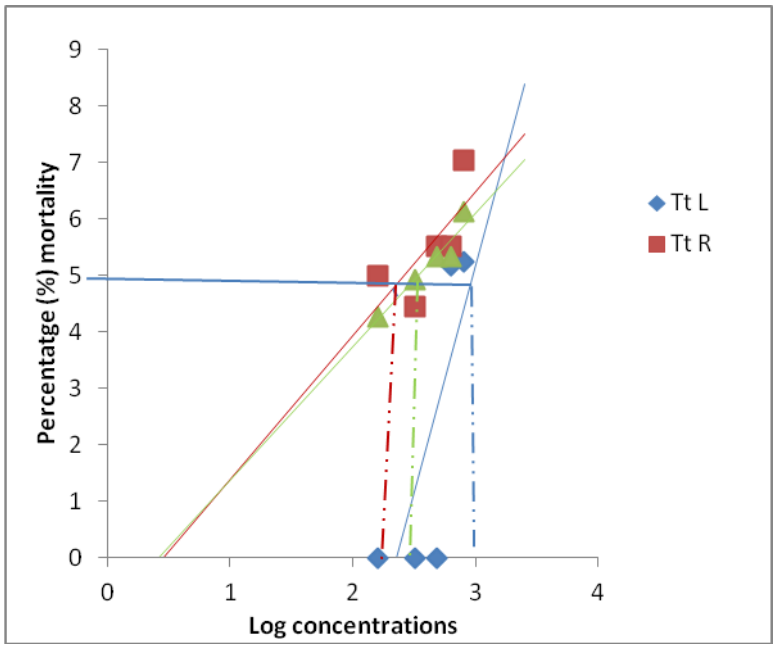
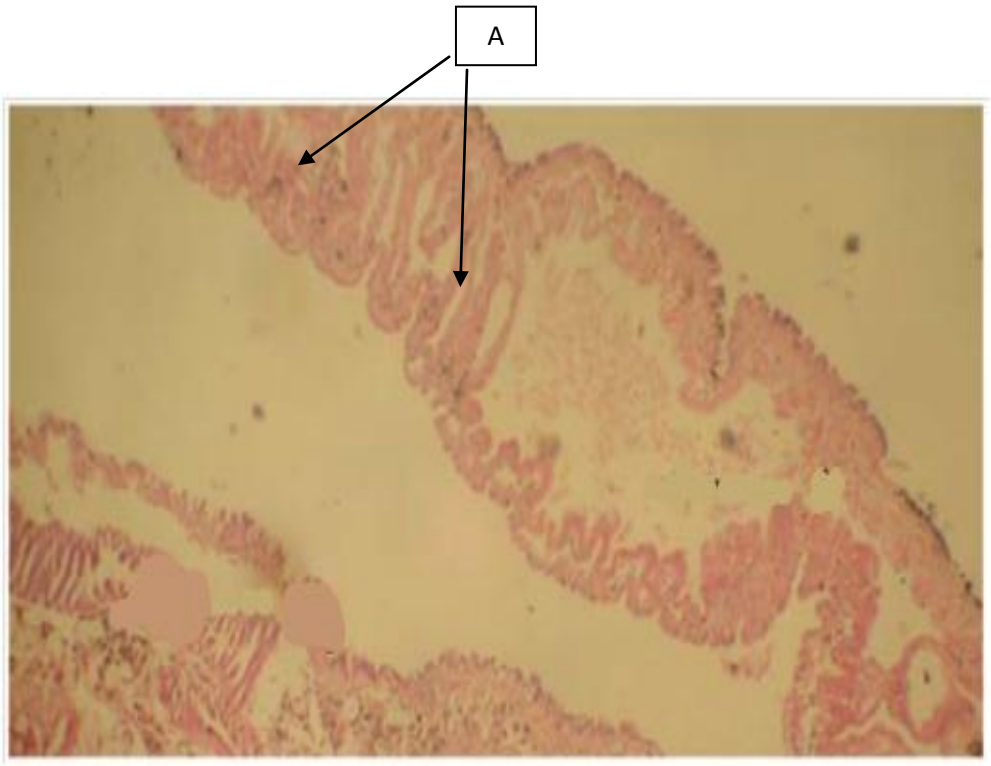
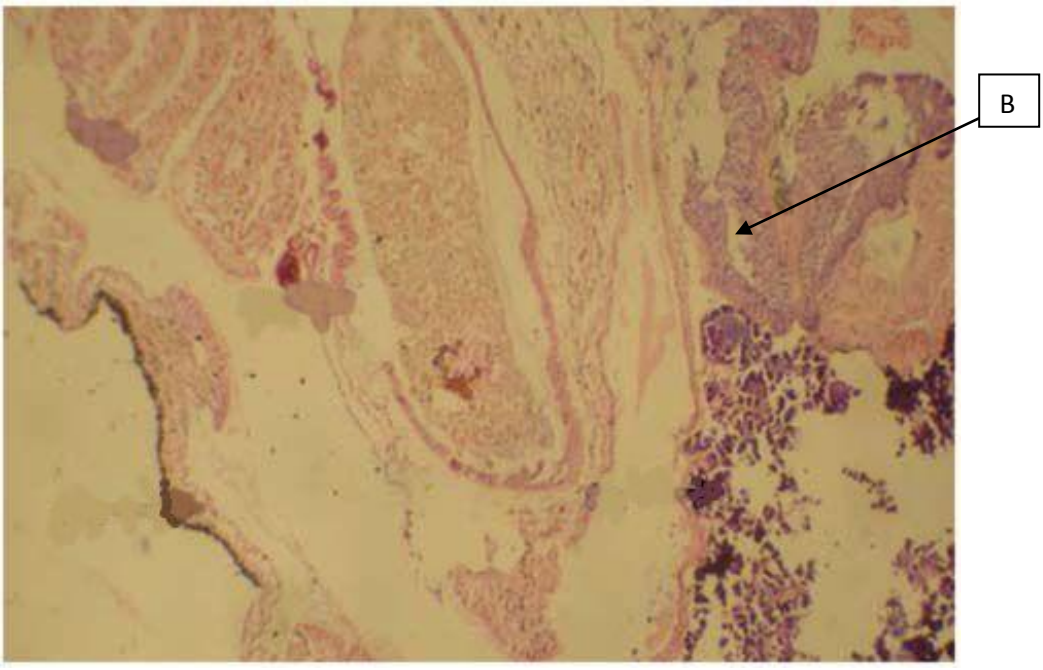


Figure 4. Median lethal concentration (LC_{50}) of *ethanolic* extracts of *Talinum triangulare* and on *B. globosus* (TtL= *T. triangulare* leaves, Tt R= *T. triangulare* roots, Tt L+R= *T. triangulare* leaf and root combination).

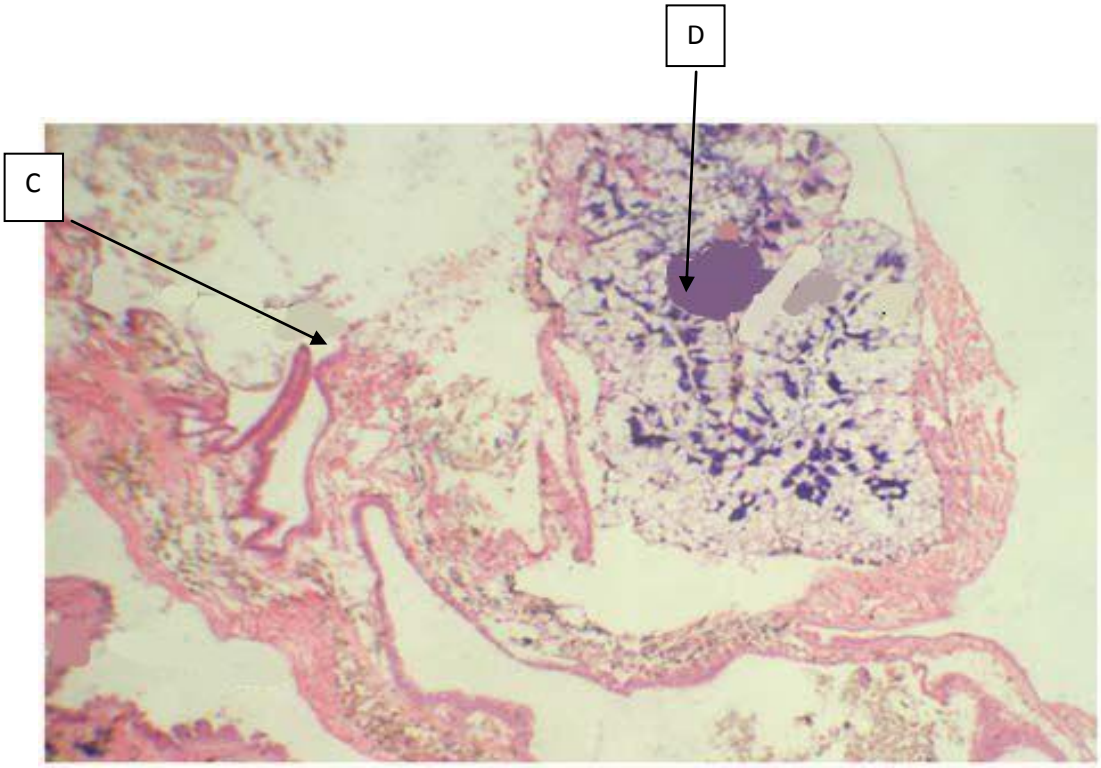


(a)

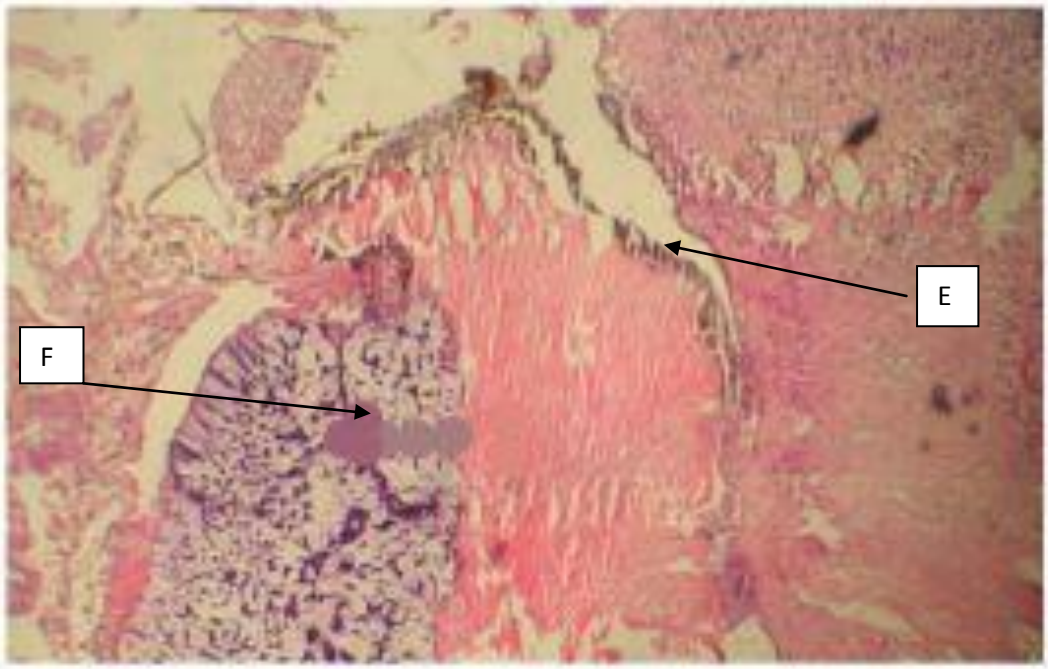


(b)

PLATE 1: Longitudinal section of digestive tissue (gland) of *B. globosus*: (a) control showing normal cytoplasm (A) and (b) exposed to ethanolic extract of *T.triangulare* leaves at 800 ppm showing disruption of cytoplasm(B**).**



(a)



(b)

PLATE 2. Longitudinal section of digestive tissue (gland) of *B. globosus* exposed to ethanolic extracts of *T.triagulare roots at* 800 ppm: (a) root extracts showing Eruption of cytoplasm (C) and necrosis (D), (b) a combination of root and leaf extracts showing deformation of cytoplasm(E), dead cell (F).

DISCUSSION

All the concentrations of ethanolic extracts of *T. triangulare* plants exerted varying degrees of mortality on the *B. globosus* snails used in this experiment when compared to the control except for the ethanolic leaf extracts that exhibited no mortality in the lower concentrations (160-480) ppm over the three days of exposure. This perhaps may be due to the tolerance of the *B. globosus* snails to these concentrations (Ming *et al.,* 2011). However, significant mean percentage mortalities were recorded from the higher concentrations (640-800 ppm) across the three days of exposure as observed in other extracts, resulting in mortality of all the exposed snails (*B. globosus*) at the end of the duration of the experiment. A similar observation was also reported for *Anonna muricata* by Parasher *et al.* (1995) against *Biomphalaria pfeifferi* snails where lower concentrations did not have any effect whereas there were significant effects were recorded at higher concentrations. Although the reasons for the molluscicidal potency and mode of action of plant substances have not been fully studied, plant substances have been reported to cause mortality of several genera of snails including *B. pfeifferi* snails (Otarigho and Olajumoke, 2012). Several authors including Kloos and McCullough (1982) and Olofintoye, 2010) have attributed the molluscicidal potency of plant substances to some active phytochemical constituents including saponins. However, these authors did not explain the biocidal activity of these phytochemicals. Cunha and Roque (2005) attributed the biocidal properties of saponins to haemolytic properties that disorganize the membrane. It is therefore thought that the saponins present in these plant substances may have exerted a haemolytic effect and subsequently leading to the death of the *B. globosus* snails in this experiment. Although there are few literature on the effect of the plants used in this study to bridge comparison, Amer and Manal (2004) verified the molluscicidal effect of *Solanum* species at concentrations similar to those utilized here and obtained similar mortality effects. They also mentioned that the saponins and glycoalkaloids present were responsible for the molluscicidal action.

According to WHO (2010) guideline on molluscicidal screening and evaluation, it is a necessary requirement to determine the lethal concentration (LC_{50}) of any plant substances before further recommendation can be made on the plants. Moreso, the guideline states that the lethal concentration (LC_{50}) of any plant extracts must not exceed 350 ppm before the plant extracts can be considered as strong molluscicidal agent. The low lethal concentration (LC_{50}) values (high potency) of a combination of ethanolic extracts of leaf and root of *T. triangulare* when compared to that of the ethanolic leaf extracts alone may be due to the synergistic effects of the phytochemical constituents of both leaves and roots. However, the overall lethal concentrations of *T. triangulare* tissues (leaves, roots and a combination of leaf and root) were lower than those observed by Schall *et al.* (1998), where results of their study on the effect of natural latex of *Euphorbia splendens* on *B. pfeifferi* demonstrated a more effective lethal concentration of 4.0 ppm. This may be due to species tolerance or phytochemical constituent. According to Olofintoye (2010), differences in the efficacy of plant molluscicides are attributed to three major factors: species-tolerance, concentrations used and phytochemical constituents of the plants.

The results of histopathological analysis of *B. globosus* snails showed normal cells in the gastro intestinal tissue of the snail from the controlled groups while there were tissue damages, necrosis, disruption of cytoplasm and nucleus the appearance of dead cells and cytolysis of cells in the treated *B. globosus* snails. Backry (2009) also reported damages in the digestive glands of *B. alexandrina* after their exposure to the methanolic extract of *Guayacum officinalis, Atriplex styllosa* and *Euphorbia splendens*. It was also observed that epithelial cells of the treated snails lost their regular shape and appeared empty while digestive tubules and connective tissues were also damaged. These damages are also similar to that observed by work of Adenusi and Odaibo (2009) who reported that the overall appearance of the tissue of *B. glabrata* exposed to methanolic extract of *T. tetraptera* caused swelling of the epithelial cells suggesting that the molluscicides may have either acted on the membrane of the cells in some ways as to alter their permeability or interfered with regulatory or metabolic processes within them.

CONCLUSION AND RECOMMENDATION

Since organic compounds are more environmentally friendly than the synthetic chemicals, these plant extracts are recommended

for further field evaluation for the control of *B. globosus*.

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