Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN 2320-3862
JMPS 2016; 4(5): 271-274

Received: 07-07-2016
Accepted: 08-08-2016

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The effect of aqueous leaves extract of *Solenostemma argel* (Del Hayne) on egg masses and neonates of *Biomphalaria pfeifferi* snails

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Abstract
Schistosomiasis is a major public health problem in Sudan. The lifecycle of the causative parasite of this disease includes intermediate snail hosts. Molluscicides of plant origin are preferred to be used for elimination of the snail vectors. Chemical molluscicides have negative impact on the environment, while plant molluscicides are ecologically friendly and not expensive.
This study was designed to test molluscicide activities of aqueous leaves extract of *Solenostemma argel* (*Del Hayne*) on *Biomphalaria pfeifferi* egg masses and neonates. The results of egg mortality (unhatchability) in the egg masses and mortality of neonates, were statistically analyzed using probit regression analysis to calculate the lethal dose that gives 50% and 95% mortalities in 24 hours, i.e (LD_{50} and LD_{95}). The values of the LD_{50} and LD_{95} of the egg masses were 2921.3 ppm, and 3515ppm, respectively, while those of the neonates were 368 ppm, and 1699 ppm, respectively.

Keywords: Schistosomiasis, molluscicide, Asclepiadaceae, snails, Sudan

1. Introduction
Schistosomiasis (also known as Bilharziasis) is a disease which is common worldwide. It is prevalent in developing countries in Africa, Asia and South America [1].The causative agents of this disease are the blood trematodes of the genus *Schistosoma*, and the most important species that infect man are *S. japonicum*, *S. mansoni*, and *S. mekongi*. Most intermediate snail hosts of human Schistosoma parasites belong to three genera, *Biomphalaria*, *Bulinus* and *Oncomelania*.
Many control strategies has been targeted by the World Health Organization [2], which included chemotherapy and control of the intermediate hosts (snails). Various methods have been tested for the elimination of snails, because this is the best control measure that could be applied, since it breaks the life cycle. Thousands of synthetic compounds had been tested. Although they were effective, but they were not entirely satisfactory. Increasing efforts are being made to find molluscicide products of plant origin. In endemic areas, plant molluscicides are prefered for control measures, because they are cheaper than synthetic molluscicides. Harjal (local name for S. argel) is a desert plant, and Sudan is considered the richest source of this plant, although it grows in other North African countries (Egypt, Libya and Algeria). In northern Sudan it grows naturally, and extends from Berber to Abu-Hamad, especially the Rubatab area [3]. Many studies had been done on the insecticidal activity of Harjal plant on several insect species [4, 5, 6]. The antimicrobial activity of *S. argel* aqueous extract against some microorganisms, was investigated by [7, 8] and many workers Less studies have been done to test its mollucicide activities.
In this study experimental trials were performed to find the effect of the aqueous extract of harjal on egg masses and neonates of B. pfeifferi snails.

2. Materials and Methods
2.1 Snails
This study was conducted on egg masses and neonates of Biomphalaria pfeifferi snails.
Collection and Sampling:
Snails were collected from water bodies in different locations around Khartoum State, and then taken to the laboratory, for breeding and maintenance. Egg-masses of *B. pfeifferi* snails were collected on polythene sheets and transferred to new-labeled aquaria for hatching. Only, the first generation was used in all laboratory experiments.

The snails were fed fresh lettuce or chard leaves every second day, and water was changed once a week, according to need. To minimize the stress on the colonies; extensive effort was made to keep the aquaria in favorable laboratory conditions, including providing food in reasonable quantities, removal of dead snails when observed. Also faeces and food residues were removed every two days. Fresh-dechlorinated tap water (strongly aerated for about 3 days to allow evaporation of chlorine and then, filled to two thirds), was added to aquaria to compensate for evaporation.

2.2 Breeding and Maintenance

Collected snails were screened for trematode infection. Infected snails were identified using the shedding method described by [9]. Each collected snail was placed in a small glass bottle, half filled with dechlorinated tap water. They were, then exposed to the day light and left for one hour or more to allow cercariae to emerge. The snails that shed cercariae were gathered in one circular glass trough, half filled with dechlorinated tap water. Healthy snails were maintained in aquaria of troughs (with a capacity of about 10 litres) with stocking density of 9 snails/L of water. The stock aquaria were interiorly covered with polyethene bags before filling with dechlorinated tap water, and were subjected to fluorescent light for a period of about 12 hours daily.

2.3 Collection and Preparation of Egg-masses and Neonate Snails

The snails were allowed to lay eggs. The polythene sheets were checked for egg-masses after 72 hours. Then, egg-masses attached to the polythene, were transferred to containers containing dechlorinated tap water, and used for the egg masses trials, where they were exposed to different extract concentrations.

For the neonates trials, egg masses were covered until eggs hatched into neonates. Then they were exposed to different extract concentrations. One week old neonates were required for the experiment.

2.4 Plant used: *Solenostemma argel* Del (Hayne)

S. argel belongs to family Asclepiadaceae, and has characters common for most of its genera, (milky juice, opposite leaves without stipules, corolla double with a small (corona) inside of various shapes, seed dispersal by hairs.

2.5 Collection and Preparation of Plant Material

Dry leaves of *S. argel* were obtained from local markets and cleaned manually to remove dust and any unwanted materials. They were then ground using an electric blender (Moulinex).The powder obtained was stored in clean, sterilized glass jars covered with plastic covers, and left at room conditions.

2.6 Preparation of Aqueous Plant Extract

A stock solution of 5% (5 grams of powder in 100 milliliters of distilled water) was prepared and kept in stopperd bottles. Mixing was carried out in a conical flask on a magnetic stirrer for 6 hours with the flask shaken every 30 minutes. After 24 hours, the solution was filtered through cotton wool, and used within 3 days.

2.7 Exploratory Trials

These were conducted in accordance with the guidelines of [10]. Widely logarithmic spaced doses (concentrations) were prepared as experimental solutions, by adding different appropriate volumes from the stock solution to 250 ml of de-chlorinated tap water in separate small Aluminum dishes. Then 90 eggs or 90 neonates were immersed in each dish. The exposure time for both egg masses and neonates was 24 hours, because no further changes were detected after that time duration, and the toxic effects of the active plant extract became evident in the tested egg masses and neonates. It was followed by a recovery period of 24 hours for both the egg masses and neonates in dechlorinated tap water. Once the extent of the toxicity range was determined, several convenient concentrations of the stock solution were prepared (dilution with dechlorinated tap water) to give mortalities between 0 to 100%. The effect of sublethal concentrations of *S. argel* on hatchability of *B. pfeifferi* eggs and neonates was investigated. There were three replicates of control for egg masses and neonates trials, in which both were exposed to dechlorinated tap water only without addition of extract. Eggs and neonates were examined daily, for development and hatching of eggs or death of neonates. Final assessment of mortalities was at the end of seven days. An embryo in an egg mass was considered dead if it did not hatch at the end of the experiment [11]. Mortalities were recorded as the number of dead/ unhatched embryos.

2.8 Potency Tests of Aqueous Plant Extract:-

For each chosen concentration of the aqueous extract, 90 neonates or 90 eggs o were placed in each dish, containing 250 ml of dechlorinated tap water. Each egg mass contains (10-13egg), so the number of egg masses in each dish was determined by counting the total number of eggs, so as to be 90 eggs, i.e. about (8-9 egg masses). The exposure and recovery periods are the same as mentioned above. Parallel control experiments were carried out using dechlorinated tap water. No food was provided during the exposure test. Thereafter; mortality counts were done and recorded. For each dilution of the extract, the above experimental steps were repeated 3times.

2.9 Statistical Analysis

The LC_{50} and LC_{95} values (with 95% confidence limits) of extracts for the egg masses and neonates were calculated by subjecting the data to analysis by probit analysis regression.

3. Results

3.1 The effect of aqueous leaves extract of *S. argel* on egg masses and neonates of *B. pfeifferi*

In this study the molluscicide activity of aqueous leaves extract of *S. argel* (Del Hayne) against the egg masses and first generation neonates of *B. pfeifferi* was evaluated at different concentrations. The upper and lower limits of the 24-hours LD_{50} and LD_{95} of the aqueous extract on the egg masses and neonates are shown in Table, 1 and Table, 2, respectively.

The plot probit of kill against log of doses provides a simple graphic representation of the dose-to- response ratio. The probit mortality shows a linear relationship with the log concentration of aqueous extract of *S. argel* leaves, on egg masses (Fig.1) and on neonates (Fig. 2).Toxicities of the tested extract of *S. argel*, as indicated by the number of egg mortalities (unhatched eggs after one week), where concentration-dependent and increased with increasing concentration of extracts.

3.2 Bench Observations

On hatching (between 6-8 days), the neonates resemble the adults in some ways, and their movement can be seen. Number of eggs / egg mass vary between (10-13), and is surrounded by gelatinous coat.

Table 1: The 24-hours LD_{50} and LD_{95} of the Aqueous leaves extract of *S. argel (Del Hayne)* on egg masses of *B. pfeifferi*

Probability	Confidence Limit for Dose		
	Estimate	Lower bound	Upper bound
LD_{50}	5.892	5.718	6.062
LD_{95}	9.703	9.299	10.191

Table 2: The 24-hours LD_{50} and LD_{95} of the aqueous leaves extract of *S. argel (Del Hayne)* on neonates of *B. pfeifferi*

Probability	Confidence Limit for Dose		
	Estimate	Lower bound	Upper bound
LD_{50}	0.736	0.503	0.934
LD_{95}	3.398	2.462	6.174

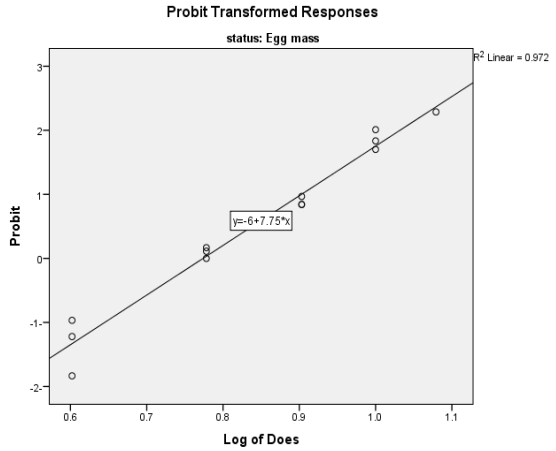

Fig 1**:** Log dose /probit regression line of *S. argel* (Del Hayne) aqueous extract of leaves on Egg masses of *B. Pfeifferi*

PROBIT (p) = Intercept + BX (Covariates X are transformed using the base 10.000logarithm.)
= 0.6 + 7.75x
Intercept = 0.6
Coefficient of dose = 0.6. the slope of the model

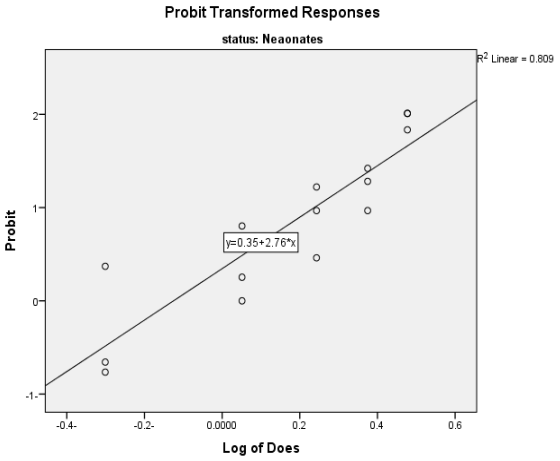

Fig 2: Log dose /probit regression line of *S. argel* (Del Hayne) aqueous extract of leaves on Neonates of *B. Pfeifferi*

PROBIT (p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)
= 0.35 + 2.76x
Intercept = 0.35
Coefficient of dose = 0.35. the slope of the model

4. Discussion

The most important control strategies for schistosomiasis is disruption of the lifecycle of the snail vector by the use of molluscicides. The study of plant molluscicides are given increasing attention by national and international institutions. Interest in plant molluscicides dates from the 1930's when [12] and [13] planted the desert palm, *Balanites aegyptiaca* and *B. maughamii*, along the water courses of the Sudan and southern Africa, respectively. The laboratory and field trials of these scientists indicated that the fruit which fell into the water inhibited the increase of snail population density.

A large number of plant families, which possess natural molluscicide activities have been identified [14, 15, 16]. Asclepiadaceae was placed among the most important flora families, which shows several bioactive plant species in Sudan.

In the present study, *S. argel* (Del Hayne) which is a member of this family was used. The obtained results revealed that egg masses and neonates of *B. pfeifferi* snails were sensitive to water extracts from leaves of Harjal plant *S. argel* (Del Hayne). Hence, water extracts of leaves of this plant, has molluscicide properties and can be used as a molluscicide in the control of schistosome-snail intermediate host.

The $LD_{50}$and LD_{95}values were used to estimate the levels of the plant toxicity. The LD_{50} for the egg masses was 294.600 ppm, and the LD_{95} was 485.150ppm. The LD_{50} for the neonates was 36.800 ppm, and the LD_{95} was169.900 ppm. The findings indicated that the neonates were more sensitive to the extract, than the egg masses. This may be attributed to the fact that the egg masses are surrounded by gelatinous coat, which protects them.

Literature revealed that *S. argel* contain different quantity and quality of active compounds [17, 18, 19, 20, 21]. The molluscicide properties of *S. argel* could be attributed to the kinds of active ingredients which occurred in this plant [22]. The experiment induced an effective control of egg masses and neonates of *B. pfeifferi* snails under laboratory conditions.

S. argel should be evaluated under field condition for proper use in control of Schistosomiasis. Also, further investigations and phytochemical studies should be done to determine the ingredients and metabolites of this plant.

5. Acknowledgements: Authors express sincere thanks to the Ministry of health for permission to conduct this study in the Bilharzia reference laboratory; Omdurman State. Thanks are also due to the staff in the laboratory for providing necessary facilities to conduct this study.

The assistance of Dr. Ehab Farah (Biostatistics & Qualitative data analyst) in analysing the data is very much acknowledged.

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