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Molluscicidal Activity and Clinico-pathological Effect of *Agave lophantha*

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Abstract □ Dry powder and different extracts of *Agave lophantha* were tested against *Biomphalaria alexandrina*. The results showed that the butanol extract has high molluscicidal activity. The activity of the dry powder has been found to be stable under the effect of some simulated field conditions. Also the toxicological effect of the plant on mice was tested through determination of certain parameters such as total protein, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and acid phosphatase enzymes as well as histopathological study on liver and kidney.

Keywords □ *Agave lophantha*, molluscicide, trematode

Schistosomiasis, the most important trematode disease of man, is a world health problem. It is one of the prevalent endemic disease in tropical and subtropical regions where it is spreading as cultivated areas increase[1]. In Egypt, the erection of irrigation projects and the opening of large areas to perennial irrigation increased the infection rate as the new areas added suitable habitat to the fresh water snails, the intermediate vectors of the parasite[2].

The chemical molluscicides are not always easily available or applied and they usually have toxic effect on water fauna, domestic animals and human beings. Therefore, the attention was drawn to use plant constituents which may have molluscicidal activity[3-6].

The present study is a trial to examine the molluscicidal activity of the dry powder and some different extracts of *Agave lophantha* (Agavaceae). On the other hand, before field application of the plant the toxicological effect of this plant on mice through determination of certain parameters such as total protein, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline and acid phosphatase as well as histopathological study on liver and kidney could be determined.

EXPERIMENTAL METHODS

Snails

Biomphalaria alexandrina snails, the intermediate host of *schistosoma mansoni* in Egypt were used for the molluscicidal test. They were collected from areas located in Giza Governorate (Egypt), and left for 3 weeks in the laboratory in dechlorinated water for acclimatization with laboratory conditions. Lettuce leaves were added daily. The snails used in each experimental concentration and control were in 2 replicates. Exposure and recovery periods were 24 hrs each unless otherwise stated according to the WHO screening[7].

Preparation of the plant extracts

The plant under investigation was collected from Orman garden, dried at room temperature and finely powdered. The dry powder of the plant was exhaustively extracted by different solvents as shown in Table I. Extracts distilled off under reduced pressure till dryness and yields of the residues estimated. For molluscicidal testing, the stock solution (500 ppm) of different extracts were prepared in distilled water on basis of weight/volume. A series of dilutions from the stock solutions that would permit

Table I. Comparative susceptibility of adult *Biomphalaria alexandrina* to the molluscicidal action of dry powder and different extracts of *Agave lophantha*

Substance	LC_{50} (ppm)	LC_{90} (ppm)	Slope
Dry powder	70 (63.44-81.20)	100	1.33
Methanol extract	32 (26.23-39.04)	52	1.22
Acetone extract	35 (21.65-29.4)	55	1.42
Ether extract	69 (59.32-82.60)	99	1.3
Chloroform extract	46 (40.35-52.44)	60	1.24
Butanol extract	17 (59.32-82.60)	32	1.82

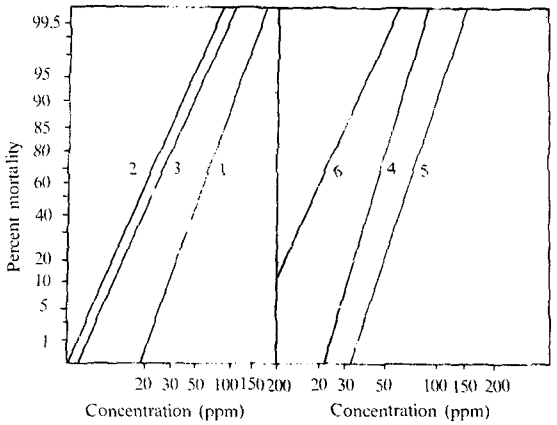

Fig. 1. Dosage mortality of adult *B. alexandrina* exposed to various test samples 1, Dry powder; 2, methanol extract; 3, acetone extract; 4, chloroform extract; 5, ether extract; 6, butanol extract after 24 hours.

the computation of LC_{50} and LC_{90} were prepared using dechlorinated water. Values of these concentrations were determined by Litchfield and Wilcoxon's method[8].

Effect of simulated field conditions on the molluscicidal activity of A. lophantha

The validity of the plant for possible field application depends on the stability of the molluscicidal activity of the plant to some factor such as different temperatures ranging from 10-30℃, different pH values from pH 4-10, sun radiations, mud particles; different exposure periods and storage. These experiments have been carried out as reported in El-Eman *et al.*[9].

Toxicological study

White mice of about 40 days old, weighing about 20 g were used in the present study. The animals were divided into 5 groups each of 10 animals. The mice were housed in polyethylene cages and having the same food. One group was kept as control (without treatment), while the four other groups were given 200 mg of plant powder/kg of body weight as aqueous suspension for 1, 2, 3 and 4 weeks. The administration was carried out daily for each mouse for the whole administration period. At the end of each period, mice sacrificed by decapitation after fasting overnight. Blood was collected in small centrifuge tubes by the help of glass funnel, centrifuged at 2000 rpm for 10 minute to obtain clear serum. The serum sample kept at −20℃ till used for estimation of total proteins, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline and acid phosphatase, using test kits supplied by Boehringer Mannheim (West Germany) and Bio Merieux (France). The methods used were those of Weichselbum[10], Reitman and Frankel[11] and Kind[12], respectively.

Statistical analysis of the data was performed according to Shedecor and Cochran[13].

Liver and kidney of treated and control mice were fixed in 10% formaline solution and embedded in paraffin wax, sectioned at 5-7 microns and stained by haematoxylin and eosin[14] for routine histophathological examination, Massone trichrone[15] for collagen and other fibrous tissue, and silver stain[16] for demonstration of reticulin fibres.

RESULTS

From the results in Table I and Fig. 1 it is evident that the butanol extract showed high molluscicidal activity (LC_{50} at 17 ppm) than other extracts. Also the dry powder of whole plant without any extraction showed high molluscicidal activity therefore, the plant become economic value for field application.

Effect of time changing of exposure periods

From Table II it is evident that the mortality disappeared below 3 hours exposure time but high mortality were recorded after 48 hours. Also the suitable time suggested for application could be after 24 hours, nearly similar for field application.

Effect of temperature changes, sun light, mud particles and storage

From Table III it was evident that:

a) At low temperatures (10℃) a remarkable decrease in the activity was recorded but at 30℃ a high increase was observed therefore summer months could be recommended as a suitable period when applying the tested plants in the field.

b) By exposing different concentrations of sun raditions for a period of 6 hours we noticed that the sun radition have no effect on the activity.

c) By preparing different concentrations of dry powders of the plant using water containing 10,000 ppm mud particles, the results in Table III indicated that no significant changes were noted in the mortality percentages of snails as compared with control experiment without mud.

d) When different concentrations of dry powders of the plant stored for about 7 days, the results showed that detectable decrease in the mortality percentage but the activity did not completely disappear.

Toxicological effect

The results displayed in Table IV showed the mean values of total protein, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline and acid phosphatase in serum of tested mice before and after treatment with *A. lophantha*. It was clear that there was slight variation in all parameters but there were no significant difference in these parameters (t-test, $p=0.05$).

The livers of all animals (control and treated) showed congestion of the central veins, sinusoid as

Table II. Time-concentrationship of the molluscicidal activity of the dry powder of *A. lophantha* against *B. alexandrina*

Concentration (ppm)	% Mortality of *B. alexandrina* after exposure periods (hr)						
	1	3	6	8	12	24	48
400	0	40	60	90	100	100	100
350	0	40	50	70	100	100	100
300	0	20	30	60	80	100	100
250	0	20	30	40	60	100	100
200	0	0	0	40	60	100	100
150	0	0	0	30	50	100	100
125	0	0	0	10	50	100	100
100	0	0	0	0	30	70	100
80	0	0	0	0	10	50	60
50	0	0	0	0	0	20	30
40	0	0	0	0	0	0	10
25	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0

Table III. Effect of temperature change pH, sun, mud and storage on the molluscicidal activity of *A. lophantha* dry powder against *B. alexandrina*

Concentration	% Mortality of *B. alexandrina* after 24 hours								
	Temperature (℃)			pH			Sun	Mud (10,000 ppm)	Storage 7 days
	10	22	30	4	7	9			
400	60	100	100	100	100	100	100	100	40
300	40	100	100	100	100	100	100	100	30
250	20	100	100	100	100	100	100	100	10
200	0	100	100	100	100	100	100	100	0
175	0	100	100	100	100	100	100	100	0
150	0	70	100	100	100	100	100	100	0
125	0	50	100	50	60	80	100	80	0
100	0	10	80	20	30	70	90	50	0
80	0	0	50	0	10	40	60	10	0
50	0	0	30	0	0	20	30	0	0
Control	0	0	0	0	0	0	0	0	0

Table IV. Mean values of studied parameters in mice's serum before and after treatment with different concentrations of *A. lophantha*

No. of groups Parameter	Control group without treatment	Group 1 1 W. Treat.	Group 2 2 W. Treat.	Group 3 3 W. Treat.	Group 4 4 W. Treat.
Total protein (g/dl)	3.4 ± 1.1	3.0 ± 0.9	3.0 ± 0.8	3.9 ± 1.0	3.6 ± 0.7
ALAT (U/l)	5.5 ± 1.6	6.4 ± 2.1	5.5 ± 1.2	5.9 ± 1.9	6.2 ± 1.3
ASAT (U/l)	7.7 ± 2.3	8.1 ± 2.0	7.3 ± 2.7	8.5 ± 3.0	9.1 ± 2.9
S. Alk. phosph. (U/dl)	6.50± 1.40	6.30± 1.20	6.80± 2.10	6.90± 1.30	7.10± 1.80
S. Acid phosph (u/dl)	2.50± 0.6	2.75± 0.40	2.50± 0.55	3.1 ± 0.9	2.70± 0.9

Table V. Pathological changes in hepatocytes according to dose of dry powder of *A. lophantha* and period of treatment

Dose (mg/kg)	Period of treatment	Hydropic degeneration	Fatty deg.	Necrosis	Cloudy swelling	Total No. of mice
200 ppm	1 week	−	−	−	−	10
	2 week	−	−	−	+	10
	3 week	±	−	−	+	10
	4 week	±	−	−	+	10

− Absent ± Occasional ++ moderate. +++ severe

Table VI. Inflammatory cellular infiltrate, central vein and sinusoidal congestion and kupffer cells hyperlplosien in mice receiving plant according to duration of treatment

Dose	Period of treatment	Inflammatory cellular infiltrate		Kupffer cells		Blood vessels	
		portal	lobular	Normal	hyperplosien	portal	congestion Sinusoidal
200 ppm	1 week	++	±	+	−	+++	++
	2 week	+	±	+	−	++	+
	3 week	+	±	−	+	++	+
	4 week	++	±	−	+	+++	+
Control		+	±	−	+	+++	++

− Absent. ± Occasional in some field. + mild. ++ moderate +++ severe

well as portal blood vessels. There was minimal parenchymatous changes, consists of cloudy swelling and mild focal areas of hydropic degeneration. Necrosis was absent. The portal tracts were mildly thicked due to infiltration with chronic inflammatory cells, (Fig. 2 and Table V,VI)

On the other hand, kidneys of all animals (control and treated) were within normal (Fig. 3 and Table VII).

DISCUSSION

Agavaceae plants which are commonly planted for ornamental purposes can easily grow in mud and sandy soils and can acclimatize in hot and cold regions, proving that the possibility of easier availability than the plant molluscicides of choice *Phytolacca dodecandra*[5].

The present work showed that the dry powder of *A. lophantha* was very toxic to *B. alexandrina* with LC_{90} at 100 ppm after 24 hours of exposure period. Comparaing the potency of dry powder of this plant with other plants studies in Egypt, it appears that it is more toxic than *Atriplex halimus*[17], *Agave ferox*[5] and *Yucca filamentosa*[18]. Moreover, the potency of the butanol extract isolated (LC_{50} at 17 ppm) from

Table VII. Pathological changes in kidney of mice in relation to duration of treatment and control group

Dose	Period of	Normal	Glomeruli		Tubules		
			Mesangial proliferation	Fibrotic	Normal	Deg.	Necrosis
200 ppm	1 week	+	−	−	+	−	−
	2 week	+	−	−	+	−	−
	3 week	+	−	−	+	−	−
	4 week	−	+	−	+	−	−
Control		−	+	−	+	−	−

− Absent, ± Occasional; + mild

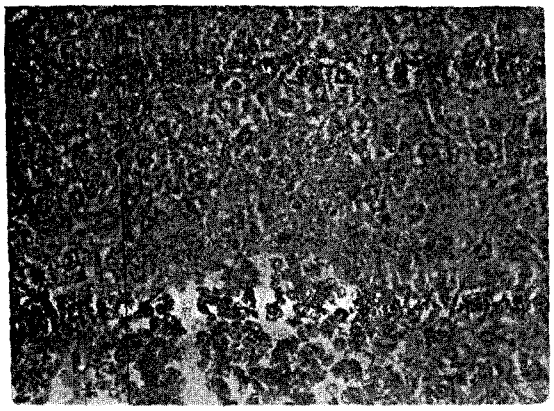

Fig. 2. Liver of mouse, after 4 weeks from treatment with *A. lophantha*. Dilatation and congestion of the contral vein with minimal parenchymatous changes, as cloudy swelling and focal areas of hydropic degeneration (H and E×120).

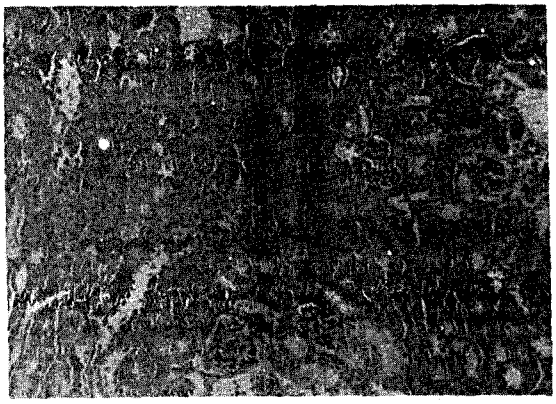

Fig. 3. Kidney specimen from mouse, after 2 weeks from treatment with *A. lophantha*, with normal glomeruli and tubules (H and E×100).

A. lophantha against *B. alexandrina* was more toxic than potency of the water extract of *Phytolacca dodecandra* (LC_{50} at 18-19)[4].

On the other hand, the stability of molluscicidal activity of the dry powder of the tested plant under different simulated field conditions as mud, sun radition and temperature can add to it's validity for possible field application. Also the application of total plant powder proved to be of economic value and possibly easy for application with simple technique.

In previous study it was reported that the synthetic molluscicide (mollotox) has an inhibitory effect on the activities of ASAT and ALAT in tested organs and hemolymph. On the other hand, it increased ACP activities and total protein concentrations. These variations were significance[19]. Other studies were carried out on inhibition of ASAT, ALAT and ACP in serum of mice by active principles of some plant[20].

It was obvious from the present study that the dry powder of *A. lophantha* has no inhibitory effect on liver cell, where was no significant changes. This results which go parallel with that cleared from our histopathological picture of liver of mice administered with plant water suspension. Also the histopathological examination of the kidney of mice treated with the plant show no pathological changes. These findings reflect the preliminary safety of these plant as molluscicides.

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