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Effect of Photosensitization Process of Carbamide Perhydrate on *Biomphalaria alexandrina* Snails and Their Infection with *Schistosoma mansoni*

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Abstract: Carbamide perhydrate, a photosensitizer, was tested as a molluscicidal agent against the medically important snails *Biomphalaria alexandrina* .In addition, the effect of its sublethal concentrations on the fecundity and infection of these snails with *Schistosoma mansoni* miracidia was carried out. The activities of glycolytic and gluconeogic enzymes e.g. lactate dehydrogenase (LDH), pyruvate kinase (PK), phosphofructokinase (PFK), glucose phosphate isomerase(GPI), hexokinase (HK), fructose-1,6 diphosphatase, phosphoenol –pyruvate carboxykinase enzymes in snail's tissues were determined. As well, total protein content, alkaline phosphatase (ALP) and transaminases enzymes (ASTand ALT) were evaluated in snail's tissues. The results indicated that LC_{90} of carbamide perhydrate against snails was 135 ppm post their exposure to direct sunlight for 4 hours at 450W/m^2. Under the same conditions, the sublethal concentrations 60 and 85 ppm inhibited snails egg laying, while those exposed to 35 ppm laid few ones. This was confirmed by histological examination that showed a severe damage in the hermaphrodite gland cells of snails exposed to these concentrations. In addition, these concentrations caused a highly significant suppressive effect on the activities of glycolytic, glyconeogenic, transminases, alkaline phosphatase enzyme activities and total protein content in snail's tissues. Treatment of snails with carbamide perhydrate markedly affected their compatibility to *S.mansoni* infection through reducing the cercarial output from snails exposed to 35 ppm during exposure to miracidia (280 cercariae / infected snail, compared to 1237 cercariae / control infected snail). Moreover, *S.mansoni* miracidia failed to infect snails exposed to 60 and 85 ppm from the experimental compound.

Key words: Photosensitization, Carbamide perhydrate, *Biomphalaria alexandrina, Schistosoma mansoni*

INTRODUCTION

Schistosomiasis is still an important public health problem in the developing countries[9]. Snail control strategies are considered a priority of the reduction of schistosomiasis transmission[20]. Although the use of molluscicides has always been considered to be a major supportive procedure in integrated schistosomiasis control. There are many restrictions of using toxic compounds (pesticides and molluscicides) with fresh water.Therefore, the safety of photosensitizes to human being is an advantage for studying their effect against the snail vectors of schistosomiasis[24].

The photosensitizer carbamide perhydrate (urea hydrogen peroxide) is commonly encountered in cosmetic dentistry, in hair dyes, to loose and remove earwax, to treat ulcers and other lesions in the mouth[19,7]. Carbamide perhydrate is a well known commercial product as antiseptic and disinfectant for a wide range of applications. It is readily biodegradable to urea as fertilizer and hydrogen peroxide which associate to water and active oxygen and environmentally safe[4]. The use of photosensitizing dyes for pathogens inactivation has been studied in both red and platelet blood components[32]. In addition, the fluorescence of some anticancer photosensitizers allows their use as tumor localizing agents who are particularly useful in precancerous conditions[32,3]. Photosensitizing dyes (Carbamide perhydrate) become toxic only when they are activated by sunlight. They are certainly characterized by a low environmental impact and a minimal risk for plant, animal and human ecosystems[4].

The aim of the present study is to evaluate the efficiency of the photosensitizer (carbamide perhydrate) as a molluscicide against *Biomphalria alexandrina* snails, survivorship and fecundity and their infection with *schistosoma mansoni*. In addition, some histological and biochemical parameters, e.g. the activities of glycolytic, glyconeogenic and liver function enzymes, in snail's tissues were determined.

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MATERIALS AND METHODS

Snails: Snails used in the present study were adult *Biomphalaria alexandrina* snails (8-10 mm diameter), collected from irrigation canals in Giza Governorate. They were maintained under laboratory conditions for several weeks, fed boiled lettuce leaves, examined for natural parasite infections and only healthy ones were used.

Schistosoma *mansoni* Miracidia and Cercariae: *Schistosoma mansoni* miracidia and cercariae were obtained from SBSC. Schistosoma eggs were extracted from the intestines of hamsters infected 45 days earlier. They were cleaned and hatched to miracidia in small amounts of dechlorinated tap water at 25 ±1 C°. Cercariae were produced from experimentally infected *B. alexandrina* snails. They were collected in small amounts of dechlorinated water and used directly after shedding.

Carbamide Perhydrate: Carbamide perhydrate (urea hydrogen peroxide, $CH_4N_2O.H_2O_2$, m.p. 85-90°C, FW 94.07), were obtained from FLUKA, is tablets and rapid-soluble in water, it contains 30% H_2O_2.

A stock solution of 1000 ppm was prepared in distilled water and kept in dark. The required dilutions were prepared by using an appropriate volume of the stock solution and complete to 200 ml with dechlorinated water.

Molluscicidal Tests: Ten *Biomphalaria alexandrina* snails (8-10mm) were added to each 200 ml of the concentrations 150, 100, 90, 70, 50, 40, 20, 10 ppm and directly maintained at sunlight (450W/m^2) for 4 hours at 25°C, then transferred to shade at the laboratory for 20 hours. Thereafter, the snails were thoroughly washed and transferred to clean dechlorinated water for another 24 hours for recovery. Three replicates were prepared for each concentration. Another three control groups of snails were run in dechlorinated water, without compound under the same experimental conditions. Death of snails was determined by changes in appearance of the shell and internal body[24].

Factors Affecting the Molluscicidal Potency of Carbamide Perhydrate:

A-Effect of Light: The same concentrations in the molluscicidal test were repeated in the laboratory at day light (12-h shade / 12-h dark), complete dark (24 hrs) or artificial light using desk lamp 100 watt at 15 cm distance (4-hrs light-lamp / 20 hrs dark).Thereafter, the snails were transferred to clean water for 24 hours for recovery and mortality was recorded.

B-Effect of Storage: The moluscicidal activity of the above concentrations was evaluated after 3 days of storage at room temperature, (25± 1°C). The snails were introduced into each concentration and directly exposed to sunlight for 4 hours and transferred to shade for 20 hours. The same test was repeated for the freshly prepared concentrations. Then, the death rate of snails was recorded after 24 hours recovery in both cases (stored and fresh preparations).

C-Effect of Incubation Period: The snails were incubated in the used concentrations, and then they were exposed to sunlight for 4 hours followed by 20 hours in shade. Thereafter they were transferred to clean water for recovery (24 hours) and the mortality was determined.

Effect of Carbamide Perhydrate on Snail's Fecundity: *B. alexandrina* snails (8-9mm) were exposed to 35, 60, and 85 ppm in (high dose of carbamide perhydrate represented by 135 ppm = LC_{90} causing high mortality rate in snails) direct sun light for 4 hours, thereafter snails were transferred to shade and maintained under laboratory conditions for 4 weeks. For each concentration a group of 50 snails in five replicates, each of 10 snails / L were used. A control group of 30 snails was used as 3 replicates each of 10 snails / L. Snails were daily fed boiled lettuce leaves, dead snails were removed and survival rate was recorded. Each aquarium was provided with polyethylene sheet for oviposition. The total number of eggs laid by treated and control snails were calculated and recorded weekly. The egg laying capacity is expressed in the form number of eggs/snail/week (E/S/W).

Effect of Carbamide Perhydrate on *S. mansoni* Miracidia and Cercariae: Five ml of water containing about 100 freshly hatched miracidia or shed cercariae were put in a small graduated Petri-dish and mixed with the same volume of double LC_{90} or LC_{50} values. About an equal number of miracidia or cercariae in 10 ml of dechlorinated water were used as control. This experiment was run under direct sun light for 20 minutes then transferred to shade for another 20 minutes. Microscopical observations of the movement and mortality of the miracidia or cercariae were performed after the exposure .Two replicates were run for both organisms. Miracidia and cercariae were considered dead when their motion ceased completely then the dead organisms were recorded. All miracidia and cercariae were killed by adding a drop of Buin's solution to each Petri-dish and their total numbers were determined. The mortality rate was then computed in each case.

Effect of Carbamide Perhydrate on Snails Infection with *Schistosoma mansoni* Miracidia and Cercarial Production: Two hundred and fifty snails (4-5mm) were divided into five groups each of 50 snails. One group was left untreated and used as control while the other four groups were treated with 35, 60, 85, and 135 ppm of carbamide perhydrate during their exposure to freshly hatched miracidia (10 miracidia /snail) under direct sun light for 4 hours followed by 20 hours in laboratory without exposure to direct light Thereafter, the snails were transferred to clean water and maintained under laboratory conditions (26± 1) for prepotency. Techniques used and conditions afforded for maintaining control and exposed snails were similar to those described by Laing. During the prepatency period dead snails were removed daily and their number was recorded. After 21 days post miracidial exposure, surviving snails were examined individually for cercarial shedding (3 hours twice weekly). The number of cerariae produced / snail was recorded throughout its life span.

Biochemical Determination: The activities of certain glycolytic, glyconeogenic liver function enzymes were determined in the tissues of snails exposed to 35, 60, 85 and 135 ppm of carbamide perhydrate for 4 hours in direct sun light followed by 20 hours in the laboratory without direct light. In addition the total protein content in snail's tissues was evaluated. In parallel, tests of control snails were carried out.

Preparation of Tissue Homogenates and Enzymes Assays:

Phosphofructokinase (PFK) and Fructose-1,6-Diphosphatase (F-1, 6-DP): A homogenate of 0.5 gm snail's tissue in 50 m MTris HCl, 1 ml M-EDTA and 5ml M-$MgSO_4$ at pH 8.2 was prepared, centrifuged (300 rpm) for 10 min at 0C^o and the supernatant was used for enzymes assay[34,26].

Hexokinase (HK): The extraction medium consists of 50 ml M Triethanolamine, 1ml M EDTA, 2ml M$MgCl_2$ and 30 ml M mercptoethanol at pH 7.5.The method of determination is according to Uyeda and Racker[31].

Glucose – Phosphate Isomerase (GPI): A homogenate of 0.5 gm snail's tissue was prepared in 2.5 ml ice-cold bidistilled water to yield 20 % homogenate using a glass homogenizer, centrifuged for 15 min at 300 rpm at 4C^o and the supernatant was used for enzyme assay[17].

Pyruvate Kinase(PK) and Phosphoenol Pyruvate Carboxykinase (PEPCK): One gram of snail's tissue was homogenized in 5ml Tris- HCl buffer pH 7.6, and then the supernatant after centrifugation (300 rpm, min) was assayed for enzyme activities[8,30].

Lactate Dehydrogenase (LDH), Asparatate Aminotransferase (AST), Alanin Aminotransferase (ALT) and Alkaline Phosphatase (ALP): Snail's tissues were homogenated in bidisttilled water at a ratio 1:10 W/V, centrifuged (300 rpm.) for 10 min and the supernatant was used for enzyme assay[6,25,16].

Total Protein: The total protein content was determined according to the method of Bradford[5], in the snail's tissue homogenate.

Enzyme activities were evaluated using end point assay method except HK and PK where the end point is the differences between the two points.

Histology: Histological preparations were done for snails exposed to 35,60 and 85 ppm of Carbamide perhydrate under direct sunlight for 4 hours followed by 20 hours in the laboratory without exposure to direct light. The hermaphrodite gland of treated snails was carefully incised using fine scissors and dropped into a fixative Bouin's solution ,then sections (5-8µm) were stained with Delafield's haematoxyline and eosin according to Mohamed and Saad[23]. Sections of control snails hermaphrodite gland were prepared simultaneously.

Statistical analysis: Computation of LC50 and LC90 values and slope function were determined utilizing the statistical program SPSS package version 7 and Litchfield & Willcoxon[21] method.

The infection and survival rate were analyzed by Chi-square values of contingency table[28].

The incubation period, duration of shedding and cercarial production of infected snails in the experimental groups and control ones were compared using Student "t" test[27].

Statistics in biological parameters is carried out using ANOVA tested and the mean differences between groups is analysed by post. Hoc (SPSS computer program).

The mean difference is significant at 0.5 levels.

RESULTS AND DISCUSSION

Results: The present data (Table 1) showed that carbamide perhydrate have a considerable molluscicidal potency against *B. alexandrina* snails. Thus, LC_{50} and LC_{90} values were 85 and 135 ppm after snails exposure to direct sun light (450 W/m^2) for 4 hours followed by

20 hours in the laboratory without exposure to light, and 24 hours of recovery. It is clear from Table (2) that there are no mortality among snails exposed to 85 ppm (LC_{50}) of carbamide perhydrate for 12 hours in shade followed by another 12 hours in dark, without exposure to sun light. The same observation was recorded for snails maintained at this concentration (85 ppm) for 24 hours in the dark, or for those exposed to artificial light (desk lamp, 100 W/15cm hight). Moreover, storage of this concentration for 3 days diminished its toxic effect against *B. alexandrina* snails. Thus, snails were not affected after exposure to this concentration in sun light for 4 hours followed by 20 hours in the laboratory (shade).On the other hand, incubation of snails for 12 hours of dark in the concentration (85ppm) followed by 4 hours in sun light resulted in 50% death of snails. The same result (50% death) was, also, recorded for snails exposed to 85 ppm freshly prepared for 4 hours in sun light followed by 20 hours in the laboratory.

It was noticed from the present results (Table 3) that carbamide perhydrate showed a long lasting toxic effect against *B. alexandrina* snails. Thus, the survivorship values of snails exposed once to 60 ppm or 85 ppm for 4 hours at sun light and maintained under laboratory conditions in clean water for 4 weeks were considerably low in comparison with that of control group, being 0.32 (=32%), 0.04 (=4%) and 0.84 (=84%) respectively. This harmful effect against the treated snails extended to deteriorate their egg-laying capacity (Mx). So, the snails survived post exposure to 10 ppm laid few eggs at the 1^{st} and 2^{nd} weeks of the experiment, being 1.7 and 0.17 eggs /snail respectively, compared to 46 and 60 eggs/control snail. Thereafter, the survived snails did not lay eggs throughout the next 2 weeks. Moreover, snails survived post exposure to 60 ppm and 85 ppm ceased egg deposition throughout the experimental period (4 weeks). This has a highly significant negative effect on the net reproductive rate ($Ro=\sum LxMx$) of the treated snails compared to that of control group, being 1.87 for snails exposed to 10 ppm after 2 weeks and 112.2 for control group.

Regarding the biocidal activity of carbamide perhydrate on *S.mansoni* miracidia and cercariae, it was proved that all used concentrations of carbamide perhydrate have neither miracidicidal nor cercaricidal activities even after their exposure to double LC_{50} or LC_{90} for 20 minutes in sun light.

The present data (Table 4) indicated that the infection rate of *B. alexandrina* snails with *S.mansoni* post their exposure to 35 ppm carbamide perhydrate for 4 hours in direct sun light followed by 20 hours in the laboratory during their exposure to miracidia was less than control group, being 41.1% and 68.0%, respectively. Moreover, under the same conditions,miracidia failed to infect snails exposed to 60 and 85 ppm of carbamide perhydrate. It was , also, noticed that the prepatent period for snail groups exposed to 35 ppm was more than that of control ones, being 49.6 and 32.3 days, respectively. This indicates a possible deterioration in the physiological parameters of treated snail which resulted in lowering the developmental rate of the parasite within these snails, shortening the duration of cercarial shedding and reducing the cercarial production/ infected snail. Thus, the results showed that the mean number of cercariae / snail in group exposed to 35 ppm was 280 cercariae compared to 1237.2 cercariae / control infected snail.

The present results (Table 5) indicated that snails exposed to sublethal concentrations of carbamide perhydrate LC_{10} =35ppm, LC_{25} =60 ppm, LC_{50}= 85 ppm and Lc_{9o} =135 ppm, significantly increased the total protein content in the snail's tissues. The highest value of total protein was recorded for snails exposed to LC $_{90}$=135 ppm, being 43.25 mg/g compared to 20.75 mg/g tissue in control group, with an increasing rate of 108.4%. A considerable increase was, also, observed for snails exposed to LC_{10}=35ppm and LC_{25}= 60ppm, being 86.7% and 43.4%, respectively.

Concerning the activities of aminotransferases (AST&ALT), the results indicate a significant reduction for this parameter in the tissues of snails exposed to LC_{25}, LC_{50}& LC_{90}.For the activity of AST at these concentrations, the recorded values were 2.32, 2.08 & 1.9 µ mol min/ mg protein compared to 2.45 µ mol/ min /mg protein for control group, with reduction rates 0f 5.3, 15.10 &22.5%, respectively. The same observation was seen for ALT activity, the reduction rates were 10.1 &22.9%, for LC_{25}&LC_{90} respectively while insignificant change is observed at LC_{50}. However, the activities of the enzymes AST and ALT in the tissues of snails exposed to LC_{10}=35 ppm exhibited significantly higher values than that of control group, being 3.1 and 5.65 µ mol/min /mg protein, compared to 2.45 and 5.10 µ mol/min / protein for control snails, respectively ($P< 0.05$).

The results also showed that the activity of ALP in snail's tissues was significantly suppressed by their exposure to LC_{10}, LC_{25} and LC_{90} of carbamide perhydrate in comparison with control group. The highest reduction rate was recorded in the case of snails exposed to LC90, being 49.7% ($P< 0.05$). On the other hand, the activity of this enzyme was significantly elevated by 16.2% ($P< 0.05$) post snails exposure to LC_{50}.

It was observed from table (6&7) and Fig. (1) that the activities of glycolytic enzymes (LDH, PK, GPI & HK) and the gluconeogenic enzymes (F-1, 6-DP & PEPCK) significantly increased in the tissues of *B. alexandrina* snails exposed to carbamide perhydrate

Table 1: Molluscicidal activity of carbamide perhydrate against *Biomphalaria alexandrina* snails (4 hours exposure to 450 W/m^2 sunlight).

LC_{10}	LC_{25}ppm	LC_{50} ppm	LC_{90} ppm	Slope
35	60	85	135	1.7

Table 2: Effect of some environmental factors on molluscicidal activity of carbamide perhydrate against *B.alexandrina* snails.

Factors	% mortality of snails exposed to 85 ppm
Day light (12hrs shad/12 hrs dark)	0.00
Dark (24 hours)	0.0
Artificial light(100w/15 cm light)	0.0
Incubation period (12hrs dark,4 hrs sun light	50
Storage (3 days,4 hrs sun light)	0.0
Fresh preparations(4 hrs sun light)	50

Table 3: Survivorship (Lx) and fecundity (Mx) of *Biomphalaria alexandrina* snails exposed to sublethal concentrations of Carbamide perhydrate (4 hours exposure to 450 W/m^2 sunlight)

Observation period (week)	Carbamide perhydrate concentrations											
	Control			35ppm			60ppm			85ppm		
	Lx	Mx	LxMx	Lx	Mx	LxMx	Lx	Mx	LxMx	Lx	Mx	LxMx
1	1.00	4.6	4.6	0.76	1.5	1.14	0.40	0.0	0.0	0.16	0.0	0.0
2	0.96	6.9	6.6	0.0	0.0	0.0	0.36	0.0	0.0	0.16	0.0	0.0
3	0.88	8.9	7.6	0.0	0.0	0.0	0.36	0.0	0.0	0.10	0.0	0.0
4	0.84	10.2	8.5	0.0	0.0	0.0	0.32	0.0	0.0	0.04	0.0	0.0
Ro after 2 weeks			27.3			1.14			0.0			0.0

Lx= Survivorship, Mx= Mean number of eggs / snail /week
R_0 = net reproductive rate = Sum of LxMx

Table 4: Susceptibility of control and treated Biomphalaria alexandrina snails to infection with Schistosoma mansoni miracidia.

Parameter		Control	35ppm	60ppm	85ppm
% Survival rate of snails at first shedding		83	56.67	13.33	11.66
Infection rate %		68	41.1	0	0
Range	27-34	47-56			
Prepatent period(days)	Mean	32.3	49.6	0	0
±S.D	3.06	4.39			
Range	7-66	1-26			
Duration of cercarial shedding(days)	Mean	18.7	8.1	0	0
±S.D	17.5	9.2			
Range	3124-372	572-28			
Mean total number of cecariae/shed snail	Mean	1237.2	280	0	0
±S.D	874.5	189.3			

Table 5: Effect of carbamide perhydrate on the total protein content, ctivities of aminotransferases (AST&ALT) and alkaline phosphatase enzymes in tissues of Biomphalaria alexandrina snails

Treatment	Control (1)	35ppm (2)	60ppm (3)	85ppm (4)	135 ppm (5)
AST(M±S.D.)	2.45±0.04	3.1±0.086	2.32±0.063	2.08±0.104	1.90±0.016
LSD	(2,3,4,5)	(1,3,4,5)	(1,2,4, 5)	(1,2, 3,5)	(1,2,3,4)
ANOVA	(0.011)	(0.000)	(0.018)	(0.002)	(0.002)
ALT(M±S.D.)	5.100±0.081	5.65±0.17	4.587±0.085	4.95±0.129	3.93±0.096

Table 5: Continue

LSD	(2,3, 5)	(1,3,4,5)	(1,2,4, 5)	(1,2, 4,5)	(1,2,3,4)
ANOVA	(0.00)	(0.000)	(0.000)	(0.000)	(0.001)
ALP(M±S.D.)	1.73±0.11	1.53±0.049	1.24±0.043	2.01±0.06	0.87±0.065
LSD	(2,3,4,5)	(1,2,3,5)	(1,2, 4,5)	(1,2, 4,5)	(1,2,3,4,)
ANOVA (0.000)	(0.000)	(0.000)	(0.000)	(0.000)	
Total Protein(M±S.D	20.75±2.21	38.7±2.98	29.75±1.7	21.25±0.96	43.25±3.95
LSD	(2,3, 5)	(1,3,4,5)	(1,2,4 5)	(1,2,3,5)	(1,2,3,4)
ANOVA	(0.000)	(0.0005)	(0.000)	(0.000)	(0.000)

•All values are expressed as µ mol min/ mg protein except protein content that is expressed in mg protein /ml.
•Statistics is carried out using ANOVA tested and the least significance difference(LSD)between groups is analyzed by post hoc(SPSS computer programmed).
•The mean difference is significant at the 0.5 level.
Mean ±S.D of 4 snails tissue homogenate in each group.

Table 6: Effect of carbamide perhydrate on the activities of some glycolytic enzymes in the tissue of Biomophlaria alexandrina snails.

Enzyme	Control (1)	35ppm (2)	60ppm (3)	85ppm (4)	135ppm (5)
LDH(M±SD)	65.26 ± 0.69	72.10±2.19	74.35±0.85	70. 39±0.21	70. 00±0.816
LSD	(2,3,4,5)	(1,3,5)	(1,2,4,5)	(1,3)	(1,2,3)
ANOVA	(0.000)	(0.015)	(0.01)	(0.000)	(0.02)
PK(M±SD)	3.39±0.13	4.00±0.0082	3.80±0.054	3.72±0.093	3.36±0.137
LSD	(2,3,4)	(1,3,4,5)	(1,2, 5)	(1,2,5)	(2,3,4)
ANOVA	(0.000)	(0.002)	(0.000)	(0.002)	(0.000)
PFK(M±SD)	7.55±0.42	9.17±0.275	6.35±0.36	8.08±0.17	5.78±0.35
LSD	(2,3,4,5)	(1,3,4,5)	(1,2,4,5)	(1,2,3,5)	(1,2,3,4)
ANOVA	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
GPI(M±SD)	119.6±6.65	225.7±17.1	173.75±13.7	174.5±9.7	137.5±5.0
LSD	(2,3,4,5)	(1,3,4,5)	(1,2,5)	(1,2,5)	(1,2,3,4)
ANOVA	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
HK(M±SD)	0.0088±0.001	0.0185±0.0024	0.017±0.004	0.0163±0.005	0.007±0.001
LSD	(2,3,4)	(1,5)	(1,5)	(1,5)	(2,3,4)
ANOVA	(0.002)	(0.002)	(0.002)	(0.000)	(0.002)

•Statistics is carried out using ANOVA tested and the least significance difference(LSD)between groups is analyzed by post hoc(SPSS computer programmed).
•The mean difference is significant at the 0.5 level. Mean ± S.D. of 4 snail, s tissue homogenates in each group.
All values are expressed as µ mol/ min/mg protein.

Table 7: Effect of carbamide perhydrate on the activities of some gluconeogenic enzymes in tissues of Biomphalaria Alexandrina snails.

Enzyme	Control (1)	35ppm (2)	60 ppm (3)	85ppm (4)	135ppm (5)
Fructose 1,6-diphosphatase					
F 1, 6-DP(M±SD)	9.17±0.40	16.53±0.57	12.13±0 .19	14.19±1.139	10.73±0.34
LSD	(2,3,4,5)	(1,3,4,5)	(1,2,4,5)	(1,2,3.5)	(1,2,3,4)
ANOVA	(0.000)	(0.001)	(0.002)	(0.001)	(0.000)
Phosphoenol pyruvate carboxykinase PEPCK (M±SD)	1.24±0.051	2.378±0.276	1.773±0.10	1.763±0.048	1.39±0.068
LSD	(2,3,4)	(1,3,4,5)	(1,2, 5)	(1,2,5)	(2,3,4)
ANOVA	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
PK /PEPCK(M±SD)	2.74±0.057	2.47±0.68	2.087±0.097	2.083±0.10	2.46±0.1
LSD	(-)	(-)	(-)	(-)	(-)

•Statistics is carried out using ANOVA tested and the least significance difference (LSD) between groups is analyzed by post hoc (SPSS computer program).
•The mean difference is significant at the 0.5 level. Mean ± S.D. of 4 snails tissue homogenates in each group.
All values are expressed as µ mol/ min/ mg protein.

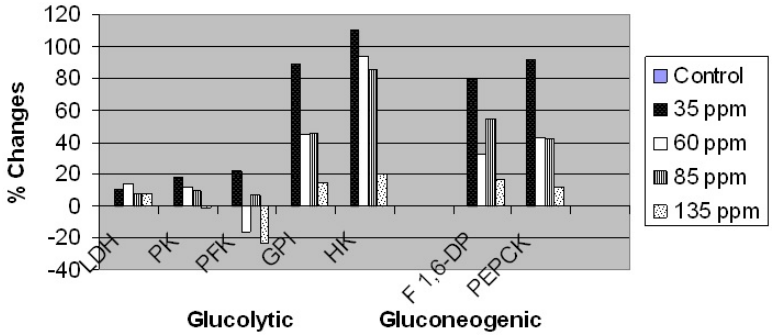

Fig 1: Changes (%) in the activities of some energetic enzymes in the tissues of *B.alexandrina* snails exposed to carbamide perhydrate.

(PK, HK and PEPCK show insignificant change at 135 ppm only of carbamide perhydrate). On the other hand, PFK shows significant increase at 35 and 85 ppm concentration level of carbamide perhydrate and significant reduction in other two concentrations in comparison with control group. The increase in activities of the tested enzymes was reduced by increasing the experimental concentration. Thus, the percentages of increase in activities of the glycolytic enzymes LDH, PK, PFK, GPI and HK of snails exposed to LC_{10} =35ppm of carbamide perhydrate were 10.49, 18.0%, 21.55, 88.7% and 110.2% respectively, which were higher than those of snails treated with LC_{50}= 85ppm (7.88,9.7%,7.02, 45.9% and 85.2%, respectively).The same trend was recorded for the activities of gluconeogenic enzymes F- 1,6-DP and PEPCK. Thus, the percentages of increase in their activities for snails at LC10=35 ppm was 80.3% and 91.8%, respectively, compared to 54.7% and 42.2% for snails at LC_{50}= 85ppm Moreover, increasing the concentration to LC_{90}=135ppm suppressed the activities of the glycolytic enzymes PK, PFK and HK in comparison with control snails. The reduction percentage in this case was 0.9, 30.07 and 20.5%, respectively.Concerning, PK/PEPCK ratio insignificant change is observed at all concentrations of carbamide perhydrate as compared to the normal control group.

Discussion: The lethal effect of carbamide perhydrate as a photosensitizer against adult *B.alexandrina* snails in the present work was supported by El-sayed and El-Sherbini[10] observations on these snails post their exposure to the hematoporphyrin as a photosensitizer .For the effect of carbamide perhydrate on survivorship (Lx) and fecundity (Mx) of *B.alexandrina* snails, the present data indicated that carbamide perhydrate showed a long lasting toxic effect against *B.alexandrina* snail and the disturbance in the all biochemical parameter measured may be attributed to the biological process that involving light may have both beneficial (photosynthesis) and destructive (photosensitization) consequences,singlest molecular oxygen ($1O_2$) and other reactive oxygen species such as hydrogen peroxide and hydroxyl radical, arise during the interaction of light with photosensitizing chemicals in the presence of molecular oxygen. $1O_2$ oxidizes macromolecules such as lipids ,nucleic acids and protein depending on its intracellular site of formation and promoted detrimental process such as lipid peroxidation, membrane damage that allows the discharge of enzymes, disturbance in their level and consequently cell death[29]. Moreover the photochemical reactive oxygen species (ROS) generating system induce the expression of several eukaryotic genes, which include stress protein (causing increase of total protein content), early response genes, matrix metalloproteinase, immunomodulatory cytokinase and adhesion molecules.these gene expression phenomena may belong to cellular defensive mechanisms or may promote further injury. In addition ROS may affect singling component in the membrane, cytosol, or nucleus leading to changes in cyclooxiginase, phospholipids, protein kinase , protein phosphtase lysosomal and some mitochondrial enzymes[29,36].

Kristian and Johan[18] found that treatment of cells with 30 and 50% of the photosensitizing dyes (sulfonated tetraphenyl porphines) causing a maximum activation and release by 40 and 10 % of lysosomal enzymes and cytosol cathepsin (L and B) based on this finding, the presence limited increase in enzyme activities may be partly related to activation in mitochondrial glycolytic and glycogneogenic enzymes after treatment with photosensitizing dyes carbamid perhydrate.

Concerning the significant decrease in the activities of the transaminases AST and ALT enzymes, significant increase was observed at 35ppm combined with significant reducation in other concentrations levels. The reduction in the tranaminases levels showed an intimate relationship to cell necrosis and /or increased cell membrane permeability[12]. The decrease in transaminases level providing additional support for the photochemical inactivation of some mitochondrial enzymes of the hepatopancreace cells of treated snails[18].

In the present study, ALP enzyme shows significant reduction in all concentration levels of carbamide perhydrate except at 85 ppm which recorded significant enhancement in enzyme activity. Irene *et al.*[15] suggests that photo-sensitizing dyes at certain concentration binds selectve to plasma membrane sites where it causes damage with high efficiency on all five plasma membrane function leading to diffusion of enzymes to the circulation.

It was stated by Abd El-Meguid[1], that photosensitizers damage the biological target by photosensitized oxidation which deactivate certain enzymes through destruction of specific amino acids (e.g.methionine, histidine and tryptophan), nucleic acid (primarily of guanine) and cell membrane by oxidation of unsaturated fatty acids and cholesterol. Also, El-Emam and Ebied[11] recorded that exposing

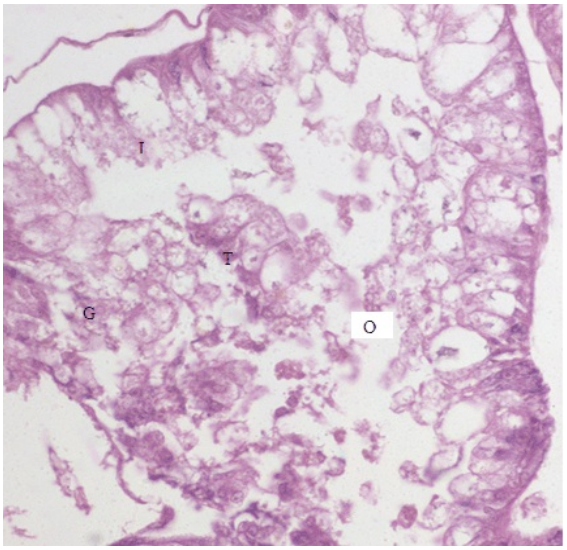

Plate 1: Section in hermaphrodite gland of unexposed Biomphalaria alexandrina snail (control), X=400
G = Spermatogonia, I = Oogonia, O =Oocyte, T =Spermatid

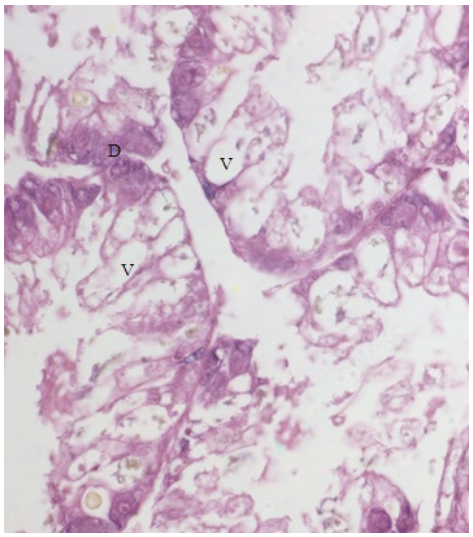

Plate 2: Section in hermaphrodite gland of Biomphalaria alexandrina snail exposed to 35 ppm of carbamide berhydrate X=400
V= vacuoles, D= degeneration

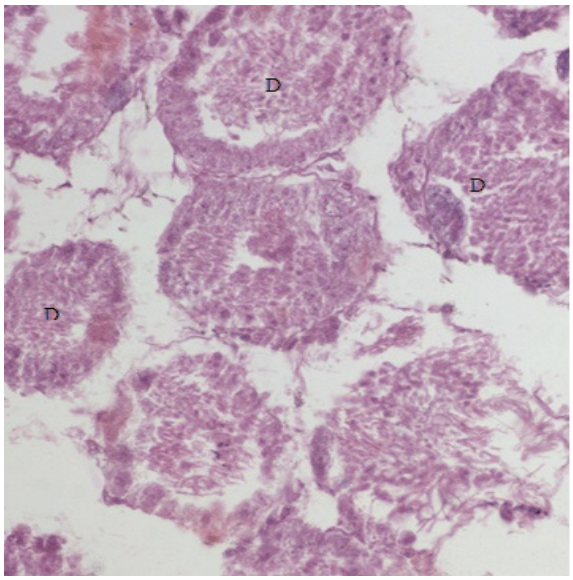

Plate 3: Section in hermaphrodite gland of Biomphalaria alexandrina snail exposed to 85 ppm of carbamide berhydrate X=400
V= vacule, D= degeneration

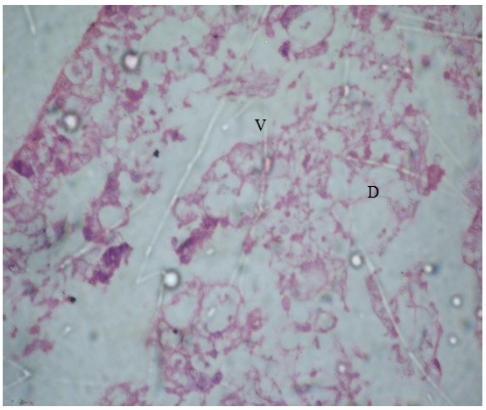

Plate 4: Section in hermaphrodite gland of Biomphalaria alexandrina snail exposed to 60ppm of carbamide berhydrate, X=400. V= vacule, D= degeneration.

B.alexandrina snails to the molluscicide Mollotox increased the activity of their acid phosphatase that has an important role in the defiance system, thus reducing the development of sporocysts and cercarial production from snails infected with *S.mansoni*.

The reproductive rate (Ro) which is the summation of LxMx of B.alexandrina snails were highly suppressed by carbamide perhydrate at the experimental conditions. These findings were supported by the present data on the negative effect of this agent on the snail's physiological activities (deteriorations in the glucolytic, gluconeogenic, transiminases and alkaline phposphatase enzymes) and reproductive system, hence reduce or stop their oviposition. This was, also supported by the histological results (plates 1, 2,&4) on the hermaphrodite gland of snails exposed to 35, 60 and 85ppm of carbamide perhydrate. These plates revealed rupturing of the gland cells and evacuation of most of its tubules from gametogenic stages. This should minimize the offspring of survived snails or stop it completely, hence interrupt or reduce schistosomiasis transmission. These observations are in accordance with the previous ones on Hematoporphyrin and Argon-ion laser[10] against *B.alexandrina* snail's oviposition. Also, Ibrahim *et.al.*[14] demonstrated a great histological damage of *B.alexandrina* ovotestis post exposure to the plants *P.repens*, *S.nigrum* and *D.kerchoveana* that stopped snail's oviposition after 4 weeks of exposure.

Concerning the effect of carbamide perhydrate, sublethal concentrations on infection of *B.alexandrina* snails with *S.mansoni* miracidia and cercarial production. The present data indicated that infection rates of *B.alexandrina* snails with *S.mansoni* post their exposure to 35 ppm carbamide perhydrate for 4 hours in direct sun light followed by 20 hours in the shade at the laboratory were significantly less than that of control group. This reduction could be due to the lethal effect of the accumulated photosensitizer in the snail's head-foot region where mother sporocysts are present[22] or could be attributed to the deteriorations that this photosensitizer has exerted on the energetic and transaminase senzymes in tissues of treated snails to be unsuitable for the developmental processes of sporocysts within these snails.[22] It was suggested that an important control point in the respiration of intermediate host existed at the level of phosphoenol pyruvate (PEP). The nature of end product formed is dependent on the competition for PEPby the two enzymes PK and PEPCK. In the present study, significant increase in PK activity in snails exposed to carbamide perhydrate ascertained the stimulation of glycolic flux previously reported by Horemans *et. al*.,[13] and Ahmed & Gad[2]. This stimulation of PK was reflected on the substrate availability of phosphoenol pyruvate (PEP) for PEPCK that resulted in its remarkable stimulation at different concentration of carbamide perhydrate that in turn led to insignificant change in PK/PEPCK ratio which is described as a potential regulatory site in the energetic metabolism and it may give an indication about a major pathway in vivo. Hence the metabolic products in this case will negatively affect the parasitic developmental stages within the tissues of treated snails. In addition, the harmful effect of carbamide perhydrate may be directly extended to affect the sporocysts.These suggestions were supported by the significantly low number of cercariae produced from snails treated with carbamide perhydrate in comparison with control ones. These findings were previously recorded by El-sayed & El-Sherbini[10] on infection of *B. alexandrina* with *S.mansoni* during their exposure to the photo synsitizer hamatoporphrin.

Conclusion: It is concluded from this work that exposing *B.alexandrina* snails to sublethal concentrations of the photosensitizer carbamide perhydrate (4 hours in sun light) reduced or stopped the snail's oviposition, hence minimize the snail population available for the parasite transmission. In addition, they caused a highly significant reduction in the out put of cercariae from infected snails. That may add to the suppression factors in schistosomiasis transmission. In addition snails treated with different

concentration of carbamide perhydrate showed significant fluctuated levels in glycolytic, glyconeogenic, AST, ALT, ALP and total protein content as compared to the normal control group. 135ppm shows significant reduction in, F-1, 6-DP, PEPCK, ALP, AST, ALT, PK, PFK and GPI as compared to other concentrations. While total protein content shows significant enhanced value. With regard to 35 ppm HK, F-1, 6-DP PEPCK carboxikinase and GPI show significant increased values as compared within different concentration groups. Concerning 60 and 85 ppm LDH and ALP exhibit significant enhanced values as compared to other concentrations.

REFERENCES

1. Abd El-Meguid, A., 1996. Effect of He-Ne laser beam on the Ommatidia in Schistsocerca gregaria. Arab. J. Nucl. Sci-& Appl., 229: 53-67.
2. Ahmed, S.A. and M.Z. Gad, 1995. Effect of schistosmal infection and its treatment on some key enzymes of glucose metabolism in mice livers. Arzeim. Forsch. 45: 1324.
3. Agnieszka, C., S. Jolanta, M. Teresa, M. Anna, M. Anna, B. Julita, P. Dorota and B. Teresa, 2003. Uptake of photofrin II, a photosensitizer used in photodynamic therapy, bytumour cells in vitro. Acta Biochimica Polonica, 50: 509-513.
4. Attin, T., C. Hannig, A. Wiegand and R. Attin, 2004. Effect of bleaching on restorative materials and restorations--a systematic review Dent Mater, 20: 852-61
5. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248.
6. Babson, A.L. and S.R. Babson, 1973. Kinetic colorimetric measurement of serum lactate dehydrogenase activity. Clin. Chem., 19: 766.
7. Brunton, P.A., R. Ellwood and R.A. Davies, 2004. A six-month study of two self-applied tooth whitening products containing carbamide peroxide. Oper. Dent., 29: 623-626.
8. Bucher, T. and G. Pfleiderer, 1975. Pyruvate kinase. In: Methods in Enzymology (Colowick, S.P. and Kaplan, N.O. Ed.) 1: 435-440. Academic Press, N.Y.
9. El-Khoby, T., N. Galal and A. Fenwick, 1998. The USAID/ Government of Egypt Schistosomiasis Research Project (SRP).Parasitol.Today., 14: 92.
10. El-Sayed, K. and El-Sherbini, 2006. Impact of hematoporphyrin and different laser sources on *Biomphalaria alexandrina* snails and their infection with *Schistosoma mansoni* J. Biol. Chem. Environ. Sci., 1(2): 320-340.
11. El-Emam, M.A. and F.A. Ebied, 1989. Effect of *Schistosoma mansoni* infection,starvation and molluscicides on acid phosphatase, transaminases and total protein in tissues and hemolymph of *Biomphalaria alexandrina*. J. Egypt. Soc. Parasitl., 19(1): 139-147.
12. El-Shazly, A.M., M. Soliman, M.R. El-Kella, H. Resk, HE El Nemr, A.E. Handouusa and M.M. Helmy, 2001. Study of soluble adhesion molecules in some intestinal and tissue heliminthes. J. Egypt. Soc. Parasitol., 31(2): 501-9.
13. Horemans, A.M.C., A.G.M. Tielens and S.G. Van Den Bergh, 1992. The reversible effect of glucose on the energy metabolism of Schistosoma mansoni cercariae and schistosomual. Mol. Biachem. Parasitol., 51: 73.
14. Ibrahim, A.M., M.A. El-Emam, S.M. El-Dafrawy and H.S. Mossalem, 2004. Impact of certain plant species on *Schistosoma mansoni-Biomphalaria alexandrina* system. Proc. 3rd Int. Conf. Sci., 3: 390-413.
15. Irene, K.E., B. Joseph and L.C. Wei, 1994. Enfluence of dye and protein location on photosensitization of the plasma membrane. Biochmica. Biophysica. Acta, 1196.
16. Kind, P.R. and E.J. King, 1954. Estimation of plasma phosphatase by determination of hydrolysed phenol (with amino-antipyrine) J. Clin. Pathol.
17. King, J., 1965. Glucose phosphate isomerase in: Practical Clinical Enzymology: pp. 1113-1117. D. Van Nostr and Co. Ltd, London.
18. Kristian, B. and M. Johan, 1994. Lysosomes as photochemical targets. Int. J. Cancer, 59: 814-822.
19. Kihn, P.W., 2000. A clinical evaluation of carbamide peroxide and hydrogen peroxide whitening agents during daytime use. J. Am. Dent. Assoc., 131: 1478-1484.
20. Lardans, V. and C. Dissous, 1998. Snail control strategies for reduction of Schistosomiasis transmission.PubMed Available online.
21. Litchfield, J.T. and F. Wilcoxon, 1949. A simplified method of evaluating Dose-effect experiments. J. Pharacol. Exp. Therap., 96: 99-113.
22. Malek, E.A., 1980. Schistosomiasis, In:Snail-transmitted parasitic diseases. ED. CRC Press, Inc., Boca Ratn, Florida, USA: 179-307.
23. Mohamed, S.H. and A.A. Saad, 1990. Histological studies on the hermaphrodite gland of *Lymnaea caillaudi* and *Biomphalaria alexandrina* upon infection with certain larval trematodes. Egypt. J. Histol., 13: 47-53.
24. Nolan, M.O., W.B. Howad, and R.M. Elizabeth, 1953. Results of laboratory screening tests of chemical compounds for molluscicidal activity. Amer. J. Trop. Med. Hyg., 22: 716-752.

25. Reitman, S. and S. Frankel, 1957. Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Path., 28: 56.
26. Sand, O., I.M. Petersen and B.K. Emmersen, 1980. Changes in some carbohydrate metabolizing enzymes and glycogen in liver. Glucose and lipid in serum during vitellogenesis and after induction by estradiol 17 B in the flounder Platichtys flesus L. Comp. Biochem. Physiol, 65: 327.
27. Sokal, R.R. and F.J. Rohlf, 1981. Biometry2Ed W.H. Freeman San Francisco.
28. Southwood, T.R.E., 1978. Ecological methods, with particular reference to study of insect populations. The English Language Book Society and Chopan-Hall.
29. Stefan, R.W. and T.M. Rex, 1998. Singlet molecular oxygen (O_2); A possible effectors of eukaryotic gene expression. Free. Radical. Biol. Med., 24: 1520-1534.
30. Suarez, R.K., M.D. Mallet, C. Doxboeck and P.W. Hochachka, 1986. Enzymes of energy metabolism and gluconeogenesis in specific blue malin, Makaira nigricans. Can. J. Zool., 64: 694.
31. Uyeda, K. and E. Racker, 1965. Regulatory mechanisms in carbohydrate metabolism. VII. Hexokinase and phosphofructokinase. J. Biol. Chem., 240: 4682.
32. Wagner, S. and A. Skripchenko, 2003. Investigation of photosensitizing dyes for pathogen reduction in red cell suspension. Biotech. Hitochem., 78: 171-177.
33. Wainuright, M., 2003. The use of dyes in modern biomedicine. Biotechnic. Histochem., 78: 147-155.
34. Zammit, V.A., I. Beis and E.A. Newsholme, 1978. Maximum activities and effects of fructose bisphosphate on pyruvate kinase from muscles of vertebrates and invertebrates in relation to the control of glycolysis. Biochm. J., 175: 989.