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RESEARCH ARTICLE

ASSESSMENT OF CERTAIN FOOD PRESERVATIVES ON BIOLOGICAL AND BIOCHEMICAL PARAMETERS OF BIOMPHALARIA ALEXANDRINA SNAILS, AS A BIOLOGICAL MODEL

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ABSTRACT

The toxicological effect of xenobiotic substances, Sodium Metabisulphite (SMS) and Potassium metabisulphite (KMS) against medically important Biomphalaria alexandrina snails were unknown. Thus, the present study was performed to test the effect of these food preservatives on some biological and biochemical activities of B. alexandrina as a novel and biological model. Exposure of snails to LC10 and LC25 of SMS during infection with Schistosoma mansoni lead to a statistically significant increase in cercarial production, as well, snails' egg production was increased by exposure of snails to the low concentration(LC10) of the two tested compounds (LC10) compared to control unexposed snails. The egg production of snails exposed to LC25 of KMS completely inhibited. The results revealed that in both concentrations of SMS an increase in the levels of the transaminase AST in the hemolymph, while ALT did not show significant variations from control snails. Alkaline phosphatase was significantly increased in the snail tissues, while ACP was significantly increased in the hemolymph of treated snails. The level of total protein was decreased in tissues of snails after treatment with tested chemicals, while albumine not affected in hemolymph. In conclusion Snails considered as a possible test model to assess the medically suitable tests and the results strongly indicate that SMS and KMS were toxic for B. alexabdrina snails, and this snail could be used for biomonitoring the environmental impact.

INTRODUCTION

In the last years, the population of the world is increasing; this increase has led to the search for new food sources and methods for increasing their productivity and protection from spoiling. Food preservatives are substances internationally added to food to extend a food's freshness or shelf life and keep it from spoiling or oxidizing. The safety of repeated exposure to permitted synthetic food additive (colorants or preservatives) has been questioned. It has been reported that certain food additives, especially antimicrobial agents are genotoxic in different test systems (Mujherjee *et al*., 1988).

Sodiom metabisulphite or sodium pyrosolphite (SMS) is an inorganic compound of chemical formula $Na_2S_2O_5$. Sodium metabisulphite is used as a disinfectant, antioxidant, and preservative agent in the food, beverage, drug industries. Sodium metabisulphite is widely used in the food and pharmaceutical industries, because of its ability to inhibit proliferation of microorganisms and its antioxidant properties (Carvalho *et al*., 2011) Also Sodium metabisulphite (SMS) is used in marine shrimp aquaculture to prevent the occurrence of black spot. (Galli *et al*., 2012).

Potassium metabisulphite,(KMS) $K_2S_2O_5$, also known as potassium pyrosulfite, is a white crystalline powder with a pungent sulfur odour. The main use is as an antioxidant and chemical sterilant. Potassium metabisulfite is a food preservative, which preserves the natural color of food and protects food against bacteria. KMS is used as an antimicrobial substance in many kinds of foods It might be concluded that KMS had a high genotoxic and cytotoxic risk, (Yavuz *et al.*, 2008**).** It is a disulfite and is chemically very similar to sodium metabisulphite, with which it is sometimes used interchangeably. Potassium metabisulphite is generally preferred out of the two as it does not contribute sodium to the diet.

The proper amount of SMS and KMS that could be safely released into the environment is unknown. To evaluate the impacts of these xenobiotics on aquatic organisms into the ecosystem, toxicity tests have been performed (Lombardi, 2004). The acute toxicity test provides a quick answer estimating the lethal effects of a particular toxicant on aquatic organisms. Xenobiotics in the environment have been shown to induce adverse effects in animals and humans by interfering with endocrine functions. These effects include increased frequencies of sex hormone-dependent cancers (breast, testis, prostate, etc.), genital abnormalities, premature puberty in females, and increased occurrence of endometriosis in humans (Gist, 1998; Swan *et al*., 2000). Freshwater mollusks of the genus *Biomphalaria* are widely distributed in several countries and considered to be good indicators for biomonitoring studies

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(Abd-Allah *et al*., 1999; Nakano *et al*., 2003; Ansaldo *et al*., 2006). *Biomphalaria* spp. snails have been extensively used as experimental biological models contributing significantly to new developments in many areas studied by biomedical scientists. In addition to medical, veterinary perspectives and the impact of *Biomphalaria* spp. on public health, these snails are also, considered as interesting biological models for studding population biology, including genetics and demography, mating systems, and biogeography (Brown 1994).

Aim of the work

This work evaluates sensitivity of some biological and biochemical parameters of *Biomphalaria alexandrina* snails to food preservatives Sodium metabisulphite and potassium metabisulphite aiming to derive recommendations for using such snail species as a standard biological model for the medically suitable biotests.

MATERIALS AND METHODS

Snails

Adult *Biomphalaria alexandrina* snails (5:7 mm) used in the present study and were obtained from the bred stock in Medical Malacology Laboratory, Theodor Bilharz Research Institute (TBRI) Egypt.

The tested compounds

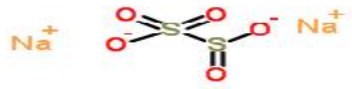

Fig.1 Sodium metabisulphite $Na_2S_2O_5$

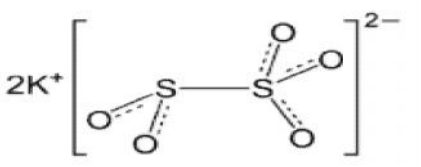

Fig 2 Potassium metabisulphite $K_2S_2O_5$.

The compounds used are: Sodium metabisulphite (SMS) $Na_2S_2O_5$ Fig (1)

And Potassium metabisulphite (KMS) $K_2S_2O_5$. (Sigma-Aldrich) Fig (2)

Toxicity tests

The potential toxic activities of both sodium metabisulphite and potassium metabisulphate were tested against adult *B. alexandrina* according to the standard method recorded by WHO (1965). Different concentrations in ppm were prepared on basis of weight/volume. For each concentration three glass containers each containing one liter dechlorinated water to which the test material was added directly on the water surface and stirred properly then 10 snails were introduced. The glass containers were covered by porous plastic sheets and maintained for 24 hours of exposure at laboratory conditions ($25^oC \pm 1^oC$). After that, the snails were washed thoroughly with dechlorinated water, transferred to jars containing fresh dechlorinated water for another 24 hours for recovery. Three replicates of control snails (10 snails /L) were prepared in dechlorinated water. Dead snails were distinguished and counted.

Further experiments were carried out in which four groups of laboratory bred *B. alexandrina* snails (each of 150 snails, 5-7 mm) were exposed continuously for three weeks to the sublethal concentrations ($LC10\&LC_{25}$) of the tested compounds; another group of 150 snails was maintained at the same conditions but without tested compound as control. The tested concentration was renewed twice weekly. Dead snails were removed daily from all experimental groups while at the end of the three weeks, the surviving ones were divided into two subgroups, one of them was subjected to withdrawal of their hemolymph and their tissues were homogenized and prepared for biochemical tests while the second subgroup was subjected to infection with *S. mansoni* miracidia.

Infection of snails

To study the effect of sublethal concentrations of Sodium meta bisulphite and potassium metabisulphite on *B. alexandrina* infection with *S. mansoni*, 50 survived from each of the groups previously exposed to LC_{10} and LC25 from the tested compounds were individually exposed to 10 miracidia / snail for 3hrs at $25C^o \pm 1$ under ceiling illumination. After that, they were washed and maintained in clean plastic aquaria with dechlorinated water and supplied with lettuce leaves. Another of 50 survivied from the control group snails were exposed to miracidia only at the same conditions. During the prepotency 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 Assay

Haemolymph samples were collected according to the previous method of Abdul-Salam and Michelson, (1983). Tissue homogenates were prepared by dissecting out the snail soft parts from their shells, then 1 g from each group was homogenized in 5 ml distilled water, then the fresh supernatant was used. All biochemical parameters were determined using reagent kits from bio-Merieux Company. Aspartate aminotransferase [AST] and alanine aminotransferase [ALT] were assayed according to reported method Reitman and Frankel, (1957). Alkaline phosphatase [ALP] and acid phosphatase [ACP] activities were tested as descried previously (Kind and King, 1954). Total protein level was estimated according to the Folin-phenol method (Lowry *et al*., 1951). The results of enzyme activities were expressed as means ± SD of the three replicates (IPM SPSS Statistics program, version 20 for Windows).

The egg laying capacity of Biomphalaria alexandrina snails under the effect of different concentrations of the tested compounds

The snails used in this test were divided into five groups. The 1st and 2nd groups were exposed to LC10 and LC25 of SMS, the 3rd and 4th were exposed to LC10 and LC25 of KMS and the fifth one was considered as a control. Snails were fed daily boiled lettuce leaves, each aquarium was provided with polyethylene sheets on which snails can lay eggs. Throughout 4 weeks of continuous exposure, the survival, and egg

production of snails were determined weekly. The tested concentration was renewed twice weekly.

Statistical analysis

The data are presented as mean ± standard `deviation. The means of the different groups were compared globally using the student's t- test **(**Soka**l** and Rohlf, 1981). Molluscicidal activity of the tested compound was calculated by probit analysis **by** SPSS Computer Program version7

RESULTS

The toxic effects of Sodium metabisulphite (SMS) and potassium metabisulphite (KMS) against *Biomphalaria alexandrina* snails showed that LC50 and LC90 were 244ppm and 426 ppm for SMS, and for KMS 506 and 634, respectively (Table I). The Sub-lethal concentrations LC10 &LC25 of the tested compounds were used in the biological and biochemical tests.

Table 1 Toxic effect of Sodium metabisulphite and potassium metabisulphite against *Biomphalaria alexandrina* snails 24hrs exposure.

compound	LC50 ppm	LC90 ppm	Sublethal concentration (ppm) LC10ppm	LC25 ppm
Sodium metabisulphite	244	426	61	147
potassium metabisulphite	506	634	378	439

Effect of Tested Compound on survival rate, infection rate and Cercarial production of B. alexandrina snails with S. mansoni:

The results showed that a high significant reduction in the survival rate at 1st cercarial shedding of snail group exposed to LC10 of SMS compared to control one. The rate for snails exposed to LC10 of SMS was 35%, compared to 58% for control one (P < 0.001). It was also noticed that survival rates of snails exposed to Lc10 of KMS were(42%) slightly higher than that of those exposed to LC10 SMS(35%), but still significant lower than that of control group(p<0.05) (Table 2).

The infection rate of snails by *S. mansoni* was affected only by the two sublethal concentration of KSM. The rates were 25% and 11.8% for snails exposed toLC10& LC25 respectively, compared to35% for control group. In contrast cercarial production/snail was significantly increased by snails' exposure to the tested SMS concentration. Thus, this parameter for snail groups exposed to LC10 SMS 5199 cercariae/snail, compared to 1921 cercariae/control snail (P < 0.001) Table (2)

increase of deposited eggs was observed when snails were exposed to the LC10 of SMS at 2nd and 3rd week reached to 8 and 7 eggs/snail/week compared to the control group which reached to 3 and 4 eggs/snail/week, respectively. Also a higher increase was observed when snails were exposed to the LC10 of KMS especially at the 3rd week, being 23 eggs/snail/week while the value for control snails was 4 eggs/snail/week. It is worthy mentioning the egg lying of snails exposed to LC25 KMS concentration was completely stopped throughout the four successive weeks of exposure (fig 3).

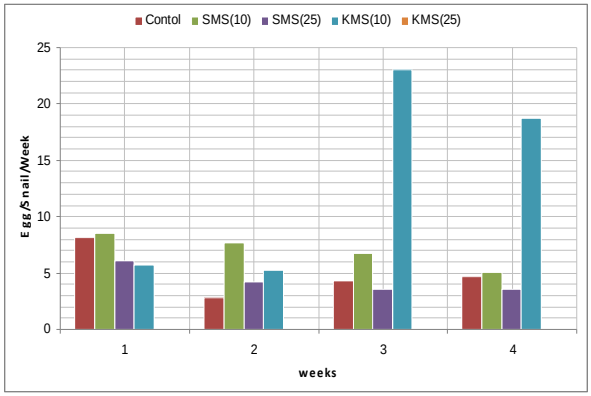

Fig 3 Egg- laying capacity of *B. alexandrina* snails after continuous exposure to the sublethal concentrations of SMS& KMS for 4 weeks.

Effect on biochemical parameters in tissues and haemolymph of B. alexandrina snails exposed to the testes compounds

The results (Table3) indicated that the activity of the enzyme ALP was significantly raised in tissues of snails exposed, to SMS and KMS compared to control snails (P < 0.001) while the ACP activity was not significantly affected in the tissues of snails exposed to LC10 of each SMS and KMS. For ALT and AST levels in soft tissue of treated snails, they were reduced in comparison with those of control group. This phenomenon was recorded for total proteins concentration in the tissues of snails exposed to SMS and KMS (P < 0.001). Similar pattern as seen with albumin concentration for snails exposed to SMS (P < 0.001), but those exposed to KMS exhibited no significant variation compared to control group. In haemolymph (Table 4) there was a significant decrease in the activity of ALP enzyme, while ACP enzyme was raised in snails exposed snails to LC10 SMS and LC25 KMS compared to control snails. For AST, its activity in hemolymph of snails exposed LC10 and LC25 of KMS and LC25 of SMS did not significantly varied from that of control snails(P < 0. 05). This

Table 2 Effect of Sodium metabisulphite and potassium metabisulphite on infection of *Biomphalaria alexandrina* snails exposed to *Schistosoma mansoni* miracidia.

Items	Control	SMS		KMS	
		LC10	Lc25	Lc10	Lc25
% Survival rate of snails at 1st shedding	58	35***	42.5*	42*	42.3*
% Infection rate	35	35.7	35.3	25*	11.8**
Total number of periodic cercariae /shedding snail(mean ±S.D)	1921± 701	5199 ±3120***	5183 ±3338***	1006 ±989	2305 ±1647*

Exposed snail: 25 **** P<0. 001

Effect of different concentrations of the tested compound on the egg laying capacity of B. alexandrina snails

The eggs laid by experimental snails were strongly affected by their exposure to LC10 of two tested compound (Fig. 3). So an

observation was recorded for ALT activity in hemolymph of snails exposed to both concentrations of each of SMS and KMS. However, total protein concentration was significantly reduced (P < 0.001) in hemolymph of snails treated with both concentration of each tested compound. This phenomenon was

observed in albumin concentration for snails treated with SMS ($P < 0.001$), but no significant variations were seen in this parameter for snails treated with KMS ($P < 0.05$)

Table 3 Biochemical parameters of *Biomphalaria alexandrina* tissues post 3 weeks of continuous exposure to sublethal concentrations of sodium and potassium metabisulphate (mean ± S.D.).

Enzyme	Alkaline phosphatase (g/u)	Acid phosphatase (g/u)	AST (g/u)	ALT (g/u)	Total protein(mg/g)	Albumin (mg/g)
Control	7.29±0.81	16.1±0.77	7.8±1.46	6.26±0.64	2.19±0.02	1.05±0.08
SMS(LC10)	25.73101***	18.40±1.42	5.8±0.55*	4.8±0.21**	1.86±0.01**	0.87±0.06***
SMS(LC25)	35.29±1***	22.6±2.6	6.3±1.04	5.2±0.31***	1.73±0.16***	0.87±0.005***
KMS (LC10)	31.88±0.9***	15.15±4.54	5.6±1.13*	5.8±0.54	1.77±0.21***	0.92±.017
K M S (L C 2 5)	31.80±1***	21.45±3.18*	5.3±0.75*	5.4±0.75*	1.93±0.16***	0.9±0.17

Table 4 Biochemical parameters of *Biomphalaria alexandrina* hemolymph post 3 weeks of continuous exposure to sublethal concentrations of sodium and potassium metabisulphate(mean ± S.D.).

Enzyme	Alkaline phosphatase(g/u)	Acid phosphatase(g/u)	AST (g/u)	ALT (g/u)	Total protein (mg/g)	Albumin (mg/g)
Control	13.98±1.16	16.50±2.33	12.66±2.32	10.33±3.28	2.25±0.21	1.23±0.05
SMS(LC10)	9.35±1.19***	12.71±1.16***	15.85±1.47**	9.33±2.82	3.44±0.18*	1.36±0.23
SMS(LC25)	11.0±11.02***	18.35±0.32*	13.66±4.28	9.66±1.07	3.15±0.6*	1.36±0.11
KMS (LC10)	6.28±1.31***	23.08±2.70***	12.01±1.06	7.67±0.84	2.34±0.06	1.26±0.11
K M S (L C 2 5)	10.44±1.24***	17.7±94.49	14.01±0.88	8.69±2.16	3.26±0.06	1.36±0.23

DISCUSSION

In toxicity tests it is common to use organisms that are early in their life stage, due to increased sensitivity of these organisms. The use of mollusks as test organisms is becoming widespread, since they are able to accumulate contaminants and facilitate the detection of minute amounts of trace contaminants (Elder and Collins, 1991). *Biomphalaria glabrata* is an aquatic gastropod mollusk (Pulmonata, Planorbidae) that can be easily maintained in aquaria, predisposing the species for use in ecotoxicological tests (Rivero-Wendt *et al.,* 2014). In the present study the toxicity effect of Sodium Metabisulphite (SMS) and Potassium metabisulphite (KMS) against *Biomphalaria alexandrina* snails was performed. The results obtained showed that the two compounds displayed pronounced toxic effects against *B. alexandrina*. After 24 hours of exposing snails to SMS, the LC50 and LC90 values were 244ppm and 426ppm, KMS was 506ppm and 634ppm respectively. In another marine species *Ucides cordatus*, (Pedale *et al* 2012) reported that the acute toxicity of sodium metabisulphite was the LC (I)50 - 96 hours estimated at 42.58 mgL-1 with lower limit of 35.64 mg.L-1 and the upper 50.89 mg.L-1. Data provided by the Information Sheet for Chemical Safety (MSDS) of Fmaia (2010) reported acute toxicity data for cladoceran (*Daphnia magna*) with LC50 (48 hours) equal to 89 mg.L1. Aragão *et al.* (2008) determined the LC50 (96 hours) for *Mysidopsis juniae* at 38.2 ± 4.7 mg.L-1. Galli *et al*., (2012) determined the LC50 (48 hours) for zoea I of the land crab *Cardisoma guanhumi* is 34 ± 1.1 mg/L, for megalopa 31.1 ± 1.9 mg/L, and for post-larvae 30.6 ± 0.5 mg/L.

The infectivity of *S. mansoni* miracidia to *B. alexandrina* was greatly reduced by LC25 of KMS. This was supported by the interruptions in biochemical parameters, as well, the activities of enzymes of treated snails that render their physiological processes unsuitable for the parasite development. Comparable results were obtained by (Bakry *et. al* 2011). However SMS and KMS (LC25) compound increased the number of cercariae shedding/snails, this is could be attributed the accumulation of Na and K in the tissue of snails and this may increase cercarial production. Mostafa and Dajem (2010) report that Na and K were detected in higher concentrations in cercariae-shedding snails than uninfected one. The reproduction of aquatic organisms is one of the endpoints currently employed to evaluate the effect of toxicants (Cervera *et al.,* 2004; Coeurdassier *et al.,* 2005). At the low concentration of SMS and KMS (LC10), the egg production of snails was increased especially at the end of exposure period (4 weeks), i-e Exposure of *B. alexandrina* to the tested compound did not alter oocyte maturation, but may influenced sperm production and number of egg masses produced. This could be due to release of sodium sulphate acid resulting in water acidification, causing a decrease in pH values (Valença 2003), and causing a stress condition for *Biomphalaria* snail. In addition to that, it has a synergistic effect increasing mortality rate of organisms exposed to this solution (Badaró- Pedroso *et al*. 2002, Aragão *et al.* 2008). Genetic toxicity was studied by the (OECD 2001) and indicates that sodium metabisulphite is equivocal in *in vitro* testing, but is not genotoxic in the *in vivo* testing.

The results of the present study also indicated that there was a significant difference in the levels of the transaminases ALT and AST in the hemolymph at the end of the third week of exposure of snails to the two tested compounds as compared to the control levels. In contrast, their tissue levels were decreased. The increased levels of transaminases in the hemolymph may be attributed to their release from the damaged tissue into the hemolymph (Bakry *et. al* 2011), and this would also explain the decreased levels in the snail tissue. AST and ALT are vital enzymes in the metabolism and generation of energy from amino acids (Tunholi *et al* 2011). Therefore, the elevated transaminases may also indicate the high energy demand of the snail under stressful conditions of intoxication. In another related study Rodriguez *et al*., (1994) reported that when the rats were given a diet supplemented with 0.25 or 2.5% sodium metabisulphite for 5 weeks, Sucrase, maltase, lactase and alkaline phosphatase were assayed in intestinal homogenates and in brush border membrane fractions while the intake of only 2.5% sulfite induced an increase in the specific activities of sucrase, maltase, and alkaline phosphatase compared to control levels ($P < 0.05$). The author revealed that, the origin of such altered enzyme activities is still unknown. Under stress conditions of intoxication of two compounds, the concentration of the total proteins in the snail tissues was

significantly decreased (P<0.001). The decreased protein content may be attributed either to the destruction of the snail tissue and impairment in protein synthesis or to the stimulation of gluconeogenesis to use proteins as a source of energy (Bakry *et al.*, 2011).

CONCLUSION

SMS and KMS have a toxic effect on *B. alexandrina* snails and its infection with miracidia of *Schistosoma mansoni*. These results highlight the importance of providing more information about the toxicity of these compounds, due to that the scientific literature lacks information about the minimum amount of this compound that can be released into the environment without harming the ecosystem. Moreover, this work showed that *B. alexandrina* could be recommended as a biological model for the medically suitable biotests.

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