Evaluation of sodium fluoride toxicity in *Schistosoma* infected snails: assessment of antioxidants, antiapoptotic, hypoprotein and hypocholesterol activities

Khaled M. M. Koriem^{1,2} · Radziyah B. Shamsuri² · Asliza M. Ubaidillah²

Received: 7 July 2015 / Accepted: 14 August 2015 / Published online: 13 January 2016
© Indian Society for Parasitology 2016

Abstract The snails' tissues represents an intermediate or secondary host for *Schistosoma* sporocysts where, germ cells within the secondary sporocyst begin to divide to produce thousands of cercariae capable of infecting humans. The aim of the study was to evaluate the toxicity of sodium fluoride in *Schistosoma* snails' tissue homogenates. A total number of 264 different *Schistosoma* snails were collected from eight drainage water resources and divided into control uninfected and infected snails; where infected snails divided into four group; the first group without any treatment while second, third and fourth groups immersed in 25, 50 and 100 mg sodium fluoride/L during the period of 4 weeks then the snails' hemolymph and tissue homogenates were prepared to evaluate the snail' tissue antioxidants, protein content, lipid profile and apoptosis. The results obtained revealed that superoxide dismutase, glutathione-S-transferase, glutathione peroxidase, catalase, reduced glutathione, glutathione reductase levels were decreased while malondialdehyde, protein carbonyl, total protein, albumin, globulin, cholesterol, low density lipoprotein and triglycerides levels were increased in *Schistosoma* infected snails' tissues. *Schistosoma* also induced apoptosis in snails' tissues homogenates. Sodium fluoride restores all the above parameters to approach the control uninfected snails levels. In conclusion, sodium

fluoride inhibits oxidative stress and apoptosis produced in *Schistosoma* infected snails and consequently it is be useful to be used in *Schistosoma* infection inhibition where sodium fluoride at higher dose was more effective than the lower two doses.

Keywords *Schistosoma* · Snails · Sodium fluoride · Oxidative stress · Protein · Lipids

Introduction

Schistosomiasis remains one of the most prevalent parasitic infections in the world. It has been estimated that more than 207 million people are infected and 779 million people are at risk of infection (Steinmann et al. 2006). It is the major health risk in the rural areas of China and South East Asia and continues to rank high in other developing countries (Bichler et al. 2001). The disease is diagnosed either by the presence of blood in the urine or, in the case of intestinal schistosomiasis, by initially atypical symptoms which can lead to serious complications involving the hepatocellular carcinoma (El-Tonsy et al. 2013) and kidney failure (Koriem et al. 2015). The life cycles of all five human schistosomes are broadly similar: parasite eggs are released into the environment from infected individuals, hatching on contact with fresh water to release the free-swimming miracidium. Miracidia infect freshwater snails by penetrating the snail's foot. After infection, the miracidium transforms into a primary (mother) sporocyst. Germ cells within the primary sporocyst will then begin dividing to produce secondary (daughter) sporocysts, which migrate to the snail's tissues. Once at the snails' tissues, germ cells within the secondary sporocyst begin to divide again, this

✉ Khaled M. M. Koriem
kkoriem@yahoo.com

¹ Medical Physiology Department, Medical Research Division, National Research Centre, 33 El-Buhouth Street, P. O. Box 12622, Dokki, Cairo, Egypt

² Integrative Medicine Cluster, Advanced Medical and Dental Institute (AMDI), Universiti Sains Malaysia (USM), Penang, Malaysia

time producing thousands of new parasites, known as cercariae, which are the larvae capable of infecting mammals. Cercariae emerge daily from the snail host in a circadian rhythm, dependent on ambient temperature and light. Young cercariae are highly mobile, alternating between vigorous upward movement and sinking to maintain their position in the water. Cercarial activity is particularly stimulated by water turbulence, and by shadows found on human skin. The most common way of getting schistosomiasis in developing countries is by wading or swimming in lakes, ponds and other bodies of water that are infested with the snails (usually of the genera *Biomphalaria*, *Bulinus*, or *Oncomelania*) that are the natural reservoirs of the *Schistosoma* pathogen.

Prevention is best accomplished by eliminating the water-dwelling snails that are the natural reservoir of the disease. For many years from the 1950s onwards, vast dams and irrigation schemes were constructed, causing a massive rise in water-borne infections from schistosomiasis. In the last decade the question how to minimise the spread of the *Schistosoma* disease had been risen to be urgent matter. Fluoride is an essential element (Disanayake 1991). It is an inorganic anion of fluorine with the chemical formula-F. Its salts and minerals are important chemical reagents and industrial chemicals, mainly used in the production of hydrogen fluoride for fluorocarbons. Fluoride ions occur on earth in several minerals, particularly fluorite. Fluoride contributes a distinctive bitter taste. It contributes no color to fluoride salts. All tea leaves contain fluoride (Chan et al. 2013); however, mature leaves contain as much as 10–20 times the fluoride levels of young leaves from the same plant (Wong et al. 2003; Gardner 2007; Malinowska et al. 2008). Fluorine, in the form of fluoride, is considered to be a micronutrient for human health, necessary to prevent dental cavities, and to promote healthy bone growth (Malinowska et al. 2008). Approximately, 50 % of absorbed fluoride is excreted renally with a 24 h period. The remainder can be retained in the oral cavity, and lower digestive tract. Fluoride salts are commonly used to inhibit the activity of phosphatases, such as serine/threonine phosphatases (Nakai and Thomas 1974). Fluoride-containing compounds, such as sodium fluoride and sodium monofluorophosphate are used in topical and systemic fluoride therapy for preventing tooth decay.

The purpose of the present study was to evaluate the toxicity of sodium fluoride in *Schistosoma* infected snails. To accomplish this, we measured several oxidative stress, protein content, lipid profile and apoptotic parameters to determine if *Schistosoma* infection can induce oxidative stress and consequently prevent by sodium fluoride.

Materials and methods

Materials

Sodium fluoride (NaF) was purchased from Merck Chemical, Inc. (Darmstadt, Germany). All kits reagents were of analytical grades and were obtained from Biomerieux company, France through Malaysian local supplier.

Collection of *Schistosoma* snails

About 264 different *Schistosoma* snails were collected from eight drainage water resources in Bertam, Kepala Batas, Penang, Malaysia during January 2013 until March 2013. Immediately after collection of the snails; they differentiated into free uninfected control and infected *Schistosoma* snails by putting the collected snails in fully water glass jar and examined under the microscope. Consequently; the infected snails were divided into four subgroups: subgroups I, II, III and IV where the sodium fluoride dose were chosen from the previous study (Eren et al. 2005; Eraslan et al. 2007).

Experimental design

The schistosoma snails were divided into the following groups as follows: 1st group: Control uninfected healthy snails. 2nd group: *Schistosoma* infected snails without any treatment. 3rd group: *Schistosoma* infected snails' immersed in 25 mg NaF/L. 4th group: *Schistosoma* infected snails' immersed in 50 mg NaF/L. 5th group: *Schistosoma* infected snails' immersed in 100 mg NaF/L. All the groups were stayed for 4 weeks of the experimental study then the snails' hemolymph and tissue homogenates were prepared.

Collection of snails' hemolymph and preparation of snails' tissue homogenates

The hemolymph was collected from the snails by the head foot retraction method (Kopáček et al. 1993) and frozen at -80°C until use. Shells were broken between two glass slides, all debris was carefully removed, and tissues were washed and homogenized in 1 ml 0.01 M phosphate buffer solution (PBS). The homogenate was frozen and thawed 3 successive times, each for 5 min then centrifuged at 16,000 rpm for 5 min. The supernatant was collected, divided into aliquots, and frozen at -80°C until use.

Determination of biochemical analysis

Glutathione-S-transferase (GST) levels in snails' tissue homogenates were assayed according to the method of

Mannervik and Guthenberg (1981) with slight modification. Determination of glutathione peroxidase (GPx) activity in snails' tissue homogenates was determined to the method of Pagalia and Valentine (1967). Catalase (CAT) activity assay in snails' tissue homogenates was measured according to the method described by Aebi (1984). Determinations of snails' tissue homogenates reduced glutathione (GSH) levels were estimated according to the method of Ellman (1959), with slight modification. Evaluation of snails' tissue homogenates glutathione reductase (GR) level was done according to Goldberg and Spooner (1983) method. Determination of malondialdehyde (MDA) levels in snails' tissue homogenates, which is a measure of lipid peroxidation, was measured by Okhawa et al. (1979) method. Determination of snails' tissue homogenates superoxide dismutase (SOD) was estimated based on the method of Suttle (1986). Determination of protein carbonyl (PC) level in snails' tissue homogenates was determined using a method by Levine et al. (1990). The determination of snails' total protein was performed according to the method of Gornall et al. (1949). Determination of albumin (Alb) in snails' tissue was according to the method of Drupt (1974). Snails' tissue homogenates globulin (Glob) and (Alb)/Glob ratio were also estimated (Latner 1975). Total cholesterol was determined using the enzymatic method according to Allain et al. (1974). Determination of snails' low-density lipoproteins (LDL) were adopted and described by Steinberg (1981). Snails' tissues triglycerides were determined according to the method of Fossati and Prencipe (1982). Snails' tissues homogenates high-density lipoproteins (HDL) were determined according to the method described by Fruchart et al. (1982).

Evaluation of apoptotic cells

Apoptotic cells were detected by terminal deoxynucleotidyl transferase mediated deoxy-UTP nick end labeling (TUNEL), using an in situ detection kit (Roche Diagnostic, Mannheim, Germany). To detect apoptotic germ cells in schistosoma snails tissue homogenates where snails tissues homogenates were fixed in formalin for 24 h and then embedded in paraffin, and tissues sections were sliced in 6 µm ribbons on a Leica microtome. Individual sections were mounted on HTC coated slides. The tissue sections were washed with 1 % phosphate buffer solution (PBS), permeabilized using 0.1 % (v/v) Triton X-100, 0.1 % (w/v) sodium citrate in 1 × PBS, and incubated at room temperature for 10 min. The presence of cell apoptosis related to DNA transferase mediated d'UTP nick-end labeling by means of the In Situ Cell Detection Kit with FITC-labeled d'UTP. A TUNEL mixture (Roche) was deployed onto the slide according to the manufacturer's instruction. Slides

were incubated for 60 min at 32 °C, in the dark, and were monitored for wetness by adding 1 % PBS solution. Slides were analyzed immediately using a fluorescent microscope (Nikon Eclipse 80i) with a Cool Snap EZ camera and NIS Elements BR3.2 software. A minimum of 20 fields of view at ×1000 magnification were randomly selected for analysis of the cells.

Statistical analysis

The results were expressed as mean ± standard error (SE). Statistical significance was determined through one-way analysis of variance (ANOVA), followed by post hoc test. *P* values less than 0.05 were considered statistically significant. **P* ≤ 0.05 significant difference compared to control uninfected snails (−ve control), ** *P* ≤ 0.01 highly significant differences compared to control uninfected snails (−ve control). ^a*P* ≤ 0.05 significant difference compared to schistosoma infected snails (+ve control) group. ^b*P* ≤ 0.01 highly significant difference compared to schistosoma infected snails (+ve control) group.

Results

Table 1 investigated SOD, GST, GPx and CAT in control and uninfected snails, *Schistosoma* infected snails, and sodium fluoride-treated groups into *Schistosoma* infected snails. Comparing to control group, the *Schistosoma* infected snails had significantly lower SOD, GST, GPx and CAT levels in snails' tissue homogenates. However, the treatment of the *Schistosoma* infected snails with sodium fluoride (25, 50 and 100 mg/L) increased significantly the SOD, GST, GPx or CAT levels in the snails' tissue homogenates. Where sodium fluoride at higher dose was more potent than the lower two doses used.

Table 2 exhibited stomach GSH, GR, MDA and PC levels in control and uninfected snails, *Schistosoma* infected snails, and sodium fluoride-treated groups into *Schistosoma* infected snails. *Schistosoma* infection induced significant decrease in antioxidant enzymes like GSH and GR levels while significantly increase lipid peroxidation by increasing the level of malondialdehyde (MDA) and PC levels in snails' tissue homogenates. The animals treated by sodium fluoride (25, 50 and 100 mg/L) significantly increase GSH and GR levels while decreasing lipid peroxidation and PC levels in the sodium fluoride treated groups. On the other hand, the higher dose of sodium fluoride was more potent than the lower two doses chosen.

Table 3 revealed that *Schistosoma* infected snails had highly significantly increased in total protein, albumin and globulin levels in snails' tissue homogenates while albumin/globulin ratio in snails' tissue homogenates was

Table 1 Effect of Sodium fluoride on SOD, GST, GPx and CAT levels in snails' tissue homogenates

Groups	Parameters			
	SOD (mU/mg tissue)	GST (μ mol/mg tissue)	GPx (mU/mg tissue)	CAT (U/g tissue)
Control uninfected snails	139.45 $\pm$ 12.19	1.94 $\pm$ 0.019	3.69 $\pm$ 0.16	3.58 $\pm$ 0.52
Schistosoma infected snails	63.57 $\pm$ 9.42**	0.56 $\pm$ 0.014**	1.20 $\pm$ 0.47**	1.17 $\pm$ 0.24**
Schistosoma infected snails + 25 mg NaF/L	125.12 $\pm$ 13.76 ^a	1.84 $\pm$ 0.032 ^a	3.58 $\pm$ 0.40 ^a	3.50 $\pm$ 0.27 ^a
Schistosoma infected snails + 50 mg NaF/L	132.68 $\pm$ 15.19 ^b	1.87 $\pm$ 0.046 ^b	3.61 $\pm$ 0.31 ^b	3.53 $\pm$ 0.28 ^b
Schistosoma infected snails + 100 mg NaF/L	135.10 $\pm$ 13.52 ^b	1.90 $\pm$ 0.035 ^b	3.64 $\pm$ 0.27 ^b	3.55 $\pm$ 0.19 ^b

Results were expressed as mean $\pm$ SE and significant difference according to control uninfected group at $p \leq 0.05$. ANOVA showed a highly significant difference between all groups at $p \leq 0.0001$. ** $p \leq 0.01$ highly significant difference compared to control uninfected snails

^a $p \leq 0.05$ significant difference compared to *Schistosoma* infected snails

^b $p \leq 0.01$ highly significant difference compared to *Schistosoma* infected snails

Table 2 Effect of sodium fluoride on GSH, GR, MDA and PC levels in snails' tissue homogenates

Groups	Parameters			
	GSH (Mmol/mg tissue)	GR (U/mg tissue)	MDA (nmol/100 mg tissue)	PC (%)
Control uninfected snails	0.73 $\pm$ 0.041	0.96 $\pm$ 0.21	29.15 $\pm$ 3.26	105.21 $\pm$ 13.82
Schistosoma infected snails	0.34 $\pm$ 0.029**	0.45 $\pm$ 0.17**	62.10 $\pm$ 4.54**	162.37 $\pm$ 20.13**
Schistosoma infected snails + 25 mg NaF/L	0.64 $\pm$ 0.033 ^a	0.85 $\pm$ 0.13 ^a	36.24 $\pm$ 3.85 ^a	112.18 $\pm$ 15.96 ^a
Schistosoma infected snails + 50 mg NaF/L	0.68 $\pm$ 0.036 ^b	0.89 $\pm$ 0.24 ^b	33.12 $\pm$ 3.22 ^b	108.63 $\pm$ 14.90 ^b
Schistosoma infected snails + 100 mg NaF/L	0.70 $\pm$ 0.027 ^b	0.92 $\pm$ 0.18 ^b	31.45 $\pm$ 2.42 ^b	105.52 $\pm$ 16.31 ^b

Results were expressed as mean $\pm$ SE and significant difference according to control uninfected group at $p \leq 0.05$. ANOVA showed a highly significant difference between all groups at $p \leq 0.0001$

** $p \leq 0.01$ highly significant difference compared to control uninfected snails

^a $p \leq 0.05$ significant difference compared to *Schistosoma* infected snails

^b $p \leq 0.01$ highly significant difference compared to *Schistosoma* infected snails

Table 3 Effect of sodium fluoride on total protein, albumin, globulin and albumin/globulin ratio in snails' tissue homogenates

Groups	Parameters			
	Total protein (mg/g tissue)	Albumin (mg/g tissue)	Globulin (mg/g tissue)	Albumin/globulin ratio
Control uninfected snails	0.72 $\pm$ 0.09	0.41 $\pm$ 0.05	0.31 $\pm$ 0.03	1.32 $\pm$ 0.20
Schistosoma infected snails	1.09 $\pm$ 0.06**	0.63 $\pm$ 0.03**	0.46 $\pm$ 0.02**	1.37 $\pm$ 0.32
Schistosoma infected snails + 25 mg NaF/L	0.78 $\pm$ 0.10 ^a	0.45 $\pm$ 0.04 ^a	0.33 $\pm$ 0.05 ^a	1.36 $\pm$ 0.23
Schistosoma infected snails + 50 mg NaF/L	0.75 $\pm$ 0.08 ^b	0.43 $\pm$ 0.05 ^b	0.32 $\pm$ 0.06 ^b	1.34 $\pm$ 0.15
Schistosoma infected snails + 100 mg NaF/L	0.70 $\pm$ 0.09 ^b	0.40 $\pm$ 0.04 ^b	0.30 $\pm$ 0.05 ^b	1.33 $\pm$ 0.19

Results were expressed as mean $\pm$ SE and significant difference according to control uninfected group at $p \leq 0.05$. ANOVA showed a highly significant difference between all groups at $p \leq 0.0001$

** $p \leq 0.01$ highly significant difference compared to control uninfected snails

^a $p \leq 0.05$ significant difference compared to *Schistosoma* infected snails

^b $p \leq 0.01$ highly significant difference compared to *Schistosoma* infected snails

unaffected parameter. While, sodium fluoride (25, 50 and 100 mg/L) decreased total protein, albumin and globulin levels to approach the control uninfected snails levels in *Schistosoma* infected snails. However, sodium fluoride at

higher dose was more potent than the lower two doses chosen for such a study.

Table 4 stated the effect of *Schistosoma* infection on snails' tissue cholesterol, LDL, triglycerides and HDL of

Table 4 Effect of Sodium fluoride on total lipid, cholesterol, LDL and triglycerides in snails' tissue homogenates

Groups	Parameters			
	Cholesterol (mg/g tissue)	LDL (mg/g tissue)	Triglycerides (mg/g tissue)	HDL (mg/mg)
Control uninfected snails	48.2 ± 3.46	20.7 ± 1.95	27.1 ± 2.01	22.1 ± 1.87
Schistosoma infected snails	62.9 ± 2.86**	30.1 ± 1.86**	41.2 ± 1.93**	24.6 ± 2.03
Schistosoma infected snails + 25 mg NaF/L	53.4 ± 4.05 ^a	23.0 ± 2.01 ^a	30.2 ± 2.46 ^a	24.3 ± 1.92
Schistosoma infected snails + 50 mg NaF/L	51.0 ± 2.94 ^b	22.4 ± 1.68 ^b	29.5 ± 1.29 ^b	22.7 ± 1.18
Schistosoma infected snails + 100 mg NaF/L	49.7 ± 3.69 ^b	21.1 ± 1.75 ^b	28.0 ± 1.48 ^b	23.0 ± 1.35

Results were expressed as mean ± SE and significant difference according to control uninfected group at $p \leq 0.05$. ANOVA showed a highly significant difference between all groups at $p \leq 0.0001$

** $p \leq 0.01$ highly significant difference compared to control uninfected snails

^a $p \leq 0.05$ significant difference compared to *Schistosoma* infected snails

^b $p \leq 0.01$ highly significant difference compared to *Schistosoma* infected snails

snail' homogenates. It is clear that there was a clear highly significant increase in cholesterol, LDL, triglycerides and HDL levels of *Schistosoma* infected snails while HDL level of snails' tissue homogenates was unaffected. Moreover, sodium fluoride at doses of 25, 50 and 100 mg/L decreased snails' tissue cholesterol, LDL and triglycerides levels to approach the control uninfected snails levels in *Schistosoma* infected snails. Furthermore, sodium fluoride at higher dose was more potent than the lower two doses used.

Figure 1 showed the cross section of the control uninfected snails (Fig. 1a), while apoptosis appeared in *Schistosoma* infected snails tissue homogenate cells (Fig. 1b), furthermore snails' tissue homogenates exposed to sodium fluoride at the level of 100 mg/L showed no apoptotic pattern like control uninfected snails (Fig. 1c) as detected using the TUNEL assay.

Discussion

In this study, the toxic effect of sodium fluoride on *Schistosoma* infected snails was investigated. The anti-apoptotic activities, decreasing protein content and lipid profile of sodium fluoride on *Schistosoma* infected snails were also studied. The Results obtained indicated that *Schistosoma* infection induced oxidative damage, possibly by scavenging existing ROS, while halted the production of further ROS, nitric oxide, lipid peroxidation, and protein carbonylation. In addition, sodium fluoride acted by increasing levels of reduced glutathione (GSH) and superoxide dismutase (SOD), as well as, the activity of the detoxification enzyme, glutathione peroxidase (GPx); where sodium fluoride at higher dose was more effect.

Schistosoma infected snails showed significant decrease in GSH in snails' tissue homogenates; indicating that exposure to *Schistosoma* induced oxidative stress in snails'

tissues. However, sodium fluoride was capable of restoring GSH levels in snails' tissues. A possible explanation for a decrease in GSH levels is reduced activity of the enzymes involved in GSH synthesis and/or oxidation of GSH to GSSG under oxidative stress. An alternative explanation could be an increase in extra-cellular levels of glutamate which, in turn, might inhibit the uptake of cystine via the glutamate–cystine exchange system, thereby reducing GSH synthesis. Our result are in line with the results previously reported by Xiong et al. (2007) and Kanbur et al. (2009) who reported that AST and ALT activities did not change due to liver protective effect of sodium fluoride as a result of exposure to fluoride in children diagnosed with dental fluorosis.

In the current study, *Schistosoma* induced a significant increase in MDA levels in snails' tissue homogenates, as compared to sodium fluorides-treated groups, thereby pointing to the role of the antioxidant role of sodium fluoride in protecting snails from *Schistosoma*-induced damage. Our results are in accordance with the previously reported increase in lipid peroxidation in response to *Schistosoma* (Koriem et al. 2014, 2015). Concomitant reduction of GSH levels might have hampered the decomposition of lipid peroxides in *Schistosoma* infected snails. Sodium fluoride was able to prevent lipid peroxidation by supplying an adequate amount of GSH as a substrate for GPx to effectively decompose lipid peroxides in snails' tissue homogenates, thus reducing MDA levels.

In this study, increased snails' tissue homogenates total protein, albumin, globulin and protein carbonylation is a reliable parameter of oxidative stress, as it lacks interference from other non-protein substances (Esterbauer et al. 1991), and measures carbonylation of various protein residues, including lysine and arginine. Our results showed that snails' tissue total protein, albumin, globulin and protein carbonylation increased in *Schistosoma* infected snails, but such increase was prevented by sodium fluoride.

Fig. 1 Showed the cross section of the control uninfected snails (a), while apoptosis appeared in *Schistosoma* infected snails tissue homogenate cells (b), furthermore snails' tissue homogenates exposed to sodium fluoride at the level of 100 mg/L showed no apoptotic pattern like control uninfected snails (c) as detected using the TUNEL assay

These results are in line with those reported previously (Xiong et al. 2007; Kanbur et al. 2009).

The increase in lipid peroxidation was accompanied by a concomitant decrease in the activity of antioxidant enzymes, such as SOD and GR. SOD is an enzyme responsible for the detoxification of highly reactive and potentially toxic radicals to less toxic hydrogen peroxide (Fridovich 1983). The results of the present study indicated an inhibition of the enzyme in snails' tissue homogenates in *Schistosoma* infected snails. These results are in agreement with those of Murphy and Hoover (1992) and Anderson et al. (1984) who demonstrated that fluoride increased Na^+/K^+ ATPase pump activity in osteoblast-like cells. Reduced inorganic phosphorus in the fluoride-treated group can be attributed to the increased activity of the Na^+/K^+ ATPase pump and the increase of inorganic phosphorus influx into the cell, which caused a decrease in phosphorus in the blood and protection was occurred; so sodium fluoride is used to treat osteoporosis.

A significant reduction in the activity of GPx, was observed after *Schistosoma* infection in this study, may have been partially due to diminished GSH levels that GPx needs as a substrate. Our results are also in accordance with the previously reported decrease in GPx activity after *Schistosoma* infection due to the interaction of *Schistosoma* with the essential selenocysteine moiety of the enzyme (Koriem et al. 2014, 2015).

Increased CAT activity was observed in *Schistosoma* infected snails. Sodium fluoride pretreatment, yet again, reversed CAT activity in *Schistosoma* infected snails. Changes in CAT activity have been observed in *Schistosoma* infected snails' tissues that were undergoing oxidative stress. Increased CAT activity in *Schistosoma* infected snails could be an adaptive response to the higher levels of H_2O_2 generated by inhibition of GPx. The response of this antioxidant enzyme to oxidative agents is tissue/organ specific and has an adaptive character. Increased CAT activity in *Schistosoma* infected snails could be an adaptive response to the higher levels of H_2O_2 generated by inhibition of GPx. The possible mechanism for the restored CAT activity in *Schistosoma* infected snails, when treated with sodium fluoride, may be the scavenging of free radicals by amide antioxidant ingredient or by providing more GSH, which is a substrate for GPx.

In the current study, anti-apoptotic activity of sodium fluoride in *Schistosoma* infected snails was recorded. *Schistosoma* induced DNA damage underwent apoptotic cell death rather than repair. These results were in agreement with that of Koriem et al. (2014) who stated that *Schistosoma* induced liver apoptosis; while sodium fluoride

prevented apoptotic action of *Schistosoma* through its protection effect (Bouaziz et al. 2006; Machalinska et al. 2002; Xiong et al. 2007).

In conclusion, the results reported here indicated that sodium fluoride toxicity induced into *Schistosoma* infected snails enhanced lipid peroxidation accompanied by decrease in the antioxidant capacity of the cell. The decreased antioxidant ability of the cell will result in the reduced disposal of oxygen-free radicals and peroxides that may exceed the cellular defense system and eventually cause cellular injury by damaging DNA structure, apoptosis, protein content, and lipid-profile and that ultimately may be responsible for toxic effect to infected snails and consequently can protect against *Schistosoma* infection. The suppressive effect of sodium fluoride on *Schistosoma* related effects appears to be through enhancement of endogenous antioxidant enzymes and disposal of free radicals as well as apoptotic, protein and lipid activities inhibition of sodium fluoride; where sodium fluoride at higher dose was more effect.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflict of interests.

References

- Aebi H (1984) Catalase in vitro. *Method Enzymol* 105:121–126
- Allain CC, Poon LS, Chan CS, Richmond W, Fu PC (1974) Enzymatic determination of total serum cholesterol. *J Clin Chem* 20:470–475
- Anderson RE, Kemp JW, Jee WSS, Woodbury DM (1984) Effects of cortisol and fluoride on ion-transporting ATPase activities in cultured osteoblast like cells. *In Vitro* 20(11):847–855
- Bichler KH, Feil G, Zumbragel A, Eipper E, Dyballa S (2001) Schistosomiasis: a critical review. *Curr Opin Urol* 11:97–101
- Bouaziz H, Ketata S, Jammoussi K (2006) Effects of sodium fluoride on hepatic toxicity in adult mice and their suckling pups. *Pest Biochem Physiol* 86(3):124–130
- Chan L, Mehra A, Saikat S, Lynch P (2013) Human exposure assessment of fluoride from tea (*Camellia sinensis* L.): a UK based issue? *Food Res Int* 51(2):564–570
- Dissanayake CB (1991) The fluoride problem in the groundwater of Sri Lanka-environmental management and health. *Inter J Environ Studies* 38(2–3):137–156
- Drupt F (1974) Determination of serum albumin by bromocresol green. *Pharm Biol* 8:77–99
- Ellman GL (1959) Tissue sulphhydryl groups. *Arch Biochem Biophys* 82:70–77
- El-Tonsy MM, Hussein HM, Helal TE, Tawfik RA, Koriem KM, Hussein HM (2013) *Schistosoma mansoni* infection: is it a risk factor for development of hepatocellular carcinoma? *Acta Trop* 128(3):542–547
- Eraslan G, Kanbur M, Silici S (2007) Evaluation of propolis effects on some biochemical parameters in rats treated with sodium fluoride. *Pest Biochem Physiol* 88(3):273–283
- Eren E, Öztürk M, Mumcu EF, Canatan D (2005) Fluorosis and its hematological effects. *Toxicol Ind Health* 21(10):255–258
- Esterbauer H, Schaur RJ, Zollner H (1991) Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic Biol Med* 1(1):81–128
- Fossati P, Principe L (1982) Serum triglyceride determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin Chem* 28:2077–2284
- Fridovich I (1983) Superoxide radical as an endogenous toxicant. *Ann Rev Pharmacol Toxicol* 23:239–257
- Fruchart JC, Kora I, Cachera C, Clavey V, Duthilleul P, Moschetto Y (1982) Simultaneous measurement of plasma apolipoproteins A-I and B by electroimmunoassay. *Clin Chem* 28:59–62
- Gardner EJ (2007) Black tea—helpful or harmful? A review of the evidence. *Eur J Clin Nutr* 61(1):3–18
- Goldberg DM, Spooner RJ (1983) In “Methods of enzymatic analysis (Bergmeyer, H. V. Ed) 3rd Ed. Verlag Chemie, Deerfield Beach, FL. 3:258–265
- Gornall AC, Bardawill CJ, David MM (1949) Determination of serum proteins by means of the Biuret-reaction. *J Biol Chem* 177:751–766
- Kanbur M, Eraslan G, Silici S, Karabacak M (2009) Effects of sodium fluoride exposure on some biochemical parameters in mice: evaluation of the ameliorative effect of royal jelly applications on these parameters. *Food Chem Toxicol* 47(6):1184–1189
- Kopáček P, Hall M, Söderhäll K (1993) Characterization of the clotting protein isolated from plasma of the freshwater crayfish *Pacifastacus leniusculus*. *Eur J Biochem* 13:591–597
- Koriem KM, Shahabudin RZ, Jamaludin RZ (2014) *Aristolochia gehrtii* inhibits liver toxicity and apoptosis in *Schistosoma malayensis* infection. *Asian Pac J Trop Med* 7(9):685–692
- Koriem KM, Idris ZH, Haron HF, Omar NA, Lazain HS (2015) Therapeutic effect of *Arctium lappa* in *Schistosoma haematobium* associated kidney disturbance: biochemical and molecular effects. *J Parasit Dis*. doi:10.1007/s12639-015-0662-4
- Latner AL (1975) Cantarow and trumper. *Clinical biochemistry*, 7th edn. Philadelphia W. B. Saunders Company, Philadelphia, pp 147–259
- Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stadtman ER (1990) Determination of carbonyl content in oxidatively modified proteins. *Method Enzymol* 186:464–478
- Machalinska A, Nowak J, Jarema A, Wiszniewska B, Machalinski B (2002) In vivo effects of sodium fluoride on bone marrow transplantation in lethally irradiated mice. *Fluoride* 35(2):81–89
- Malinowska E, Inkielewicz I, Czarnowski W, Szefer P (2008) Assessment of fluoride concentration and daily intake by human from tea and herbal infusions. *Food Chem Toxicol* 46(3):1055–1061
- Mannervik B, Guthenberg C (1981) Glutathione-S-transferase (human placenta). *Methods Enzymol* 77:231–235
- Murphy AJ, Hoover JC (1992) Inhibition of the Na, K-ATPase by fluoride. Parallels with its inhibition of the sarcoplasmic reticulum Ca-ATPase. *J Biol Chem* 267(24):16995–17000
- Nakai C, Thomas JA (1974) Properties of a phosphoprotein phosphatase from bovine heart with activity on glycogen synthase, phosphorylase, and histone. *J Biol Chem* 249(20):6459–6467
- Okhawa H, Ohishi N, Yagi K (1979) Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal Biochem* 95:351–358
- Pagalía DE, Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 70:158–169
- Steinberg D (1981) Metabolism of lipoproteins at the cellular level in relation to atherogenesis. *Lipoprot Atheroscler Coron Heart Dis* 1:24–29
- Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J (2006) Schistosomiasis and water resources development: systematic

- review meta-analysis, and estimates of people at risk. *Lancet Infect Dis* 6:411–425
- Suttle NF (1986) Copper deficiency in ruminants; recent developments. *Vet Rec* 119:519–522
- Wong MH, Fung KF, Carr HP (2003) Aluminium and fluoride contents of tea, with emphasis on brick tea and their health implications. *Rev Toxicol Lett* 137(12):111–120
- Xiong X, Liu J, He W (2007) Dose-effect relationship between drinking water fluoride levels and damage to liver and kidney functions in children. *Environ Res* 103(1):112–116