Therapeutic effect of *Arctium lappa* in *Schistosoma haematobium* associated kidney disturbance: biochemical and molecular effects

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Abstract *Schistosoma haematobium* (*S. haematobium*) infection has been found to be strongly associated with bladder cancer, which necessitates for discover of a natural new therapeutic agent. The aim of this study was to evaluate the therapeutic effect of *Arctium lappa* seed extract in *S. haematobium* associated kidney disturbance. Forty male albino mice were used and divided into four equal groups; group 1 control includes non-infected healthy mice, groups 2, 3 and 4 subcutaneous infected with *S. haematobium* cercariae. Groups 3 co-treated daily with oral dose of *A. lappa* seed extract (300 mg/kg, bwt) for 15 days in the same time of *S. haematobium* infection. Groups 4 post-treated daily for 15 days with oral dose of *A. lappa* seed extract (300 mg/kg, bwt) after 15 days of *S. haematobium* infection. The results obtained revealed that *S. haematobium* significantly decreased kidney weight and serum sodium, potassium and chloride, but increased urinary volume, urinary excretion of

sodium, potassium and chloride, serum urea, creatinine and uric acid. *Schistosoma haematobium* also significantly decreased kidney superoxide dismutase, glutathione peroxidase and reduced glutathione levels while increased kidney lipid peroxidation level. Co- and post-treatment with *A. lappa* seed extract restore all the above parameters to approach the normal values. These results were supported with histopathological examinations. In conclusion, *A. lappa* seed extract has therapeutic effect in kidney disturbance caused by *S. haematobium* where co-treatment of *A. lappa* seed extract was more effective than post-treatment of the extract.

Keywords *Arctium lappa* · Asteraceae ·
Schistosoma haematobium · Electrolytes · DNA ·
Mice

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). The main forms of human schistosomiasis are caused by five species of the flatworm, or blood flukes, known as Schistosomes: *Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosoma mekongi*, *Schistosoma intercalatum* and *Schistosoma haematobium*. The last species, *S. haematobium* causes urinary schistosomiasis and affects millions of

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people in the developing countries. *Schistosoma haematobium* infection has been found to be strongly associated with bladder cancer and hepatocellular carcinoma, respectively (Khurana et al. 2005) where international agency for research on cancer (IARC) considers *S. haematobium* infection a definitive cause of urinary bladder cancer with an associated fivefold risk (Pisani et al. 1997; Kouriba et al. 2005). Renal involvement occurs mostly with *S. haematobium* infection as a consequence of fibrosis and calcification of tissue-trapped ova in the lower urinary tract, leading to obstruction, reflux, infection, and stone formation and the resulting interstitial nephritis present with tubular dysfunction syndrome before it progresses to end-stage renal disease (Barsoum 2003; Vennervald and Polman 2009).

The research for a new and natural source to be used as a treatment for Schistosomiasis infection was increased in the last decade. The medicinal plants provide a new, available and cheap source for developing new drugs nowadays. *Arctium lappa* (family: Asteraceae) commonly called greater burdock, gobō, edible burdock, lappa, or beggar's buttons (Rose 1981); it is an edible biennial plant. Greater Burdock is rather tall, reaching as much as 9 feet. It has large, alternating, cordiform leaves that have a long petiole and are pubescent on the underside. The flowers are purple and grouped in globular capitula and appear in mid-summer, from July to September. This species is native to the temperate and tropical regions of the world, from Scandinavia to the Mediterranean, and from the British Isles through Russia, and the Middle East to China and Japan, including India (Yin-Yang 2013). In the second half of the 20th century, burdock achieved international recognition for its use due to the increasing popularity of the macrobiotic diet, which advocates its consumption. The root contains a fair amount of dietary fiber (6 g per 100 g), calcium, potassium, amino acids (Rose 1981) and is low calorie. It contains polyphenols that causes darkened surface and muddy harshness by formation of tannin-iron complexes. Those polyphenols are caffeoylquinic acid derivatives (Kemper 2010). They are used in Western folk medicine as a diaphoretic, and a blood purifying agent (Chan et al. 2010). *Arctium lappa* as an ingredient in Essiac tea is used for the alternative treatment of some cancers (Matsumoto et al. 2006). It is a component of natural cosmetics, shampoos and hair care products. It is used to prevent baldness and to treat rheumatoid arthritis, skin infections, acne, boils, bites, eczema, herpes, impetigo, rashes, ringworm and sore throat (Chan et al. 2010; Lee et al. 2011). The seeds of greater burdock are employed for skin cancer and have anticancer activity in vitro (Matsumoto et al. 2006; Kyoko et al. 2010).

The selection of *A. lappa* seed for the current study was due to its major bioactive polyphenols constituents (Kemper 2010), where polyphenols have antioxidant activity

(Raederstorff 2009). The aim of the present study was to discover a natural adjunctive new therapeutic agent to treated *S. haematobium*-like infection complications in kidney tissue. To accomplish this, we measured several oxidative stress parameters to determine if *S. haematobium* induces oxidative stress in kidney and if this stress could be prevented by the use of *A. lappa* seed extract.

Materials and methods

Plant material

Arctium lappa seeds were provided from Penang Botanical Garden, Penang, Malaysia in January 2013. The plant was botanically identified authenticated at School of Pharmaceutical Science, Universiti Sains Malaysia, Malaysia. Voucher specimen of each plant was deposited at the herbarium of the Pharmacy School, Universiti Sains Malaysia, Malaysia. The seeds were crushed, pulverized and then weighed and prepared for extraction.

Preparation of aqueous extract of *Arctium lappa* L. seeds

Fresh *Arctium lappa* L. seeds were washed in tap water and air-dried in the shade. Four kilograms of seeds were extracted with warm distilled water (DW) (DW: material, 10:1, v/w) twice in an incubator at 80 °C for 1.5 h. The hot-water extract was filtered through Whatman filter paper, and the extraction was repeated. The combined filtrates were concentrated in a vacuum at 60 °C, and the resulting filtrates were freeze-dried (Boyikang Refrigerated Vapor Trap, SD-1A-50) and weighed to determine the yield of soluble constituents. The sample was stored at −20 °C (yield 12.2 % w/w dry weight basis) until use.

Estimation of lethal dose₅₀ (LD₅₀) of the extract

Thirty-six mice were orally administered the seeds extract with different seed extract concentrations. Six rats were kept as control group throughout the entire experimental period. Mortality was assessed and counted in the different groups. The LD₅₀ was calculated according to Behrens and Karber (1953) and 1/10 of the LD₅₀ was found to be safe (Koriem et al. 2010).

Test for purity, quality and stability of the extract

Purity tests (microbiological, pesticide residues, heavy metals, radioactive residues, chemical, foreign organic matter and sulfated ash) were performed in accordance with the Malaysian accepted protocol requirements and

accredited to ISO/IEC 17025 consulting WHO guidelines on stability and quality controls methods for medicinal plants (Farnsworth 2001; Leung and Foster 1996). The seed extract was stored in a tightly cooling (-4°C) sealed container away from heat and light that prevent a loss of extract solvent and entry of water; where plant extract was dissolved in 1 ml DW and given orally to individual mice.

Animals

Forty male albino mice (20–25 g) were obtained from the animal house colony of the advanced medical and dental institute, Universiti Sains Malaysia, Malaysia and were kept in special plastic cages. The animals were maintained on a commercial balanced diet and tap water. The experiments were performed after approval from the ethics committee of the Universiti Sains Malaysia, Malaysia and assigned approval number (FWA/IRB Registration number: 00009723/00005682).

Experimental design

The mice model had been established and divided into four equal (ten mice each) groups as follows; Control group: This group includes non-infected healthy, parasite free mice as a control group. *Schistosoma haematobium* infected group: This group was represented by subcutaneously infected with 50 cercariae of *S. haematobium*. *Arctium lappa* seeds extract co-treated group: This group comprises *S. haematobium* infected group co-treated daily with oral dose of *Arctium lappa* seed extract (300 mg/kg, bwt) for 15 days in the same time of *schistosoma* infection. *Arctium lappa* seeds extract post-treated group: This group includes *S. haematobium* infected group post-treated daily for 15 days with oral dose of *Arctium lappa* seed extract (300 mg/kg, bwt) after 15 days of *schistosoma* infection.

All mice were anesthetized 24 h after the last oral administration by inhalation with diethyl ether solution. All mice were massed at the beginning and at the end of the study.

Assessment of diuretic activity

Diuretic activity was determined according to the method described by Kau et al. (1984), with minor modifications. Each animal was placed in an individual metabolic cage 24 h before the beginning of the experiment for environmental adaptation. Mice were fasted overnight with free access to water. To impose a uniform water and salt load, all animals received physiological saline (0.9 % NaCl) before treatment, at an oral dose of 5 ml/100 g body weight. Urine was collected at the end of the experiment and its volume was measured.

Blood sampling and handling

Blood samples were collected from retro-orbital plexus of mice using capillary tubes (Schermer 1967) into clean centrifuge tubes. Part of blood sample was collected in the presence of using EDTA as an anticoagulant for blood while the other part of the blood sample was allowed to coagulate and centrifuged at 4000 rpm for 15 min to separate blood serum which stored at -20°C .

Tissue preparation

The animals were decapitated and then dissected, whereby the kidney tissues were obtained, washed in cold saline, and dried between filter papers. They were weighed, homogenized and kept at -80°C for further investigation; 0.5 g of kidney tissues were dissolved in 2.5 ml of Tris buffer solution, then homogenated in the homogenizer for exactly 30 min. Then they were centrifuged for exactly 20 min at 7000 rpm, which separated the supernatants, which used for antioxidant activities and DNA content determination in kidney tissue.

Biochemical analysis

Kidney function

Serum urea, creatinine and uric acid were determined calorimetrically using kits reagents.

Electrolytes levels

Serum and urinary sodium (Na^{+}), potassium (K^{+}) and chloride (Cl^{-}) levels were determined calorimetrically using kits reagents.

Antioxidants enzymes

Kidney tissue superoxide dismutase (SOD), glutathione peroxidase (GPx), reduced glutathione (GSH) and lipid peroxidation (MDA) levels were determined calorimetrically using kits reagents.

Image analysis

Feulgen-stained slides were prepared for the nuclear DNA analysis using the Leica Qwin 500 Image Analyzer (LEICA Imaging Systems Ltd, Cambridge, UK). The system was calibrated before each measurement session using the calibration slides provided with the system at high power magnification (400 $\times$). The optical density of the selected nuclei in each microscopic field was measured and automatically converted by the system into DNA content.

Many fields were selected by the desired number of nuclei (100–150) has been measured. The results were displayed as a frequency histogram on the monitor generated by plotting the DNA content versus the number of nuclei counted. The DNA histograms were classified according to Danqu et al. (1993) as diploid (DNA index ranging from 0.9 to 1.1), tetraploid (DNA index ranging from 1.8 to 2.2), and aneuploid (when at least 10 % of the total events show a distinct abnormal peak outside the 2C or 4C position) based on the amount of DNA relative to the normal control.

Histopathological examinations

Specimens of kidney were fixed in 10 % neutral formalin solution, and then processed for routine technique for embedding in paraffin. Blocks were sectioned at a thickness of 5 µm and stained with hematoxylin and eosin for histopathological examination, which were performed under light microscopy and documented by an Olympus microphotocamera.

Statistical analysis

The results were expressed as mean $\pm$ standard error (SE). Statistical significance was determined through one-way analysis of variance (ANOVA), using the SPSS 13 software package for Windows (Chicago, IL). Post hoc testing was performed for inter-group comparisons using the least significance difference (Tukey) test. * $P \leq 0.05$ significant difference compared to control (–ve control), ** $P \leq 0.01$ highly significant differences compared to control (–ve control). ^a $P \leq 0.05$ significant difference compared to *S. haematobium* (+ve control) group. ^b $P \leq 0.01$ highly significant difference compared to *S. haematobium* (+ve control) group.

Results

Body weight, food and water intake and LD₅₀ of the extract

In the present study, there were observable differences of body weight, food or drink intake in *S. haematobium* infected mice; where there are a significant decrease in body weight, food or drink intake in *S. haematobium* infected mice than control mice; while co-treated and post-treated with *A. lappa* seed extract approach the normal level in body weight, food or drink intake during experimental period of the study. The maximum non-lethal dose of *A. lappa* seed extract was found to be 3000 mg/kg; hence 1/10 of the dose was taken as effective dose (300 mg/kg, bwt).

Kidney function and weight

Table 1 illustrated the kidney function and weight. It showed the co-treatment and post-treatment of *A. lappa* seed extract on serum urea, creatinine and uric acid levels, as well as, kidney weight in *S. haematobium* infected mice. It is clear that there is a significant increase in serum urea, creatinine and uric acid levels in *S. haematobium* infected mice compared to control. On the other hand, kidney weight was decreased in *S. haematobium* infected mice. The co-treatment and post-treatment of *A. lappa* seed extract to *S. haematobium* infected mice restores all the above parameters to approach the normal values where co-treatment was more potent in all the above parameters than the post-treatment with *A. lappa* seed extract.

Serum sodium, potassium and chloride levels

Table 2 investigated the serum sodium, potassium and chloride electrolytes in normal, *S. haematobium* infected mice, both co-treated and post-treated of *A. lappa* seed extract to *S. haematobium* infected mice. It is clear that there is a significant decrease in serum sodium, potassium and chloride electrolytes in *S. haematobium* infected mice compared to control ones. The co-treatment and post-treatment of *A. lappa* seed extract to *S. haematobium* infected mice push serum sodium, potassium and chloride electrolytes towards the normal values where co-treatment was more effect in all the above parameters than the post-treatment with *A. lappa* seed extract.

Urinary volume and electrolytes

Table 3 revealed the effect of co-treatment and post-treatment with *A. lappa* seed extract in *S. haematobium* infected mice for 15 days period on urinary volume and electrolytes. It is clear that there is a significant increase in urinary volume and electrolytes excretion in *S. haematobium* infected mice. The co-treatment and post-treatment of *A. lappa* seed extract to *S. haematobium* infected mice restores all the above parameters towards the normal values where co-treatment was more potent in all the above parameters than the post-treatment with *A. lappa* seed extract.

Kidney antioxidants

Table 4 exhibited kidney SOD, GPx, GSH and MDA in normal, *S. haematobium* infected mice, both co-treated and post-treated of *A. lappa* seed extract to *S. haematobium* infected mice. Compared to the control, the *S. haematobium* infected mice had significantly lower kidney SOD, GPx and GSH levels in the kidney studied. On the contrary, kidney MDA level was significantly increased in *S.*

Table 1 Effect of *Arctium lappa* seed extract on serum urea, creatinine, uric acid and kidney weight of *S. haematobium* infected mice

Groups	Parameters			
	Serum urea (mg/dl)	Serum creatinine (mg/dl)	Serum uric acid (mg/dl)	Kidney weight (g/100 g bwt)
Control	26.5 ± 2.83	0.75 ± 0.08	8.21 ± 0.43	0.72 ± 0.06
<i>S. haematobium</i>	34.0 ± 2.59*	0.98 ± 0.09*	17.65 ± 0.82**	0.54 ± 0.04**
<i>Arctium lappa</i> co-treatment with <i>S. haematobium</i>	28.2 ± 2.74 ^a	0.79 ± 0.09 ^a	9.16 ± 0.58 ^b	0.71 ± 0.08 ^b
<i>Arctium lappa</i> post-treatment with <i>S. haematobium</i>	29.7 ± 2.95 ^b	0.85 ± 0.07 ^a	10.74 ± 0.91 ^b	0.68 ± 0.09 ^b

Results were expressed as mean ± SEM and significant difference according to control group at $P < 0.05$, ANOVA showed a highly significant difference between all groups at $P < 0.0001$

* $P < 0.05$ significant difference compared to control (–ve control), ** $P < 0.01$ highly significant differences compared to control (–ve control)

^a $P < 0.05$ significant difference compared to *S. haematobium* (+ve control) group

^b $P < 0.01$ highly significant difference compared to *S. haematobium* (+ve control) group

Table 2 Effect of *Arctium lappa* seed extract on serum electrolytes of *S. haematobium* infected mice

Groups	Serum electrolyte level		Saluretic index		Serum Na ⁺ /K ⁺	Serum Cl [–] (mmol/L)
	Na ⁺ (mmol/L)	K ⁺ (mmol/L)	Na ⁺	K ⁺		
Control	154.60 ± 3.29	5.62 ± 0.19	–	–	–	102.83 ± 2.19
<i>S. haematobium</i>	110.24 ± 4.16**	2.71 ± 0.28**	0.71	0.48	1.48	81.47 ± 1.85**
<i>Arctium lappa</i> co-treatment with <i>S. haematobium</i>	144.9 ± 3.28 ^b	5.43 ± 0.18 ^a	0.94	0.97	0.97	101.26 ± 2.39 ^a
<i>Arctium lappa</i> post-treatment with <i>S. haematobium</i>	141.26 ± 3.6 ^b	5.39 ± 0.19 ^a	0.91	1.05	0.87	99.86 ± 2.18 ^a

Results were expressed as mean ± SEM and significant difference according to control group at $P < 0.05$, ANOVA showed a highly significant difference between all groups at $P < 0.0001$. Saluretic Na⁺/K⁺ index: Serum Na⁺/K⁺ of treated/Serum Na⁺/K⁺ of negative control

* $P < 0.05$ significant difference compared to control (–ve control), ** $P < 0.01$ highly significant differences compared to control (–ve control)

^a $P < 0.05$ significant difference compared to *S. haematobium* (+ve control) group

^b $P < 0.01$ highly significant difference compared to *S. haematobium* (+ve control) group

Table 3 Effect of *Arctium lappa* seed extract on urinary volume and electrolyte excretion of *S. haematobium* infected mice

Groups	Urine volume (ml/100 g)	Diuretic index	Urinary Na ⁺ (mmol/l)	Urinary K ⁺ (mmol/l)	Saluretic index		Urinary Na ⁺ /K ⁺	Urinary Cl [–]
					Na ⁺	K ⁺		
Control	0.98 ± 0.12	–	99.13 ± 5.73	58.00 ± 2.79	–	–	–	7.52 ± 0.23
<i>S. haematobium</i>	1.26 ± 0.25**	1.29	128.62 ± 7.15**	80.16 ± 3.05**	1.30	1.38	1.60	11.36 ± 0.19**
<i>Arctium lappa</i> co-treatment with <i>S. haematobium</i>	1.06 ± 0.15 ^a	1.08	107.91 ± 5.64 ^a	62.59 ± 2.41 ^b	1.09	1.08	1.01	7.72 ± 0.29 ^a
<i>Arctium lappa</i> post-treatment with <i>S. haematobium</i>	1.08 ± 0.18 ^a	1.10	110.26 ± 5.14 ^a	65.23 ± 3.19 ^b	1.11	1.13	0.98	7.85 ± 0.24 ^a

Results were expressed as mean ± SEM and significant difference according to control group at $P < 0.05$, ANOVA showed a highly significant difference between all groups at $P < 0.0001$. Diuretic index: Urine volume of treated/Urine volume of negative control, Saluretic Na⁺/K⁺ index: Urinary Na⁺/K⁺ of treated/Urinary Na⁺/K⁺ of negative control

* $P < 0.05$ significant difference compared to control (–ve control), ** $P < 0.01$ highly significant differences compared to control (–ve control)

^a $P < 0.05$ significant difference compared to *S. haematobium* (+ve control) group

^b $P < 0.01$ highly significant difference compared to *S. haematobium* (+ve control) group

Table 4 Effect of *Arctium lappa* seed extract on SOD, GPx, GSH and MDA levels in the kidney tissues of *S. haematobium* infected mice

Groups	Parameters			
	SOD (mU/mg)	GPx (mU/mg)	GSH (Mmol/mg)	MDA (nmol/100 mg)
Control	139.45 ± 12.19	3.57 ± 0.16	0.54 ± 0.031	28.59 ± 3.16
<i>S. haematobium</i>	63.57 ± 9.42**	1.09 ± 0.41**	0.27 ± 0.016**	57.06 ± 4.15**
<i>Arctium lappa</i> co-treatment with <i>S. haematobium</i>	135.67 ± 12.64 ^b	3.51 ± 0.20 ^b	0.49 ± 0.033 ^b	31.69 ± 2.47 ^b
<i>Arctium lappa</i> post-treatment with <i>S. haematobium</i>	128.56 ± 13.16 ^a	3.49 ± 0.37 ^b	0.49 ± 0.033 ^b	36.21 ± 3.85 ^a

Results were expressed as mean ± SE and significant difference according to control 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

^a $p \leq 0.05$ significant difference compared to *S. haematobium*

^b $p \leq 0.01$ highly significant difference compared to *S. haematobium*

haematobium infected mice. However, the co-treatment and post-treatment of *A. lappa* seed extract to *S. haematobium* infected mice push the kidney SOD, GPx, GSH and MDA towards the normal values where co-treatment was more effect in all the above parameters than the post-treatment with *A. lappa* seed extract.

Kidney DNA

Table 5 showed that the *S. haematobium* decreased the number of haploid (1C) cells and increased the number of diploid (2C), S phase, and tetraploid (4C) cells; where the kidney of control group showed 72.8 % (2C) diploid cells, 14.9 % (3C) triploid cells (medium proliferation index), 0 % (4C) tetraploid cells and 0 % (>5C) aneuploid cells (diploid-medium proliferation index). In *S. haematobium* infected group, the kidney showed the kidney showed 19.8 % (2C) diploid cells, 31.1 % (3C) triploid cells (high proliferation index), 28.3 % (4C) tetraploid cells, and 4.7 % (>5C) aneuploid cells (tetraploid-high proliferation index). In rats co-treated or post-treated with *A. lappa* seed extract to *S. haematobium* infected mice, the kidney showed 37.6 % (2C) diploid cells, 18.8 % (3C) triploid cells (medium proliferation index), 1.9 % (4C) tetraploid cells, and 0 % (>5C) aneuploid cells (diploid-medium proliferation index).

Kidney histology

Figure 1 revealed the kidney of control mouse, had normal renal structure which showed a normal structure of renal glomeruli. The proximal convoluted tubules are lined with typical thick cubic epithelium. The distal convoluted tubules show considerably lower cubic epithelium. The tubules have a relatively regular distinct lumen (Fig. 1a). *Schistosoma haematobium* caused marked and widespread necrosis with dilatation, vacuolar degeneration, epithelial desquamation and intraluminal cast formation in the

proximal convoluted tubules (Fig. 1b). The histopathological renal damage induced by *S. haematobium* was markedly ameliorated in animals co-treated or post-treated with *A. lappa* seed extract (Fig. 1c, d).

Discussion

The results of the present study revealed highly significant decrease in kidney weight, serum sodium, potassium and chloride but highly significant increase in urinary volume, urinary excretion of sodium, potassium and chloride, as well as, serum urea, creatinine and uric acid in *S. haematobium* infected mice. On the other hand, co-treatment and post-treatment with *A. lappa* seed extract push serum electrolytes, kidney function, urinary electrolyte excretion kidney weight and volume towards normal values.

The results indicate that *A. lappa* seed extract prevented oxidative damage, possibly by scavenging existing ROS, while halting the production of further ROS, nitric oxide, lipid peroxidation, and protein carbonylation. In addition, *A. lappa* seed extract acted by increasing levels of kidney SOD, GPx and GSH while decreasing kidney MDA level in *S. haematobium* infection.

The *S. haematobium* showed significant decrease in GSH in the kidney organ tested; indicating that exposure to *S. haematobium* infection induced oxidative stress in kidney tissues. However, *A. lappa* seed extract was capable of restoring GSH levels in these 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 extracellular 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 Sheweita et al. (2003) who observed a depletion of GSH level in kidney and bladder by 72 and 54 % at 8 and

Table 5 Kidney DNA ploidy in mice treated with *Arctium lappa* seed extract to *S. haematobium* infected mice

Control				<i>S. haematobium</i>				<i>Arctium lappa</i> seed extract + <i>S. haematobium</i>			
Range	Total cells	% cells (%)	DNA index	Range	Total cells	% cells (%)	DNA index	Range	Total cells	% cells (%)	DNA index
All	107	100.0	1.00	All	106	100.0	2.37	All	109	100.0	1.1
5cER	0	0.0	–	5cER	5	4.7	2.95	5cER	0	0.0	–
<1.5c	13	12.2	0.65	<1.5c	6	5.7	–	<1.5c	9	8.26	0.7
1.5c–2.5c	78	72.9	0.98	1.5c–2.5c	21	19.8	1.05	1.5c–2.5c	63	57.8	1.0
2.5c–3.5c	16	15.0	1.36	2.5c–3.5c	33	31.1	1.56	2.5c–3.5c	32	29.4	1.4
3.5c–4.5c	0	0.0	–	3.5c–4.5c	30	28.3	2.03	3.5c–4.5c	5	4.6	1.8
>4.5c	0	0.0	–	>4.5c	16	15.1	2.71	>4.5c	0	0.0	–

Fig. 1 A Photomicrographs of mouse kidney (H&E) from: **a**, 200× control group showing normal renal architecture. **b**, 200× *S. haematobium* infected-group showing widespread coagulative necrosis with dilatation, vacuolar degeneration, epithelial desquamation and intraluminal cast formation. **c**, 200× *S. haematobium* infected-group co-treated with *Arctium lappa* seed extract (300 mg/kg) group

displaying marked improvement in the histological picture which is comparable to that of the control group. **d**, 200× *S. haematobium* infected-group post-treated with *Arctium lappa* seed extract (300 mg/kg) group displaying marked improvement in the histological picture which is comparable to that of the control group

10 weeks post-infected with *S. haematobium*. Also, Diab et al. (2013) showed a highly significant decrease in GSH level as a result of schistosomiasis in the selected organs, on the other hand, nitrite/nitrate and MDA levels were increased significantly. *A. lappa* seed extract treated kidney

disturbance by increase GSH biosynthesis by reducing extracellular cystine to cysteine, and/or by supplying the sulfhydryl groups that can stimulate GSH biosynthesis, or by conversion of GSSG to GSH by non-enzymatic thiol disulfide exchange (Koriem et al. 2014).

In the current study, *S. haematobium* induced a significant increase in MDA levels in kidney tissue of these animals, as compared to *A. lappa* seed extract-treated group, thereby pointing the antioxidant role of *A. lappa* in protecting animals from *S. haematobium*-induced damage. Our results are in accordance with the previously reported increases in lipid peroxidation in response to *S. haematobium* (Halliwell and Gutteridge 2001; Kouriba et al. 2005). Concomitant reduction of GSH levels might have hampered the decomposition of lipid peroxides in *S. haematobium*-treated animals. *Arctium lappa* seed extract was able to prevent lipid peroxidation by supplying an adequate amount of GSH as a substrate for GPx to effectively decompose lipid peroxides in the mice, thus reducing MDA level. The increase in lipid peroxidation was accompanied by a concomitant decrease in the activity of antioxidant enzymes, such as SOD and GPx. 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 kidney of *S. haematobium* treated animals. These results are in agreement with those of El-Shenawy and Soliman (2003), El-Shenawy et al. (2008) who Schistosomiasis causes a reduction in the levels of protective endogenous antioxidants and increases generation of free radicals. This case of imbalance in the antioxidant-oxidants system was creating an oxidative stress so there is a decline in the activity of SOD and attributed this decrease to the *S. haematobium*-induced copper deficiency, as copper plays a catalytic role in this enzyme (Fridovich 1983). A significant reduction in the activity of GPx, observed after *S. haematobium* 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 *S. haematobium* due to the combination of *S. haematobium* with the essential selenocysteine moiety of the enzyme (Halliwell and Gutteridge 2001).

The present results showed that *S. haematobium* caused an increase in the DNA content of the kidney cells, but not reaching aneuploidy. Co-treatment or post-treatment with *A. lappa* seed extract to *S. haematobium* infected mice; the DNA content of kidney cells restored to the normal diploid value. Many aneugenic compounds are known to affect on the mitotic apparatus leading to an erroneous migration of chromosomes. Mal-segregation occurs when a chromosome (or a chromatid) fails to migrate and remains at the metaphase plate. Non-disjunction implies the lack of dissociation between sister chromatids and the migration of both together to the same pole. Mal-segregation induced by *S. haematobium*. Microtubules are the prime components involved in chromosomal segregation, their functional accuracy ensuring maintenance of the normal karyotype in

the progeny. *Schistosoma haematobium*-induced disruption of microtubules during mitosis can lead to aneuploidy (Voutsinas et al. 1997; Seoane et al. 2002).

Histopathological studies of kidney tissues in *S. haematobium* infection or co-treatment or post-treatment of *A. lappa* seed extract + *S. haematobium* infected mice indicate that *A. lappa* seed extract has cytoprotective properties.

Conclusion

The *S. haematobium*-treated mice 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, proteins, and lipids and that ultimately may be responsible for kidney disturbance. The suppressive effect of *A. lappa* seed extract on *S. haematobium*-related effects appears to be through enhancement of endogenous antioxidant enzymes and disposal of free radicals.

Conflict of interest The authors declare that there is no conflict of interests regarding the publication of this article.

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