

Therapeutic effects of *Schistosoma mansoni* soluble egg antigen in high fat diet induced dyslipidemia, hepatic and cardiovascular pathology in mice

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Abstract. High-fat diet (HFD) can cause hyperlipidemia, fatty liver and cardiovascular disorders. Herein, we evaluated therapeutic effects and possible underlying mechanisms of actions of *Schistosoma mansoni* soluble egg antigen (SEA) against experimental HFD induced dyslipidemia, hepatic and cardiovascular pathology. Forty Swiss albino mice were divided into four groups (10 each); mice fed standard diet (SD), mice fed HFD, mice fed HFD for 8 weeks then infected by *S. mansoni* cercaria (HFD+I) and mice fed HFD for 8 weeks then treated with SEA (HFD+SEA), all mice were euthanized 16 weeks after starting the experiment. HFD+SEA mice showed significantly ($p<0.001$) reduced total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG), and significantly ($p<0.05$) increased high-density lipoprotein cholesterol (HDL-C) comparing to HFD mice with non-significant difference with HFD+I mice group. Doppler flowmetry showed significantly ($p<0.01$) lower arterial resistance and significantly ($p<0.05$) higher blood flow velocity in HFD+SEA and HFD+I mice groups than HFD mice. HFD+SEA mice revealed improving in liver and aortic pathology and these were better than HFD+I mice group. HFD+SEA and HFD+I mice groups had less myocardium lipid deposits, but still showing some congested blood vessels. HFD myocardium revealed strong CD34⁺ expression on immunohistochemistry study, while that of HFD+SEA showed weak and HFD+I mice had moderate expressions. HFD+SEA mice had significantly ($p<0.01$) lower serum IL-1 β and vascular endothelial growth factor (VEGF) and significantly ($p<0.001$) higher serum transforming growth factor beta 1 (TGF- β 1) and IL-10 than HFD mice with non-significant difference with HFD+I mice. In conclusion, SEA lowered serum lipids, improved aortic function, decreased liver and cardiovascular pathology in HFD mice, so, it is recommended to purify active molecules from SEA to develop anti-dyslipidemic treatment.

INTRODUCTION

High-fat diet is an important risk factor for development of dyslipidemia, obesity, non-alcoholic fatty liver (NAFL) and cardiovascular disorders (Savini *et al.*, 2013). In 2014, about 1.9 billion people were obese worldwide mainly because of consuming carbohydrate and fat rich diet (WHO, 2015). Obesity is usually associated with chronic inflammation resulting in elevated levels of pro-inflammatory cytokines and reactive

oxygen species (D'Souza *et al.*, 2015). NAFL is one of the most common forms of hepatic disorders worldwide. The disease is characterized by accumulation of enormous intrahepatic fat without extreme alcohol consumption or any other secondary cause, and it can progress slowly leading to liver fibrosis, cirrhosis and even hepatoma (Loomba & Sanyal, 2013).

Atherosclerosis is a growing health problem, and it causes about one third of all deaths worldwide (WHO, 2015). It is a

chronic inflammatory disorder affecting walls of large and medium arteries, starting by formation of foam cells through local accumulation of lipoproteins within the macrophages and ending with fatty plaque formation and narrowing of arterial lumen. Macrophages in intima of the affected arteries differentiate into either classically activated M1 or alternatively activated M2 phenotypes. M1 macrophages release pro-inflammatory cytokines, such as IL-1 β and TNF- α inducing a Th1 immune response, while M2 macrophages produce anti-inflammatory cytokines, like TGF- β 1 and IL-10 initiating Th2 immune response which has role in controlling inflammation (Moss & Ramji, 2016; Pirillo *et al.*, 2018). Other hallmarks of atherosclerosis include proliferation of smooth muscle cells, endothelial necrosis, and arterial bed fibrosis (Wu *et al.*, 2017).

Different cytokines can influence all stages of atherosclerosis formation (Fatkhullina *et al.*, 2016). The endothelial dysfunction and injury resulting from HFD cause tissue ischemia, thereof increasing production of proangiogenic substances, such as VEGF, which in turn induces recruitment of CD34⁺ progenitor endothelial cells, which are mainly derived from bone marrow, to maintain the vascular integrity (Ricottini *et al.*, 2016). Mobilization of CD34⁺ endothelial cells into the circulation depend on many factors, including cytokines (Altabas *et al.*, 2016).

Schistosoma mansoni, a blood-dwelling trematode, is one of the most prevalent parasites in many tropical countries (WHO, 2016). After depositing their eggs, *Schistosoma* derives immune response of the affected host from Th1 toward Th2 with subsequent induction of regulatory T cells and production of TGF- β 1 and IL-10 (Pearce & MacDonald, 2002). It was found that schistosomes and their prepared soluble egg antigens (SEA) can alleviate severity of many inflammatory diseases, for example multiple sclerosis (Libbey *et al.*, 2014), and diabetes (Osada *et al.*, 2017). Also, schistosomes can reduce serum lipids in experimental animals (Doenhoff *et al.*, 2002; Wolfs *et al.*, 2014)

and in human (Dimenstein *et al.*, 1992) lowering the risk of atherosclerosis (Shen *et al.*, 2015). Therefore, the aim of the present study was to evaluate therapeutic effects and possible underlying mechanisms of actions of *Schistosoma* SEA in HFD-fed mice.

MATERIALS AND METHODS

Animals

Forty male Swiss albino mice (6-8 weeks old), weighing approximately 25 \pm 2.3 grams were recruited in the present study. The study was conducted in the Schistosome Biological Supply Program (SBSP), Theodor Bilharz Research Institute (TBRI), Giza, Egypt. The mice were housed on a 12-h light-dark cycle, with optimum conditions of humidity (60 \pm 10%) and temperature (21 \pm 1 $^{\circ}$ C), into polypropylene cages having cover screens made of stainless steel. The experiments were carried out following the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85-23, revised in 1996).

Diets

Mice were either fed SD containing 12% fat, 28% protein and 60% carbohydrate (4.6 kilo calories/g daily), or HFD containing 29% fat, 24% protein, 47% carbohydrate enriched with lard, egg yolks, wheat flour, corn starch, casein, vitamins and minerals mixture (5.7 kilo calories/g daily) (Neves *et al.*, 2007). Diet was prepared freshly each week and stored at room temperature. Water and food were allowed to the animals *ad libitum*.

S. mansoni infection

Cercariae used in this work were of the Egyptian strain of *S. mansoni* collected from laboratory bred infected *Biomphalaria alexandrina* snails at TBRI. The infected mice group were subcutaneously infected with 50 \pm 5 cercariae (Alencar *et al.*, 2009).

Preparation of *S. mansoni* SEA

SEA was obtained from SBSP unit, TBRI. The antigen was prepared by homo-

genization of *S. mansoni* eggs that were purified from liver and intestine of infected mice. Then, it was centrifuged at 100,000 g for 90 minutes (min) at 4°C and the supernatant was taken (Boros & Warren, 1970). SEA was dissolved in sterile endotoxin-free PBS, its total protein content was determined with Bradford protein assay Kite (Sigma, USA), and kept at -20°C until use. Mice were injected subcutaneously with SEA in a dose of 50 µg weekly for 5 weeks (Wolfs *et al.*, 2014).

Experimental animal groups

After acclimatization for 1 week, 40 mice were divided into four groups (10 mice, each) as the followings; (1) SD fed: mice were fed SD for 16 weeks and this group served as the control group, (2) HFD fed: mice were fed HFD for 16 weeks, (3) HFD+I: mice were fed HFD for 8 weeks, and then infected by *S. mansoni* cercariae and (4) HFD+SEA: mice fed HFD for 8 weeks, and then treated with SEA. All the mice groups were euthanized 16 weeks after starting the experiment.

Doppler flowmetry

Before sacrificing of the mice, assessment of the vascular hemodynamics was achieved by measuring artery resistance and blood flow velocity using pulsed Doppler flowmeter (Hayashi Denki Co. Ltd., Japan). Briefly, the mice were anesthetized with 25% urethane (0.6 ml/100gm, intraperitoneal injection). A midline laparotomy was performed exposing the abdominal aorta. After tip of the probe was filled with coupling gel, the probe was placed over the aorta until a stable record could be achieved.

Serum preparation

All the mice were fast overnight and after accomplishing the Doppler flowmetry, they were euthanized by cardiac puncture. The blood was allowed to coagulate for 30 min at room temperature, then centrifuged at 2000 rpm for 10 min to separate serum samples which were stored at -20°C until use.

Serum lipids assays

Serum levels of TC, HDL-C, and TG were determined by fixed rate colorimetric methods on a Jenway Genova autoanalyzer (UK) using commercial enzymatic kits (Sigma-Aldrich, USA). LDL-C concentration was calculated according to Friedewald *et al.* (1972) equation, where $LDL-C = TC - (HDL-C + TG/5)$.

Histopathological examination

At autopsy, liver, aorta and heart specimens were obtained from each mouse. The tissues were immediately washed with saline, then fixed in 10% buffered formalin. After embedding the specimens in paraffin, the deparaffinised sections were stained with hematoxylin and eosin (H & E) and examined microscopically following standard procedures.

CD 34+ immunohistochemical staining

The procedure was performed using anti-CD34+ antibody kit (Abcam, UK) according to the manufacturer's protocol. Briefly, the paraffin embedded myocardium sections (4 µm in thickness) were fixed on slides, deparaffinised and dehydrated. The blocking serum was loaded on the sections. Then, the sections were incubated with monoclonal mouse CD34+ antibody for 2 h and washed with phosphate buffer solution (PBS). After that, biotinylated secondary antibody (Dako, Denmark) was added for 30 min followed by washing step. Peroxidase labelled streptavidin was put on the sections for 20 min, and the slides were washed. Chromogen substrate (3,3'-diaminobenzidine-tetrahydrochloride) mixture was incubated with the sections for 10 min, which were then dipped with distilled water and counterstained with Myer's hematoxylin (Sigma, USA). Finally, the slides were washed by tap water, and covered for microscopic examination. Immunohistochemical evaluation was performed qualitatively according to the staining intensity and classified into negative, weak, moderate and strong (Fedchenko & Reifenrath, 2014).

Cytokines and VEGF ELISAs

Serum levels of IL-1 β , TGF- β 1, IL-10 and VEGF (Quantikine[®] ELISA, R&D Systems Inc., MN, USA) were determined by quantitative sandwich enzyme immunoassay technique using an automatic optical reader (SUNRISE Touchscreen, TECAN, Salzburg, Austria).

Statistical analysis

Statistical analysis was performed using SPSS version 20 (SPSS Inc., Illinois, USA). Data were presented as mean \pm standard deviation (sd). The mice groups were compared with the control or with each other using unpaired Student's *t*-test. Values of $p < 0.05$, $p < 0.01$ and $p < 0.001$ were considered as statistically significant.

RESULTS

Serum lipid concentrations

Serum concentrations of TC, HDL-C, LDL-C and TG, in the studied mice groups are shown in Table 1. Mice fed HFD showed high significant ($p < 0.001$) increase in serum levels of TC (166.17 \pm 4.77 mg/dl), LDL-C (99.04 \pm 5.19 mg/dl) and TG (131.52 \pm 9.82 mg/dl) with significant ($p < 0.01$) decrease in serum level of HDL-C (40.83 \pm 1.74 mg/dl) when compared to SD mice (TC, HDL-C, LDL-C and TG; 86.48 \pm 2.43, 50.66 \pm 1.95, 22.34 \pm 3.69 and 67.36 \pm 5.59, mg/dl, respectively). HFD+SEA mice showed high significant ($p < 0.001$) reduction of TC (102.37 \pm 5.26 mg/dl), LDL-C (39.7 \pm 3.65 mg/dl) and TG (88.62 \pm 6.71 mg/dl) with significant ($p < 0.05$) increase of HDL-C

(44.92 \pm 1.6 mg/dl) when compared to HFD mice. However, serum levels of TC, LDL-C and TG were still significantly higher ($p < 0.001$) and HDL-C level was significantly lower ($p < 0.05$) than that of SD mice. Serum lipid levels of *S. mansoni* infected mice (HFD+I) showed similar pattern as in SEA treated mice, where HFD+I mice showed high significant ($p < 0.001$) reduction in serum TC (105.31 \pm 6.17 mg/dl), LDL-C (41.6 \pm 3.54 mg/dl) and TG (89.58 \pm 7.93 mg/dl) and significant ($p < 0.05$) increase of HDL-C (45.79 \pm 1.84 mg/dl) when compared to HFD mice. However, serum levels of TC, TG and LDL-C were still significantly higher ($p < 0.001$) and HDL-C level was significantly lower ($p < 0.05$) when compared with SD mice. There was non-significant ($p > 0.05$) difference between the lipid concentrations of HFD+SEA and HFD+I mice.

Doppler examinations

Examination of the aortic artery using Doppler flowmetry (Table 2) showed that arterial resistance increased (1.31 \pm 0.05 peripheral resistance unit (PRU)) significantly ($p < 0.001$), whereas, velocity of blood flow decreased (5.31 \pm 0.03 cm/sec) significantly ($p < 0.001$) in HFD mice when compared to SD mice (0.61 \pm 0.04 PRU & 7.42 \pm 0.32 cm/sec, respectively). Arterial resistance in HFD+SEA and HFD+I mice groups (0.91 \pm 0.02 and 0.93 \pm 0.06 PRU, respectively) was significantly lower ($p < 0.01$) than that of HFD mice, but it was still significantly higher ($p < 0.01$) than that of SD mice. On the other hand, blood flow velocity of HFD+SEA and HFD+I mice groups (6.58 \pm 0.45 and 6.34 \pm 0.14 cm/sec,

Table 1. The effects of SEA treatment on the serum lipids

Serum lipids (mg/dl)	SD	Mice groups		
		HFD	HFD+I	HFD+SEA
TC	86.48 \pm 2.43	166.17 \pm 4.77***	105.31 \pm 6.17****††	102.37 \pm 5.26****††
HDL-C	50.66 \pm 1.95	40.83 \pm 1.74**	45.79 \pm 1.84*†	44.92 \pm 1.6*†
LDL-C	22.34 \pm 3.69	99.04 \pm 5.19**	41.6 \pm 3.54****††	39.7 \pm 3.65****††
TG	67.36 \pm 5.59	131.52 \pm 9.82***	89.58 \pm 7.93****††	88.62 \pm 6.71****††

Data are expressed as means \pm sd. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ are of significant difference in comparison with SD mice, whereas, † $p < 0.05$, †† $p < 0.01$ and ††† $p < 0.001$ are of significant difference in comparison with HFD mice. TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol and TG: triglycerides.

Table 2. The effects of SEA treatment on the artery resistance and the blood flow velocity

Doppler parameters	SD	Mice groups		
		HFD	HFD+I	HFD+SEA
Artery Resistance (PRU)	0.61±0.04	1.31±0.05***	0.93±0.06**††	0.91±0.02***††
Blood Flow Velocity (cm/sec)	7.42±0.32	5.31±0.03***	6.34±0.14*†	6.58±0.45*†

Data are expressed as means ± sd. **p<0.01 and ***p<0.001 are of significant difference in comparison with SD mice, whereas, †p<0.05 and ††p<0.01 are of significant difference in comparison with HFD mice. PRU: peripheral resistance unit.

respectively) was significantly higher (p<0.05) than HFD, but significantly lower (p<0.05) than that of SD mice groups.

Histopathological results

Liver sections of SD mice (Figure 1-a) showed normal architecture of hepatic lobules without any remarkable pathological changes. The HFD mice had pale, greasy and enlarged livers in comparison with the control group when examined

grossly. Liver sections of that group revealed blood congestion in the portal vein, dilatation of sinusoids with inflammatory infiltrates, while the hepatocytes showed cytoplasmic vacuolation, extensive intra-hepatic fatty deposition whether micro- or macrovesicular steatosis, hepatocytes enlargement, besides to that some hepatocytes were binuclear (Figure 1-b). HFD+I mice revealed a lot of *Schistosoma* granulomas consisting of the eggs

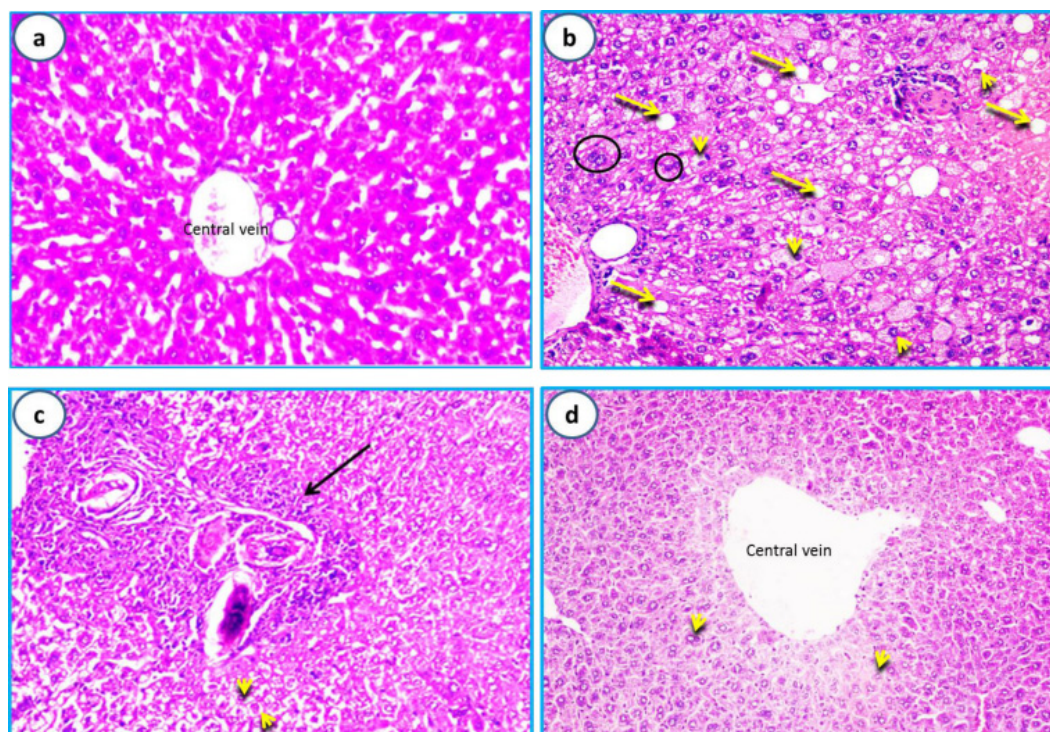


Figure 1. Liver section stained with H&E; (a) SD mice showing normal liver with no lipid deposits (x400), (b) HFD mice having binucleated cells (black circles), macrovesicular steatosis (yellow arrow) and microvesicular steatosis (yellow arrowheads) (x200), (c) HFD+I mice revealing *Schistosoma* granuloma (black arrow) consisted of eggs surrounded by chronic inflammatory infiltrates and microvesicular steatosis (yellow arrowheads) outside the granuloma (x200) and (d) HFD+SEA mice showing less microvesicular steatosis (yellow arrowheads) (x200).

surrounded by chronic inflammatory infiltrates, in addition to congestion, dilatation of portal tracts with inflammatory cellular infiltrations, necrosis, fibrosis and loss of normal architecture of the hepatocytes. Also, there was no fatty infiltration in vicinity of the granuloma with mild microvesicular steatosis of the hepatocytes outside the granuloma (Figure 1-c). The SEA treated mice showed better improvement in the liver architecture than that occurred in HFD+I with few microvesicular steatosis and less lymphocytes infiltration (Figure 1-d).

Cut sections of the aortic arch of SD mice showed normal histological appearance regarding endothelium, sub-endothelium, media and adventitia (Figure 2-a). HFD mice showed denuded endothelium, vacuolar changes and foamy macrophages in sub-endothelium, increased thickness of media with degenerative cystic

changes in media with presence of sub-endothelial lipid clefts and congested blood vessels in vasa vasorum (Figure 2-b). On examining aortic sections of HFD+I mice, there were observable improvement in atherosclerotic changes in the vascular wall with mild sub-intimal vacuulations, few foamy macrophages and mild inflammatory infiltrates in sub-adventitial tissue (Figure 2-c). HFD+SEA mice showed a progressive improvement in the aortic sections, where only few sub-intimal foamy macrophages and few vacuolation were detected (Figure 2-d).

Hearts of SD mice were of normal size and shape on gross study and without any abnormal pathology changes on routine H & E histopathological examination of myocardium (Figure 3-a). In HFD mice, the heart revealed cardiac hypertrophy in gross examination and the heart sections showed severe blood vessels congestions

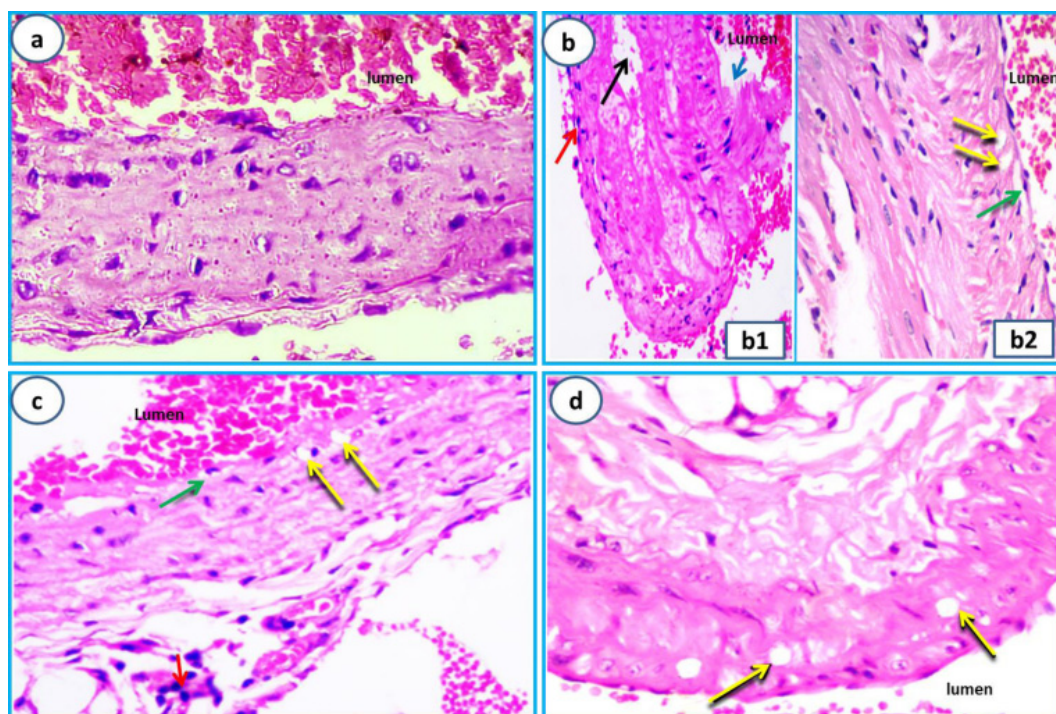


Figure 2. Aortic sections stained with H&E (x400); (a) SD mice showing normal aortic tissue with no atherosclerotic changes, (b) HFD mice having denuded endothelial cells (b1, blue arrow), cystic degeneration (b1, black arrow), inflammatory cells (b1, red arrow), foamy cells (b2, yellow arrow) and fat cleft (b2, green arrow), (c) HFD+I mice revealing less inflammatory cells (red arrow), foamy cells (yellow arrow), smaller fat cleft (green arrow), than HFD mice and (d) HFD+SEA mice showing vacuolar changes (yellow arrow), with nearly absence of other atherosclerotic changes.

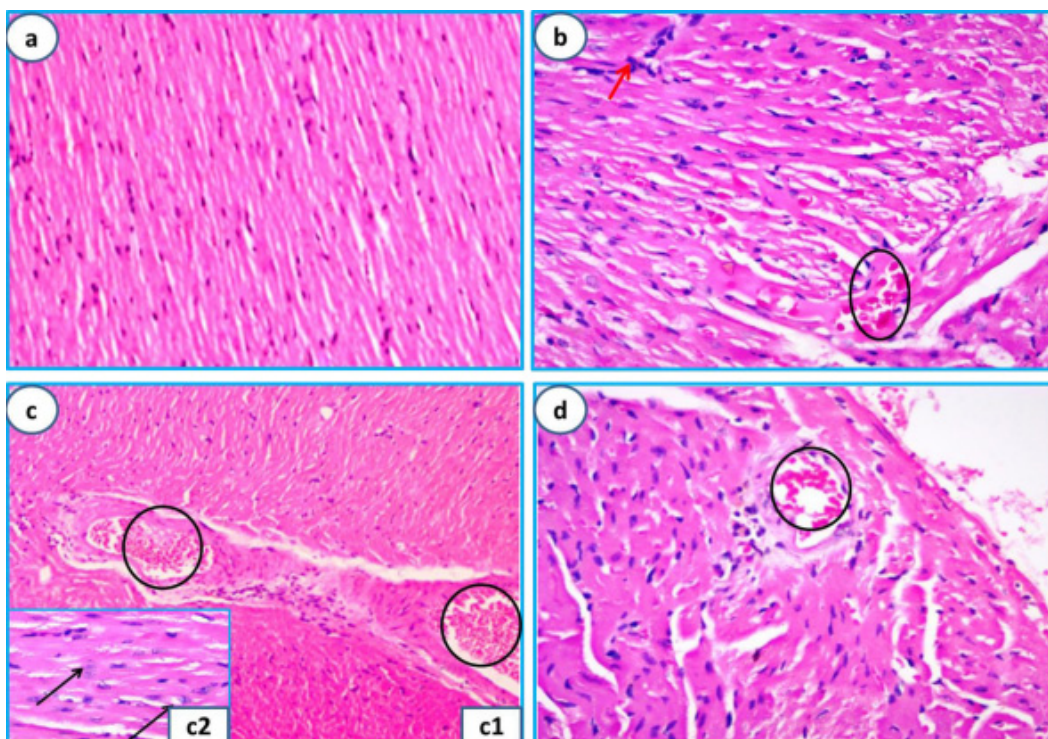


Figure 3. Myocardium sections stained with H&E; (a) SD mice showing normal myocardium tissue (x200), (b) HFD mice having neovascularization (angiogenesis) (black ring) and scattered fat cells and inflammatory cells (red arrows) (x400), (c) HFD+I mice revealing fat cells scattered among myocardial fibres, congestion (c1, black ring) and perivascular inflammatory infiltrates (x200) and histiocytes (c2, black arrows) (x400), and (d) HFD+SEA mice showing less neovascularization (black ring), less inflammatory infiltrates and less fat deposits than HFD mice (x400).

and neovascularization. Many histiocytes and myofibroblasts were identified with perivascular inflammatory cellular infiltrates. Loss of many cardiomyocytes, fibroblast proliferation, disruption of the cardiac tissue were also seen in these sections, in addition to many adipocytes deposits were detected (Figure 3-b). In the heart sections of HFD+I (Figure 3-c) and HFD+SEA (Figure 3-d) mice groups, there were less lipid deposits than HFD group, with slight improvement in the pathological lesions, but still there were congested myocardial blood vessels, many myofibroblasts and inflammatory cellular infiltrates.

Immunohistochemistry

On immunohistochemical staining of the myocardium with anti-CD34⁺ antibodies, sections from HFD mice revealed strong positive CD34⁺ expression, while myo-

cardium sections from the SEA treated mice showed weak positive expression and that of HFD *S. mansoni* infected mice had moderate expression of CD34⁺ (Figure 4 a-d).

Serum cytokines and VEGF

Serum level of IL-1 β increased significantly ($p < 0.001$) in HFD mice (38.93 ± 2.84 pg/ml) in comparison to SD mice (18.62 ± 3.97 pg/ml). HFD+SEA and HFD+I mice groups showed significant ($p < 0.01$) low level of this cytokine (21.91 ± 4.86 and 23.76 ± 5.85 pg/ml, respectively) when compared to HFD mice. Both of these mice groups had high non-significant ($p > 0.05$) high level of IL-1 β in comparing to that of SD mice. SEA treatment decreased the cytokine in a non-significant ($p > 0.05$) level than the *Schistosoma* infection (Figure 5).

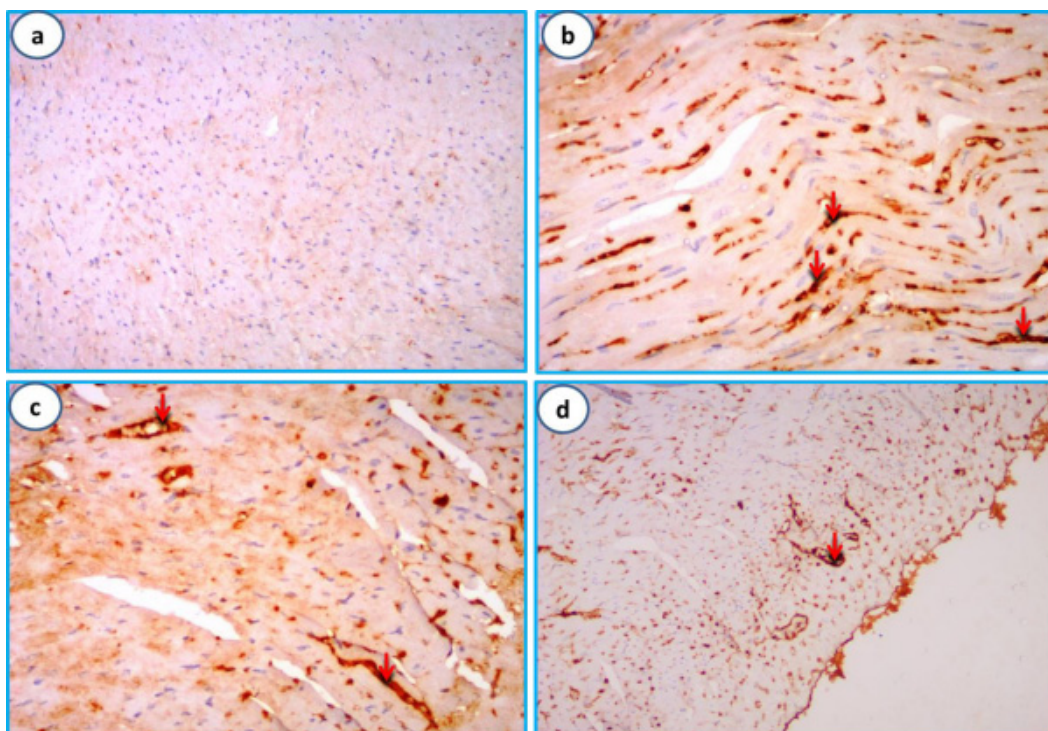


Figure 4. Myocardium sections stained immunohistochemically with anti-CD34⁺ antibodies (x200); (a) SD mice showing negative, (b) HFD mice having strong, (c) HFD+I mice revealing moderate, while (d) HFD+SEA mice showing weak expression of CD34⁺ (red short arrows).

Serum level of TGF- β 1 (23.2 ± 3.02 pg/ml) decreased significantly ($p<0.05$) in HFD mice when compared to SD mice (34.47 ± 3.87 pg/ml). SEA treatment caused higher significant ($p<0.001$) level of TGF- β 1, reaching 97.15 ± 4.62 pg/ml, than HFD or SD mice groups, also, HFD+I mice revealed 93.85 ± 5.2 pg/ml of TGF- β 1 with significant ($p<0.001$) increase in comparison with SD or HFD mice groups. There was non-significant ($p>0.05$) difference between HFD+SEA and HFD+I mice groups concerning this cytokine level (Figure 5).

Serum level of IL-10 (35.7 ± 1.1 pg/ml) decreased significantly ($p<0.05$) in HFD mice when compared to SD mice (41.23 ± 2.1 pg/ml). The level of this cytokine increased significantly ($p<0.001$) in HFD+SEA (62.3 ± 3.2 pg/ml) and HFD+I mice groups (57.61 ± 5.2 pg/ml) when compared with SD or HFD mice group, but without significant difference between HFD+SEA and HFD+I mice groups ($p>0.05$) (Fig. 5).

VEGF increased significantly ($p<0.001$) in HFD in comparison to SD mice group (7.68 ± 0.21 vs 4.17 ± 0.19 pg/ml). SEA treatment decreased significantly ($p<0.01$) VEGF serum level in comparison with HFD mice reaching 5.62 ± 0.25 pg/ml, but it was significantly ($p<0.05$) higher than that of SD mice. Also, *Schistosoma* infection caused significant ($p<0.05$) lowering of VEGF level (6.18 ± 0.34 pg/ml) when compared to HFD mice, however VEGF level was still significantly ($p<0.01$) higher than SD mice group (Figure 5).

DISCUSSION

Feeding of HFD for long time may lead to many medical disorders like dyslipidaemia, steatohepatitis and cardiovascular disease. Herein, we studied efficacy of SEA in improving the pathological and the immuno-

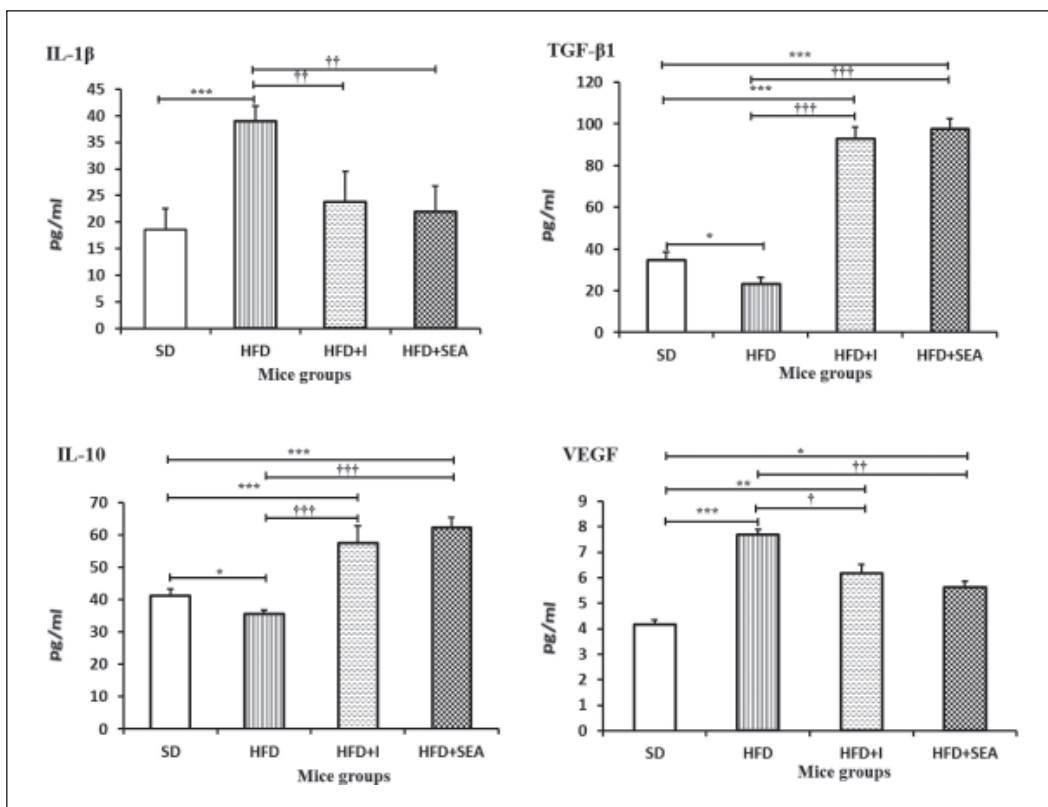


Figure 5. Effect of SEA treatment on serum levels of IL-1 β , TGF- β 1, IL-10 and VEGF which their levels were measured by sandwich ELISAs. Data are represented as mean \pm sd. * p <0.05, ** p <0.01 and *** p <0.001 are of significant difference in comparison to SD mice. † p <0.05, †† p <0.01 and ††† p <0.001 are of significant difference in comparison to HFD mice.

logical effects of long administration of fat on liver and heart in experimental mice.

Mice fed HFD had significant increase in serum levels of TC, LDL-C and TG and decrease in HDL-C level in comparison with SD mice, and these findings are in accordance with other workers (La Flamme *et al.*, 2007; Wolfs *et al.*, 2014). On giving SEA, the mice revealed significant reduction in serum levels of TC, LDL-C and TG with significant increase in HDL-C level comparing with HFD mice. It was noticed that the *Schistosoma* infection (HFD+I) achieved nearly similar effects on the lipids levels as SEA. These results were in parallel with Wolfs *et al.* (2014) who found that SEA significantly reduced plasma TC level in low-density lipoprotein receptor knockout (LDLR $^{-/-}$) mice, La Flamme *et al.* (2007) reported that *Schistosoma* eggs reduced serum levels of TC and TG in apolipoprotein

E knockout (ApoE $^{-/-}$) mice, also, *S. mansoni* infection reduced serum lipids in experimental mice (Doenhoff *et al.*, 2002; Stanley *et al.*, 2009) and in human (Dimenstein *et al.*, 1992; Shen *et al.*, 2015).

There are many mechanisms by which schistosomes can cause hypolipidemia. *Schistosoma* can inhibit cholesterol esterifying in the infected liver (Soares & Borges, 1990). The adult worms cannot synthesize their own cholesterol and they internalize LDL-C from their host (Tempone *et al.*, 1997). The shed glycosylphosphatidylinositol-anchored proteins by the worms into circulation may have a role in reduction of the serum lipoproteins (Sprong *et al.*, 2006). La Flamme *et al.* (2007) found that *Schistosoma* eggs enhance macrophages to uptake serum LDL-C.

In this study, Doppler flowmetry was applied to measure arterial resistance and blood flow velocity reflecting arterial stiffening and endothelial dysfunction that are early markers for developing vascular disorders, such as atherosclerosis (Hilgers & Das, 2015). Significant improvement in blood velocity and decreasing arterial resistance were recorded in HFD+SEA and HFD+I mice groups compared to HFD group and this could be related to lowering serum lipid and this may refer clinically to the benefits of decreasing serum TC and LDL-C on the vascular functions.

Liver sections of HFD mice revealed micro- and macrovesicular steatosis and this was similar to other studies (Neves *et al.*, 2007; Stanley *et al.*, 2009; Lee *et al.*, 2015). SEA treatment caused less microvesicular steatosis and low hepatic fat deposition mostly due to decreasing plasma lipoproteins concentration. Although HFD+I mice revealed low fat content in the region of granuloma, they still showed *Schistosoma* granulomas with loss of normal architecture of the hepatic structure and this is in accordance with Neves *et al.* (2007) and Stanley *et al.* (2009).

Aortic sections obtained from HFD mice revealed variable atherosclerotic changes, but the typical atherosclerotic lesion did not develop in this study because we used wild mice in which HDL-C is the primary lipoprotein that hinders the development of the full atherosclerosis lesion, added to that these mice do not have cholesteryl ester transfer protein, however, ApoE^{-/-} and LDLR^{-/-} mice show the typical atherosclerosis lesions (Getz & Reardon, 2012). SEA treatment improved pathological features in the aortic sections and these were better than that achieved in HFD+I mice. This improvement is in parallel with Wolf *et al.* (2014) who reported that the size of atherosclerotic plaque in SEA treated LDLR^{-/-} mice was less by 44% than in control mice with obvious improvement in severity of the lesion, also, Donehoff *et al.* (2002) reported that atherogenic plaque in aortic arch from *S. mansoni* infected ApoE^{-/-} mice were smaller than that found in the control

animals with 50% inhibition of development of atherosclerosis.

Pathological findings in myocardium of HFD mice are consistent with other reports (Goes *et al.*, 2012). SEA administration lowered lipid deposits in the heart tissues than in HFD mice, but they still had some congested blood vessels and many myofibroblasts. HFD mice subjected to the parasite showed similar results. This slight improvement in both mice groups was due to lowering serum lipids hence decreasing the heart tissues contents of lipids, however, the full recovery of the myocardium might occur over a long time later on. It is worthy to mention that this is the first report that studied the effects of SEA on the myocardium of HFD mice histologically.

In the current work, we measured serum levels of IL-1 β , TGF- β 1 and IL-10 to shed more light on the possible mechanisms used by SEA to decrease serum lipids and improve the HFD induced pathological lesions in liver, aorta and myocardium. HFD mice showed high IL-1 β and low levels of both TGF- β 1 and IL-10 in comparison to SD mice as increased consumption of HFD is associated with increase in production of pro-inflammatory cytokines and Th1 cells activation (Santana *et al.*, 2014). Herein, SEA caused significant decrease in serum level of IL-1 β and increase in levels of TGF- β 1 and IL-10 when compared with HFD mice. This was in accordance with Zhang *et al.* (2012) who found that SEA of *S. japonicum* had anti-atherosclerosis activity in ApoE^{-/-} mice through increasing regulatory T cells proportion and inhibiting production of pro-inflammatory cytokines, also, Wolfs *et al.* (2014) found that the peritoneal macrophages isolated from SEA-treated HFD LDLR^{-/-} mice produced significant high IL-10.

IL-1 β is involved in the vascular wall inflammation and it mediates development of atherosclerotic lesions where its production depends on activation of NLRP3 inflammasome caused by cholesterol crystals (Sheedy *et al.*, 2013), whereas its deficiency reduces atherosclerosis progression (Kirii *et al.*, 2003). TGF- β 1 and

IL-10 have anti-atherogenic effects (Xue-Mei *et al.*, 2017). TGF- β inhibits differentiation of Th1 and Th2 cells, but it promotes differentiation of regulatory T cells (Ait-Oufella *et al.*, 2011). It can inhibit expression of key genes involved in the process of lipoproteins uptake by macrophages (Michael *et al.*, 2012), as well as, TGF- β suppresses fibroblast growth factor of vascular smooth muscle cells, so decreasing plaque size of atherosclerosis (Chen *et al.*, 2016). Overexpression of IL-10 in LDLR^{-/-} mice increases cholesterol efflux from the macrophages and reduces pro-inflammatory cytokines production lowering the atherosclerotic lesion size (Han *et al.*, 2010).

Collectively, the anti-atherosclerotic effects of SEA could be attributed mainly to induction of prominent Th2 immunological profile; increasing TGF- β 1 and IL-10, and alleviating Th1 one; reducing IL-1 β levels. During Th-2 immune response, anti-oxidized LDL IgM antibodies are produced and they bind to oxidized LDL-C particles hindering its uptake by the macrophages preventing foam cells formation (Tsimikas *et al.*, 2007). The anti-inflammatory cytokines, induced by SEA, can promote differentiation of the macrophages in intima of the affected arteries into anti-inflammatory M2 phenotype (Chinetti-Gbaguidi *et al.*, 2015), which are less adherent to the activated endothelium (Wolf *et al.*, 2014).

HFD mice, here, recorded the highest serum VEGF level followed by HFD+I then HFD+SEA mice. Consequently to the endothelial dysfunction caused by HFD and the resulting tissue ischemia which is the main inducer of angiogenesis (Elpek, 2015), there was an increase in level of VEGF accompanied with increasing endothelial progenitor cells CD34⁺ cells in the myocardium tissues in order to maintain vascular integrity (Kawamoto & Losordo, 2008).

Level of VEGF was higher in HFD+I and HFD+SEA mice groups than in SD group and this was in consistence with other studies denoting that *Schistosoma* infection and SEA have ability to induce angiogenesis, through overexpression of VEGF in com-

parison to the controls. SEA increased human umbilical vein endothelial cells proliferation with expression of messenger RNA of VEGF (Luo *et al.*, 2017). *S. japonicum* eggs promoted VEGF production in vicinity of the granuloma (Liu *et al.*, 2014), and the hepatocytes of *S. mansoni* infected mice expressed strongly VEGF (Abd El-Aal *et al.*, 2017). VEGF plays an important role in the angiogenesis process as it regulates vasodilatation, vascular permeability and migration of the endothelial cells, and its expression occur mainly by the vascular endothelial cells and several epithelial cells (Hoeben *et al.*, 2004).

This study is the first one which examined CD34⁺ immunohistochemically in the myocardium of HFD mice model. CD34⁺ is a marker of the vascular endothelial progenitors cells (Fina *et al.*, 1990). The angiogenic molecules, including VEGF, can induce migration of these cells mainly from bone marrow to the injured endothelium (Altabas *et al.*, 2016). CD34⁺ was negative in the normal myocardium tissues, but its expression increased during taken HFD and this denoted prominent angiogenesis in the HFD mice as a consequence of impaired blood flow because of the vascular dysfunction resulted from hyperlipidaemia. It was reported that HFD causes angiogenesis (Silvennoinen *et al.*, 2013; Fang & Tang, 2017). SEA treatment caused an obvious decrease in CD34⁺ expression indicating low angiogenesis, also HFD+I mice showed lower CD34⁺ expression than HFD ones, but still higher than HFD+SEA mice group. This finding could be attributable to improvement of the vascular pathology because of decreasing the lipids in serum and in cardiac tissue, with subsequence improvement of blood flow to the cardiac tissue, hence decreasing serum level of VEGF that is incriminated in attraction of CD34⁺ cells to the injured endothelium.

In conclusion, SEA reduced serum lipids, improved aortic function as indicated by Doppler flowmetry, decreased pathological changes in liver, myocardium, alleviated atherosclerotic changes and declined angiogenesis in HFD mice and

these seem to be through immunomodulation against hyperlipidaemia inducing Th2 pro-inflammatory condition, so it is recommended to purify active molecules from SEA offering new visions to generate novel therapeutics against dyslipidaemia.

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CONFLICT OF INTEREST

None.

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