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ORIGINAL ARTICLE

Antioxidant and cardioprotective activity of *Stachys schimperi Vatke* against doxorubicin-induced cardiotoxicity

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Abstract Cardiotoxicity is one of the major side effects of anthracycline antibiotics. Most studies implicated increased oxidative stress as the major determinant of doxorubicin (DOX) cardiotoxicity. The aim of the current investigation was to study the possible cardioprotective effect of *Stachys schimperi Vatke* (family Lamiaceae) on DOX-induced cardiotoxicity in rats based on biochemical and histopathological parameters. The phenolic profile of the methanol extract was determined qualitatively by HPLC. Isoscutellarein 7-*O*-[2″-*O*-(6‴-acetyl)-β-D-allopyranosyl]-β-D-glucopyranoside (compound **1**) was isolated and identified from EB fraction as a major constituent for the first time from this *Stachys* species. The methanolic extract and the combined EtOAc and n-butanol fractions (EB) as well as compound **1** showed prominent free radical scavenging activity when assessed by the DPPH method. The methanolic extract showed moderate protection against DOX-induced alteration in cardiac oxidative stress markers; GSH and MDA, and cardiac serum markers; CK-MB and LDH activities. Additionally, histopathological study denoted mild protection against DOX-induced cardiotoxicity.

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It was concluded that *Stachys schimperi Vatke* methanolic extract protected against DOX-induced cardiotoxicity, at least in part, by virtue of its antioxidant activity.

1. Introduction

Doxorubicin (DOX) is one of the most effective antitumor antibiotics belonging to the class of anthracyclines. However, its use is limited by a high incidence of cardiotoxicity.[1] With the increase of its use, an acute cardiotoxicity has been recognized as a severe complication of DOX chemotherapy.[2] Although numerous mechanisms have been proposed, most studies supported that an increase in oxidative stress, along with reductions in the levels of antioxidants, which play a key role in the pathogenesis of DOX-induced cardiomyopathy.[3,4] Therefore, the use of natural or synthetic antioxidants might protect against the oxidative stress caused by DOX and other cytotoxic drugs.[5] Diets rich in fruits and vegetables have been associated with decreased risks of several chronic diseases, such as coronary heart disease and some cancers.[6–8] These protective effects have been attributed partly to the various antioxidant compounds, e.g. vitamins C and E, β-carotene and polyphenolics.[9] Lycopene, a carotenoid occurring in tomatoes[10] and gingerols in *Zingiber officinale*[11] were found to protect against DOX-induced nephrotoxicity. The antioxidant properties of flavonoids were shown to reduce DOX-toxicity due to their ability to chelate free radical.[12–14] Investigations on the antioxidant activity of herbs that are used for treating cancer show that polyphenols are the carriers of these properties.[15,16] Phenolic compounds are known to act as antioxidants not only because of their ability to donate hydrogen or electrons but also because they are stable radical intermediates.[17] Probably the most important natural phenolics are flavonoids because of their broad spectrum of chemical and biological activities including radical scavenging properties.[14] Previous reports on *Stachys* species have shown the presence of flavonoids and phenolic acids,[18–22] indicating that these plants could possess antioxidant activity.[22]

Genus *Stachys* is one of the largest genera of the family Lamiaceae. Plants of this genus have been used in folk medicine for centuries to treat genital tumors, sclerosis of the spleen, inflammatory diseases, cough and ulcers.[23] In the course of our interest in DOX-cardioprotection from Saudi plants,[13,14] *Stachys schimperi* was selected to determine its polyphenols profile, *in vitro* and *in vivo* antioxidant activity, in addition to its possible cardioprotective activity.

2. Experimental

2.1. General

^{1}H NMR spectra were measured on Bruker DRX-400, instrument (^{1}H NMR, 400 MHz, ^{13}C, 100 MHz) in CD_3OD and chemical shifts were given in ppm relative to TMS as the internal standard. The spectrophotometric measurements were carried out using a Carry 300 Scan spectrophotometer (Varian, USA). Standard phenolic acid, flavonoidal aglycones and glycosides and other chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA) or from Carl Roth GmbH (Buchenau, Germany). All solvents used are of analytical grade or HPLC grade and were purchased from Merck (Darmstadt, Germany).

2.2. Plant material

The plant was collected from Al-Taif-Al-Baha road in May 2009. A herbarium specimen was deposited at the Herbarium of the Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University (#SS1095). The collected plant was identified by the staff of the Department of Biology, Faculty of Science, King Abdulaziz University. Aerial parts were air-dried and subjected to grinding, then kept in dark air-tight closed containers until the extraction step.

2.3. Extraction and isolation

The air-dried powdered materials (700 g) were extracted with methanol (3 × 1500 ml) at room temperature using Ultra turrax T25 homogenizer (Janke and Kunkel, IKA Laboratories, Stauten, Germany). The crude extract (120 g) was fractionated with petroleum ether, chloroform, ethyl acetate and *n*-butanol, followed by the determination of their free radical scavenging activity. The EtOAc and *n*-butanol showed similar chromatographic patterns and were combined together to give EB fraction. The EB fraction (with highest antioxidant activity) was subjected to fractionation over Si gel 60 H column (160 g, 4 × 100 cm) using EtOAc–MeOH–H_2O (17.5:10.5:1.0) to give three main fractions (frs **A**–**C**). Fraction **B** (822 mg) was subjected to chromatography on a Sephadex LH-20 column (3 × 22 cm), using a mixture of MeOH–H_2O (1:1) as mobile phase to afford 10 major fractions (**B-1** to **B-10**). Fr. **B-2** (121 mg) was further purified on a reversed-phase RP-18 Si gel (1 × 20 cm, flow rate 4 ml/min, 10 ml fraction) using MeOH–H_2O (11:9) as eluent. The eluted fractions were monitored by TLC using the following systems, S1: *n*-butanol–acetic acid–water (3:1:1) and S2: EtOAc–MeOH–H_2O (17.5:10.5:1.0). TLC chromatograms were visualized under UV-light and by spraying with $AlCl_3$, as well as by *p*-anisaldehyde. Fractions eluted between 19 and 27 afforded 25 mg of pure compound **1**.

2.4. HPLC analysis

HPLC analysis was performed on Hewlett Packard 1100, equipped with a quaternary pump, and connected to diode-array detector (DAD) and managed by a HP 9000 workstation (Agilent Technologies, Palo Alto, CA, USA). Hibar® Prepacked column RT (RP-18, 250 × 4.6 mm, 5 μm, Merck, Darmstadt, Germany) maintained at 26 °C was used in HPLC analysis. The mobile phases are (A) aqueous formic acid solution (pH 3.2) and (B) acetonitrile. The following multi-step linear gradient was applied: from 13% to 15% B/A in 10 min, 15% to 25% B/A in 10 min, isocratic 25% B/A for 5 min; 25% to 60 % B/A in 12 min and a final step of 5 min to initial conditions.

Flow rate was 0.8 ml/min, and oven temperature was 26 °C. The Diode-detector (UV–VIS) spectra were recorded between wave length 220 and 500 nm and the chromatograms followed at 254 nm for isoflavonoids, 280 for phenolics, and 330 nm for flavonoids. All authentic and test samples were injected in triplicate (20 µl). Identification of the major constituents of the MeOH extract was performed using HPLC-DAD based on comparison of the retention times and superimposed spectra with reference samples.

2.5. In vitro antioxidant activity

2.5.1. Reducing power

The reducing power of the methanolic extract was determined according to the method of Oyaizu.[24] The sample solution (50, 100 or 200 µg/ml) in methanol (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide and the mixture was incubated at 50 °C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid were added, and the mixture was centrifuged at 200 g for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride. Finally the absorbance was measured at 700 nm against a blank. Ascorbic acid was used as a positive control.

2.5.2. Total antioxidant activity

The total antioxidant activity of the methanolic extract was assessed using the linoleic acid system.[25] The sample solution (100 µg/ml; 0.2 ml) was added to a solution of linoleic acid (0.13 ml), 99.8% ethanol (10 ml), and 0.2 M phosphate buffer (pH 7.0, 10 ml). The total volume was adjusted to 25 ml with distilled water. The reaction mixture was incubated at 40 °C, and the degree of oxidation was measured according to the thiocyanate method[26] by sequentially adding ethanol (10 ml, 75%), ammonium thiocyanate (0.2 ml, 30%), sample solution (0.2 ml), and ferrous chloride (20 mM in 3.5% HCl) solution (0.2 ml). After the mixture was stirred for 3 min, the peroxide value was determined by reading the absorbance at 500 nm, and the percentage inhibition of linoleic acid peroxidation, 100 − [(absorbance increase of sample/absorbance increase of control) × 100], was calculated to express antioxidant activity. All tests and analyses were run in triplicate and averaged. BHA was used as a positive control.

2.5.3. DPPH radical scavenging activity

The free radical scavenging activity of the methanolic extract of *S. schimperi*, as well as its fractions (petroleum ether, chloroform and EB fractions) thereof was determined based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) using the method described by Braca et al.[27] An aliquot (0.1 ml) of test samples (50, 100 or 200 µg/ml) was mixed with 3 ml of a 0.004% MeOH solution of DPPH and kept in the dark for 30 min. The absorbance was determined at 517 nm, and the percentage inhibition activity was calculated from the following equation:

$$[(A_0 - A_1)/A_0]/100,$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract or standard. The DPPH solution without sample solution was used as control. All tests were run in triplicate. Ascorbic acid was used as positive control.

2.6. In vivo studies

2.6.1. Animals and experimental protocol

Male Wister rats, weighing 250–300 g were used in the study in accordance with the guidelines of the Biochemical and Research Ethical Committee at King Abdulaziz University, Jeddah, Saudi Arabia. Animals were housed in a well-ventilated, temperature-controlled room at 22 ± 3 °C with 12 h light-dark cycle. Food consisted of normal rat chow and water was provided *ad libitum*. Care was taken to avoid stressful conditions. All experimental procedures were performed between 8 and 10 a.m. Rats were randomly assigned into four groups (12 rats each). Group I received CMC (0.5%) (1 ml/200 g body weight/day) orally for 10 consecutive days. Group II received CMC orally for 10 consecutive days and a single dose of DOX (15 mg/kg, i.p.) (EBWE Pharma, A-4866 Unterach, Austria) on day 7.[28] Group III received the methanolic extract of *S. schimperi*; suspended in 0.5% CMC (100 mg/kg; orally once daily for 10 consecutive days). Group IV received the plant extract combined with DOX in the previously mentioned doses. The plant extract was administered for 10 consecutive days and DOX was administered once on day 7. Twenty-four hours after the plant extract or CMC treatment (day 11), rats were anesthetized with thiopentone (35 mg/kg; i.p.). Blood samples were collected by orbital puncture in serum separating tubes. The blood was centrifuged at 3000*g* for 15 min to separate the sera that were kept at −70 °C for biochemical analyses. Abdomen of each rat was opened and hearts were rapidly dissected out, washed in ice-cold isotonic saline and blotted between two filter papers. Four hearts from each group were fixed in 10% formalin for histopathological examination and the remaining hearts from each group were homogenized in ice-cold 0.1 M potassium phosphate puffer (pH 7.4) and stored at −70 °C for subsequent analyses.

2.6.2. Cardiac biochemical assay

Cardiac GSH content was determined according to the method of Adams et al.[29] GSSG level was assessed according to the method of Hissin and Hilf[30] and values were expressed as nmol/mg protein. Lipid peroxidation products were determined by measuring malondialdehyde (MDA) content in tissue homogenates according to the method of Mihara and Uchiyama.[31] The MDA content was measured spectrophotometrically at 532 nm. The MDA content was calculated based on a standard curve using 1,1,3,3-tetraethoxypropane as a standard. Values are expressed as nmol/g protein.

2.6.3. Serum biochemical assay

Creatine kinase isoenzyme-MB (CK-MB) and lactate dehydrogenase (LDH) activities were determined according to standard methods using diagnostic kits from BioSystems S.A. (Barcelona, Spain). CK-MB activity was assayed by measuring the rate of NADPH formation at 340 nm.[32] LDH activity was determined by measuring the rate of nicotinamide adenine dinucleotide reduced form (NADH) formation at 340 nm.[33]

2.6.4. Histopathological study

Hearts were cut at 0.5 µm, mounted on slides, stained with hematoxylin and eosin (H&E) and examined under a light microscope (Olympus BX-50 Olympus Corporation, Tokyo, Japan).

2.7. Statistical analysis

Results are expressed as means ± SEM. Assessment of results was performed using one-way analysis of variance (ANOVA), followed by Tukey–Kramer test for multiple comparisons using Graph Pad In Stat software, Version 4 (Graph Pad Software Inc., La Jolla, CA, USA). The statistical significance was accepted at p value below 0.05.

2.8. Isoscutellarein 7-O-[2″-O-(6‴-acetyl)-β-D-allopyranosyl -β-D-glucopyranoside

Yellow amorphous powder, mp 260–262 °C (uncorrected); UV/Vis λ_{max} (MeOH) nm: 276, 306, 327, (NaOMe): 247, 275, 377; (AlCl3): 280, 322, 347; ($AlCl_3$ + HCl): 281, 322, 345 (sh); (NaOAc): 275, 304, 329 (sh), 386; (NaOAc + H_3BO_3): 276, 305, 327 (sh); $_1$H NMR (400 MHz, CD_3OD): δ7.92 (2H, d, J = 8.40 Hz, H-2′, 6′), 6.95 (2H, d, J = 8.4 Hz, H-3′, 5′), 6.63 (1H, s, H-3), 6.78 (1H, brs, H-6), 4.93 (1H, d, J = 6.9 Hz, H-1″), 5.07 (1H, d, J = 6.9 Hz, H-1‴), 2.1 (3H, s, CO$\underline{CH_3}$), rest of sugar protons 3.5–4.2. ^{13}C NMR (100 MHz, CD_3OD): δ 184.64 (C-4),173.11 (CO), 166.83 (C-2), 163.16(C-4′), 154.23 (C-5), 152.23 (C-7), 145.46 (C-9), 129.96 (C-2′,6′), 129.87 (C-8), 123.3 (C-1′), 117.18(C-3′,5′), 107.86 (C-10), 103.87 (C-3), 104.35 (C-1‴),103.06 (C-1″), 84.14 (C-2″), 78.62 (C-5″), 77.70 (C-3″), 73.54 (C-5‴), 73.23 (C-2‴), 72.73 (C-3‴), 70.9 (C-4″), 68.62 (C-4‴), 65.15 (C-6‴), 62.42 (C-6″), 20.98 (Ac).

3. Results

The present study describes the chemical and biological (cardioprotective effect) evaluation of the methanolic extract of the aerial parts of *S. schimperi*.

3.1. HPLC analysis

Qualitative HPLC profile of the methanolic extract of *S. schimperi* was performed using HPLC coupled with DAD detector and monitored at wave lengths 254, 280 and 330 nm. Qualitative HPLC analysis of the major peaks of the methanolic extract was based on the comparison of their retention times and superimposed spectra with reference standards. The following flavonoids were identified: rutin, kaempferol, isorhamnetin, luteolin, hypersoid, and isoflavonoid daidzein; phenolic acids: (*E*)-hydroxyl cinnamic acid, hydroxy-4-phenylbutanoic acid, syringic acid, vanillic acid, ferulic acid and p-coumaric acid; in addition to the isolated isoscutellarein 7-*O*-[2″-*O*-(6‴-acetyl)-β-D-allopyranosyl-β-D-glucopyranoside flavonoid glycoside from EB fraction.

3.2. Compound 1

Compound **1** was identified using spectral analysis (UV, 1D and 2D NMR). UV analysis in methanol (λ_{max} nm: 276, 306, 327) and after addition of different shift reagents revealed a flavone skeleton.[34] The presence of two anomeric protons and carbons (^{1}H and ^{13}C NMR) indicated a diglycoside flavone. The sugar moieties were identified as allose and glucose based on extensive study of its NMR spectra (2D NMR: H–H COSY, HSQC, HMBC) and by comparison to the data in literature.[35,36] The presence of an acetyl group was confirmed from signals at δ_H 2.1 (check) and δ_C 20.98 which attached to C-6 of the allose moiety (HMBC). The aglycon moiety was identified as isoscutellarein based on its NMR data and by comparison with reported data.[35] From the previous data and by comparison to the reported data, compound **1** was identified as isoscutellarein7-*O*-[2″-*O*-(6‴-acetyl)-β-D-allopyranosyl-β-D-glucopyranoside.[35,36]

3.3. In vitro antioxidant activity

3.3.1. Reducing power

Table 1 shows the reducing power of methanol extract compared to ascorbic acid based on the conversion of the Fe^{3+} into Fe^{2+} in the presence of extract samples using the method followed by Oyaizu.[24] The concentrations 50, 100, and 200 μg/ml were used in the *in vitro* evaluation of the reducing capacity. The reducing power of methanol extracts increases with increasing concentration of sample. The methanolic extract showed a moderate reducing power of 0.61 at a concentration of 200 μg/ml.

3.3.2. Total antioxidant activity

Antioxidant activity of the methanol extract of *S. schimperi* was assessed using the linoleic acid system. The degree of oxidation was measured according to the thiocyanate method. Butylated hydroxyanisole (BHA) was used as a reference standard antioxidant. The results (Table 2) indicate that the methanolic extract at concentration of 100 μg/ml exhibited obvious antioxidant activity (60%) but was not superior to BHA (96%).

3.3.3. DPPH radical scavenging activity

Based on the previous results, the antioxidant activity of the methanolic extract as well as its fractions and compound **1** were examined by exploring the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Table 3). The IC_{50} of all tested samples were determined. The reduction capability of the extract to DPPH radical is determined by the decrease in absorbance at 517 nm induced by antioxidants. Ascorbic acid is commonly used as a reference standard. All samples were able to reduce the stable radical DPPH to the yellow colored diphenylpicrylhydrazine. Thus, all samples exhibited observable free radicle scavenging activity in a dose-related manner (Table 3).

The results of scavenging activity of DPPH free radical (IC_{50}) of the methanol extract, EB fraction and compound **1** were as follows: 62.1, 10.3 μg/ml and 4.95 ng/ml, respectively.

3.4. In vivo studies

3.4.1. Effects of the methanolic extract of S. schimperi on tissue and serum cardiac markers

The data in Table 4 show the effect of administration of the tested extract (100 mg/kg/day) on the oxidant status in cardiac

Table 1 Reducing power of the methanol extract of *S. schimperi*.

	Absorbance at 700 nm		
	50 μg/ml	100 μg/ml	200 μg/ml
S. schimperi	0.31 ± 0.01	0.35 ± 0.02	0.61 ± 0.05
Ascorbic acid	1.53 ± 0.06	1.87 ± 0.08	2.87 ± 0.09

Data are the mean ± SEM of six replicates.

Table 2 The antioxidant activity of the methanol extract of *S. schimperi*.

	% Inhibition (100 μg/ml)
S. schimperi	60 ± 0.09
BHA	96 ± 0.12

Data are the mean ± SEM of six replicates; BHA: butylated hydroxyanisole.

Table 3 DPPH free radical scavenging activity of compound **1**, methanol extract and EB fraction.

IC_{50} μg/ml			
Qurecetin	MeOH extract	EB fraction	Compound **1** (ng/ml)
8.04 ± 0.18	62.1 ± 0.30	10.3 ± 0.13	4.95 ± 0.60

Data are the mean ± SEM of six replicates; EB: combined EtOAc and *n*-butanol fractions.

tissues as well as the serum activities of CK-MB and LDH as markers of cardiac injury. The extract exhibited no significant alteration in the assessed parameters as compared to the control (saline-treated) group. This clearly indicates that methanol extract of *S. schimperi* was devoid of any cardiotoxicity under our experimental conditions.

3.4.2. The effect of methanol extract of S. schimperi on DOX-induced alterations in cardiac tissue oxidative stress

The data in (Table 5) indicated that the treatment of rats with DOX resulted in a severe oxidative stress in cardiac tissues as evidenced by significant GSH depletion as well as accumulation of lipid peroxides as marked by MDA. The treatment with *S. schimperi* extract significantly protected against DOX-induced GSH depletion and MDA elevation.

3.4.3. Activity of cardiac serum markers

Activities of serum CK-MB and LDH (Table 5) were assessed as markers of cardiac injury. DOX insult resulted in significant elevation of the activities of both CK-MB and LDH as compared to the control group. Treatment with *S. schimperi* extract showed minor decreases of the level of both LDH and CK-MB with insignificant protection against DOX-induced cardiac injury.

3.5. Histopathological study

Cardiotoxicity induced by DOX was further assessed using hematoxylin and eosin stained sections. Hearts from control group (I) and group received plant methanolic extract only (groups III) showed regular cell distribution and normal myocardium architecture (Fig. 1a and b).

Histological examination of the rat hearts from DOX-only treated rats (group II) revealed severe cytoplasmic vascular degeneration, interstitial edema and fibrotic bands (Fig. 2a). Administration of the methanolic extract in addition to DOX showed mild improvement in the altered histopathological pattern (Fig. 2b) as compared to the DOX group.

4. Discussion

Typical phytochemical constituents of the genus *Stachys* were essential oils,[37,38] C9-iridoidal glycosides,[39] labdane, neo-clerodane,[40] diterpenoids, flavonoids,[41] phenylpropanoids,[42] and phenylethanoids.[43] Some of these constituents were considered worth investigation for potential protection against DOX cardiotoxicity. Of special concern were labdane and flavonoids. Forskolin, a labdane diterpenoid was reported to possess antihypertensive, positive inotropic, platelet aggregation inhibitory, and adenyl atecyclase activating properties.[44] It was shown to improve experimentally-induced heart failure.[45] Flavonoids, like quercetin and rutin were shown to protect against ischemia-reperfusion-induced myocardial infarction

Table 4 Effects of the methanol extract of *S. schimperi* on tissue and serum cardiac markers.

	Cardiac tissue		Serum cardiac markers	
	GSH (μmol/g protein)	MDA (nmol/g protein)	CK-MB (IU/mg protein)	LDH (IU/mg protein)
Control	4.42 ± 0.13	55.3 ± 1.80	93.0 ± 6.70	122 ± 9.80
S. schimperi	4.23 ± 0.21	54.2 ± 2.20	93.5 ± 4.07	119 ± 5.08

Data are the mean ± SEM of six rats.

Table 5 The effect of methanol extract of *S. schimperi* on doxorubicin (DOX)-induced alterations in cardiac tissue oxidative stress; GSH and malondialdehyde (MDA) and serum cardiac markers; CK-MB and LDH activities.

	Cardiac tissue		Serum Cardiac markers	
	GSH (μmol/g protein)	MDA (nmol/g protein)	CK-MB (IU/mg protein)	LDH (IU/mg protein)
Control	4.42 ± 0.13	55.3 ± 1.80	93.0 ± 6.70	122 ± 9.80
DOX	1.93[a] ± 0.06	154[a] ± 5.30	189.1[a] ± 10.0	211[a] ± 10.30
DOX + *S. schimperi*	2.58[a,b] ± 0.11	121[a,b] ± 4.70	155.5[a] ± 7.00	182.4[a] ± 7.70

Data are the mean ± SEM of six rats.
[a] $p < 0.05$ vs. corresponding control group.
[b] $p < 0.05$ vs. corresponding DOX group.

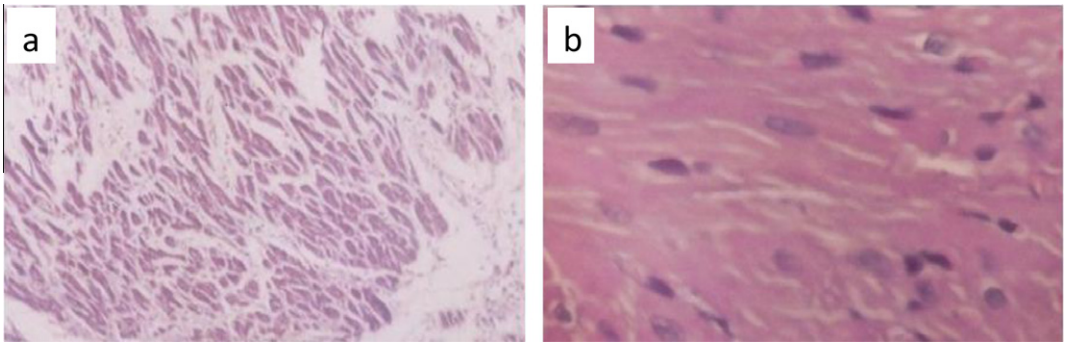

Figure 1 Effect of methanolic extract of *S. schimperi* on cardiac histological pattern. Heart sections showing normal histological pattern (regular cell distribution and normal myocardium architecture) from control group (a) which received carboxymethylcellulose (CMC; 0.5%) for 10 consecutive days and rats which received MeOH extract of *S. schimperi*, (100 mg/kg/day) suspended in CMC (0.5%) for 10 consecutive days (b) (H&E 125×).

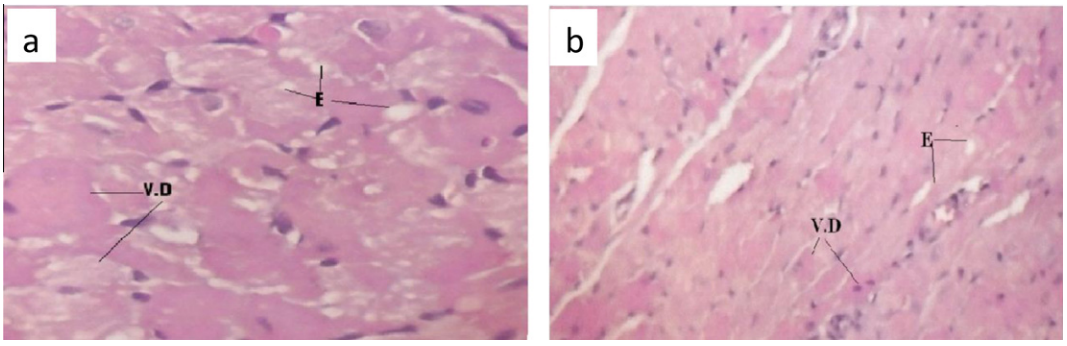

Figure 2 Effect of methanolic extract of *S. schimperi* on doxorubicin (DOX)-induced alterations in cardiac histopathological pattern. Plant methanolic extract (100 mg/kg/day for 10 consecutive days) or carboxymethylcellulose (CMC; 0.5%) were administered orally to rats and DOX (15 mg/kg; i.p.) was administered on the seventh day. (a) heart sections from rats which received CMC and DOX showing severe cytoplasmic vacular degeneration of the cardiac muscle (V.D), interstitial edema (E) and fibrotic bands, administration of the methanolic extract in addition to DOX improved the altered histopathological pattern, (b) heart sections from rats which received *S. schimperi* extract and DOX showing focal vacular degeneration of the cardiac muscle (V.D) and mild interstitial edema (E), a marginal influence (H&E 125×).

in both normal and type 1 diabetic rats.[46] The current results indicated that *Stachys schimperi* methanolic extract possesses a moderate radical scavenging and antioxidant activity with a modest activity against DOX cardiotoxicity as indicated by the biochemical markers. However, histopathological examination indicated minor improvement. The limited cardioprotective effects of the extract can be explained on the ground basis of the schedule of dosing or the need of higher doses. Examining individual constituents or different extract fractions and/or modifying the dosing regimen may disclose better protection against DOX-induced cardiotoxicity. Meanwhile, mechanisms other than antioxidation are worth considering.

5. Conclusion

In this study, although the DPPH radical-scavenging ability of the petroleum ether and chloroform extracts of *S. schimperi* was insignificant, it was evident that the methanolic and EB fractions did show strong, significant proton-donating ability and could serve as free radical-scavenging. Therefore, we studied *S. schimperi* for possible protection against cardiotoxicity induced by DOX. Although the results are not satisfactory, further study will be required by modifying the dose and investigating the cardioprotective effect of the polar *n*-butanol fraction.

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