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Regulatory efficacy of scopoletin, a biocoumarin on aortic oxido lipidemic stress through antioxidant potency as well as suppression of mRNA expression of inos gene in hypercholesterolemic rats

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ABSTRACT

Preceding work in our laboratory has revealed that scopoletin, one of the main bioactive coumarin from fruits of Morinda citrifolia, exerts anti-diabetic activity in in vitro partly by averting a glucosidase and a amylase action. However, its aorto/vaso protective impact-based research is still incomprehensible. The present study looks into the regulatory efficacy of scopoletin on aortic lipid profile, radical scavenging status, endothelial factors and aortic morphology in dyslipidemic rats. Rats fed with normal diet serve as control [Group (G) 1], rats fed with cholesterol-enriched diet (CED) (4 % cholesterol and 1 % cholic acid) for 45 days (G2), rats fed with CED for 45 days + scopoletin (10 mg/kg, body weight/day orally) for the last 30 days (G3) and scopoletin alone rats (G4). Blood and aortic tissue were taken immediately and used for various biochemical, histological and molecular analyses. A pronounced increase in the levels of aortic lipid profile, lipid peroxidation along with substantial suppression in the activities of aortic antioxidant and endothelial factor was observed in G2. The mRNA expression levels of iNOS gene were significantly up-regulated in aortic tissue of G2. On treatment with scopoletin, all the levels were reverted to near normalcy G3. Morphology of aorta in G2 indicated numerous foam cells with intimal changes, whereas the aorta of G3 exhibited fewer foam cells with normal intima. These results support that scopoletin expressively represses the aortic oxido-lipidemic stress and thereby upholding normal morphology of the aorta, and thus minimizing the peril of CVD.

Keywords: Scopoletin; Endothelial factor; Cholesterol; Radical scavenging; mRNA expression.

INTRODUCTION

Atherosclerosis is a process that leads to the narrowing or complete occlusion of the arterial lumen myocardial infarction, stroke, or peripheral vascular disease. Atherogenesis is the process of development of atherosclerotic plaques. The key early event in atherosclerosis is damage to the endothelium. The endothelium loses its cell-repellent quality and admits inflammatory cells into the vascular wall. It also becomes more permeable to lipoproteins which deposit in the intima. This may be caused by excess of lipoproteins, hypertension and diabetes or by components of cigarette smoke. Initially the damage is more functional than structural; later, there is structural damage or a complete destruction of endothelial cells [1–3].

Hypercholesterolemia stimulates atherogenesis by damaging the endothelial wall [4] owing to a sizable production of free radicals [5]. Too much production of free radicals outstrips endogenous free radical scavenging mechanisms

and attacks biological macromolecules such as lipids, proteins and DNA. This state has commonly been denoted as oxidative stress. An increasing body of evidence proposes that oxidative stress is involved in the pathogenesis of many cardiovascular diseases, including atherosclerosis and hypertension [6].

Superfluous oxidative stress is caused by a disparity between oxidant and free radical scavengers, leading to an overproduction of free radicals, including superoxide, hydroxyl radicals and lipid radicals, which may impair cellular components, interfering with normal function [7]. During hypercholesterolemia, the reactions between oxygen radicals, or enzymatic oxidation and lipoproteins or more specifically phospholipids can lead to production of lipid radicals (OxLDL and OxPL). This OxPL can interact with membrane receptors to accumulate within the cellular membrane, disrupting normal cellular function through a reduced bioavailability of NO (by enhancing iNOS activity), eliciting an immune response, leading to poor vascular function and ultimately atherosclerosis [8].

Several reports have shown that the use of various secondary metabolites, in addition to conventional medical drugs, can help in regulating lipid levels and have therapeutic effects [9,10]. One such phytoconstituent is *scopoletin* isolated from fruit of Indian mulberry, which belongs to the rubiaceae family and has been utilized in ayurvedic medicine for several indications including memory decline, inflammation, pain, pyrexia, epilepsy and as a sedative. *Scopoletin* has been registered to possess potent-free radical scavenging properties [11]. Besides, it also exhibits hepatoprotective [12] and anti-inflammatory [13] properties.

As mentioned above an alcoholic extract of *Indian mulberry* can act as anti-dyslipidemic drug by attenuating renal oxidative stress on diabetic nephropathy-induced rats [14]. However, the regulatory effect of *scopoletin* on aortic lipid profile, radical scavenging status, endothelial factors, mRNA expression of iNOS and morphological changes in cholesterol-enriched diet (CED) induced dyslipidemic rats has not been evaluated so far.

MATERIALS AND METHODS

Chemicals

Lipid profile kit and diagnostic enzymatic kits are purchased from Spin React (Spain). Trizol, One step RNA isolation kit was purchased from Medox Biotech, India. One Step RT-PCR kit was purchased from Qiagen, Germany. Primers were designed and provided by MWG Biotech, Ebersberg, Germany. *Scopoletin*, cholesterol, cholic acid and adenosine tri phosphate (ATP) were purchased from Sigma Aldrich, (Linco Research, Inc. St. Charles, MO). All other chemicals and solvents used in the present study were of analytical grade and highest purity.

Analytical instruments

The UV–visible spectrum of the isolated compound was recorded on a Perkin-Elmer UV-Visible spectrophotometer with quartz cells of 1-cm path length, at 25°C. IR spectra studies were carried out in the solid state as pressed KBr pellets using a Perkin-Elmer FT-IR spectrophotometer in the range of 400–4,000 cm⁻¹. The mass spectrum of the complex was obtained using Jeol Gcmate. The ¹H NMR and C¹³NMR were obtained using Bruker AM-500 instrument at 500.13 and 125.758 MHz, respectively. The spectra were recorded without any correction for instrumental characteristics.

Preparation of solvent fractions and isolation of the compound

Deseeded, dried and grinded pulp of fruits of Indian mulberry (2.8 kg) were soaked in hexane in the ratio of 1:3 parts of sample to solvent for 2 h in a 60 °C water bath, then filtered and concentrated with a rotary evaporator (Buchi, R-200 Switzerland). This was repeated thrice times. Thereafter, the pulp was left to air dry completely for 3 days before repeating the whole process with chloroform and then ethanol, respectively. The yield for the hexane, chloroform and ethanol extracts of *Indian mulberry* were 1.25 %, 1.11 %, and 6.45 %, respectively. The ethanol extract of the fruits (80 g) was then partitioned with petroleum ether, chloroform and water to yield the respective solvent extracts.

The chloroform extract (5 g) was further purified by silica gel chromatography ($4 \text{ cm} \times 90 \text{ cm}$, 0.063-0.200 mesh) and eluted with a chloroform/methanol gradient elution (the ratio from 100:0 to 8:100). Thirteen column fractions were collected and analyzed by TLC (chloroform/methanol). Fractions with similar TLC pattern were combined to a total of four fractions. Fraction 2 yielded from chloroform/methanol ratio 100:4 was rechromatographed on a preparative TLC (2 mm thickness) with solvent system chloroform/methanol (ratio of 1000:15) yielding a total of seven bands. Band three was collected and rechromatographed on preparative TLC (0.5 mm thickness) with solvent

system chloroform/methanol (ratio of 89:11) to yield four bands, with band two yielding 7-hydroxy-6 methoxycoumarin (49 mg). The purity of the whitish semi-crystalline colourless needles obtained was checked by analytical TLC.

Characterization of the isolated compound

The isolated compound identified as *scopoletin* (7-hydroxy-6-methoxycoumarin) was investigated using various analytical spectral studies such as UV–Visible, IR, Mass and NMR and compared with those previously reported [15].

Dosage Fixation studies

Dosage fixation studies were carried out by administering graded doses of scopoletin (5, 10, 15 and 20 mg/kg b.w) to determine the dose-dependent effect in dyslipidemia induced rats. We found that the optimum dosage was 10 mg/kg b.w. It was found that *scopoletin* showed maximum free radical scavenging and anti dyslipidemic efficacy at a concentration of 10 mg/kg body weight administered orally for 30 days.

Animal Grouping

Healthy male albino rats of Wistar strain weighing 180–200 g were housed in large spacious cages. Food and water were given *ad libitum*. The animal house was ventilated with a 12 h light/dark cycle, throughout the experimental period. All experiments and protocols described in this study were approved by the Institutional Animal Ethics Committee (UNISZA/AEC/02/14/006 dt 30/09/2014).

The rats were randomly divided into four groups of six rats each.

Group 1 Control rats fed with normal diet receiving 10 % dimethyl sulphoxide (DMSO) orally (feed contained 8 % fat, 25 % protein, 55 % carbohydrate, 4 % fibre (w/w) with adequate mineral and vitamin) for 45 days.

Group 2 Rats fed with CED receiving 10 % DMSO orally for 45 days [rat chow supplemented with 4 % cholesterol (w/w) and 1 % cholic acid (w/w)].

Group 3 Rats fed with CED for 45 days + *scopoletin* in 10 % DMSO (10 mg/kg. body weight/day by oral gavage) for the last 30 days.

Group 4 G 1 diet for 45 days + *scopoletin* in 10 % DMSO orally (10 mg/kg, body weight/day by oral gavage) for the last 30 days.

Autopsy Sample

The animals were fasted overnight and killed by cervical decapitation under mild anesthesia at the end of the experimental period (46^{th} day). Blood was collected in a separate tube with anticoagulant-EDTA for the separation of plasma. The aortic tissue was excised immediately with heart and washed with ice-cold saline and then dried with filter paper. The slice of aorta tissue was fixed with 10 % formalin and stained with hematoxylin and eosin (H&E) stain for histopathological studies. A 10 % homogenate of aorta were prepared using 0.1 M Tris HCl buffer pH 7.4. The above said samples were used for biochemical and molecular analysis.

Biochemical analysis

In order to screen the regulatory activity of *scopoletin* on aortic lipid profile, radical scavenging status, endothelial factors and aortic morphology in dyslipidemic rats, the *scopoletin* is dissolved in DMSO, in a final concentration of at most 0.1 %. Plasma total cholesterol (TC), LDL-C and HDL-C levels were measured using commercial kits from Spin react (Girona, Spain) according to the manufacturer's specifications. Aortic total lipids (TL) were extracted with chloroform: methanol (2:1) and purified by Folch's wash procedure [16] and aliquot were used for the estimation of aortic TC [17], triglyceride (TG) [18] and phospholipids (PL) [19] (with slight modification). Measurement of aortic ROS was done by the method of Chade et al. [20] by measuring the intensity of chemiluminescence (CL) probe using a luminescence reader apparatus. The aortic radical scavengers, namely, superoxide dismutase (SOD) [21], catalase (CAT) [22], glutathione peroxidase (GPx) [23], vitamin-C [24] and vitamin-E [25] were estimated. The LPO in the aorta was measured [26]. Nitric oxide in plasma was estimated by the method of Green et al. [27] (Greiss reagent). Protein was estimated by the method of Lowry et al. [28].

Reverse Transcriptase-polymerase chain reaction (RT-PCR) analysis

Total RNA was isolated from the aortic tissue, according to the manufacturer's instruction (Trizol, One step RNA isolation kit, Medox Biotech Pvt Ltd.). The purity and yield of RNA was quantified by measuring the absorbance of the RNA solution at 260 and 280 nm (absorbance ratio of 260/280 ranges from 1.6 to 1.8 was taken for further

reaction). RT-PCR for iNOS mRNA expression was performed according to manufacturer's guidelines (Qiagen One Step RT-PCR mix). Briefly, the reaction mixture contained 10 μ l of 5x Qiagen One step RT-PCR buffer containing a final concentration of 2.5 mM MgCl₂, 2 μ l of dNTP Mix, 5 μ l of each sense and antisense primers of iNOS and 5 μ l of sense and antisense primers of housekeeping β -actin, 1.0 μ g of template RNA, 2 μ l of Qiagen one step RT-PCR enzyme mix and made up to 50 μ l with RNase free water. β -actin was used as an internal control (house keeping gene).

Primers were designed by primer3 software and confirmed by BLAST analysis. The following primers were used: iNOS Forward: 5'-TGG AGC GAG TTG TGG ATT-3',

and Reverse: 5'-ATC TCG GGT GCG ATA GGT-3';

β-actin Forward: 5'-GAG CGG GAA ATC GTG CGT GAC-3',

and Reverse: 5'-GCC TAG AAG CAT TTG CGG TGG-3' (MWG Biotech, Ebersberg, Germany).

Amplification conditions used in this study consisted of an initial denaturation at 94 °C for 5 min followed by denaturation at 94 °C for 2 min. Annealing was at 58 °C 30 s and extension at 72 °C for 2 min for 30 cycles. The cycles followed incubation at 72 °C for 7 min. To compare with the amount of steady-state mRNA, 5 μ l of each PCR product was resolved onto 2 % agarose gel using TBE buffer after electrophoresis; the gel was viewed under UV light and digital images were captured on Bio-Rad gel documentation system. Densitometric analysis of the bands was expressed as net intensity ratio corrected for the corresponding β -actin contents.

Histomorphological Studies

A midline thoracotomy was performed and the portion of thoracic aorta was quickly removed with heart from control and experimental animal and fixed in 10 % formalin, then dehydrated in the descending grades of isopropanol and xylene. The aorta was then embedded in molten paraffin wax sectioned at 5 μ m thickness and stained with hematoxylin and eosin (H&E). Tissue sections were then viewed under a light microscope (Nikon microscope ECLIPSE E400, model 115, Japan) for morphological changes.

Statistical analysis

Statistical analysis was performed with the SPSS software (Version 17). Differences between groups were assessed by one-way analysis of variance (ANOVA). The values were expressed as mean \pm SD (n = 6) for six animals in each group. *Post hoc* testing was performed for intergroup comparisons using the least significant difference (LSD) test at the level of p < 0.05 and p < 0.01.

RESULTS AND DISCUSSION

The contemporary exploration was steered to elucidate the effect of *scopoletin* on aortic lipid profile, radical scavenging prominence, endothelial factors and mRNA expression of iNOS as well as histopathological changes in CED rats. Dyslipidemia is identified as caused from the aberrations in lipid metabolism and diet plays an essential role in the instigation and advancement of dyslipidemia-induced atherogenesis. In the animal model feeding of CED produces severe dyslipidemia and vascular atherosclerotic lesions (aortic lesion) by elevated oxidative stress [29, 30]. Table 1 shows the levels of plasma lipid profile (TC, HDL and LDL) in control and experimental rats. The levels of plasma TC and LDL were found to be significantly increased (p < 0.01); whereas the levels of HDL showed a significant decrease (p < 0.01) in CED induced rats. Treatment with *scopoletin* reverted all the lipid profile levels to near normalcy.

The levels of aortic lipid profile (TC, TG, and PLs) in control and experimental rats are depicted in Fig. 1. Cholesterol feeding triggered a substantial accumulation of TC, TG, and PL in various cells [31, 32]. The levels of aortic lipid profile such as TC, TG, and PL were markedly increased (p < 0.01) in CED fed rats compared to control rats in accounts to upsurge plasma lipid levels which has been reported in various studies [33, 34]. On treatment with *scopoletin*, the levels of aortic TC, TG (p < 0.05) and PLs were extensively attenuated (p < 0.01) in comparison with CED-induced rats. Recently, we reported that *Indian mulberry* rich in *scopoletin* as well as *avocado* fruit can lessen TC absorption, and lessen the TG and PL in renal tissue [14, 35] and hence the authors postulate that the presence of these *coumarin* rich *Indian mulberry* fruit is directly also involved in the decrement of lipid levels in aortic tissue (TC, TG, and PLs).

Dyslipidemia upsurges endothelial O_2^- production and vascular oxidative stress, which may in turn contribute to the impaired endothelial damage and atherogenesis [36]. The levels of aortic ROS were found to be ominously raised (p < 0.01) in CED induced rats; however, on supplementation with *scopoletin*, the levels of ROS were prominently blunted (p < 0.01) by diminishing NADPH oxidase activity (Table 2). Previous studies [37, 38] have reported that *scopoletin* possesses radical scavenging effects via decreasing NADPH oxidase-dependent ROS production. The activities of aortic free radical scavengers in control and experimental rats are shown in Tables 2 and 3. SOD catalyzes the dismutation of O_2^- radical anions to H_2O_2 and O_2 [39], in turn, these H_2O_2 are encountered by CAT or GPx and thereby dilute the degree of cellular damage. In addition, GSH serves as a substrate for the enzyme GPx, and it has been proposed that it is through the activity of this enzyme that GSH protects the various cells against oxidative damage. Apart from enzymatic antioxidants, the non-enzymatic radical scavengers such as GSH, vitamins C and E play a vivacious role in shielding cells from oxidative damage.

Activities of aortic enzymic and non-enzymic antioxidants were significantly dropped (p < 0.01) in CED induced rats due to a concomitant escalation in the levels of ROS, which in turn elicits the oxidative stress and finally lands up in weakened activities of these aortic antioxidants [40]. The *scopoletin* treated rats revealed a marked increase (p < 0.01) in the activities of these antioxidants in the aorta owing to inhibition of the enzymatic activity of 5-lipoxygenase and acetyl cholinesterase, and confronted oxidation in the ABTS, DPPH, FRAP and β -carotene bleaching assay [41]. They have further proved that *scopoletin* modulates the expression of certain antioxidant enzymes (SOD, CAT, and GPx) as well as enzymes involved in the generation of ROS in the brain tissue [42–44].

Various studies had shown that intake of CED leads to an increase in free radical production, which elevates lipid peroxides [45]. The levels of aortic LPO of CED induced control and experimental rats are shown in Fig. 2. A notable increase (p < 0.01) in LPO levels of aortic tissues was observed in CED-fed groups compared to the control group. This is in corroboration with earlier reports [46, 47]. The levels of LPO products were meaningfully refurbished (p < 0.01) to normal levels of treatment with *scopoletin*.

Dyslipidemia is a central pathogenic factor of endothelial dysfunction caused by an impairment of endothelial NO. NO is synthesized from the amino acid L-arginine through the enzyme nitric oxide synthase (NOS), and has been widely considered as an endothelial dependent regulator of vascular tone (endothelial factor), with additional roles in preventing platelet activation, inhibiting oxidative stress, cell growth and inflammation [48, 49].

Figure 3 illustrates the levels of NO in plasma of control and experimental rats. The levels of NO in plasma of CED fed rats were significantly weakened (p < 0.01) compared to normal rats. This could be due to the boosted production of O_2^- , which then reacts with NO to form augmented ONOO⁻ which in turn activates iNOS mRNA expression. Deepa and Varlakshmi [49] and Wu et al. [4] report that the levels of NO of dyslipidemic rats were significantly reduced due to a pronounced increase in the level of ONOO⁻ [50]. Oral administration with *scopoletin* brought back the NO level to normalcy, analogous to CED rats. V. de Sandro *et al.* [50] demonstrated that *scopoletin* has an impact on the oxidative stress cascade by scavenging ROS, especially O_2^- [51]. Hence, in our study, we infer that the increased bioavailability of NO level in *scopoletin* treated rats is due to scavenging ROS and is comparable with previous studies [52].By contrast, *scopoletin* alone treated group did not show any sizable change in aortic lipid profile, antioxidant status and endothelial factor, compared with control rats.

NO produced by isoforms of endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS) participates in neurotransmission and cardiovascular signaling, whereas NO produced by inducible nitric oxide synthase (iNOS) is an important mediator of acute or chronic inflammation [53]. The inducible isoform, iNOS is expressed in various cell types including macrophages, hepatocytes and vascular smooth muscle cells in response to cytokines. The iNOS-derived NO overproduction appears to be a ubiquitous mediator of vascular inflammatory conditions, including atherogenesis by enhancing the production of ONOO⁻, which has been accepted as central mediator of cytotoxic effect in various cells, especially endothelial cells [54].

Figure 4 shows the mRNA expression of iNOS in aortic tissue of CED-induced control and experimental rats. Various studies have described that increased iNOS level in rats fed with hypercholesterolemic diet [55, 56] is due to amplified generation of ROS, which in turn enhances the activity of NF- κ B. The increased activated NF- κ B translocates to the nucleus, where it binds to the promoter regions of various genes that include iNOS [57]. Hence iNOS mRNA expression is up-regulated during dyslipidemic condition. Similar to the above discussed line, we observed substantial up-regulation (p < 0.01) in the mRNA expression level of iNOS in aortic tissue of CED-

induced rats (lane 2) compared to normal rats (lane 1). However, treatment with scopoletin (lane 3) markedly downregulated (p < 0.01) the mRNA expression level of iNOS likely due to its free radical scavenging activity [52,58] as well as anti-inflammatory effect [13]. *Scopoletin* only treated rats (lane 4) do not indicate any alterations in expression levels of iNOS.

The effect of *scopoletin* on histopathological changes with hematoxylin and eosin (H&E) staining in thoracic aorta of control and experimental rats are shownin Fig. 5. Transection of control aorta showed the normal architecture of the vascular intima (Fig 5a), whereas the aorta of CED rats revealed the existence of numerous foam cells with loss of normal arrangement of vascular intima when matched with control rats (Fig 5b). Similarly, Wu et al. [4] and Sohn et al. [58] proved that increased accumulation of foam cells with vascular intimal changes were observed in high cholesterol/fat diet-induced rats. Treatment with *scopoletin* presented fewer appearance of foam cells count with normal vascular intima (Fig. 5c), which may be due to its antidyslipidemic and free radical scavenging capacity. *Scopoletin* only treated rats (Fig. 5D) showed the normal architecture of the vascular intima (H&E, $100 \times$).



Figure 1. Effect of scopoletin on aortic lipid status of HCD induced hypercholesterolemia in control and experimental rats. Values were expressed as mean $\pm S$. D for 6 rats in each group. *P<0.05, **P<0.01 and inter-comparison between the groups are represented as ^a Compared with Control and ^b Compared with HCD.



Figure 2. Effect of scopoletin on the levels in aortic LPO of HCD induced hypercholesterolemia in control and experimental rats. Values were expressed as mean \pm S. D for 6 rats in each group. **P<0.01 and inter-comparison between the groups are represented as a Compared with Control and bCompared with HCD.



Figure 3. Effect of scopoletin on NO in plasma of HCD induced hypercholesterolemia in control and experimental rats. Values were expressed as mean $\pm S$. D for 6 rats in each group. **P<0.01 and inter-comparison between the groups are represented as aCompared with Control and bCompared with HCD.



Figure 4. Effect of scopoletin on mRNA expression levels of iNOS in aortic tissue of control and experimental rats.

Lane 1 represents a control group; Lane 2 represents the HCD group; Lane 3 represents a HCD+scopoletin treatment group; Lane 4 represents scopoletin alone group. Densitometric analysis of the bands is expressed as a net intensity ratio corrected for the corresponding β -actin contents. Values were expressed as mean \pm S.D for 6 rats in each group. **P<0.01 and inter-comparison between the groups are represented as aCompared with Control and bCompared with HCD.



Figure 5. portraits the morphological changes in aortic tissue of HCD induced control and experimental rats.

Transection of aortic tissue (control) showed the normal architecture of the vascular intima (H&E, 100X) (Fig 5A). Transection of HCD induced rats showed numerous foam cells with loss of the normal arrangement of vascular intima were noted (Fig 5B). Transection of HCD+ scopoletin groups showed less appearance of foam cells count with normal vascular intima (Fig 5C). scopoletin alone (Fig 5D) treated rats showed normal intima without any foam cell accumulation, and was similar to that of control group (H&E, 100X) (Scale bar-50µm).

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Group	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control	98.86 ± 8.79	32.96 ± 3.44	46.67 ± 5.03
НСD	$157.94 \pm 14.25 \ a^{**}$	27.34 ±3.13 a**	109.97 ±11.56 a**
HCD+ Scopoletin	$109.38 \pm 10.92 \text{ b } **$	$30.45 \pm 3.05 b^{**}$	$61.14 \pm 6.38 \ b^{**}$
Scopoletin	101.29 ± 9.81	$31.44.16 \pm 2.95$	50.12 ± 4.69

Table 1. Effect of *scopoletin* on plasma lipid profile in control and experimental rats.

Values were expressed as mean \pm S.D for 6 rats in each group. Statistical Significance (P value): **P<0.01^a Compared with Control, bCompared with HCD.

Table 2. Effect of Scopoletin on the activities of aortic ROS and enzymic antioxidants in control and experimental rats.

Groups	ROS	SOD	CAT	GPx
Control	256.23 ± 22.13	33.42 ± 3.51	38.45 ±3.67	1.49±0.12
НСД	$328.18 \pm 35.22 a^{**}$	$22.24 \pm 2.56 a^{**}$	21.51 ± 2.56 a**	1.05±0.15 a**
HCD+ Scopoletin	$287.45 \pm 29.56 b^{**}$	$29.28 \pm 2.41 \text{ b}^{**}$	$33.59 \pm 3.92 \text{ b}^{**}$	1.23±0.10 b**
Scopoletin	248.78 ± 21.34	32.19 ± 3.30	39.66 ± 4.03	1.48±0.12

Values were expressed as mean \pm S.D for 6 rats in each group. Statistical Significance (P value): **P<0.01 aCompared with Control, bCompared with HCD.

ROS: Counts/mg protein/min, SOD: Units/µg protein, one unit is equal to the amount of enzyme that inhibits the auto-oxidation reaction by 50%, CAT: µmoles of H2O2 consumed/min/µg protein, GPx: µg of GSH oxidized/min/µg protein

Table 3. Effect of scopoletin on the activities of aortic non-enzymic antioxidants of HCD induced hypercholesterolemia in control and
experimental rats.

Groups	GSH	Vit-C	Vit-E
Control	5.67 ± 0.64	1.34±0.12	1.24±0.14
НСD	$4.13 \pm 0.48 a^{**}$	0.68±0.04 a**	0.59±0.07 a**
HCD+ Scopoletin	$5.05 \pm 0.41 \ b^{**}$	1.06±0.11 b**	1.04±0.10 b**
Scopoletin	5.98 ± 0.61	1.26±0.12	1.21±0.11

Values were expressed as mean \pm S.D for 6 rats in each group. Statistical Significance (P value): **P<0.01 aCompared with Control, bCompared with HCD. GSH, Vit C, Vit E: μ g/mg protein.

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CONCLUSION

The findings of the present study show that *scopoletin* could reduce oxido-lipidemic stress by elevating the activity of antioxidants as well as extenuating the lipid profile levels. It is further hypothesized that *scopoletin* could additionally normalize the endothelial factor (NO) by suppressing mRNA expression of iNOS gene, through which, it is assumed to maintain the normal morphology of the aorta. Accordingly, it prevents or delays the development of lipid accumulation and plummets the onset of CVD risk. Henceforth, *scopoletin* may be beneficial as aorto/vasoprotective drug in dyslipidemic ailment.

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