Therapeutic potential of *Dracaena cinnabari* Balf. f. on high fat diet fed rats

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ABSTRACT

Coronary artery disease (CAD) is related to the interruption of blood flow in coronary arteries of heart caused by the initiation of arterial inflammation due to fat deposition (plaque). The present study was undertaken to study the altered protein expression of rat plasma after the treatment with natural medicinal extract *Dracaena cinnabari* Balf. The high fat diet (HFD) rat (Wistar) models that mimic the CAD condition were generated. Wistar rats were fed with HFD followed by treatment with *Dracaena cinnabari* Balf. f., curcumin and standard drug (atorvastatin) for one month. Our study explores the effect of *Dracaena cinnabari* Balf. f. compared to curcumin and standard drug atorvastatin to reduce the disease severity in HFD fed rats. Total 8 differentially expressed proteins (DEP’s) were identified using proteomics technique by running two-dimensional gel electrophoresis (2-DE) followed by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-ToF MS/MS). Amongst which 7 DEP’s were distinct and apolipoprotein A-1 (apo A-1) has been reported to play a role in regulation of lipid transfer and metabolism, involved in the pathogenesis of CAD. Apo A-1 was thus screened to see its effect after the treatment with *Dracaena cinnabari* Balf. f., curcumin and standard drug and thus validated by western and enzyme-linked immunosorbent assay (ELISA). The study revealed that expression level of apo A-1 in CAD was restored to normal levels by *Dracaena cinnabari* Balf. f. treatment, providing the way towards the treatment of CAD.

Key words: Resin; rat plasma; proteomics; wistar rats; high fat diet.

ABBREVIATIONS

CAD, coronary artery disease; LDLc, low density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; HDL, high density lipoprotein; HMG-CoA, 3hydroxy3methyl glutaryl coenzyme A; apo-A1, apolipoprotein A-1; LCAT, Lecithin-Cholesterol Acyltransferase; HFD, high fat diet; 2-DE, two-dimensional gel electrophoresis; MALDI-TOF-MS/MS, matrix-assisted laser desorption/ionization-time of flight mass spectrometry; RT, room temperature; EDTA, ethylene diamine tetra acetic acid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; IEF, isoelectric focusing; analysis of variance; PLTP, Phospholipid Transfer Protein.

INTRODUCTION

Coronary artery disease (CAD) occurs mainly due to blockage of coronary arteries and atherosclerosis is the major event in the development of CAD occurring due to deposition of cholesterol in coronary arteries (Liao et al., 2015). According to WHO report, cardiovascular diseases (CVD) is the leading cause of death affecting 17.9 million (31%) population in 2016 worldwide in which CAD affects 7.4 million globally and burden increases rapidly (American Heart Association report, 2017). CAD is characterized by endothelial dysfunction, vascular inflammation and the deposition of lipids, cholesterol, calcium and cellular debris within the walls of coronary arteries (Hadi et al., 2013). The
major risk factors are stress, smoking, high alcohol consumption, hypertension, diabetes mellitus, age, obesity etc. (Dunn et al, 1970) causing inflammation, leading to plaque deposition and onset of CAD. Lipoprotein molecules entered into the endothelium, modified and become inflammatory, creating foam cells or fatty streaks. This is the beginning of atherosclerosis which leads to various manifestations of vascular diseases (Mahmoud et al, 2014). But neither the exact mechanisms responsible for the onset of CAD are known nor is there any specific biomarker for the early diagnosis of disease. Till now various blood-based biomarkers are reportedly used to predict the disease either alone or in combination (Brown and Bittner, 2008). C-reactive protein (CRP) inflammatory cytokines like TNF-α, IL-6 etc. are currently used as diagnostic marker of inflammation but is not specific to CAD (Sproston and Ashworth, 2018). Currently there is an absolute need of specific candidate molecules/biomarkers that are indicative of the diseased condition and can be taken as a target for the treatment.

The objective of this work was therefore to study the effect of differential protein of plasma after the treatment of HFD rat model with new medicinal extract of Dracaena cinnabari Balf. f. for treating CAD along with curcumin (Ayurvedic medicine as positive control) and Atorvastatin (standard drug as control). Dracaena cinnabari Balf. f. (Dragon’s blood) is a red exudate, collected in the form of resin or latex produced by various plant species such as Daemonorops, Dracaena, Croton and Pterocarpus. It carries valuable medicinal properties and is used for traditional treatment (Jura-Morawiec and Tulik, 2016) because of its antifungal, antibacterial, antiviral, antitumor, anti-inflammatory, antioxidant, etc. properties and high therapeutic potential (Figure 1) (Gupta et al, 2008; Ibraheem et al, 2018). Curcumin (diferuloylmethane) is an active component of turmeric commonly used as a food coloring agent, cosmetics, spices and as medicines because of its antioxidant and anti-inflammatory properties (Gupta et al, 2013). It is reported to be associated with a variety of chronic pathological complications such as cancer, atherosclerosis, neurodegenerative diseases and aging (Menon and Sudheer, 2007). Atorvastatin is a synthetic compound consisting of 3hydroxy3methyl glutaryl coenzyme A (HMG CoA) reductase inhibitor and is used to lower the plasma cholesterol levels (Lea and McTavish, 1997).

To do this study High Fat Diet (HFD) fed animal models were generated that is, a robust model as obesity, insulin resistance, fasting, hyperglycemia, dyslipidemia was easy to induce (Zhou and Xie, 2015). Rats were then treated with selected medicinal extracts and sacrificed plasma samples were collected followed by identification of proteins using proteomic approach. Among all the identified differential proteins, one of the downregulated proteins, apolipoprotein-A1 (apoA-1) was screened since it was profoundly known in the disease condition of CAD. It is the main protein of High-density lipoprotein (HDL) consisting of 243 amino acid mainly synthesized by liver and has the ability to reverse cholesterol transport (RCT) that convert nascent HDL into mature form. It also activates lecithin cholesterol acyltransferase (LCAT) which convert cholesterol into cholesterol esters (Liao, 2015; Sissel and

![Figure 1: A representative picture of Dracaena cinnabari.](image-url)
MATERIALS AND METHODS

Chemicals

Curcumin was commercially obtained from Hi-Media Laboratories Pvt. Ltd. (Mumbai, India) and Dracaena cinnabari Balf. f. was procured from the Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi, India. HFD was purchased from Research Diets, Inc. (New Brunswick, USA). Chow diet was purchased from Golden Feeds (Mehrauli, New Delhi). Atorvastatin was collected from Ranbaxy Laboratories, India. All other chemicals used for two-dimensional gel electrophoresis (2-DE) and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS/MS) analysis were of highest analytical grade (Bio-Rad, USA; Sigma-Aldrich, USA).

Preparation of extract of Dracaena cinnabari Balf. f.

Dracaena cinnabari Balf. f. was collected in resin form, crushed to make powder. The powdered resin (100 gm) was successively extracted with 250 ml methanol in soxhlet apparatus (Borosil, India) at room temperature (RT). The process was carried out over a period of 48 hrs. Extract was filtered through whatman filter paper and the solvent was vacuum distilled at 65°C in a rotary evaporator (IKA, India). Red semi-solid methanolic extract (dry weight 90%) thus obtained was kept at 4°C until further use (Baumer and Dietmann, 2010).

Animals and treatment

Male Wistar rats were purchased from National Institute of Nutrition (NIN), Hyderabad, India. Study was followed strictly under the guidelines approved by the animal ethical Committee of Council of Scientific and Industrial Research (CSIR)-Institute of Genomics and Integrative Biology, Delhi University Campus, Mall Road, Delhi, India (Reference no. IGB/IAEC/OCT2018/01) for the purpose of control, supervision, handling and maintenance of experimental animals. They were maintained under standard conditions (12/12-hour light/dark cycle at 25±5°C). Rats were divided into five groups having six rats per cage and were allowed to acclimatize for 1 week under standard conditions. HFD was composed of 22% protein; 55% carbohydrate; 5.02% fat; 4% crude fiber; 6% ash; 1% calcium; 0.5% phosphorous; 1.2% lysine; 0.9% methionine; and different vitamins (Gold Mohur feeds Ltd, New Delhi, India).

Experimental design

A pilot study was carried out to find out the effect of Dracaena cinnabari Balf. f. in comparison with pure compound curcumin and standard drug atorvastatin. Atherosclerosis like condition was induced in rats by feeding HFD for one month whereas control rats were given normal chow diet. The diet was given to all five groups of rats as follows;

Group 1 (G1) - Healthy control (only chow diet)
Group 2 (G2) - HFD 150 mg/kg body weight and curcumin (300 mg/kg/day)
Group 3 (G3) - HFD 150 mg/kg body weight and methanolic extract of Dracaena cinnabari Balf. f. resin (300 mg/kg/day)
Group 4 (G4) - HFD 150 mg/kg body weight and standard drug atorvastatin (30 mg/kg/day)
Group 5 (G5) - CAD Control with only HFD150mg/kg body weight.

Drugs (Dracaena cinnabari Balf. f., curcumin and atorvastatin) were dissolved in Milli-Q water and were orally administered to rats. After thirty days of administration of the respective drug, all the rats (experimental and control) were sacrificed and blood samples were collected into vacutainers containing ethylene diamine tetra acetic acid (EDTA). Samples were then processed for identification of differential proteins using 2-DE and MALDI-TOF MS/MS analysis.

Sample preparation for 2-DE

The blood samples were centrifuged at 1,500 rpm for 15 min at 4°C; plasma samples were separated and stored at 80°C. Sample preparation was carried out by removing salts from the plasma by centrifugation and concentrated using 3 kDa amicon columns (Merck Millipore, USA) (Tu et al., 2010).

Two-dimensional gel electrophoresis (2-DE)

Total protein concentration of plasma was estimated by Bradford method (Bio-Rad, USA) (Ernst and Zor, 2010) and separated by 2-DE. In the first step, proteins (110 µg) were added to the rehydration buffer (7 M urea, 2 M thiourea, 2% 3-[3-cholamidopropyl] dimethylammonio]-1-propanesulfonate (CHAPS)) containing 50 mM dithiothreitol and 0.2% ampholyte, incubated for 2 h and then applied to immobilized pH gradient (1PG) strips overnight (12 -16 hr). First dimension separation of proteins was carried out by using IPG strips (4–7 pH) in an isoelectric focusing (IEF) unit (Bio-Rad, USA) at 200 V for 1 h, 500 V for 1 h, ramping at 1000 V for 1 h and final focusing at 8000 V for a total of 13000 VH. It is followed by
second dimension separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). IPG strips were equilibrated in equilibrium buffer (50 mM Tris-HCl, pH 8.8, 6 M urea, 4% (w/v) SDS, 20% (w/v) glycerol) containing 2% (w/v) DTT for 20 min and then again for 20 min in the equilibrium buffer containing 2.5% (w/v) iodoacetamide instead of DTT. After equilibration of IPG strips, SDS-PAGE (12%) was performed in a Mini-Protean II slab cell vertical system (Bio-Rad, USA). Gels were run for 2 h for protein separation on the basis of molecular weight (MW) followed by silver nitrate (Merck Chemicals, USA) staining (Biswas et al., 2013).

Silver staining and Trypsin digestion

Immediately after 2-DE, silver staining of gels were carried out. Briefly, gels were placed in fixative for 1 h, sensitized for 1 min in 2% solution of sodium sulfite (Na$_2$SO$_3$) followed by silver staining for 20 min and development with sodium carbonate (Na$_2$CO$_3$) and formaldehyde (HCHO). The reaction was then stopped with 6% acetic acid solution and scanned using chemiDoc™ MP imaging system (Bio-Rad, USA). Protein spots were visible in the gel as dark brown spots, excised in 1 mm cubes, destained using 1:1 solution of 30 mM potassium ferricyanide (Sigma-Aldrich, USA) and 100 mM sodium thiosulphate (Sigma-Aldrich, USA) and dehydrated with acetonitrile (ACN). The gel pieces were then equilibrated with 100 mM ammonium bicarbonate and trypsin digested with trypsin gold (Promega, USA) by overnight incubation at 37°C. The supernatant was collected and dried in a vacuum centrifuge. The peptides were separated from gel pieces by varying the concentration of ACN and trifluoro acetic acid (TFA, Sigma-Aldrich, USA), dried in a speed-vac to expel TFA/ACN and stored at -20°C till further use (Biswas et al., 2013; Wirth and Vesterberg, 1988).

Peptide mass fingerprinting by MALDI-TOF MS/MS

In-gel digestion and peptide extraction was carried out by MALDI-TOF-MS/MS (Applied Biosystems, USA) analysis as mentioned earlier. Peptide mixture was desalted using ZipTips (C18 resin; Millipore) and mixed with an equal volume of a saturated solution of α-cyano-4-hydroxycinnamic acid (CHCA) in 0.1% TFA onto the MALDI target plate and air dried for 10 min. MS/MS spectra were procured over the mass scope of 10,000-20,000Da in reflector positive mode with 5600 laser shots force (25 Shots/Sub-Spectrum for 500 aggregate Shots/Spectrum). Protein identification was only considered with a statistically significant probability based Mowse score (p<0.05). Proteins with more than 2 peptides match were only considered for further study (Wirth and Vesterberg, 1988).

In-Silico analysis

STRING database (Radivojac et al., 2010) has been used to find out the interacting partners of apoA-1. It comprises both physical and functional protein-protein interactions. It holds a unique scoring-framework built on diverse types of associations against a common reference set. The full explanation of a protein’s function requires knowledge of all partner proteins with which it specifically interacts. The server also predicts the non-physical interaction such as participation in the same metabolic pathway or cellular process or modulated expression pattern in similar conditions. STRING provides a graphical representation of protein interaction network that gives a high-level view of functional association and facilitates the analysis of biological processes at system level.

Western blot analysis

The expressions of apoA-1 in all five groups (rat model) were validated by Western blotting (Biswas et al., 2013). Briefly, 20μg protein sample was run in SDS-PAGE (12%) and transferred to the nitrocellulose membrane (Millipore, USA) using semi dry trans blot (BioRad, USA). Membrane was then blocked overnight with 5% bovine serum albumin (BSA, Sigma, USA) at 4°C. Membranes were then incubated with primary antibody anti-apoA-1 (1:2000 dilution) for 2 h at RT followed by secondary antibody horseradish peroxidase (HRP) conjugated anti-rabbit antibody (dilution 1:5000) for 1hr at RT, developed with enhanced chemiluminescence assay (ECL) (Thermo Scientific, USA), using ChemiDoc™ MP Imaging system (Bio-Rad, USA). After each step, membranes were washed 3 times for 10 mins with wash buffer (sterile phosphate buffer saline (PBS) and 0.05% Tween-20). Experiments were carried out in triplicate.

Validation by enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) was carried out in a 96 well plate (Biswas et al., 2013). Plasma samples were diluted (1 μl/200 μl) in a coating buffer (0.01M Na$_2$CO$_3$ and 0.035M NaHCO$_3$, pH 9.6) and incubated over night at 4°C. Plates were washed thrice with PBS, blocked with 1% BSA for 1 h, treated with diluted (1:2000) anti-apoA-1 (Santa Cruz, USA) for 2 h at RT. Wells were washed again and incubated with 100 μl diluted (1:1000) HRP-conjugated secondary anti-rabbit antibody (Jackson, USA) at RT for 1 h and developed with ortho-phenylene diamine (1 mg/ml). The reactions were terminated by adding 50 μl of 3N H$_2$SO$_4$ to stop solution in each well. Optical density was then observed with absorbance at 492 nm in an ELISA reader (Spectra Max plus 384, molecular devices).
RESULT

In the present study, HFD rat models were generated and treated with methanolic extract of *Dracaena cinnabari Balf.* f. (Table 1) (Yehia et al., 2013) along with standard drug control atorvastatin and curcumin as a known natural antioxidant. We used proteomic techniques to study the differentially expressed proteins of plasma samples of HFD rat model using 2-DE and MALDI-TOFMS/MS analysis and identified 8 differentially expressed proteins. These are, Alpha-1-macroglobulin, Haptoglobin (two isoforms), Alpha-1-antiproteinase, Group specific component, Serum albumin, Apolipoprotein A-1 and Anionic trypsin-1. Protein name, Accession Number, peptide match, score etc are given in Table 2. Haptoglobin was identified in 2 different spots, may be isoforms, thus 7 distinct differential proteins were identified. Amongst these identified proteins, ApoA-1 has been reported to be majorly involved in CAD. The predicted protein-protein interacting partners of apoA-1 were analyzed by String analysis. Apo-A1 has been found to be functionally associated with Lecithin-Cholesterol Acyltransferase (LCAT) (Figure 2), screened for further study due to its exclusive involvement in lipid metabolism. In the animal model experiments, with the exposure of various drug treatments, alteration in the level of apoA-1 in the plasma samples of rats were observed. Levels of apoA-1 were considered as a key to predict the effectiveness of given plant extract.

Comparison of protein profile patterns in control group G1, and various drug treated groups, G2 (curcumin); G3 (*Dracaena cinnabari Balf.* f.); G4 (Atorvastatin) and diseased group G5 (HFD) were analyzed. ApoA-1 (spot 7) was found to be significantly down regulated in diseased case (G5) when compared to control group G1 (Figure 3a and 3b). Densitometric analysis of 2-DE spot of apo A-1 revealed 0.93-fold down regulation in G5 compared to G1 (healthy control). Levels of apoA-1 in other groups were upregulated by 1.29 fold (G2 group), 2.05 fold (G3 group), 1.70 fold (G4 group) compared to G5 group (Figure 3c and 3d). The expression differences of apoA-1 in all 5 groups were further validated by western blotting and ELISA and their differential expression was confirmed by densitometry analysis. The expression level of apoA-1 in G5 (diseased case) by Western analysis revealed 0.87-fold downregulated compared to G1 (healthy control). After the drug treatment, the level of apoA-1 was upregulated by 1.33-fold in G2 group, 1.56-fold in G3 group, and 1.89-fold in G4 group in comparison to HFD fed rat plasma (G5 group)(Figure 4). ELISA result of apoA-1 was found to be highly significant with p<0.0001 after the drug treatment with 1.00-fold upregulated expression in G3 group (*Dracaena cinnabari Balf.* f. group) as compared to HFD fed rat plasma (G5 group) showing the effectiveness of given natural extract as drug (Figure 5).

DISCUSSION

CAD is interrupted blood flow in heart due to blockage in coronary arteries. The main cause of CAD is atherosclerosis which is a lipid driven disease indicating that HFD is majorly involved in creating the conditions of CAD (Liao et al., 2015). Currently there is no specific biomarker available to diagnose the disease at an early stage. Proteomic technologies have a great potential to identify biomarkers for diagnosis, monitoring disease progression, and to identify therapeutic targets. During diseased condition, protein alteration occurs in accordance with their conformation and concentration that may act as biomarkers to diagnose the onset of disease (Brown and Bittner, 2008). These candidate protein biomarkers are indicators of the diseased condition and can be taken for treatment target. In the present study, we identified 7 distinct differential plasma proteins of HFD fed rats.

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Table 1: Phytochemical screening of methanolic extract of *Dracaena cinnabari.*

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Reducing Sugar</td>
<td>+</td>
</tr>
</tbody>
</table>

(Yehia et al, 2013)
Table 2: Proteins identified by MALDI-TOF MS/MS analysis from rat plasma.

<table>
<thead>
<tr>
<th>Spot No.</th>
<th>Proteins identified</th>
<th>Accession No.</th>
<th>M.W. (Da)</th>
<th>Peptide matched</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alpha-1-macroglobulin OS=Rattus norvegicus</td>
<td>sp</td>
<td>Q63041</td>
<td>A1M_RAT</td>
<td>167,125</td>
</tr>
<tr>
<td></td>
<td>OX=10116 GN=A1m</td>
<td>PE=1 SV=1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Haptoglobin OS=Rattus norvegicus OX=10116</td>
<td>tr</td>
<td>A0A0H2UHM3</td>
<td>A0A0H2UHM3_RAT</td>
<td>38,432</td>
</tr>
<tr>
<td></td>
<td>GN=Hp</td>
<td>PE=1 SV=1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Haptoglobin OS=Rattus norvegicus OX=10116</td>
<td>tr</td>
<td>A0A0H2UHM3</td>
<td>A0A0H2UHM3_RAT</td>
<td>38,432</td>
</tr>
<tr>
<td></td>
<td>GN=Hp</td>
<td>PE=1 SV=1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Alpha-1-antiproteinase OS=Rattus norvegicus</td>
<td>tr</td>
<td>A0A0G2</td>
<td>J73</td>
<td>A0A0G2J73_RAT</td>
</tr>
<tr>
<td></td>
<td>OX=10116 GN=Serpina1</td>
<td>PE=1 SV=1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Group specific component OS=Rattus norvegicus</td>
<td>tr</td>
<td>Q68FY4</td>
<td>Q68FY4_RAT</td>
<td>53,518</td>
</tr>
<tr>
<td></td>
<td>OX=10116 GN=Gc</td>
<td>PE=1 SV=1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Serum albumin OS=Rattus norvegicus OX=10116</td>
<td>tr</td>
<td>A0A0G2</td>
<td>SH5</td>
<td>A0A0G2SH5_RAT</td>
</tr>
<tr>
<td></td>
<td>GN=Alb</td>
<td>PE=1 SV=1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Apolipoprotein A-I OS=Rattus norvegicus</td>
<td>sp</td>
<td>P04639</td>
<td>APOA1_RAT</td>
<td>30,062</td>
</tr>
<tr>
<td></td>
<td>OX=10116 GN=Apoa1</td>
<td>PE=1 SV=2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Anionic trypsin-1 OS=Rattus norvegicus</td>
<td>sp</td>
<td>P00762</td>
<td>TRY1_RAT</td>
<td>25,959</td>
</tr>
<tr>
<td></td>
<td>OX=10116 GN=Prss1</td>
<td>PE=1 SV=1</td>
<td></td>
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</table>

Amongst all the identified proteins, apoA-1 has been reported to be majorly related to the cholesterol pathway, involved in HDL protein complex (Zhang et al., 2014). In-silico analysis of this protein (apo-A1) suggested that apolipoprotein family is associated with LCAT and other proteins of apo family like apoA2, apo B, apo C etc. (Figure 2). These proteins are profoundly related to cholesterol pathway. Further, we found that Apo-A1 was significantly
Figure 3: Two-dimensional gel electrophoresis of plasma proteins of control group G1, various drug treated groups G2 (curcumin); G3 (Dracaena cinnabari); G4 (Atorvastatin) and diseased group G5 (HFD fed rat) - (a) Tryptic digested protein spots.

Figure 3b: ApoA-1 identified protein (spot no.7) in all groups.
Figure 3c: The representative 2-DE gel showing densitometry analysis of ApoA-1 (spot no.7) carried out by PDQuest basic 8.0.1 version.

Figure 3d: The bar diagram showing the mean densitometry values of the respective group 1 to 5 of ApoA-1.
Figure 4: Western blot analysis showing the expression level of apoA-1, confirmed by densitometry analysis revealed 0.87 fold downregulation in G5 comparison to G1. Level of apoA-1 was upregulated by 1.33 fold (G2 group), 1.56 fold (G3 group), and 1.89 fold (G4 group) in comparison to HFD fed rat plasma (G5 group).

Figure 5: Enzyme linked immunosorbent assay of apoA-1 in plasma showing significant p<0.0001 with 1.00 fold upregulated expression of apoA-1 in G3 group (Dracaena cinnabari group) in comparison with HFD fed rat plasma (G5 group).
down regulated in HFD fed (Group 5) rat plasma indicating that apoA-1 has its own relevance in lipid metabolism and have associative role in CAD disease.

Our interest was therefore to see the effect of apoA-1 after the treatment with medicinal extract. Apo A-1 is majorly helping to clear fats, including cholesterol from white blood cells and arterial wall to prevent them from fat overload. Its molecular weight is 28kDa, produced in hepatocytes, plays crucial role in cholesterol metabolism and is an integral part of Phospholipid Transfer Proteins (PLTP) and Reverse cholesterol transport (RCT) (Chirackal Manavalan et al., 2014). ApoA-1 is not only a component of HDL but also work as a recognition molecule and activator of the other enzymes like LCAT and PLTP of RCT pathway. As the level of apoA-1 decreases, it inhibits the PLTP and LCAT activity. PLTP is involved in the pathogenesis of atherosclerosis causing CAD (Zhang et al., 2014) and is intricately involved with HDL in the movement of cholesterol as it activates LCAT. Binding of apoA-1 to lipid molecules (chylomicrons, phospholipid (PL) and free cholesterol (FC)) form pre βHDL. It activates the enzyme LCAT which esterifies the lipid molecule and helps in the formation of spherical HDL which finally sequestered by liver by PLTP activity. Here apo A-1 is regularizing the transportation of lipid molecules and turn LDL to HDL (Figure 6) (Mani et al., 2014). The other study reported that a prominent drug statin increases the level of HDL that gives less significant improvement in CAD condition, however raising the levels of apoA-1 provides prominent reduction of CAD (Vergeer et al., 2010). The level of apo A-1 is therefore inversely associated with risk of CAD. Previous studies showed that level of HDL-C and apo-A1 was significantly increased in response to Rhus coriaria methanolic extracts in patients with hyperlipidemia (Hajmohammadi et al., 2017). Mostly medicinal research efforts in the field of complementary and alternative medicines (CAM) had shown least side effects. The resin extract of Dracaena cinnabari Balf. f. is used extensively as a powerful medicinal herb endemically for the treatment of skin diseases, stomach pain, inflamed eye, internal and external bleeding and gastrointestinal diseases (Al-Fatimi, 2018; Ibraheam et al., 2018).

Due to its antifungal, antioxidant and cytotoxicity activity it can be used to treat inflammatory disease like CAD, arthritis, respiratory diseases etc. Recently it has been shown that in experimental rats, Dracaena have hypoglycemic and hypolipidemic activity (Al-Baoqai et al., 2018). Our studies involved the use of Dracaena and curcumin over the synthetic drug, atorvastatin to evaluate the efficacy of methanolic extract of Dracaena cinnabari Balf. f. in CAD treatment. It is also reported that the methanolic extract of Dracaena resin could be tolerated up to the dose 2000 mg/kg body weight (Al-Affifi et al., 2018). Curcumin (diferuloylmethane), an active component of turmeric commonly used as a food coloring agent, cosmetics, spices and medicines because of its antioxidant and anti-inflammatory properties (Menon and Sudheer, 2007), used as positive control in our studies. Atorvastatin, used as standard control in our study is a synthetic compound, consists of 3hydroxy3methyl glaryl coenzyme A (HMG CoA) reductase inhibitor, used to lower the plasma cholesterol levels (Lea and McTavish, 1997). With the exposure of various drug treatments, 2.0 fold up-regulation level of apoA-1 in G3 group ((Dracaena cinnabari Balf.) by
2-DE was validated by western and ELISA, revealed 1.56-fold upregulation, and 1.00-fold upregulation respectively (p<0.0001) in comparison to G5 group (HFD). Thus, resulting into the conclusion that Dracaena played a decent role in increasing the level of apoA-1 and is comparable to other standard drug (atorvastatin) and well known natural anti-inflammatory drug, curcumin. Evaluation of the produced data of the plant based medicinal extract over the standard commercial synthetic drug provides the information that Dracaena extract equally increases the level of apoA-1 like that of standard drug (atorvastatin). These significant results proved the proposed hypothesis that the plant extract of Dracaena is more valuable and beneficial to regulate disease severity positively than synthetically produced medicine (atorvastatin in this study) without much side effects and hence can be safely used to treat CAD. However more study is required to the above conclusion.

CONCLUSION

We conclude that protein apoA-1 is differentially expressed when HFD induced rat models were treated with Dracaena cinnabari Balf. f. extract. Overall, our data showed that drug exposure induces changes in the plasma protein (apo-A1) expression profile that closely correlated with the initial onset of physiological changes of the cardiovascular disease. The changes in proteins could be of critical importance in the pathogenesis of atherosclerosis development. Extract of Dragon’s blood resin and curcumin could be considered as a possible source for the treatment of atherosclerosis. Further studies are needed to evaluate the prospective use of drug and dose optimization of Dracaena cinnabari Balf. f. in order to find out its best potential effect against CAD.

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