Evaluation of possible mechanisms of *Cordia dichotoma* fruits for hyperlipidemia controlling in Wistar albino rats

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ARTICLE INFO

Article history:
Received 4 April 2018
Revision 6 May 2018
Accepted 10 June 2018
Available online 28 June 2018

Keywords:
*Cordia dichotoma* fruits
Antioxidants activities
Cholesterol absorption

ABSTRACT

**Objective:** To investigate the antioxidant capacity of aqueous extract from *Cordia dichotoma* (*C. dichotoma*) fruits *in–vitro* and their effect on nutritional parameters in rats fed on high-fat diet.

**Methods:** *In–vitro* antioxidant capacity of *C. dichotoma* extract were evaluated and compared to two standard materials, ascorbic acid and butylated hydroxytoluene. Metabolic experiments were set out using rats fed on high-fat diet. The extract was tested with two dosages: 0.5 and 1.0 g/kg body weight/ day for four weeks. Lipid constituents of diet and faeces and lipid profile of serum and liver were determined.

**Results:** The administration of the *C. dichotoma* extract with two dosages caused a significant improvement in the lipid metabolism of rats, compared to the hyperlipidemic control which showed significant disturbance in lipid profile. *C. dichotoma* extract reduced total body weight gain and total feed intake, and enhanced the fresh and dry weight of faecal excretion. The superior effect was recorded with the high dosage of extract. *C. dichotoma* minimized fat and cholesterol intake significantly and maximized those in faecal excretions in comparison with hyperlipidemic control values, and low dosage was better than the high one. *C. dichotoma* extract at two dosages normalized the lipid profile of the serum and liver compared with hyperlipidemic control.

**Conclusions:** The protective effect of *C. dichotoma* extract against hyperlipidemia may be attributed to the reduced ability of an animal to ingest and absorb fat and cholesterol, and enhanced ability to get rid of them in faecal excretion.

1. Introduction

In cells, the production of reactive oxygen species (ROS) during metabolism is a normal and necessary process. ROS have important physiological functions, some ROS acting as cellular messengers in redox signaling. Oxidative stress is an imponderables between the systemic production of ROS and a biological system’s ability to remove and detoxify the reactive intermediates or to remedy the produced damage, which produces peroxides and free radicals. The peroxides or free radicals have a toxic effect and damage the proteins, lipids, and DNA of cells. Many human diseases have


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How to cite this article: El-Newary SA, Ibrahim AY, Osman SM, Wink M. Evaluation of possible mechanisms of *Cordia dichotoma* fruits for hyperlipidemia controlling in Wister albino rats. Asian Pac J Trop Biomed 2018; 8(6): 302-312.
been attributed with oxidative stress, including atherosclerosis, heart failure and myocardial infarction[1]. ROS can damage proteins or change their functions by damaging metal-binding sites and disulfide bonds, fragmentation and cross-linking. An increase in the intracellular generation of ROS plays an essential role in the atherosclerosis incidence[2]. Hypercholesterolemia causes an increment in low density lipoprotein (LDL) in the artery wall, which is followed by oxidative modifications. Therefore, antioxidant substances can stop the initiation and propagation of atherosclerosis[3]. The atherosclerosis process starts by the oxidation of accumulated LDL in arteries. The presence of Ox-LDL is associated with a production of superoxide anion, hydrogen peroxide (H$_2$O$_2$), and peroxynitrite, which make up the evidence for the oxidation of lipids in human atheroma lesions[4].

Many plants have a powerful antioxidant capacity, such as Cordia dichotoma (C. dichotoma) Forst. (Boraginaceae), also known as sebestan plum or soap berry; and it has been known in Egypt as a mokhate[5]. The fruit is divided into a pulpy part and one kernel seed. The kernel seed is surrounded by a viscid sweetish transparent pulp. Traditionally, C. dichotoma has been used for treating anemia, impotence, gastric pain, asthma, mouth ulcers, bronchitis, diarrhea, rheumatism, and dental caries; it has also been used as an anti-inflammatory agent, an antioxidant, an anticancer agent, an expectorant, as a demulcent, an anti-arthritis agent, an antidepressant, an anti-ulcer, an anti-fertility, a cosmetic agent and also used in healing wounds. Fruits have ulcer protective, hepatoprotective and wound-healing characteristics, showing antidiabetic and anthelmintic activities as well as hypolipidemic ability[6]. C. dichotoma fruit contains many vital components, such as polyphenols, flavonoids, alkaloids and mucilage[5,6]. Rosmarinic acid, chlorogenic acid, caffeic acid, rutin, quercetin, and kaempferol are the main polyphenols that occur in viscid layer aqueous extract[6]. According to our results which have been published in 2016[6], it was found that the hypolipidemic effect of the aqueous extract of C. dichotoma fruits including decreasing total cholesterol (TC), triglycerides (TG) and low density lipoprotein cholesterol (LDL-C) levels as well as increasing high density lipoprotein cholesterol (HDL-C) level in rats fed on high fat diet for 10 weeks.

So, the current work was carried out to complete the previous study to investigate the possible mechanisms of C. dichotoma fruits extract for hyperlipidemia control in high-fat diet induced hyperlipidemic Wistar albino rats.

2. Materials and methods

2.1. Chemicals

Ferrous chloride, potassium hexacyanoferrate, trichloroacetic acid, 1,1-diphenyl-2-picryl-hydrayl (DPPH), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine), nicotinamide adenine dinucleotide, phenazine methosulphate, nitroblue tetrazolium salt, butylatedhydroxytoluene (BHT), ascorbic acid (Vit C), 2,2-azinobis (3-ethylbenzthiazolin-sulphonic acid) diammonium salt, peroxidase, cholesterol and cholic acid were obtained from the Sigma-Aldrich Company (St. Louis, MO, USA). A chow-based commercial diet was purchased from the animal house of National Research Centre, Dokki, Giza, Egypt. Camel fat was obtained from local market. All chemicals used were of analytical grade.

2.2. Plant material and preparation of the extract

Fruits were obtained from Cordia trees in the Sharkia area, Egypt, on August 2015. Fruits were washed and were separated into two parts; the external part (pulp) and internal part (the kernel seed surrounded with a viscid layer enriched with mucilage). Using cold soaking by water the viscid layer was extracted at room temperature for a period of 24 h. The aqueous extract was concentrated in rotary evaporators (Buchi Rotavapor)[6] and then was lyophilized. The remaining powder was kept on -20 °C until use.

2.3. In-vitro study

Determination of the antioxidant activity of C. dichotoma extract was carried out with five methods as free radicals scavenging, reducing power, ferrous ion chelating activity, superoxide anion scavenging activity, and total antioxidant capacity. The antioxidant activities of the extract were compared with those of ascorbic acid and BHT which were taken as reference materials. Four concentrations of each material and the extract were used as 1 000, 500, 250 and 100 μg/mL. IC$_{50}$, the concentration of the extract that inhibited the 50% of free radicals, was calculated using obvious concentrations.

2.3.1. Free radical scavenging activity

The effect of C. dichotoma aqueous extract and standards (ascorbic acid and BHT) on DPPH radicals was determined using the method of Yamaguchi et al.[7].

2.3.2. Reduction capability

The reduction capability of the aqueous extract and standard materials were determined by using the method of Oyaizut[8].

2.3.3. Ferrous ion chelating activity

The ferrous ion chelating effect of the extract and standard compounds were assayed according to Dinis et al.[9].

2.3.4. Superoxide anion scavenging activity

The measurement of the superoxide anion scavenging activity of
the extract was based on the method described by Liu et al.[10].

2.3.5. Total antioxidant capacity
The total antioxidant capacity of the extract and standard compounds was estimated based on the methods of Miller and Rice-Evans[11] and Armao et al.[12].

2.4. In-vivo study

2.4.1. Ethical and animals
The experimental protocol was carried out based on the institutional guidelines for the care and use of laboratory animals, National Research Centre, Dokki, Giza, Egypt. This study was approved by the Medical Research Ethics Committee, National Research Centre, Egypt, under registration No. 16/ 032. The experiment was performed in the animal house of the National Research Centre, Dokki, Giza, Egypt.

Adult male Wistar albino rats (thirty-six), weighing between 140 and 160 g, were used and were maintained at (25±2) °C, moisture 60%-65% with a 12-h light: 12-h dark cycle. The animals were individually deposited in wire-bottomed metabolic cages and maintained at room conditions. The animals were fed standard laboratory rat food, with free access to tap water. Rats were weighed and randomly assigned to the different experimental groups (six animals per group).

2.4.2. Diet
The standard diet was a chow-based commercial diet containing: 4.60% fat, 25.00% crude protein, 4.78% crude fiber and 6.71% crude ash (the diet composition analyzed according to AOAC[13]). High-fat diet was an obvious standard diet with fat modification to 20% using camel fat, and supplemented with 1% cholesterol and 0.25% cholic acid[6].

2.4.3. Experimental design
The animals were acclimatized for 7 d under laboratory conditions before the experiment period. The rats were divided into six groups, each group with six rats.

Group I: Rats fed standard diet for four weeks and were kept as a negative control.
Group II: Rats fed standard diet and were force fed extract at dosage 0.5 g/kg/day for four weeks and were kept as positive control for this dosage.
Group III: Rats fed standard diet and were force fed extract at dosage 1.0 g/kg/day for four weeks and were kept as positive control for this dosage.
Group IV: Rats fed high-fat diet for four weeks and were kept as a hyperlipidemic control.
Group V: Rats fed high-fat diet and were co-administrated extract at dosage 0.5 g/kg/day orally for four weeks and were kept as protective group.
Group VI: Rats fed high-fat diet and were co-administrated extract at dosage 1.0 g/kg/day orally for four-weeks and were kept as protective group.

After four weeks, the animals were fasted and maintained with tap water over night. To achieve anesthesia rats were injected 87 mg ketamine/ kg of body weight and 13 mg/ xylazine, beginning 10-15 min after simultaneous injection intraperitoneal and lasting 15-30 min. The two drugs were dissolved in normal saline and each rats received 0.2 mL/100 g body weight[14]. Animals were scarified after anesthesia and the blood samples were collected from the retro-orbital plexus as well as the liver for biochemical analysis. Sera were collected after blood centrifugation at 4000 g for 10 min by using Sigma Laborzentrifugen (Osterode am Harz, Germany). Livers were weighed and were used to prepare liver homogenate using tissue homogenizer ultrasonic, and then centrifuged at 4000 g, at 4 °C for 15 min[15]. Supernatants were removed to use for the determination of antioxidant parameters and oxidative stress parameters.

2.4.4. Nutritional parameters
Over the experimental period, faeces were collected daily and weighed before and after drying; body weight and food intake were also monitored daily. Feed efficiency is evidence of the extent of benefit from ingested food, or how much of the food eaten has benefitted the animal was calculated. Diets and faeces protein, fat and TC were analyzed. Crude protein and fat were determined according to the methods adapted in AOAC[13]. The diet and faecal TC was estimated by the procedure developed by Folch et al.[16]. TC concentration was analyzed with the same enzymatic kits as used in the serum analysis.

2.4.5. Biochemical analysis
The lipid profile was determined in serum samples. The determination of TC, HDL-C and TG was carried out according to the methods of Allain et al.[17], Naito and Kaplan[18] and Fossati and Prencipe[19], respectively using kits obtained from Biodigonestico Co (Egypt). The calculation of LDL-C [LDL-C= TC-(VLDL + HDL)], very low density lipoprotein cholesterol (VLDL-C) (VLDL-C= TG/5) and the risk ratio (LDL-C/ HDL-C) was carried out according to Friedewald et al.[20], Naito and Kaplan[18] and Kikuchi et al.[21] respectively.

Glutathione (GSH) concentration and antioxidant enzymes activities of liver, such as glutathione reductase (GR), glutathione-S-transferase (GST), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD), were estimated in liver homogenate according to Griffith[22], Goldberg and Spooner[23]; Habig et al.[24]; Paglia and Valentine[25]; Beers and Sizer[26] and Fridovich[27], respectively.
using kits obtained from Biodigonestic Co (Egypt). Oxidative stress parameters: malondialdehyde (MDA) and H$_2$O$_2$ in the liver were analyzed according to Ohkawa et al.[28] and Chance and Maehly[29], respectively using kits obtained from Biodigonestic Co (Egypt).

Liver TC and TG were extracted, using the procedure developed by Folch et al.[16]. The concentrations of TC and TG in the liver were measured, using kits obtained from Biodigonestic Co (Egypt).

2.5. Statistical analysis

All studied data were statistically analyzed using the Co-Stat 6.303 Software Computer Program 2004 hypothesis; testing methods included one-way analysis of variance (ANOVA) test.

3. Results

3.1. In-vitro results

3.1.1. DPPH radical scavenging activity

_C. dichotoma_ extract showed weak dose-dependent radical scavenging. A high concentration of extract, 1 000 μg/mL, showed low DPPH radical scavenging, compared with ascorbic acid and BHT, respectively. IC$_{50}$ value of the extract was 2 235 μg/mL, compared with 41.25 and 48.66 μg/mL by ascorbic acid and BHT, respectively. IC$_{50}$ value was 522.90 μg/mL, compared with IC$_{50}$ of ascorbic acid (35.80 μg/mL) and BHT (42.10 μg/mL).

3.1.2. Reducing power

In the reducing power assay, the reducing power of _C. dichotoma_ extract was higher than that of ascorbic acid and BHT (Table 1). IC$_{50}$ value of the extract was 2 235 μg/mL, compared with 41.25 and 48.66 μg/mL by ascorbic acid and BHT, respectively (Table 1).

3.1.3. Ferrous ion chelating activity

The ferrozine method was used to determine the ability of _C. dichotoma_ extract to bind to Fe$^{2+}$ ion catalyzing oxidation; as data shown in Table 1. The extract showed Fe$^{2+}$ chelation ranged between 23.86% at the lowest concentration (100 μg/mL) to 71.30% at the highest one (1 000 μg/mL). IC$_{50}$ value was 522.90 μg/mL, compared with IC$_{50}$ of ascorbic acid (35.80 μg/mL) and BHT (42.10 μg/mL).

3.1.4. Superoxide anion radical scavenging activity

_C. dichotoma_ extract showed potent scavenging properties against superoxide radicals (O$_2^-$) in a concentration-dependent manner (Table 1). Extract (IC$_{50}$: 178.41 μg/mL) was required to inhibit 50% O$_2^-$ generated in a phenazine methosulphate-nicotinamide adenine dinucleotide system, compared with 55.50 μg/mL for ascorbic acid and 52.00 μg/mL for BHT.

3.1.5. Total antioxidant capacity

The total antioxidant capacity of the extract was evaluated according to the ABTS/ H$_2$O$_2$ discoloration method, corresponding to standard material (ascorbic acid and BHT) data in Table 1. _C. dichotoma_ at different concentrations displayed a noteworthy antioxidant capacity. The total antioxidant capacity, represented as IC$_{50}$ of the extract, was 37.43 μg/mL compared with the natural antioxidants, ascorbic acid (41.88 μg/mL), and synthetic ones BHT (33.10 μg/mL).

3.2. In-vivo results

3.2.1. Nutritional parameters of rats co-administrated _C. dichotoma_ extract

3.2.1.1. Total body weight gain (g/4 weeks)

Administration of high-fat diet elevated the total body weight gain of hyperlipidemic control rats [(59.60±1.30) g/4 weeks], compared to the negative control [(59.60±1.30) g/4 weeks]. The co-administration of the extract was in two dosages: 0.5 and 1.0 g/kg; this reduced the total body weight gain of rats fed high-fat diet.

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Table 1

Antioxidants characters of _C. dichotoma_ extract and standard compounds.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Concentration (μg/mL)</th>
<th>DPPH scavenging activity (%)</th>
<th>Reducing power (Absorbance)</th>
<th>Fe$^{2+}$ chelating effect (%)</th>
<th>O$_2^-$ ion scavenging (%)</th>
<th>Total antioxidant capacity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. dichotoma</em></td>
<td>100</td>
<td>2.95±1.36$^{a,b}$</td>
<td>90±0.03$^{a,b}$</td>
<td>23.86±0.70$^{a,b}$</td>
<td>36.60±1.50$^{a,b}$</td>
<td>55.82±0.90$^{a,b}$</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>8.55±0.03$^{a,b}$</td>
<td>95±0.05$^{a,b}$</td>
<td>29.95±0.40$^{a,b}$</td>
<td>66.18±1.90$^{a,b}$</td>
<td>70.05±1.55$^{a,b}$</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>13.34±0.03$^{a,b}$</td>
<td>97±0.05$^{a,b}$</td>
<td>49.20±2.20$^{a,b}$</td>
<td>80.88±2.33$^{a,b}$</td>
<td>72.65±1.70$^{a,b}$</td>
</tr>
<tr>
<td></td>
<td>1 000</td>
<td>26.22±1.45$^{a,b}$</td>
<td>97±0.05$^{a,b}$</td>
<td>71.30±1.30$^{a,b}$</td>
<td>97.52±2.23$^{a,b}$</td>
<td>73.50±1.31$^{a,b}$</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>100</td>
<td>60.00±1.84$^{a,b}$</td>
<td>19±0.05</td>
<td>60.65±0.85</td>
<td>57.21±1.80$^{a,b}$</td>
<td>60.50±2.30$^{a,b}$</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>73.31±1.87$^{a,b}$</td>
<td>58±0.03</td>
<td>81.33±1.13</td>
<td>80.37±1.57$^{a,b}$</td>
<td>77.94±2.40$^{a,b}$</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>78.28±2.00$^{a,b}$</td>
<td>64±0.04</td>
<td>90.52±0.70</td>
<td>95.34±1.45$^{a,b}$</td>
<td>88.75±0.12$^{a,b}$</td>
</tr>
<tr>
<td>BHT</td>
<td>1 000</td>
<td>94.00±1.96$^{a,b}$</td>
<td>78±0.06</td>
<td>98.19±1.60</td>
<td>97.75±1.80$^{a,b}$</td>
<td>97.00±1.00$^{a,b}$</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>60.46±1.01$^{a,b}$</td>
<td>19±0.05</td>
<td>59.45±1.60</td>
<td>60.22±1.14$^{a,b}$</td>
<td>61.50±2.40$^{a,b}$</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>70.10±2.02$^{a,b}$</td>
<td>49±0.04</td>
<td>87.93±1.45</td>
<td>73.61±0.40$^{a,b}$</td>
<td>75.85±0.09$^{a,b}$</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>79.88±1.60$^{a,b}$</td>
<td>53±0.03</td>
<td>94.16±2.14</td>
<td>93.21±0.80$^{a,b}$</td>
<td>85.50±0.80$^{a,b}$</td>
</tr>
<tr>
<td></td>
<td>1 000</td>
<td>96.32±1.50$^{a,b}$</td>
<td>60±0.05</td>
<td>99.09±0.30</td>
<td>100.0±1.89$^{a,b}$</td>
<td>99.50±0.19$^{a,b}$</td>
</tr>
</tbody>
</table>

Data are presented as mean of triplicates ± SE, t-test was used for data analysis (n=3, P<0.05). Data are followed with small letter; a means significant difference with ascorbic acid, b means significant difference with BHT.
by (79.80±3.40) and (60.14±6.37) g/4 weeks, respectively, compared with the hyperlipidemic control. In contrast, the C. dichotoma extract insignificantly increased the total body weight gain of positive control rats compared to the negative control.

3.2.1.2. Total food intake (g/4 weeks)

Rats fed high-fat diet consumed more to reach 10.61% over that consumed by the negative control rats ([367.7±9.9] g/4 weeks). Co-administration of C. dichotoma reduced the amount of high-fat diet which rats consumed ([383.9±11.5] and [381.7±11.5] g/4 weeks, respectively) at different dosages, compared with the hyperlipidemic control. The total feed intake of positive controls, which was administered C. dichotoma extract, nearly remained the same that of the negative control.

3.2.1.3. Feed efficiency

Feed efficiency was significantly raised as a response to the administration of high-fat diet (0.24±0.02)% compared with the negative control at (0.16±0.01)% High dosages of C. dichotoma extract reduced feed efficiency significantly (0.16±0.03)% while low dosages did not change it significantly compared to the hyperlipidemic control. The feed efficiency of positive control rats did not show any significant change compared with the negative control.

3.2.1.4. Total faecal excretion (g/4 weeks)

Neither the fresh nor dry weight of faecal excretion significantly changed with the administration of high-fat diet compared with that of the negative control: (68.8±1.3) and (37.6±2.0) g/4 weeks (Table 2). Fresh faecal excretion was significantly elevated by the co-administration of C. dichotoma extract with two dosages, 0.5 and 1.0 g/kg, by about (113.0±3.7) and (101.9±2.5) g/4 weeks, respectively, compared to the hyperlipidemic control. Dry faecal excretion was also significantly higher in the rats which were fed high-fat diet and co-administered C. dichotoma extract [(72.7±3.5) and (58.8±2.5) g/4 weeks, respectively]. In the positive control rats, C. dichotoma extract with two dosages (0.5 and 1.0 g/kg) raised fresh and dry faecal excretion compared to the negative control.

3.2.2. Intake and excretion of fat, TC and protein

3.2.2.1. Fat

A significant increase was recorded in the fat intake during 4 weeks (+411.38%), fat faecal excretion (+171.43%), fat faecal percentage (+150.0%), and apparent fat digestibility (AD) (+22.0%) in the hyperlipidemic control as a response to the high-fat diet administration, in comparison with the negative group (Table 3). Co-administration of C. dichotoma extract at 0.5 and 1.0 g/kg in concurrence with feeding high-fat diet significantly reduced fat intake [(80.6±3.7) and (80.1±1.4) g/4 weeks, respectively], compared with that of the hyperlipidemic control rats [(85.4±1.1) g/4 weeks] (P<0.05). The fat intake of positive control rats force fed C. dichotoma at two dosages was not significantly influenced by C. dichotoma administration compared with the negative control.
Administration of low dosage of the *C. dichotoma* extract (0.5 g/kg) magnified fat faecal excretion percentages in rats fed high-fat diet or fed a standard diet, with (5.5±0.3)% and (2.2±0.3)%, respectively, compared with fat excreted in the hyperlipidemic and the negative control rats, respectively. High dosage did not affect the fat faecal excretion percentages of rats fed high-fat diet and positive diet with respect to each comparison group.

Rats fed high-fat diet and administrated with *C. dichotoma* at two dosages [(4.0±0.1) and (2.6±0.2) g/4 weeks, respectively] excreted fat much higher than that excreted by rats of the hyperlipidemic control [(1.9±0.1) g/4 weeks]. The same was recorded in the positive control rats, where *C. dichotoma* extract elevated fat faecal excretion, compared with the negative control, *P*<0.05.

*C. dichotoma* administration decreased the fat AD either in rats fed high-fat diet or in rats of positive control, compared to each control (*P*<0.05).

### 3.2.2.2. TC

The TC intake was much higher in animals fed high-fat diet and co-administered low and high dosage of *C. dichotoma* extract (Table 3), with (15.80±0.10) and (16.40±0.60) g/4 weeks, respectively in comparison with TC intake of the hyperlipidemic control [(17.70±0.20) g/4 weeks]. The TC intake by the positive control rats was close to that of the negative control.

Both TC faecal content and TC faecal excretion were significantly increased when rats were fed high-fat diet and administrated with *C. dichotoma* extract, compared to the hyperlipidemic control. TC faecal content and excretion of rats force-fed the low dosage of the extract was much higher, with (31.6±1.7) mg/g faeces and (2.4±0.1) g/4 weeks, respectively, than that of rats co-administered the high dosage [(27.9±1.2) mg/g faeces and (1.65±0.04) g/4 weeks, respectively] compared with hyperlipidemic control [(22.5±0.8) mg/g faeces and (0.92±0.10) g/4 weeks, respectively]. Compared to the negative control, *C. dichotoma* extract with two dosages elevated the TC faecal excretion of the positive control rats.

Therefore, *C. dichotoma* extract significantly decreased the AD of TC on rats fed high-fat diet or positive control rats in comparison with the hyperlipidemic and negative controls, respectively.

### 3.2.2.3. Protein

Rats fed high-fat diet registered a significant elevation of protein intake and protein efficiency ratio (PER) in comparison with the negative control value. Only the percentage of the faecal excretion of protein was reduced significantly, corresponding to the negative control (Table 3). The faecal excretion of protein and the AD of protein did not change significantly on the administration high-fat *C. dichotoma* extract at two dosages (0.5 and 1.0) g/kg reduced protein intake of rats fed high-fat diet as a result of decreased food intake basically. No significant difference was noticed between the protein intake of all positive control groups and the negative control.

Low dosages of extract significantly decreased the faecal percentages of protein in hyperlipidemic rats, while high dosages did not show this effect compared with the hyperlipidemic control. In positive control of rats administrated *C. dichotoma* extract with two dosages, the faecal percentage of protein was decreased compared with negative control (*P*<0.05).

In addition, the faecal excretion of protein was significantly elevated in hyperlipidemic rats force fed high dosages of extract, while two dosages of extract significantly elevated faecal excretion of protein in positive control rats as a result of increasing faecal excretion in general.

According to the data mentioned above, *C. dichotoma* extract at low and high dosages remarkably decreased AD in hyperlipidemic rats and positive control rats as a response to elevated faecal excretion, in comparison with hyperlipidemic and negative control, respectively.

*C. dichotoma* extract decreased the PER of hyperlipidemic rats as a result of decreasing total body weight. In an opposite trend of positive control rats, force feeding of high dosages significantly increased PER of rats as a response to total body weight gain in positive control rats, compared with the negative control.

### 3.2.3. Hypolipidemic parameters of serum and liver of rats co-administered *C. dichotoma* extract

#### 3.2.3.1. Serum lipid pattern

The group fed high-fat diet caused a significant hyperlipidemia (data presented in Table 4). Serum lipid profile of hyperlipidemic control rats showed elevated levels of serum TC, TG and VLDL-C and LDL-C and decreased level of HDL-C compared with those

#### Table 4

Effect of force-feeding of *C. dichotoma* extract on lipid profile in rats fed high-fat diet after 4 weeks.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mg/dL)</th>
<th>HDL-C (mg/dL)</th>
<th>LDL-C (mg/dL)</th>
<th>TG (mg/dL)</th>
<th>VLDL-C (mg/dL)</th>
<th>Risk ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative group</td>
<td>87.89±2.69</td>
<td>40.85±1.69</td>
<td>29.70±3.10</td>
<td>86.68±2.75</td>
<td>17.34±0.55</td>
<td>0.73±0.09</td>
</tr>
<tr>
<td>Positive control</td>
<td>73.56±3.50</td>
<td>40.81±1.60</td>
<td>15.03±1.89</td>
<td>88.62±1.82</td>
<td>17.72±0.36</td>
<td>0.37±0.07</td>
</tr>
<tr>
<td></td>
<td>77.75±1.60</td>
<td>46.64±1.77</td>
<td>16.74±0.85</td>
<td>71.87±2.60</td>
<td>14.37±0.57</td>
<td>0.36±0.03</td>
</tr>
<tr>
<td>Hyperlipidemic control</td>
<td>234.39±4.92</td>
<td>25.19±1.57</td>
<td>181.68±4.71</td>
<td>137.62±4.04</td>
<td>27.52±0.82</td>
<td>7.23±0.42</td>
</tr>
<tr>
<td>Protective group</td>
<td>125.30±1.69</td>
<td>40.98±0.46</td>
<td>60.96±2.15</td>
<td>116.85±4.01</td>
<td>23.37±0.80</td>
<td>1.48±0.04</td>
</tr>
<tr>
<td></td>
<td>138.29±2.87</td>
<td>41.13±1.06</td>
<td>78.42±2.98</td>
<td>93.75±2.03</td>
<td>18.75±0.41</td>
<td>1.92±0.19</td>
</tr>
</tbody>
</table>

Data are represented as mean of six replicates ± SE. Data analyzed with ANOVA one-way. value with the same letter is not significant but value with different letter is significant at *P*<0.05. Risk ratio= LDL-C/HDL-C.
respectively, the risk ratio of hyperlipidemic control (7.23±0.42)% were elevated significantly compared to the negative control (0.73±0.09)%.

TC levels were much lower in rats fed high-fat diet and co-administrated 

C. dichotoma extract at low and high dosages with (125.30±3.69) and (138.29±2.87) mg/dL, respectively, in comparison with (234.39±4.92) mg/dL TC in the hyperlipidemic control rats. Also, TC of the positive controls was lower than that of the negative control at (73.56±3.50), (77.75±1.60) and (87.89±2.69) mg/dL, respectively, 
P<0.05.

The TG and VLDL-C of animals fed high-fat diet and co-

administrated 

C. dichotoma extract at low and high dosages were significantly decreased with (116.85±4.01) and (93.75±2.03) mg/dL for TG and (23.37±0.80) and (18.75±0.41) mg/dL for VLDL-C, respectively, compared with those of the hyperlipidemic control [(137.62±4.04) and (27.52±0.82) mg/dL]. Low extract dosages did not change the TG and VLDL-C of positive control rats, while high dosages reduced these significantly, with (71.87±2.60) and (14.37±0.57) mg/dL, respectively, compared with the negative control [(86.68±2.75) and (17.34±0.55) mg/dL] (P<0.05).

On the other hand, HDL-C levels were much higher in the animals ingested high-fat diet and co-administrated with two extract dosages of 0.5 and 1.0 g/kg, with (40.98±0.46) and (41.13±0.06) mg/dL, respectively, in comparison with (25.19±1.57) mg/dL in the hyperlipidemic control. In the positive control rats, high dosages of 

C. dichotoma extract raised HDL-C significantly, while the low dosage had not significant effect on HDL-C compared with the negative group.

Based on the previous results, LDL-C levels were significantly lowered, when rats were force-fed with 0.5 and 1.0 g/kg extract and fed high-fat diet, to (60.96±2.15) and (78.42±2.98) mg/dL, respectively, compared to (181.68±4.71) mg/dL showed in the hyperlipidemic control. LDL-C levels of the positive control rats showed the nearly same results in comparison with the negative group.

Accordingly, the risk ratio was significantly reduced, when hyperlipidemic rats were force-fed by two dosages of extract; (1.48±0.04)% and (1.92±0.19)%, respectively, compared with the hyperlipidemic control (7.23±0.42)%.

The risk ratios of positive control rats receiving low and high dosages of extract were force-fed by two dosages of extract; (1.48±0.04)% and (1.92±0.19)%, respectively, compared with the hyperlipidemic control (7.23±0.42)%.

3.2.3.2. Hepatic cholesterol and TG

Administration of high-fat diet significantly increased relative weight of rat’s liver compared to that of the negative control. 

C. dichotoma extract did not exert an effect on relative weight of liver of rats fed standard diet or high-fat diet compared with the negative or hyperlipidemic controls.

In comparison with animals administrated a standard diet, rats fed high-fat diet showed significant elevation of hepatic TC and TG, respectively (Table 5). The TC and TG stored in the hepatic tissue were much lower in the rats fed high-fat diet and co-administrated 

C. dichotoma extract in low and high dosages ([44.84±2.63] and (49.25±1.49) mg/g liver tissue for TC and (50.88±2.51) and (55.47±4.22) mg/g liver tissue for TG, respectively) compared to (83.04±4.43) and (84.13±4.19) mg/g liver tissue that were recorded in the hyperlipidemic control, (P<0.05).

In positive control rats, neither TC nor TG changed significantly when rats were administrated extract with two dosages 0.5 and 1.0 g/kg compared with the negative group.

Table 5

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver % TC (mg/g)</th>
<th>TG (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>3.00±0.11</td>
<td>38.61±3.99</td>
</tr>
<tr>
<td>Positive control</td>
<td>0.50 g extract/kg</td>
<td>2.97±0.18</td>
</tr>
<tr>
<td></td>
<td>1.00 g extract/kg</td>
<td>3.25±0.20</td>
</tr>
<tr>
<td>Hyperlipidemic control</td>
<td>3.70±0.10</td>
<td>43.30±4.43</td>
</tr>
<tr>
<td>Protective group</td>
<td>0.50 g extract/kg</td>
<td>3.75±0.21</td>
</tr>
<tr>
<td></td>
<td>1.00 g extract/kg</td>
<td>3.82±0.21</td>
</tr>
</tbody>
</table>

Data are represented as mean±SE. Mean of six replicates. Data analyzed by ANOVA, value with the same letter is not significant but value with different letter is significant at (P<0.05). TC: Total cholesterol; TG: triglycerides.

3.2.4. Antioxidant parameters of liver of rats co-

administrated 

C. dichotoma extract

Liver GSH concentration and antioxidant enzymes activities, GR, GST, GPx, CAT and SOD in all groups were documented in Table 6. Feeding high-fat diet caused a significant reduction of GSH concentration and GR, GST, GPx, CAT and SOD activities in comparison with the negative group (P<0.05).

Co-administration of the extract restored GSH concentration of hyperlipidemic rats higher than GSH of hyperlipidemic control by about 73.41% and 82.96% with low and high dosage respectively. Significant increase was observed in GSH of rats fed standard diet and co-administrated the extract compared with the negative control.

The GR activity was much higher in rats fed high-fat diet and co-administrated with low and high dosages of extract, with (7.83±0.50) and (8.26±0.32) μmol/mg protein/ min, respectively, compared with (4.52±0.52) μmol/mg protein/min of the hyperlipidemic control.

A moderately higher GR activity was observed in the positive control rats [(9.65±0.52) and (8.96±0.33) μmol/mg protein/min at two dosages, respectively] compared with the negative group: (6.85±0.42) μmol/mg protein/min.

The GST activity was significantly increased in rats fed high-fat diet and co-administrated with low and high dosages of C. dichotoma extract at low and high dosages [(3.95±0.25) and (83.04±4.43) mg/g liver tissue showed in the hyperlipidemic control, (P<0.05).
value with different letter is significant at glutathione reductase (GR). Data are represented as mean ± SD. Liver antioxidants include glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione–S-transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GR). Data are represented as mean±SE. Data analyzed by ANOVA, value with the same letter is not significant but value with different letter is significant at P<0.05. H2O2: hydrogen peroxide; MDA: malondialdehyde.

Table 7
Effect of C. dichotoma extract on liver oxidative stress of hyperlipidemic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>H2O2 (mmol/g tissue)</th>
<th>MDA (mmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>21.88±1.35</td>
<td>5.44±0.61</td>
</tr>
<tr>
<td>Positive control</td>
<td>15.48±1.47</td>
<td>2.76±0.27</td>
</tr>
<tr>
<td>Hyperlipidemic control</td>
<td>10.14±1.26</td>
<td>5.94±0.40</td>
</tr>
<tr>
<td>Protective group</td>
<td>36.37±2.36</td>
<td>8.78±0.48</td>
</tr>
</tbody>
</table>

Data are represented as mean±SE. Mean of six replicates. Data analyzed by ANOVA, value with the same letter is not significant but value with different letter is significant at P<0.05.

4. Discussion

This study was conducted to complete our previous study on the hypolipidemic effect of C. dichotoma aqueous extract of the viscid sweetish transparent layer, which surrounds the kernel seeds. We studied the hypolipidemic mechanism of this extract. The rats fed high-fat diet only was characterized by elevated TC, TG, VLDL and LDL levels and a reduced HDL level, compared to normal lipid profile of negative control.

Feeding rats on high-fat diet showed a significant rise in total body weight and total food intake, and did not affect the fresh and dry weight of faecal excretion. The force-feeding of the extract decreased the total gain in body weight and the total food intake, and did not affect the fresh and dry faecal excretion weight, but did not show any significant effect on body weight gain or feed intake. These data are supported by those of Lecumberri et al.[30] on cocoa fiber, and El-Newary et al.[6] on C. dichotoma.

The decrease in body weight of rats fed high-fat diet and co-administrated C. dichotoma extract may be elucidated by a decreasing food, fat, and TC intake with an increase in faecal excretion and fat, and TC faecal excretion. This reduction may be associated with the

Table 6
Effect of C. dichotoma extract on GSH concentration and antioxidant enzymes activities in liver homogenate of hyperlipidemic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (mmol/g tissue)</th>
<th>GR (μmol/mg protein/min)</th>
<th>GST (μmol/mg protein/min)</th>
<th>GPx (μmol/mg protein/min)</th>
<th>CAT (U/mg protein)</th>
<th>SOD (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>8.11±0.76</td>
<td>6.85±0.42</td>
<td>5.42±0.33</td>
<td>3.94±0.32</td>
<td>12.77±0.63</td>
<td>7.69±0.15</td>
</tr>
<tr>
<td>Positive control</td>
<td>11.40±0.52</td>
<td>9.65±0.52</td>
<td>6.30±0.53</td>
<td>4.94±0.46</td>
<td>11.94±0.60</td>
<td>10.64±0.24</td>
</tr>
<tr>
<td>Hyperlipidemic control</td>
<td>10.59±0.39</td>
<td>8.96±0.33</td>
<td>6.86±0.16</td>
<td>5.46±0.14</td>
<td>16.33±0.95</td>
<td>9.70±0.06</td>
</tr>
<tr>
<td>Protective group</td>
<td>5.34±0.62</td>
<td>4.52±0.52</td>
<td>3.19±0.12</td>
<td>5.34±0.17</td>
<td>4.66±0.11</td>
<td>3.06±0.18</td>
</tr>
</tbody>
</table>

Liver antioxidants include glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione–S-transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GR). Data are represented as mean±SD. Mean of six replicates. Data analyzed by ANOVA, value with the same letter is not significant but value with different letter is significant at P<0.05.
mucilage content in the extract: 20.18% as viscous dietary fibers[6], and a reduction of the availability of diet energy, which is decreased by increasing dietary fiber consumption. Viscous fiber increased the food glueyness and size in the stomach, which induces satiety and sense of fullness and a delay in stomach drainage; this all resulted in decrease in caloric intake[31]. Metabolizable energy is the difference between the total income energy and the outcome energy through faeces, urine and combustible gases which is decreased by increasing dietary fiber consumption. Undoubtedly, phenols such as caffeine suppress appetite and equally stimulate thermogenesis, a physical reaction that increases the number of calories the body burns. Caffeine may cause temporary weight loss due to its diuretic effect. Quercetin causes a reduction in body weight and liver fat accumulation[32]. C. dichotoma extract contains quercetin and caffeic acid at about 4.90 and 13.5 mg/g extract, respectively[6].

The decrease in amount of ingested food might also be attributed to the C. dichotoma extract mucilage. Soluble fiber as mucilage was not absorbed in the small intestine, and fermented in the large intestine, which produces glucagon-like peptide (GLP-1) and peptide YY (PYY). Both GLP-1 and PYY are gut hormones, which affect satiety. Viscous fibers have been shown to lead to a simple distention of the gastric antrum. Therefore, the feeling of satiety was elevated and stopping of eating through meal times. Viscous fiber delayed a postprandial rise of ghrelin, resulting in the slow distention of the gastric antrum. Therefore, the feeling of satiety was elevated and stopping of eating through meal times. Viscous fiber delayed a postprandial rise of ghrelin, resulting in the slow absorption of glucose and amino acids[33].

The administration of C. dichotoma extract caused significant improvement in the lipid profile of rats fed high-fat diet and co-administrated extract with two doses towards normalization. Significant drops in TC, LDL-C, TG and VLDL-C, as well as an elevation of HDL-C were recorded. The risk ratio of these rats was decreased. The same observation was registered in positive rats. We concluded that fat and TC in the diet are not absorbed in the intestines, and hence the delivery from them to the liver is small.

The TC and TG of liver tissue significantly lessened when positive rats and rats fed high-fat diet were force-fed C. dichotoma extract. These results may be due to the content of C. dichotoma extract from polyphenols, flavonoids, alkaloids and mucilage, as well as its improved effect on HDL-C.

Mucilage formed a viscous matrix that trapped bile salts in the gut, forcing the liver to use more cholesterol to synthesis more bile acids. Mucilage reduced the digestion and absorption of dietary fats, causing a decrease in cholesterol delivery to the liver by chylomicron remnants. This caused up-regulation of the LDL receptor and decreased lipoprotein secretion to maintain cholesterol homeostasis in the liver. Mucilage was not digested in the stomach and passed through the small intestines without absorption, but it was fermented as a work of colonic microflora, composing short-chain fatty acids like acetate, propionate, and butyric acids. Propionates hinder cholesterol and fatty acids synthesis in the liver[30]. The extract under study contains 20.18% mucilage.

On the other hand, the extract contains 2.47% flavonoids which demounded as a hypolipidemic[6]. Quercetin, kaempferol and catechin flavonoids are documented as hypolipidemic agents. Quercetin has anti-dyslipidemia effect, induces lipolysis activity, reduces oxidative stress, and up-regulates the adipocyte gene expression which increases the lipids’ beta oxidation[36]. Quercetin suppressed LDL oxidation and exerted significant vaso-relaxation[37]. Kaempferol reduced oxidative stress[38], preventing arteriosclerosis by the inhibition of LDL oxidation. Flavonoids inhibit acyl coenzyme A, cholesterol O-acyltransferase and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase in rats[39]. C. dichotoma extract contains quercetin and kaempferol at about 4.90 and 24.10 mg/g extract, respectively[6]. Polyphenols lowered serum TG levels and contributed to the inhibition of pancreatic lipase, which decreased the intestinal absorption of TG and secretion of apolipoprotein-B, and raised lipoprotein lipase activity[30]. The extract contains 5.52% polyphenols[6]. The lipoprotein lipase has an important role in lipid metabolism, it hydrolyzes the core TG from circulating chylomicrons and VLDLs. Lipoprotein lipase activity is positively correlated with the intake of antioxidants[39].

The protective effect of C. dichotoma may due to its effect on HDL-C. HDL-C represents a key cardioprotective factor which is given its role in reverse cholesterol transport, its effects on endothelial cells, and its antioxidant activity. Administration of C. dichotoma extract restored HDL-C of rats fed high-fat diet within normal levels[40].

In the cardiovascular system, cells constantly generate ROS that are utilized as signaling molecules. Oxidant stress promotes atherosclerosis. Oxidant stress causes endothelial dysfunction; activates inflammation, immune responses, and thrombus formation; oxidizes lipids; and initiates a cascade of vascular events that is permissive for the formation of atherosclerotic plaques. The endothelial dysfunction...
increases the permeation of LDL through the intima layer, resulting in oxidation and formation of atherosclerotic damage. The complexity of the cardiovascular antioxidant system is highlighted by the enzymatic and non-enzymatic antioxidants that reduce cellular ROS. There are a several key cellular and circulating antioxidant systems, including the superoxide dismutases, glutathione peroxidases, and catalase that collectively reduce superoxide/hydrogen peroxide (or lipid hydroperoxides) to water (or lipid hydroxides). There are also many important small-molecule antioxidants such as reduced GSH\(^{[41,42]}\). 

*C. dichotoma* extract showed a high to moderate reducing power, Fe\(^{2+}\) chelation ability, free radical scavenging and O\(^2-\) scavenging activity *in vitro*. Also, administration of *C. dichotoma* extract with two doses ameliorated oxidative stress and enhanced antioxidant defence system in rats fed high-fat diet and co-administrated the extract. These results agreed with that of Singh et al.\(^{[43]}\) on *Cinnamomum tamala*.

We found that the *C. dichotoma* extract with two doses (0.5 and 1.0 g/kg/day) normalized lipid profile of rats fed on high-fat diet, and the risk ratio of these rats also significantly decreased. The protective effect of extract against hyperlipidemia could be due to the increment of faecal excretion and fat, and TC in the faecal excretion which indicated missing absorption of fat, and TC in intestine. The extract lowered the ability of rats fed high-fat diet to ingest and absorb fat and cholesterol, and enhanced the ability to get rid of them in faecal excretion.

**Conflict of interest statement**

The authors would like to confirm the absence of any conflict of interest associated with the implementation or the publication of this work.

**References**


