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Hypolipidemic Effect of *Cordia dichotoma* Forst. Pulp in High-fat Diet-Fed Rats

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Abstract: The fruits of *Cordia dichotoma* Forst., family Boraginaceae are used traditionally as analgesic, anti-inflammatory, hepatoprotective, diuretic, aphrodisiac and anthelmintic. *Cordia dichotoma* Forst. pulp (CDP) were obtained after separation of seeds and dried at 40°C. Pulp powder contained 81.06% moisture (wet weight)), 8.86 % protein, 7.93% crude ash, 0.57% crude lipid, 10.17% crude fiber and 72.47% total carbohydrates (dry weight). The major hydrocarbon, sterol and fatty acids were eicosane-C20; 27.34%, beta-sitosterol; 7.82% and oleic acid; 46.88%. Phytochemical screening of dried pulp indicated the presence of carbohydrates, tannins, flavonoids, saponins, triterpenes, sterols, mucilage and coumarins. The results indicated that, dried pulp contained polyphenolics 2.64 g gallic/100 g, flavonoids 1.42 g quercetin/100 g, tannins 0.45g catechin/100 g and mucilage 10.74 g/100 g. IC_{50} of CDP powder, ethanolic and petroleum ether extract were 67.70, 40.05 and 104.07 g/L, respectively, compared with IC_{50} of ascorbic acid (7.95 g/L). In hyperlipidemic experiment, male albino rats were fed high-fat diet (HFD) for 10 weeks. Hyperlipidemic rats were divided into three groups; rats fed HFD only as HFD-control group, rats fed HFD and supplemented with CDP in two levels 10% (HFD-$CDP_{10\%}$ group) and 20% (HFD-$CDP_{20\%}$ group). In normal rats, ND-$CDP_{20\%}$ caused significantly decreased of risk ration to about 57.9% with respect of ND-control. Liver and kidney functions of hyperlipidemic rats were improved with two levels supplemented of *Cordia dichotoma* Forst. pulp. The results of pathological indicted that Increasing of CDP to 20% caused decrease in fatty changes in the liver of rats.

Key words: *Cordia dichotoma* fruits · Hypolipidemic action · Lipid profile · Liver functions · Kidney functions · Enzymes

INTRODUCTION

2.8 million People die each year as a result of being overweight or obese. Risks of heart disease, strokes, cancer and diabetes increase steadily with increasing body mass index (BMI). The prevalence of overweight is highest in upper-middle-income countries but very high levels are also reported from some lower-middle income countries. In European region, the Eastern Mediterranean region and the region of the Americas, over 50% of women are overweight. Raised cholesterol is estimated to cause 2.6 million deaths annually; it increases the risks of heart disease and stroke [1]. In Egypt about 15.5% of the adult population was obese (12.573.767 adult) [2]. These results are mainly due to changes in eating habits: high-fat and low-fiber diets. The elevation of serum total cholesterol and low-density lipoprotein (LDL) cholesterol as well as alteration of other lipid parameters has been implicated as a primary risk factor for cardiovascular disease [3, 4]. Therefore, reducing serum cholesterol levels, especially low-density lipoprotein (LDL) cholesterol levels in patients with hypercholesterolemia, is desirable. Current approaches in reducing blood LDL-cholesterol espouse inhibiting cholesterol synthesis, [5] blocking the absorption of dietary cholesterol [6]. Varieties of plants have been used traditionally in the treatment of various cardiovascular diseases. *Cordia dichotoma* Forst. (Boraginaceae) is tree of tropical and subtropical regions, commonly known as Lasaura/Lasura and in Egypt the tree called mokhat. The fruits are globos,

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yellowish, brown, pink or black and pulpy [7]. All organs of *C. dichotoma*; leaves, fruits (pulp or seeds pulp) and bark stems contained useful compound and many biological activities. Fruits are important source for fibers, minerals, protein, vitamins and carbohydrates. Also, fruits contained high polyphenolics content, flavonoids, tannins and saponins [8, 9]. Cordia is used as immunomodulator, antidiabetic, anthelmintic, diuretic, hepatoprotective, treatment of inflammation, bronchitis, fever, astringent, wound healing and demulcent in folklore medicine [8]. Fruits were documented as anthelmintic, to promote wound healing, to relief severe colic pain, to improve memory, to treat anxiety, anti-inflammatory, antimicrobial, hypoglycemic and treat eczema [10-13]. *C. dichotoma* seeds cotain á-amyrine, betulin, octacosanol, lupeol-3-rhamnoside, β-sitosterol, β-sitosterol-3-glucoside, hentricontanol, hentricontane, taxifolin-3, 5-dirhmnoside and hesperitin-7-rhamnoside. The seeds is important source of oleic and linoleic fatty acids [14].

The present study aims to estimate the chemical composition of *C. dichotoma* pulp, as well as to investigate the hypolipidemic action of *C. dichotoma* pulp in hyperlipidemic rats.

MATERIALS AND METHODS

Preparation of Samples: *Cordia dichotoma* fruits were collected from Cordia trees in Sharkiya Governorate, Egypt during August, 2012. The tree was identified by Agric. Eng. Tereza Labib in Orman Botanical Garden, Giza, Egypt. The fruits were separated into pulp (external parts) and seeds pulp (internal parts). The external pulp parts were dried at 40°C in drying oven. The dried pulp were grinded with blander. Grinded pulp was kept in packet at 5±1°C until using.

Chemical Composition of *C. dichotoma* Pulp: Approximate Analysis: Approximate analysis findings; moisture, crude protein, fat, fiber and ash were determined according to the methods described by A.O.A.C. [15]. Total carbohydrates were calculated by the difference.

Unsaponifable and Saponifable Matter Composition: Unsaponifable and saponifable matter were separated and identified according to method of A.O.A.C. [15] by GLC. Fatty acid methyl esters of *C. dichotoma* pulp oil were determined according to A.O.A.C standard method No. 969/33 [16]. The non-saponifable matter was prepared according to A.O.A.C standard method No. 933/08 [15].

Phytochemical Screening: Phytochemical screening was performed using standard procedures according to Balbaa *et al.* [17].

Determination of Total Phenolics Content: Total phenolics contents were determined by the Folin-Ciocalteau method with some modifications [18] Total phenol values were expressed in terms of g gallic/100 g dry weight.

Determination of Flavonoids Content: The total flavonoids content was determined according to the aluminum chloride colorimetric method [19]. Total flavonoids values were expressed as g rutin /100 g dry weight.

Determination of Condensed Tannin Contents: Condensed tannins were determined according to the method of Julkunen-Titto [20]. The results were expressed as mg catechin equivalents g catechin /100 g dry weight.

Determination of Total Mucilage: Total mucilage content was determined according to the method of Khuller *et al.* [21] and was expressed as g mucilage/100 g dry weight.

Determination Free Radicals Scavenging Activity Against 1, 1-Diphenyl-2-picrylhydrazyl (DPPH): The ability of pulp powder and petroleum ether extracts to scavenge DPPH radicals was assessed as described by Ohinishi *et al.* [22]. The inhibition% was plotted against the sample extract concentration in order to calculate the IC50 values (g/L). Ascorbic acid was used as a reference.

Biological Activity of *C. dichotoma* Pulp:

Experimental Design: Two experiments were carried out to evaluate the hypolipidemic effect of CDP in normal and hyperlipidemic rats. Male albino rats of western strain (36 rats) weighted between 120-140 g was obtained from Central Animal House of National Research Centre, Dokki, Giza, Egypt. Animals were housed in plastic cages under standard laboratory conditions (20-25°C, 40-60% humidity, 10-12 hours light/dark cycle) at the animal facility of animal house at National Research Centre, Dokki, Giza, Egypt. Water was available *ad libitum* and weighed food was free over 10 week period. Normal diet was standard diet was prepared according to Campbell [23]. High-fat diet was obvious normal diet supplemented with 20% camel fat, 1% cholesterol and 0.25% bile acids. Two sets diets from each diet were prepared by

Table 1: The diets composition.

	Normal diet			High-fat diet		
Containing	ND-control	ND-CDP-10%	ND-CDP-20%	HFD-control	FHD-CDP-10%	HFD-CDP-20%
Casein	20%	20%	20%	20%	20%	20%
Corn oil	5%	5%	5%	5%	5%	5%
Mineral complex	4%	4%	4%	4%	4%	4%
Vitamin complex	1%	1%	1%	1%	1%	1%
Cellulose	5%	5%	5%	5%	5%	5%
Cholesterol	-	-	-	1%	1%	1%
Fat camel	-	-	-	15%	15%	15%
Colic acid	-	-	-	0.25%	0.25%	0.25%
Starch	65%	55%	45%	48.75%	38.75%	28.75%
CDP powder	-	10%	20%	-	10%	20%

Table 2: Design of experiment.

Normal rats group (ND-rats)			
Subgroup 1	Rats fed on normal diet	ND-control	Ve- control
Subgroup 2	Rats fed on normal supplemented with 10% CDP	ND-CDP 10%	Ve+ control
Subgroup 3	Rats fed on normal supplemented with 20% CDP	ND-CDP 20%	Ve+ control
Hyperlipidemic rats group (HFD-rats)			
Subgroup 1	Rats fed on high-fat diet	HFD-control	Ve+control
Subgroup 2	Rats fed on high-fat diet supplemented with 10% CDP	HFD-CDP 10%	Treated
Subgroup 3	Rats fed on high-fat diet supplemented with 20% CDP	HFD-CDP 20%	Treated

supplementing the diet with 10 and 20% of CDP powder as Table 1. After adaptation period (7 days) animals were divided into two groups, (each group 18 rats). The first group was the normal rats, which fed on chow-based diet (ND-rats). The second group was the hyperlipidemic rats, which fed on high-fat diet (HFD-rats). Each group divided into three subgroups as shown in the Table 2.

At the end of the experimental period (10 week), rats were fasted overnight, then sacrificed under diethyl ether anesthesia. Then, the blood samples from retro-orbital venous plexus. Blood was centrifuged (4000 rpm, 10 min, 4°C by using Sigma labor zentrifugen) and serum was separated. Liver, kidney, heart, spleen and brain were collected from each animal and washed in ice-cold 1.15% KCl solution, blotted and weighed. A piece of liver from each rat was separately homogenized in ice-cold Tris-HCl buffer (0.1M, pH 7.4) using a potter Elvehjem type homogenizer. The resulting homogenate was centrifuged at 4,000 rpm for 15 minutes by using Sigma labor zentrifugen [24] and used for antioxidants analysis. Another piece of liver, kidney and heart were kept in 15% formalin for pathological examination.

Biochemical Analysis: Total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-C) were estimated according to the methods of Allain *et al.*[25], Fossati and Prencipe [26] and Naito and Kaplan [27], respectively using kits obtained from ElITECH (ELITech Group 12-12 bis rue Jean Jaurès 92800 Puteaux France). Low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and risk ratio (RR) were calculated according to Friedewald *et al.* [28], Naito and Kaplan [27] and Kikuchi *et al.* [29], respectively. Liver functions; total protein (TP), albumin (Alb) and aspartate transaminase (AST) and alanine transaminase (ALT) were determined using kits obtained from DIAMOND (Diamond Diagnostics-333 Fiske Street Holliston, MA 01746, USA) according to methods of Henry [30], Doumas *et al.* [31] and Reitman and Frankel [32], respectively. Globulin (Glo) was calculated by the difference between total protein and albumin according to Reinhold [33] Kidney functions; urea, uric and creatinine were estimated according to the methods of Tabacco *et al.* [34], Gochman and Schmitz [35] and Faulkner and King [36], respectively. Reduced L-glutathione (GSH), catalase (CAT) and malondialdehide (MDA) of liver were analyzed by kits obtained from BIODIGONESTIC according to methods of Beuthler [37], Aebi [38] and Vashney and Kale [39], respectively.

Pathological Study: Gross pathological observations in different organs of different groups were recorded. Histopathologically tissue specimens from liver, kidney and heart were collected, fixed in 10% neutral buffer formalin solution for 48 hours then dehydrated in increasing concentrations of alcohol, cleaned in pure

xylene and embedded in paraffin wax (Automatic tissue processor, Leice, TP 1050, Germany). Paraffin blocks were prepared and 4-6μ thin sections were obtained by a rotative microtome (Leice, Germany), stained with haematoxylin and eosin (H & E) and exanimated microscopically [40].

Statistical Analysis: All studied data were statistically analyzed using Co-Stat 6.303 Software Computer Program 2004) hypothesis testing methods included one way analysis of variance (ANOVA) using dancnn Test [41].

RESULTS

Chemical Composition of CDP: Data presented in Table 3 show that CDP contains 81.06% moisture, 7.93% crude ash, 0.57crude fats, 8.86% crude protein, 10.17% crude fiber and 72.47% total carbohydrates.

Lipid Composition of CDP: Unsaponifable matter of CDP consisted of 87.34% hydrocarbons and 12.66% sterols. Total hydrocarbons ranged between C15 (pentadecane, 0.163%) and C20 (eicosane, 27.34%). In sterols, major component was beta-sitosterol (7.819 %), while the minor sterol was cholesterol; 1.230 % (Table 4). Fatty acid composition of CDP was represented in Table 5. Three saturated fatty acids and four unsaturated others were identified (18.17 and 76.73%, respectively). The major saturated fatty acids were palmetic acid (10.39%), followed by hexacoanoic acid (7.46%). Unsaturated fatty acids classified into monounsaturated fatty acids; 57.82% and polyunsaturated fatty acids; 18.90%. The major monounsaturated fatty acid was oleic acid (46.88%) followed by palmetoyl acid (10.94%). Polyunsaturated fatty acids occurred as linoleic and linolenic acids (10.72 and 8.17%, respectively).

Phytochemical Screening of CDP: Qualitative analysis was carried out to investigate compounds groups of CDP (Table 6). The pulp was contained carbohydrates, tannins, mucilage, saponins, flavonoids (combined and free), terpenoids and/or sterols and alkaloids. While, coumarins were traces. Pulp did not contain volatile oil.

Phytoconstituents Content of CDP: CDP contained many useful components and data were shown in Table 7. It contained polyphenolics (2.46 g gallic/100 g powder), flavonoids (1.42 g quercetin/100 g powder), tannins (0.45 g catechin/100 g powder) and mucilage (10.74 g/100g powder).

Table 3: Approximate analysis findings of C. dichotoma pulp powder.

Major components	
Parameters	g/100g
Moisture*	81.06±0.48
Total carbohydrates	72.47±0.54
Crude ash	7.93±0.11
Crude protein	8.86±0.50
Crude fiber	10.17±0.95
crude fat	0.57±0.06

* Wet weight

Mean of three replicates.

Table 4: GLC analysis of Unsaponifable matter compounds of C. dichotoma pulp.

Components	Area %
C5 (pentane)	0.619
C6 (hexane)	0.568
C14 (Tetradecane)	1.240
C15 (Pentadecane)	0.163
C16 (Hexadecane)	0.241
C17 (Heptadecane)	0.853
C19 (Nonadecane)	10.839
C20 (Eicosane)	27.341
C22 (Decosane)	0.389
C23 (Triocosane)	1.819
C24 (Tetracosane)	1.143
C25 (Pentacosane)	2.033
C26 (Hexacosane)	2.00
C27 (Heptacosane)	4.635
C28 (Octacosane)	3.934
C29 (Nonacosane)	2.300
C30 (γ- sitosterol)	2.672
Holesterol	1.230
Campasterol	1.607
Stigmasterol	2.007
Beta-sitosterol	7.816
Known %	74.181
Unknown %	25.819

Table 5: GLC analysis of unsaponifable matter compounds of C. dichotoma pulp.

Component name	Rt.	Area %
C16:0 (Palmetic acid)	16.645	10.386
C16:1 (Palmetoyl acid)	17.079	10.940
C18:0 (Stearic acid)	18.802	0.318
C18:1 (Oleic acid)	21.062	46.880
C18:2 (Linoleic acid)	21.707	10.720
C18:3 (Linolenic acid)	22.630	8.172
C26:0 (Hexacoanoic acid)	33.021	7.462
Crude oil %	0.56	
Total Saturated acids	18.17	
Unsaturated fatty acids	Mono-unsaturated acids	57.82
	Poly-unsaturated acid	18.90
	Total unsaturated acids	76.71
Known fatty acids %	94.878	
Unknown fatty acids %	5.122	

Table 6: Phytochemical screening of C. dichotoma pulp.

Compounds	Observation
1. Test carbohydrates and/ or glycosides	+ ve
2. Test for Tannins	+ ve
3. Test for alkaloids	+ ve
4. Test for mucilage	+ ve
5. Test for flavonoids	+ ve
6. Test for saponins	+ ve
7. Test for coumarins	Traces
8. Test for triterpenes and/or sterols	+ ve
10. Test for volatile oil	- ve

-Absent
+ Present.

Table 7: Phytoconstituents contents (g/100 g dried pulp) of Cordia dichotoma pulp.

Compound	(g/100g)
Total Polyphenolics	2.46±0.24
Total Flavonoids	1.42±0.09
Crude Mucilage	10.74±0.57
Condensed Tannins	0.45±0.09

Table 8: IC 50 antioxidant (g/L.) of Cordia dichotoma dried pulp.

IC50 antioxidant (g/L)	Pulp powder	67.70±2.02
	Ethanolic extract	40.05±7.51
	Petroleum ether extract	104.07
	Ascorbic acid	7.95±0.79

Mean of three replicates.

Free Radicals Scavenging Activity Against 1, 1-Diphenyl-2-picrylhydrazyl (DPPH): The DPPH radical-scavenging capacity after one hour reaction time for CDP powder and petroleum ether extracts was evaluated. Parameter used to measure the IC50 value, defined as the concentration of antioxidant required for 50% scavenging of DPPH radicals in this specified time period. The IC_{50} value of CDP powder, ethanolic and petroleum ether extract and ascorbic acid were shown in Table 8. IC_{50} of CDP powder, ethanolic and petroleum ether extract were 67.70, 40.05 and 104.07 g/L, respectively, compared with IC_{50} of ascorbic acid (7.95 g/L.).

Hypolipidemic Effect of CDP in Normal and Hyperlipidemic Rats:

Daily Food Intake, daily body weight Gain and Food Efficiency: Daily body weight gain (DBWG) and daily food intake (DFI), of rats feeding on ND-$CDP_{10\%}$ and ND-$CDP_{20\%}$ showed no significant differences, except ND-$CDP_{20\%}$, which caused significant decrease in DBWG (21.89%), compared to ND-control (Table 9). Feeding on high-fat diet caused significant increase on DBWG (24.46%) and non-significant increase in DFI (5.78%), compared with ND-control (2.33±0.57 and 15.92±0.89 g, respectively). Feeding on HFD-$CDP_{10\%}$ or HFD-$CDP_{20\%}$ decreased DBWG (19.67 and 40.34% respectively) and DFI (4.81 and 14.90% respectively) of hyperlipidemic rats, compared to HFD-control (2.90±0.36 g/day and 16.84 g/day, respectively) ($P \leq 0.05$). Food efficiency (FER) was calculated by dividing DBWG on DFI. No significant difference was noticed between FER of all groups, except group fed on high level 20% in normal and hyperlipidemic rats ($P \leq 0.05$).

Table 9: Effect of feeding on C. dichotoma pulp on daily food intake, daily body weight gain and food efficiency of normal and hyperlipidemic rats during 10 weeks.

	Normal rats			Hyperlipidemic rats			
Parameters	Control	10% CDP	20% CDP	Control	10% CDP	20% CDP	LSD
DBWG	2.33b±0.57	2.58ab±0.03	1.82c±0.57	2.90a±0.36	2.28b±0.11	1.73c±0.20	0.30
DFI	15.92ab±0.89	15.89ab±1.70	15.44b±1.23	16.84a±0.46	16.03ab±1.30	14.33c±1.40	0.90
FER	0.15ab±0.03	0.16a±0.02	0.13bc±0.05	0.16a±0.02	0.15ab±0.01	0.11c±0.02	0.02

DBWG= daily body weight daily, DFI= daily food intake and FER=food efficiency.
Mean of three replicates.
$P \leq 0.05$, Value with the same letter has no significant but value with different letter has significant at 0.05

Table 10: Effect of administration of Cordia dichotoma pulp on relative weight of organs (g/100) in hyperlipidemic and normal rats during 10 weeks.

	Normal rats			Hyperlipidemic rats			
Parameters	Control	10% CDP	20% CDP	Control	10% CDP	20% CDP	LSD
liver	3.15c±0.15	2.52d±0.38	3.26c±0.07	4.90a±0.14	4.30b±0.18	5.20a±0.39	0.490
Heart	0.50a±0.09	0.34b±0.11	0.50a±0.06	0.55a±0.02	0.48a±0.06	0.52a±0.03	0.123
Kidney	0.32b±0.06	0.31b±0.01	0. 40a±0.05	0.27b±0.04	0.29b±0.01	0.43a±0.05	0.076
Spleen	0.42b±0.02	0.37b±0.04	0.57ab±0.14	0.47ab±0.05	0.41b±0.05	0.70a±0.19	0.200
brain	0.55ab±0.08	0.51ab±0.06	0.68a±0.14	0.46b±0.04	0.60ab±0.09	0.57ab±0.04	0.125

Mean of three replicates.
$P \leq 0.05$, Value with the same letter has no significant but value with different letter has significant at 0.05.

Table 11: Effect of administration of Cordia dichotoma pulp on lipid profile in normal and hyperlipidemic rats during 10 weeks.

	Normal rats			Hyperlipidemic rats			
Parameters	Control	10% CDP	20% CDP	Control	10% CDP	20% CDP	LSD
TC	89.90e±1.34	99.73d±4.98	79.95f±1.09	184.34a±3.19	143.10b±3.60	123.57c±5.95	6.75
HDL-C	50.86ab±0.66	45.88b±2.24	52.50a±1.47	31.25d±3.60	39.09c±1.76	49.05ab±2.98	4.32
LDL-C	19.16e±0.56	36.34d±5.50	8.54f±1.08	115.29a±3.93	73.52b±3.72	57.16c±3.07	6.36
VLDL-C	19.88c±1.18	17.51c±1.01	18.92c±0.71	37.81a±1.32	30.49b±1.72	17.36c±0.90	2.11
TG	99.39c±5.88	87.56c±5.06	94.60c±3.56	189.03a±6.59	152.44b±8.61	86.79c±4.49	10.54
RR	0.38d±0.01	0.81c±0.15	0.16d±0.03	3.71a±0.49	1.89b±0.18	1.17c±0.06	0.41

Mean of three replicates.

P≤0.05, Value with the same letter has no significant but value with different letter has significant at 0.05

Data in Relative Weight of Organs: Table 10 revealed to that feeding on high-fat diet caused significant increase in relative weight of liver of HFD-control rats, compared to ND-control. Other organs (heart, kidney, spleen and brain) did not affect significantly (P≤0.05). Normal and hyperlipidemic rats, which fed in ND-$CDP_{10\%}$ and HFD-$CDP_{10\%}$ decreased relative weight of liver significantly (2.52±0.38 and 4.30±0.18, respectively), while both of ND-$CDP_{20\%}$ and HFD-$CDP_{20\%}$ caused non-significant increase (3.26±0.07 and 5.20±0.39 respectively), compared to ND-control and HFD-control respectively. Both of ND-$CDP_{20\%}$ and HFD-$CDP_{20\%}$ caused significant increase on relative weight of kidney (0.40±0.05 and 0.43±0.05 respectively) of normal and hyperlipidemic rats, while ND-$CDP_{10\%}$ and HFD-$CDP_{20\%}$ did not cause any change (0.31±0.01 and 0.29±0.01, respectively). No significant difference was noticed on relative weight of brain on normal or hyperlipidemic rats. Relative weight of spleen did not change in normal rats, but increased significantly by $CDP_{20\%}$ in hyperlipidemic rats.

Serum Lipid Profile: Administration high-fat diet elevated TC, LDL-C, VLDL-C and TG significantly (105.05, 501.73, 90.19 and 90.19%) compared with rats fed on normal diet (89.90±1.34, 19.16±0.56, 19.88±1.48 and 99.39±5.88 mg/dl, respectively). On the other hand, HDL-C was decreased (38.56%) significantly (Table 11). TC was decreased significantly by administration CDP in hyperlipidemic rats, compared to HFD-control. HFD-$CDP_{10\%}$ and HFD-$CDP_{20\%}$ caused significant reduction on TC (22.37 and 33%, respectively) on hyperlipidemic rats. No significant effect was noticed on TC of all normal groups. HDL-C the good cholesterol was increased significantly when rats fed on HFD-$CDP_{10\%}$ and HFD-$CDP_{20\%}$ about 25.09 and 56.96% respectively, compared to HFD-control (31.25±3.60 mg/dl). No significant difference was recorded between HDL-C in all normal groups. LDL-C was calculated by equation of LDL-C= T.C-(HDL+VLDL). In hyperlipidemic rats LDL-C was decreased significantly by HFD-$CDP_{10\%}$ and HFD-$CDP_{20\%}$ (36.23 and 50.42% respectively), compared with HFD-control (115.29±3.93 mg/dl). In normal rats, ND-$CDP_{10\%}$ increased LDL-C level significantly, while ND-$CDP_{20\%}$ caused significant decrease on LDL-C (55.43%), compared to ND-control. Risk ratio was calculated by equation; risk ratio= LDL-C/HDL-C. Risk ratio was decreased in hyperlipidemic rats fed on HFD-$CDP_{10\%}$ and HFD-$CDP_{20\%}$ (57.41 and 63.09%, respectively), compared with HFD-control. In normal rats, ND-$CDP_{10\%}$ increased risk ratio significantly about 113.16%, but ND-$CDP_{20\%}$ decreased it significantly about 57.9%, with respect of ND-control. HFD-$CDP_{10\%}$ and HFD-$CDP_{20\%}$ caused significant decline in TG and VLDL-C in hyperlipidemic rats, compared with HFD-control. HFD-$CDP_{20\%}$ was more effective than $CDP_{10\%}$ (with reduction about 19.36% and 54.09%, respectively), compared with HFD-control. Neither TG nor VLDL-C of normal rats was affected by CDP.

Liver Functions: The liver is the main organ involved in lipid metabolism and prone to potential oxidative damage in conditions of hyperlipidemia, we measured the levels of AST, ALT, TP, Glo and Alb in serum. Data in Table 12 indicated that, feeding on high-fat diet did not affect significantly in TP and Glo, while Alb was decreased significantly (16.71%), compared with ND-control; 5.97±0.06, 2.20±0.20 and 3.77±0.20 mg/dl, respectively. Both of AST and ALT increased significantly (11.60 and 12.85%, respectively), with respect of ND-control, 82.83±5.30 and 35.50±1.99 mg/dl, respectively. CDP 10% or 20% increased TP significantly in normal and hyperlipidemic rats, compared with ND-control and HFD-control respectively. In hyperlipidemic rats, no significant difference was recorded in TP of rats fed on HFD-$CDP_{10\%}$ or HFD-$CDP_{20\%}$. But HFD-$CDP_{20\%}$ increased TP in normal rats. Alb was increased non-significantly (14.65 and 15.93%, respectively) when rats fed on

Table 12: Effect of administration of Cordia dichotoma pulp on liver function in normal and hyperlipidemic rats during 10 weeks.

	Normal rats			Hyperlipidemic rats			
Parameters	Control	10% CDP	20% CDP	Control	10% CDP	20% CDP	LSD
TP	5.97c±0.06	6.45b±0.26	7.16a±0.36	5.77c±0.07	6.85ab±0.50	6.95ab±0.10	0.475
Alb	3.77a±0.20	3.76a±0.04	3.51ab±0.42	3.14b±0.70	3.60ab±0.18	3.64ab±0.09	0.374
Glo	2.20c±0.20	2.70bc±0.22	3.65a±0.41	2.62bc±0.09	3.25ab±0.29	3.31ab±0.19	0.620
Alb/Glo	1.73a±0.24	1.41b±0.10	1.00b±0.32	1.20b±0.06	1.11b±0.06	1.10b±0.09	0.312
AST	82.83b±5.30	82.61b±1.47	55.82d±3.51	92.44a±4.13	85.91b±3.05	67.65c±3.43	6.524
ALT	35.50bc±1.99	36.46abc±3.26	32.41c±0.46	40.06a±1.85	35.61bc±3.30	37.74ab±2.22	3.09
AST/ALT	2.34a±0.14	2.28a±0.23	1.72b±0.13	2.31a±0.07	2.42a±0.15	1.80b±0.19	0.224

Mean of three replicates.

P≤0.05, Value with the same letter has no significant but value with different letter has significant at 0.05

Table 13: Effect of administration of Cordia dichotoma pulp on kidney functions in normal and hyperlipidemic rats during 10 weeks.

	Normal rats			Hyperlipidemic rats			
Parameters	Control	10% CDP	20% CDP	Control	10% CDP	20% CDP	LSD
Uric acid	2.93b± 0.07	2.46c ±0.07	2.10c±0.11	3.730a± 0.30	3.77a ± 0.17	2.27c ±0.16	0.30
Urea	49.86ab±4.09	45.56bc±3.75	55.31a±1.47	56.00a±2.77	41.15c±1.88	49.01b±2.15	5.07
Creatinine	1.22b±0.21	1.60b±0.22	1.56b±0.13	2.16a±0.30	1.51b±0.21	1.70b±0.46	0.347

Mean of three replicates.

P≤0.05, Value with the same letter has no significant but value with different letter has significant at 0.05

HFD-$CDP_{10\%}$ and HFD-$CDP_{20\%}$, compared with HFD-control. No significant difference was noticed between control rats and others fed on ND- $CDP_{10\%}$ and ND-$CDP_{20\%}$. Glo was calculated by the difference between TP and Alb. Glo in normal or hyperlipidemic rats was increased after CDP treatments, compared with ND-control and HFD-control respectively. Alb/Glo ratio was decreased significantly in all groups, except ND-control. Normal rats fed on ND-CDP-20% showed the lowest ratio (1.00±0.32), compared with ND-control. No significant difference was noticed between the ratios of all hyperlipidemic rats. AST was reduced significantly by CDP, compared to HFD-control. $CDP_{10\%}$ and $CDP_{20\%}$ reduced AST about 7.06% and 26.80% respectively. Rats fed in ND-$CDP_{10\%}$ did not change on AST, while that fed on ND-$CDP_{20\%}$ recorded highly significant reduction on AST (32.61%), compared to ND-control. ALT in rats, fed in HFD-$CDP_{10\%}$ reduced significantly by 11.11%, while ALT of rats fed on HFD-$CDP_{20\%}$ showed non- significant reduction (5.80%), compared to HFD-control rats. An opposite trend was noticed in normal rats, ND-$CDP_{10\%}$ did not cause change in ALT, while level ND-$CDP_{20\%}$ caused significant reduction (8.70%), compared with ND-control. No significant difference was noticed between the effect of low and high level on ALT, in normal and hyperlipidemic rats. AST/ALT ratio was reduced significantly by $CDP_{20\%}$ in normal and hyperlipidemic rats. Other groups did not record any significant differences on the ratio.

Kidney Functions: Feeding on high-fat diet caused significant increase on uric acid, urea and creatinine of HFD-control rats (27.30, 12.31and 43.52%), compared with ND-control rats; 2.93±0.07, 49.86±4.09 and 1.22±0.21 mg/dl, respectively (Table 13). Uric acid did not change in hyperlipidemic rats fed on HFD-$CDP_{10\%}$, while HFD-$CDP_{20\%}$ caused significant reduction about 38.65%, compared with HFD-control. The significant reduction on uric acid of normal rats was lower than hyperlipidemic rats, compared to ND-control. No significant difference was noticed between two levels of CDP. Urea concentration was decreased significantly when rats fed on HFD-$CDP_{10\%}$ and HFD-$CDP_{20\%}$ (33 and 12.45% respectively), compared to HFD-control. In normal rats, no significant difference was noticed between urea concentrations in all normal rats. Rats fed on HFD-$CDP_{10\%}$ recorded the lowest urea concentration, while the highest concentration recorded in HFD-$CDP_{20\%}$.

Antioxidant of Liver Homogenate: Antioxidants such as reduced L. glutathione (GSH) and catalase (CAT) protect cells against oxidative stress. Malondialdehyde (MDA), a naturally occurring end product of membrane lipid peroxidation, is one of the most frequently used biomarker for free radical mediated damage. Data in Table 14 show that GSH was reduced in rats fed on ND-$CDP_{10\%}$ and ND-$CDP_{20\%}$ (about 22.16 and 35.94% respectively), compared with ND-control. Both of CAT and MDA did not record any significant difference between all normal

Table 14: Effect of feeding of C. dichotoma pulp on GSH, CAT and MDA of normal and hyperlipidemic rats during 10 weeks.

	Normal rats			Hyperlipidemic rats			
Parameters	Control	10% CDP	20% CDP	Control	10% CDP	20% CDP	LSD
GSH	9.62a±1.26	7.49ab±0.61	6.17b±0.31	8.03ab±1.20	8.06ab±1.13	5.87b±0.34	1.60
CAT 0.38a±0.06	0.38a±0.07	0.31ab±0.03	0.27b±0.06	0.29ab±0.06	0.22 b±0.05	0.08	
MDA	2.84b±0.52	2.70b±0.27	3.31ab±0.30	3.70a±0.25	3.04ab±0.25	3.20ab±0.27	0.58

Mean of three replicates.

P≤ 0.05, Value with the same letter has no significant but value with different letter has significant at 0.05.

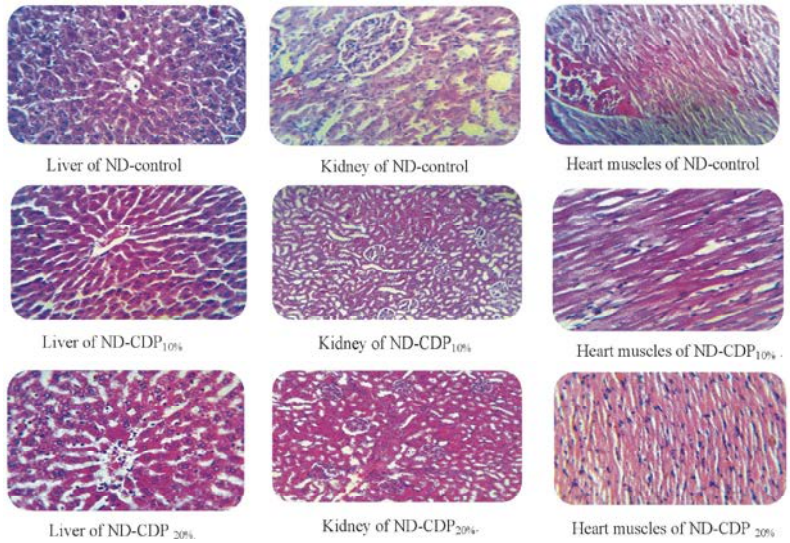

Fig 1: H&EX300 of liver, kidney and heart muscles of rat feed on ND and ND -CDP 10 and 20%.

groups (P≤0.05). In hyperlipidemic rats, feeding on high-fat diet caused non-significant reduction in GSH and significant reduction on CAT; 16.52 and 29.69%, respectively, in compression of ND-control; 9.62±1.26 nmol/g liver and 0.384±0.06 U/mg liver respectively. MDA recorded an opposite trend, it was increased significantly (about 30.28%), with respect of ND-control, 2.84±0.52 nmol/mg liver (Table 14). Feeding on HFD-$CDP_{10\%}$ and HFD-$CDP_{20\%}$ did not cause significant change on GSH, CAT and MDA, compared with HFD-control, except GSH of HFD-$CDP_{20\%}$ group. No significant difference was noticed between MDA of HFD-$CDP_{10\%}$ and HFD-$CDP_{20\%}$ groups and ND-control (P≤0.05).

Pathological Studies: The liver of rats fed in normal diet (ND-control) has a relatively dark-red color. Microscopically it revealed normal hepatic lobules with normal central veins hepatic cords; sinusoids; and portal tracts (normal hepatic artery; portal vein and bile duct). Fatty change appeared in some cases. Kidney showed a normal glomerulus by light microscopy. The glomerular capillary loops are thin and delicate. Endothelial and mesangial cells are normal in number. The surrounding tubules are normal. Life is good. Heart, showed normal appearance of myocardial fibers in longitudinal section. Note the central nuclei and the syncytial arrangement of the fibers, some of which have pale pink intercalated disks (Fig. 1). When rats were fed on normal diet supplemented with 10 and 20% CDP along 10 weeks an improvement was recorded in kideny, liver and heart, compared with organs of normal control. Feeding on HF-diet for 10 weeks caused fatty changes in the body of rats. The liver of HFD-control rats has a yellowish color. This liver was highly enlarged (the relative weight of liver was 4.90±0.14 g live/100 body weight, compared with ND-control; 3.15±0.15 g live/100 body weight) and has a pale yellow appearance, this uniform change is consistent with fatty metamorphosis (fatty change). This was the histopathologic appearance of hepatic fatty change. The lipid accumulates in the hepatocytes as vacuoles. These vacuoles have a clear appearance with H&E staining. Liver of HFD-fed rats illustrated poor cellularity with extensive lipid depositions and enlarged hepatocytes. Macro-steatosis represented by single large vocal replacing the cytoplasm and pushing the nucleus to the periphery (signet ring appearance). The portal area

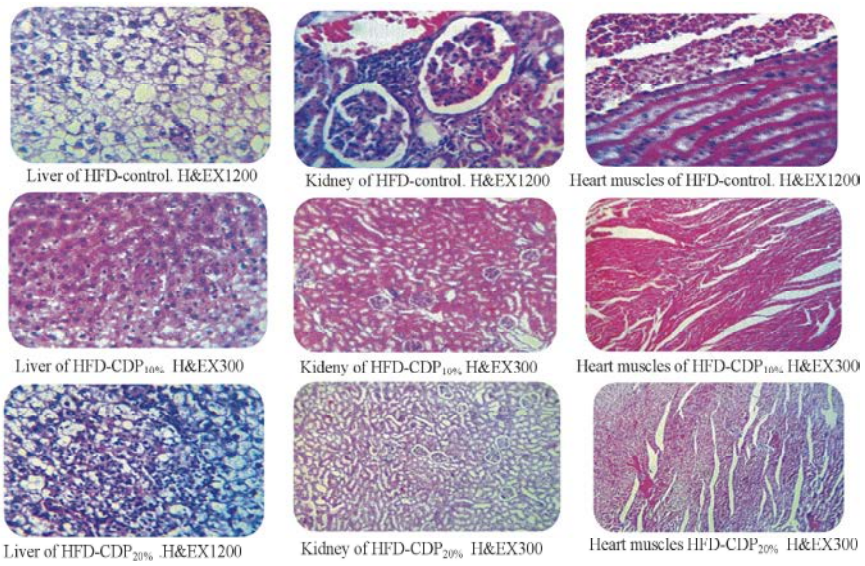

Fig 2: Liver, kidney and heart muscles of rat feed on HFD and HFD - CDP 10 and 20%.

showed portal infiltration of lymphocytes and macrophages. Focal distortion of some hepatic cells and replacement by macro fatty vessels as shown in Fig. 2. Rats of HFD-$CDP_{10\%}$ their liver, heart and kideny were protected. Liver of this group was not as much enlarged as that in HCD-fed rat. Such liver showed micro-steatosis of most of hepatic cells, In HFD-$CDP_{10\%}$ fed rats, a lesser degree of lipid deposition and hepatocytes enlargement was observed. Kidney and heart were normal, compared with HFD-control and ND-control. Increasing CDP to 20% caused decrease in fatty changes in the liver of rats. Kidney did not affect and appeared with normal structure. Liver showed micro vesicular steatosis of almost hepatic cells and macro vesicular steatosis of some hepatic cells. Focal replacement of degenerated hepatic cells by macrophage was appeared. This treatment caused an improvement in liver, but the liver did return to its normal case. Heart showed normal muscles structure.

DISCUSSION

To our knowledge, this is a first report in which the effect of the CDP consumption on lipid metabolism in normal and hyperlipidemic rats was studied. The aim of the study was to determine favorable effect of CDP consumption on lipids, metabolism, liver and kidney functions and oxidative stress. CDP contained a large amount from crude fiber, polyphenolics compounds, flavonoids, tannins and mucilage. Hyperlipidemia, including hypercholesterolemia and hypertriglyceridemia is a major risk factor for the development of cardiovascular diseases. Oxidatively damaged LDLs are taken up by macrophages, which accumulate in the endothelial wall as lipid-laden foam cells in the initial phases of atherosclerotic fatty streak lesions. Therefore, reduction of circulating TGs and total and LDL cholesterol are a primary step in the prevention of vascular disease. Also, prevention of LDL oxidation by dietary antioxidants could delay the development of atherosclerosis [42]. In the present study, high-fat diet increased daily body weight gain, TG, TC and LDL-C levels, with decreasing circulating HDL cholesterol levels, thus providing a model for dietary hyperlipidemia. GSH, which plays a key role in coordinating the body's antioxidant defense processes, was decreased. Similarly CAT in HFD-rats was reduced, compared with ND-control. Pathological examination shown fatty changes in liver, heart muscles, aorta and kidney in hyperlipidemic rats. This model was used to study the potential hypolipidemic effect of a *C. dichotoma* pulp. Administration of *C. dichotoma* pulp with the two levels; 10 and 20% showed a beneficial effect in reversing the hyperlipidemic condition. These had a strong hypolipidemic action, with reduction of TC, TG and LDL-C and elevation on HDL-C. Moreover, liver lipoperoxidation were non-significantly decreased as indicated by the lower levels of MDA. The fatty changes were improved significantly; kidney show with normal structure, compared with HFD-control.

In normal rats, feeding on CDP with level 20% decreased daily body weight gain, daily feed intake, TC, LDL-C and risk ratio of normal rats significantly, with no changes on TG, VLDL-C and HDL-C. Both of CAT and

MDA did not changed significantly, while GSH was decreased significantly with respect of ND-control. Pathological observations were coordinated with the above data. Obtained results may be due to polyphenolics, flavonoids, tannins and dietary fibers of CDP, which were documented as hypolipidemic agents. Polyphenol-rich foods and beverages had a strong hypolipidemic effect such as red wine, tea and apple [43-45]. Lower serum TG levels by polyphenol-rich foods has been associated with decreased intestinal absorption of TG by inhibition of pancreatic lipase [44] or with a lower microsomal transfer protein activity and apolipoprotein B-secretion and increased lipoprotein lipase activity [43, 46, 47]. This could result in modified LDL levels, which in turn would lead to decreased LDL cholesterol concentrations. Also, upregulation of hepatic LDL receptor expression and activity by polyphenols might account for their hypocholesterolemic effect [46, 48]. *C. dichotoma* pulp is rich in polyphenols compounds 2.46g gallic acid/100 powder pulp. Flavonoids have been shown to possess a variety of biochemical and pharmacological activities, including hypolipidemic effects, cardio-protective and antioxidant properties. Choi *et al.* [49] and Koshy *et al.* [50] reported that flavonoids decreased TC, LDL-C and VLDL-C but increased HDL-C may be due to increase lipolysis more than lypogenisis. CDP contains 1.42g quercetin/100 g pulp powder. Tannins have also reported to increase faecal bile acid excretion, thereby leading to reduction in cholesterol absorption [51], CDP contained tannins about 0.45g catechin/100 pulp powder.

Dietary fibers, especially viscous soluble polysaccharides, are well known for their effect lowering total and LDL cholesterol [52]. Viscous soluble fibers hinder digestion and absorption of dietary fats, resulting in lower cholesterol delivery to the liver by chylomicron remnants, with a concomitant up-regulation of LDL receptor and decreased lipoprotein secretion to maintain cholesterol homeostasis in the liver. Further, bile salts are trapped in the viscous matrix formed by soluble dietary fibers polysaccharides in the gut. To compensate for the increased fecal excretion of bile salts, cholesterol is derived to the synthesis of bile acids. Moreover, soluble fibers are fermented by the colonic micro flora generating short-chain fatty acids (acetic, propionic and butyric acids). Hypolipidemic effects have been associated with propionate by inhibition of cholesterol and fatty acids synthesis in the liver. Moreover, insoluble dietary fibers through its effect diluting gastrointestinal contents may hinder digestion and absorption of dietary fats, thus contributing to the effects of soluble dietary fibers. All these mechanisms lead to lower serum levels of cholesterol and TG, subjacent to the reduced risk of cardiovascular disease associated to dietary fibers intake [52, 53]. CDP contains 10.19 g/100 g pulp powder insoluble fibers and 10.74 g mucilage/100 g pulp powder as viscous soluble polysaccharides may account for the observed hypolipidemic effects elicited by *C. dichotoma* fibers. In conclusion the findings of the study suggested that *C. dichotoma* pulp has a strong hypolipidemic action and for the need for further studies in order to evaluate the impact of nutritional components of different extracts, identification of active constituents and investigate the hypolipidemic mechanism of *C. dichotoma* fruits.

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