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Evaluation Hypolipidemic and Antioxidant Characters of *Cordia dichotoma* Fruits Mucilage in High Fat Diet-induced Hyperlipidemic Rats

Samah A El-Newary[1], Abd-Elrahman M Sulieman[2], Mohsen S Asker[3], Manal G Mahmoud[3], Sayed R El-Attar[4]

[1]Department of Medicinal and Aromatic Plants Research, National Research Centre, Dokki-12622, Giza, Egypt
[2]Department of Food Science, Zagazig University, Egypt
[3]Department of Microbial Biotechnology, National Research Centre, Dokki, Giza, Egypt
[4]Department of Pathology, Zagazige University, Egypt

ABSTRACT

Mucilage of Cordia dichotoma fruits (Boraginaceae) has been used as an expectorant and treatment of lung diseases and gonorrhea. This study isolated and identified C. dichotoma mucilage as a hypolipidemic agent on blood lipid and antioxidant stress status using hyperlipidemic male albino rats. C. dichotoma mucilage was isolated and its physical and chemical characteristics were evaluated. Mucilage was tested at two doses; 0.5 and 1 g/kg (as 0.05 and 0.1 of determined LD_{50}) orally for 10 weeks. Dietary hyperlipidemia model was used to investigate the potential hypolipidemic. Mucilage has a good swelling index (76.35%) and contains galactose, arabinose and glucouronic acid at molar ratio 5.0:3.7:1.0, respectively. In-vivo hypolipidemic experiment, feeding on high-fat diet caused significant increase in serum lipid profile; Total Cholesterol (TC), Triglycerides (TG), Very Low Density Lipoprotein Cholesterol (VLDL-C) and Low Density Lipoprotein Cholesterol (LDL-C) as well as significant decrease on High Density Lipoprotein Cholesterol (HDL-C). Treatment of two doses of mucilage showed promising hypolipidemic activity represented as normalization all obvious abnormalities lipid profile. Antioxidants enzymes activities; Glutathione Reductase (GR), Glutathione-S-transferase (GST), Glutathione Peroxidase (GPx), Catalase (CAT) and Superoxide Dismutase (SOD) were significantly decreased by administration high-fat diet. Meanwhile, mucilage administration significantly improved them. Mucilage treating significantly improved fatty changes shown in hyperlipidemic rat's liver. Mucilage has a potential effect to prevent hyperlipidemia and oxidative stress with a good safety margin.

Keywords: *Cordia dichotoma*, Mucilage, Molar ratio, Lipid profile, Liver functions, Liver histology

INTRODUCTION

Mucilage is a viscous polysaccharide substance used in medicine and adhesive. Currently mucilage is used in the pharmaceutical industries, as thickeners, water retention agents, emulsion stabilizers, suspending agents, binders and film formers. Traditionally, mucilage has been used to treat cough, demulcent and relieve irritation of mucous membranes [1]. Natural mucilage is cheaper, available, nontoxic and non-irritating, so it is preferable than synthetic and semisynthetic agent [2]. Mucilage exists in many plants as *Opuntia ficus indica* L. [3], Fenugreek seeds [4], *Cordia* fruits [5], flaxseeds [6], papaya seed [7], Chia, *Salvia hispanica* L. [8], hibiscus species [9] and *Cordia dichotoma* [10,11].

The mucilage in the present study was extracted from *C. dichotoma* Forst. (Boraginaceae) is known as Indian cherry, lasura, Sekendal, Kendal Paw man [12] and mokhate in Egypt [10]. The fruits of *C. dichotoma* are globose, yellowish-brown, pink or black and pulpy. This fruit is divided into pulpy part and one kernel seed. The kernel seed is surrounded by viscid sweetish transparent pulp [11]. In folk medicine fruits have been used as cooling, astringent, diuretic, aphrodisiac, emollient, expectorant, anthelmintic, laxative and purgative [13,14]. *C. dichotoma* mucilage has been used as a gum for pasting sheets of paper and cardboard. And mucilage has been used in treating the lung diseases, gonorrhea, uterus and urethra disorders [14,15]. Many pharmacological properties of *C. dichotoma* fruits were documented as anti-ulcer [16], hepatoprotective [17], wound healing and anti-inflammatory [12], antidiabetic activity [18] and anti-hyperlipidemia [10,11]. The viscid sweetish transparent layer surrounding the kernel seed is enriched with mucilage 20.18% [11]. Mucilage consists of arabinose, galactose, and glucouronic acid (10:7:2 moles) and traces of rhamnose [19].

Excessively consumption of saturated fat and cholesterol, due to fasting diet and disruption in lifestyle lead to taking less healthy food. They cause lipid peroxidation of bio-membranes of cells resulting obesity, hyperlipidemia, hyperglycemia, cardiovascular diseases, cancer and aging [20]. Hyperlipidemia is risky factor for cardiovascular diseases.

Numerous drugs that were approved for the treatment of hyperlipidemia have serious adverse effects. Therefore, many studies have been conducted to find dietary supplements of medicinal plants to minimize the synthetic drugs side effects. Many plants have been documented as a hypolipidemic plants such as coca fibers [21], flax seeds and pumpkin seeds [22], *Portulaca oleracea* seeds and stems [23,24], *Artemisia capillaris* [25], *Anthoxylum zanthoxyloid* [26], *Tinospora cardifolia* [27]. In obvious studies the hypolipidemic and antioxidant properties of *C. dichotoma* pulp powder and *C. dichotoma* seeds pulp aqueous extract has been documented [10,11].

The aims of this study are isolation and identification the mucilage of viscid sweetish layer surrounding the kernel seeds. And evaluate their hypolipidemic effect and protective effect against oxidative stress.

MATERIAL AND METHODS

Chemicals

Folin-Ciocalteu reagent, gallic acid, cholesterol and colic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Kits for lipid profile; Total Cholesterol (TC), High Density Lipoprotein (HDL) and Triglycerides (TG), as well as liver functions; total protein, albumin, Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were obtained from ElITECH. (ELITech Group 12-12 bis rue Jean Jaurès 92800 Puteaux, France). Kits for antioxidant enzymes; Catalase (CAT), Glutathione Reductase (GR), glutathione peroxidase (GPx), Glutathione-*S*-transferase (GST) and Superoxide Dismutase (SOD) were bought from BIODIGENOS TIC Company, Dokki, Egypt. Ethanol and other chemicals were analytical grade.

Chemical study

Extraction of mucilage

C. dichotoma fruits were collected from Cordia trees in Sharkia Governorate, Egypt during August, 2015 [11]. The fruits were washed and outer covering was removed. Seeds surrounded with viscid layer (1 kg) were placed in a stopper container with the cold water as 1-20 and allowed to stand at room temperature at least 24 h [28].

Isolation of mucilage using ethanol

To the filtrate, absolute ethanol was added in 1:3 proportions to precipitate out mucilage. The obtained mucilage so obtained was then subjected to air drying for sufficient period of time and further dried in vacuum dryer at 40°C.

Purification of the isolated mucilage

The well dried mucilage was powdered with the help of mortar and pestle and passed through sieve number 60 then the powdered mucilage was re-dissolved in distilled water. The concentrated solution was precipitated by ethanol. The precipitate was separated and dried at 50°C. The dried mucilage was powdered and was stored in tightly closed bottle and was kept at -20°C.

Physicochemical characteristics of mucilage

Organoleptic evaluation of mucilage

The isolated mucilage was characterized for organoleptic properties such as color, odor, taste, fracture and texture [29].

Chemical composition of mucilage

The total carbohydrates and reducing sugars were determined by phenol-H_2SO_4 method of Dubois et al. [30] using glucose as a standard. Ash value was determined according to AOAC [31]. Protein content was determined by the dye binding assay according to Bradford [32] using bovine serum albumin as a standard protein. Total phenolic contents were determined by the Folin-Ciocalteau method Gorinstein et al. [33] and expressed in terms of equivalent gallic acid.

Solubility behavior mucilage

One part of dry mucilage powder was shaken with different solvents and the solubility was determined [34].

pH of mucilage

The mucilage was weighed and dissolved in water separately to get a 1% w/v solution. The pH of solution was determined using digital pH meter [34].

Swelling Index of mucilage

Swelling index of the powdered mucilage was calculated by weighing a butter paper of size 22 cm. then the butter paper was dipped in a petridish containing water and was reweighed [34]. After this 10 mg of the powdered sample was kept in a butter paper placing this on a petridish containing 15 ml of water and the swelling index was calculated at different intervals i.e., 15, 30, 45, 60, 120, 240, 360 min and the final result was calculated using the formulae:

$$\text{Swelling Index \% (SI)} = (\text{Final weight} - \text{Initial Weight})/\text{Initial Weight} \times 100$$

Viscosity

Ostwald viscometer was used to determine the viscosity of isolated mucilage. In which, viscosity of 1% mucilage solution was measured by comparing the flow times of isolated mucilage solution with that of liquid whose viscosity is known [34].

Fourier-transform infrared (FT-IR)

The major structural groups of the purified mucilage EPS was detected using FTIR. The FTIR spectrum of mucilage was obtained by the KBr method. The mucilage sample was pressed into KBr pellets at a sample-KBr ratio of 1:100. The FTIR spectra were recorded on a Bucker scientific 500-IR spectrophotometer in the region of 4000–400 cm^{-1} [35].

Determination of sugars

Twenty milligram of mucilage was hydrolyzed in 2 ml of 2 M triflouroacetic acid at 105°C in a sealed-tube for 5 h, followed by evaporation on a water bath at 40°C, co-distilled with water and dissolved in 0.2 ml deionized water. The monosaccharides contents were quantified by HPLC on a Shimadzu Shim-Pack SCR-101N column using deionized water as the mobile phase and refractive index detection [36].

Biological study

The LD_{50} assay

The LD_{50} of *C. dichotoma* mucilage was determined in Swiss albino mice (n=8) by carried adopting the method of Bruce [37]**.** Mice were administered with different doses of the mucilage by increasing or decreasing the dose according to the response of animal. The dosing pattern was 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 g/kg body weight orally while the control group received only the normal saline. All groups were observed to determine changes in parameters for assessing toxicity. All rats were kept under observation for 24 h for recording any symptoms of toxicity or mortality, after 24 h live animals were kept for further 14 days and were observed daily for behavioral and body weight changes. The obtained results revealed that, no mortality in mice up to 10 g/kg mucilage, which in turn demonstrated that mucilage, was safe up to 10 g/kg.

Experimental animals

The experiment was conducted with Wistar male Albino rats (36 rats, 140 ± 20 g) were obtained from central animal house of National Research Centre, Dokki, Giza, Egypt. The animals were housed under conditions of controlled temperature (25 ± 2°C) and humidity (50% ± 5) with 10-12 h dark/light cycle. Water was available *ad libitum* and food was free over 10-week period.

The experimental protocol was developed according to the institution's guideline for the care and use of laboratory animals National Research Centre, Dokki, Giza, Egypt. This study was approved by Medical Research Ethics Committee, National Research Center, Egypt, under registration No. 15/191.

Diet

An ordinary diet was obtained from laboratory animals National Research Centre, Dokki, Giza, Egypt, containing 4.60% fat, 26.60% crude protein, 4.78% crude fiber and 6.71% crude ash was used as standard diet [31]. High-fat diet was obvious ordinary diet was substituted to contain 20.0% fat, 26.60% crude protein, 4.78% crude fiber and 6.71% crude ash and supplemented with 1.0% cholesterol, 0.25% colic acid.

Experimental design

Two doses of *C. dichotoma* mucilage were tested as the results of LD_{50}. Low and high dose (0.5 and 1.0 g/kg body weight as 0.05 and 0.1 of LD_{50}) were prepared by dissolving in worm distilled water by using Sigma sonicator bath ultrasonic. After adaptation period (4 days) animals were divided into three main groups. The first main group was negative control group (-ve control) fed on ordinary diet and force fed with distilled water for 10 weeks. The second main group was positive control group, which was divided into three subgroups: first and second subgroup were rats fed on ordinary diet and force fed by low and high dose of mucilage (0.5 and 1.0 g/kg body weight/day for 10 weeks) respectively, while third one was rats fed on high-fat diet and force fed with distilled water for 10 weeks (hyperlipidemic control). The third main group was treated group, which was divided into two subgroups fed high-fat diet and force fed with low and high dose of mucilage (0.5 and 1.0 g/kg body weight/day for 10 weeks), respectively.

Samples

At the end of the experimental periods (10 weeks), rats were deprived of food for overnight and then they were slightly anesthetized then, blood samples were collected from the eye venous plexuses by capillary tube into glass tubes. Serum was obtained from blood samples after centrifugation (4000 g, 10 min by using Sigma labor zentrifugen). Organs were collected, washed in ice saline solution and were weighted freshly. A piece of each liver was used to make liver homogenate, which was prepared by homogenized a weight of liver in homogenizing buffer (ice-cold Tris-HCl buffer, 0.1 M, pH 7.4), using tissue homogenizer ultrasonic. The resulting homogenate in each case was centrifuged at 4000 g for 15 min at 4°C by using Sigma labor centrifuge [38] for antioxidants enzymes. Another piece of liver was kept immediately in formalin 10% for histopathological examination.

Analytical procedure of samples

The lipid profile and liver functions were determined spectrophotometrically in sera samples, meanwhile antioxidants enzymes activities were determined in liver homogenate. Total Cholesterol (TC), High-Density Lipoprotein Cholesterol (HDL-C) and Triglycerides (TG) were determined according to Allain et al. [39], Naito and Kaplan [40], Fossati and Prencipe [41] respectively. Low Density Lipoprotein Cholesterol (LDL-C), Very Low Density Lipoprotein Cholesterol (VLDL-C) and Risk Ratio were calculated according to Friedewald et al. [42], Naito and Kaplan [40], Kikuchi et al. [43], respectively.

Liver function; total protein, Albumin and liver enzymes Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) activities were measured according to Henry [44], Doumas et al. [45], Reitman and Frankel [46]. Globulin was calculated by the difference between total protein and albumin according to Reinhold [47].

Antioxidant enzymes activities Catalase (CAT), Glutathione Reductase (GR), Glutathione-*S*-transferase (GST), Glutathione Peroxidase (GPx) and Superoxide Dismutase (SOD) activities were determined according to Beers and Sizer [48], Goldberg and Spooner [49], Habig et al. [50] Paglia and Valentine [51] and Fridovich [52], respectively.

Pathological study

Histopathological studies were carried out in by pathology unit at Department of Pathology, Faculty of Veterinary Medicine, Zagazig University. Tissue of liver was examined microscopically using procedure of Bancroft and Gamble [53].

Statistical analysis

Data were reported as mean ± standard deviation for three rats per group. Comparisons across groups were performed by one-way analysis of variance ANOVA test at $P \leq 0.05$.

RESULTS

Physicochemical characteristics of C. dichotoma mucilage

The mucilage isolated from *C. dichotoma* fruits is a light brown colored powder. With ruthenium red and corallin soda, the particles did not stain pink and a gelatinous mass is not formed when the powder was heated with distilled water. All these tests indicated that the isolated compound is mucilage. In the iodine test, the particles did not stain blue indicating the absence of starch. Mucilage is soluble in hot water forming colloidal solution and practically insoluble inorganic solvents. The pH of 1% solution of mucilage is found to be near neutral. It showed good swelling and water absorption capacity (Table 1). It contained a high amount of carbohydrates; 87.29% and reducing sugars; 6.77%. As well as total phenols about 4.17%. Protein and ash are existing in mucilage (2.52 and 2.63%, respectively). Mucilage evaluated for organoleptic properties. It is tasteless and has characteristic odor. Fracture and texture is found to be rough and irregular. Mucilage is soluble in warm water and slightly soluble in cold water and insoluble in inorganic solvents.

Table 1: Micromeritic study data of isolated mucilage

Parameters	Result
Swelling index (%)	76.35
pH	7.5
Viscosity	8.24
Ash value (%)	2.63
Carbohydrates (%)	87. 29
Protein (%)	2.52
Reducing sugars (%)	6.77
Phenolic compounds (%)	4.17

The monosaccharide composition of the mucilage was investigated by High Performance Liquid Chromatography (HPLC) analysis. The hydrolysis of mucilage exposed the consisting of galactose, arabinose and glucouronic acid in a molar ratio of 5.0:3.7:1.0, respectively. The results revealed the presence of more complex peak patterns ranging from 3000-800 cm^{-1}. The mucilage contains a significant number of OH groups, which exhibited an intense broad stretching peak at around 3464 cm^{-1}. The absorption in that region had the rounded trait typical of OH group. The FTIR spectra of mucilage exposed functional characteristic, as well as a broad-stretching OH group at 3464 cm^{-1}, two weak C-H stretching peaks at 2964 and 2935 cm^{-1} consequent to CH_3 and CH_2 groups. The strong absorption experiential at 1664.23 cm^{-1} corresponded to the amide stretch. Furthermore, the peak at 1405 cm^{-1} might be assigned to the >C=O stretch of the COO^- groups and the C-O link from COO^-. There was no peak around 1700-1775 cm^{-1}, suggesting that neither glucuronic acid nor diacyl ester was present in the mucilage. The peak about 1652 cm^{-1} suggested the presence of the COO^-. The characteristic absorptions at 832.19 cm^{-1} in the IR spectra indicated that α-configurations were simultaneously present in mucilage.

The hypolipidemic effect of *Cordia dichotoma* mucilage

Effect on food intake and body weight gain

A significant increment was recorded on daily body weight gain (g/day) of rats (40.0%), when rats fed high-fat diet corresponding to -ve control (Table 2). Mucilage at high dose significantly decreased daily body weight gain of treated rats (25.10%) compared to hyperlipidemic control ($P<0.05$). daily body weight gains of +ve controls did not significantly affect with respect to -ve control (LSD; 0.50).

Feeding on high-fat diet caused insignificant increment on feed intake (g/day) about 10.17%, compare with -ve control. Rats treated with low dose of mucilage ingested food amount less than those treated with high dose and hyperlipidemic control ($P<0.05$, LSD; 1.82). In +ve control of mucilage, both of body weight gain and feed intake did not significantly change by *C. dichotoma* mucilage.

Table 2: Daily feed intake and body weight gain of normal and hyperlipidemic Wistar Albino rats treated with *Cordia dichotoma* mucilage at two doses (0.5 and 1 g mucilage/kg body weight)

Groups	Subgroups	Feed intake	Body weight gain
Negative control group	-ve control	$15.72^{ab} \pm 0.67$	$2.25^{b} \pm 0.26$
Positive control group	Mucilage-0.5 (+ve)	$16.07^{ab} \pm 1.02$	$2.63^{ab} \pm 0.26$
	Mucilage-1.0 (+ve)	$15.57^{ab} \pm 0.89$	$2.49^{ab} \pm 0.22$
	Hyperlipidemic control	$17.5^{a} \pm 1.28$	$3.15^{a} \pm 0.25$
Treated group	Mucilage-0.5	$13.93^{b} \pm 1.01$	$3.09^{a} \pm 0.47$
	Mucilage-1.0	$15.22^{ab} \pm 1.61$	$2.36^{b} \pm 0.17$
	LSD	1.82	0.5

Data presented as a mean of three replicates. Data analyzed by one-way ANOVA, $P \leq 0.05$, Value with the same letter has no significant but value with different letter has significant at 0.05

Effect on serum lipid profile

Feeding high-fat diet resulted in the development of hyperlipidemia in experimental rats, as is evident from Table 3. Hyperlipidemia in hyperlipidemic control was characterized by remarkable increment in T.C, 98.73%, T.G and VLDL-C, 76.05%, LDL-C, 483.73% and reduction on HDL-C, 31.52%, compared with -ve control. Risk ratio of hyperlipidemic control group which calculated by the equation: risk ratio=LDL-C/HDL-C, was significantly increased over eight times, compared with -ve control.

Mucilage with two doses; 0.5 and 1.0 g/kg body weight/day showed hypolipidemic effect on hyperlipidemic rats explained by the amelioration in all abnormalities parameters, compared with hyperlipidemic control rats, P<0.05. TC levels significantly declined when rats treated with low and high dose of mucilage (32.51 and 31.41%, respectively) in comparison to hyperlipidemic control (P<0.05, LSD; 8.68).

Table 3: Lipid profile and risk ratio of normal and hyperlipidemic Wistar Albino rats treated with *Cordia dichotoma* mucilage at two doses (0.5 and 1 g mucilage/ kg body weight)

Main groups	Subgroups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	Risk ratio %
Negative control	-ve control	$92.76^{c} \pm 3.79$	$112.81^{b} \pm 4.05$	$51.49^{a} \pm 3.03$	$18.71^{de} \pm 4.13$	$22.56^{b} \pm 0.81$	$0.36^{d} \pm 0.05$
Positive control group	Mucilage-0.5 (+ve)	$92.93^{c} \pm 7.81$	$126.537^{b} \pm 2.3$	$47.97^{ab} \pm 1.44$	$19.66^{d} \pm 7.56$	$25.31^{b} \pm 0.46$	$0.41^{d} \pm 0.04$
	Mucilage-1.0 (+ve)	$79.35^{c} \pm 1.67$	$123.42^{b} \pm 7.18$	$43.73^{b} \pm 1.06$	$10.94^{e} \pm 1.95$	$24.68^{b} \pm 1.45$	$0.25^{d} \pm 0.05$
	Hyperlipidemic control (+ve)	$184.34^{a} \pm 7.64$	$198.60^{a} \pm 1.99$	$35.26^{c} \pm 2.14$	$109.39^{a} \pm 3.30$	$39.72^{a} \pm 0.40$	$3.11^{a} \pm 0.07$
Treated group	Mucilage-0.5	$124.41^{b} \pm 5.06$	$121.83^{b} \pm 7.4$	$51.96^{a} \pm 2.02$	$48.07^{c} \pm 4.76$	$24.37^{b} \pm 1.47$	$0.93^{c} \pm 0.11$
	Mucilage-1.0	$126.43^{b} \pm 12.87$	$118.78^{b} \pm 11.7$	$40.52^{b} \pm 2.71$	$62.16^{b} \pm 8.32$	$23.76^{b} \pm 2.34$	$1.54^{b} \pm 0.29$
	LSD	8.68	11.92	3.41	10.05	2.38	0.26

Data analyzed by one-way ANOVA, $P \leq 0.05$, Value with the same letter has no significant but value with different letter has significant at 0.05

At the same, TG and VLDL-C (TG/5) recorded significant reduction by about 38.66 and 40.19 % sequentially as a response for administration low and high dose of mucilage corresponding to hyperlipidemic control. On contrary, HDL-C the good cholesterol of rats was returned to normal case with administration low dose of mucilage and equal with ˉve control value (P<0.05, LSD; 3.41). Resulting on previous results, force fed mucilage with two doses; 0.5 and 1.0 g/kg body weight/day significantly reduced LDL-C by about 56.06 and 43.18%, respectively with respect to hyperlipidemic control, (P<0.05, LSD; 10.05). Consequently, risk ratio significantly reduced to return towards normalization as a response for administration low and high doses of mucilage (70.42 and 50.50%, respectively) in comparison with hyperlipidemic control (P<0.05, LSD; 0.26)**.** In +ve controls, administration mucilage did not record any significant change on TG and VLDL-C of rats, compared with -ve control. Mucilage with low level (0.5 g/kg body weight/day) had not significant influence on TC, HDL-C and LDL-C of rats with respect to -ve control. While, increasing dose of mucilage to 1.0 g/kg body weight/day recorded significant reduction on TC (14.46%), LDL-C (41.62%) and HDL-C (15.07%), compared with -ve control**.** But the significant decrease, in HDL-C values did not influence in risk ratio seriously. The TC: HDL-C ratio of +ve control rats treated with high dose of mucilage (55.01%) was equaled with that of -ve control (55.51%). So the risk ratio (LDL-C: HDL-C) of this group was the lowest value (0.25 ± 0.02%), compared with that of -ve control (0.36 ± 0.02%).

Effect on serum liver functions

Hyperlipidemia induces liver damage and changes in serum AST and ALT activities, which are markers of hepatotoxicity. Hyperlipidemia is associated with fatty changes in hepatocytes of hyperlipidemic control rats, causing elevation in AST and ALT activities (13.45 and 13.45%, respectively) as mentioned in Table 4. Mucilage treatment improved these liver parameters; AST and ALT activities, total protein, albumin and globulin significantly in rats fed high-fat diet and force fed with mucilage with respect to hyperlipidemic control (P<0.05) showing the hepatoprotective properties.

Table 4: Liver functions of normal and hyperlipidemic Wistar Albino rats treated with *Cordia dichotoma* mucilage at two doses (0.5 and 1 g mucilage/kg body weight)

Main groups	Subgroups	Total protein (mg/dl)	Albumin (mg/dl)	Globulin (mg/dl)	ALT (U/l)	AST (U/l)
Negative control group	ˉve control	$5.8^{c} \pm 0.2$	$3.8^{a} \pm 0.2$	$2.1^{b} \pm 0.3$	$35.3^{bc} \pm 1.7$	$82.8^{b} \pm 5.3$
Positive control group	Mucilage-0.5 ($^{+}$ve)	$7.2^{ab} \pm 0.4$	$3.5^{a} \pm 0.2$	$3.8^{a} \pm 0.6$	$33.2^{bc} \pm 0.8$	$66.2^{e} \pm 0.67$
	Mucilage-1.0 ($^{+}$ve)	$7.6^{ab} \pm 0.6$	$3.5^{a} \pm 0.3$	$4.1^{a} \pm 0.4$	$32.0^{c} \pm 0.8$	$72.5^{cd} \pm 1.2$
	Hyperlipidemic control ($^{+}$ve)	$5.8^{c} \pm 0.4$	$3.1^{a} \pm 0.4$	$2.6^{b} \pm 0.6$	$40.1^{a} \pm 1.9$	$92.4^{a} \pm 8.4$
Treated group	Mucilage-0.5	$8.0^{a} \pm 0.1$	$3.8^{a} \pm 0.2$	$4.2^{a} \pm 0.1$	$32.1^{c} \pm 2.3$	$69.3^{de} \pm 2.2$
	Mucilage-1.0	$7.1^{b} \pm 0.4$	$3.5^{a} \pm 0.4$	$3.5^{a} \pm 0.6$	$36.4^{b} \pm 1.4$	$77.1^{c} \pm 3.4$
	LSD	0.68	ns	0.76	2.79	4.66

Data represented as Mean ±SD. Mean of three replicates. Data analyzed by one-way ANOVA, $P \leq 0.05$, Value with the same letter has no significant but value with different letter has significant at 0.05

Elevated AST and ALT activities were significantly decreased when rats treated with mucilage, compared with hyperlipidemic control. Mucilage with two doses caused significant increment on production of total protein and globulin of treated rats. As for, albumin not significantly change by neither high fat-diet nor mucilage treatment. In +ve control rats, liver performance was in healthy rates, and mucilage improved it more, with respect to -ve control. The biochemical parameters studied did not show any of the adverse effect of mucilage on experimental animals.

Effect on antioxidants enzymes activities of liver

As for the antioxidant enzymes activities, their activities in the liver were reported to decrease as a response to stress situations elicited by hyperlipidemia (Table 5). In hyperlipidemic control rat's antioxidant enzymes activities were diminished; GR (28.75%), GST (47.52%), GPx (46.78%), SOD (33.31%) and CAT (20.35%) with respect to -ve control. Conversely, increased activity of these enzymes as a result of administration *C. dichotoma* mucilage was reported in this study in comparison with hyperlipidemic control ($P<0.05$).

GR activity of rats fed high-fat diet and treated with low and high doses of mucilage showed significant increment by about 292.21 and 238.76%, respectively corresponding to hyperlipidemic control. The same observation recorded in +ve control rats treated with two doses of mucilage compared to -ve control ($P<0.05$, LSD; 1.55).

Given the GST activity find significant magnify, when rats force fed high-fat or ordinary diets and co-administrated low dose (216.03 and 31.35% respectively) and high one (105.36 and 74.27%, sequentially) of mucilage in comparison with hyperlipidemic and -ve controls, respectively ($P<0.05$, LSD; 1.53).

Table 5: Activities of antioxidant enzymes of normal and hyperlipidemic Wistar Albino rats treated with *Cordia dichotoma* mucilage at two doses (0.5 and 1 g mucilage/kg body weight)

Main groups	Subgroups	GR (U/ gm protein)	GST (U/ g protein)	GPx (U/ g protein)	CAT (U/gm protein)	SOD (U/ mg protein)
Negative control group	$^{-}$ve control	$7.93^{c} \pm 0.42$	$9.25^{c} \pm 0.45$	$6.91^{c} \pm 0.76$	$9.24^{d} \pm 0.62$	$11.80^{ab} \pm 0.23$
Positive control group	Mucilage-0.5 ($^{+}$ve)	$21.43^{a} \pm 1.0$	$12.15^{b} \pm 0.84$	$9.00^{b} \pm 0.97$	$16.74^{b} \pm 0.41$	$11.48^{b} \pm 0.86$
	Mucilage-1.0 ($^{+}$ve)	$19.56^{ab} \pm 0.9$	$16.12^{a} \pm 1.13$	$12.00^{a} \pm 0.82$	$16.41^{b} \pm 0.41$	$13.05^{a} \pm 0.68$
	Hyperlipidemic control ($^{+}$ve)	$5.65^{d} \pm 0.6$	$4.85^{d} \pm 0.23$	$3.21^{d} \pm 0.58$	$7.36^{e} \pm 0.12$	$7.87^{d} \pm 0.59$
Treated group	Mucilage-0.5	$22.16^{a} \pm 1.07$	$15.34^{a} \pm 1.22$	$9.80^{b} \pm 0.42$	$19.50^{a} \pm 0.36$	$12.82^{a} \pm 0.47$
	Mucilage-1.0	$19.14^{b} \pm 1.00$	$9.96^{c} \pm 0.85$	$6.48^{c} \pm 0.67$	$10.74^{c} \pm 0.33$	$9.83^{c} \pm 0.51$
	LSD	1.55	1.53	1.29	0.79	1.05

GR; glutathione reductase, GPx; glutathione peroxidase, GST; glutathione S transferase, CAT; catalase and SOD; superoxide dismutase. Data presented as a mean of three replicates. Data analyzed by one-way ANOVA, $P \leq 0.05$, Value with the same letter has no significant but value with different letter has significant at 0.05

GPx activity was significantly magnified by force feeding *C. dichotoma* mucilage and recorded increase percent by about 205.30 and 101.87%, respectively with low and high dose of mucilage respect to hyperlipidemic control. In +ve controls rats, GPx activity was higher than that of -ve control ($P<0.05$, LSD; 1.29).

CAT activity was significantly increased by mucilage treatment at low and high dose by about 164.95 and 45.92%, respectively, compared to hyperlipidemic control. The +ve controls CAT activity recorded the same observation, corresponding with $^{-}$ve control ($P<0.05$, LSD; 0.79).

SOD activity was significantly increased as a response for treating with mucilage at two doses; 0.5 and 1.0 g/kg body weight/day (62.90 and 24.90%, respectively) in comparison to hyperlipidemic control. SOD of +ve controls of mucilage did not significantly influence by force feeding mucilage compared to -ve control ($P<0.05$, LSD; 1.05).

Pathological evaluation of the effect of Cordia dichotoma mucilage

Histopathological examination is a good tool to evaluate the effect of high fat diet on experimental rats and the effect of hypolipidemic plant extract**.** The liver of -ve control rats and +ve controls of low and high dose of mucilage groups appeared with a relatively dark-red color or light brown color respectively. The examined Liver sections of control rats (force fed mucilage or -ve control) revealed normal hepatic lobules, central veins and hepatic cords (Figures 1A, 1B and 2A). Feeding on high fat-diet led to fatty infiltration and fatty metamorphosis (fatty changes) in the body of rats.

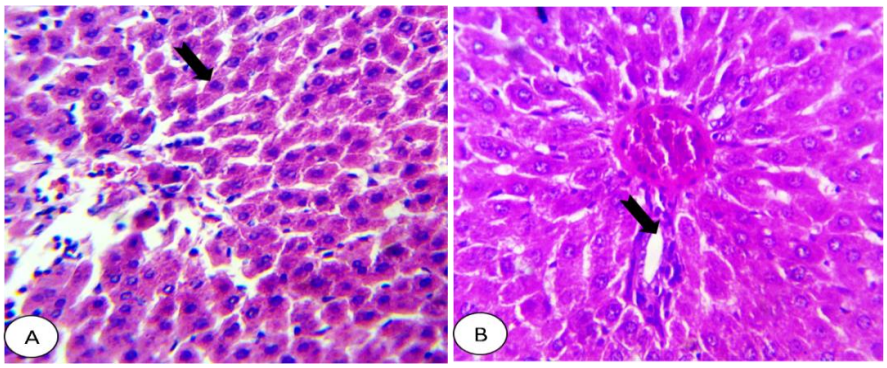

Figure 1: Liver of +ve controls of low and high dose of mucilage groups showing normal hepatic structures. H & E X400

The liver of hyperlipidemic control rats appeared with a yellowish color. Such livers were highly enlarged (5%) and had a pale yellow tint appearance (fatty change). The lipids were accumulated in the hepatocytes as tiny many small circumscribed vacuoles or a single large one (Macro and micro-steatosis) (Figure 2B).

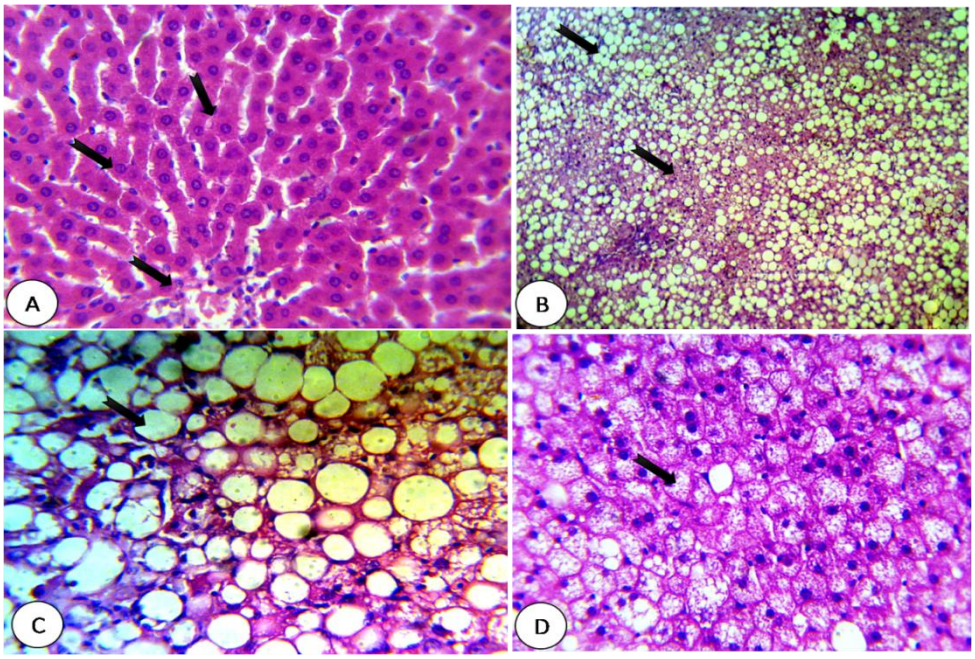

Figure 2: Livers of -ve control group(A) and hyperlipidemic control rats (B, C and D). H&EX 120&400

Macro-steatosis was represented by single large vocal replacing the cytoplasm and pushing the nuclei to the periphery (signet ring) (Figure 2C) Micro-steatosis appeared as some small circumscribed vacuoles of nearly the same size and evenly distributed in the hepatocyte cytoplasm (Figure 2D). The portal area showed portal infiltration of lymphocytes and macrophages (Figure 3A).

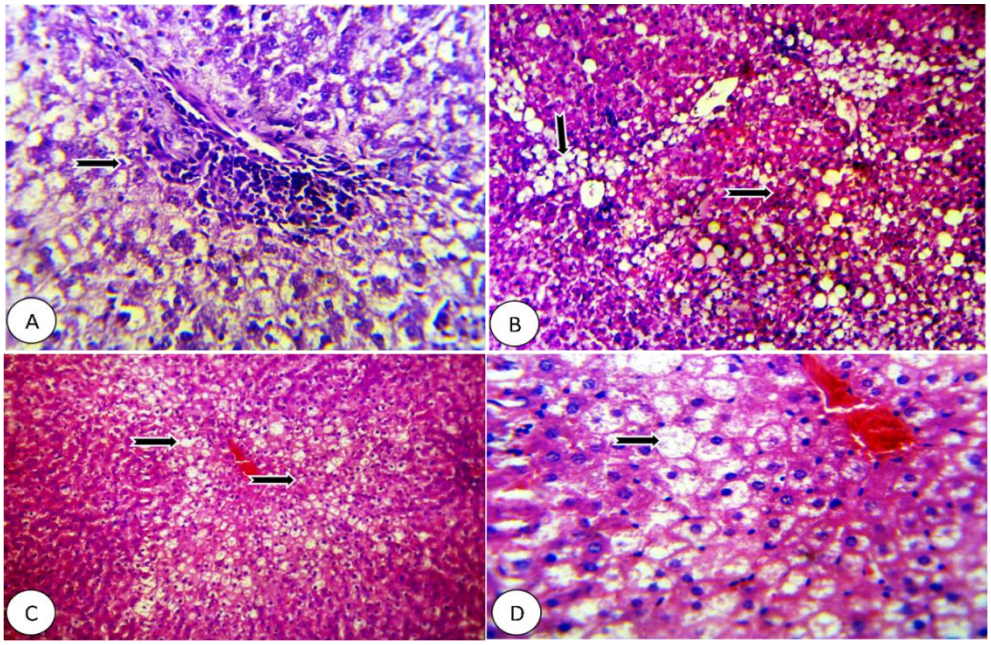

Figure 3: Represented portal infiltration of lymphocytes and macrophages in liver of hyperlipidemic control group (A), rats fed on high-fat diet and force fed with low dose of mucilage (0.5 g/kg body weight/day) (B) and rats fed on high-fat diet and force fed with high dose of mucilage (1.0 g/kg body weight/day) (Figures C and D H & EX 400 & 120)

There was also Focal destruction of some hepatic cells and replacement by large fatty cysts. Livers of rats fed high-fat diet and force fed with low dose of mucilage (0.5 g/kg body weight/day) group were more enhanced and healthier than that of hyperlipidemic control. The liver regained its normal reddish color appearance, nearly similar to that of -ve control livers, however it still assuming an enlarged size (4.33%) but less than that of hyperlipidemic control. Microscopically liver cells of some rats showed hepatic vacuolation and moderate macro-steatosis and micro-steatosis (Figure 3B), but almost hepatocytes cells were apparently healthy with respect of hyperlipidemic control. Liver rats fed high-fat diet and force fed with high dose of mucilage (1.0 g/kg body weight/day group showed more advanced improvement, compared with hyperlipidemic control. The liver color of this group got tended reddish over a yellowish appearance.

This liver was of an average normal size (3.90%), compared with hyperlipidemic control. Macro and micro-steatosis were seen in some hepatocytes (about 10-20%) (Figure 3C and D), but the majority of hepatocytes were normal. Livers of these groups were nearly the same as the -ve control. *C. dichotoma* mucilage decreased fat accumulation in livers of rats leading to improvement in the liver functions.

DISCUSSION

Cordia dichotoma mucilage is a glow brown colored with ruthenium red and coralline soda and a gelatinous mass was not formed when the mucilage was heated with water. The hydrolysis of the mucilage revealed the consisting of galactose, arabinose and glucouronic acid in a molar ratio of 5.0:3.7:1.0, respectively. The mucilage consists of arabinose, galactose, and glucouronic acid (10:7:2 mol) and traces of rhamnose [19]. The absorption in that region had the rounded trait typical of OH group [54], suggestive of that the substance was a mucilage. The FTIR spectra of the mucilage showing functional feature, as well as a broad-stretching OH group at 3464 cm^{-1}, two weak C-H stretching peaks at 2964 and 2935 cm^{-1} resulting to CH_3 and CH_2. The strong assimilation observed at 1664.23 cm^{-1} corresponded to the amide stretch. Also, the peak at 1405 cm^{-1} strength is assigned to the COO^- groups and the C=O link from COO^- groups [55-56]. There was no peak around 1700-1775 cm^{-1} suggestive of that neither glucuronic acid was present in the mucilage. The peak about 1652 cm^{-1} suggested the occurrence of the C-O group [54,57]. A broad stretch of C-O-C, C-O was observed at 1000-1200 cm^{-1}, thus suggestive of the presence of polysaccharides [57,58]. The strongest absorption band at 1075 cm^{-1} was analytical that the substance was a polysaccharide [59]. Moreover, the spectrum shows the presence of COO^- and OH, which is attributable to credit for the flocculation process, a result similar to the results previously reported [60]. The typical absorptions at 832.19 cm^{-1} in the IR spectra indicated that α-configurations were concurrently current in mucilage.

Atherosclerosis is a chronic disease in which precipitates cholesterol, cholesteryl esters and cellular debris in the inner surface of large and medium size arteries, which leading to reduce or even stop the flow of blood increasing risk of ischemic heart diseases [61]. One of the side effects of most hyperlipidemia drugs is decrease each of bad cholesterol (LDL-C) and good cholesterol (HDL-C). This study carried out to find natural components decrease bad cholesterol (LDL-C) and increase good cholesterol (HDL-C) resulting decrease risk of heart diseases. In the current study dietary hyperlipidemia model was used to study the hypolipidemic and antioxidant effect of mucilage isolated from *C. dichotoma* fruits. Hyperlipidemic model characterized by elevated daily body weight gain, TC, TG, VLDL-C and LDL-C and reduced HDL-C. Administration mucilage at two doses (0.5 and 1.0 g/kg body weight/day) for 10 weeks caused remarkably depletion on daily body weight gain, TC, LDL-C, TG and VLDL-C, concurrent with significant elevation on HDL-C concentration compared with hyperlipidemic control. Risk ratio defines as a ratio of bad cholesterol (LDL-C) and good cholesterol (HDL-C) is believed to be an important risk factor of atherosclerosis. Mucilage significantly decreased the ratio, which is indicated the anti-atherogenic effect of mucilage.

C. dichotoma mucilage decreased body weight gain by decreasing food intake, which may be caused a decrease in body fat weight. These results may be due to increase daily dietary fibers intake, which in turn decreased energy absorption by diluting a diet's energy availability [62]. The soluble fibers don't digest but it ferments in the large intestine producing two gut hormones; Glucagon-like Peptide (GLP-1) and Peptide YY (PYY) [63]. These hormones increase satiety feeling in brain. GLP-1 hormone increases insulin secretion by increasing beta cells mass, insulin gene expression and decreases glucagon secretion, which increases lipolysis than lipogenesis [64]. Weickert and Pfeiffer [65] concluded that increase viscosity in intestine retards absorption of fats. In the same direction Struthers [66] demonstrated that mucilage hydrates and can swell in the intestine which causes an increase in volume and viscosity of gastrointestinal content, leading to enhances intestinal transit and decreased gastrointestinal transit time. Confirmation of the above, mucilage in this study has swelling index about 76.35%.

Viscous soluble fibers inhibit digestion and absorption of dietary fats causing decrease in chylomicron remnants, which transports cholesterol to the liver. The decreasing on cholesterol delivery in liver pays it to upregulate LDL-C receptor and decreasing lipoprotein secretion to maintain cholesterol homeostasis. Viscous soluble fibers trap bile salts in the viscous matrix in the gut. So, cholesterol is derived to the synthesis of bile acids, resulting decrease in cholesterol reserve in liver. Moreover, soluble fibers are fermented by the colonic micro flora generating short-chain fatty acids (acetic, propionic, and butyric acids). Propionates inhibit cholesterol and fatty acids synthesis in the liver [21]. Our obvious results were in accordance of these pronounced by Struthers [66], Anderson et al. [67] on whole grain foods, Lecumberri et al. [21] on coca fibers, Makni et al. [22] on flax seeds Sulieman and El-Newary [10]; El-Newary et al. [11] on aqueous extract of *C. dichotoma* seeds pulp.

HDL plays a key role in the protection against oxidative damage of membranes [68]. HDL-C levels could potentially contribute to its anti-atherogenic properties, including its capacity to inhibit LDL-oxidation and protect endothelial cells from the cytotoxic effects of oxidized LDL-C [69]. In our study *C. dichotoma* mucilage increased HDL-C and decreased LDL-C of hyperlipidemic rats. Elevated level of HDL-C of rats treated with mucilage at two doses; 0.5 and 1.0 g/kg body weight/day for 10 weeks could be due to increase in activity of lecithin cholesterol acyl transferase (LCAT), which contributes to regulation of blood lipids. LCAT plays a key role in incorporating free cholesterol into HDL and transferring back to VLDL and IDL, which is taken back by liver cells [70]. GPx have an important role in the prevention of oxidative stress it is considered to be an anti-atherogenic enzyme [71]. Mucilage recorded an increment in GPx of hyperlipidemic rats about 205.30 and 101.87% by low and high dose respectively. These results supported by those of Sanmi et al. [26] on Black tea and *Zanthoxylum zanthoxyloid* individually or together and El-Newary et al. [11] on *C. dichotoma* seeds pulp aqueous extract.

CONCLUSION

During the experimentation, Wistar albino rats did not show any mortality or any other adverse effects when the rats fed orally with *C. dichotoma* mucilage at the doses of 0.5 or 1.0 g/kg/day. Mucilage caused markedly amelioration in lipid profile and antioxidant system of liver without any adverse effect in normal and hyperlipidemic rats. Mucilage recorded an improvement in liver functions and liver histology. It is indicating that *C. dichotoma* mucilage have a good margin of safety. The results concluded that *C. dichotoma* mucilage have hypolipidemic, antioxidant and hepatopr-otective effect.

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