



RESEARCH ARTICLE

ANTIDIABETIC AND ANTIHYPERLIPIDEMIC ACTIVITY OF *DRACAENA CINNABARI* BALF. RESIN ETHANOLIC EXTRACT OF SOQATRA ISLAND IN EXPERIMENTAL ANIMALS

Nahed Al-Baoqai^{*1}, Hassan Al-Mahbashi², Adnan Al-Adhal²

¹Department of Pharmacology and Therapeutics, Faculty of Medicine and Health Sciences, Sana'a University, Yemen.

²Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Sana'a University, Yemen.

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*Address for Correspondence:

Nahed Al- Baoqai, Department of Pharmacology and Therapeutics, Faculty of Medicine and Health Sciences, Sana'a University, Yemen.
 E-mail: Nahed.pharmacist@hotmail.com

Abstract

Objective: *Dracaena cinnabari* balf is a species plant in *Agavaceae* family. It is a tree endemic to the Island of Socotra, Yemen. It was used as a dye and medicine in Socotra and the Mediterranean basin. Some researches demonstrated both hypoglycemic and hypolipidemic of its resin of *Dracaena cochinchinensis*, a traditional Chinese tree. Accordingly, this study aimed to evaluate the antidiabetic potential of the ethanolic extract of *Dracaena cinnabari* balf resin (DCBR), endemic in Socotra Island, in Alloxan induced diabetes, and evaluation of possible antihyperlipidemic activity of the same extract in high-cholesterol diet-induced hyperlipidemia in male albino rats.

Methods: Experimental diabetes was induced in rats with intraperitoneal injection a single dose of Alloxan (150 mg/Kg). Hyperlipidemia was induced in rats by administration of 1% cholesterol in diet and 0.5 % Hydrogen Peroxide in drinking water for 14 days. Fasting blood glucose levels were measured at 1st day, 7th day and 14th day. Pancreas histopathology was done at the end. Estimation of serum lipid profile and liver histopathology was done at the end of 14 day. The results were expressed as mean±SD and differences among the groups of animals were compared using one-way ANOVA.

Results: Administration of ethanolic extract of resin of two doses (100 and 300mg/Kg) in alloxan induce diabetic rats resulted in a significant decrease in FBG levels with a recovery in destruction of pancreas cell compared with untreated group. Also the same doses of extract showed significant decrease in triglycerides, total cholesterol, low density lipoprotein, very low density lipoprotein and Atherogenic index in comparing with untreated group.

Conclusion: From the results of present study we can conclude the ethanolic extract of *Dracaena cinnabari* Balf. have a hypoglycemic and hypolipidemic activity in experimental animals, these activity are time and dose dependent.

Keywords: alloxan, blood glucose, diabetes, *Dracaena cinnabari*, flavonoids, glibenclamide, resin.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by elevated levels of glucose in blood (hyperglycemia) due to insufficient production of insulin or because cells do not respond to insulin produced¹. It is estimated that about 350 million people have diabetes, the prevalence being similar in both high and low income countries and it is forecasted that global diabetes prevalence will increase by 50% in 2030². The disease is characterized by increase production of oxygen-free radicals such as superoxide (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxide (OH⁻) radicals and lack in antioxidant defense mechanisms

which lead to increase oxidative stress and development of diabetes complications³. Management strategies of diabetes include life style intervention through diet modification and exercise and the use of oral hypoglycemic therapy and insulin treatment⁴.

Hyperlipidemia related to increased oxidative stress causing significant production of oxygen free radicals, which may lead to oxidative modifications in low-density lipoproteins, which present a significant function in the initiation and progression of atherosclerosis and associated cardiovascular diseases⁵. The treatments for hyperlipidemia vary according to factors that include heart disease risk, lipid levels and a patient's overall health. Potential treatments for lipid

disorders include dietary changes, weight loss, regular exercise, quitting smoking, medications and periodic lipid screenings⁶. The chemical drugs used for treatment of hyperlipidemia are: statin, fibrates, In addition, other drugs, such as ezetimibe, colesvelam, torcetrapib, avasimibe, implitapide, and niacin are also being considered to manage hyperlipidemia⁷. Although the drugs are available in the market, long term use may cause a number of side effects. Hence a large number of studies are in progress to find natural sources, which are effective in reducing the intensity of diabetes and hyperlipidemia. World Health Organization (WHO) approved the use of plant drugs for different diseases⁸ including diabetes mellitus⁹ and hyperlipidemia¹⁰. One of these plants is *Dracaena cinnabari*, known as *Dam Al Akhawain*. It is one of the rare and blessed tree in islet of Socotra, Yemen. *D. cinnabari* balf is a species plant in *Agavaceae* family. It is a tree endemic to the Island of Socotra, Yemen¹¹ the evocatively named dragon's blood tree has a unique and bizarre appearance, its upturned, densely-packed crown having the shape of an upside-down umbrella¹². The name *Dracaena* is derived from the Greek word 'drakainia' meaning a female dragon). The most striking source is the *D. cinnabari* balf, which is endemic to the island of Socotra (Yemen)¹³. There are a lot of researches that have been worked on *D. cinnabari* balf resin and approved its effectiveness as a antimicrobial, antiviral¹⁴, antitumor and cytotoxic¹⁵. It is also found that, it is a potent analgesic¹⁶, antioxidant¹⁷ and anti-inflammatory¹⁸. No previous studies about *in vivo* antidiabetic and antihyperlipidemic activity of *D. cinnabari* balf resin that is a species plant endemic to the Island of Socotra, Yemen. Accordingly, the current study was done to prove the antidiabetic and antihyperlipidemic activity of *D. cinnabari* balf resin ethanolic extract in experimental animals.

METHODS

Chemicals and Instruments

Alloxan 98% (Hydrate) which was obtained from Oxford laboratory, India. Glibenclamide which obtained from DSM Sinochem pharmaceuticals, India. Auto analyzer performa- accuCheck Advantage II, Roche), Glucose kit from Roche, Germany. Cholesterol powder obtained from BDH chemicals LTD, England. Atorvastatin which obtained from DSM Sinochem pharmaceuticals, India. Hydrogen peroxide 30% obtained from EMPLURA (500 ml) MERCK, India. Cholesterol. Triglyceride, HDL and LDL kits. HumaLyzer 3500 photometers, Germany.

Plant material

D. cinnabari balf resin is exuded and collected from incisions of the trunk of *D. cinnabari* Balf.

Preparation of ethanolic extract

One kilogram of the Resin was washed thoroughly with distilled water, air-dried, powdered with an electrical grinder, and soaked in 99% ethanol (1:10) at room temperature (25°C) over period of 48 hr and were shaken several times (maceration method). The ethanol containing the extract was then filtered through Whitman paper then the solvent was vacuum distilled

at 40°C in rotary evaporator. Final extract was red semi-solid in percentage dry weight 90%. This ethanol extract was kept at 4°C until use.

Experimental animals

Healthy male albino rats of Wistar strain weighing 150-250 gm. They were obtained from the Central Animal House, Sana'a University. The present study was approved before start off experiment by the animal Ethics committee, 00243, Government of Yemen constituted, Sana'a University. The animals were housed in standard polypropylene cages and maintained within the same room temperature and humidity with 12:12 hour light and dark cycle. All the rats were given a 14 days period of acclimatization before starting the experiment.

Induction of diabetes

Diabetes was induced by intraperitoneal administration of a single dose of alloxan (150 mg/kg)²⁰. Male albino Rats were fasted for 18 h. The fasting blood sugar (FBS) levels of the rats were determined with blood from the rats' tail vein using an auto analyzer (Accu Check Performa Advantage II, Roche). Freshly prepared Alloxan monohydrate dissolved in normal saline (0.9% w/v NaCl) and injected as a single dose of 150 mg/kg intraperitoneally to induce hyperglycemia. To avoid fatal hypoglycemia as a result of massive pancreatic insulin release, rats were Kept for the next 24 h on 5% glucose²¹. After 72 hours, rats with fasting blood glucose Level more than 250 mg/dL were considered to be diabetic and selected for studies²². The experiment was approved by the Institutional Ethical Committee, Faculty of Medicine and Health Sciences, Sana'a University.

Experimental design

Evaluation of antidiabetic activity of DCBR methanolic extract in alloxan-induced diabetic rats

The albino rats were allocated randomly into five groups, each containing six rats:-

Group 1: Normal control, without treatment rats.

Group 2: Diabetic control, without treatment rats.

Group 3: Diabetic rats received Ethanolic extract (100 mg/Kg/day, p.o.)²³.

Group 4: Diabetic rats received Ethanolic extract (300 mg/Kg/day, p.o.)²³

Group 5: Diabetic rats received glibenclamide (2.5 mg/Kg/day, p.o.)²⁴.

After 72 hours from administration of alloxan rats with hyperglycemia more than 250 mg/dl were selected and used for experiment. The rats were administered the extract (dissolved in Tween 80) and chemical drug for two weeks orally by using a feeding cannula. The experimental rats were weighed every day to compare the body weight change in different groups through the experimental period. FBG was estimated before the start of treatment, on 7th day and 14th days of treatment. All rats were later sacrificed on 14th days by diethyl ether and the Pancreases were weighted separately then collected in 10% formalin for histopathological examination.

Histopathological examination of Pancreas

For histological study, specimens of pancreases were immersed in neutral buffered formalin (10%) for 48 hours. Then tissue dehydration was done using

ascending grades of alcohol (ethanol), followed by tissue clearing using xylene. The tissues were transferred to molten paraffin for impregnation and embedded in paraffin blocks. After fine sectioning, staining was done using hematoxylin and eosin (H and E) stain and examined under a microscope²⁵. All the sections were examined under a light microscope under X400 magnifications. Photomicrographs of lesions were taken for observations and documentation of histopathological lesions. **Induction of hyperlipidemia**

Hyperlipidemia was induced in rats by administration of 1% cholesterol in diet and 0.5 % Hydrogen Peroxide in drinking water for 14 days²⁶.

Experimental design

Evaluation of antihyperlipidemic activity of DCBR methanolic extract in experimental rats.

The albino rats were allocated randomly into five groups, each containing six rats :-

Group 1: Normal control, without treatment.

Group 2: Hyperlipidemic rats received 1% cholesterol + 0.5 % Hydrogen Peroxide

Group 3: Hyperlipidemic rats received 1% cholesterol + 0.5 % Hydrogen Peroxide and Ethanolic extract (100 mg/Kg/day, p.o)

Group 4: Hyperlipidemic rats received 1% cholesterol + 0.5 % Hydrogen Peroxide and Ethanolic extract (300 mg/Kg/day, p.o).

Group 5: Hyperlipidemic rats received 1% cholesterol+0.5% Hydrogen Peroxide and Atorvastatin (10 mg/Kg/day, p.o)²⁷.

After experimental period, and after an overnight fasting, animals in different groups were anesthetic under mild diethyl ether and the blood was withdrawn by retro-orbital method to determine serum lipid profile. Serum obtained by immediate centrifugation of blood samples using centrifuge at 3000 rpm for 15 min. at room temperature. The concentration of total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) was determined by using enzymatic commercial kits marketed by QCA Ltd, Spain. While VLDL and atherogenic index in plasma was calculated as per Friedewald estimation²⁸. Then, the animals were sacrificed to take the liver and aorta specimens for histopathological examination.

Histopathological examination of liver and Aorta

Specimens of liver and aorta were immersed in neutral buffered formalin (10%) for 48 hours. Then tissue dehydration was done using ascending grades of alcohol (ethanol), followed by tissue clearing using xylene. The tissues were transferred to molten paraffin for impregnation and embedded in paraffin blocks. After fine sectioning, staining was done using hematoxylin and eosin (H and E) stain and examined under a microscope²⁵. All the sections were examined under a light microscope under X400 magnifications. Photomicrographs of lesions were taken for observation and documentation of histopathological lesions.

Statistical analysis

The results were expressed as mean±SD and differences among the groups of animals were

compared using one-way ANOVA with post-hoc LSD's test. Statistical significance was set at $p \leq 0.05$. Statistical analysis was performed using Microsoft excel, and SPSS software version 20.

RESULTS

Result of antidiabetic activity of DCBR methanolic extract in alloxan-induced diabetic rats.

Relative change in body weight

Measurement of the weight of all animals from the first day to the last day of the experiment showed an irregular change and differences in the groups as shown in Table 1. The weight of group 1 increased significantly from 1st day to 14th day in opposite to group 2. While, the groups that treated with 100 mg/Kg (group 3) and 300 mg/Kg DCBR extract (group 4) showed noticeable improvement to normal weight during treatment period whereas the weight were dropped dramatically because of Alloxan injection. Group 5 that was treated with 2.5 mg/Kg Glibenclamide kept the same weight level during treatment period.

Table 1: The effect of DCBR extract on change in the body weight (g) of Alloxan -induced diabetic rats.

| Groups | 1 st Body weight(g) | 7 th day Body weight(g) | 14 th day Body weight(g) |
|---------|--------------------------------|------------------------------------|-------------------------------------|
| Group 1 | 165.41±43.8 | 196.46±32.7 | 219.5±35.7 |
| Group 2 | 178.33±17.4 | 158.66±32.3 | 146.73±36.3* |
| Group 3 | 175.93±25.7 | 184.48±26.5 | 196.05±27.5 |
| Group 4 | 153.16±43.1 | 172.28±40.5 | 187.73±39.0 |
| Group 5 | 153±14.0 | 150.56±19.9 | 150.03±18.5* |

N=6, Values are expressed as Mean ±SD; * $p \leq 0.05$ vs. group 1. DCBR: *Dracanea cinnabari* balf Resin.

Relative change in weight of pancreas:

As shown in Table 2, after 14 day of treatment, there was a significant decrease in pancreas weight in group 2, in comparison to group 1 (p -value ≤ 0.05). Group 3 which was treated with 100 mg/Kg DCBR extract showed an increase in pancreas weight. We got the same result with group 5 which was treated with 2.5 mg/Kg Glibenclamide, whereas group 4 which was treated with 300 mg/Kg DCBR extract, showed a significant increase in the pancreas weight in comparison to group 2.

Table 2: The effect of DCBR extract on change in the pancreas weight (g) of Alloxan-induced diabetic rats.

| Groups | pancreas weight (g) mean ±SD | % of change vs. group 1 |
|---------|------------------------------|-------------------------|
| Group 1 | 1.57±0.43 | |
| Group 2 | 0.48±0.08* | 69.4 % |
| Group 3 | 1.03±0.42 | 34.3% |
| Group 4 | 1.33±0.43** | 15.3% |
| Group 5 | 0.92±0.21* | 41.4% |

N=6, Values are expressed as Mean ±SD; * $p \leq 0.05$ vs. group 1, ** $p \leq 0.05$ vs. Group 2. DCBR: *Dracanea cinnabari* balf Resin.

Biochemical investigation (FBG)

The groups 2, 3, 4 and 5 which were injected with 150 mg/Kg alloxan showed a significant increase in the FBG values more than 200 mg/dl (p -value ≤ 0.05) in comparison to group 1. After 7 days of treatment, the dose of 100 mg/Kg DCBR extract (group 3), 300 mg/Kg DCBR extract (group 4) and 2.5 mg/Kg

Glibenclamide (group 5) showed a significant change in comparison to group 1 and group 2 (p -value ≥ 0.05), as well as after 14 days of treatment. By the way, group 4 did not show a significant change in FBG in comparable to group 1, which means that 300 mg mg/Kg DCBR extract dose, achieved the target normal value of FBG (Table 3).

Table 3: Effects of DCBR extract on the FBG values (mg/dl) of Alloxan-induced diabetic rats.

| Groups | Basal Value (mg/dl) | 7 th day (mg/dl) | % of FBG change vs. Basal Value after 7 days | 14 th day (mg/dl) | % of FBG change vs. Basal Value after 14 day |
|---------|---------------------|--------------------------------|--|--------------------------------|--|
| Group 1 | 80.16 ± 9.7 | 88.16 ± 4.2 | | 90.3 ± 6.7 | |
| Group 2 | 495.0 ± 32.7* | 522.16 ± 31.1*, ^Δ | | 508.4 ± 34.9*, ^Δ | |
| Group 3 | 381.6 ± 22.2*,** | 291.8 ± 18.1*,**, ^Δ | 24% | 180.8 ± 14.9*,**, ^Δ | 53% |
| Group 4 | 473.6 ± 14.7* | 186.6 ± 4.4*,**, ^Δ | 60.5% | 101.6 ± 3.1**, ^Δ | 79% |
| Group 5 | 481.5 ± 11.4* | 415.0 ± 13.7*,**, ^Δ | 14% | 217.0 ± 10.7*,**, ^Δ | 55% |

N=6, Values are expressed as Mean ± SD ; * $p \leq 0.05$ vs. group 1 for each column, ** $P \leq 0.05$ vs. group 2 for each column. ^Δ $P \leq 0.05$ vs. basal value for each row. DCBR: *Dracanea cinnabari* balf Resin, FBG: Fasting Blood Glucose.

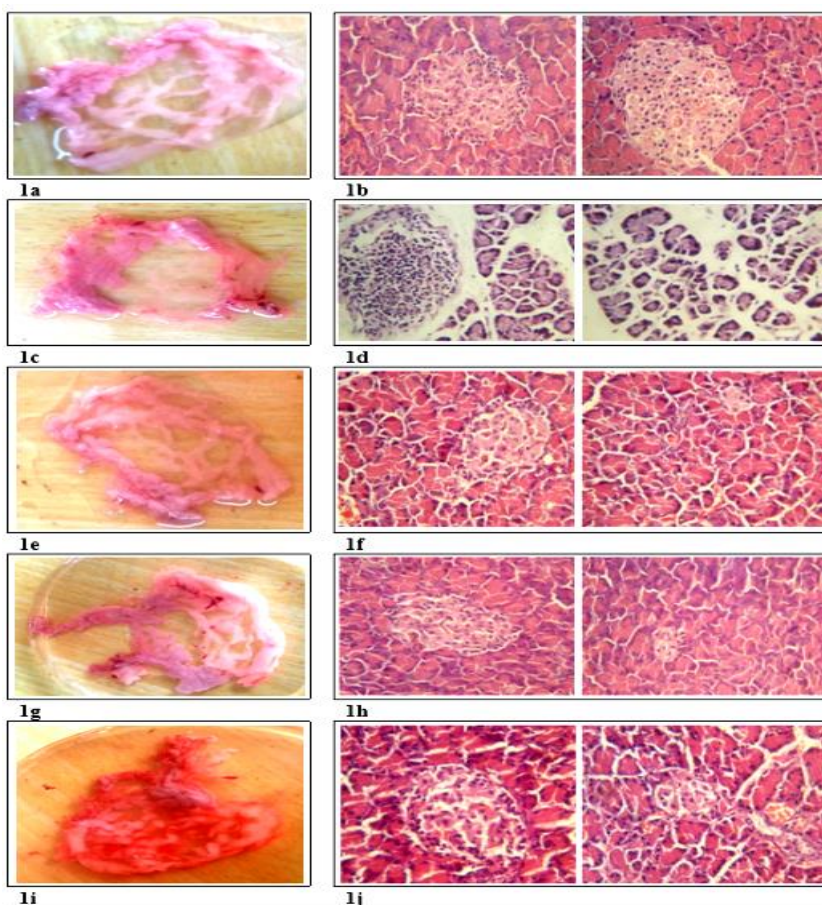


Figure 1: Macroscopic and microscopic observation of pancreas in rats.

Macroscopic images: 1a: group 1, 1c: group 2, 1e: group 3, 1g: group 4, 1i: group 5

Microscopic images: Hematoxylin and eosin staining of pancreatic islets of group 1, group 2, group 3, group 4 and group 5 (1b), (1d), (1f), (1h), respectively. Observe islet cells with defined boundaries (black arrow) in (1b), (1f), and (1j), when compared with (1d) X400

Table 4: The effect of DCBR extract on change in the body weight (g) of hyperlipidemic rats.

| Groups | Initial Body weight (g) | 7 th day body weight (g) | 14 th day Body weight (g) |
|---------|-------------------------|-------------------------------------|--------------------------------------|
| Group 1 | 251.33 ± 22.5 | 271.48 ± 19.7 | 295.01 ± 15.5 |
| Group 2 | 277.9 ± 6.6 | 279.25 ± 2.1 | 287.3 ± 0.6 |
| Group 3 | 295.4 ± 32.1 | 293.4 ± 34.2 | 305.46 ± 32.6 |
| Group 4 | 321.3 ± 43.3 | 279.41 ± 37.5 | 280.98 ± 42.4 |
| Group 5 | 306.53 ± 35.9 | 300.26 ± 30.4 | 316.38 ± 34 |

N=6, Values are expressed as Mean ± SD; * $p \leq 0.05$ vs. group 1. DCBR: *Dracanea cinnabari* balf Resin.

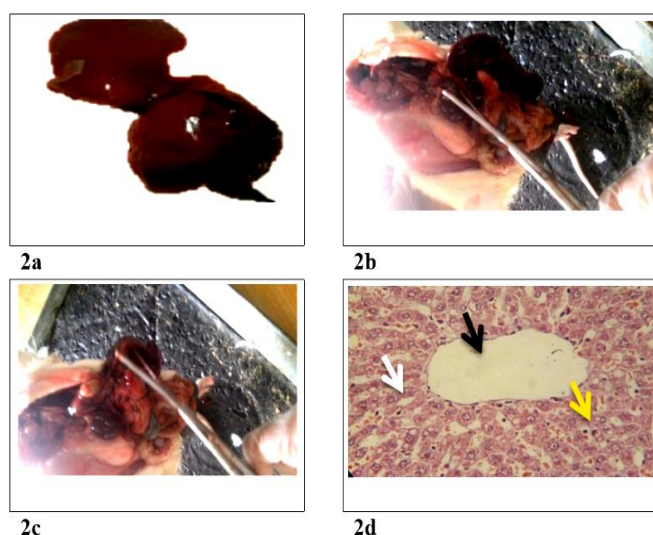


Figure 2: Macroscopic and microscopic observation of liver in rats (group 1).

2a: Macroscopic image of normal liver; **2b and 2c:** Adipose tissue around the liver (black arrow); **2d:** Microscopic section of normal liver stained by hematoxylin-eosin shows portal vein (black arrow), hepatic cord (white arrow) and hepatic sinusoid (yellow arrow) X400.

Histopathological examination of pancreas

Macroscopic observations

The pancreas was subjected to macroscopic and microscopic investigation to detect irregularities and abnormalities of the structure. Macroscopic analysis of the control group demonstrated normal pinkish appearance (Figure 1a) in comparison to the pancreas of untreated diabetic, depletion large area of the pancreas as a result of alloxan injection 150 mg/Kg, (Figure 5c). Treated groups with 300 mg/Kg extract, 100 mg/Kg extract, and 2.5 mg/Kg Glibenclamide showed clear preserved tissue (Figure 1e, Figure 1g, and Figure 1i) respectively).

Microscopic observations

The microscopic appearance of the control group islet cells (group 1) was normal (Figure 5b). Untreated diabetic group (group 2) revealed a breakdown of micro-anatomical features including necrosis as in (Figure: 1d). The comparison of the untreated diabetic group to normal group, there was destruction in the islet cells with irregular shape and atrophy (Figure: 1d). (Figure: 1f, 1h and 1j) figures showed treated diabetic rats with 100 mg/Kg extract (group 3), 300 mg/Kg extract (group 4) and 2.5 mg/Kg Glibenclamide (group 5) respectively. The comparison of these three groups with the untreated diabetic rats, there was an evidence of recovery of Langerhans islets, more obvious islet pattern with well outlined boundaries, vacuolation was reduced or absent in many islets.

Result of antihyperlipidemic activity of DCBR methanolic extract in experimental rats.

▪ Relative change in body weight

Based on the result reflected on Table 4, we could say that the rats of groups 2,3,5 (hyperlipidemic without treatment, 100 mg/Kg DCBR extract, 10 mg/Kg Atorvastatin respectively, showed an increased growing in body weight throughout the entire period of treatment. However, there was a decrease in group 4 which was treated with 300 mg/Kg DCBR extract.

Table 5: The effect of DCBR extract on change in the liver weight (g) of hyperlipidemic rats.

| Groups | liver weight (g) mean \pm SD | % of change vs. group 1 |
|---------|-----------------------------------|----------------------------|
| Group 1 | 9.65 \pm 0.25 | |
| Group 2 | 16.9 \pm 0.91* | 43% |
| Group 3 | 10.4 \pm 1.08** | 7.8% |
| Group 4 | 11.13 \pm 1.09** | 13% |
| Group 5 | 13.21 \pm 2.36**&*** | 27% |

N=6, Values are Mean \pm SD; * $p \leq 0.05$ vs. group 1, ** $p \leq 0.05$ vs. group 2. DCBR: *Dracanea cinnabari* balf Resin

▪ Relative change in weight of liver

As shown in Table 5, after 14 days of treatment, there was a significant change in the liver weight of group 2 in comparison to group 1 (p -value ≤ 0.05). Also, other three groups of treatment with 100mg/Kg DCBR extract (group 3), 300 mg/Kg DCBR extract (group 4) and 10mg/Kg Atorvastatin (group 5) showed a significant change and decrease the liver weight in comparison to group 2 (p -value ≤ 0.05).

Biochemical parameters: TC, TG, HDL, LDL, VLDL, and AI

As in Table 6 the rats when fed with high-fat diet (1% cholesterol) showed a remarkable hyperlipidemia. For the whole group, there was a significant increase in TC, TG, LDL, VLDL AI, and a decrease in HDL in comparison to group1 (p -value ≤ 0.05). When we compare the treated groups with group 2, we noticed that: the treated group with 100 mg/Kg DCBR extract (group 3) showed a significant decrease in TC, TG, LDL, VLDL and AI and a significant increase in HDL (p -value ≤ 0.05), (where the change in TC, TG, LDL was insignificant, and VLDL was significant). The higher dose of 300 mg/Kg DCBR extract (group 4) showed a significant effect in decreasing TC, TG, LDL, and AI. In addition, there is no effect on HDL. The last group which was treated with 10 mg/Kg Atorvastatin showed no effect on TC, TG. But, also showed a significant increase in HDL and no significant effect on VLDL and AI.

Table 6: Effects of DCBR extract on the lipid profile mg/dl of high fat diet hyperlipidemic rats after 14 days.

| Groups | TC | TG | HDL | LDL | VLDL | AI |
|---------|--------------|---------------|-------------|---------------|--------------|-------------|
| Group 1 | 59.2±13.51 | 103.98±20.5 | 52.48±5.83 | 73.5±14.54 | 20.79±4.1 | 1.13±0.25 |
| Group 2 | 111.5±9.31* | 136±2.19* | 28±1.09* | 130.5±7.12* | 27.2±0.43* | 3.97±0.17* |
| Group 3 | 93.46±9.98** | 107±8.19** | 36±3.89** | 119.66±12.2 | 21.4±1.63** | 2.63±0.51** |
| Group 4 | 77.01±8.84** | 96.08±23.61** | 28±0.63 | 102.16±4.75** | 19.21±4.72** | 2.74±0.28** |
| Group 5 | 113.05±11.83 | 134±3.63 | 37.5±3.27** | 121±10.31 | 26.8±0.72 | 3.02±0.28** |

N=6, Values are expressed as Mean ±SD; *p≤0.05 vs. group 1, **p≤0.05 vs. group 2. DCBR: *Dracanea cinnabari* balf Resin, TC: Total Cholesterol, TG: Triglyceride, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, VLDL: Very Low Density Lipoprotein, AI: Atherogenic Index.

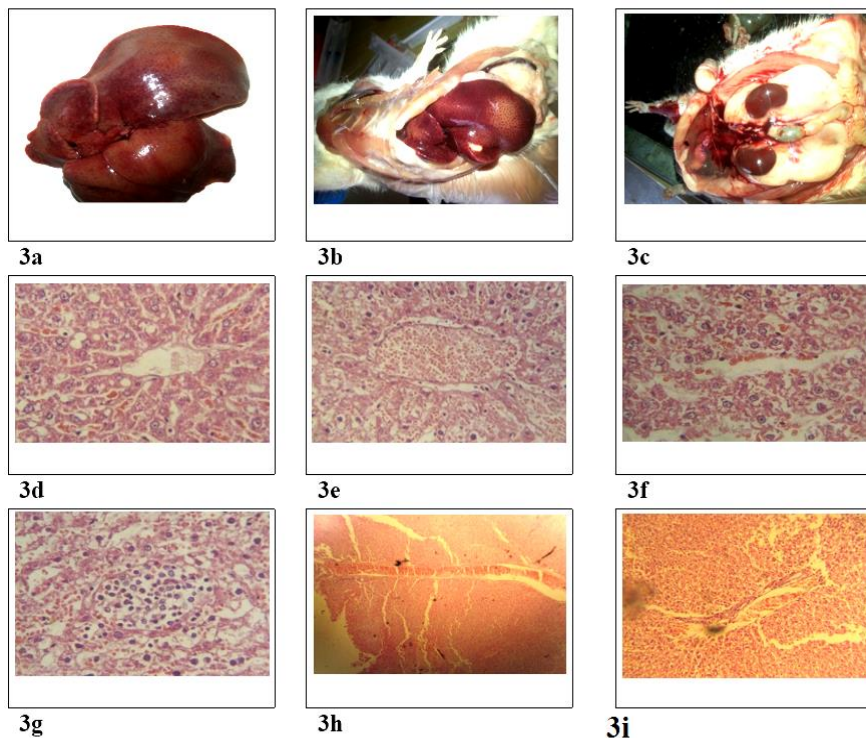


Figure 3: Macroscopic and microscopic observation of liver in rats (group 2).

3a and 3b: Macroscopic image of high fat liver; **3c:** Adipose tissue around the liver; **3d, 3e, 3g, 3g, 3h, and 3i:** Microscopic image shows abnormal liver structure i.e. **3d:** Fatty changes, **3e:** Haemorrhage, **3f:** Hydrobic changes, **3g:** Inflammation, **3h:** Vasodilatation, **3i:** Infiltration, X400

Histopathological examination of liver and Aorta
Macroscopic observations

The liver was subjected to macroscopic and microscopic investigation to detect irregularities and abnormalities of the structure. Macroscopic analysis of the liver in hyperlipidemic group (group 2) demonstrated major light brown color changes of the liver lobes (Figure 3a, and Figure 3b). While the liver of the normal group (group 1) looks in a normal appearance i.e. deep brown color with smooth surface (Figure 2a). The hyperlipidemic groups treated with 100mg/Kg DCBR extract (group 3), 300 mg/Kg DC extract (group 4), and 10 mg/Kg Atorvastatin (group 5) showed pale brown coloration of the hepatic soft tissue (Figure: 4a, 4d and 4g). In addition, inducing high fat diet shows a clear accumulation of fat in the adipose tissue inside the rat abdomen. Which is clear in group 2 (Figure: 3b and 3c). The treated group with 100mg/Kg DC extract shows no big difference in the amount of the accumulation of the fat in adipose tissue

(Figure: 4b). Whereas other doses of 300 mg/Kg DC extract and 10 mg/Kg Atorvastatin shows a notable decrease Figure 4.

Microscopic observations

The normal liver tissue section of group 1 shows normal structure i.e portal vein, hepatic cord, and hepatic sinuside (Figure 2d). Whereas the hyperlipidemic rat liver tissue section (group 2) shows distortion in the arrangement of cells around the central vein, inflammation Figure 3g, infiltration Figure 3i, fatty changes Figure 3d, hemorrhage Figure 3e, hydrobic change Figure 3f, vasodilation Figure 3h. The hyperlipidemic groups treated with 100 mg/Kg DC extract (group 3), 300 mg/Kg DC extract (group 4) and 10 mg/Kg Atorvastatin (Group 5) brought back the cellular arrangement around the vein in some parts of the tissue that lead to bring the blood vessels to the normal conditions (Figure 4c, Figure 4f and Figure 4i respectively). Histopathological picture of Aorta all groups does not show any abnormalities Figure 5.

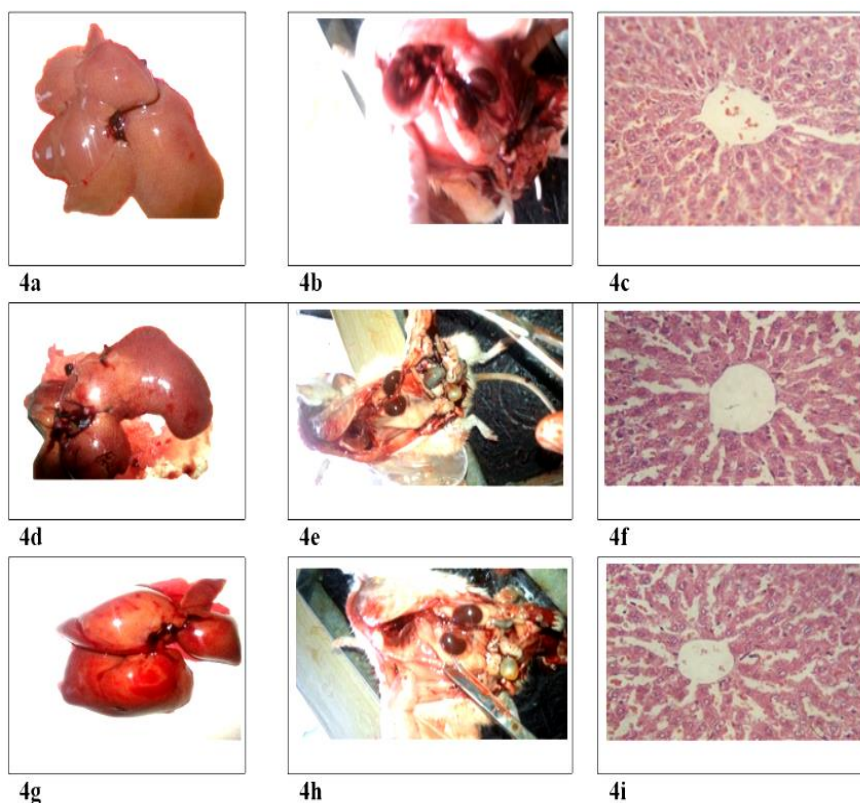


Figure 4: Macroscopic and microscopic observation of liver in rats (group 3, 4 and 5).

4a, 4d, 4g: Macroscopic images of liver of group 3, 4, and 5.

4b, 4e, 4h: Adipose tissue around the liver of group 3, 4, 5 respectively.

4c, 4f, 4i: Microscopic image shows regeneration of abnormal liver structure with the same sequence group 3, 4, and 5. X400

DISCUSSION

Alloxan can induce diabetes by destruction of the beta cells of the islets of Langerhans of the pancreas²⁹. This results in a decrease of endogenous insulin secretion which paves way for the decreased utilization of glucose by body tissues and consequently elevation of blood glucose level³⁰. In current study and after alloxan injection, elevation of blood glucose was observed in group 2, 3, 4 and 5. When treated them with the ethanolic extract of *D. cinnabari*, it was indicated that all of them reduced the blood glucose level to an extent. The dose 100 mg/Kg decreased FBG significantly ($p \leq 0.05$) up to 24% and 53% after 7, 14 day respectively. Also, the dose 300 mg/Kg showed more decrease in FBG significantly ($p \leq 0.05$) up to 60.5% and 79% after 7 and 14 days respectively. These results were compatible with Chen *et al.*,^{31,23}.

They demonstrated hypoglycemic effect of total flavonoids of *Dracaena cochinchinensis* in type 2 diabetes mellitus rats³¹. Also, Gu *et al.*, showed an antidiabetic effect of *D. cochinchinensis* by inhibition on alpha-glucosidase activity and suppressing intestinal carbohydrate absorption and thereby reducing the postprandial increase of blood glucose²³. In an *in vitro* study reported the ethyl acetate

extract of *Dragon cinnabari* resin has antidiabetic properties with standard glucose uptake procedure against MCF-7 cell line¹⁹. The mechanism of antidiabetic properties of the extract is not well known. But, it is returned likely to the phytochemical

constituents including flavonoids, alkaloids, tannins, and others. These constituents also obtained from various plant sources and they have been reported to be potent hypoglycemic agents³². Flavonoids are the main chemical constituents of *Dracaena* species^{31,33,34}.

Flavonoids derivative from the methanolic extract of *Origanum majorana* L. (family: Lamiaceae) leaves have α -glucosidase enzyme inhibitory activity³⁸. Another flavonoids from *Cecropia obtusifolia* (family: Cecropiaceae) exhibited potent hypoglycemic activity comparable to that of glibenclamide at a dose of 3 mg/kg in diabetic rats³⁹. Other flavonoids were isolated from the dried leaves of *Myrcia multiflora* DC. (family: Myrtaceae) inhibited the activity of the rat lens aldose reductase enzyme so decrease diabetic complications⁴⁰. Flavonoids isolated from *Eysenhardtia platycarpa* (family: Leguminosae) were evaluated to possess promising anti-hyperglycemic activity by decreasing glucose level of streptozotocin (STZ)- induced diabetic rats (31 mg/Kg of body weight, $P < 0.05$)⁴¹.

In addition, leaves of *Eucommia ulmoides* (family: Eucommiaceae) has flavonoid which inhibit glycation end-product formation, one of main molecular mechanisms implicated in diabetic complications⁴². Flavonoids can decrease the blood glucose by pancreatic and extra pancreatic mechanisms. They can act as insulin secretagogues which increase insulin secretion *in vitro* and *in vivo* systems^{43,38}, in similar to Sulfonylureas such as glibenclamide⁴⁴ which was used as the reference drug in this study. They also can inhibit α -glucosidase activity in the intestine and reduce glucose absorption from intestine²³.

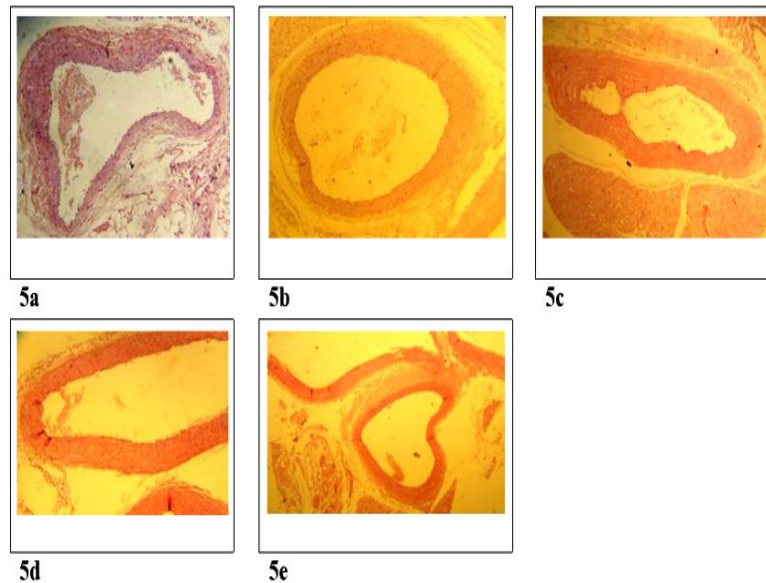


Figure 5: Microscopic observation of aorta in rats.
5a: group 1, 5b: group 2, 5c: group 3, 5d: group 4, 5e: group 5. X400

There are convincing experimental and clinical evidence that the generation of ROS is increased in both Type 1 and Type 2 diabetes and that the onset of diabetes is closely associated with oxidative stress⁴⁵. Flavonoids are rich in phenol groups which work as antioxidant which may have slowed or terminated the production of ROS thereby reversing the diabetic condition^{17,46,47,48}. In the histopathology, the degeneration observed in the pancreas of alloxan-induced diabetic rats may be due to necrotic action of alloxan on the beta cells^{49,50}.

The degenerative changes induced by alloxan in rats pancreas were recovered by DCBR extract and glibenclamide which was followed by regeneration of the islet cells and increased tissue density with attendant improved insulin production and reduction of FBG in rats. This effect was more pronounced at 300 mg/kg DCBR extract. It has been demonstrated that beta cells can regenerate from stem cells located in pancreatic ducts or from progenitor cells residing inside murine islets⁵¹. Regeneration of the islet cells by DCBR extract may involve promotion of the above mechanisms.

Also, Flavonoids are reported to regenerate the damaged pancreatic β -cells in diabetic animals which were acting as antioxidant and reducing the cells damage by alloxan⁵². Furthermore, the current study showed antihyperlipidemic effect of DCBR extract which is agreed with Fan *et al.*,³⁴. High fat diet administered to rats raised the lipid profile and with treatment by extract, lipids are decreased by 16%, 31% TC, 21%, 29% TG, 8%, 22% LDL, 21%, 29% VLDL with 100 mg/kg and 300 mg/Kg respectively and decrease atherogenic index which is an indicator to decrease the atherosclerosis risks. Increase HDL to 25% with 100 mg/Kg dose with no effect with the dose 300 mg/kg. 10 mg/Kg of atorvastatin which was used as a reference drug did not show effect on TC and TG. atorvastatin works by inhibiting HMG-CoA reductase, an enzyme found in liver tissue that plays a key role in

production of cholesterol in the body. While the outer source of lipid was continuous in food, may this interpret the lack effect of atorvastatin. Various mechanisms can be explained how flavonoids, which is the main component of *D. cinnabari*, decreased the lipid profile. Hyperlipidemia related to increased oxidative stress causing significant production of oxygen free radicals, which may lead to oxidative modifications in low-density lipoproteins, which present a significant function in the initiation and progression of atherosclerosis and associated cardiovascular diseases⁵. Flavonoids can act directly as scavenging some radical species, thus acting as antioxidants and inhibit the progression of atherosclerosis⁴⁷.

Flavonoids can bind with bile acids. Since these resin are not absorbed, bile acids are excreted. This results in a decreasing bile acid uptake by the liver, and therefore in a higher conversion of cholesterol to bile acids in the liver⁵³. Also, flavonoids can inhibit Pancreatic cholesterol esterase enzyme, therefore limit the absorption of dietary cholesterol^{54,55,53} then reduce the solubility of cholesterol in micelles which may result in delayed cholesterol absorption^{56,54}. The histopathological finding of liver showed an enhancement in some parts of the tissue, because of the effect of the extract in reducing blood lipid. By the way, long-term treatment is required for regeneration the whole tissue.

CONCLUSIONS

In conclusion, ethanolic DCBR extract has demonstrated significant antidiabetic and antihyperlipidemic activity. This effect is dose and time dependent. This effect is may be due to the presence of flavonoids in the whole extract, which are working by different mechanisms. However, further studies are recommended to isolate and elucidate the bioactive compound(s) responsible for its antidiabetic

and antihyperlipidemic activity and its molecular mechanism of action.

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AUTHOR'S CONTRIBUTION

Al-Baoqai N: writing original draft, conceptualization, methodology, investigation. **Al-Mahbashi H:** writing, review, and editing, supervision, resources. **Al-Adhal A:** writing, review, and editing. All authors revised the article and approved the final version.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

None to declare.

REFERENCES

- Barik R, Jain S, Qwatra D, Joshi A, Tripathi GS, Goyal R. Antidiabetic activity of aqueous root extract of *Ichnocarpus frutescens* in streptozotocin-nicotinamide induced type-II diabetes in rats. *Indian J Pharmacol* 2008; 40(1):19–22. <https://doi.org/10.4103/0253-7613.40484>
- Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004; 27(5): 1047–53. <https://doi.org/10.2337/diacare.27.5.1047>
- Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res*. 2010; 107(9): 1058–70. <https://doi.org/10.1161/circresaha.110.223545>
- Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HAW. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med* 2008; 359(15):1577–89. <https://doi.org/10.1056/NEJMoa0806470>
- Mishra PR, Panda PK, Chowdary KA. Evaluation of acute hypolipidemic activity of different plant extracts in Triton Wr-1339 I induced hyperlipidemia in albino rats 2011; 934:925–934. <https://doi.org/10.1016/j.apjtm.2016.03.038>
- Rosenthal RL. Effectiveness of altering serum cholesterol levels without drugs. *Proc (Bayl Univ Med Cent)* 2000; 13(4):351–5. <https://doi.org/10.1080/08998280.2000.11927704>
- Katzung B, Masters S, Trevor A. *Basic and Clinical Pharmacology*. 2012; 12:770–772.
- Tang TY, Li FZ, Afseth J. Review of the regulations for clinical research in herbal medicines in USA. *Chin J Integr Med* 2014; 20(12): 883–93. <https://doi.org/10.1007/s11655-014-2024-y>
- Gupta RK, Kesari AN, Murthy PS, Chandra R, Tandon V, Watal G. Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of *Annona squamosa* L. in experimental animals. *J Ethnoph* 2005; 99(1):75–81. <https://doi.org/10.1016/j.jep.2005.01.048>
- Ahmed D, Kumar V, Verma A, Shukla GS, Sharma M. Antidiabetic, antioxidant, antihyperlipidemic effect of extract of *Euryale ferox* salisb. with enhanced histopathology of pancreas, liver and kidney in streptozotocin induced diabetic rats. *Springerplus* 2015; 4: 315. <https://doi.org/10.1186/s40064-015-1059-7>
- Bellakhdar J. La pharmacopée Marocain Traditionnelle. Médecin Arabe Ancien et *Savoirs populaires*, Ibis Prés, Paris, in *Revue d'histoire de la pharmacie*, Persée - Portail des revues scientifiques en SHS 1997;86:764. <https://doi.org/10.22270/ujpr.v3i5.194>
- Milburn M. Dragon's blood in east and west Africa, Arabian and The Canary Islands. *Africa (Lond)* 1984; 39: 486–493. <https://doi.org/10.1016/j.jep.2007.10.018>
- Stern WT. *Dictionary of Plant Names for Gardeners*. London: Cassell Publishers Ltd., 1992.
- Mothana RAA, Mentel R, Reiss C, Lindequist U. Phytochemical screening and antiviral activity of some medicinal plants from the island Soqatra. *Phytother Res* 2006; 20(4):298–302. <https://doi.org/10.1002/ptr.1858>
- Vachálková A, Novotný L, Nejedlíková M, Suchý V. Potential carcinogenicity of homoisoflavonoids and flavonoids from *Resina sanguinis draconis (Dracaena cinnabari* Balf.). *Neoplasma* 1995; 42(6):313–6. PMID: 8592573
- Liu X. Effects of dragon ? blood resin and its component loureirin B on tetrodotoxin-sensitive voltage-gated sodium currents in rat dorsal root ganglion neurons. *Sci China Ser. C* 2004; 47(4):340. <https://doi.org/10.1360/03yc0146>
- Juránek I, Suchý V, Stará D, Mašterova I, Grancaiová Z. Antioxidative activity of homoisoflavonoids from *Muscari racemosum* and *Dracaena cinnabari*. *Pharmazie* 1993; 48(4): 310–1. PMID: 8321885
- Alwashli A, Alaoui K, Al-Sobarry M. Anti-inflammatory and Analgesic effects of ethanolic extract of *Dracaena cinnabari* Balf as endemic plant in Yemen. *Int J Pharma Bio Sci* 2012; 3(2): 96–106. <https://doi.org/10.1.1.587.1239>
- Mohammed YHE, Khanum SA. Anti-Diabetic Activity of *Dracaena cinnabari* Balf Extracts from Resin in Socotra Island-Yemen. *J Plant Biochem Physiol* 2016; 4:1. <https://doi.org/10.4172/2329-9029.1000162>
- Prince PSM, Menon VP, Pari L. Hypoglycaemic activity of *Syzgium cumini* seeds: Effect on lipid peroxidation in alloxan diabetic rats. *J Ethnopharmacol* 1998; 61(1):1–7. [https://doi.org/10.1016/S0378-8741\(98\)00002-6](https://doi.org/10.1016/S0378-8741(98)00002-6)
- Dhandapani S, Subramanian VR, Rajagopal S, Namasivayam N. Hypolipidemic Effect of *Cuminum cyminum* L. On Alloxan-Induced Diabetic Rats. *Pharmacol Res* 2002; 46(3):251–255. [https://doi.org/10.1016/S1043-6618\(02\)00131-7](https://doi.org/10.1016/S1043-6618(02)00131-7)
- El-Hilaly J, Tahraoui A, Israili ZH, Lyoussi B. Hypolipidemic effects of acute and sub-chronic administration of an aqueous extract of *Ajuga iva* L. whole plant in normal and diabetic rats. *J Ethnopharmacol* 2006; 105(3): 441–8. <https://doi.org/10.1016/j.jep.2005.11.023>
- Gu HJ, Lv JC, Yong KL, Chen X, Liu PP, Zhang XB. Antidiabetic effect of an active fraction extracted from dragon's blood (*Dracaena cochinchinensis*). *J Enzyme Inhib Med Chem* 2009; 24(1):136–9. <https://doi.org/10.1080/14756360801936399>
- Datta A, Bagchi C, Das S, Mitra A, De Pati A, Tripathi SK. Antidiabetic and antihyperlipidemic activity of hydroalcoholic extract of *Withania coagulans* Dunal dried fruit in experimental rat models. *J Ayurveda Integr Med* 2013; 4(2):99–106. <https://doi.org/10.4103/0975-9476.113880>
- Mohan H. Harsh Mohan - *Pathology Practical Book, 2nd Edition.pdf* - Google Drive. *The Indian J Pathol Micro* 2007.
- Sharaf K, Ali J Hypolipidemic effect of Kuub (*Gundelia tournefortii* A.) oil and clofibrate on lipid profile of atherosclerotic rats. *Vet Arh* 2004; 74(5): 359–369. <https://doi.org/10.3923/pjbs.2013.1238.1247>
- Gosain S, et al. Hypolipidemic effect of ethanolic extract from the leaves of *Hibiscus sabdariffa* L. in hyperlipidemic rats. *Acta Pol Pharm* 2010; 67(2):179–84. PMID: 20369795
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in

- plasma, without use of the preparative ultracentrifuge. Clin. Chem 1972; 18(6):499–502.
<https://doi.org/10.1093/clinchem/18.6.499>
29. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia 2008; 51(2):216–26.
<https://doi.org/10.1007/s00125-007-0886-7>
 30. Dhanabal SP, Raja MKMM, Ramanathan M, Suresh B. Hypoglycemic activity of *Nymphaea stellata* leaves ethanolic extract in alloxan induced diabetic rats. Fitoterapia. 2007; 78(4): 288–91.
<https://doi.org/10.1016/j.fitote.2007.02.009>
 31. F. Chen, H. Xiong, J. Wang, X. Ding, G. Shu, Z. Mei, Antidiabetic effect of total flavonoids from *Sanguis draxonis* in type 2 diabetic rats. J Ethnopharmacol 2013; 149(3): 729–36. <https://doi.org/10.1016/j.jep.2013.07.035>
 32. Al-Qattan K, Thomson M, Ali M. Garlic (*Allium sativum*) and ginger (*Zingiber officinale*) attenuate structural nephropathy progression in streptozotocin-induced diabetic rats. E Spen Eur E J Clin Nutr Metab 2008; 3(2):e62–e71.
<https://doi.org/10.1016/j.eclnm.2007.12.001>
 33. Gupta D, Bleakley B, Gupta RK. Dragon's blood: Botany, chemistry and therapeutic uses. J Ethnopharmacol 2007; 115:361–380. <https://doi.org/10.1016/j.jep.2007.10.018>
 34. Fan JY, et al. A systematic review of the botanical, phytochemical and pharmacological profile of *Dracaena cochinchinensis*, a plant source of the ethnomedicine 'dragon's blood. Molecules 2014;19(7):10650–69.
<https://doi.org/10.3390/molecules190710650>
 35. Choi JS, Yokozawa T, Oura H. Improvement of hyperglycemia and hyperlipemia in streptozotocin-diabetic rats by a methanolic extract of *Prunus davidiana* stems and its main component, prunin. Planta Med 1991; 57(3):208–11. <https://doi.org/10.1055/s-2006-960075>
 36. Kamalakkannan N, Prince PSM. Antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid, in streptozotocin-induced diabetic wistar rats. Basic Clin. Pharmacol Toxicol 2006; 98(1): 97–103.
https://doi.org/10.1111/j.1742-7843.2006.pto_241.x
 37. Jung UJ, Lee MK, Jeong KS, Choi MS. The hypoglycemic effects of hesperidin and naringin are partly mediated by hepatic glucose-regulating enzymes in C57BL/KsJ-db/db mice. J Nutr 2004; 134(10):2499–503.
<https://doi.org/10.1093/jn/134.10.2499>
 38. Kawabata J, Mizuhata K, Sato E, Nishioka T, Aoyama Y, Kasai T. 6-hydroxyflavonoids as alpha-glucosidase inhibitors from marjoram (*Origanum majorana*) leaves. Biosci Biotechnol Biochem 2003; 67(2):445–7.
<https://doi.org/10.1271/bbb.67.445>
 39. Andrade-Cetto A, Wiedenfeld H. Hypoglycemic effect of *Cecropia obtusifolia* on streptozotocin diabetic rats. J Ethnopharmacol 2001; 78(2–3):145–9.
[https://doi.org/10.1016/S0378-8741\(01\)00335-X](https://doi.org/10.1016/S0378-8741(01)00335-X)
 40. Matsuda H, Nishida N, Yoshikawa M. Antidiabetic principles of natural medicines. V. Aldose reductase inhibitors from *Myrcia multiflora* DC. (2): Structures of myrciacitrins III, IV, and V. Chem Pharm Bull (Tokyo) 2002; 50(3):429–31. <https://doi.org/10.1248/cpb.50.429>
 41. Narvaez-Mastache JM, Garduño-Ramírez ML, Alvarez L, Delgado G. Antihyperglycemic activity and chemical constituents of *Eysenhardtia platycarpa*. J Nat Prod 2006; 69(12):1687–91. <https://doi.org/10.1021/np060166z>
 42. Kim HY, Moon BH, Lee HJ, Choi DH. Flavonol glycosides from the leaves of *Eucommia ulmoides* O. with glycation inhibitory activity. J Ethnopharmacol 2004; 93(2–3):227–30. <https://doi.org/10.1016/j.jep.2004.03.047>
 43. Pinent M, Castell A, Baiges I, Montagut G, Arola L, Ardévol A. Bioactivity of flavonoids on insulin-secreting cells. Compr Rev Food Sci Food Saf 2008; 7(4):299–308.
<https://doi.org/10.1007/s11130-016-0571-4>
 44. Distefano JK, Watanabe RM. Pharmacogenetics of anti-diabetes drugs. Pharmaceuticals (Basel) 2010; 3(8):2610–2646. <https://doi.org/10.3390/ph3082610>
 45. Johansen JS, Harris AK, Rychly DJ, Ergul A. Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. Cardiovasc Diabetol 2005;4: 5.
<https://dx.doi.org/10.1186%2F1475-2840-4-5>
 46. Abu-Taleb Y, Alzowahi F, Tukaram K, Shaikh R. *In vitro* evaluation of antimicrobial and antioxidant activity of Dragon's blood tree (*Dracaena cinnabari* Balf) of Socotra Island (Yemen). J Coast Life Med 2013; 123–129.
<https://doi.org/10.1186/1472-6882-11-13>
 47. Gupta D, Gupta RK. Bioprotective properties of Dragon's blood resin: *in vitro* evaluation of antioxidant activity and antimicrobial activity. BMC Complement Altern Med. 2011; 11(1):13. <https://doi.org/10.1186/1472-6882-11-13>
 48. Pietta PG. Flavonoids as antioxidants. J Nat Prod 2000; 63(7):1035–42. <https://doi.org/10.1021/np9904509>
 49. Koffi N, Ernest A, Dodiomon S. Effect of aqueous extract of *Persea Americana* Seeds on the glycemia of diabetic rabbits. Eur J Sci Res 2009; 26(3):376.
 50. Yasir M, Das S, Kharya MD. The phytochemical and pharmacological profile of *Persea Americana* Mill. Pharmacogn Rev 2010; 4(7):77–84.
<https://doi.org/10.4103/0973-7847.65332>
 51. Liu H, Guz Y, Kedees MH, Winkler J, Teitelman G. Precursor cells in mouse islets generate new beta-cells *in vivo* during aging and after islet injury. Endocrinol 2010; 151(2):520–8. <https://doi.org/10.1210/en.2009-0992>
 52. Syiem D, Syngai G, Khup PZ, Khongvir BS, Kharbuli B, Kayang H. Hypoglycemic effects of *Potentilla fulgens* L in normal and alloxan-induced diabetic mice. J Ethnopharmacol 2002; 83(1–2):55–61.
[https://doi.org/10.1016/S0378-8741\(02\)00190-3](https://doi.org/10.1016/S0378-8741(02)00190-3)
 53. Tania O, Kelly R, Márcia A, Marcelo C, Tanus N. Hypolipidemic Effect of flavonoids and cholestyramine in rats. Lat Am J Pharm 2007; 26 (3):407–10.
 54. Ngamukote S, Mäkynen K, Thilawech T, Adisakwattana S. Cholesterol-lowering activity of the major polyphenols in grape seed. Molecules 2011; 16(6):5054–61.
<https://doi.org/10.3390/molecules16065054>
 55. Sivashanmugam T, et al. Discovery of potential cholesterol esterase inhibitors using *in silico* docking studies. Bangladesh J Pharmacol 2013; 8(3):223–229.
<https://doi.org/10.3329/bjp.v8i3.14521>
 56. Chávez-Santoscoy RA, Gutiérrez-Urbe JA, Serna-Saldívar SO. Effect of flavonoids and saponins extracted from black bean (*Phaseolus vulgaris* L.) seed coats as cholesterol micelle disruptors. Plant Foods Hum Nutr 2013; 68(4): 416–23. <https://doi.org/10.1017/S0007114514001536>