

IN VITRO EVALUATION OF HYPOLIPIDEMIC EFFECT OF EXTRACTS OF MEDICINAL *DRACAENA CINNABARI* BALF. F. RESIN**YASSER HUSSEIN EISSA MOHAMMED^{1,2}, DEEPIKA HS¹, FARES HEZAM AL-OSTOOT^{1,3}, ZABIULLA¹, ANILAKUMAR⁴, SHAUKATH ARA KHANUM^{1*}**

¹Department of Chemistry, Yuvaraja's College, University of Mysore, Mysuru, Karnataka, India. ²Department of Biochemistry, Faculty of Applied Science College, University of Hajjah, Hajjah, Yemen. ³Department of Biochemistry, Faculty of Education and Science, Al-Baydha University, Albaydha, Yemen. ⁴Applied Nutrition Division, Defence Food Research Laboratory, Mysuru, India. Email: shaukathara@yahoo.co.in

Received: 09 March 2019, Revised and Accepted: 03 August 2019

ABSTRACT

Objective: The objective of the study was to *in vitro* evaluate of hypolipidemic effect of extracts of medicinal *Dracaena cinnabari* Balf. f. resin.

Methods: About 800 g of dry powder of the resin of dracaena cinnabar was taken in a Soxhlet apparatus and subjected for sequential extraction of solvents from non-polar to polar end (hexane, benzene, diethyl ether, dichloromethane, chloroform, ethyl acetate, acetone, ethanol, methanol, and water); the extract samples were kept at 4°C for further assays. All the extracts were subjected to glucose uptake assay.

Results: The ethanol extract showed significant ($p < 0.05$) hypolipidemic effect by decreasing the activity of enzyme such as significant reduction in the pancreatic lipase enzyme, malic dehydrogenase enzyme, and glucose-6-phosphate dehydrogenase enzyme with $IC_{50} \sim 13$, ~ 13 , and ~ 14 , respectively. This results were similar to the standard drug atorvastatin with $IC_{50} \sim 12$, ~ 16 , and ~ 17 , respectively. Ethanol extract exhibited significant atherogenic index and percentage protection against hyperlipidemia. The potential biological activity of ethanol extract may be attributed to the highest polarity which needs further investigation.

Conclusion:

Keywords: *Dracaena cinnabari*, Enzyme inhibition, Glucose-6-phosphate dehydrogenase, Malate dehydrogenase, Soxhlet.

© 2019 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2019.v12i10.32984>

INTRODUCTION

One of the most common vital threats for the accelerated atherosclerosis and cardiovascular diseases is hyperlipidemia [1,2]. It is considered as one of the diseases which have its own problematic consequences and impact on the health of a human. This disease has a direct relation with the food system that a person adopts in his daily life. To avoid being threatened by this disease, a person can do some exercises and eating healthy food. The formation of plaques in the walls of coronary arteries is one of the characteristics of atherosclerosis by reducing the flow of blood, leading to coronary artery disease. Hypercholesterolemia contributes to the increased prevalence of coronary artery diseases [3]. To reduce the risk of coronary artery diseases, so many studies were conducted and most of them stated that lowering of serum cholesterol is one of those beneficial ways for the reduction of risk [4]. Ayurveda which is an Indian traditional medicine includes a large number of plants which are categorized as hypolipidemic. Three main pathways are responsible for the synthesis and transport of lipids within the body: Exogenous, endogenous, and reverse cholesterol transport. The pictorial representation is given in Fig. 1 [5]. Dragon's blood tree is a non-specific name for dark red resinous exudations from different plant species endemic to various regions around the globe that belongs to four genera *Dracaena* spp. [6]. A wide-ranging research over the decades on this plant indicated that it is a persuasive therapeutic agent against various diseases. Dragon's blood has been found to be safe even at very high doses in human clinical trials [7]. It has so many treatment uses, but due to the bioavailability of this tree, it has no effective therapeutic molecule [8].

MATERIALS AND METHODS**Materials**

The commercially available refined avocado oil was used for extraction from Sigma Chemical Company, USA. Other chemicals used in the studies were of analytical grade and purchased from SDFCL, Mumbai, India.

The enzyme, NADP-malic enzyme (EC 1.1.1.40) was obtained from Sigma-Aldrich, Bengaluru. The other enzymes glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and pancreatic lipase (EC 3.1.1.3) were purchased from SRL, India.

Methods*Collection of plant material*

The resin of dragon's blood tree (*Dracaena cinnabari*) was brought from the origin of this tree from Socotra Island, Republic of Yemen. It has a matchless and strange appearance, described as upturned, densely packed crown having the shape of an umbrella [4].

Preparation of extracts

About 800 g of dry powder of the resin of dracaena cinnabar was taken in a Soxhlet apparatus and subjected for sequential extraction of solvents from non-polar to polar end (hexane, benzene, diethyl ether, dichloromethane, chloroform, ethyl acetate, acetone, ethanol, methanol, and water); the extract samples were kept at 4°C for further assays. All the extracts were subjected to glucose uptake assay [7].

Inhibition of pancreatic lipase activity

The hypolipidemic ability of plant extracts was tested by assessing the pancreatic lipase inhibition assay [9]. Plant extracts were individually incubated with 1 ml of reaction mixture containing 1 unit of lipase enzyme and 100 mM phosphate buffer (pH 7.2) with Triton-X-100 (0.5%). The enzyme reaction was started by adding p-nitrophenyl butyrate (5 mM in acetonitrile) and monitored at 340 nm. A control tube was maintained and the result was expressed as percent activity.

Inhibition of glucose-6-phosphate dehydrogenase activity

Glucose-6-phosphate dehydrogenase activity was measured by monitoring reduction of NADP at 340 nm [10]. Plant extracts were

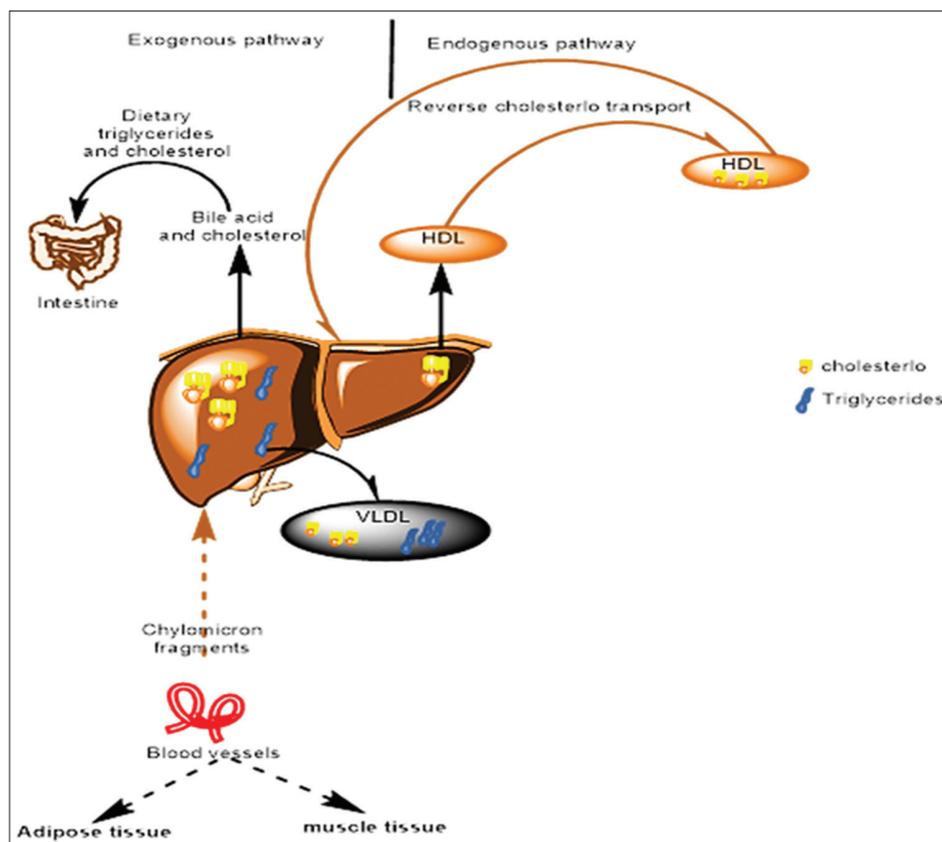


Fig. 1: Pathways of lipid metabolism

Table 1: Inhibitory concentration of plant extracts at 25–75 μM concentrations

Sample description parts solvent	Concentrations 25–75 μM	Pancreatic lipase (μM)	Malic dehydrogenase (μM)	Glucose-6-phosphate dehydrogenase (μM)
Hexane	25	35.3 0.01	30.3±0.07	40.1±0.14
	50	30.2±0.07	29.6±0.05	31.3±0.01
	75	25.0±0.17	22.1±0.07	26.6±0.07
Benzene	25	55.2±1.12	50.4±0.12	66.1±0.14
	50	45.2±0.02	50.9±6.02	72.4±2.09
	75	50.2±2.00	44.2±1.00	62.1±0.03
Diethyl ether	25	41.9±0.17	70.0±0.11	52.5±0.01
	50	33.9±0.05	63.0±1.4	42.5±0.09
	75	31.9±3.07	55.0±0.21	45.1±1.50
Chloroform	25	48.9±9.00	69.0±0.20	55.9±0.10
	50	45.2±0.01	52.2±0.21	53.1±3.00
	75	39.9±0.11	49.9±0.40	50.0±0.8
Ethyl acetate	25	38.2±0.20	44.4±0.01	49.0±1.07
	50	36.9±2.00	41.6±9.01	44.9±1.20
	75	29.2±0.09	39.1±2.00	41.5±0.06
Dichloromethane	25	35.1±1.00	48.1±1.30	43.2±0.02
	50	33.0±0.01	41.0±0.03	39.9±0.08
	75	30.6±6.00	42.9±9.00	36.0±1.02
Acetone	25	35.3±0.01	36.6±0.25	31.0±0.21
	50	28.9±9.11	34.2±2.05	30.9±5.00
	75	25.0±2.00	31.8±0.20	30.0±0.01
Ethanol	25	14.2±1.10	17.1±0.05*	17.4±0.04*
	50	13.0±1.05*	12.1±1.00	14.0±0.02*
	75	12.9±0.02*	11.1±0.05*	13.9±0.04*
Methanol	25	29.4±0.02	29.9±0.08	30.1±0.032
	50	24.0±9.00	24.3±0.02	27.4±0.09
	75	23.0±0.09	24.4±0.11	24.9±0.12
Water	25	35.1±0.07	31.9±0.09	30.2±1.00
	50	31.8±1.02	29.2±0.82	23.3±0.07
	75	31.0±9.00	27.0±0.08	20.1±4.07
Standard atorvastatin	25	13.1±0.10	17.1±0.09	20.1±0.20
	50	13.0±1.10	19.1±0.01**	17.0±0.22
	75	12.1±9.09	14.1±1.00	16.4±1.00

Statistically significant values are expressed as *p<0.05 and **p<0.01

individually incubated with 1 ml of reaction mixture containing 0.5 units of enzyme, 50 mM tris HCl (pH 7.4), and 0.1 mM glucose-6-phosphate. The enzyme reaction was started by adding 0.15 mM NADP and monitored at 340 nm. A control tube was maintained and the result was expressed as percent activity.

Inhibition of malic dehydrogenase activity

Malic dehydrogenase activity was measured by monitoring reduction of NADP at 340 nm [11,12]. Plant extracts were incubated with 1 ml of tris-HCl (100 mM, pH 7.4) containing 0.5 unit of malic dehydrogenase enzyme, L-malate (10 mM), and MnCl₂ (2 mM). The enzyme reaction was started by adding 2 mM NADP and monitored at 340 nm. A control tube was maintained and the result was expressed as percent activity.

Statistical analysis

Values were expressed as mean \pm standard deviation. MS Excel 8.1 version software was used for data analysis; statistical significance was evaluated by one-way analysis of variance followed by two-tailed Student's t-test. Statistically significant values were expressed as *p<0.05 and **p<0.01.

RESULTS AND DISCUSSION

Hypolipidemic decrease by the effect of the pancreatic lipase, malic dehydrogenase, and glucose-6-phosphate dehydrogenase enzymes

The enzyme inhibition studies of the glucose-6-phosphate dehydrogenase were evaluated at different concentrations (25, 50, and 75 μ M) of the resin of dragon's blood. It was noticed that while performing the test for the enzyme inhibition of the glucose-6-phosphate dehydrogenase, the other extracts showed moderate and insignificant effects compared to the ethanol extract which showed significant hypolipidemic effect by decreasing the activity of enzyme with IC₅₀ ~13. Furthermore, the significant effect of ethanol extract was obviously noticed by reduction in the pancreatic lipase enzyme with IC₅₀ ~13 and these results gave us motivation to pursue our work. Compared to the previous investigations, the malic dehydrogenase enzyme was also reduced by the effect of ethanol extract with IC₅₀ ~14. It was proved that the results of this investigation were similar to the standard drug atorvastatin with IC₅₀ ~12, ~16, and ~17, respectively. Ethanol extract exhibited significant atherogenic index and percentage protection against hyperlipidemia (Table 1). The potential biological activity of ethanol extract may be attributed to the highest polarity which needs further investigation.

CONCLUSION

This study of the extraction of *D. cinnabari* Balf. f. was conducted to estimate hypoglycemic effects of the extracts by the inhibition assays of the enzymes pancreatic lipase, glucose-6-phosphate dehydrogenase, and malate dehydrogenase. All the assessments performed for the extracts exhibited hypolipidemic activity by attenuating the activity of the enzymes studied. Ethanol extract was found to be a more potent enzyme inhibitor. From our present investigations, ethanol extract could be concluded as promising hypolipidemic agent.

ACKNOWLEDGMENTS

Yasser Hussein Eissa Mohammed is thankful to the University of Hajja, Yemen, for providing financial assistance under the teacher's fellowship. Fares Hezam Al-Ostoot is thankful to Al-Baydha University, Yemen, for providing financial assistance under the teacher's fellowship. Zabiulla gratefully acknowledges the financial support provided by the Department of Science and Technology, New Delhi, under INSPIRE-Fellowship scheme (IF140407). Shaukath Ara Khanum expresses sincere gratitude to the Government of Karnataka, Vision Group on Science and Technology, Bangalore, for the financial assistance and support (VGST/CISEE/2012-13/282 dated March 16, 2013) and UGC, New Delhi, under the Major Research Project Scheme (F.39/737/2010 [SR]). Authors are also thankful to Director, Defence Food Research Laboratory, Mysuru, India, for support to carry out the analytical work.

AUTHORS' CONTRIBUTIONS

The author declares that this work was done by the authors named in this article.

CONFLICTS OF INTEREST

No conflicts of interest are associated with this work.

REFERENCES

1. Chouhan H, Purohit A. The protective effect of cumin (*Cuminum cyminum* L.) seed extract on cardiovascular system toxicity and hematology on hyperlipidemic rabbits: An experimental study. *Asian J Pharm Clin Res* 2018;11:353-6.
2. Subashini S, Kripa Kg, Pugalendi Kv. Hypolipidemic potential of methanolic extract of *Gracilaria corticata* on streptozotocin-induced diabetic rats. *Asian J Pharm Clin Res* 2017;10:402-5.
3. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation* 2002;105:1135-43.
4. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: The scandinavian simvastatin survival study (4S). *Lancet* 1994;344:1383-9.
5. Patwardhan B, Vaidya AD, Chorghade M. Ayurveda and natural products drug discovery. *Curr Sci* 2004;86:789-99.
6. Mohammed YH, Khanum SA. Anti-diabetic activity of *Dracaena cinnabari* Balf.f extracts from resin in Socotra Island-Yemen. *J Plant Biochem Physiol* 2016;4:162.
7. Mohammed YH. *In vitro* anti-cancer activity of extracts *Dracaena cinnabari* Balf.f resin from Socotra Island in Yemen republic. *Biochem Anal Biochem* 2016;5:296.
8. Su J, Wang H, Ma C, Liu C, Rahman MT, Gao C, et al. Hypolipidemic mechanism of gypenosides via inhibition of pancreatic lipase and reduction in cholesterol micellar solubility. *Eur Food Res Technol* 2016;242:305-12.
9. Puneeth HR, Ananda H, Kumar KS, Rangappa KS, Sharada AC. Synthesis and antiproliferative studies of curcumin pyrazole derivatives. *Med Chem Res* 2016;25:1842-51.
10. Xu Y, Osborne BW, Stanton RC. Diabetes causes inhibition of Glucose-6-phosphate dehydrogenase via activation of PKA, which contributes to oxidative stress in rat kidney cortex. *Am J Physiol Renal Physiol* 2005;289:F1040-7.
11. Roobol A, Alleyne GA. A study of stabilization of gluconeogenic activity in rat liver slices by calcium and manganese ions. *Biochem J* 1972;129:231-9.
12. Kong C, Nimmo L, Elatrozy T, Anyaoku V, Hughes C, Robinson S, et al. Smoking is associated with increased hepatic lipase activity insulin resistance dyslipidaemia and early atherosclerosis in Type 2 diabetes. *Atherosclerosis* 2001;156:373-8.