International Journal of Food Science and Nutrition
ISSN: 2455-4898
Impact Factor: RJIF 5.14
www.foodsciencejournal.com
Volume 3; Issue 5; September 2018; Page No. 39-43

Cardio protective activity of *Abelmoschus esculentus* (Okra)

Vindika Sumudunie Kuruwitaarachchige[1], Deepthi Inoka Uluwaduge[2*], Sirimal Premakumara[3], Jayantha Wijayabandara[4]
[1] Faculty of Graduate Studies, University of Sri Jayewardenepura, Nugegoda, 10250, Sri Lanka
[2, 4] Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, 10250, Sri Lanka
[3] Industrial Technology Institute, Colombo 07, 00700, Sri Lanka

Abstract
Okra or Lady's fingers (*Abelmoschus esculentus*) is a popular vegetable of the Malvaceae family. Several pharmaceutical benefits of okra fruit have been documented. This study investigated the effect of mucilage of okra (crude water extract and water fraction) fruit on lipid parameters in a high- fat diet fed rats.
Okra crude water extract (500 and 1000 mg/kg body weight) or water fraction (50 and 100 mg/kg body weight) was provided with a high fat diet to hypercholesterolemic rats for one week. The effect of treatment of okra fruit on lipid parameters of hypercholesterolemic rats were evaluated and compared with the negative control and positive control rats.
Crude water extract (1000 mg) and the water fraction of okra (50 mg and 100 mg) had the potential to reduce ($p< 0.01$) different lipid fractions (total cholesterol, triglycerides, LDL and VLDL) and atherogenic index in the test group. The mucilage had the potential to increase the HDL fraction ($p< 0.01$) of the test group.
These results suggest that crude water extract and the water fraction of the okra fruit modulate the blood lipid levels favorably and have the potential to be used as a "heart friendly" vegetable.

Keywords: Okra, *Abelmoschus esculentus*, lipid lowering, hypercholesterolaemia

Introduction
According to the World Health Organization (WHO) reports in 2017 Cardio Vascular Diseases (CVDs) are the main cause of death in most of the countries. It has been estimated that 17.7 million people died from CVDs in 2015, representing 31% of all global deaths [1]. The underlying primary cause of CVD is believed to be arteriosclerosis, a progressive multifactorial disease of the arterial wall. Central to the pathogenesis of arteriosclerosis is deposition of cholesterol in the arterial wall [2]. Drug management of hypercholesterolaemia without associated untoward effect has remained a challenge for orthodox medical practice. This has necessitated exploration and screening of plant based products with hypocholesterolaemic activity. Of the array of such plants with hypocholesterolaemic potentials are *Abelmoschus esculentus* which the lipid lowering ability is not scientifically validated and documented into a greater extent. It is valued for its edible green seed pods which serve as a delicious vegetable worldwide.
A*belmoschus esculentus* L. (Moench) or okra (Synonym; *Hibiscus esculentus*, Family: Malvaceae, Sinhala: Bandakka, English: Lady's fingers, Tamil: Shabdkosh) is a flowering plant and is cultivated throughout the tropical, sub-tropical and warm temperate regions around the world [3, 4].
Okra is widely used in ethno medicine in diverse cultures [5, 6, 7]. Different parts of the plant are employed in the treatment of human diseases throughout the world. It has been documented that the infusion of the fruit mucilage has relieving effect on dysentery in acute inflammation and irritation of the stomach [8]. Antihyperglycemic effect of okra has been widely studied by several workers during the recent past. A significant reduction in blood glucose levels of diabetic rats on administration of peel and seed powder of the fruit of okra has been reported by Sabitha *et al*., [6]. On administering the water soluble fraction of fruit of okra, a significant reduction in glucose absorption was noted by Khatun *et al*., [9]. Further it was revealed that the presence of two major flavonioid glucosides [isoquercetin and quercetin-3-O-beta-glucopyranosyl-(1-6) glucoside] in okra seeds exhibit the α-glucosidase inhibitory activity and this may responsible for the reduction in blood glucose in experimental rats [10]. The effect of okra mucilage on the plasma cholesterol level in rats were reported in 1977 [11]. The fruit of okra is widely consumed in Africa and investigated as a potential candidate to decrease cholesterol [12]. The extracts from total plant by dichloromethane or methanol have shown the hypolipidemic activity in tyloxapol-induced hyperlipidemic mice [13].
Scientific literature does not provide in depth details on the lipid lowering potential of fruit of okra despite its wide use as a vegetable in many countries. Therefore the present study was focused on the effect of mucilage of okra fruit (crude extract and its water fraction) on serum total cholesterol and other lipid components (triglycerides, VLDL, LDL and HDL) of Wistar rats.
In the most commonly used method for testing the hypocholesterolaemic effect of unknown compounds, the experimental animals are made hypercholesterolemic by feeding them with cholesterol mixed with the diet. The high cost of pure cholesterol, is a major drawback in this type of animal model. Therefore our research group developed a

modified cost effective hypercholesterolemic rat model by feeding low cost butter incorporated rat feed pellets.

2. Material and methods

Collection of plant materials

The fruits of A*belmoschus esculentus* were obtained from the local market of Kelaniya, Sri Lanka. A specimen of the plant and fruit was deposited in the National Herbarium, Department of National Botanic Gardens, Peradeniya, Sri Lanka, after identification of the plant by a botanist (voucher No. 6/01/H/03).

Aqueous crude extraction procedure

The fresh pods were washed in running tap water and heated for 10-15 minutes at about 60˚C in a clay pot. The mixture was filtered using a thin layer of cotton to remove the insoluble matters and mucilaginous filtrate was collected. Water extract (filtrate) was freeze-dried to obtain a dry sample. The dry residue was weighed and stored in air and water proof container and kept in a refrigerator at 4˚C. From this stock further experiments were conducted.

Solvent fractionation from the crude water extract

Freeze- dried water extract was successively fractionated with hexane, dichloromethane and ethyl acetate. The liquid fractions were concentrated under vacuum to yield dry powder. The highest yield was observed with the aqueous (water) fraction remaining after successive extractions with organic solvents.

Experimental animals and their management

After obtaining the permission from the Ethics Review Committee of the Faculty of Medical Sciences (approved Ethical Review Committee of SIDCER, Strategic Initiative for Developing Capacity in Ethical Review), University of Sri Jayewardenepura, Sri Lanka, experiments were conducted on male Wistar rats (obtained from Medical Research Institute, Sri Lanka) weighing approximately 250-280 g (protocol approval No. 538/11). The rats were allowed one week of acclimatization under standard laboratory conditions. The rats were maintained on standard rat feed and water *ad libitum* during this period.

Induction of hypercholesterolaemia in experimental animals

Hypercholesterolaemia was induced by feeding WHO recommended rat feed pellets prepared by incorporating butter (10%). The WHO standard feeding formula was obtained from the Medical Research Institute (MRI), Sri Lanka [14]. Induction of hypercholesterolaemia was verified after eight weeks of the feeding trial by measuring fasting cholesterol levels. These hypercholesterolemic rats were used as the positive control and the test groups where as normal healthy rats were used as the negative control.

Hypercholesterolaemia was confirmed when the fasting serum cholesterol levels of the test rats were significantly higher ($p<0.05$, 95.7±23.0 and 128.3±5.5 in control and test groups respectively) when compared with the control rats after a feeding trial of eight weeks.

Experimental design

All the animals were weighed and divided into following groups of six each. Doses were determined by considering the daily intake of okra by an adult human subject (100 g/75 kg body weight) and dry extract powder of the crude water extract or aqueous fraction in milligram (mg) to the body weight (kg) of rats. Conversion of human dose to experimental rat dose was achieved by the method described by Dhawan and Srimal [15]. The oral administration was done by using Sondi needles. Freeze - dried powder of the crude water extract or water fraction were dissolved in distilled water to make the extractives for oral feeding.

Determination of the hypocholesterolaemic effect of the crude water extract of okra on cholesterol induced rats

***Negative control 1*:** Normal healthy rats fed on WHO recommended rat feed pellets.

***Positive control 1*:** These animals were chosen from the hypercholesterolemic rats and were fed butter containing feed pellets (hypercholesterolemic diet) continuously for another one week.

***Test group 1*:** Animals of this group (hypercholesterolemic rats) were fed hypercholesterolemic diet and crude water extract of the okra at a dose of 500 mg/kg body weight for one week.

***Test group 2*:** Animals of this group (hypercholesterolemic rats) were fed hypercholesterolemic diet and crude water extract of the okra at a dose of 1000 mg/kg body weight for one week.

Determination of the hypocholesterolaemic effect of the water fraction of okra on cholesterol induced rats

***Negative control 2*:** Normal healthy rats fed on WHO recommended rat feed pellets.

***Positive control 2*:** These animals were chosen from the hypercholesterolemic rats and were fed butter containing feed pellets (hypercholesterolemic diet) continuously for another one week.

***Test group 3*:** Animals of this group (hypercholesterolemic rats) were fed hypercholesterolemic diet and water fraction of the okra at an oral dose of 50 mg/kg body weight for one week.

***Test group 4*:** Animals of this group (hypercholesterolemic rats) were fed hypercholesterolemic diet and water fraction of the okra at an oral dose of 100 mg/kg body weight for one week.

Blood collection and bioassays

Prior to termination of the experiments on day eight, the rats were fasted overnight (12-14 hours) and distilled water was made available *ad libitum*. Under aseptic precautions, using light ether anesthesia, blood samples (1.0 ml) were collected by tail vein puncture. Clear, non – haemolyzed serum was separated by centrifugation at 3000 rpm for 10 minutes using a centrifuge (Jawaki CFM-100, Japan). Total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL) and low density lipoprotein cholesterol (LDL) concentrations were determined by enzymatic assay method using analytical kits (Pointe cholesterol reagent kits, Canton, USA) while very low density lipoprotein-cholesterol (VLDL) and atherogenic index (AI) was calculated using the following formula of Friedewald *et al*., [16] and Abbot *et al*., [17] respectively.

VLDL= Triglycerides/5
Atherogenic Index (AI) = LDL cholesterol/ HDL cholesterol

Statistical analysis
Results were presented as Mean ±S.E.M for six observations and statistically analyzed using two-way analysis of variance on statistical computer soft wear program. Results were considered significant when $p < 0.01$ or 0.05.

3. Results

Effect of oral administration of crude water extract of okra on fasting serum cholesterol levels of rats.
The results are summarized in Table 2. As shown, 1000 mg/ kg body weight dose significantly reduced the fasting serum cholesterol levels of rats of the test group following oral administration of the extract compared with the positive control ($p<0.01$, percentage reduction 49.96%).

Table 1: Effect of oral administration of crude water extract of okra (500, 1000 mg/ kg body weight) on fasting serum cholesterol levels of rats (mean±SEM).

Experimental groups	Fasting serum cholesterol levels (mg/dL)	
	I	II
Negative control 1	93.6±2.2[a]	103.8±5.3[b]
Positive control 1	178.2±8.6[a,d]	190.6±10.2[b, c]
Test group 1 dose: 500 mg	173.5±10.9[d]	-
Test group 2 dose: 1000 mg	-	101.2±10.2[c]

n= 6, column I represents the serum cholesterol levels relevant to experiments conducted with test group 1 (dose, 500 mg), Column II represents the values relevant to test group 2 (dose, 1000 mg). Values are significant at, *$p<0.01$, compared with respective negative controls and positive controls [(a,b)], compared with positive control 1 and test group 2[(c)], non-significant comparison ($p>0.05$) was observed with positive control 1 and test group 1[(d)].

Effect of oral administration of water fraction of okra (50, 100 mg/ kg body weight) on lipid parameters of rats.
The results are depicted in Table 2. A significant increase ($p< 0.01$) in the level of TC, TG, LDL and VLDL were shown by the rats of the positive control group which received the high fat diet. Further they had a significantly low HDL level ($p< 0.01$).
Administration of water fraction of both doses of okra (50 mg and 100 mg) showed a significant reduction ($p< 0.01$) of TC, TG, LDL and VLDL of test groups when compared with positive control rats. Comparatively their HDL levels were higher than the positive control group. The observed lipid parameters of the okra treated groups were almost same as those values of the negative control group.
Further it was observed that the atherogenic index of the okra treated groups were comparatively less (approximately 50% reduction) when compared those values with the positive control rats.

Table 2: Effect of oral administration of water fraction of okra (50, 100 mg/ kg body weight) on lipid parameters of rats (mean±SEM).

Experimental groups	Lipid parameter					
	TC (mg/dL)	TG (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	HDL (mg/dL)	AI
Negative control 2	95.4±7.5[a]	94.65±11.9[a]	73.8±7.7[a]	25.6 ±1.8[a]	26.6 ±2.4[a]	2.8
Positive control 2	183.8±26.2 [a,b]	148.7 ±13.3[a,b]	119.7±8.7 [a,b]	31.8±3.7 [a,b]	19.71±1.6 [a,b]	6.0
Test group 3 dose: 50 mg	103.2 ±9.9[b]	108.8±12.1[b]	79.7±5.4 [b]	24.9±1.6 [b]	27.2±1.6 [b]	2.9
Test group 4 dose: 100 mg	92.9 ±7.9[b]	93.7 ±7.2 [b]	80.8±9.6 [b]	23.5±2.5 [b]	27.7±1.4 [b]	2.9

n= 6, Values of lipid parameters observed for negative control and for both test groups are significantly different (low for TC, TG, LDL, VLDL and high for HDL) at *$p<0.01$ compared with corresponding values of positive control [(a,b)].

The percentage reduction of different lipid fractions (TC, TG, VLDL and LDL) and percentage increase in HDL in okra treated group when compared with the corresponding values of the positive control is shown in table 3. The highest reduction in the corresponding lipid fractions (TC, TG, VLDL and LDL) were observed with the dose of 100 mg/ kg body weight. Greater elevation of the HDL levels was recorded with the same dose (100 mg/ kg body weight). Therefore, the most effective dose is the highest dose (100 mg/ kg body weight) though both tested doses (100 mg/ kg body weight and 50 mg/ kg body weight) are effective in modulating blood lipid levels favorably.

Table 3: Percentage reduction of TC, TG. LDL, VLDL and percentage increase in HDL: okra treated group vs. positive control

Experimental groups	% reduction				% increase
	TC	TG	LDL	VLDL	HDL
Test group 3 dose: 50 mg	43.8	26.8	33.4	21.9	37.7
Test group 4 dose: 100 mg	49.8	36.9	32.4	26.0	40.3

4. Discussion

Hyperlipidaemia is a major health problem among populations in affluent and less affluent societies alike [18, 19]. It is considered as an important risk factor for the development of atherosclerosis and subsequent cardiovascular diseases and stroke [2]. Thus most people would benefit by reducing blood cholesterol levels. Non pharmacological measures like dietary restriction and exercise may help in lowering blood cholesterol [2]. When such therapies fail, drug therapy is indicated. The available most effective drugs like fibrates,

statins and bile acid sequestrants have a spectrum of adverse effects in patients and they are costly [20]. Therefore it is important to investigate and develop novel therapeutically active anti- hypercholesterolemic agents mainly from natural herbal resources [21].

Evidence based studies suggest that the high intake of fresh fruits and vegetables have the ability to curtail a number of chronic debilitating diseases such as atherosclerosis and cancer [21, 22]. However only few reports are available to prove the hypolipidemic potential of the okra fruit [6, 13]. Therefore this study was undertaken to explore the lipid regulating power of okra.

The results clearly showed, for the first time, which the water extract of okra fruit (mucilage) possess antihyperlipidemic activity. It was noted that the crude water extract and the water fraction of okra fruit was effective in reducing plasma cholesterol levels of hypercholesterolemic rats. Further it was evident that the active anti- hypercholesterolemic compound bears polar groups since the activity was found in the water fraction. In contrast, Ngoc *et al*., [13] have observed the hypocholesterolaemic effect of methanol and dichloromethane fractions of okra plant and fruit. Possibly, more than one fraction may show the lipid regulating properties. However since there were no evidence on the active principle/s there is a great potential of further studies to elucidate the chemical nature of the active compound/s.

HDL is known as the good cholesterol since it carries cholesterol away from the peripheral tissues and drops it off at the liver [2]. The water fraction of the okra fruit was successful in elevating the blood HDL levels demonstrating the anti-atherogenic effect. The observed significant reduction in the serum TG, LDL, VLDL and atherogenic index with both doses strongly suggest the potential therapeutic efficacy of okra fruit as an antihyperlipidemic agent.

A previous investigation [6] has confirmed the antihyperlipidemic potential of okra peel and seed powder in streptozotocin induced diabetic rats. In the present study the fruit was prepared obtaining only the mucilage ensuring that the seeds and fiber containing peel is not included in the extract. Perhaps if the whole fruit is applied, a greater lipid reduction may observe as a summation of different active principles from different parts of the fruit.

The crude water extract of okra fruit had shown elevated total bilirubin levels in blood of diabetic albino rats [23]. Possibly, low blood cholesterol level and other lipid parameters are due to catabolism of cholesterol into bilirubin. Although the mechanism of action of hypolipidemic activity of okra remains speculation until it is subjected to further scientific validation.

In the present study boiled edible portion of the okra fruit has used to simulate the cooking method of this vegetable. In Sri Lankan culinary the fruit is prepared into different recipes. Cooking the fruit with coconut milk after the addition of spices, frying in oil with spices, made into soups are some recipes and there are many other methods which may adopt in other countries. Since the green pod is used by many parts of the world as a day today dish, the therapeutic use of the vegetable can be applied widely even on a daily basis. Currently research is going on to justify the antihyperlipidemic effect of fruit of okra on human subjects and to validate its therapeutic efficacy.

5. Conclusions

The study justified that the crude water extract and the crude water fraction of fruit of okra possesses significant lipid regulating ability in cholesterol induced rats and therefore could have the potential to be used as a “heart friendly” vegetable.

Acknowledgements

The authors are grateful to the staff of the Animal House, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka for the technical assistance.

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

References

1. Cardiovascular diseases (CVDs), Fact sheet WHO. 2017; [http://www.who.int/mediacentre/factsheets/fs317/en/. Accessed 10 May 2018].
2. Kumar P, Clark M Kumar. and Clark's Clinical Medicine, 9th ed. Elsevier Saunders; 2016.
3. Lamont W. Okra a versatile vegetable crop. Horticulture Technology. 1999; 9:179-184.
4. Kumar S, Dagnoko S, Haougui A, Ratnadass A, Pasternak D, Couame C *et al*., Okra (*Abelmoschus* sp) in West and Central Africa: potential and progress on its improvement. Afr. J Agric. Res. 2010; 5:3590-3598.
5. Kumar DS, Tony DE, Kumar AP, Kumar KA, Bramha D, Rao S, Nadendla R *et al*., A review on: *Abelmoschus Esculentus* (Okra). Int. Res J Pharm. App Sci. 2013; 3:129-132.
6. Sabitha V, Ramachandran S, Naveen KR, Panneerselvam K. Antidiabetic and antihyperlipidemic potential of *Abelmoschus esculentus* (L.) Moench in streptozotocin-induced diabetic rats. J. Pharm. Bioallied Sci 2011; 3:397-402. https://doi.org/10.4103/0975-7406.84447
7. Roy A, Shrivastava SL, Mandal SM. Functional properties of okra *Abelmoschus Esculentus* L. (Moench): traditional claims and scientific evidences, Plant Science Today. 2014; 1:121-130. https://doi.org/10.14719/pst.2014.1.3.63
8. Mamidi SA, Nadendla RR, Matangi SP, Jakka LP. Avowed assortment of flora as efficacious for diabetes. World J Pharm Pharm Sci. 2014; 3:405-417.
9. Khatun H, Rahman A, Biswas M, Islam AU. Water soluble fraction of *Abelmoschus Esculentus* L. interacts with glucose and Metformin hydrochloride and alters their co administration in rats. ISRN Pharmaceutics, 2011. https://doi.org/10.5402/2011/260537
10. Thanakosai W, Phuwapraisirisan P. First identification of α-glucosidase inhibitors from okra (*Abelmoschus Esculentus*) seeds. Natural product Communications. 2013; 8:1085-1088.
11. Woolfe JA. The effect of okra mucilage (*Hibiscus esculentus*) on the plasma cholesterol level in rats. The Proceedings of the Nutrition Society. 1977; 36:59A.
12. Bangana A, Dossou N. Cholesterol lowering effects of Okra (*Hibiscus esculentus*) in Senegalese adult men. Ann Nutr Metab. 2005; 18:199.

13. Ngoc TH, Van AT, Phung NV. Hypolipidemic effect of extracts from *Abelmoschus esculentus* L. (Malvaceae) on Tyloxapol- induced hyperlipidemia in mice. Journal of Mahidol University of Pharmaceutical Sciences. 2008; 35:42-46.
14. Sabourdy MA. Breeding and care of laboratory animals. WHO/Lab/88. 1988; 1:45.
15. Dhawan BN, Srimal RC. Laboratory Manual for Pharmacological Evaluation of Natural Products. Published by United Nations Industrial Development Organization (UNIDO) and International Centre for Science and High Technology (ICS), 2010.
16. Friedewald WT, Levy RI, Friedrickson DS. Estimation of VLDL and LDL- cholesterol in plasma, without use of preparative ultracentrifuge. Clin. Chem. 1972; 18:499-502.
17. Abott RD, Wilson PW, Kannel WB, Castelli WP. High density lipoprotein-cholesterol, total cholesterol screening and myocardial infarction. The Framingham Study. Arteriosclerosis. 1988; 8:207-211.
18. Neil HA, Mant D, Jones L, Morgan B, Mann JL. Lipid screening: Is it enough to measure total cholesterol concentration? BMJ. 1990; 301:584-587.
19. Smith GD, Song F, Shedon TA. Cholesterol lowering and mortality: The importance of considering initial level of risk. BMJ. 1993; 306:1367-1373
20. British National Formulary. Royal Pharmaceutical Society of Great Britain, 2017.
21. Gosslau A, Chen KY. Nutraceuticals, apoptosis and disease prevention. Nutrition. 2004; 20:95-102
22. Ohr L. Dietary antioxidants. Food Technol. 2004; 58:67-74.
23. Uraku AJ, Ajah PM, Okaka AN, Ibiam UA, Onu PN. Effects of crude extract of *Abelmoschus esculentus* on albumin and total bilirubin of diabetic albino rats. Int J Sci Nat. 2010; 1:38-41.