Research Article

In Vitro Antioxidant, Anti-Inflammatory and Lipid Lowering Activities of *Artocarpus lakoocha* Fruit Extract and Its Implication on Treatment of Dyslipidemia

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ABSTRACT

Reactive oxygen species (ROS) are generated by the mitochondria in aerobic organisms during physiological and pathophysiological conditions. The ROS can react with a wide variety of biomolecules such as Lipids and proteins leading to diseases. Dislipidemia is a metabolic condition where oxidative stress plays an important role in causing atherosclerosis, which leads to a variety of diseases of the heart and the blood vessels. Protective effects exhibited by certain foods have been attributed to their ability to reduce oxidative stress. In this study, the antioxidant activities of methanol extract of *Artocarpus lakoocha* fruit were assessed in an effort to validate the medicinal potential of fruit extract in inhibition of inflammatory enzymes such as Cyclooxygenase, Lipoxygenase and Myeloperoxidase and their effect on lipid peroxidation; also inhibition of enzymes involve in denovo lipid biosynthesis which are important in treatment of dyslipidemia. *Artocarpus lakoocha* Roxb, Moraceae, is a tropical tree species native to India. The fruit is a forest product which is sliced and sun dried and sold as a souring agent in culinary preparations and is believed to lower blood lipid. Phytochemical Screening reveals the presence of Sterols, Terpenoids, Flavonoids and Phenolic compounds. Results of the antioxidant activity of the Artocarpus extract was compared with ascorbic acid and vitamin E by two methods, namely ferric ion reduction and phosphomolybdate reduction. 1.94 ± 0.78 mg/gm of extract was equivalent with 2.56 ± 0.34 mg/gm Ascorbic acid and 9.64 ± 1.04 mg/gm of extract was equivalent with 0.67 ± 0.05 mg/gm Vitamin E. The extract showed antioxidative properties and free radical scavenging activities as well as inhibited enzymes involve in fatty acid synthesis and pro-inflammatory enzymes. The extract also inhibited oxidation of HDL and LDL in vitro and showed anticoagulant activity. The bioactive phytochemical(s) in A.lakoocha extract need to be identified, to be able to establish a mechanism of hyperlipidemia.

Keywords: *Artocarpus lakoocha*, Phytochemical, hyperlipidemia, fatty acid, lipid peroxidation.

INTRODUCTION

Dyslipidemia is a major risk factor for atherosclerotic cardiovascular disease, which is the leading cause of mortality worldwide. Elevation in total cholesterol, LDL-C and total triglycerides have been considered as indicators of dyslipidemia and they have been the main target for therapy.[1]

Oxygen free radicals and reactive oxygen species are byproducts of aerobic metabolism can be generated endogenously and can be formed from exogenous sources.[2] ROS include the superoxide anion (O_2−), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH·). They are often associated with oxidative stress which leads to ROS induce pathology by damaging lipids, proteins, and DNA.[3] The antioxidative defense system functions to counteract the potentially damaging effects of oxidative stress by the production of antioxidants.[4] Antioxidants can be broadly classified into two classes, namely preventive antioxidants, which intercept the oxidizing species before damage can be done and chain breaking antioxidants that slow or stop the oxidative process after it has started. The preventive antioxidants have many mechanisms by which they prevent the initial oxidant attack. For example, they can prevent the metal ion buildup such as free iron. They can remove hydro peroxides such as Catalase and glutathione peroxidase and by quenching the oxidant, like singlet oxygen by carotene, lycopene and bilirubin.[5-6]

Recent Studies has revealed that lipids have an important role in the activation of inflammatory pathways, increasing the production of inflammatory cytokines, mainly tumor necrosis factor alpha, interleukin 6 and 1β.[7] On the other hand, cytokines can promote disruption of lipid metabolism, specially reverse cholesterol transport, which is linked to development of atherosclerosis. Therefore, study of inhibiting activity of membrane-bound inflammatory enzymes such as Cyclooxygenase, Lipoxygenase and Myeloperoxidase and their susceptibility to lipid peroxidation have an importance in this filed.[8-9]

In view of the current scenario on global drug market for dyslipidemia, plant based therapies are an attractive alternate in the treatment of lipid disorders. Phytosterols present in the fruits and vegetables have been shown to decrease cholesterol absorption from intestine.[10-11] Very little information is available on phytochemicals that can inhibit denovo lipid biosynthesis or their storage as triglycerides in the adipose tissue.

Artocarpus lakoocha fruit is a commercially exploited fruit in Dakshina Kannada (DK), Uttara Kannada and Shimoga districts of Karnataka state, among other places in India. The fruit is a forest product, which is sliced, and sun dried

and sold as a souring agent. The dried fruit was purchased from the market in Mangalore (DK). It was identified by local experts as well as by persons using it as souring agent. To the best of our knowledge, there has been no reported pharmacological effect of *Artocarpus lakoocha*. The plant has widely been used by indigenous people in culinary preparations.

Therefore, the objective of this study was to assess the antioxidant and other biological properties of the extract in vitro, to see if *Artocarpus lakoocha* has the ability to inhibit different key regulatory enzymes in lipid biosynthesis pathways and inflammation, in order to determine its relevance for the treatment of dyslipidemia.

MATERIALS AND METHODS

ABTS, DPPH, Linoleic acid, Amplex Red dye, P-Nitro phenyl butyrate, 3 Hydroxy 3-Methyl glutaryl-CoA, (HMG-CoA), Malonyl-CoA, were purchased from Sigma Chemical Company, USA. NBT, NADH, PMS, Acetyl CoA, CoASH, Bovine serum albumin, NADP+, Dithiothritol (DTT), MOPS, sodium Arsenite, 5,5'-dithiobis (2-nitro) benzoic acid (DTNB) and EDTA disodium-salt, were purchased from SR Laboratories, Mumbai, India. Cis-Parinaric acid was from Cayman Chemicals, USA. All other chemicals, solvents and reagents were purchased from local suppliers of Rankem, SRL Fine Chemicals, and Himedia and were of Analytical Reagent Grade. Dried Artocarpus fruit was purchased from the local stores.

Preparation of the plant extract

50 gm dried Artocarpus fruit was refluxed with 500 ml methanol for 1 hour and then it was filtered. The filtrate was distilled to remove methanol. The volume was reduced to 50 ml. Further evaporation was carried out under reduced pressure and volume reduced to 20 ml. It was stored in an amber bottle in +4°C.

Preliminary phytochemical screening

Preliminary phytochemical tests were performed for the *Artocarpus lakoocha* methanolic extract to detect the presence of phytochemicals by following the standard methods described in the practical pharmacognosy of Kokate and Khandelwal.

Evaluation of total antioxidant capacity by Ferricyanide method

The reducing power assay of methanolic extract of Artocarpus was determined by ferricyanide method.[12] Substances, which have reduction potential, react with potassium ferricyanide (Fe^{+3}) to form potassium ferocyanide (Fe^{+2}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorbance at 700 nm. Various concentrations of extracts (0.1-1.5mg) in 1 ml of methanol were mixed with phosphate buffer (2.5 ml). The mixture was incubated at 50°C for 20 min; Aliquots of tricholoroacetic acid (2.5ml) were added to mixture, which was then centrifuged at 3000 rpm for 10 min. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5ml). The absorbance was measured at 700 nm, Ascorbic acid, BHT and Vitamin E (0.1-1.0mg) were used as standards. Increased absorbance of the reaction mixture indicates increase in reducing power.

Evaluation of total antioxidant capacity by Phosphomolibdate method

The total anti-oxidant capacity of the Artocarpus extract was determined by a spectrophotometric method.[13] The assay is based on the reduction of MO (VI) to MO (V) by the sample and the subsequent formation of a greenish blue phosphate /MO (V) complex at acidic pH. An aliquot of 0.1 ml of sample solution containing a reducing species was combined in an Eppendorf tube with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium Phosphate and 4 mM ammonium molybdate); the tubes were capped and incubated in a thermal block at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. Lipid soluble and water soluble antioxidant capacities were expressed as equivalent of vitamin E and ascorbic acid, respectively .[14]

1, 1-Diphenyl-2 picryl-hydrazil (DPPH) free radical scavenging activity

Antioxidant reacts with DPPH*, which is a stable free radical and is reduced to the DPPH and as a consequence the absorbance's decreased from the DPPH* radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds of extracts in terms of hydrogen donating ability. The assay was carried out as follows: 4 ml of extract solution made in methanol was added to 1 ml of DPPH* solution (1mM). The mixture was shaken and allowed to stand at 20°c for 30 min. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm.[15]

Hydroxyl radical scavenging activity

Hydroxyl radicals generated by reaction of an iron-EDTA complex with H_2O_2, in the presence of ascorbic acid, attack deoxyribose to form products, which upon heating with thiobarbituric acid at low pH, yield a pink chromogen. The total volume of the reaction mixture is 3.5 ml, it contained plant extract 100-200µg, Deoxyribose 15 mM, hydrogen peroxide 1mM, FeCl3 100µM, EDTA 100µM, Ascorbic acid 100µm. These are mixed in potassium phosphate buffer pH 7.4, (0.022M) and incubated at 37ºc for 1 hr. To the above reaction Mixture, 1 ml of 1% TBA (Thiobarbutric acid) and 1 ml of 2.8% TCA were added and heated in a boiling water bath for 30 min. The OD was read at 532 nm.[16] The experiment was also carried out with 0, 100, 200µg of BHT in place of plant extract. Controls contained no BHT or plant extract.

ABTS radical scavenging assay

The spectrophotometric analysis of ABTS*+ radical scavenging activity was determined. The ABTS*+ cation radical was produced by the reaction between 7 mM

ABTS in H2O and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 h. The ABTS*+ solution was diluted to get an absorbance of 0.700 at 734 nm with phosphate buffer (0.1M, pH 7.4).[17] To the diluted ABTS*+ solution (1 ml) 3 ml of Artocarpus extract in Methanol was added which contained different concentration (10-20 µg /ml) of the extract. After 30 min the percentage inhibition at 734 nm was calculated for each concentration relative to a blank absorbance (using only methanol). The scavenging capacity of ABTS*+ was calculated using the following equation: ABTS*+ scavenging effect (%) = {(A control - A sample)/A control} ×100**.** Where A control is the initial concentration of ABTS*+ and A sample is absorbance of remaining concentration of ABTS*+ in the presence of Artocarpus.

Superoxide anion radical scavenging activity in PMS-NADH/NBT system

Superoxide radicals are generated in PMS-NADH system by oxidation of NADH assayed by the reduction of NBT. In this experiment, the superoxide radicals were generated in 1.5 ml of Tris-HCl buffer (16 mM, pH 8) containing 0.5 ml of NBT (50µM) solution, 0.5 ml NADH (78 µM) solution and sample solution of Artocarpus (30µg/ml). The reaction was started by adding 0.5 ml of PMS solution (10µM) to the mixture. The reaction mixture was incubated at 25°C for 5 min and the absorbance was measured at 560 nm against a blank. Ascorbic acid was used as a control. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity.[18] The inhibition percentage of superoxide anion generation was calculated by using the following formula: %Inhibition= (A control- A sample)/Acontrol] X 100 Where A-control is the absorbance of the L-Ascorbic acid and A-sample the absorbance of Artocarpus or standards.

Assay of in vitro lipid oxidation inhibitory activity

Isolation of human LDL

LDL and HDL were prepared from either pooled serum by density gradient centrifugation. A stepwise gradient was prepared as follows: 10 ml of serum was mixed with solid KBr such that the density was about 1.3 g/ml. 30 ml of saline, pH 7, was layered on top of the serum sample. The tubes were centrifuged in a Sorvall ultra centrifuge in F-50L fixed angle rotor for 3hours, at 40000 rpm (200 000 g) at 4°C. After centrifugation, the tubes were carefully removed from the rotor and placed in the vertical position. The fractions were aspirated from the top. The LDL fraction appeared as a yellow layer in the center of the tube, and HDL appeared as an orange-layer at the bottom of the tube. The LDL and HDL fraction were collected separately. LDL and HDL containing fractions were dialyzed in the dark for 6 to 8 hours against double distilled water.[19]

Determination of lipoprotein oxidation

Lipid oxidation in lipoproteins was assessed by spectrophotometric monitoring of conjugated diene formation. LDL and HDL were diluted 1:50 with PBS. 100 µl of LDL or HDL was mixed with 850 µl PBS and 50 µl of 5 µM copper sulfate. The OD of the mixture was monitored at 234 nm, every 10 min for up to 90 min. The optical density was plotted against time.[20]

In vitro anti-inflammatory activity

Cyclooxygenase Enzyme inhibition

The Cox isoenzymes both have sequential cyclooxygenase and peroxidase activities within the same holoenzyme. When oxidized by Cox-l or Cox-2 in the presence of arachidonic acid or linoleic acid the colorless and non-florescent Ampler Red Reagent is converted to the highly florescent dye resorufin (7-hydroxyphenoxazone).. A Cycloxygenase enzyme was assayed using rat peritoneal macrophages. The rat peritoneal macrophages were isolated. The macrophages were lysed and the lysate was used as an enzyme source. In the presence of cycloxygenase the non-fluorescent reagent is converted to resorufin, a fluorescent molecule. The assay reaction was carried out in final volume of 1ml containing 100 µM Linoleic acid and 10 µM Amplex red reagent (prepared in pure DMSO and stored at (-20°C) in 50 mM Tris, HCL buffer pH 9. The reaction mixture was incubated for 5 min at 37°C and the relative fluorescence intensity was measured in a fluorimeter using appropriate blanks. The excitation was at 563 nm and emission was at 587 nm. The cycloxygenase inhibitor assay was carried out by pre incubating the enzyme with the Artocarpus extract for 15 min prior to determining its COX activity. The results are expressed as percent inhibition of the COX activity. The results were compared with standard phytochemicals.[21-22]

Lipoxygenase inhibiting activity

Lipoxygenase enzymes were assayed using EAT Cell Extract and soybean extract as a source of Lipoxygenases. EAT lysate was used as a source of Lipoxygenase. The reaction was carried out in a final volume of 2 ml containing 50 µM DTT, 200 µM ATP, 300 µM Calcium chloride and 150 µM Linoleic acid. The reaction was started by adding 50 µl of the enzyme preparation. The formation of 5-HETE was followed for 3 min at 234 nm at room temperature. The LOX inhibiting activity of the Artocarpus extract was determined by pre incubating the enzyme with the extract prior to determining its LOX activity. The results are expressed as percent inhibition of the LOX activity. The reference phytochemicals were also used for their inhibitory activity on LOX.[23]

Soybean Lipoxygenase inhibitory activity

The soybean lipoxygenase was partially purified. Briefly soybean seeds were obtained from National Seed Collection Centre and powdered with a pestle and mortar. The powder was suspended in petroleum ether (60-80) in cold and extracted three times. The defatted powder was dried in air and suspended in 0.2 M sodium acetate buffer pH 4.5 at 10% concentration (w/v) and stirred at 4°C for 1 hr. The suspension was allowed to

settle and then decanted. It was then centrifuged in a refrigerated centrifuge at 4° C for 10 min at 5000 rpm. The clear supernatant was used as the source of enzyme. The reaction was carried out in a final volume of 3 ml containing 2.9 ml of 0.1 M borate buffer pH 9 and 50 µl of 10 mM Linoleic acid. The reaction was started by the addition of 50 µl of the soybean enzyme extract. The enzyme activity was measured by following the formation of the product, at 234 nm for up to 1min. The enzyme inhibition was determined by pre incubating the enzyme with the plant extract or standard phytochemicals prior to determining its LOX activity. The results are expressed as percent inhibition of the LOX activity.[24]

Inhibition of Myeloperoxidase

Myeloperoxidase oxidizes Guaiacol in the presence of H_2O_2 to a colored chromogen, which has an absorption maximum at 470 nm. The assay was carried out as Follows: To a spectrophotometric cuvette, 3 ml of reagent A was added (Reagent A:50 mM potassium phosphate buffer with 100 mM Guaiacol and 0.0017 hydrogen peroxide, pH 7.0 at 25◦C. A blank was prepared similarly by taking 3 ml of Reagent A To the test, 0.035 ml of Reagent B was added (B: Myeloperoxidase enzyme solution from EAT cells). The tube was immediately mixed by inversion and at exactly one minute, the absorbance was read at 470 nm. For the blank, instead of reagent B, 0.035 ml of water was taken. The Artocarpus extract was pre-incubated with the Myeloperoxidase prior to determining its activity to measure its inhibitory activity.[25]

Preparation of Myeloperoxidase from EAT cells

Eat bearing mice were scarified by cervical dislocation, 2ml of 0.9% saline was injected intraperitonially. The peritoneum open and ascites fluid was collected and transferred to 15 ml polypropylene tube and centrifuge at 3000 rpm for 5 min. Supernatant ascites-free of cells was collected for further studies. Calculations:

$$\text{Unit/ml enzyme} = \frac{\text{A test at 1 min-A blank at 1min X (df)}}{(0.035)}$$

df = dilution factor

035 = volume of enzyme (ml)

Anticoagulation Assay

The anti-coagulation assay using whole blood was performed in the absence and presence of the Artocarpus extract. To 340 µl of whole blood, 20 µl of 1:5 diluted Artocarpus extract (2 mg/ml) and 20 µl of calcium chloride were added and the coagulation was monitored.[26]

Lipid metabolism enzyme Inhibitory activities in vitro

Preparation of rat liver microsomes

Rat liver microsomes were prepared. Briefly, the rats were killed by cervical dislocation and the livers were excised immediately. They were placed in cold Tris-HCl buffer (pH 7.4, 100 mM) containing 0.02 M EDTA. The liver was weighed and homogenized in a Potter–Elvehjem homogenizer (3.5 ml/gm). The homogenate was centrifuged at 12,000 x g for 10 min. The supernatant was centrifuged at 60,000 g for 1 hr. The precipitate obtained was redissolved with 100 mM Tris HCl buffer pH 7.4 containing 0.02 M EDTA and frozen at –20 C°. Before use, the pellet was resuspended in 100 mM Tri HCl buffer pH 7.4 containing 0.02 M EDTA and 10 mM dithiotritol solution. The resuspended microsomes were diluted if necessary to give a protein concentration of 5-10 mg/ml and used.[27]

Fatty Acid Synthase (EC 2.3.1.85)

The fatty acid synthase activity was assayed by measuring the Malonyl CoA-dependent oxidation of NADPH. The reaction was carried out in a total volume of 1 ml, containing 100 mM potassium phosphate buffer pH 6.5, 1 µmol of EDTA, 10 µmol of acetyl CoA, 0.075 µmol of NADPH and 60 µl of 1:10 diluted enzyme (Liver homogenate). The reaction was initiated by the addition of 100 µl of 0.075 µM Malonyl CoA and the change in absorbance was measured at 340 mm. A control was run without the addition of Malonyl CoA to check the background activity. The activity was expressed as µmol of NDAPH utilized/min. Extinction coefficient of 6.22 mM^{-1} cm^{-1} was used to calculate the µmol of NADPH utilized.[28]

HMG-CoA Reductase (EC 1.1.1.34)

The assay was carried out in a final volume of 1 ml containing 150 nmol of HMG-CoA, 2µmol of NADPH, 100 mM triethanolamine buffer pH 7.4 containing 0.02 M EDTA. The reaction is stated by the addition rat liver microsomes (1 mg protein). The reaction mixture was incubated at 27°C for 30 min. After incubation 20 µl of 0.01 M sod. arsenite solution was added. The reaction was terminated by the addition of 0.1 ml of 2 M citrate buffer pH 3.5 containing 3% sod. tungstate, to give a final pH of 4.0. At this pH, the microsomes precipitate. The precipitated microsomes were removed by centrifugation at 25,000 g for 15 min. 1 ml of the supernatant was transferred to a stoppered tube. The pH was brought to 8.0 by the addition of 0.2 ml of 2 M Tris buffer pH 10.6 and 0.1 ml of 2 M Tris buffer pH 8.0. The formation of arsenite dithiol is completed within 3-4 min after the addition of sod. arsenite. The concentration of monothiols was determined by reacting with DTNB. To 1 ml of the reaction mixture in a 1ml Cuvette, 20 µl of 2 mM DTNB in 0.1 M triethanolamine buffer pH 7.4 containing 0.02 M EDTA was added mixed and the absorbance was measured at 412 nm for 4 min. Monothiols react with DTNB rapidly whereas dithiols react slowly. The concentration of monothiols reacting with DTNB was calculated from the linear portion of the curve. The molar extinction coefficient of 1.36 x 10^4 was used to calculate the CoA formed per min as follows.[29]

$$\text{Activity (nmol / min)} = \frac{(\text{Areaction} - \text{Acontrol}) \times \text{dilution factor}}{0.0136 \times \text{Time}}$$

The dilution factor for the reaction was 1.43. The reaction taking place in the absence of NADP is HMG-CoA deacetylase and is used as control to subtract from the experimental.

Determination of IC_{50} value

IC_{50} was determined for the Artocarpus extract by taking different concentration of the extract in the enzyme assays. The IC_{50} for the inhibition of enzymes was calculated by plotting log concentration in µg/ml against percent activity. A linear regression was done using the software built in to Excel. From the equation y = mx + c was equated to 50% and the concentration x, was calculated in µg/ml

Protein estimation

The amount of protein was estimated using Lowry's method, taking bovine serum albumin as standard.[30]

Statistics

The results are expressed as Mean ± SD of at least three independent determinations.

RESULTS AND DISCUSSION

Artocarpus lakoocha when extracted with methanol gave about 8% extract by weight. The phytochemicals in the methanol extract are shown in table 1. Phytochemical Screening reveals the presence of Sterols, Terpenoids, Flavonoids and Phenolic compounds. HPLC Analysis of the extract was published previously.[31] That have shown the presence of phenolics and flavonoids. Results of the antioxidant activity of the Artocarpus extract was compared with ascorbic acid and vitamin E by two methods, namely ferric ion reduction and phosphomolybdate reduction and is shown in the table 2.

The free radical scavenging activities of the A.lakoocha extract were tested against ABTS radical, DPPH radical, hydroxyl radical and superoxide anion radical scavenging activity is shown in the table 3. The IC_{50} for the inhibition of the Pro-oxidant (Pro-inflammatory) enzymes by the extract are shown in table 4. IC_{50} values for the in vitro inhibition of enzymes of lipid biosynthesis, is given in table 5. Results are mean± SD (n=4). Figure 1 represents the inhibition of in vitro oxidation of HDL and LDL. With increasing concentration of the extract there was an inhibition in the oxidation of HDL and LDL in a dose dependent manner. The anti-coagulant activity of *Artocarpus lakoocha* extract is shown in figure 2. The Recalcification time had significantly increased and the fibrinolysis had increased resulting in a 50% decrease in the fibrin. Oxidative stress is the underlying cause of many diseases like diabetes, cardiovascular diseases and cancer.[5,9] Oxidative stress is produced in different tissues which is responsible for the tissue specific disorder. Increased oxidative stress in vascular walls leads to pathogenesis of hypertension and atheroschrosis.[8,32]

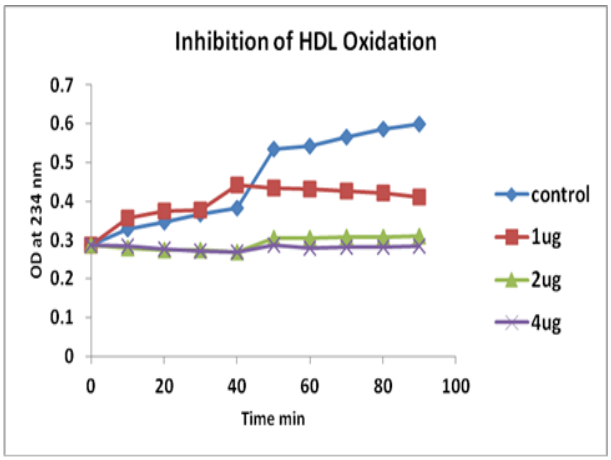
White adipose tissue of obese rats had higher expression of NADPH oxidase and reduced levels of antioxidant enzymes.[6] Treatment with NADPH oxidase inhibitor apocytin reduced ROS production in adipose tissue of KKAY mice and also improved the hyperglycemia, hypertriglyceridemia and hyper insulinemia.[6] Adipose tissue, in addition to being a storage organ for lipids, is also metabolically active endocrine organ. Inflammatory cytokines like TNFα and IL-6 are produced by the human adipose tissue. In healthy individuals, systemic IL-6 concentrations increase with adiposity. It has been estimated that about one third of total circulating IL-6 originates from the adipose tissue.[33] The hepatic synthesis of acute phase protein, C - reactive protein is largely regulated by IL-6.[7] Inflammation is a source of oxidative stress. Increased production of ROS may also enhance inflammatory response by activating redox sensitive nuclear transcription factors such as AP-I and NFkB.[34]

The idea that dyslipidemia is a state of chronic oxidative stress and inflammation even in the absence of open CVD risk factors makes it necessary to look for antioxidant therapy in dyslipidemia to reduce the complications arising out of high fat level. Plants being rich in antioxidant phytochemicals have been extensively tested for their antioxidant properties and free radical scavenging activities. *Artocarpus lakoocha* was shown to have phenolics and flavonoids which have antioxidant properties. Flavonoids are the largest group of phytochemicals with over 8000 individual compounds identified .[35] In addition to their role as antioxidants in plants they are known to exhibit biological activities in animals that consume them. They have been shown to have anti-inflammatory, antiviral and vasodiation effects.

Ingested flavonoids are generally degraded to their phenolic acids. These phenolic acids still possess antioxidant and radical scavenging activities. Artocarpus species have been shown to have wide variety of phytochemicals including flavanols, flavones and xanthones. The ability of flavonoids to act as antioxidants depends on their molecular structure. The position OH group and other features of chemical structure of flavonoids influence their antioxidant and free radical scavenging activity. The total antioxidant capacity of A.lakoocha extract determined by Ferric ion reduction and phosphomolybdate reduction showed similar results when compared with ascorbic acid, whereas, when the antioxidant capacity was compared with that of vitamin E, the results showed an order of magnitude difference. This may be because of the presence of different molecules with different chemical structure and

reactivity. The DPPH and ABTS radical scavenging activities of A.lakoocha extract were comparable, whereas the extract was very efficient in scavenging superoxide anion radicals. However, it was not efficient in scavenging hydroxyl radicals. In vivo the half-life of hydroxyl radical is approximately 10–9 sec. Enzymes cannot eliminate hydroxyl radical, since the half-life of this ion would be less than the diffusion rate. Hence, it will oxidize any oxidizable molecules in its viscidity. Although there would be no molecule theoretically capable of completely eliminating the OH radical, phytochemicals can reduce the harmful effect of OH radicals. The Myeloperoxidase and lipoxygenase inhibiting activities of A.lakoocha extracts were comparable. However, the extract was less efficient in inhibiting cyclooxygenase as well as soybean lipoxygenase enzymes. The extract was able to prevent LDL and HDL oxidation in a dose dependent manner in vitro. Whether it would inhibit LDL oxidation in vivo needs to be verified in in vivo experiments. Phytochemical Composition of A.lakoocha; The A.lakoocha extract showed about 6 major components accounting for about 75-95% of the total compounds. However, their retention times did not exactly match those of the reference compounds suggesting that the structure of the compounds in the extract may be different, but belonging to the same family of molecules.[36]

The Artocarpus extract showed anti-coagulant activity in vitro by increasing fibrin degradation. Whether it would have anti-coagulant activity in vivo is not known. If it also shows anti-coagulant activity in vivo, it could be used for thinning blood of patients with risk of thrombosis. The ability of Artocarpus extract to inhibit enzymes of lipid biosynthesis suggests that the Artocarpus extract may have components that can act as inhibitors of lipid biosynthesis. Appropriate bioactive molecule and it target identification would be a challenging task.



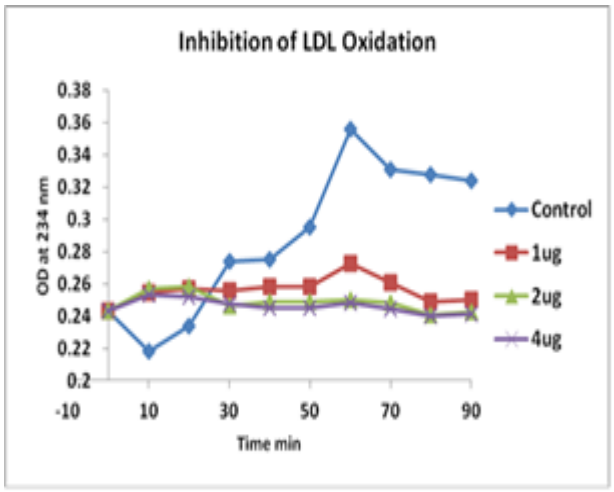
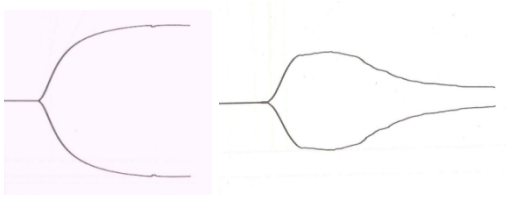


Figure 1: *In vitro* Lipid oxidation Inhibitory activity. Inhibition of in vitro oxidation of HDL and LDL by A.lakoocha extract. HDL and LDL both, was isolated from pooled human serum. It was subjected it in vitro oxidation using Cu++ in the absence or presence of increasing concentration of Artocarpus extract as described in methods.



(A) Control (B) with A.lakoocha extract

Figure 2: Anticoagulant activity of Artocarpus extract Recalcification time of human blood was determined in the absence and presence of Artocarpus extract as described in the methods

Table 1: Phytochemical Screening

Phytochemical Class	Present / Absent
Alkaloids	Absent
Sterols	Present
Terpenoids	Present
Flavonoids	Present
Phenolics	Present

Ethanol extract was tested by qualitative analysis for the presence of phytochemicals as described in the methods

Table 2: Total Antioxidant Capacity of *A. lakoocha*

Ascorbic acid equivalent	1.94 ± 0.78 mg/gm	2.56 ± 0.34 mg/gm
Vitamin E equivalent	9.64 ± 1.04 mg/gm	0.67 ± 0.05 mg/gm

A calibration curve was prepared using ascorbic acid and vitamin E in the two assay systems. The total antioxidant capacity of the extract was compared with that of

Ascorbic acid and Vitamin E. Results are mean ± S.D (n=3).

Table 3: Radical Scavenging Activity of *A.lakoocha* Extract

Radical	Scavenging Activity Equivalent
ABTS	2.32 ± 1.27 mg Ascorbic acid / gm
DPPH	6.72 ± 4.70 mg Ascorbic acid / gm
Hydroxyl	41.35 ± 11.75 mg BHT/ gm
Superoxide anion	0.47 ± 0.13 mg Ascorbic acid / gm

Radical Scavenging Activity of A.lakoocha extract was compared with ascorbic acid or BHT and expressed as mg equivalent /gm of A.lakoocha. Results are mean ± SD (n=4)

Table 4: IC_{50} values for Inhibition of Pro-oxidant (Pro-inflammatory) Enzymes

Pro-oxidant Enzyme	IC_{50} µg
EAT cell lipoxygenase	4.79 ± 1.50
Soybean lipoxygenase	25.7 ± 0.85
Myeloperoxidase	3.1 ± 0.66
Cyclooxygenase	31.40 ± 2.89

IC_{50} for the pro-oxidant enzyme was determined as described in the methods. The results are mean ± SD (n = 3).

Table 5: IC_{50} values for the inhibition of Enzymes of Lipid Biosynthesis

Enzyme	IC_{50} µg
HMGCoA reductase	9.08 ± 0.46
Fatty acid synthase	2.61 ± 0.06

The enzyme inhibition was studied in vitro. Results are mean ± SD (n=4)

CONCLUSION

Since plants contain a wide variety of phytochemicals possessing antioxidant and anti-inflammatory properties, they seem to be attractive candidates for the treatment of diseases caused by oxidative stress and inflammation. Dislipidemia is a metabolic syndrome where oxidative stress plays an important role. Hence, antioxidative plant extracts may also have hypolipidemic effects. In view of this *Artocarpus lakoocha* was tested for its anti-oxidant and lipid lowering properties in vitro. It showed antioxidative properties and free radical scavenging activities as well as inhibited enzymes involve in cholesterol and fatty acid synthesis. The bioactive phytochemical in A.lakoocha extract need to be identified, to be able to establish a mechanism of hyperlipidemia.

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