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EVALUATION OF ANTIOXIDANT AND ANTIHYPERLIPIDEMIC ACTIVITY OF EXTRACT OF *GARCINIA INDICA*

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ABSTRACT

Garcinia indica is well spread in tropical rain forest of Western Ghats of India. Extract of dried fruit rind of kokum was prepared using methanol. Extract was evaluated for antioxidant and antihyperlipidemic activity. *Garcinia* was tested for antihyperlipidemic activity using cholesterol induced hyperlipidemic model in Albino Wistar rats. It exhibited significant reduction in total cholesterol, triglycerides, LDL-C, VLDL-C levels and increase in level of HDL-C when compared to Lovastatin. Artherogenic index and LDL-C: HDL-C ratio was also reduced to significant extent. Significant antioxidant and antihyperlipidemic activity may be due to the presence of polyphenols like Garcinol and hydroxycitricacid in *Garcinia*.

INTRODUCTION: *Garcinia indica* is well spread in evergreen tropical rain forest of Western Ghats of India from Kokan to Mysore. The dried outer rind

of fruits of *Garcinia indica* is popularly known as kokum and is used for imparting flavor and taste to curries. The fresh fruits are steeped in sugar syrup to make amrutkokum - a healthy soft drink to relive sunstroke, which is popular during summer [1, 2, 3]. Many therapeutic effects of the fruit have been described in traditional medicine based on Ayurveda. These include its usefulness as an infusion, in skin ailments such as rashes caused by allergies; treatment of burns, scalds and chaffed skin; to relieve sunstroke; remedy for dysentery and mucous diarrhea; as a cardiotonic and for bleeding, piles, dysentery, tumors and heart diseases [4]. The present study was carried out to understand the antioxidant and antihyperlipidemic activity of the extract.

Experimental:

Collection of plant material and Extraction: *Garcinia indica* fresh fruits were collected from the forest area of Ratnagiri district of Maharashtra, India in the month of April. Authentication of the collected material was carried out from Agharkar Research Institute, Pune by Dr. A. M. Majumdar and its identity was confirmed to be *Garcinia indica Choisy*, family Clusiaceae. A voucher specimen (A-09) has been deposited at our Pharmacognosy Laboratory. The fruits were cut into two pieces and after removal of seeds the rinds were sun dried. The dried material was powdered and was subjected to soxhlet extraction using methanol as a solvent. The extract was concentrated under vaccum at temperature not more than 60^0C.

Chemoprofiling of the extract with High Performance Liquid Chromatography (HPLC) method: The chemoprofiling of the extract was carried out using HPLC analysis. One of the major constituents present in the extract was isolated using preparative Thin Layer Chromatography. Precoated TLC silica gel 60 F_{254} (Merck) plates were used as stationary phase. The mobile phase comprised of Toluene: Ethyl Acetate: Formic Acid (4: 1: 0.5). The phyoconstituent was eluted from the stationary phase by using methanol as a solvent and after evaporation of the solvent; the residue was subjected to spectral analysis using UV and IR spectral studies.

The total extract and the isolated constituent were subjected to HPLC analysis using Isocratic HPLC system (Jasco, Japan) with the following specifications;

Column: Qualisil BDS C18 Column
Flow Rate: 1 ml/min.
Mobile Phase: Methanol.
Detector: UV detector (280 nm)

Animals: For antioxidant activity, Swiss Albino mice (25-30 gm) of either sex were obtained from Haffkin Biopharmaceutical Corporation Ltd., Mumbai. For antihyperlipidemic study, albino wistar rats (120-150 gm) of either sex were obtained from Glenmark Pharmaceuticals Ltd., Mumbai. All the animals were housed under good hygienic conditions in the departmental animal house of Prin. K. M. Kundnani College of Pharmacy. Animals were maintained under standard environmental conditions (22-28^0C, 60-70% relative humidity, 12 hr L: D cycle) and fed with standard diet and water *ad libitum*. Animals were allowed to acclimatize to experimental conditions by housing them for 8-10 days period prior to experimental study.

Evaluation of Antioxidant Activity: The experimental protocol was approved by the Institutional Animal Ethics Committee (Protocol No-091011) and the experimental work was carried out as per Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidlines. The antioxidant activity of plant was evaluated by two different methods.

Antioxidant activity by Lipid Peroxidation Method: The antioxidant activity of plant was evaluated by calculating inhibition of lipid peroxidation which was performed using liver homogenate of mice. Mice liver homogenate (10%) was prepared by homogenizing the fresh liver in 0.15 M KCl solution. This was mixed with 0.15 M KCL and tris hydrochloride buffer. The various extracts of *Garcinia indica* were then added in different concentrations. Curcumin was used as standard. *In vitro* lipid peroxidation was initiated by addition of 100 µM ferrous sulphate and 100 µM ascorbic acid. After incubation for 1 hour at 37^0C, the reaction was terminated by addition of thiobarbituric acid and then boiled at 95^0C for 15 minutes for development of colored complex. On cooling test tubes were centrifuged at 4000 rpm for 10 minutes. Absorbance of supernatant was determined colorimetrically at 532 nm and percent inhibition of formation of thiobarbituric acid reactive substances (TBARs) was calculated with respect to control in which no test sample was added [5, 6, 7]. The IC_{50} values were calculated for all the test materials by subjecting the results to linear regression.

Antioxidant activity by Deoxyribose Method: The antioxidant activity was performed using free radical scavenging by Deoxyribose method. The reaction mixture consisted of Deoxyribose (3mM), Fecl3 (0.1 mM), Ascorbic acid (0.1 mM), H_2O_2 (2 mM) in phosphate buffer (pH-7.4). To this various concentration of test extracts were added. After incubation for 30 minutes at 37^0C, TCA (5%, 0.5 ml) and TBA (1%, 0.5 ml) were added. The Reaction mixture was kept in boiling water for 30 minutes, cooled and absorbance were measured at 532 nm. Quercetin being phenolic compound was used as positive control. The hydroxyl Radical scavenging activity of the extract is reported as % inhibition of deoxyribose degradation. The results were subjected to linear regression and IC_{50} value was calculated [8].

Evaluation of Antihyperlipidemic Activity: The experimental protocol was approved by the Institutional Animal Ethics Committee and the experimental work was carried out as per CPCSEA guidelines. The procedure was followed as per the method of Dhuley *et al*., [9]. Albino Wistar rats (120-150 gm) of either sex were randomly divided into five different groups each containing 6 animals as follows:

Group 1- Control: Vehicle control.

Group 2- Hyperlipidemic: Cholesterol in coconut oil (25 mg/kg/ day) was administered each rat of this group

Group 3- Standard: Lovastatin (10 mg/kg/day) was administered along with cholesterol in oil.

Group 4- Test: Rats of this group were administered with *Garcinia indica* whole methanolic extract reconstituted in water, (200 mg/kg/day) along with cholesterol in oil.

Group 5- Test: Rats of this group were administered with *Garcinia indica* whole methanolic extract reconstituted in water, (400 mg/kg/day) along with cholesterol in oil.

The cholesterol in oil was administered to each animal except the vehicle control group daily at 10.00 am. The solution of extract and standard drug were prepared freshly every day and administered to the test animals in their respective groups at 3.00 pm. This process was followed for 22 days and the amount of food intake was monitored daily. At the end of the experimental studies animals were fasted for 12 hrs and the blood was collected by cardiac puncture under light ether anesthesia. Animals were then sacrificed and the livers were isolated and preserved in 10% formalin solution [10]. The biochemical parameters that were evaluated using Span Diagnostic Kits were Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C), Very

Low Density Lipoprotein Cholesterol (VLDL-C), High Density Lipoprotein Cholesterol (HDL) and Triglycerides (TAG) from the serum. The Artherogenic Index and LDL-C: HDL-C ratios were calculated to determine the cardiac risk factors [11]. The activities of marker enzymes like Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT) were also estimated. All the results were subjected to statistical analysis using one way Annova followed by Dunnet's multiple comparison tests against the hyperlipidemic group. The p values < 0.01 were considered statistically significant. All the values were expressed as Mean±SEM and compared with control group for estimation of antihyperlipidemic activity of test extracts

RESULTS AND DISCUSSION:

HPLC Chemoprofiling: The phytoconstituents isolated by preparative TLC from whole extract exhibited λ_{max} at 280 nm when subjected for UV spectral analysis. Its IR spectral analysis exhibited peaks at 3528, 3480, 3321, 3200, 2951, 1590, 1117 cm^{-1} wave numbers. From the reported literature data [12] its identity was confirmed to be garcinol. The HPLC chromatogram (**Figure 1A**) of garcinol depicted retention time at 7.43 mins, which matched with one of the major constituents present in the whole extract (**Figure 1B**) confirming the presence of garcinol in the same.

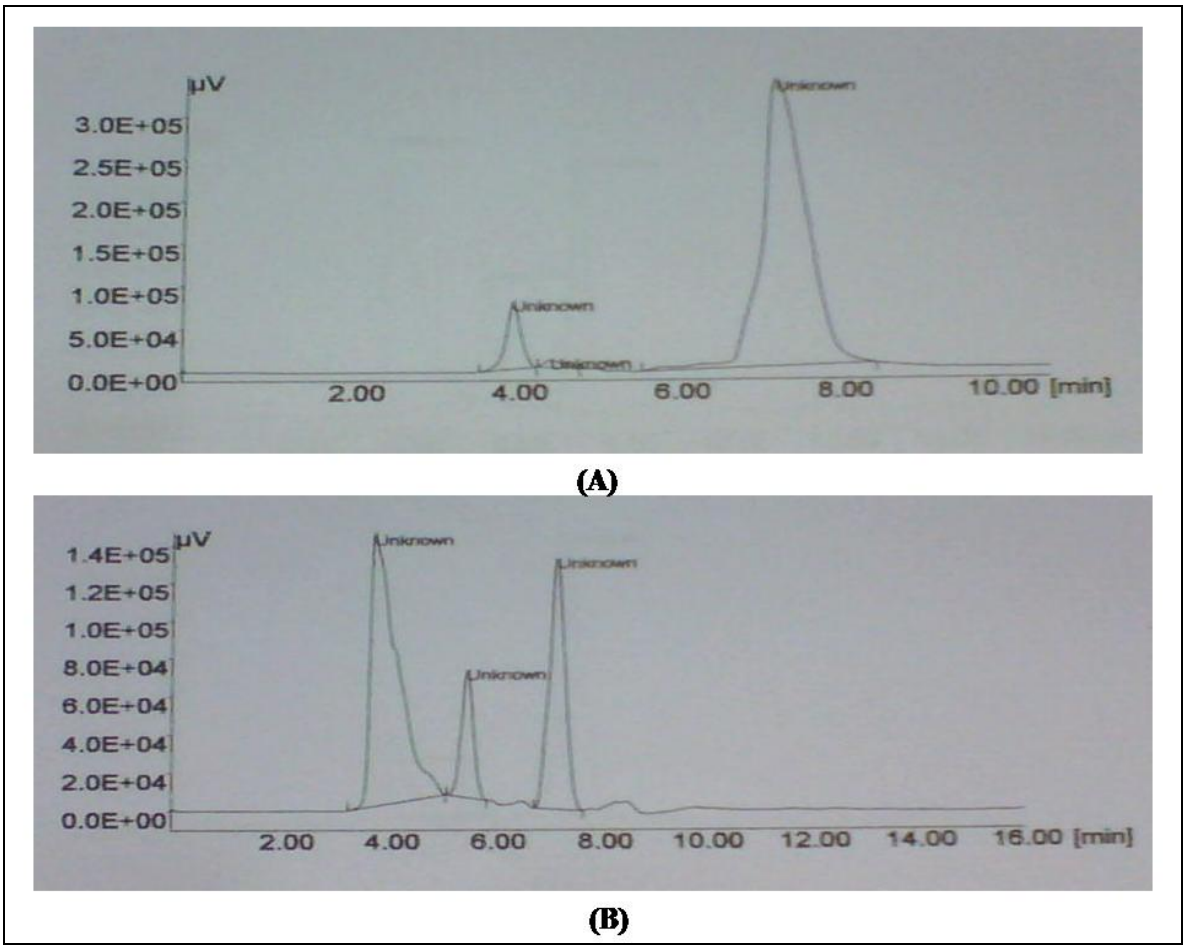

FIG. 1: (A) HPLC CHROMATOGRAPH OF ISOLATED GARCINOL (B) HPLC CHROMATOGRAPH OF *GARCINIA INDICA* EXTRACT

Antioxidant activity: The antioxidant activity evaluation using *in vitro* methods revealed significant activity for *Garcinia indica* extract (**Table 1**). The observed activity may be mainly due to the presence of phytochemicals like garcinol and hydroxy citric acid (HCA) [13]. Garcinol is polyisoprenylated benzophenone which has structural similarity with curcumin which is a well known antioxidant. Garcinol has a phenolic hydroxyl group which is mainly responsible for its antioxidant activity [14]. Apart from this extract also contains citric acid, maleic acid, polyphenols, carbohydrates, anthocyanin pigments and ascorbic acid [4, 15].

TABLE 1: ANTIOXIDANT ACTIVITY OF EXTRACT OF *GARCINIA INDICA* BY *IN VITRO* LIPID PEROXIDATION

Extracts	IC_{50}(µg/ml)
By lipid peroxidation method	
Garcinia indica extract	99.53
Curcumin (Standard)	49.73
By Deoxyribose method	
Garcinia indica extract	106.83
Quercetin (Standard)	33.32

Antihyperlipidemic activity: In the present study, the whole methanolic extract of *Garcinia indica* exhibited a significant antihyperlipidemic activity in cholesterol induced hyperlipidemic model in Albino Wistar rats which was better than that observed with standard drug Lovastatin. The activity was observed in a dose dependent manner for all the treated groups. The levels of TC, LDL-C, VLDL-C and TAG were found to be considerably less than standard Lovastatin drug (**Table 2**). The atherogenic index and LDL- C: HDL-C ratio was found to be less than Lovastatin group (**Table 3**) which leads to reduction in cardio-vascular risk factor.

A major culprit in the development of atherosclerosis is oxidized LDL. Free radicals may contribute to atherogenesis by oxidizing low density lipoproteins (LDL) which then damage arterial walls. The oxidation of LDL cholesterol is suspected to occur at the initial stages of atherosclerosis, and antioxidants have been shown to inhibit this oxidative reaction [16]. The significant antioxidant activity demonstrated by the extract of *Garcinia indica* could be also one of the mechanisms for lowering the levels of TC, LDL-C, VLDL-C and TAG.

TABLE 2: EFFECT OF *GARCINIA INDICA* EXTRACTS ON THE SERUM LIPID PROFILE

Groups	TC (mg/dl)	TAG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Control group	61.00±1.14*	48.80±4.12*	39.60±0.58*	12.60±1.23*	8.640±1.42*
Hyperlipidemic group	120.00±1.41	190.6±3.61	16.36±0.53	65.52±1.48	38.12±0.72
Standard group lovastatin (10 mg/kg/day)	73.60±2.58*	90.00±6.23*	24.46±0.71*	31.13±2.34*	18.00±1.24*
Whole methanolic extract (200 mg/kg/day)	77.60±2.50*	76.20±9.15*	30.43±0.64*	31.93±2.77*	15.24±1.83*
Whole methanolic extract (400mg/kg/day)	74.60±3.58*	72.40±4.34*	34.28±0.69*	25.57±3.89*	14.48±0.86*

N=6, Values are expressed as Mean± S.E.; *p < 0.01 vs. Hyperlipidemic group

TABLE 3: EFFECT OF EXTRACTS OF *GARCINIA INDICA* ON PARAMETERS LIKE AI & LDL-C: HDL-C RATIO AND ON ENZYME ACTIVITY LIKE SGOT AND SGPT

Groups	AI	LDL-C / HDL-C	SGPT	SGOT
Control group	1.540±0.01*	0.317±0.03*	35.55±6.48*	174.7±9.968*
Hyperlipidemic group	7.369±0.28	4.026±0.19	156.1±7.94	250.8±8.728
Standard group lovastatin (10 mg/kg/day)	3.017±0.12*	1.253±0.10*	43.64±4.75*	204.5±9.848*
Whole methanolic extract (200 mg/kg/day)	2.550±0.08*	1.050±0.09*	58.96±1.06*	93.32±5.315*
Whole methanolic extract (400mg/kg/day)	2.174±0.08*	0.742±0.10*	67.36±6.39*	142.9±2.452*

N=6, Values are expressed as Mean± S.E.; *p < 0.01 vs. Hyperlipidemic group

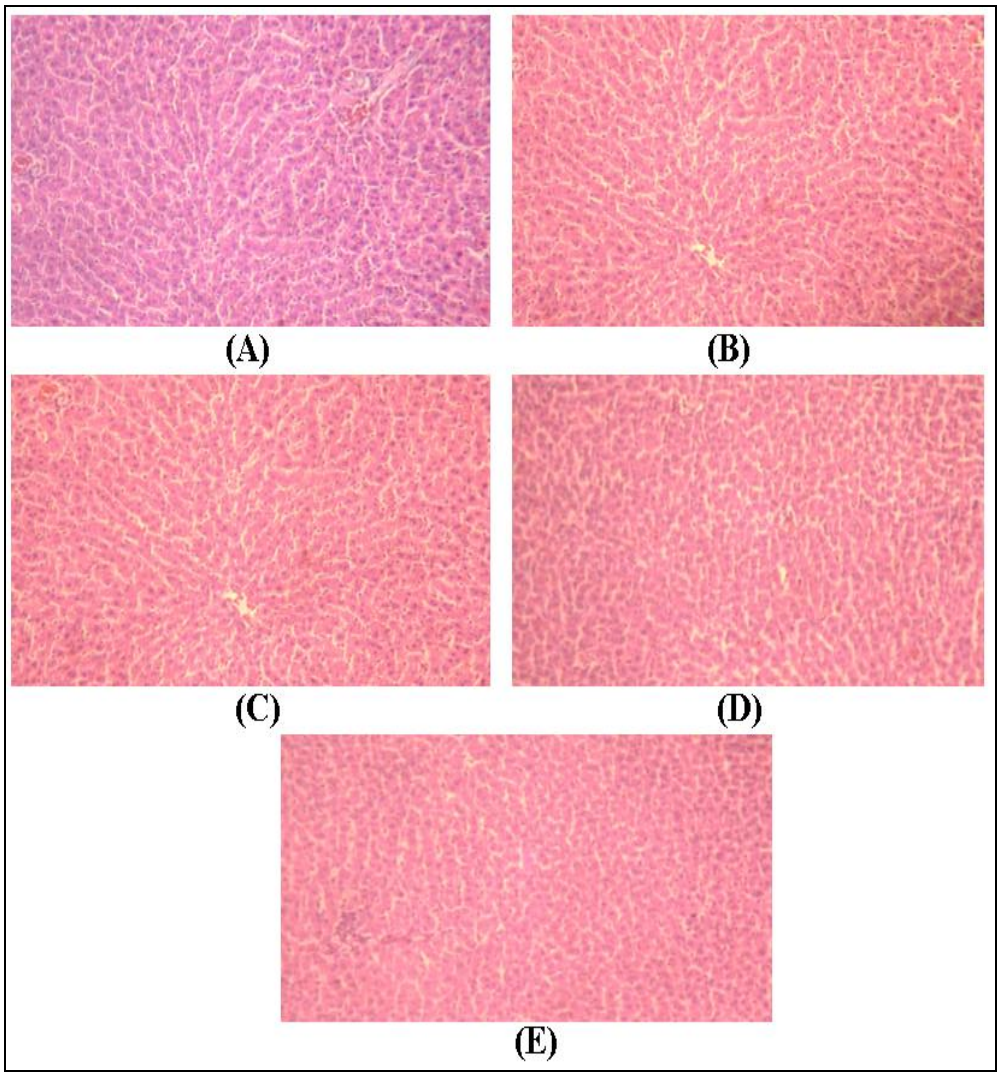

FIG. 2: HEPATOCYTES OF RATS STAINED WITH HEMATOXYLIN AND EOSIN (100 X MAGNIFICATION)
(A) CONTROL GROUP SHOWING NORMAL ARCHITECTURE;
(B) HYPERLIPIDEMIC GROUP SHOWING GRANULAR DEGENERATION;
(C) LOVASTATIN GROUP SHOWING MILD DIFFUSE GRANULAR DEGENERATION;
(D) *GARCINIA INDICA* WHOLE METHANOLIC EXTRACT (200MG/KG/DAY) SHOWING NO EVIDENCE OF ANY SPECIFIC TOXICITY;
(E) *GARCINIA INDICA* WHOLE METHANOLIC EXTRACT (400MG/KG/DAY) SHOWING NO EVIDENCE OF ANY SPECIFIC TOXICITY

In hyperlipidemic model, the purpose of inclusion of cholesterol and coconut oil is attributed to the very well established findings that addition of dietary cholesterol along with saturated fats results in accumulation of intracellular cholesterol and its ester in the body tissues as coconut oil contains approximately 92% of saturated fatty acids (FA): of short chain 15%, medium chain 64.2% and long chain 12.2% [11, 18]. Antihyperlipidemic agents which are active in cholesterol induced hyperlipidemic model function by one or more mechanisms. Earlier researchers have reported the role of hydroxy citric acid as an appetite suppressant which is one of the constituents of the extract of *Garcinia indica*. The activity also could be the result of synergism between several constituents like garcinol, anthocyanins along with hydroxyl citric acid present in the extract.

The maximum activity against hyperlipidemia was exhibited by whole methanolic extract of *Garcinia indica* at the dose of 400 mg/kg/day. The present findings leading to the significant antioxidant and antihyperlipidemic activity can be explored further with respect to its mechanism of action to develop good antihyperlipidemic agents.

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