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Research article

Hypolipidemic evaluation of *Averrhoa bilimbi* leaf ethanolic extracts on streptozotocin induced diabetic rats

Azeem A K*[1], B M Vrushabendraswami[2]

[1]J J T University, Research Scholar.
[2]Professor, East Point College of Pharmacy, Bangalore.

Abstract

Averrhoa bilimbi belongs to the family *Oxalidaceae* is a common plants found in the southern part of India. Their fruits were edible and it is consumed by the public. Traditionally various parts of these plants especially fruits and leaves were widely used by the ethnic communities in the treatment of various disorders. Coronary heart disease is a major cause of death at least in the developed countries because of various reasons including changing food habits and various other factors. The role of lipids like Total cholesterol, Triglycerides, Low density lipoproteins, very low density lipoproteins and high density lipoproteins in this disorder is widely established. Here an attempt is made to screen the leaf ethanolic extract of this plant towards anti hyperlipidemic property on streptozotocin induced diabetic rats. The plants were collected and authentified and dried with special care. Extraction was done by using soxhlet apparatus with 90% ethanol as the solvent. Qualitative phytochemical evaluation revealed the presence of glycoside, tannins and phenolics, Flavonoids, saponins, triterpenes and carbohydrates. In the antihyperlipidemic activity on Streptozotocin induced diabetic rat model all the doses of extracts tested showed significant results. In fact in some parameters the ethanolic fraction at a dose of 400mg/kg body weight was found to be more potent than the standard drug Atorvastatin on various parameters.

Key words: *Averrhoa bilimbi,* Streptozotocin, Hypolipidemic property.

***Corresponding author: A K Azeem**, Dept of Pharmacognosy, Al Shifa College of Pharmacy, Poonthavanam Post, Perinthalmanna, Malappuram, Kerala, India.

1. Introduction

Plants were widely used as medicines from the beginning of the early civilization itself. In fact in this developed modern world also nearly 80% of the world population is dependable to medicines which were obtained from the plant sources. This fact is approved by WHO[1, 2]. Coronary heart disease is a major cause of death at least in the developed countries because of various reasons including changing food habits and various other factors. The role of lipids like Total cholesterol, Triglycerides, Low density lipoproteins, very low density lipoproteins and high density lipoproteins in this disorder is widely established. Hyperlipidemia is a condition where excess

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of fatty substances called lipids, predominantly cholesterol and triglycerides are present in the blood. It is also known as hyperlipoproteinemia because these fatty materials travel in the blood attached to proteins[3, 4]. Averrhoa bilimbi is a small attractive tree which belongs to the family Oxalidaceae and it produces many fruits which are sour in taste but edible[5, 6]. The plant was commonly known as bilimbi in English and having a wide range of ethnomedical uses. The tree is very attractive, long lived and may reaches from 5-10 m in height. It has a short trunk dividing into a number of upright branches. The synthetic drugs available for the treatment of hyperlipidemia are not showing good patient compliance because of their cost and possible adverse effects. Here an attempt is done towards the screening of the leaf ethanolic extract of Averrhoa bilimbi towards hypolipidemic evaluation using streptozotocin induced diabetic rats.

2. Methodology

Plant Collection

The leaves of *Averrhoa bilimbi* were collected from Nedumangadu Taluk of Kerala state just before the commencement of rainy season. It was authenticated and a voucher specimen (ACP/COG/142) was deposited in the herbarium of Department of Pharmacognosy, Al Shifa College of Pharmacy, Perinthalmanna.

Drying and Powdering

After collection, the leaves of *Averrhoa bilimbi* were washed thoroughly with running water separately to get rid of all the impurities adhered with it. The leaves were separated and dried under shade and then using hot air oven at a temperature not exceeding 60^{o} C. Precautions were taken to avoid the contamination and chances of fungal and insect attacks during drying. After drying the leaves of the plants were powdered using a blender and sieved to a coarse form.

Ethanol Extraction

The air dried and coarsely powdered leaves of *Averrhoa bilimbi* (500grams) was extracted with petroleum ether in a soxhlet apparatus. The soxhlet extraction process was carried out until the solvent found to be colourless. Then the solvent was filtered and concentrated under reduced pressure to get *Averrhoa bilimbi* leaf ethanolic ether extract (ABLE). The percentage yield was calculated with reference to the air dried drug.

Qualitative Phyto Chemical Evaluation

The extract obtained by soxhlet extraction procedure is subjected to preliminary phytochemical tests in order to identify the nature of chemical constituents.

Hyperlipidemia in Streptozotocin induced diabetic rats

Diabetes mellitus is a complex disorder which is characterized by hyperglycemia resulting from malfunction in insulin secretion or insulin action. Both will results in the impaired metabolism of glucose, lipids and protein. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs of our body. In diabetic rats, the utilization of impaired carbohydrates may leads to accelerate lipolysis that ultimately leads to hyperlipidemia. The long term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of

autonomic dysfunction, including sexual dysfunction etc. People with diabetes are at an increased risk of cardiovascular, peripheral vascular and cerebrovascular disease. Several pathogenetic involvements are processed in the development of diabetes. These include processes which destroy the beta cells of the pancreas with subsequent insulin deficiency, and others which may results in resistance to insulin action. The abnormalities of carbohydrate, fat and protein metabolism are due to deficiency in the action of insulin on target tissues resulting from insensitivity or lack of insulin.

Experimental procedure for *Averrhoaa bilimbi* [7]

Wister Albino rats of either sex weighing between 200-250g were used for the study. They were obtained from the animal house of Al Shifa College of Pharmacy and housed in dark and light phase of equal hours. They were provided with standard pellet diet and water prior to the experiment. Streptozotocin (STZ) was dissolved in cold normal saline immediately before use. Diabetes was induced in rats by intraperitoneal administration of streptozotocin at a dose of 50 mg/kg. Forty eight hours after streptozotocin administration, blood samples were withdrawn from tail tip and glucose levels were determined to confirm diabetes. The rats were divided into five groups as follows. The treatment was done daily for 21days. Blood was collected from the tail for glucose estimation, just before drug administration on 1st day and 1 h after sample administration on the 21st day. Triglycerides, cholesterol, HDL cholesterol, and LDL-cholesterol were estimated from the serum biochemically using standard kits.

Group 1 serves as control receives 5%CMC and Normal saline
Group 2 Received Streptozotocin 50mg/kg ip
Group 3 STZ + ABLE 200mg/kg body weight
Group 4 STZ + ABLE 400mg/kg body weight
Group 5 STZ + Atorvastatin 10mg/kg body weight

3. Results and Discussion

Preliminary phytochemical evaluation of the ethanolic extract of *Averrhoa bilimbi* revealed the presence of glycosides, tannin and phenolics, saponins, flavonoids, terpenoids and carbohydrates. Antihyperlipidemic evaluation was done by using streptozotocin induced diabetic rats. Here the animals were divided into five groups and the extract tested was on the dose 200 and 400mg/kg body weight. Lipid profiles were estimated biochemically. All the extracts tested showed good response but the extent of activity varies with different parameters. In some cases like HDL it was found to be A significant reduction in the total cholesterol and triglycerides was found in ABLE 400mg/kg. In the case of HDL which is said to be good cholesterol there showed significant increase by ABLE 400mg/kg. In LDL and VLDL the positive response was indicated by ABLE 400mg/kg. The results were illustrated in table no. 1.

Statistical Analysis

All the values expressed are in Mean ± Standard Error mean, the number of animals used is 6. The comparison was done with one way analysis of variance followed by Dunnets multiple comparison test against control with SPSS software. P values >0.01 was considered statistically significant.

Table No. 1. Anti hyperlipidemic activity of *Averrhoa bilimbi* leaf ethanolic extract on Streptozotozin induced diabetic rats.

Test	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL(mg/dl)
Control	88.66±1.21	79.91±3.32	39.68±0.97	30.87±0.24	15.97±0.18
Diabetic Control	214.65±2.44	167.76±2.43	21.22±0.39	153.94±0.67	33.93±0.15
STZ + ABLE 200mg/kg	182.70±1.45	150.66±1.79	30.87±0.49*	57.14±1.07*	25.89±0.47
STZ + ABLE 400mg/kg	105.65±3.21**	109.23±2.14*	36.50±0.15**	54.55±1.14*	27.07±0.31
STZ + Std 10mg/kg	117.8±5.32*	110.83±3.64*	35.87±0.45*	59.05±0.98*	22.34±0.45**

Values are expressed in Mean ± Standard Error Mean for six animals in each group *=P <0.05, **=P<0.01 on comparision with control

Conclusion

Flavonoids, Phenolic compounds as well as some glycosides were reported to have anti hyperlipidemic property. These compounds were idendified in the various extracts of *Averrhoa carambola* and *Averrhoa bilimbi*. Here the evidenced activity may be due to the presence of flavonois. The isolated compounds were also having the flavonoidal nature. However a detailed study is required to elucidate the possible mechanism for the above mentioned activity as well as isolate more constituents from this plant, which will help the society in getting a new anti hyperlipidemic drug from the nature with better patient acceptance.

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