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Hypolipidemic activity of *Piper betel* in high fat diet induced hyperlipidemic rat

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ABSTRACT

Objective: To evaluate the hypolipidemic effect of *Piper betel* (*P. betel*) in high fat diet induced hyperlipidemia rat. **Methods:** The methanol leaf extract was tested for hypolipidemic effect in the albino rats at the selected optimum dosage of 250 mg/kg body weight and administered orally. Adult male albino rats of six numbers in each group were undertaken study and evaluated. **Results:** In group II animals, the activity levels of serum total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and very low density lipoprotein-cholesterol (VLDL) were significantly enhanced when compared to that of normal rat. **Conclusion:** It could be said that the methanolic leaf extract of *P. betel* exhibited a significant hypolipidemic effect.

1. Introduction

Diseases of the cardiovascular system are the most common cause of death. Lifestyle changes have a significant impact on the health of the people. The modernization of societies appears to result in a dietary pattern that is high in saturated fats and refined sugars and is low in fibres content[1]. It is now established that hyperlipidemia represents a major risk factor for the premature development of atherosclerosis and its cardiovascular complications. Hyperlipidemia is a disorder characterized by the increase in blood lipoprotein or cholesterol levels. Atherosclerosis is a common condition in both developed and developing countries and is now recognised to be an inflammatory condition leading to the development of ischemic heart diseases, cerebrovascular diseases and peripheral vascular diseases. Cardiovascular diseases (CVD) account for 29% deaths worldwide in 2005[2]. The major risk factor for CVD is hypertension, hypercholesterolemia, diabetics and obesity. Hypertensive individual number in India is projected to increase two fold by 2025[3].

Hyperlipidemia contributes drastic threat towards the spread and expansion of atherosclerosis and coronary heart diseases (CHD). Significant impairment of lipid profiles is responsible for the onset of CHD. Ischemic heart disease is a major risk factor in the pathogenesis of preoperative adverse cardiovascular events which lead to significant morbidity and mortality within the high risk surgical patient population[4]. Recent studies have shown that lipid associated disorders are not only attributed to the total serum cholesterol, but also to its distribution among different lipoproteins. The low density lipoproteins (LDL) are the major carriers of cholesterol towards tissues having atherogenic potential, while the high density lipoproteins (HDL) carry cholesterol from peripheral tissues to the liver. HDL thus gives protection against many cardiac problems and obesity. Although genetic factors recline behind these lipid disorders[5]. The treatment of hyperlipidemia depends

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on the patient's cholesterol profile. Many antihyperlipidemic agents like statin, fibrates, niacin, bile acids, ezitimibe etc reduce cholesterol level with different condition[6]. Currently available hypolipidemic drugs have been associated with a number of side effects. The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function[7]. Since ancient times plants have been exemplary sources of medicine. Plants still constitute one of the major raw materials in drugs for treating various ailments of human being, although there has been significant development in the fields of synthetic drug chemistry and antibiotics. During the last two decades, considerable changes have taken place in the medicinal system all over the world. Because of the general awareness of the widespread toxicity and harmful after-effects associated with the long term use of synthetic drugs and antibiotics, drugs from natural sources are being preferred. According to the World Health Organization[8], a medicinal plant is any plant in which, one or more of its organs contains substances that can be used for the synthesis of useful drugs. This definition distinguishes those plants whose therapeutics properties and constituents have been established scientifically from plants that are regarded as medicinal but which have not yet been subjected to thorough investigation. The term herbal drug determines the part/parts of a plant used for preparing medicines (for examples: leaves, flowers, seeds, roots, barks, stems, *etc*.). Furthermore, WHO[9], defines medicinal plant as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products. Medicinal plants contain biologically active chemical substances such as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds[10,11], (which have curative properties. These complex chemical substances of different compositions are found as secondary plant metabolites in one or more of these plants. Tyler[12], has reported that plants also contain certain other compounds that moderate the effects of the active ingredients. Plants and many plant derived preparations have long been used as traditional remedies and in folklore medicine for the treatment of hyperlipidemia in many parts of the world. There are many plants and their products that have been reputedly and repeatedly used in Indian traditional system of medicine. Recently, the search for appropriate antihyperlipidemic agents have been again focused on plants because of less toxicity, easy availability and easy absorption in the body that may be better treatment than currently used drugs. Traditional system of medicine like Ayurveda, Unani and Chinese prescribe numerous herbal drugs for cardio vascular disorders. Recently herbal hypolipidemics have gained importance to fill the lacunae created by the allopathic drugs. Plant products are generally considered to be less toxic and less prone to side effects than drugs manufactured by chemical synthesis. The potential therapeutic and preventive benefits of plant-based medications have been the subject of extensive studies, and many natural constituents have been uncovered with significant pharmacologic activity[13,14], including agents with antiglycemic, hypolipidemic and antihypertensive properties. *Piper betel* (*P. betel*) L. (Piperaceae) leaves is widely used as a mouth freshener after meal. This plant is extensively grown in Bangladesh, India, Sri Lanka, Malaysia, Thailand, Taiwan and other Southeast Asian countries. Its common name is betel in English, paan in India and Vettrilai in Tamilnadu. The oil consists of phenols and terpenes. It was also reported that the leaves contain vitamins and significant amounts of all the essential amino acids except lysine, histidine and arginine which occur in traces[15]. *P. betel* leaves possess activity like antidiabetic, antiulcer, antiplatelet aggregation, antifertility, cardiotonic; antitumour, antimutagenic, respiratory depressant and antihelmenthetic[16], wound healing property. *P. betel* is used to treat alcoholism, bronchitis, asthma, leprosy and dyspepsia, antihistaminic, antioxidant property[17], antimicrobial activity, anti-inflammatory[18], radioprotective and immunomodulatory property[19-21].

In the present study, we investigated the effect of *P. betle* methanol leaf extracts on the reduction of cholesterol level in serum.

2. Materials and methods

2.1. Plant material

The leaves of *P. betle* L. (Piperaceae) were collected during the month of February-August, (2012) from in and around Vellore District, Tamilnadu, India. The plant material was cleaned with distilled water and shade dried at room temperature. The leaves were cleaned and shade dried at room temperature and authenticated and a voucher specimen is kept at the Department of Botany, Voorhees

College, Vellore - 632 001, Tamilnadu, India.

2.2. Plant extracts preparation

The shade dried leaves were powdered in an electric blender and was extracted separately to exhaustion in a soxhalet apparatus using methanol solvent system. Leaves extract was filtered through a cotton plug followed by Whatmann filter paper No.1 and then concentrated by using a rotary evaporator at low temperature 40-50 ℃. Extract was preserved in air tight container and kept at 4-5 ℃ until further use. Preliminary screening test was performed at dosage of 50, 100, 150, 200, 250 mg/kg body weight. The methanol leaf extract was tested for hypolipidemic effect in the albino rats at the selected optimum dosage of 250 mg/kg body weight and administered orally.

2.3. Animals

Adult male albino rats of Wistar strain weighing around 180-200 g were purchased from Tamilnadu Veterinary and Animal Sciences University, Chennai, India. The animals were kept in polypropylene cages (three in each cage) at an ambient temperature of (25±2) ℃ and 55%-65% relative humidity. A (12±1) h light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions. They were fed with commercially available rat chow (Hindustan Lever Ltd., Bangalore. India) and had free access to water. The experiments were designed and conducted in accordance with the institutional guidelines.

2.4. Development of high fat diet fed rats

Rat was fed with two dietary regimes such as Normal pellet Diet (NPD) and High fat Diet (HFD). The rat was feeding either NPD or HFD (58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal) ad libitum, respectively, for the initial period of 2 weeks[22]. The composition and preparation of HFD as were described elsewhere[23].

2.5. Experimental design

The studies were conducted in the four groups of animals

Group I: Normal rats.

Group II: Experimental rats (Fat diet food) for 2 weeks

Group III: Methanol leaf extract of *P. betel* at an optimum dosage of 250 mg/kg body wt. for 2 weeks (Normal rat feed)

Group IV: Methanol leaf extract of *P. betel* at an optimum dosage of 250 mg/kg body wt. for 2 weeks (High Fat Diet feed)

2.6. Preparation of serum samples

After the experimental period, the rats in the different groups were sacrificed by decapitation. Blood was collected from the animals and centrifuged. The serum samples were collected in separate containers for biochemical estimations.

2.7. Estimation of biochemical analysis

The biochemical estimation was carried out in our lab by using the following methods. Serum Total cholesterol[24], Triglycerides[25], Serum high density lipoprotein, Serum low density lipoprotein, Serum very low density lipoprotein[26].

2.8. Statistical analysis

The results were expressed in mean ± standard deviation. Statistical analysis was carried out by using one way ANOVA as in standard statistical software package of social science (SPSS).

3. Results

To study the hypolipidemic effect of the methanol leaf extract of *P. betel* on the high fat diet induced male albino rats. The activity levels of serum total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), Serum high density lipoprotein (HDL) and very low density lipoprotein-cholesterol (VLDL-C) were observed in normal

Table 1

Effect of the methanol leaf extract of *P. betel* on serum lipid profile levels (mg/dL), in HFD induced rat.

Groups	Total cholesterol(mg/dL)	Triglycerides(mg/dL)	HDL(mg/dL)	LDL(mg/dL)	VLDL(mg/dL)
Group-I	82.12±1.06	84.26±1.21	35.12±1.83	29.13±0.14	13.90±0.24
Group-II	154.23±2.48*	157.14±2.17*	21.49±0.88*	56.04±2.11*	29.12±1.89*
Group-III	83.27±1.23NS	85.33±1.48NS	36.19±1.21NS	32.15±0.74NS	14.26±0.99NS
Group-IV	89.55±1.65*	98.33±2.45*	30.17±1.51*	41.31±2.81*	17.61±2.89*

Data are expressed as Mean ±SD of 6 individual observations. Statistical significance *P<0.001, NS - Non significant.

and experimental animal. In group II animals, the activity levels of serum total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and very low density lipoprotein-cholesterol (VLDL) were significantly elevated when compared to that of normal groups (Table 1). On the other hand the serum level of Serum high density lipoproteins (HDL) were significantly depleted in the HFD fed rat.

Lipid profiles Viz., total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and very low density lipoprotein-cholesterol (VLDL) were estimated in normal, experimental and plant treated animals. In group-III animals the methanol leaf extract of *P. betel* was administered. *P. betel* alone administered group-III rat did not show any significant alterations in their activities as compared to that of normal animals. In group- IV *P. betel* (250 mg/kg body weight) supplemented later with HFD fed rat showed a significant depletion in the activity levels of total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and very low density lipoprotein-cholesterol (VLDL) as compared to normal group (Table:1). The activity level of HDL was significantly augmented in plant treated group.

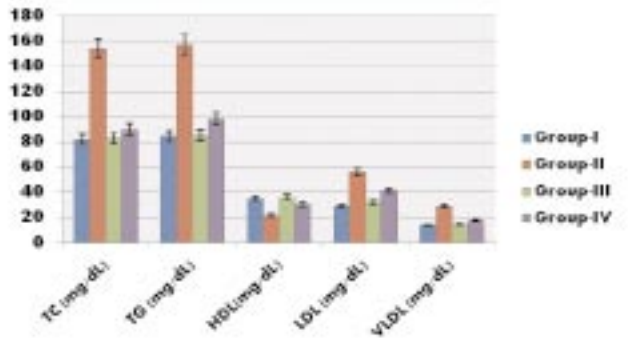

Figure 1. Effect of the methanol leaf extract of *P. betel* on serum lipid profile levels (mg/dL), in HFD induced rat.

4. Discussion

P. betel a well known traditional medicinal plants possesses diverse biological activities and pharmacological function including reducing blood glucose and serum lipids. It has long been used to treat diabetes mellitus and related hyperlipidemia. Hypercholesterolemia, a high cholesterol diet and oxidative stress increase serum LDL levels resulting in increased risk for development of atherosclerosis[27]. Cholesterol is synthesized in all animal tissue. It is important to relate to its role in the stabilization of membrane structures because of its rigid planar structure. It also as a precursor for the synthesis of steroid hormones. Increased amount of cholesterol leads to cardiovascular disease particularly coronary heart disease (CHD)[28].

In the present study, feeding rats with diets rich in cholesterol resulted in increased TC, TG and LDL cholesterol levels. This model was used to study the potential of hypolipidemic effect of supplementations of *P. betel* that contained significant amounts of antioxidants properties. From this study, we found that daily oral administration *P. betel* supplements shows a positive result on significantly reduced total cholesterol levels in plasma after 2 weeks of supplementation. This result agrees with literature where depleted level of HFD fed hyperlipdemia. HDL is directly anti-androgenic and it is believed to remove cholesterol from the developing lesions. LDL is a risk factor and plays a role in several steps of atherosclerosis. A decrease in oxidative stress and protection of LDL from oxidation might therefore be a strategy with great promise for prevention of atherosclerosis associated cardiovascular disease. The intense interest in this area results in part from the generally low toxicity of antioxidants and the hope that treatment with antioxidants might be additive with cholesterol lowering regimes. VLDL particles are smaller than the chylomicrons and also are rich triglycerides though to a lesser extent VLDL particles sizes vary widely, with a concomitant variation of the chemical composition; the larger particles are rich in triglycerides and in apo-c and the smaller particles depleted of TG and surface materials result from the hydrolysis of VLDL by lipoprotein lipase activity. VLDL is the main carrier if triglycerides and it is less harmful than but still can damage the arterial lining. In the present study serum TG levels were significantly elevated in HFD rat. The excess of fat diet increased the TG level which is one of the causes of hardening of arteries.

In conclusion, it could be said that the methanolic leaf extract of *P. betel* exhibited a significant hypolipidemic activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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