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Research Article

HYPOLIPIDEMIC ACTIVITY OF *PSIDIUM GUAJAVA LINN* LEAVES EXTRACTS IN HYPERLIPIDEMIC RATS

SHUBHANGI SHINDE[1*], NIRANJAN CHIVATE[2], PRAMODINEE KULKARNI[3], NILOFER NAIKWADE[4]

[1,2,3]KCT'S Krishna College of Pharmacy, Karad, Dist-Satara, 415110, Maharashtra, India, [4]Aappasaheb Birnale College of Pharmacy, Sangli, Dist-Sangli, 416416, Maharashtra, India. Email: shubhangipatil20@gmail.com

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ABSTRACT

Objective: The objective of present investigation was to evaluate hypolipidemic activity of Psidium *guajava Linn* leaves extracts in hyperlipidemic rats.

Materials and Methods: Hyperlipidemia condition was induced by High Cholesterol Diet (HCD) in wistar rats and these rats were treated with aqueous and methanolic extract of Psidium *guajava leaves* for ten days. At the end of experiment blood serum level of TC, TG and HDL were measured and LDL and VLDL were calculated.

Result: Blood serum level of TC, TG, LDL were elevated and HDL level was reduced in HCD controlled group but brought at control level in hyperlipidemic rats treated with methanolic extract 400mg/kg.body weight p.o.

Conclusion: Aqueous and methanolic extract of leaves of *Psidium guajava L.*exerted a hypolipidemic activity in laboratory animals.

Keywords: Hypolipidemic, HCD, TC, TG and HDL

INTRODUCTION

Excessive quantities or improper types of lipid-intake may result in hyperlipidemia which is characterized by an abnormal elevation in one or more of the serum lipids such as total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C) and triglycerides (TG). Hyperlipidemia is considered to be a major risk factor for cardiovascular diseases including atherosclerosis, myocardial infarction, heart attacks, and cerebrovascular diseases [1].

Today in most of the developed and developing countries, hyperlipidemia and thereby atherosclerosis is the leading cause of cardiac illness and deaths.

Hypercholesterolemia and hypertriglyceridemia are independent risk factors that alone or together can accelerate the development of atherosclerosis and progression of atherosclerotic lesions [2].

Plant *Psidium guajava leaves* belonging to family Myrtaceae commonly known as guava is a shrub often grows naturally in the house gardens in the tropical regions.

Traditionally the leaves of the guava tree are used to treat diarrhea, gastric disorders, wound healing, cancer, epilepsy etc.

The leaves of guava are rich in tannins, phenols, triterpines, essential oils, saponins, vitamins. The major active constituents of the drug are flavonoids like quercetin. On the above evidence, the present investigation was planned to study the effect of aqueous and methanolic extracts of *Psidium guajava Linn* leaves in hyperlipidemia induced rats.

MATERIALS AND METHODS

Collection and authentication of plant material

The fresh leaves of plant *Psidium guajava* Lin*n.* used in this study were collected from local area of Sangli, Maharashtra, during the month of September and authenticated by botanist at Department of Botany Kasturbai Walchand College, Sangli, where voucher specimen has been preserved for further identification.

Preparation of aqueous extract

The leaves were dried under shade and powdered to obtain course powder. The powdered leaves were placed in conical flask containing distilled water and chloroform (9:1) for 7 days. Then it was filtered using a piece of clean, sterile, white cotton cloth and evaporated to dryness to yield aqueous (water) extract. The brown colour extract Psidium guajava L .obtained was stored in an airtight container in refrigerator for further experimental studies.

Aqueous extract solution referred to as aqueous extract of *Psidium guajava* L. (AEPG) and prepared by dissolving in distilled water and used for further experimental studies.

Preparation of methanolic extract

The leaves were dried under shade and dried leaves were subjected to size reduction to a course powder by using mixer grinder. This powder was defatted with petroleum ether then filtered. The residue was allowed to dry at room temperature. The leaves powder was exhaustively extracted using methanol in a Soxhlet extractor for 72h. The extract was dried at room temperature till semisolid mass was obtained. The dark yellowish green coloured semisolid residue formed after the complete dryness, was kept in an air-tight and water proof container, which is stored in refrigerator. From this, a fresh stock for daily use was prepared.

Methanolic extract solution referred to as methanolic extract of *Psidium guajava* L. (MEPG) and prepared by dissolving in distilled water and used for further experimental studies.

Animals

Fifty male wistar rats weighing 100-120 g were obtained from the animal house of Appasaheb Birnale College of Pharmacy, Sangli. They were maintained at standard housing conditions and fed with commercial standard diet (Amrut agro Ltd., Sangli) and provided with water *ad libitum* during the experiment.

Acute Oral Toxicity study of Aqueous and Methanolic extract of leaves of *Psidium guajava* L.

Acute oral toxicity study was carried out according to the OECD guidelines.

Female rat were randomly selected housed individually in polypropylene cages maintained under standard condition (12h light and 12h dark cycle; 30-60% humidity).The animals were fed with standard rat pellet diet & provided water ad. libitum. Animals were fasted 24 hrs prior to dosing. Test substances were administered in a single dose by gavage using orogastric tube. Test substance is

administered to the set of five female rats at predetermined doses e.g. (2000 mg/kg and 5000 mg/kg body wt. for limit test). Animals were observed individually after dosing at least once during first 30 minutes, periodically during the first 24 hrs, with special attention giving during the first 4 hrs [3,10].

Absence of the compound related mortality will determine the next step. No mortality was seen with this dose. 1/10th of this dose was used in subsequent study. As per OECD guidelines the substance might be considered to have an LD50 value above 2000 mg/kg and 5000 mg/kg body wt.

Dose selection of aqueous and methanolic extract of leaves of *Psidium guajava L.*

From results of Acute oral toxicity study, it was found that an aqueous and methanolic extract of *Psidium guajava* L. was safe at limit dose 5000 mg/kg and 2000 mg/kg, No mortality was seen with this dose. Therefore doses below 1/10th of this dose *i.e.* 400 mg/kg and 200 mg/kg for both aqueous and methanolic extract were used in subsequent study.

High Cholesterol Diet (HCD) induced hyperlipidemia in rat

Preparation of High Cholesterol Diet (HCD)

Diet cocktail contained 100 g cholesterol and 50 g cholic acid in 1 liter of coconut oil. All ingredients were of the highest analytical grade and supplemented with egg yolk [4,5,6,7]. Hypercholesterolemia was induced in rats by daily intragastric administration of cocktail diet (1ml/100gm) and also fed egg yolk. Serum cholesterol level was monitored after a 14 hrs fast by collecting blood samples by retro-orbital method under light anesthesia with diethyl ether. Body weight was recorded on every 5th day. The amount of food intake was measured every day. After induction of hypercholesterolemia confirmed at a serum cholesterol level greater than 140 mg/dl, the rats were further randomly subdivided into six groups.

Experimental Groups

Each group contained six animals (n=6). Inducing control group receives cocktail diet. (1 ml/100gm) for 20 consecutive days. Group I served as control received normal diet from 1st to 20th day. GroupII received only HCD from 1st to 20th day. Group III received HCD from 1st to 20th day & simvastatin (10 mg/kg p.o.) from 11th to 20th day. Group IV & V received HCD from 1st to 20th day & also AEPG 200(mg/kg p.o.) & 400(mg/kg p.o.) received from 11th to 20th day rasp. Group VI & VII received HCD from 1st to 20th day & also MEPG 200(mg/kg p.o.) & 400(mg/kg p.o.) received from 11th to 20th day resp.

Estimation of blood cholesterol

Rats were fasted 08 hours after final sample treatment; blood was collected by retro orbital sinus puncture under light ether anesthesia. The blood was centrifuged at 3000 rpm for 10 minutes. Serum was separated and estimated for lipid parameters such as cholesterol, triglycerides, LDL, HDL and VLDL and analysed by colorimeter.

Estimation of lipid profile in Serum

Total Cholesterol, Triglyceride, HDL Cholesterol was estimated by using available diagnostic kits and expressed as mg/dl. And VLDL, LDL Cholesterol, A.I. were calculated by formula.

1) LDL Cholesterol [8]

2) LDL Cholesterol was calculated by using Friedewald's formula.

LDL Cholesterol = Total Cholesterol – (HDL Cholesterol – VLDL Cholesterol)

LDL Cholesterol level in serum was expressed as mg/dl**.**

2) VLDL Cholesterol

VLDL Cholesterol was calculated Friedewald's formula

$$\text{VLDL Cholesterol} = \frac{\text{Triglycerides}}{5}$$

VLDL Cholesterol level in serum was expressed as mg/dl.

3) Atherogenic Index

The atherogenic index was calculated using the formula.

$$\text{Atherogenic Index} = \frac{\text{Total Cholesterol} - \text{HDL Cholesterol}}{\text{HDL Cholesterol}}$$

Statistical Analysis

All the results were expressed as mean ± SEM. The Statistical significance between means was analyzed using one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison post-test using Graph Pad software. *P-values < 0.05* were considered significant.

RESULT

Effect on blood cholesterol level

In the present study, the result obtained from exclusive high cholesterol fed rats were compared with those of the normal group. While the values for the group that were fed HCD plus extract of Psidium guajava leaves were compared with those of HCD group. (Table No. 1)

Table 1: Effect of Psidium guajava Linn. leaves extracts on serum lipid profile in HCD induced hyperlipidemia in rat

S. No.	Group	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	A.I.
1.	Normal Control	77.77± 4.06	70.15± 6.16	34.81± 1.59	14.03 ± 1.23	27.08± 2.91	1.24 ± 0.11
2.	HCD Control	183.33± 6.67# (↑135.73)	148.91± 6.53# (↑112.30)	18.51± 1.34# (↓46.32)	29.78± 1.31# (↑112.18)	135.04± 5.48# (↑398.67)	9.06± 0.53# (↑630.64)
3.	HCD+ Simvastatin 10mg/kg.	103.33± 7.14* (↓43.64)	97.22± 5.28* (↓34.16)	31.48± 1.53* (↑70.07)	19.60± 1.04* (↓34.18)	52.09± 4.14* (↓61.43)	2.31± 0.15* (↓74.50)
4.	HCD+ MEPG, 400 mg/kg.	133.33± 11.46* (↓27.28)	112.74± 6.34* (↓24.29)	28.88± 2.50* (↑56.02)	22.55± 1.27* (↓24.28)	85.56± 12.23* (↓36.64)	3.63± 0.19* (↓59.93)
5.	HCD + MEPG, 200 mg/kg.	150± 5.77* (↓18.19)	125.49± 4.72* (↓15.73)	26.29± 2.10* (↑42.03)	25.09± 0.94* (↓15.75)	98.61± 3.35* (↓26.98)	4.79± 0.27* (↓45.16)
6.	HCD + AEPG, 400 mg/kg.	146.66± 8.03* (↓20.01)	118.62± 5.57* (↓20.35)	27.04± 1.94* (↑46.08)	23.72± 1.11* (↓20.35)	96.02± 5.31* (↓28.89)	4.46± 0.14* (↓50.77)
7.	HCD + AEPG, 200 mg/kg.	153.33± 4.94* (↓16.37)	133.32± 4.48 (↓10.48)	22.59± 1.57 (↑22.04)	26.67± 0.89* (↓10.44)	104.08± 4.57* (↓22.92)	5.92± 0.45* (↓34.66)

Values are presented as the mean ± S.E.M.The data were evaluated by one-way ANOVA using Dunett's test. *p<0.05 was taken as the criterion of significance. Values in brackets indicate % increase or decrease.

#p<0.05 when compared to Normal (untreated) control; *p<0.05 when compared to HCD control

It was observed that feeding rats with HCD significantly increased the TC, TG, and LDL-C level in serum as compare to rats on normal diet.

However dramatic decrease of serum TC and TG levels were observed in all *Psidium guajava* extracts and simvastatin dosing group compare to those of HCD control with dose dependent pattern. The serum TC level of simvastatin, ME 400, ME 200, AE 400, AE 200 mg/kg of PG extracts dosing group were decreased by 43.64, 27.28, 18.19, 20.01, 16.37% compared to HCD control rat respectively. Serum TG level of simvastatin, ME 400, ME 200, AE 400, AE 200 mg/kg of *Psidium guajava* extracts dosing group were decreased by 34.19, 24.29, 15.73, 20.35, 10.48% compared to HCD control rat respectively.

A significant increase of serum LDL-C level i.e.398.67% was detected in HCD group compared to that of animals on normal diet. However dramatic decrease of serum LDL-C levels were observed in groups treated with PG extract and Simvastatin. groups compared to those of HCD group with dose dependent pattern. The serum LDL-C levels on Simvastatin, ME 400, ME 200, AE 400, AE 200 mg/kg of Psidium guajava extracts dosing group were decreased by 61.43, 36.64, 26.98, 28.89, 22.92%. compared to HCD control group respectively.

A significant decrease of serum HDL-C level i.e. 97.13% was detected in HCD group compare to that of animals on normal diet. However there was marked increase in serum HDL-C level in all *Psidium guajava* extract and simvastatin dosing groups. HDL-C level of Simvastatin ME 400, ME 200, AE 400, AE 200 mg/kg of *Psidium guajava* extract treated groups were increased by 70.02, 56.02, 42.03, 46.08, 22.04% compared to HCD group respectively. A significant increase in A.I. i.e. 630.64% was detected in HCD group when compared to vehicle group and significant decrease in A.I.($p<0.05$) were observed in all *Psidium guajava* extracts treated as well as Simvastatin dosing groups in dose dependent pattern.

DISCUSSION

Hyperlipidemia is a major risk factor that can facilitate the development of coronary artery diseases and progression of atherosclerotic lesions. As increase in lipid profiles is a contributing factor to the pathogenesis of atherosclerosis associated cardiac disorders. [1]

The induction of hyperlipidemia, particularly hypercholesterolemia by feeding experimental animals a high cholesterol diet (HCD), has been suggested by many scientists as a reliable model for atherosclerosis in humans.

HCD causes marked hypercholesterolemia i.e. increased level TC, LDL-C, VLDL-C. Elevated lipid level specially hypercholesterolemia results due to increased absorption in the gut or endogenous synthesis [9].

Therefore in this study we evaluated the hypolipidemic and antioxidant activity of *Psidium guajava* L. leaves extract in wistar rat model in which hyperlipidemia was induced by HCD

The increase in body weight after hyperlipidemia is generally known and it can be used as one of the aspect of the animal models to develop antiobesity agents similar to those of previous study. A significant increase in body weight was detected in HCD control group compare to normal control. In present study, however no favorable changes in body weight were detected after *Psidium guajava* L. leaves extract dosing.

Rat fed with cholesterol rich diet showed elevated TG, TC and LDL-C levels, as well as increased A.I. Furthermore the HDL-C levels in these rats were reduced. In the present study, Simvastatin was used as positive control because it is potent hypolipidemic drug with known mechanism of action and effects including inhibition of HMG-COA reductase, the rate limiting step in cholesterol biosynthesis, and resultant increase in LDL receptors. In addition it possesses an inhibitory effect on the inhibition of LDL oxidation. . Simvastatin was shown significant reduction in TG, TC, and LDL-C whereas HDL-C was markedly increased.

However the treatment of hyperlipidemic rats with methanolic and aqueous extract of *Psidium guajava* L. at a different doses 200 mg/kg, and 400 mg/kg of body wt. along with cholesterol diet shown significantly decrease ($p<0.05$) in serum TG, TC and LDL-C levels. As well as significant decrease in A.I.value whereas HDL-C level was significantly decreased ($p<0.05$).

Furthermore methanolic extract at a dose of 400 mg/kg of body wt. showed highly significant effect than all other treatment groups.

CONCLUSION

The present study demonstrated that, aqueous and methanolic extract of leaves of *Psidium guajava* L. exerted a hypolipidemic activity in laboratory animals. The administration of these extracts significantly decreases the serum levels of TC, TG and LDL-C. These results suggest that *Psidium guajava* L. leaves extract might prevent hyperlipidemic atherosclerosis and reduce the risk of coronary heart disease**.**

From the results obtained in this study, it is concluded that *Psidium guajava* L. leaves possesses hypolipidemic effect. Furthermore methanolic extract is more potent than aqueous extract. Flavonoids of *Psidium guajava* L. leaves play a promising role in the treatment of hyperlipidemia and atherosclerosis. Further studies on the activity guided isolation of the extract of this plant may yield valuable therapeutic compounds which may be useful for developing powerful hypolipidemic agent.

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