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Effect of Vanillin on lipid profile in a model of hyperlipidemia, a preliminary study

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To evaluate the effect of vanillin on the lipid profile of high fat diet induced hyperlipidemia in rats, the hyperlipidemia was induced by feeding cholesterol-rich high fat diet for 45 days in wistar rats of either sex. The reduction in the triglycerides and VLDL-C was significant at 200 & 400 mg/kg dose of vanillin compared to atorvastatin group. Reduction in total cholesterol was significant at 200 and 400 mg/kg doses compared to hyperlipidemic control. The results demonstrate that vanillin at a dose of 200 and 400 mg/kg body weight lowers the serum triglyceride, VLDL-C and total cholesterol level significantly in high fat diet induced hyperlipidemic rats. However there was no significant effect on the lipid profile at 100 mg/kg dose. There were no statistically significant changes in the HDL-C and LDL-C levels at any of the given doses.

Keywords: Hypertriglyceridemia, Lipid profile, Vanillin

Hyperlipidemia is a condition associated with increased level of lipids and cholesterol in plasma leading to various disorders including coronary artery disease. Hyperlipidemia is a highly predictive risk factor for atherosclerosis, coronary artery disease and cerebrovascular disease[1]. Statins form the main stay of the treatment. However they are associated with side effects like headache, bowel upset, nausea, muscle tenderness and sleep disturbances. Rise in creatinine phosphokinase and serum transaminase levels can occur, hence monitoring of these parameters is necessary with statin therapy. Fibrates, bile acid sequestrants and nicotinic acid constitute other modalities of treatment but control of lipid levels is far from satisfactory[2]. Hence research is being conducted to pursue better drugs in this regard.

Vanilla (*Vanilla planifolia*), a monocotyledonous orchid native of Central America, is grown for the attractive aroma produced by its fruit[3]. Because vanilla is so much in demand, and expensive, synthetic vanilla is often used instead of natural vanilla. In fact, 97% of vanilla used as a flavor and fragrance is synthetic. Synthetic vanilla contains only one organic component, vanillin, that possesses the flavor and fragrance that we most associate with vanilla. Natural vanilla extract is a mixture of several hundred different compounds in addition to vanillin. Vanillin is one of the primary chemical components of the extract of the vanilla bean. It is a pleasant aromatic compound that occurs naturally in vanilla beans; it is a fine, white to slightly yellow crystal, usually needle-like, having an odour and taste suggestive of vanilla. Synthetic vanillin is used as a flavoring agent in foods, beverages, and pharmaceuticals[4]. Studies on vanillin have demonstrated that it has antimutagenic[5], anti-invasive and antimetastatic[6,7] properties. Free radical scavenging activity of vanillin has been recently demonstrated. Inhibition of oxidation of human low-density lipoproteins by this phenolic substance has also been reported[8]. Hence this preliminary study has been conducted to evaluate the effect of vanillin on the lipid profile of high fat diet induced hyperlipidemic rats.

Materials and Methods

Animals—Albino wistar rats of either sex, inbred in the institutional central animal house were used. Rats were housed in clean polypropylene cages, three rats in each cage, in a controlled environment (24-26°C) with a 12:12 h L:D cycle with standard chow containing fat 4.15%, protein 22.15%, carbohydrates 4% (supplied by Amruth Laboratory Animal Feed manufactured by Pranav Agro Industries Ltd., Sangli) and water *ad libitum*. The rats were

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allowed to acclimatize to these conditions for one week. The experiment was performed during the light phase of the cycle (1000-1700 hrs). The animals were maintained as per the CPCSEA guidelines and regulations. The study was approved by the Institutional Animal Ethics Committee.

Study drugs—Vanillin [IUPAC name 4-hydroxy-3-methoxybenzaldehyde, chemical formula $(CH_3O)(OH)C_6H_3CHO$, molecular weight of 152.15] was obtained from Hi Media Laboratories. Atorvastatin produced by Biochem (India) Limited, Hyderabad was used as standard drug.

Induction of hyperlipidemia—Hyperlipidemia was induced by feeding the rats with cholesterol-rich high fat diet for 45 days.

Preparation of cholesterol rich high fat diet—Deoxycholic acid (5 g) was mixed thoroughly with 700 g of powdered rat chow diet. Simultaneously cholesterol (5 g) was dissolved in 300 g butter (Amul). This mixture of cholesterol and butter was added slowly into the powdered mixture of deoxycholic acid and rat chow to obtain a soft homogenous cake. This cholesterol-rich high-fat diet (HFD) was molded into pellets of about 3 g each and was used to feed the animals *ad libitum*[9,10].

Experimental design—Rats were randomly assigned to five groups of six rats each. For 45 days they were fed on a high fat diet. The animals did not receive any treatment for the first 15 days. This was done to ensure that the rats become hyperlipidemic before the initiation of treatment. During the latter 30 days the animals were treated with drug/vehicle. The feeding and treatment schedule for all the groups are shown in Table 1. Vanillin was administered in three incremental doses based on previous toxicity study reports[11]. Blood samples were obtained by cardiac puncture of anaesthetized rats after 30 days of treatment. They were collected in simple glass tubes (for separation of serum). Serum was analyzed for total cholesterol, triglycerides and HDL-C according to standard method[12]. LDL-C and VLDL-C were calculated using Friedewald formula[13].

Table 1—Feeding and treatment schedule

Group	Treatment
I	Normal diet (for 45 days)
II	High fat diet (for 45 days)
III	High fat diet (for 45 days) plus atorvastatin 10mg/kg/day (for the latter 30 days)
IV	High fat diet (for 45 days) plus Vanillin 100mg/kg/day (for the latter 30 days)
V	High fat diet (for 45 days) plus Vanillin 200mg/kg/day (for the latter 30 days)
VI	High fat diet (for 45 days) plus Vanillin 400mg/kg/day (for the latter 30 days)

Statistical analysis—The data were presented as mean ± SE and analyzed using One-way ANOVA followed by Tukey's multiple comparison test.

Results

The results are presented in Table 2.

Feeding of HFD significantly increased total cholesterol, serum triglyceride, serum VLDL-C and serum LDL-C compared to normal group over a period of 45 days. Atorvastatin (10 mg/kg) and vanillin (200 and 400 mg/kg) significantly reduced total cholesterol levels compared to HFD control ($P<0.001$).

Vanillin (200 and 400 mg/kg) showed a significant reduction in serum triglyceride and serum VLDL-C levels compared to HFD control and atorvastatin group ($P<0.001$). There were no significant changes in the total cholesterol, serum triglyceride and VLDL-C levels with vanillin (100 mg/kg).

There were no significant changes in serum HDL-C levels in any of the treatment groups. Atorvastatin significantly reduced serum LDL-C compared to HFD control but vanillin failed to produce significant changes in serum LDL-C levels.

Discussion

Hyperlipidemia indicates the onset of abnormalities in lipid metabolism secondary to the manifestation and progression of atherosclerosis. Hypertriglyceridemia is characterized by elevated triglyceride levels (>200 mg/dL)[14]. An increase of 89 mg/dL in the triglyceride level is associated with a 30% increase in coronary heart disease in men and a 70% increase in women[15]. As predictors of risk of cardiovascular disease, levels of triglycerides are independent of HDL-C and total cholesterol[16] but the risk is highest when LDL-C levels are lower[15]. Monotherapy with statins has failed in controlling isolated hypertriglyceridemia. Niacin and statin combinations are recommended by National Cholesterol Education Program (NCEP) for patients with high triglycerides[17]. However use of niacin is associated with side effects, including flushing, dizziness, palpitation, tachycardia, hyperglycemia and gout[2]. Use of medicinal plants as a pharmacologic modality in preventing alteration in lipid metabolism

Table 2—Effect of Vanillin on the lipid profile in normal and high fat diet fed rats
[Values are mean±SE]

Group	Treatment	Total cholesterol (mg/dL)	S. triglyceride (mg/dL)	S. VLDL (mg/dL)	S. HDL (mg/dL)	S. LDL (mg/dL)
I	Normal Diet	70.50±0.957	87.33±6.227	17.66±1.166	21.67±0.494	31.166±1.712
II	High Fat Diet(HFD)	130.67±2.929[ae]	187.0±6.981[a]	36.833±1.335[a]	30.50±3.879	59.166±1.627[a]
III	HFD+Atorvastatin (10 mg/kg)	105.83.±2.574[abef]	164.67±6.125[a]	32.70±1.273[a]	30.00±1.183	43.133±2.875[b]
IV	HFD+Vanillin (100 mg/kg)	120.83±4.037[ace]	200.50±22.745[a]	40.10±4.548[a]	34.17±2.007[a]	46.566±6.779[a]
V	HFD+Vanillin (200 mg/kg)	93.50±2.277[abdefh]	83.00±6.09[bcdfgh]	16.500±1.223[bcdfgh]	29.33±1.406	47.333±0.610[a]
VI	HFD+ Vanillin (400 mg/kg)	89.28±3.133[abdefh]	77.65±6.224[bcdf]	15.53±1.004[bcdfg]	27.33±1.406	46.333±4.743[a]

$P<0.05$ compared to: [a] group I, [b] group II, [c] group III, [d] group IV

$P<0.001$ compared to: [e] group I, [f] group II, [g] group III, [h] group IV

has received wide attention from several workers[18]. Rats fed with a diet supplemented with 5 g cholesterol and 5 g deoxycholic acid in butter for 45 days served as the experimental model[9]. The mechanism of action of deoxycholic acid is two-fold: an increase in cholesterol absorption and a concomitant suppression of cholesterol 7α-hydroxlyase activity that results in decreased cholesterol excretion. Deoxycholic acid improves cholesterol absorption by its emulsifying property[19]. From the obtained result it was observed that feeding the animals on HFD significantly increased the total cholesterol, triglyceride, VLDL-C and LDL-C level in serum ($P<0.05$) as compared to rats on normal diet. When vanillin was co-administered with HFD at a dose of 200 and 400 mg/kg during the latter 30 days of study, there was a significant decline in total cholesterol, triglyceride and VLDL-C levels compared to HFD control and atorvastatin group. However no significant changes were noted in HDL-C and LDL-C levels. No significant changes were noted at 100 mg/kg dose of vanillin in any of the parameters. Free radical scavenging could be the possible mechanism for lowering of triglycerides & VLDL-C levels. This property of vanillin can have potential benefits in familial hypertriglyceridemia[20]. Rats treated with atorvastatin also showed marked reduction in all serum lipoproteins as compared with HFD group.

The study could have had more insights into the effects of vanillin in dyslipidemia if

(a) the HFD had reduced HDL levels (which were not reduced); and

(b) there was one more group receiving a combination of vanillin and atorvastatin.

Results of the present study reveal that 200 and 400 mg/kg of vanillin improved the serum lipid profile in rats by decreasing serum total cholesterol, triglyceride and VLDL-C. This finding provides some biochemical basis for the use of vanillin as an anti-hypertriglyceridemic agent having preventive and curative effect against hypertriglyceridemia. Further studies are required to gain more insight into the possible mechanism of action of vanillin.

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