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Lowering of platelet aggregation and serum eicosanoid levels in rats fed with a diet containing coconut oil blends with rice bran oil or sesame oil

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ABSTRACT

The present investigation was undertaken to study the effects of feeding a diet containing blended and interesterified fat to rats on thrombotic parameters such as platelet aggregation and eicosanoid levels in blood serum. Male Wistar rats were fed with a diet containing 10% fat from native; coconut oil (CNO), rice bran oil (RBO), sesame oil (SESO), blended; (CNO+RBO blend (B), CNO+SESO(B), or interesterified oils; CNO+RBO interesterified (I), CNO+SESO(I) for a period of 60 days. Rats given a diet containing blended oil of CNO+RBO(B) or CNO+SESO(B) showed a decrease in rate of ADP induced aggregation of platelets by 34% and 30%, respectively, compared to those fed with CNO. Aggregation induced by collagen was also reduced similarly in rats given blended or interesterified oils of CNO with RBO or SESO. Feeding interesterified oil CNO+RBO(I), and CNO+SESO(I) to rats also resulted in decrease in rate of ADP induced platelet aggregation by 37% and 34%, respectively, compared to rats fed with CNO. The prostacyclin/thromboxane ratio in serum was increased in rats fed with blended and interesterified oil compared to those fed with CNO. These results indicated that CNO when blended or interesterified with RBO or SESO exhibit antithrombotic effects as compared to the effect observed by feeding rats with CNO.

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1. Introduction

Atherosclerosis and thrombosis are two important events responsible for the development and progression of cardiovascular disease (CVD). The interactions of circulating platelets with vascular endothelium play a significant role in the formation of coronary thrombosis [1,2]. Platelet hyperactivity is considered to be a risk factor for thrombosis [1]. Polyunsaturated fatty acids influence thrombotic events and atherogenesis. Dietary lipids modify the fatty acid composition of platelets and thereby influence platelet aggregation [3].

Saturated fatty acids are atherogenic and favor platelet aggregation by decreasing prostacyclin production and increasing thromboxane production. They can thus be considered prothrombotic substances. Polyunsaturated fatty acids reduce platelet and thrombogenic activity of the arterial wall [4,5]. The type and level of eicosanoids synthesized in the system influence the platelet aggregation and thereby modulate thrombotic tendency [6].

The effects of individual fatty acids on thrombotic events have been evaluated in animal models and in human subjects. Studies on human volunteers have shown that *ex vivo* platelet aggregation time as measured by filtragometry was favorably prolonged during intake of diets containing linoleic acid as compared to those taking diet containing stearic or oleic acid [7]. In rats, arterial thrombosis tendency as measured with the aortic loop technique, was lower when given n-6 and n-3 PUFA, whereas SFA with 12–16 carbon atoms promoted arterial thrombus formation. The effects of oleic acid containing diet on platelet aggregation were neutral or antithrombotic compared to SFA diet [8].

Influence of dietary fatty acids on platelet aggregation and vascular function has been linked to prostaglandins generated by platelet and endothelial cells. Thromboxane A_2 is a vasoconstrictor and a powerful inducer of platelet aggregation. This is produced by platelets. Prostacyclin is an antagonist for platelet aggregation and is synthesized by endothelial cells. Both these compounds are synthesized by cyclooxygenase pathway from arachidonic acid and the ratio of these eicosanoids is considered to control thrombosis [9]. When acted up on by phospholipases, arachidonic acid is released from phospholipids. This in turn is used by the enzyme cyclooxygenase and is rapidly converted to the labile cyclic endoperoxides, PGG_2 and PGH_2. Thromboxane

Abbreviations: CNO, coconut oil; RBO, rice bran oil; SESO, sesame oil; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CVD, cardiovascular disease; PPP, platelet poor plasma; PRP, platelet rich plasma; PG, prostaglandin; TX, thromboxane; MDA, malondialdehyde; LPO, lipid peroxide; FFA, free fatty acid

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synthetase and prostacyclin synthetase convert the endoperoxides to biologically active thromboxane A_2 and PGI_2. Although linoleic acid is rapidly converted to arachidonic acid in the liver and in cells such as fibroblast, similar elongation and desaturation does not occur in platelet membranes. A diet high in linoleic acid leads to increased concentrations of linoleic acid in the platelet membrane but decreased arachidonic acid [6].

CNO is commonly used in many parts of the world as cooking oil or is largely consumed by locals in countries which produce the oil. However, in the last few years CNO has been negatively associated with cardiovascular health. To address this issue it is important to investigate the possibility of blending and interesterification of CNO with PUFA or MUFA. Coconut oil, as saturated oil, could lend more stability to the product in terms of oxidation, whereas the PUFA and MUFA could add the health benefits.

We have earlier demonstrated that the atherogenic potentials of coconut oil (CNO), which contained saturated fatty acids to an extent of about 90% could be significantly reduced by blending it with oils containing unsaturated fatty acids such as those found in rice bran oil (RBO) or sesame oil (SESO). We further noticed that the efficacy of these blended oils to reduce serum cholesterol in rats was significantly enhanced when fatty acids were rearranged in triglycerides by lipase catalyzed interesterification reaction [10]. The blended or interesterified oils used in our studies contained 29–33% linoleic acid whereas coconut oil *per se* contained 2% linoleic acid. The increased amounts of linoleic acid in modified oils in turn may affect thrombotic parameters. To evaluate this, the effect of feeding a diet containing CNO, blended oils of CNO+RBO, CNO+SESO and interesterified oils containing CNO+RBO, CNO+SESO on platelet aggregation and eicosanoid levels in rats were studied.

2. Materials and methods

Coconut oil (CNO) and sesame oil (SESO) were purchased from a local super market. Physically refined rice bran oil (RBO) was provided by A.P. Solvex Limited, Dhuri, India. Lipozyme IM-RM was a gift from NOVO Nordisk Bioindustrial Inc. (Danbury, CT, USA). BF_3 in methanol, collagen, adenosine diphosphate (disodium salt ADP), bovine serum albumin, heparin, thiobarbituric acid were purchased from Sigma Chemical Co, St. Louis, MO, USA. Ascorbic acid and trichloroacetic acid were purchased from SISCO Laboratory Pvt. Ltd., Mumbai, India. All the solvents used were of analytical grade and distilled prior to use.

2.1. Preparation of blended and interesterified oils

Blended and interesterified oils were prepared as described earlier [11]. Briefly, blended oils were prepared to obtain SFA:MUFA:PUFA approximately in the ratio of 1:1:1. The blending was carried out by mixing the required amounts of oils and stirring for 1 h at 40 °C on a magnetic stirrer after flushing with nitrogen. The mixing efficiency was monitored by estimating fatty acid composition of the blend periodically. The theoretical and experimental fatty acid values were compared to determine the mixing efficiency.

Interesterified oil was prepared by incubating the blended oils with immobilized lipase (*Rhizomucor miehei* lipozyme IM-RM) at 1% (w/w) and agitating at a speed of 160 rpm in a shaking water bath (BS-31, Tokyo, Japan) for 72 h at 37 °C. The oil was decanted, and enzyme recovered was washed with hexane for reuse. The quality of interesterified oil was then evaluated by measuring peroxide value and free fatty acid content. Interesterification did not affect the quality, nutraceutical contents and total fatty acid composition of blended oils [11].

2.2. Fatty acid composition of oils

Fatty acid composition of native, blend and interesterified oils were analyzed as methyl esters by gas chromatography [12]. The fatty acid methyl esters were prepared by saponifying oils with 0.5 M KOH and later methylating with 40% BF_3 in methanol, and separated using a fused silica capillary column 25 m × 0.25 mm (Parma bond FFAP-DF-0.25: Machery-Negal Gm BH co. Duren, Germany) connected to GC (Shimadzu 14B fitted with FID) The operating conditions were: initial column temperature 120 °C, raised by 15 °C per min to 220 °C, injection temperature 230 °C and detector temperature 240 °C. Nitrogen was used as the carrier gas. Individual fatty acids were identified by comparing with the retention times of standards (Nuchek Prep, Elysin, MN, USA) and quantified using Clarity Lite software.

2.3. Animal experiments

All the experiments involving animals were approved by the institutional ethical committee. Male Wistar rats [OUTB-Wistar, IND-cft (2c)] (*Rattus norvegicus*) weighing 40–45 g were grouped (6 rats in each group) by random distribution and housed in individual cages, under a 12 h light/dark cycle, in an approved animal house facility at the Central Food Technological Research Institute, Mysore, India. Rats were fed with fresh diet daily, and left over diets were weighed and discarded. The gain in body weight of rats was monitored at regular intervals. The rats had free access to food and water throughout the study. Each group of rats were fed with, AIN-76 diet containing 10% fat from either CNO, RBO, SESO, CNO+RBO(B), CNO+SESO(B), CNO+RBO(I) or CNO+SESO(I), sucrose 60%, casein 20%, cellulose 5%, mineral mix 3.5%, vitamin mix 1.0%, choline chloride 0.2%, and methionine 0.3% [13]. Rats were fed on test diets for 60 days and following an overnight fast on day 60, were anesthetized, sacrificed and blood was collected. The liver, heart and brain were removed, rinsed with ice-cold saline, blotted and weighed.

2.4. Preparation of platelet rich plasma and platelet poor plasma

Blood was collected by cardiac puncture from anesthetized rats in heparin as anticoagulant (50 IU/mL, blood). It was centrifuged at 150*g* for 20 min at room temperature to separate platelets from erythrocytes and leukocytes. The platelet rich plasma (PRP) thus obtained was then centrifuged at 150*g* for 20 min at room temperature to remove any residual erythrocytes and leukocytes. PRP was then utilized as source of platelets to study aggregation. Platelet poor plasma (PPP) was prepared by centrifugation of anticoagulated blood or PRP at 5000*g* for 10 min at 4 °C [14]. The platelet count was adjusted to 400,000/μL.

2.5. Platelet aggregation

The aggregation experiments were performed within 2 h of blood collection. Platelet poor plasma was used as the blank. After setting the baseline with 450 μL of PRP and PPP in their respective cuvettes the aggregation of platelets were followed by adding 10 μL of 25 μM ADP or 15 μL of collagen (5 mg/10 mL in 0.1 N acetic acid) in a Chronolog Dual Channel platelet aggregometer (Denmark) at 37 °C with constant stirring at 1000 rpm. Aggregation was followed for at least 5 min. The light transmission was recorded on a chart paper. The platelet aggregation was quantitated as the maximum change in light transmittance

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