# A Study on Protective Effects of low Dietary PUFA levels in Hypertensive Rats

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Abstracts: Background & objectives: Short term dietary supplementation of eicosapentanoic acid [EPA, 20:5(ω-3)] and docosahexanoic acid [DHA, 22:6(ω-3)] in high amounts to prevent cardiovascular diseases have been reported. The present study investigates the effects of long term dietary supplementation of low PUFAs in a cereal-legume diet on plasma fatty acid composition, platelet function and cadmium induced hypertension in male *Charles foster* rats. Materials and Methods: Rats were fed with 3% of safflower oil, groundnut oil or linseed oil, in a cereal-legume based diet (total fat content, 5.5%) for a period of six months. Plasma fatty acid composition, platelet aggregation and blood pressure of four groups with fifteen rats each namely Safflower (S), Groundnut (G), Linseed (L) and Control (C) group were studied . Blood pressure was measured after administration of intraperitoneal cadmium chloride (i.p. dose, 1mg/kg body weight) for fifteen days. Results and Interpretation: The ω-3 fatty acids were found to be significantly higher (T-test, $p<0.005$) in the plasma of rats fed on Linseed oil. There was an increased platelet aggregatory response to adenosine diphosphate (ADP) in rats fed with groundnut oil (G group). The platelet aggregation (%) and rate of aggregation between groups were found to be significantly low in the Linseed oil group indicating the impact of diet on membrane function. The systolic blood pressure was found to be significantly different between groups (ANOVA test) after cadmium treatment at level of $p<0.05$. Incidence of blood pressure was highest in rats supplemented with Groundnut oil (G, 66%) followed by control group (C, 50%). The incidence was least in the groups supplemented with Safflower and Linseed oils (S & L- 33%) in cadmium treated showing protective effect of PUFAs on blood pressure. Conclusion: It can be concluded from the study that long term supplementation of low dietary PUFA shows significant changes in plasma fatty acid composition, platelet aggregation thereby affecting the blood pressure. As cereals and legumes contain cardioprotective flavonoids, the combined effect of PUFA and flavonoids needs to be evaluated. [Chatterjee B et al NJIRM 2012; 3(3) : 75-80]

**Key Words:** PUFA, Tocopherols, Hypertension, visible fat, Invisible fats.

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**Introduction:** Hypertension has become a major cause of morbidity and mortality worldwide and it is now ranked third as a cause of disability adjusted –adjusted life years. The estimated total number of people with hypertension in India in 2000 was 60.4 million males and 57.8 million females and projected to increase to 107.3 million and 106.2 million respectively in 2025[1]. The risk factors include improper diet leading to hypercholesterolemia and Hypertriglyceridemia along with other risk factors such as physical inactivity, smoking, tobacco, alcoholism etc[1-4].

Excessive fats consumed elevate cholesterol levels in blood, which may be responsible for CVDs[5]. Dietary intake of various fats may have different effects on blood pressure. Higher intake of saturated FAs, monounsaturated FAs (MUFAs), and trans-fatty acids are each associated with increased risk of hypertension. Polyunsaturated fatty acids (PUFAs) on one hand are the culprits in the pathogenesis of hypertension (a CVD) as they are susceptible to oxidative stress due to lipid peroxidation. On the other hand PUFA supplementation is known to have cardioprotective effect by improving lipid profile, and blood pressure[5-10]. Fish oil containing eicosapentanoic acid (EPA), docosahexanoic acid (DHA) and oleic acid in olive oil have been found to be effective in lowering blood pressure[7-10]. PUFAs should be in an optimal ratio in the diet to avoid various health hazards. A desirable ω-6/ω-3 ratio is between 5-10. A ratio above 50 is injurious to health[5]. An adequate intake of both linoleic acid and linolenic acid (precursors of long chain PUFA) is important for physiological functions—lipoprotein transport, blood clotting, inflammation, immune functions and blood pressure regulation [11-15]. Therefore fats are an important constituent of any diet and play an important role in both health and disease. The current study aims to find out the effect of low PUFA supplementation in a cereal-legume diet on plasma fatty acid composition, platelet aggregation and hypertension.

**Material and Methods:** Plasma fatty acid composition, platelet aggregation and blood pressure (B.P.) were determined in rats to study the effect of diet having varying fatty acid composition. Studies were carried out in accordance with institutional ethical guidelines for the care of laboratory animals. The duration of the study was six months.

Second generation male weanling rats of *Charles foster* strain were maintained under standard laboratory conditions [temperature (22°C ± 2°C) and humidity (45°C ± 5°C)] with 12:12 hour day-night cycle]. Each group had fifteen rats. Animals fed on standard laboratory pellet diet (table 1) were called the Control (C) group and were used for comparison. The remaining animals were divided into 3 study groups viz., Safflower (S), Groundnut (G), Linseed (L) groups depending on the oil supplemented in a cereal legume based diet (Table1).

**Table 1: Composition of Diet Given to Rats**

| Cereal -Legume diet | | Pellet Diet | |
|---|---|---|---|
| Ingredients | Gram % | Ingredients | Gram % |
| Wheat flour | 40 | Crude Protein | 21.95 |
| Millet Flour | 11 | Crude oil | 3.95 |
| BengalGram Flour | 12 | Crude Fibre | 2.85 |
| Cow peas | 4.5 | Ash | 7 |
| Green Gram | 4.5 | | |
| Skimmed Milk | 23 | | |
| CalciumCarbonate | 1 | | |
| Common Salt | 0.7 | | |

Rats were fed with 3% oil in the diet for a period of six months. The animals were allowed to drink water ad libitum. Blood samples of rats were collected after the rats achieved the body weight of 225 grams to study plasma fatty acid composition after preparing fatty acid methyl esters (FAMES) by gas chromatography (Schimadzu, GC 1S)[6]. Platelet rich plasma (PRP) was separated (Megafuge 2.0 R) at to see the effect of diet on platelet aggregation (Chronolog Aggregometer) with ADP (20µM) as an inducer. PRP of two rats was pooled ($10^9$platelets/ml) to carry out the studies[6]. A fifteen day cadmium treatment was given to the animals intraperitoneally (I.P. dose: 1mg/kg body weight) to induce hypertension. Rats from control group were saline treated. Systolic blood pressure of these rats was recorded on a Polyrite recorder under urethane anesthesia (dose 1200 mg/kg body weight). Blood pressure was recorded through continuous tracings of arterial blood pressure which was recorded on a Polyrite recorder through a Stathum transducer (T301 pressure transducer, range-30 to +300 mmHg). Stabilization period for systolic B.P was 5 minutes. The process was standardized with known pressures before cannulating each rat. A pilot study was carried out for each set of experiments.

**Table2: Comparison between the body weights (gms) before and after Cadmium treatment.**

| | C | S | L | G |
|---|---|---|---|---|
| Cadmium Untreated N=15 | 268 ± 16 | 264 ± 13.4 | 269 ± 10 | 272 ± 11 |
| Cadmium Treated N=12 | 253 ± 22 | 240 ± 9 | 245 ± 6 | 252 ± 6 |

Significance was tested using ANOVA test at the level of p<0.05 and a significant difference was observed between groups

**Results and Interpretation:** Significant difference in body weight before and after cadmium treatment was observed between groups using ANOVA test at the level of p<0.05. Table 3 shows that the plasma fatty acid composition are comparable and differ from L group significantly. In L group, PUFA of ω-3 series was significantly higher compared to other groups. There was an increased platelet aggregatory response to ADP in rats fed with groundnut oil (G group) followed by control group, S group and L group in descending order. The difference in plasma PUFA content brings about significant difference in the rate of platelet aggregation (table4).

**Table 3: Plasma Fatty Acid Composition**

| Fatty Acids | C (n=10) | S (n=10) | G (n=10) | L (n=10) |
|---|---|---|---|---|
| 16:0+ 16:1 | 25.7±0.55 | 24.6±0.86 | 27.8±2.2 | 26.1±0.35 |
| 18:0+ 18:1 | 19.5±0.795 | 18.2±1.104 | 22.85±1.485 c | 22.08±0.96 bc |
| 18:2 (ω-6) | 29.7±1.5 | 34.8±1.4 b | 30.4±4.2 | 29.4±0.7 c |
| 20:4 (ω-6) | 23.2±0.3 | 20.1±1.5 | 23.3±0.71 d | 13.3±0.7 bc |
| 20:5 (ω-3) | n.d* | n.d | n.d | 5.6±1.15 bcd |
| 22:5 (ω-6) | 1.0±0.43 | 0.76±0.26 | 1.25±0.46 | n.d |
| 22:6 (ω-3) | 1.08±0.24 | 0.92±0.2 | 1.3±0.34 d | 2.7±0.48 bc |

Significance was checked with students T-test at level of $p<0.05$ , b- significant when compared with C, c- significant when compared with S, d- significant when G is compared with L. *n.d.: not detected.

**Table 4: Platelet aggregation (%) and rate of aggregation ($t_{1/2}$ sec ) induced by ADP in rats fed diets with varying ω-6/ω-3 ratio.**

| | % Aggregation | T 1/2 (sec) |
|---|---|---|
| | ADP | ADP |
| C | 61.5 ± 6 | 42 ± 3 |
| S | 64.8 ± 2.7 | 34 ± 0.5 |
| G | 68.5 ± 2.7 | 30 ± 0.5 |
| L | 65 ± 2.7 | 44 ± 0.5 bc |
| ANOVA p-value | N.S. | $p < 0.05$ |

'b' indicates $p<0.05$ when C compared to L; 'c' indicates $p<0.05$ when S compared to L.

The differences in serum lipid profile, platelet membrane fatty acid composition as well as platelet aggregation with collagen have already been reported in our previous paper[6]. Significance was tested using ANOVA test at the level of $p<0.05$ and a significant difference was observed between groups and the % incidence of hypertension was highest in G group followed by C group rats after cadmium treatment. The incidence was least in the groups supplemented with Safflower and Linseed oils (S & L- 30%), groups consuming higher amount of total PUFAs indicating a greater resistance to cadmium induced hypertension as shown in table [5].

**Table5: Effect of Diet supplemented with different oils on blood pressure (mm/ Hg) before and after $CdCl_2$ exposure**

| | C | S | G | L |
|---|---|---|---|---|
| N | 10 | 10 | 10 | 10 |
| B.P. before CdCl2 Exposure (mm Hg) | 118.5 ±1.3 | 109.5 ±1.8 | 115 ±2.2 | 115.6 ±2.2 |
| B.P.after CdCl2 Exposure (mm Hg)* | 130 ±11.1 | 122 ±9.3 | 126 ±11.6 | 122.8 ±7.1 |
| Incidence of hypertension (%) | 50 | 30 | 70 | 30 |
| Total PUFA Content in diet (%) | 1.9 | 3.57 | 2.07 | 3.37 |

*Significance was tested using ANOVA test at the level of $p<0.05$ and a significant difference was observed between groups for B.P.

The cereal legume based diet and vegetable oils given in low concentrations (3%) to rats have shown cardio protective functions when fed for a long time. Both safflower oil and Linseed oils are rich in PUFA.

**Discussion:** There are primarily two types of fats in the diet namely visible fats and invisible fats. Visible fat comes from animal and vegetable oil sources. Invisible fat is present as an integral part of the food we eat and it contributes significantly to the total fat content of food[5]. Clinical and epidemiological studies have shown that the food we eat affects fatty acid composition of plasma and therefore the membrane functions. Membrane composition has been found to be more responsive to ω-6 and ω-3 polyunsaturated fatty acid (PUFA) levels in the diet[16]. Beneficial effects of high amount of fish oil supplementation on platelet aggregation and blood pressure which is one of the most critical factors involved in cardiovascular pathologies such as atherosclerosis or stroke have been reported in both humans as well as animals[17-23].

In the present study, LCPUFA (18:2, ω-6 FA) was significantly lower in plasma samples of L group. The highest amount was seen in S group followed by the samples of G group. On the other hand, LCPUFA of ω-3 series was significantly higher in L group. Linseed oil is known to have highest concentration of ω-3 PUFA. The plasma fatty acid composition indicates the differences in types of PUFA found in plasma in relation to dietary intake of PUFA, therefore on platelet function and hypertension. The incidence of hypertension was least in both L and S group animals because of higher amount of total PUFAs (ω-3 and ω-6 FA) in their diet.

Studies have also reported the cardioprotective effects of high flavonoid (cereals, legumes -the chief staple of India) consumers[24-27.] The tocols (tocopherols and tocotrienols), carotenes and other antioxidants which are known to be found in cereals and legumes could have had an additive effect on the beneficial effects of PUFA in the current experimental groups which needs to be evaluated in further studies.

Conclusion: The present study demonstrates that a long-term of low PUFA in a cereal-legume diet shows its beneficial effects. Even a low fat diet can change plasma fatty acid composition, platelet membrane function (i.e. aggregation and rate of aggregation (and blood pressure which have a role in CVDs and hypertension. As shown in table 5, the incidence of hypertension was found to be low in groups consuming higher amount of total PUFAs (S & L), indicating a greater resistance to cadmium induced hypertension.

The cereal legume based diet and vegetable oils also have tocols (tocopherols and tocotrienols), carotenes and other antioxidants which have are now known to have cardioprotective functions. Further studies are required to be carried out to find out the combined effect of PUFA and tocols on hypertension and other cardiovascular disorders as Indian diet is rich in cereals, legumes and vegetables which are good sources of tocols.

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