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Dietary Effects of Punicic Acid on the Composition and Peroxidation of Rat Plasma Lipid

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Abstract: The purpose of the present study is to examine the oxidative behavior of punicic acid (9 *cis*, 11 *trans*, 13 *cis*-18:3 octadecatrienoic fatty acid) under *in vivo* condition using male albino rats after blending with dietary soybean oil. Male weanling Charles Foster rats (n = four to eight), weighing 65-68 grams, were allocated for fourteen weeks to diets containing 0.6, 1.2 and 2.4% (by weight) punicic acid obtained from snake gourd seed oil (*Trichosanthes anguina*) after mixing with soybean oil, *vis-à-vis* control group consisting of 100% soybean oil, containing 50.3% C18:2, 6.2% 9 *cis*, 12 *cis*, 15 *cis*-18:3 (linolenic acid) and does not contain punicic acid. Increase in weight remained higher than control in rats fed with 0.6 and 1.2% punicic acid throughout the experimental period. There was significant lowering in plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) in the groups fed with 2.4% punicic acid. Lipoprotein oxidation susceptibility or LOS expressed as nmoles of malondialdehyde (MDA) per mg of non-high density lipoprotein cholesterol (non-HDL-C) and plasma lipid peroxidation or PLP expressed as nmoles of MDA per ml of plasma exhibited maximum antioxidant efficiency at 1.2% punicic acid, being equivalent to that of control (100% soybean oil). While LOS expressed as nmoles of MDA decreased significantly (except at 2.4% punicic acid level) with the least oxidation at 0.6% punicic acid, with respect to control. Accordingly, punicic acid seemed to be acting both as pro-oxidant (at 1.2% punicic acid level) and antioxidant (at 0.6% punicic acid level), with significant decrease in TC and LDL-C at 2.4% punicic acid level when compared to control.

Key words: punicic acid, snake gourd, *Trichosanthes anguina*, lipoprotein oxidation susceptibility and plasma lipid peroxidation

1 Introduction

Punicic Acid is a conjugated trienoic fatty acid of the constitution *cis*-9-*trans*-11-*cis*-13-octadecatrienoic fatty acid. It is found in the seed fat of Snake gourd plant, *Trichosanthes anguina*, belonging to the natural order Cucurbitaceae. It maybe stated that the natives consume the fruits and seeds of the snake gourd plant mostly as vegetables. The seeds contain about 40-70% oil, with punicic acid content to be more or less equivalent to 40 % (1). Conjugated polyunsaturated fatty acids including conjugated linoleic acid (CLA) usually constitutes less than 1% in natural products or dairy products and it has been found to have antiatherosclerotic and anticarcinogenic effects owing to its antioxidant activity (2). Dhar *et. al.* has examined the oxidative behaviour of α-elaeostearic acid obtained from the seed oil of karela, *Momordica charantia*, theoretically consisting of 33%

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cis and 66% *trans* molecular composition and has claimed the antioxidant activity of the same in the plasma lipid of rats (3). No such study has been made incase of punicic acid, which has 66% *cis* and 33% *trans* configuration.

Punicic acid, a stereoisomer of α-elaeostearic acid has melting point 43.5-44℃ (4).

The present study is therefore specifically aimed to examine the oxidative behavior of punicic acid (9*c*, 11*t*, 13*c*-18:3 conjugated fatty acid) considering its antagonistic *cis-trans* molecular arrangement with reference to (α-elaeostearic acid (9*c*, 11*t*, 13*t*-18:3 conjugated fatty acid), after blending with soybean oil having non-conjugated octadecadienoic and octadecatrienoic fatty acids of all cis configuration, in the plasma lipid of male albino rats, under *in vivo* conditions.

2 Experimental

2·1 Extraction from Dietary Fat Sources, Refining and Bleaching

The fresh and ripe seeds of *Trichosanthes anguina* obtained from local market at "Sealdah", Calcutta, India, were grinded with anhydrous Na_2SO_4, extracted several times with solvent petroleum ether (40°-60℃, Analytical Reagent or AR and free from sulphur compounds) at room temperature. The extracts of the sample were filtered and concentrated by evaporation in vacuum. After removal of the solvent, dark reddish green oil having Free Fatty Acid (FFA) content of 3.58% was obtained. The FFA present was removed by using 10% NaOH solution (20% excess of the required theoretical amount). The refined oil was bleached with tonsil earth optimum (1% w/w), obtained from P.T. Sud-Chemic (Jakarta, Indonesia) and activated carbon (0.5% w/w), obtained from E. Merck India Pvt. Ltd., (Mumbai, India) at room temperature for 30 minutes. The oil was recovered by filtration and desolventised under vacuum at about 37℃. The refined and bleached oil was flushed with nitrogen and stored in a refrigerator until further use.

2·2 Characterization of the Oil

The fatty acid compositions of the dietary oils were determined by converting the oils (triglycerides) into their methyl esters, separating them by thin layer chromatography (TLC) and finally analyzing them by gas liquid chromatography (GLC) techniques (5).

The position of punicic acid, *cis-trans-cis* isomer was identified by dissolving it in cyclohexane (analytical reagent or AR grade) and observed over wavelength range of 200-300 nm using a Shimadzu UV-Vis Spectrophotometer (Tokyo, Japan). The wavelengths 265.8, 275.6 and 286.8 nm corresponded to *cis-trans-cis* chromophore of punicic acid, respectively (6, 7).

The Infra Red (IR) Spectrum yielded two peaks at 932 cm^{-1} and 981 cm^{-1} corresponding absorptivity at 10.13 μ and 10.67 μ respectively, due to *trans* unsaturation of punicic acid (8, 9).

Punicic Acid is confirmed to be *cis*-9-*trans*-11-*cis*-13-octadecatrienoic acid $CH_3.(CH_2)_3.CH{=}CH.CH{=}CH.CH{=}CH.(CH_2)_7.COOH$.

The fatty acid compositions of the dietary oils were determined by converting the oils (triglycerides) into their methyl esters, according to the standard method of Litchfield (10), which were separated by TLC (Silica gel-G, 20×20 cms, solvent system-hexane/ diethyl ether/ acetic acid-80:20:10, by vol.) according to the general procedure by Mangold (11). Finally the methylated fatty acids were separated and identified and were analyzed using GLC techniques in a Hewllet and Packard Model 5890 Å with flame ionization detector and N_2 as the carrier gas with flow rate of 30 ml per minute, glass column 1/8 inch in diameter and six feet in length had been packed with 10% diethylene glycol succinate (DEGS). The oven, injection port and the detector block temperatures were maintained at 190, 230 and 240℃ respectively. About 0.1-1.0 (μg of sample was injected with the help of a Hamiltonian syringe, when the required chromatogram was obtained. The quantification of the fatty acids was done by comparing the retention time (RT) of GLC grade standard methyl esters of varying chain length and unsaturation (5). Fatty acid composition of the dietary oils and their blends are illustrated in **Table 1**.

2·3 Dietary Fat Blends

Soybean oil, trade name Vital Refined Soybean Oil, SM Dyechem Ltd., SM Center, Mumbai, India, was mixed with 1.5, 3.0, and 6.0% Snake Gourd oil, contributing 0.6, 1.2 and 2.4% punicic acid respectively, along with a control consisting of 100% Soybean oil, so as to make the final concentration of 20% fat in the diet of each of the four groups.

2·4 Animals and Diet

The animal experiments have been designed and carried out as per reports published from the laboratory earlier (3, 12). Thirty-two male albino rats were divided into four groups each of average body weight and kept in wire-bottomed cages of size 27×21×14 cm^3, with free access to food and water. Male albino rats of Charles Foster strain were chosen for the experiment (13). Everyday rats were fed fresh water and diet *ad libitum* along with 20% fat, 55% starch, 18% casein, 3% cellulose, 4% salt mixture (Bernhart Tommarelli modified supplied by SISCO Research Laboratories Pvt. Ltd. Mumbai-400060, India.), along with one multivitamin and multimineral capsule per kg of diet; the composition of multivitamin capsule had been-vitamin A IP (as acetate) 10,000 IU, cholecalciferol IP (vitamin D3) 1,000 IU, thiamine mononitrate IP 10.0 mg, riboflavine IP 10.0 mg, pyridoxine hydrochloride IP 3.0 mg, cyanacobalamin IP 15.0 mg, nicotinamide IP 100 mg, calcium pantothenate IP 16.3 mg, ascorbic acid IP 150.0 mg, α-tocopheryl acetate IP 25.0 mg, biotin USP 0.25 mg; composition of multiminerals as follows-calcium phosphate IP 129.0 mg, magnesium oxide light IP 60.0 mg, dried ferrous sulphate IP 32.04 mg, magnesium sulphate BP 2.03 mg, total phosphorus in the preparation 25.8 mg; trace elements were-copper sulphate IP 3.39 mg, zinc sulphate IP 2.20 mg, sodium molybdate 0.25 mg, sodium borate IP 0.88 mg. The diets were adequate in all nutrients. The amount of food consumed per day along with weekly increase in body weight was noted for each rat. The animals were maintained on their respective diets for 14 weeks. At the end of the feeding experiment the rats were kept fasting for 12 hours and sacrificed under mild anesthesia, blood was collected by heart puncture and after sealing properly, kept in the refrigerator until further analysis.

2·5 Lipid Analysis in Plasma

The estimations of Total Cholesterol or TC (14), Triglyceride or TG (15), High Density Lipoprotein Cholesterol or HDL-C (16, 17, 18), and Low Density Lipoprotein Cholesterol or LDL-C (19) of plasma were carried out using enzymatic kits supplied by Span Diagnostics Ltd., Surat, India and Crest Biosystems, Goa, India. Plasma lipid peroxide was measured by the assay of thiobarbituric reactive substances (TBARS) according to the standard method (20). The amount of malondialdehyde (MDA) formed had been calculated taking the extinction coefficient of MDA as 1.56×10^5 M^{-1} cm^{-1}. Lipoprotein Oxidation Susceptibility Test (LOS) had been carried out by the precipitation of non-HDL-C i.e. apoB lipoproteins-LDL and Very Low Density Lipoprotein (VLDL) according to standard methods described by Phelps *et al*. (21).

2·6 Statistics

Data are expressed as mean ± standard error of mean (SEM). Comparison was made using the one-

Table 1 Fatty Acid Composition of Dietary Oils and Oil Blends.

Dietary Fat	Fatty Acid Compositions (% area)[a]					
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$ [b]	$C_{18:3}$ [c]
Snake gourd oil	6.1	6.0	27.0	21.0	39.9	—
[d] Soybean oil	11.7	2.9	28.8	50.3	—	6.2
[e] Soybean oil + Snake gourd oil (98.5 : 1.5, w / w)	11.6	3.0	28.7	49.9	0.6	6.1
[f] Soybean oil + Snake gourd oil (97 : 3, w / w)	11.5	3.0	28.8	49.4	1.2	6.0
[g] Soybean oil + Snake gourd oil (94 : 6, w / w)	11.4	3.1	28.7	48.5	2.4	5.8

[a] Obtained from gas liquid chromatography (GLC).

[b] Punicic acid or conjugated linolenic acid of 9 *cis*, 11 *trans*, 13 *cis*-18:3 fatty acid configuration.

[c] Linolenic acid of 9 *cis*,12 *cis*,15 *cis*-18:3 fatty acid configuration.

[d] Control group A

[e] Experimental group B

[f] Experimental group C

[g] Experimental group D

way analysis of variance (ANOVA) followed by multiple comparison 't' test (22).

3 Results and Discussion

3·1 Body Weight Gain

The mean body weight gain in rats fed with the 0.6% (group B), 1.2% (group C) and 2.4% (group D) 9*c*, 11*t*, 13*c*-18:3 (punicic acid) dietary groups for 14 weeks remained more or less steady, without much decline in trend (**Fig. 1**.). Smedman *et al*. also claimed that conjugated linoleic acid (CLA) had no major effects on body weight (23). But the increase in weight of the 1.2% (group C) and 0.6% (group B) punicic acid seemed to be higher than the control group A (soybean), throughout the experimental period. Dhar *et al*. showed evidence of improved body weight gain in rats fed with α-eleostearic acid (3).

3·2 Lipoprotein Oxidation Susceptibility or LOS in Plasma and Plasma Lipid Peroxidation or PLP

LOS (nmoles of MDA) was found to increase with increase in the percentage of punicic acid with the maximum reduction in oxidative susceptibility at 0.6% punicic acid (group B) and the minimum reduction in oxidative susceptibility with 2.4% punicic acid (group D) as illustrated in **Table 2**. However, the overall statistical significance was obtained at $p < 0.01$. On multiple 't' test comparison, significance (at $p < 0.01$) was obtained between the groups administered with dietary oils containing 1.2% punicic acid (group C) and 2.4% punicic acid (group D). Significance was further observed at $p < 0.01$ between experimental groups D (2.4% punicic acid) and B (0.6% punicic acid) at $p < 0.001$ (**Table 2**). Thus maximum antioxidant activity was achieved with 0.6% punicic acid (group B).

When LOS expressed as nmoles of MDA per mg of non-HDL-C (**Table 2**), maximum antioxidant efficiency was obtained with control group A (soybean oil) followed by experimental groups C (1.2% punicic acid) and B (0.6% punicic acid), while peroxidation reached its peak incase of group D (2.4% punicic acid) (**Table 2**). In this case the statistical significance was obtained at $p < 0.01$ using ANOVA. On multiple 't' test comparison, significance was obtained at $p < 0.001$ between

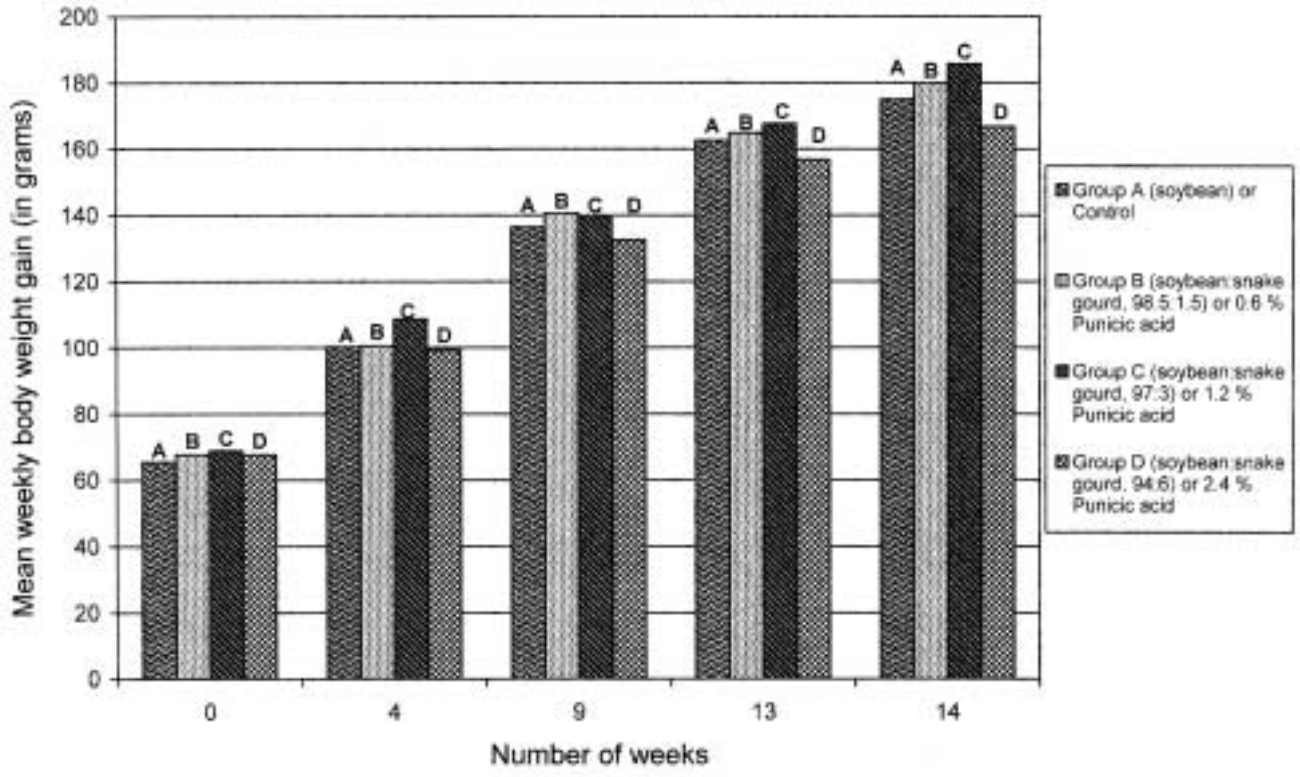


Fig. 1 Average Weekly Body Weight Gain in Rats Fed with the Dietary Oil Blends for 14 Weeks (n=4 to 8).

Table 2 [a]LOS of Rats fed with Control and Experimental Dietary Oil Blends.

% of Punicic Acid in Dietary Oil Blends	Lipoprotein Oxidation Susceptibility (LOS) (nmoles of MDA/ mg of non-HDL Cholesterol)	LOS (nmoles of malonaldehyde or MDA)
0.0 % 9*c*, 11*t*, 13*c*-18:3 (group A or control)[b]	0.08 ± 0.014[c, d, e]	13.36 ± 3.2[c, i, j]
0.6 % 9*c*, 11*t*, 13*c*-18:3 (group B)[b]	0.16 ± 0.008[c, e, g, h]	7.36 ± 0.96[c, l, j]
1.2 % 9*c*, 11*t*, 13*c*-18:3 (group C)[b]	0.1 ± 0.0007[c, f, g]	9.59 ± 1.04[c, k]
2.4 % 9*c*, 11*t*, 13*c*-18:3 (group D)[b]	0.25 ± 0.024[c, d, f, h]	20.67 ± 2.93[c, k, i, l]

[a] Expressed as means ± SEM; n=four to eight rats per group.

[b] Rats maintained on dietary blends containing 0.0% (group A or control oil), 0.6% (group B), 1.2% (group C) and 2.4% (group D) punicic acid, respectively.

[c] Denotes a significant difference with other numbers in the same column ($p < 0.01$).

[d] Significant difference with control and 2.4% 9 *c*, 11 *t*, 13 *c*-18:3 at $p < 0.001$.

[e] Significant difference with control and 0.6% 9 *c*, 11 *t*, 13 *c*-18:3 at $p < 0.02$.

[f] Significant difference with 1.2% 9 *c*, 11 *t*, 13 *c*-18:3 and 2.4% 9 *c*, 11 *t*, 13 *c*-18:3 at $p < 0.001$.

[g] Significant difference with 1.2% 9 *c*, 11 *t*, 13 *c*-18:3 and 0.6% 9 *c*, 11 *t*, 13 *c*-18:3 at $p < 0.05$.

[h] Significant difference with 2.4% 9 *c*, 11 *t*, 13 *c*-18:3 and 0.6% 9 *c*, 11 *t*, 13 *c*-18:3 at $p < 0.01$.

[i] Significant difference with control and 2.4% 9 *c*, 11 *t*, 13 *c*-18:3 at $p < 0.05$.

[j] Significant difference with control and 0.6% 9 *c*, 11 *t*, 13 *c*-18:3 at $p < 0.1$.

[k] Significant difference with 1.2% 9 *c*, 11 *t*, 13 *c*-18:3 and 2.4% 9 *c*, 11 *t*, 13 *c*-18:3 at $p < 0.01$.

[l] Significant difference with 2.4% 9 *c*, 11 *t*, 13 *c*-18:3 and 0.6% 9 *c*, 11 *t*, 13 *c*-18:3 at $p < 0.001$.

group A (control-soybean) and group D (2.4% punicic acid); $p < 0.02$ between control (group A) and group B (0.6% punicic acid); between group C (1.2% punicic acid) and group D (2.4% punicic acid) significance was obtained at $p < 0.001$, while that among group D (2.4 % punicic acid) and group B (0.6% punicic acid) it was $p < 0.01$. Thus maximum antioxidant activity (LOS expressed as nmoles of MDA per mg of non-HDL-C) among the experimental groups [0.6% (group B), 1.2% (group C) and 2.4% (group D) punicic acid] was achieved with 1.2% punicic acid (group C) as evident from **Table 2**.

PLP expressed as nmoles of MDA per ml of plasma was maximally reduced with control, containing soybean oil (group A). This was nearly the same to that of 1.2% punicic acid (group C), showing the maximum antioxidant efficacy with respect to other experimental groups (**Fig. 2**). No statistical significance was obtained.

UV spectrophotometric study of the plasma lipid of rats raised on 0.6% punicic acid (group B) showed absorbance at wavelengths ranging from 269.5 to 275 nm, in cyclohexane solvent, suggesting the presence of conjugated trienoic fatty acid which maybe punicic acid or isomers of α-elaeostearic acid; while that of 1.2% punicic acid (group C) at (λ_{max} 230.0 nm maybe due to conjugated dienoic fatty acid. Spectrophotometric studies of plasma lipid of group B (0.6% punicic acid) show that some trienoic fatty acid in conjugated form might have been retained in plasma as evident from the UV spectra. According to Allen and Kummerow (24), the amount of triene conjugation lost and amount of diene conjugation formed are proportional to the amount of oxygen absorbed. Some conjugated dienoic fatty acids function by being prone to more rapid oxidation, picking up more free radicals, eliminating or reducing the formation of hydroperoxides. PLP (expressed as nmoles of MDA per ml of plasma) and LOS (expressed as nmoles of MDA per mg of non-HDL-C) were the least at 1.2% Punicic acid level (group C). In general, incase of PLP (expressed as nmoles of MDA per ml of plasma) and LOS (expressed as nmoles of MDA per mg of non-HDL-C) there appeared to have some dose-dependent protection at levels of 1.2% punicic acid, but no further beneficial effect was evident at levels above 1.2 % punicic acid or below it (**Fig. 2** and **Table 2**). Whereas, in the case of LOS (as nmoles of MDA) there appeared to be a dose-dependent protection at 1.2% punicic acid

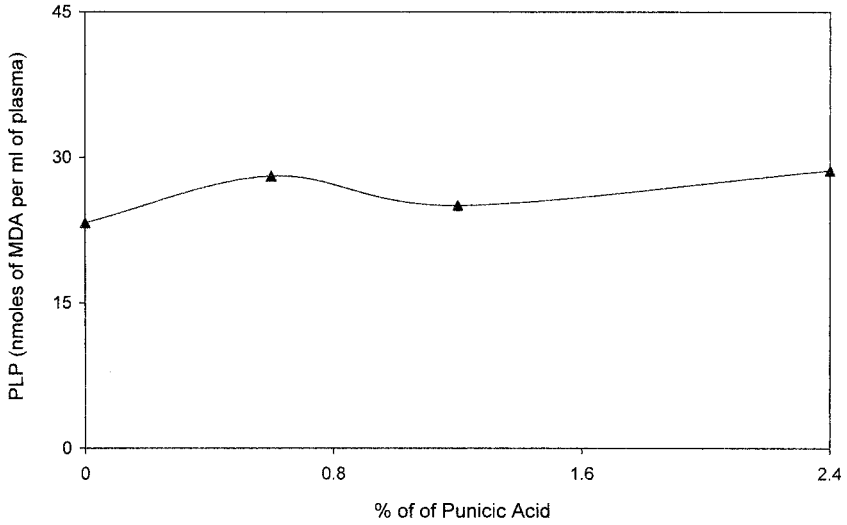


Fig. 2 PLP (nmoles of MDA per ml of plasma) in the Rats with respect to Increase in Percentage of Punicic Acid.
Each data point represents mean ± standard error (n= 4 to 8 Rats).
▲; plasma lipid peroxidation (nmoles of malonaldehyde per ml of plasma).

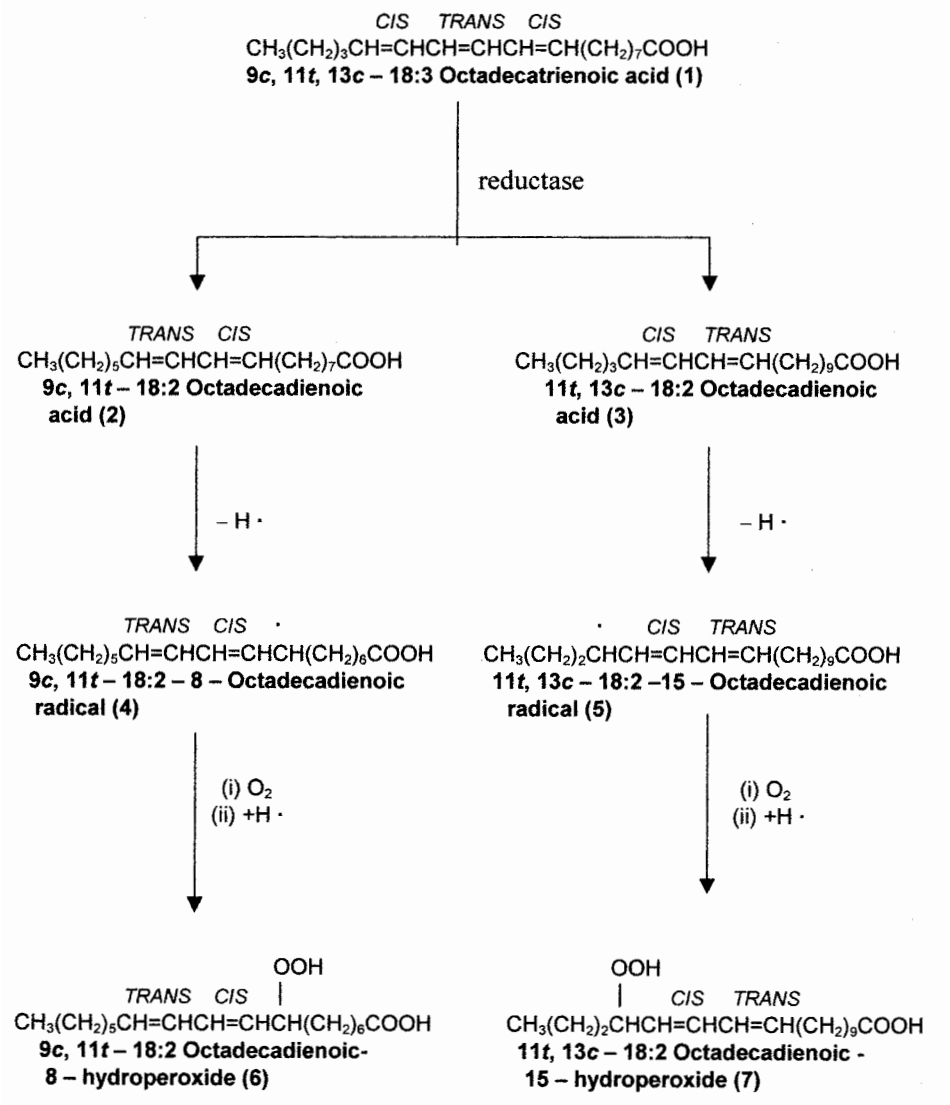
and below it, but no further beneficial effect was evident at levels above 1.2% punicic acid (**Table 2**). Thus, *in vivo* conditions the conjugated linolenic fatty acid, here punicic acid, might have inhibited the hydroperoxide formation by lowering free radical generation and peroxidation of PUFA (3). Another possible explanation may be that the biohydrogenation or free radical addition to one of the conjugated double bonds of punicic acid might have taken place, resulting in the formation of conjugated dienes that could have possibly acted as antioxidants (25). The possible mechanism involved in the hydroperoxide formation and biohydrogenation of punicic acid is thus illustrated in **Scheme 1**.

3·3 Total Cholesterol, High Density Lipoprotein, Low Density Lipoprotein Cholesterol, Triglyceride (TG) and Very Low Density Lipoprotein (VLDL) in Plasma

Total cholesterol (TC) in plasma of rats was the lowest when fed with 2.4% punicic acid (group D) and the highest when fed with 1.2% punicic acid (group C). It is found to be statistically significant at $p < 0.05$ (**Table 3**), as against α-elaeostearic acid, where no significant difference in TC and various forms of cholesterol between experimental and control groups of rats was evidenced (3). This can also be attributed to the findings of Sambaiah *et al*., showing the degree of hydrogenation (saturation) of dietary unsaturated fatty acids in the fore stomach of ruminant animals by bacterial fermentation, which presumably led to increased formation of *trans* fatty acids of edible oils thus increasing its capacity to enhance serum cholesterol levels in a dose dependent manner (26).

As against the findings of α-elaeostearic acid (3) high-density lipoprotein cholesterol (HDL-C) was the maximum with 0.6% punicic acid (group B) and minimum with 2.4% punicic acid (group D), when administered to rats in the diet, although HDL-C was predominant incase of control group A (100% soybean oil). However no statistical significance was established (**Table 3**).

LDL-C was the maximum at 1.2% punicic acid (group C) and minima both at 2.4% (group D) and 0.6 % (group B) punicic acid. It is found to be statistically significant at $P < 0.01$, groups A (control) and C (1.2 % punicic acid) yielded significance at $p < 0.01$, while significant values were obtained at $P < 0.001$ incase of experimental groups C (1.2% punicic acid) and D (2.4 % punicic acid). Also, significant values were obtained at $P < 0.01$ incase of experimental groups C (1.2% punicic acid) and B (0.6 % punicic acid) (**Table 3**).



Scheme 1

Interestingly, the Atherogenic Index (AI) ratio [(TC /HDL-C)-1] was significantly ($p < 0.01$) reduced at 0.6 % punicic acid (group B) and 2.4% punicic acid (group D) levels respectively (**Fig. 3**). Though not significant, the same trend was observed in case of LDL-C to HDL-C (LDL-C/HDL-C) ratio. It may be due to the fact that in punicic acid isomer the total *cis* and *trans* molecular configuration increased with increase in the amount of punicic acid from 0.6% in group B to 2.4% in group D through 1.2% in group C (**Fig. 4**).

Punicic acid when administered at 2.4% level (group D) in the dietary blend lowered TC and LDL-C; similar findings were reported with conjugated linoleic acid (CLA) that lowered levels of TC and LDL-C in the blood of rabbits and hamsters (27).

TG and VLDL in plasma have lower values than the control with no statistical significance (**Table 3**).

These results may also be attributed to the increase in the *cis* configuration being 66% as against 33% *trans* in the conjugated molecule of punicic acid (the configuration of punicic acid being *cis-trans-cis*).

4 Conclusion

In conclusion, the present study unfolds the activity of 9*c*, 11*t*, 13*c*-18: 3 fatty acid (Punicic Acid) upon administration in the diet of rats at various concentrations. It is found to be efficient as an antioxidant (when

Table 3 Plasma Lipid Profile of Rats fed Dietary Oil Blends Containing 0.0% (group A), 0.6% (group B), 1.2% (group C), and 2.4% (group D) Punicic Acid (Conjugated 9*c*, 11*t*, 13*c* -18:3).

[a] Parameters	[b]Group A	[c]Group B	[d]Group C	[e]Group D
Total Cholesterol or TC (mg/dl)	130.85 ± 4.1[f]	112.54 ± 8.7[f, h]	153.81 ± 13.7[f, g, h]	109.50 ± 11.6[f, g]
Triglyceride or TG (mg/dl)	88.34 ± 13.0	79.77 ± 10.0	78.4 ± 12.5	76.91 ± 15.7
HDL-Cholesterol or HDL-C (mg/dl)	42.70 ± 4.6	37.20 ± 2.8	33.37 ± 2.7	31.80 ± 3.0
VLDL (mg/dl)	17.67 ± 2.6	15.95 ± 2.0	15.68 ± 2.5	15.38 ± 3.1
LDL-Cholesterol or LDL-C (mg/dl)	66.53 ± 4.7[i, j]	59.59 ± 6.7[i, l]	100.18 ± 11.8[i, j, k, l]	56.51 ± 4.9[i, k]

[a] Expressed as means ± SEM; n=four to eight rats per group.
[b] Rats maintained on dietary oils containing 0.0% punicic acid.
[c] Rats maintained on dietary oils containing 0.6% punicic acid.
[d] Rats maintained on dietary oils containing 1.2% punicic acid.
[e] Rats maintained on dietary oils containing 2.4% punicic acid.
[f] Denotes a significant difference with other numbers in the same row ($p < 0.05$).
[g] Significant difference with 1.2% and 2.4% punicic acid at $p < 0.01$.
[h] Significant difference with 1.2% and 0.6% punicic acid at $p < 0.02$.
[i] Denotes a significant difference with other numbers in the same row ($p < 0.01$).
[j] Significant difference with control and 1.2% punicic acid at $p < 0.01$.
[k] Significant difference with 1.2 and 2.4% punicic acid at $p < 0.001$.
[l] Significant difference with 1.2 and 0.6% punicic acid at $p < 0.01$.

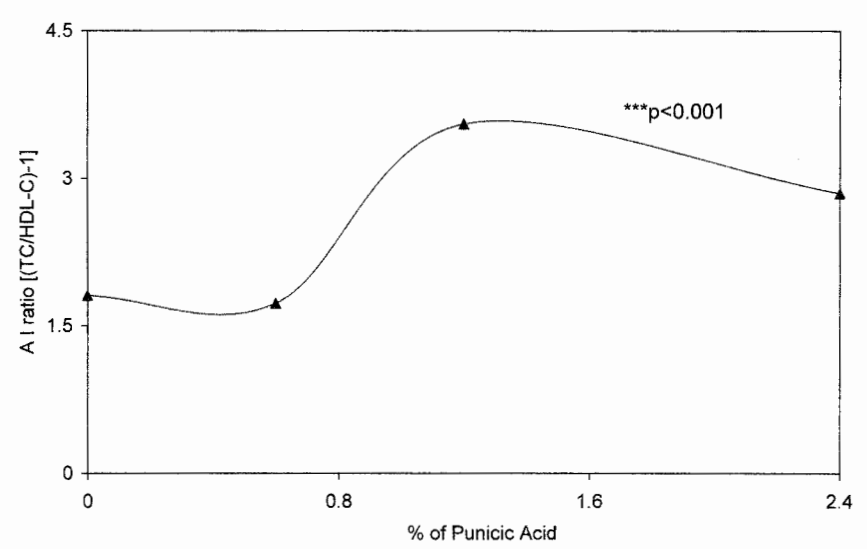


Fig. 3 AI ratio [(TC/HDL-C)-1] in the Plasma of Rats with respect to Increase in Percentage of Punicic Acid.
Each data point represents mean ± standard error (n = 4 to 8 Rats).
▲; atherogenic index ratio [(total cholesterol/high density lipoprotein cholesterol)-1].
*** Significant difference between control (group A) and three experimental groups (B, C, and D consisting of 0.6%, 1.2% and 2.4% 9*c*, 11*t*, 13*c*-18:3 conjugated fatty acid respectively) at $p<0.01$, by one way ANOVA followed by multiple comparison 't' test, between groups A and D at $p<0.02$, between groups C and B at $p<0.01$, between groups D and B at $p<0.01$.

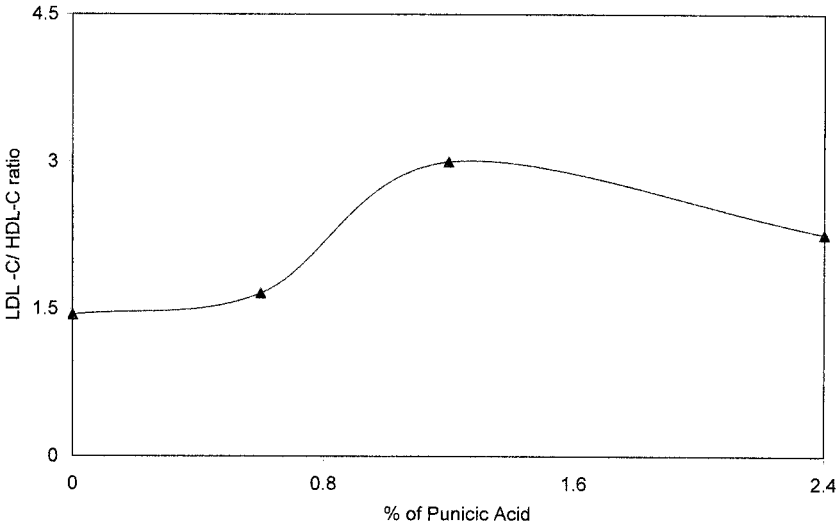


Fig. 4 LDL-C/HDL-C ratio in the Plasma of Rats with respect to Increase in Percentage of Punicic Acid.
Each data point represents mean ± standard error (n= 4 to 8 Rats).
▲; low density lipoprotein cholesterol /high density lipoprotein cholesterol ratio.

expressed as nmoles of MDA) both at 0.6% (group B) and 1.2% levels (group C) but is found to be a marginal prooxidant at 1.2% level (group C) (when expressed as nmoles of MDA/mg of non-HDL cholesterol and nmoles of MDA/ml of plasma). There is significant decrease in TC and LDL-C at 2.4% level (group D), accompanied by a significant decrease in LDL /HDL and atherogenic index (AI) ratio at 0.6% 9*c*, 11*t*, 13*c*-18:3 conjugated fatty acid (group B) concentrations. The dietary groups were nutritionally equivalent, and the PUFA content of the four dietary groups were more than adequate, almost equal in content, and the percentage of punicic acid were low.

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