Diet containing partially hydrogenated vegetable fat enhances the carrageenan induced paw inflammation but not oxidative stress markers in liver of rats

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Abstract In the present study, we investigated the effect of feeding Partially hydrogenated vegetable fat (PHVF) on carrageenan induced paw inflammation and oxidative stress markers in liver of rats. In addition, the effect of feeding rats with Linseed Oil (LSO, α -linolenic acid, n-3 PUFA) or PHVF blended with incremental amounts of LSO on these markers were also monitored. Rats weighing 200 g were given 1 mL of different oils (PHVF, Groundnut Oil; GNO, Olive Oil; OO and LSO) per day for 15 days. Rats given PHVF showed higher levels of paw inflammation in response to carrageenan injection. Rats given LSO showed least amounts of paw inflammation when injected with carrageenan. A second set of experiment was conducted by feeding weaning rats with AIN-93 purified diet supplemented with PHVF or PHVF with incremental amounts of LSO for 60 days. The rats fed PHVF showed higher degree of carrageenan induced inflammation as compared to rats given GNO and LSO. However, the rats fed PHVF showed lower levels of lipid peroxides, protein carbonyls, 8-hydroxy guanine and antioxidant enzyme activities in liver homogenate as compared to those given LSO. In conclusion dietary PHVF rendered the rats prone to higher levels of carrageenan induced inflammation which can be reduced by giving PHVF blended with LSO. However, oxidative stress markers found to be higher levels in rats given LSO or PHVF blended with LSO as compared to rats given PHVF as sole source of fat.

Keywords Partially hydrogenated vegetable fat · Elaidic acid · Linseed Oil · α -Linolenic Acid · Oxidative stress markers · Paw Inflammation

Introduction

Trans fats contain unsaturated fatty acids with at least one double bond in the trans configuration. Unlike cis isomers, the trans fats show deleterious effects on cardiovascular functions (Kummerow 2009). The major source for Trans fatty acids (TFA) in Indian diet is from industrially produced Partially Hydrogenated Vegetable Fat (PHVF). It contains elaidic acid (EA, C18:1 t9) as the main TFA component. The consumption of TFA in India is estimated to be ~2 g/person/day from fried foods, bakery products and ready to eat foods (Amruthakala 2012). Based on many studies conducted on adverse effects of TFA, the US Food and Drug Administration has advised that the consumption of TFA should be kept below 1 % of total energy intake (Wendy Marcason 2006). However in India TFA containing fats are used liberally for cooking.

TFA is known to affect essential fatty acid metabolism. It also enhances the production of many pro-inflammatory mediators such as tumor necrosis factor- α , interleukins, prostaglandins and leukotrienes (Teng et al. 2010). Inflammation is an independent risk factor for atherosclerosis, sudden death from cardiac cases and heart failure. The process of inflammation involves multiple systems in which immune system play a central role. Inflammation also results in higher production of Reactive oxygen species (ROS). Antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione transferase normally defend the host against the damaging effects of ROS. TFA intake enhances the levels of ROS and also increasing the formation of oxidized LDL.

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These cumulated events are known as oxidative stress. The prolonged exposure to oxidative stress has been implicated in the development or acceleration of several diseases such as cardiovascular disease and autoimmune diseases (Estadella et al. 2013).

Fatty acids play important roles in immune and inflammatory responses. While saturated fatty acids and TFA enhance the inflammatory responses, n-3 fatty acids exhibit anti-inflammatory activity (Harvey et al. 2008; Rao and Lokesh 2014). Linseed Oil (LSO) is a rich source for α -linolenic acid (ALA, n-3 PUFA). It has beneficial effects in the prevention and treatment of autoimmune diseases like arthritis (Calder 2010). Studies have also shown that n-3 PUFA lower inflammatory conditions by enhancing the activities of endogenous antioxidant enzyme systems in rats (Ramaprasad et al. 2005). However, the effect of n-3 PUFA in countering inflammatory conditions exacerbated by TFA has received less attention.

The present investigation was designed to study the effect of ALA (18:3, n-3 PUFA) containing LSO on carrageenan induced paw inflammation and oxidative stress markers that may be altered by the consumption of PHVF containing EA. Carrageenan induced inflammation in rats is a widely used model for studying chronic inflammatory processes (Morris 2003). This model was used in the current study for monitoring the effect of feeding PHVF per se and PHVF mixed with LSO on inflammation in rats.

Materials and methods

Materials

Groundnut Oil (GNO) and PHVF, Olive Oil (OO) and corn starch were purchased from local super market, Mysore, India. Linseed Oil (LSO) was a generous gift from Kamani Oil Industries Pvt Ltd, Mumbai, India. Glutathione reduced, carrageenan λ (Irish moss, type IV), 8-hydroxy-2-deoxyguanosine, xanthine oxidase, cytochrome-C were obtained from Sigma Chemical Company, St. Louis, Mo. USA. The general chemicals used in all the analysis were obtained from Sisco Research Laboratory (Mumbai, India). All dietary ingredients were purchased from Himedia Laboratories (Mumbai, India).

Experimental animals

First set of experiments were carried to understand the effect of different oils given to rats in 1 mL dose per day by gavage on carrageenan induced inflammation. Male Wistar rats [IND-CFT (2c)] weighing 200 ± 6 g were given rodent pellets (Sai Durga Feeds, Bangalore, India). In addition, rats were given

daily 1 mL of following oils by gavage for a period of 15 days. GNO (1 mL); PHVF (1 mL); OO (1 mL); LSO (1 mL); PHVF+GNO (0.5+0.5 mL); PHVF+OO (0.5+0.5 mL); PHVF+LSO (0.5+0.5 mL). Paw inflammation was induced in these rats by injecting carrageenan as described in the following section.

In the second set of experiments, male wistar rats weighing 45–50 g were fed AIN-93 purified diet (Reeves et al. 1993) supplemented with following oils. PHVF (10 wt%), LSO (10 wt%), PHVF mixed with LSO at 2.5, 5.0 and 7.5 wt%. The final concentration of the fat in the diet was 10 wt%. The basal composition of the diet (g/kg diet) was corn starch; 500, casein; 200, sucrose; 100, fat; 100, cellulose; 50, mineral mix; 35, vitamin mix; 10, L-cysteine; 3, choline bitartrate; 2. The diet in powder form was fed to rats for a period of 60 days. The food intake and growth of the animals were monitored at regular intervals. Animals had free access to food and water. One set of rats from this group was used for inducing the inflammation in paw by injecting carrageenan and the second set of animals were used for analysis of lipid parameters such as fatty acid composition, antioxidant enzyme activities and lipid profile. Blood was drawn by cardiac puncture and centrifuged at $700 \times g$ for 15 min at 4°C to get serum which was used for further analysis.

The experimental protocol was approved by Institute Animal Ethical Committee (Reg.no: 49/1999/CPCSEA). The rats were housed in individual cages in a room where temperature was maintained at $25 \pm 2^\circ\text{C}$ with a 12-h light/dark cycle in an approved animal house facility in Central Food Technological Research Institute, Mysore, India.

Methods

Induction of inflammation

Inflammatory responses in the rats were followed by measuring the increase in paw volume after injecting carrageenan (Singh and Mourya 1972). Briefly, carrageenan (2.5 mg/kg body weight) in 200 μL sterile saline was injected in to the right hind paw under plantar aponeurosis. An equal volume of saline was injected in to the left paw which served as the control. At different intervals of time, the increase in the paw volume in response to carrageenan injection was measured by mercury displacement method as described earlier (Pulla Reddy and Lokesh 1994). The volume of the left paw injected with saline was also measured at different time intervals.

The area under the time curve ($\text{AUC}_{0-24\text{h}}$) was calculated by the trapezoidal method with linear interpolation, whereas the peak swelling (C_{max}) and the time to reach C_{max} (T_{max}) were directly taken from the analysed data with 'mi' denoting the single measurements, 'ti' denoting the time distance

between the measurements, and 'n' denoting the total number of measurements.

$$AUC = \left(\sum_{i=1}^{n-1} \frac{(m(i+1) + m_i).t_i}{2} \right)$$

Lipid analysis

Rats were sacrificed by giving diethyl ether anesthesia. Liver was removed from rats and rinsed in ice-cold saline, blotted and stored at -80°C till further analysis. Liver lipid was extracted using the method described by Folch, Lees, and Stanley (1957). One gram of liver was homogenised with 1.0 mL of 0.74 % potassium chloride and 20 mL of chloroform-methanol (2:1, v/v) for two min. The extract was left at 4°C overnight and filtered with Whatmann no: 1 filter paper. Potassium chloride (0.74 %, 3.0 mL) was added and mixed well. The solution was allowed to stand at room temperature until phase separation. The upper aqueous layer was removed carefully and then the lower phase was washed with 3 mL of chloroform/methanol/water (3:48:47 v/v) mixture. The chloroform layer was taken out used for lipid analysis.

The fatty acid methyl esters of dietary and liver lipids were prepared using 14 % BF_3 in methanol (Morrison and Smith 1964). The fatty acid methyl esters were separated using an SP-2560 fused silica capillary column (100 m $\times$ 0.25 mm i.d., 0.2 μm thickness: Supelco Chemical Co. St. Louis, MO, U.S.A). Individual fatty acid was identified by comparing with the retention times of standards (Sigma, St. Louis, MO, U.S.A.) and was quantified by an online Chromatopac CR-6A integrator. Pentadecanoic acid was used as internal standard.

Estimation of oxidative stress markers

Lipid peroxide

The lipid peroxides were estimated as malonaldehyde by the method of Buege and Aust (1978). One gram of liver was homogenized in 10 mL of 20 mM phosphate buffer, pH 7.4 using glass homogenizer. The homogenate was centrifuged at $600\times g$ for 15 min and supernatant was treated with thiobarbituric acid reagent. The malondialdehyde formed was measured at 535 nm and quantitated using extinction coefficient of $1.56 \times 10^{-5} \text{ cm}^{-1}$.

Protein carbonyl

Protein carbonyl content in the liver homogenate was determined as described by Levine et al. (1994). An aliquot of liver homogenate used in lipid peroxidation assay was mixed with 0.5 mL of 10 mM dinitrophenylhydrazine and incubated for 1 h. Protein was precipitated by adding 500 μL of 20 % TCA. The pellet was washed with acetone followed by centrifugation at $10,000\times g$ for 10 min. 1 mL of 2 % sodium dodecylsulphate (w/v) was added to the pellet and absorbance was read at 360 nm. The protein carbonyl content was calculated in terms of nmol/mg protein.

8-hydroxy-2-deoxyguanosine

The 8-hydroxy-2-deoxyguanosine in liver was estimated by the method of Abu-Quare and Abou-Donia (2000). One gram of liver was homogenized in 10 mL of 20 mM phosphate buffer, pH 7.4 using glass homogenizer. The homogenate was centrifuged at 600 g for 15 min and supernatant was acidified with 0.1 N acetic acid (pH: 5). The solution was passed through Sep Pak C-18 column (Waters, Millipore Corp., Milford, MA, USA) and 8-hydroxy-2-deoxyguanosine was eluted using methanol. The methanol extracts were evaporated under nitrogen and loaded on RP-C 18 column (Supelco, Discovery, 5 μM , 25 cm $\times$ 4.6 mm, Bellefonte, USA) fitted to a SPD- 20A HPLC (Shimadzu Corp., Tokyo, Japan). 8-hydroxy-2-deoxyguanosine was eluted with water (adjusted pH to 3 using 0.1 M Acetic acid): Acetonitrile (83:17 v/v) at a flow rate of 0.5 mL/min and monitored at 254 nm. The 8-hydroxy-2-deoxyguanosine levels were calculated from a curve generated with a standard and expressed as the nmole per mg protein.

Hepatic antioxidant enzymes

One gram of liver was homogenized in 10 mL of 20 mM phosphate buffer, pH 7.4 using glass homogenizer. The homogenate was centrifuged at 600 g for 15 min and supernatant was used for analysis of enzyme activities. Superoxide dismutase (SOD) activity was measured by the inhibition of cytochrome-C reduction mediated via superoxide anions generated by the xanthine/ xanthine oxidase system and monitored at 550 nm (Flohe and Otting 1984). Catalase activity was assayed according to the method of Aebi (1984) by following the decomposition of hydrogen peroxide, measured at 240 nm. Glutathione peroxidase (GPx) activity was determined by NADPH oxidation in a coupled reaction system, consisting of oxidized glutathione (Tappel 1978). Glutathione transferase (GST) was measured with 1-chloro 2, 4 dinitrobenzene as the substrate, and the enzyme activity was expressed as μmoles of chlorodinitrobenzoic acid-

glutathione conjugates formed per minute per mg protein (Jensson et al. 1985).

Estimation of protein

Total protein of liver homogenate was estimated by the method of Lowry et al. (1951) using bovine serum albumin as reference standard.

Statistical analysis

Results are presented as Mean $\pm$ Standard Deviation for each group. The data was analyzed by one way ANOVA followed by a post hoc Tukey test was used to measure statistical significance of data from different groups. *P*-values less than 0.05 were considered as statistically significant. All statistical analysis was performed using SPSS statistical software package version 17.0.

Results

Fatty acid composition of back ground diet

The composition of the back ground diet (wt%) consisted of carbohydrates; 64, protein; 20, Fat; 3, fiber; 6, mineral mix; 6, vitamin mix; 1. The Fatty acid composition (%) of this diet was 16:0; 25, 18:0; 5, 18:1; 33, and 18:2; 37. Fatty acid composition of oils and fats given to rats by gavage is shown in Table 1. PHVF contained 17.5 % of EA which was not observed in

other oils. 18:1Cis (41.9 %) and 18:2 n-6 PUFA (32.4 %) are the major fatty acids found in GNO. OO contained 18:1Cis (70.9 %) as the major fatty acid component. LSO contained 21.4 % 18:1Cis, 13.6 % of 18:2 n-6 PUFA and 51.7 % 18:3 n-3 PUFA. Thus major fatty acids present in oils given to rats differed significantly in terms of monounsaturated and polyunsaturated fatty acids. Mixing of PHVF with different proportions of LSO significantly reduced EA content in the oil and also significantly increased 18:3 levels (Table 1). These oils were given to rats for a period of 15 days at 1 mL per day by gavage. These rats were then injected with carrageenan in foot pads to induce inflammation.

Paw inflammation

Increase in paw volume was observed when carrageenan was injected to foot pad of rats. The paw increase in paw reached maximum at 3 h after injecting carrageenan. It however, declined gradually over a period of time. Rats injected with saline showed a marginal increase in paw volume which subsided within a 1 h of injecting saline. The rate and extent of paw inflammation observed in rats given carrageenan injection, however dependent on the type of oils fed to rats. Rats given PHVF and injected with carrageenan showed maximum increase in paw volume as compared to rats given other oils (Table 2 & Fig. 1a). Rats given PHVF by gavage for 15 days showed higher levels of inflammation by 14, 37 and 175 % at 3 h as compared to rats given GNO, OO and LSO. Therefore, rats given n-3 PUFA rich LSO exhibited significant anti-inflammatory property when injected with carrageenan as compared to those given PHVF.

Table 1 Fatty acid composition (wt%) of oils and fats given to rats by gavage at 1 mL per day for 15 days

Fatty acids	GNO (1mL)	PHVF (1mL)	OO (1mL)	LSO (1mL)	PHVF+GNO (0.5+0.5mL)	PHVF+OO (0.5+0.5mL)	PHVF+LSO (0.5+0.5mL)
14:0	nd	2.6 $\pm$ 0.2 ^a	nd	1.8 $\pm$ 0.2 ^a	1.8 $\pm$ 0.3 ^a	1.6 $\pm$ 0.2 ^a	1.8 $\pm$ 0.3 ^a
16:0	22.1 $\pm$ 1.2 ^c	39.9 $\pm$ 2.8 ^e	16.8 $\pm$ 1.3 ^b	8.3 $\pm$ 0.6 ^a	30.6 $\pm$ 1.9 ^d	31.2 $\pm$ 1.8 ^d	24.4 $\pm$ 1.2 ^c
18:0	3.6 $\pm$ 0.3 ^a	4.8 $\pm$ 0.4 ^a	3.5 $\pm$ 0.4 ^a	3.2 $\pm$ 0.4 ^a	4.1 $\pm$ 0.5 ^a	4.0 $\pm$ 0.4 ^a	4.4 $\pm$ 0.6 ^a
18:1 <i>trans</i>	nd	17.5 $\pm$ 1.4 ^d	nd	nd	8.8 $\pm$ 0.7 ^b	8.3 $\pm$ 0.8 ^b	8.5 $\pm$ 0.8 ^b
18:1 <i>cis</i>	41.9 $\pm$ 2.5 ^d	32.1 $\pm$ 1.3 ^c	70.9 $\pm$ 3.4 ^f	21.4 $\pm$ 1.1 ^a	36.4 $\pm$ 2.7 ^c	48.2 $\pm$ 1.5 ^c	27.7 $\pm$ 1.8 ^b
18:2	32.4 $\pm$ 3.1 ^f	3.1 $\pm$ 0.2 ^a	8.8 $\pm$ 0.6 ^c	13.6 $\pm$ 1.3 ^d	18.3 $\pm$ 1.2 ^e	6.7 $\pm$ 0.7 ^c	8.1 $\pm$ 0.4 ^c
18:3	nd	nd	nd	51.7 $\pm$ 3.2 ^d	nd	nd	25.1 $\pm$ 1.3 ^b
SFA	25.7 $\pm$ 1.5 ^c	47.3 $\pm$ 3.4 ^f	20.3 $\pm$ 1.7 ^b	13.3 $\pm$ 1.2 ^a	36.5 $\pm$ 2.7 ^c	36.8 $\pm$ 2.4 ^c	30.6 $\pm$ 2.1 ^d
MUFA	41.9 $\pm$ 2.5 ^d	49.6 $\pm$ 2.7 ^e	70.9 $\pm$ 3.4 ^g	21.4 $\pm$ 1.1 ^a	45.2 $\pm$ 3.4 ^c	56.5 $\pm$ 2.3 ^f	36.2 $\pm$ 2.6 ^c
PUFA	32.4 $\pm$ 3.1 ^e	3.1 $\pm$ 0.2 ^a	8.8 $\pm$ 0.6 ^c	65.3 $\pm$ 4.5 ^g	18.3 $\pm$ 1.2 ^d	6.7 $\pm$ 0.7 ^b	33.2 $\pm$ 1.7 ^e
P/S ratio	1.26 $\pm$ 0.29 ^d	0.06 $\pm$ 0.01 ^a	0.43 $\pm$ 0.05 ^c	4.91 $\pm$ 0.53 ^f	0.50 $\pm$ 0.06 ^c	0.18 $\pm$ 0.04 ^b	1.08 $\pm$ 0.11 ^d

GNO Ground nut oil, PHVF Partially hydrogenated vegetable fat, OO olive oil, LSO Linseed oil, SFA Saturated fatty acids, MUFA Monounsaturated fatty acids, PUFA Polyunsaturated fatty acids, P/S Polyunsaturated fatty acids/ saturated fatty acids, nd: not detected, Each value represents the mean $\pm$ SD of triplicate samples; Values in a row with same superscript are not statistically significant at *P*<0.05

Table 2 Carrageenan induced inflammation in rats given different oils for 15 days by gavage

Oils given by gavage	Inflammation (change in paw volume, mL)	% Decrease in paw volume as compared to rats given PHVF	AUC _(0–24h) (Change in paw volume, mL)
GNO (1 mL)	1.9±0.06 ^c	14	11.6±0.6 ^d
PHVF (1 mL)	2.2±0.1 ^g	–	15.3±1.1 ^g
OO (1 mL)	1.6±0.05 ^c	27	10.5±0.8 ^c
LSO (1 mL)	0.8±0.05 ^a	64	4.9±0.3 ^a
PHVF+GNO (0.5+0.5 mL)	2.0±0.07 ^f	9	13.4±1.2 ^f
PHVF+OO (0.5+0.5 mL)	1.8±0.06 ^d	18	12.9±1.3 ^e
PHVF+LSO (0.5+0.5 mL)	1.2±0.06 ^b	45	6.7±0.6 ^b

The second set of rats was given PHVF mixed with GNO or OO or LSO in equal amounts. Rats receiving the mix of PHVF+GNO, PHVF+OO and PHVF+LSO in equal proportions showed lesser increase of paw swelling in response to carrageenan injection by 9, 18 and 45 % respectively, as compared to those given PHVF alone (Table 2 & Fig. 1a). This study indicated that rats given PHVF by gavage are primed for higher levels of paw inflammation when injected with carrageenan. However, rats given PHVF blended with equal proportions of GNO, OO or LSO showed reduced levels of paw inflammation when injected with carrageenan as compared to rats given PHVF alone by gavage. Among the oils used for blending with PHVF, rats given PHVF blended with LSO showed maximum reduction in paw inflammation when injected with carrageenan. Therefore, in the next set of experiments the diet was prepared by mixing PHVF with incremental amounts of LSO and fed to rats for a period of 60 days before injecting carrageenan to foot pads for inducing inflammation.

Dietary studies

Fatty acid composition of dietary lipids

PHVF contained about 17 % EA, which was not detected in diets consuming GNO and LSO. LSO contained about 52 % of ALA. PHVF blended with LSO at 2.5, 5.0 and 7.5 wt% levels showed increased ALA levels to 13, 26 and 39 %, respectively. The diets containing PHVF blended with LSO at 2.5, 5.0 and 7.5 wt% levels showed a decrease in EA levels to 13, 8 and 4 %, respectively. These diets were fed to the rats for a period 60 days. The amount of food consumed by rats from

a Studies using rats given specific oils by gavage

b Dietary studies

Fig. 1 a Effect of feeding different lipids to rats for period of 15 days on carrageenan-induced inflammation b Effect of feeding PHVF and PHVF with incremental amounts of LSO to rats for 60 days on carrageenan induced inflammation. GNO: Groundnut oil, PHVF: Partially hydrogenated vegetable fat, LSO: Linseed oil

different groups was similar. No significant changes were observed in the food efficiency ratio measured by the gain in body weight to the amount of food consumed (data not shown). This study indicated that dietary PHVF and PHVF blended with LSO had no adverse effect on growth of animals.

Effect of feeding rats with PHVF, PHVF with incremental amounts of LSO on carrageenan induced paw inflammation

PHVF given in combination with LSO significantly reduced paw swelling in rats injected with carrageenan when the oils were provided through oral route. To validate these results through dietary intervention studies, rats (45–50 g) were fed a diet containing mixture of PHVF with LSO at different proportions. The final amount of fat present in the diet was 10 wt%. After 60 days of feeding these diet rats were injected with carrageenan in the foot pads as described in the methods to induce inflammation. Increase in paw swelling was

measured at different intervals of time (Fig. 1b). The rats given PHVF showed higher levels of paw inflammation by 45 and 175 % when compared to rats fed GNO and LSO, respectively. Paw swelling was reduced significantly by 32, 50 and 59 %, respectively in rats given mix of PHVF with LSO at 2.5, 5.0 and 7.5 wt% as compared to rats given PHVF alone. These studies indicated that n-3 PUFA rich oils such as LSO can significantly prevent the effect of PHVF on carrageenan induced inflammation in rats. These studies demonstrated that rats given PHVF (either by gavage or by inclusion in the diet) was primed to show higher degree of inflammation in foot pads when injected with carrageenan. This effect can be significantly reduced when rats were fed a diet containing PHVF mixed with incremental amounts of LSO. In order to determine whether other parameters including oxidative stress markers and antioxidant enzymes systems are also primed in a similar fashion, these parameters were monitored in liver of rats given PHVF or PHVF mixed with incremental amounts of LSO.

Fatty acid composition of liver lipids in rats given PHVF and PHVF with incremental amounts of LSO

The fatty acid composition of liver lipids reflected on the nature of dietary fats fed to the animals (Table 3). The EA was present to an extent of 5.6 % in liver lipids of rats fed PHVF alone, but it was decreased in rats fed mix of PHVF with LSO at 2.5, 5.0 and 7.5 wt% to 3.7, 2.8 and 1.7 % respectively. The ALA in liver lipids of rats given LSO alone was found to be 3.9 %. Rats given blends of PHVF with LSO at 2.5, 5.0 and 7.5 wt% levels showed ALA levels at 1.9, 2.8

and 3.7 %, respectively. Thus ALA in LSO can compete with EA from PHVF and reduce its incorporation in to liver lipids.

Effect of feeding rats with PHVF, PHVF with incremental amounts of LSO on oxidative stress markers

Oxidative stress has a significant influence on inflammation. Many of the oxidative stress markers linked with inflammation can be monitored in liver. In the present investigation the effect of feeding PHVF or PHVF after mixing with incremental amounts LSO to rats on liver lipid peroxides, protein carbonyls, 8-hydroxy-2-deoxyguanosine and activities of antioxidant enzymes were monitored in liver to evaluate the effect of dietary lipids on oxidative stress markers.

The liver lipid peroxide levels were found to be lower by 33 and 61 % in rats given PHVF as compared to rats given GNO and LSO, respectively (Fig. 2a). The liver lipid peroxide levels in rats fed mix of PHVF with LSO at 2.5, 5.0 and 7.5 wt% were increased by 35, 80 and 120 % when compared to rats given PHVF alone (Fig. 2a). The protein carbonyls in PHVF fed animals were found to be lower by 26 and 52 % as compared to rats given GNO and LSO, respectively (Fig. 2b). The protein carbonyls levels in rats fed mix of PHVF with LSO at 2.5, 5.0 and 7.5 wt% were increased by 21, 50 and 83 % respectively as compared to rats given PHVF alone (Fig. 2b). In addition to lipid peroxides, 8-hydroxy-2-deoxyguanosine is also an important biomarker for assessing the oxidative stress. The 8-hydroxy-2-deoxyguanosine levels in PHVF fed animals were found to be lower by 25 and 67 % as compared to that observed in rats given GNO and LSO, respectively (Fig. 2c). The 8-hydroxy-2-deoxyguanosine

Table 3 Fatty acid composition (wt%) of liver lipids in rats fed PHVF and PHVF mixed with incremental amounts of LSO

Fatty acids	GNO (10 wt%)	PHVF (10 wt%)	PHVF+LSO (7.5+2.5 wt%)	PHVF+LSO (5.0+5.0 wt%)	PHVF+LSO (2.5+7.5 wt%)	LSO (10 wt%)
14:0	2.1±0.2 ^a	2.8±0.3 ^a	2.2±0.2 ^a	2.7±0.3 ^a	2.5±0.2 ^a	2.9±0.2 ^a
16:0	26.2±1.3 ^a	30.5±1.7 ^b	29.3±1.5 ^b	24.1±2.2 ^a	25.9±2.1 ^a	24.1±0.6 ^a
16:1	1.8±0.3 ^{ab}	2.4±0.2 ^b	1.2±0.2 ^a	1.2±0.3 ^a	1.4±0.2 ^a	2.3±0.3 ^b
18:0	17.5±1.4 ^a	16.8±0.9 ^a	16.2±1.1 ^a	15.7±0.6 ^a	15.0±0.5 ^a	13.7±0.4 ^a
18:1 ^{trans}	nd	5.6±0.4 ^d	3.7±0.2 ^c	2.8±0.3 ^b	1.7±0.3 ^a	nd
18:1 ^{cis}	21.7±1.5 ^a	23.9±1.4 ^{ab}	25.0±1.5 ^b	25.6±1.8 ^b	24.7±1.2 ^b	25.3±1.7 ^b
18:2	16.8±1.2 ^c	10.9±0.5 ^a	12.0±0.5 ^a	13.1±0.4 ^{ab}	14.0±0.4 ^b	14.9±0.8 ^b
18:3	nd	nd	1.9±0.2 ^a	2.8±0.3 ^b	3.7±0.2 ^c	3.9±0.4 ^c
20:4	13.9±1.4 ^c	7.1±0.4 ^a	8.5±0.6 ^b	9.7±0.3 ^b	11.1±0.6 ^c	12.9±0.8 ^c
SFA	45.8±2.9 ^{ab}	50.1±2.9 ^b	47.7±2.8 ^b	42.5±3.1 ^a	43.4±2.8 ^a	40.7±1.2 ^a
MUFA	23.5±1.8 ^a	31.9±2.0 ^b	29.9±1.9 ^b	29.6±2.4 ^b	27.8±1.7 ^b	27.6±2.0 ^b
PUFA	30.7±2.6 ^c	18.0±0.9 ^a	22.4±1.3 ^b	25.6±1.0 ^b	28.8±1.2 ^c	31.7±2.0 ^c
P/S ratio	0.67±0.1 ^c	0.36±0.03 ^a	0.47±0.05 ^a	0.60±0.03 ^c	0.66±0.04 ^c	0.78±0.18 ^d

GNO Groundnut oil, PHVF Partially hydrogenated vegetable fat, LSO: Linseed oil, SFA Saturated fatty acids, MUFA Monounsaturated fatty acids, PUFA Polyunsaturated fatty acids, P/S Polyunsaturated fatty acids/ saturated fatty acids, nd: not detected. Each value represents the mean±SD of six rats per group. Values in a row with same superscript are not statistically significant at $P<0.05$

Fig 2 Effect of feeding rats with GNO, LSO, PHVF and PHVF with incremental amounts of LSO for 60 days on **a** lipid peroxidation **b** protein carbonyls **c** 8-hydroxy-2-deoxyguanosine in liver homogenate. GNO: Groundnut oil, PHVF: Partially hydrogenated vegetable fat, LSO: Linseed oil. Results are expressed as means $\pm$ SD of six rats per group. Means sharing the same superscript are not statistically significant at $P < 0.05$

levels in rats fed mix of PHVF with LSO at 2.5, 5.0 and 7.5 wt% were increased by 27, 53 and 80 % respectively as compared to rats given PHVF alone (Fig. 2c). These studies indicated that diets containing PUFA rich oils increased the lipid peroxides, protein carbonyls levels and 8-hydroxy-2-deoxyguanosine in liver of rats as compared to those given PHVF alone.

Hepatic antioxidant enzymes

The oxidative stress biomarkers such as lipid peroxide levels were found to be higher in rats given GNO, LSO indicating susceptibility of PUFA to oxidation. However inbuilt antioxidant systems are normally geared up to cope with the increased oxidative stress (Ramaprasad et al. 2005). Hence the levels of antioxidant enzymes were monitored in rats given PHVF and PHVF in combination with different levels of LSO. The specific activities of antioxidant enzymes superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione transferase (GST) were found to be lower by 17, 14, 16 and 18 %, respectively in rats fed PHVF, compared to rats fed GNO (Table 4). The specific activities of SOD, catalase, GPx and GST in rats fed LSO was found to be lower by 42, 33, 36 and 38 % respectively in rats given PHVF as compared to rats given LSO. SOD activity was however increased by 13, 29 and 54 % in rats fed mix of PHVF with LSO at 2.5, 5.0 and 7.5 wt% respectively, when compared to that in rats fed PHVF alone. Catalase activity was increased by 12, 24 and 33 % in rats fed blends of PHVF with LSO at 2.5, 5.0 and 7.5 wt%, respectively, when compared to rats fed PHVF

alone. GPx activity was increased by 15, 27 and 44 % in rats fed mix of PHVF with LSO at 2.5, 5.0 and 7.5 wt% respectively, when compared to rats fed PHVF alone. GST activity was increased by 15, 29 and 46 % in rats fed mix of PHVF with LSO at 2.5, 5.0 and 7.5 wt% respectively, when compared to rats fed PHVF alone (Table 4). Thus antioxidant enzyme activities in rats given PHVF significantly lowered when compared to that observed in rats fed PHVF as sole source of fat, but enhanced when rats were given PHVF mixed incremental amounts of LSO.

Discussion

The objective of this study was to evaluate the effect of feeding trans fatty acid (EA) containing fats like PHVF on carrageenan induced inflammation and oxidative stress markers in rats. The effect of feeding mixture of PHVF with n-3 PUFA rich oils such as LSO was also monitored on these markers in rats.

The current study demonstrated that rats fed PHVF showed higher levels of paw swelling when injected with carrageenan as compared to rats fed MUFA or PUFA rich oils. However, when PHVF was mixed with equal amounts of oils containing PUFA (n-6 PUFA or n-3 PUFA) and fed to rats there was a significant prevention in carrageenan induced paw swelling as compared to rats given PHVF alone. In this regard the effectiveness of n-3 PUFA rich oil was more beneficial as compared to other oils. Dietary trans fats promotes inflammation. Our current study indicated that dietary PHVF containing TFA enhanced carrageenan induced inflammation in rats. Pulla reddy and Lokesh (1994) have shown that carrageenan induced inflammation in rats fed coconut oil which is rich in saturated fatty acids is significantly higher as compared to those fed PUFA rich oils. Our present study also indicated that PUFA containing oils can reduce the extent of carrageenan induced paw inflammation in rats. These studies indicated that PHVF consumption renders the animals more vulnerable to inflammation as compared to those given oils containing cis-unsaturated fatty acids. However, PUFA is also known to downregulates the production of inflammatory mediators like interleukins, CRP and metallo proteases (Mazière et al. 1999). The n-3 PUFA can effectively decrease the arachidonic acid content in the membranes and limit its availability for the synthesis of pro inflammatory eicosanoids (Calder 2010). Our unpublished results also indicated that dietary PHVF elevates the levels of pro-inflammatory eicosnoids such as PGE₂, LTB₄ as compared to rats given n-3 PUFA rich ALA. Further we also observed upregulation of genes for PLA₂, COX-2, NF-κB and downregulation of PPARγ in PHVF fed rats.

Oxidative stress is considered to be an important promoter for inflammation (Iwata et al. 2011). Some of the oxidative

Table 4 Effect of feeding rats with PHVF and PHVF mixed with incremental amounts of LSO on antioxidant enzyme activities in liver homogenate

Dietary lipids	SOD (unit/min/ mg protein)	Catalase (μmol/min/ mg protein)	GPx (μmol/min/ mg protein)	GST (μmol/min/ mg protein)
GNO (10 wt%)	47.2±1.9 ^b	58.1±1.7 ^b	67.2±1.8 ^b	57.6±1.9 ^b
PHVF (10 wt%)	39.4±1.8 ^a	49.7±2.3 ^a	56.2±2.9 ^a	47.5±1.2 ^a
PHVF+LSO (7.5+2.5 wt%)	44.6±1.7 ^b	55.8±1.4 ^b	64.8±2.8 ^b	54.8±2.8 ^b
(5.0+5.0 wt%)	50.8±1.9 ^c	61.7±1.2 ^c	71.1±2.1 ^c	61.2±3.2 ^c
(2.5+7.5 wt%)	60.6±2.4 ^d	66.0±1.6 ^d	80.9±2.4 ^d	69.4±1.8 ^d
LSO (10 wt%)	67.5±2.1 ^e	73.8±2.0 ^e	87.8±3.6 ^e	76.8±2.4 ^e

GNO Groundnut oil, PHVF Partially hydrogenated vegetable fat, LSO: Linseed oil, SOD: superoxide dismutase, GPx: glutathione peroxidase, GST glutathione transferase. Each value represents the mean ± SD of six rats per group. Mean in a column with different superscript differ significantly at $P < 0.05$

stress markers can be monitored in liver. In order to assess whether oxidative stress has any influence on higher levels of inflammation observed in rats, we fed the animals with PHVF or PHVF containing LSO in incremental amounts. Rats fed PHVF as sole source of fat incorporated significant amount of 18 % trans fatty acid in hepatic lipids. 18:1trans in hepatic lipids. However when PHVF was mixed with incremental amounts of LSO and fed to rats, there was a progressive decrease in the levels of EA and an increase in the levels of ALA incorporated into liver lipids. Therefore ALA can compete with EA for incorporation into liver lipids. Similar results were obtained with peritoneal macrophages (Rao and Lokesh 2014). Rats fed diets containing LSO showed higher levels of oxidative stress markers such as lipid peroxides, protein carbonyls and 8-hydroxy-2-deoxyguanosine as compared to rats given PHVF. This indicated vulnerability of PUFA from LSO to oxidation. Since, PHVF containing fatty acids which are not vulnerable to oxidation, the animals on PHVF diet showed lower levels of conventional oxidative stress markers. Seppanen et al. (2013) also observed that rats given a diet high in PUFA showed higher levels of lipid peroxidation products in liver as compared to rats given a diet with low in PUFA. Sargis and Subbaiah (2003) studied the effect of trans and cis fatty acids on LDL oxidation. They enriched the LDL with cis and trans forms of 16:1 and 18:2. The LDL with trans fatty acids showed lesser vulnerability to oxidation by 19 % when compared to the ones containing cis fatty acids.

It is observed that vulnerability of PUFA to oxidation also results in enhanced antioxidant defense mechanisms in the body to minimize the oxidative damage to the system. The rats fed LSO showed higher activities of antioxidant enzymes such as SOD, catalase, GPx and GST. This may partly compensate for the high susceptibility of PUFA to oxidation and protect such lipids to some extent. The dietary PUFA are known to activate the nuclear factor kappa B (NF- κ B) receptor inducing NADPH oxidase through PPAR-dependent mechanism. The enzyme NADPH oxidase plays an important role in nonspecific host defense during infection by generating large quantities of superoxide anions. It has been demonstrated that vascular NADH/NADPH oxidase significantly contributes to superoxide production, a mechanism through which PUFA is thought to enhance the antioxidant enzyme activities (Mazière et al. 1999).

Ojeh et al. (2009) observed that the rats fed hydrogenated palm oil showed significantly reduced levels of GST, glutathione reductase, GPx activities compared to rats fed unhydrogenated palm oil. Dhibi et al. (2011) studied the effect of unmodified and hydrogenated soyabean oil on endogenous antioxidant enzyme activities in rat liver. They observed lower activities for SOD, catalase, GPx in liver homogenate of rats given hydrogenated fat. Therefore, hydrogenated fats do not evaluate antioxidant systems in the body unlike that observed when PUFA rich oils are fed (Nagaraju and Lokesh 2008). These studies indirectly indicated that dietary PHVF as compared to PUFA rich diets enhances the

vulnerability of rats to paw inflammation when injected with carrageenan. But dietary PHVF did not elevate oxidative stress markers which were observed in rats given cis unsaturated fatty acid containing oils. Inflammation can also be mediated through another pathway involving pro inflammatory cytokines and eicosanoids. TFA enhance intrinsic signaling mechanisms leading to a chronic, pro inflammatory state. Bryk et al. (2011) reported that the human aortic endothelial cells incubated with 100 μ mol/L of EA increased leukocyte adhesion to the endothelium through the ROS dependent NF- κ B activation. The diet high in hydrogenated fat increased production of inflammatory cytokines. Higher intakes of TFA increased concentrations of inflammatory molecules such as interleukin-6, tumor necrosis factor- α , C-reactive protein, and monocyte chemo attractant protein-1 (Iwata et al. 2011).

In summary rats fed PHVF showed higher levels of paw swelling when injected with carrageenan as compared to rats given LSO. Rats given PHVF mixed with different amounts of LSO significantly prevented inflammatory responses to carrageenan. However, lipid peroxides, protein carbonyls and 8-hydroxy-2-deoxyguanosine were found to increase in liver of rats given LSO as compared to that given PHVF. The antioxidant defense enzymes such as SOD, catalase, GPx and GST showed higher activity in rats given LSO or when PHVF was fed as a mix with LSO as compared to those given PHVF. Therefore, though PHVF intake elevated the susceptibility of rats to carrageenan induced inflammation, but it did not increase lipid peroxidation, protein carbonyls and 8-hydroxy-2-deoxyguanosine levels as compared to rats given n-3 PUFA containing LSO. The effect of PHVF in elevating susceptibility of rats to inflammation can be reduced by judiciously mixing PHVF with LSO. The effect of dietary PHVF on other inflammatory mediators such as interleukins, TNF- α , metallo proteases need to be evaluated to understand whether the susceptibility of rats to carrageenan induced inflammation can be mediated by a mechanism other than that of oxidative stress.

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Conflict of Interest The authors declare that they have no conflict of interest.

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