Journal of Oleo Science
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doi : 10.5650/jos.ess14128
J. Oleo Sci. **63**, *(11) 1117-1124 (2014)*

Antioxidative Effect of Rice Bran Oil and Medium Chain Fatty Acid Rich Rice Bran Oil in Arsenite Induced Oxidative Stress in Rats

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Abstract: The present study was adopted to evaluate the antioxidant efficacy of medium chain fatty acid (caprylic, capric and lauric) rich rice bran oils in comparison to rice bran oil in terms of altered biochemical parameters of oxidative stress following sodium arsenite treatment in rats. Animals were divided into ten groups; five normal groups and five arsenite treated groups. Results showed that activities of antioxidant enzymes in liver, brain and erythrocyte membrane increased with the administration of rice bran oil and MCFA rich rice bran oils both in normal and arsenite treated cases. Lipid peroxidation increased with the administration of sodium arsenite, but again administration of rice bran oil and MCFA rich rice bran oils decreased the lipid peroxidation. Caprylic acid rich rice bran oil showed the best ameliorative effects.

Key words: medium chain fatty acids, rice bran oil, antioxidant enzymes, peroxidation, erythrocyte membrane

1 Introduction

In many countries, the levels of arsenic (As) in the environment have turned out to be one of concern and many studies have recognized various adverse health effects on populations[1]. In modern days, exposure to sufficiently high concentrations of inorganic As in natural environments such as in water, sediment and soil has proved to be harmful to the organisms[2, 3]. The main pathways of exposure to the human beings include ingestion of drinking water and consumption of foods and to a lesser extent, inhalation of air[4]. Residual damage from the exposures to arsenic has been reported to lead to toxic consequences in the body and these toxic effects in the patient's body may lead to the development of secondary cancers[5, 6]. In view of the global health problems associated in drinking water and its impacts on the society, it is important to prevent the bioavailability of As in humans.

The dissolved As compounds are readily absorbed through the gastrointestinal tract after ingestion and distributed in the blood to different organs like liver, brain, kidney and so on, i.e., it affects almost entire organ systems of the body in particular, arsenic induces oxidative DNA damage and lipid peroxidation. A number of studies have shown arsenic-induced formation of reactive oxygen and nitrogen species as well as elevated DNA oxidation. Thus, arsenite increases the generation of superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2) in diverse cellular systems[7]. Liver is an important target organ for arsenic toxicity during its cycles between different oxidation states; arsenic generates reactive oxygen species (ROS) and causes organ-toxicity. ROS directly react with cellular biomolecules, damage lipids, proteins and DNA in cells and that can ultimately lead to cell death[8]. Arsenic binds with thiol groups on functional proteins and causes a primary imbalance between pro-oxidant and antioxidant homeostasis in biological systems[9]. It also induces oxidative tissue damage through interference with glutathione reductase (GR) utilization[10]. Inorganic arsenic has been shown to inhibit several of the antioxidant systems in the body, such as catalase, glutathione peroxidase and superoxide dismutase[11]. Thus, increasing the antioxidant levels in the body may protect against arsenic-induced toxicity. There are few reports in which certain antioxidant compounds like α-tocopherol, ascorbic acid and quercetin were used to repair or inhibit the oxidative damage induced by sodium

Abbreviations: As, arsenic; O_2^-, superoxide anions; H_2O_2, hydrogen peroxide; GR, reduced glutathione; ROS, reactive oxygen species; RBO, rice bran oil; CAT, catalase; SOD, superoxide dismutase; GC, gas chromatography; GPx, glutathione peroxidase.

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Accepted July 22, 2014 (received for review June 16, 2014)
Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
http://www.jstage.jst.go.jp/browse/jos/ http://mc.manusriptcentral.com/jjocs

arsenite[12, 13].

Reactive oxygen species (ROS) can cause cell and tissue damage and lipid peroxidation, leading to impaired cellular function and alterations in the physico-chemical properties of cell membranes, which in turn disrupt vital functions[14]. Medium chain fatty acids are likely to be highly resistant to peroxidation[15, 16]. Antioxidant enzymes provide protection against ROS and, similar to many other biochemical systems, their effectiveness varies with the stage of development and other physiological aspects of the organism[17, 18]. The most important antioxidant enzymes are superoxide dismutase, catalase and glutathione peroxidase[19]. Thus, the extent of lipid peroxidation is likely to depend in part on both the activity of antioxidant enzymes and the mix of fatty acids present in a target for peroxidation[20].

In our previous study we observed the antioxidant effect of capric acid rich mustard oil in comparison to mustard oil. Thus the aim of our study is to observe the antioxidative effect of caprylic acid, capric acid and lauric acid rich rice bran oils (RBO) in comparison to rice bran oil fed to oxidative stress generated rats.

2 Materials and Methods

2.1 Chemicals

Sodium arsenite ($NaAsO_2$) which was chosen as the source of arsenic, was purchased from S.D. Fine-Chem limited, Mumbai, India. All other chemicals used in the experiment were of analytical grade. The dose of Sodium arsenite (10 mg/kg BW) was chosen on the basis of the previous studies[12, 21]. The medium chain fatty acids were purchased from Sisco Research Loboratories Pvt. Ltd.

2.2 Production of medium chain fatty acid rich RBO

The reaction between caprylic acid, capric acid and lauric acid with rice bran oil was carried out in a packed-bed bioreactor. The reactor consisted of a tubular glass column of 10 mm ID and was 50 cm long. It was also provided with a water jacket for temperature control. The immobilised enzyme (*Rhizomucor mehei*) packed into the reactor was retained in place by means of a sintered plate. The substrates were fed from the top and the products were collected at the bottom. The substrates were previously blended and well mixed at the reaction temperature before conducting the packed-bed reaction and were poured into the enzyme bed, maintaining a fixed sample head. Water from a constant temperature bath was circulated through the jacket by a peristaltic pump. A partial suction was given to maintain the constant flow rate (0.4 mL/min; optimized in the previous study); 20 g of enzyme was closely packed into the column by repeated tapping to avoid any air gaps. Transesterification reactions were then carried out by passing the substrate through the column. The temperature was maintained at the desired value of 60℃ by passing water through the column jacket. The product mixture was collected at the outlet and the fatty acid composition of the oils was determined by gas chromatography (GC).

2.3 Chromatographic analysis of oils

Fatty acid compositions of native and medium chain fatty acid-enriched rice bran oil were analysed by GC. The oils were saponified with 0·5 M KOH and methylated with boron trifluoride in methanol. The gas chromatograph (Agilent 6890 N; J&W Scientific, Wilmington, DE, USA) was fitted with a DB-Wax capillary column (30m × 0·32mm × 0·25 mm) and a flame ionization detector. N_2, H_2 and airflow rate were maintained at 1, 30 and 300 ml/min, respectively. Inlet and detector temperatures were kept at 250℃ and the oven temperature was programmed to increase from 150 to 190℃ at a rate of 15℃/min, then to hold for 5 min, and then to increase to 230℃ at a rate of 48℃/min, and then again to hold for 10 min.

2.4 Animal Treatment

Adult male albino rats of Wistar strain were housed and given food and water ad libidum. The duration of the experimental period was 32 days. The rats were fed balanced diet having the following composition: fat free casein-18% (protein source), fat-20%, starch-55% (carbohydrate source), Salt mixture 4% [composition of salt mixture No.12 (wt in gm): NaCl-292.5; KH_2PO_4-816.6; $MgSO_4$-120.3; $CaCO_3$-800.8; $FeSO_4$, $7H_2O$-56.6; KCl-1.66; $MnSO_4$, $2H_2O$-9.35; $ZnCl_2$-0.5452; $CuSO_4$, $5H_2O$-0.9988; $CoCl_2$-, $6H_2O$-0.0476]; cellulose-3%; and one multivitamin capsule (Vitamin A.I.P. 10,000 units, thiamine mononitrate I.P.5 mg, vitamin B.I.P. 5 mg, calcium pantothenate USP 5 mg, niacinamide I.P. 50 mg, ascorbic acid I.P. 400 units, cholecalciferol USP 15 units, menadione I.P-9.1 mg, folic acid I.P -1 mg and vitamin E USP 0.1 mg) per kg of diet. The diet was adequate in all nutrients. The animals were divided into ten groups with six rats in each: Group I: vehicle treated control animals, Group II: rats fed with rice bran oil instead of control oil, Group III: rats fed with caprylic acid rich rice bran oil, Group IV: rats fed with capric acid rich rice bran oil, Group V: rats fed with lauric acid rich rice bran oil, Group VI: rats were fed with sodium arsenite (10 mg/kg BW) for 14 days, Group VII: rats received rice bran oil along with sodium arsenite (10 mg/kg BW) for 14 days, Group VIII: rats received caprylic acid rich rice bran oil along with sodium arsenite (10 mg/kg BW) for 14 days, Group IX: rats received capric acid rich rice bran oil along with sodium arsenite (10 mg/kg BW) for 14 days, Group X: rats received lauric acid rich rice bran oil along with sodium arsenite (10 mg/kg BW) for 14 days; Groundnut oil was used as the vehicle and given to all the groups as the level of 20% of the total diet. At the end of the experiment

the feeding of rats was stopped and after 12 h fasting, the rats were anesthetized by chloroform and 5 ml of blood was taken from the heart in heparinized tubes. The plasma was obtained by centrifugation of the blood. The liver, brain and mesentery was removed, rinsed with ice-cold saline, blotted, weighed and stored at −20℃ until analyzed.

2.5 Analysis of plasma lipid concentrations

Total cholesterol, HDL-cholesterol and triglyceride concentrations were determined using enzyme kits supplied by Merck India Limited.

2.6 Enzyme assays

Measured amounts of liver and brain were homogenised in phosphate buffer. The samples were then centrifuged and the supernatants were used for the enzyme assay. The activity of catalase (CAT) was determined spectrometrically by the method of Aebi[22]. Superoxide dismutase (SOD) activity was assayed by measuring the auto-oxidation of haematoxylin as described by Martin *et al.*[23]. Glutathione reductase (GR) was determined by the method of Ellman[24]. Total activity of glutathione peroxidase (GPx) was determined in the tissue homogenates and plasma according to Flohe & Gunzler[25]. All enzyme activities are expressed as enzyme units per mg protein. Protein content was determined using the method of Lowry *et al.*[26].

2.7 Preparation of erythrocyte membranes

All procedures were done at 0–5℃ (typically on ice), and all centrifugations were performed in a Sorvall SS-34 rotor at 15,000 rpm unless specified. Rat red cells and hemoglobin-free ghosts were prepared as described in the literature[10], except that the hemolysis buffer was 5 mM * NaPi (pH 8), 0.01 mM $MgSO_4$, and the membranes were suspended for 10 min in this buffer before each centrifugation to allow hemoglobin to exit fully.

2.8 Antioxidant Enzyme Assay of erythrocyte membrane

Measured amounts of erythrocyte membrane were homogenized in phosphate buffer. The samples were then centrifuged and the supernatants were used for enzyme assay. The activity of CAT, SOD, GR and GPx were determined using the same methods described previously.

2.9 Products of lipid peroxidation

For lipid peroxide measurement, approximately 1 g of liver or 0·4ml of plasma was placed into a glass centrifuge tube (70 ml) for 2 min in a solvent mixture consisting of 10 ml chloroform and 20 ml methanol, and homogenised on ice. Then, 10 ml of chloroform was added and homogenization continued for another 30 s. Finally, 10 ml of redistilled water were added and the mixture was homogenised for 30 s. The tubes were then centrifuged for 20 min at 4000 rpm, and the chloroform layer was separated[27]. Thiobarbituric acidreactive substances were measured according to the method described by Schmedes & Hølmer[28]. Malondialdehyde (MDA) concentration was calculated by taking the extinction coefficient of MDA to be 1.56×10^5/M cm[29].

2.10 Statistical analysis

All the data are presented as means with their standard errors. Statistical comparisons between groups were performed using one way ANOVA by Tukey test.

3 Results

3.1 Changes in fatty acid composition

Analysis of the medium chain fatty acid rich RBO showed that caprylic acid rich RBO contained 14.00% caprylic acid (C8), capric acid rich RBO contained 13.73% capric acid (C10) and lauric acid rich RBO contained 13.11% lauric acid (C12). The fatty acid compositions of the MCFA rich RBOs, are given in Table 1.

3.2 Changes in plasma lipid profile

Lipid profiles of plasma of different rats fed with different dietary oils are presented in Table 2. In the absence of added sodium arsenite, plasma total cholesterol, non-HDL-cholesterol and triglyceride concentrations were lower in rats fed the MCFA-enriched RBO compared with those fed the native RBO (Table 2). Conversely, HDL-cholesterol concentration was higher in rats fed the MCFA-enriched RBO (Table 2). Adding sodium arsenite to the diet increased plasma total cholesterol, non-HDL-cholesterol and triglyceride concentrations and decreased HDL-cholesterol concentration (Table 2). However, the plasma lipid profile was better when rats received the MCFA-enriched RBO

Table 1 Fatty acid composition of native rice bran oil and medium chain fatty acid rice rice bran oils.

Fatty acid / Sample	Fatty Acid (% w/w)								
	$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Rice Bran Oil	–	–	–	0.28	17.10	1.90	48.27	31.35	1.1
Caprylic acid rich RBO	**14.00**	–	–	0.29	13.77	1.90	40.76	28.28	1.0
Capric acid rich RBO	–	**13.43**	–	0.27	10.44	1.89	43.39	29.58	1.0
Lauric acid rich RBO	–	–	**13.11**	0.27	10.56	1.88	45.29	27.89	1.0

Table 2 Plasma lipid concentrations (mg/dL) in rats fed the different diets.

Parameters	Normal Control		Rice bran oil		Caprylic acid-enriched rice bran oil		Capric acid-enriched rice bran oil		Lauric acid-enriched rice bran oil	
	No added Na Arsenite	+ Na Arsenite	No added Na Arsenite	+ Na Arsenite	No added Na Arsenite	+ Na Arsenite	No added Na Arsenite	+ Na Arsenite	No added Na Arsenite	+ Na Arsenite
Total cholesterol	150.12 ± 1.20	340.60 ± 0.98[b]	130.30 ± 2.70[a]	214.67 ± 2.98[c]	65.33 ± 0.23[a,d]	139.31 ± 1.89[c,d]	73.33 ± 0.11[a,d,e]	154.00 ± 1.23[c,d,e]	106.67 ± 0.56[a,d,e]	166.17 ± 1.22[c,d,e]
HDL-cholesterol	18.69 ± 0.23	11.21 ± 0.11[b]	23.00 ± 1.21[a]	16.92 ± 1.11[c]	30.62 ± 1.82[a,d]	24.00 ± 1.85[c,d]	28.24 ± 1.02[a,d,e]	22.33 ± 0.66[c,d,e]	26.27 ± 0.34[a,d,e]	17.67 ± 0.21[c,d,e]
Non-HDL cholesterol	118.72 ± 2.13	305.82 ± 1.88[b]	96.65 ± 1.78[a]	177.63 ± 2.31[c]	28.60 ± 1.01[a,d]	101.47 ± 0.77[c,d]	36.27 ± 1.23[a,d,e]	116.98 ± 2.30[c,d,e]	70.65 ± 1.02[a,d,e]	132.03 ± 1.30[c,d,e]
Triacylglycerol	63.57 ± 1.20	117.87 ± 0.45[b]	53.23 ± 0.20[a]	100.60 ± 1.29[c]	30.57 ± 0.34[a,d]	69.20 ± 0.10[c,d]	44.11 ± 1.00[a,d,e]	73.45 ± 1.20[c,d,e]	48.76 ± 0.87[a,d,e]	82.36 ± 0.20[c,d,e]

Values are mean ± S.E.M. (n=10 rats per diet group)
[a] Comparison between control group and other groups in normal case (p<0.05)
[b] Comparison between normal control and Na arsenite treated control group (p<0.05)
[c] Comparison between Na arsenite treated control group and other groups in Na arsenite treated group (p<0.05)
[d] Comparison between RBO group and MCFA-rich RBO treated groups both in normal and Na arsenite treated group (p<0.05)
[e] Comparison between caprylic acid rich RBO group and other MCFA-rich RBO treated groups both in normal and Na arsenite treated group (p<0.05)

Table 3 Antioxidant activity (U/min/mg protein) of liver tissue.

Antioxidant enzymes	Normal Control		Rice bran oil		Caprylic acid-enriched rice bran oil		Capric acid-enriched rice bran oil		Lauric acid-enriched rice bran oil	
	No added Na Arsenite	+ Na Arsenite	No added Na Arsenite	+ Na Arsenite	No added Na Arsenite	+ Na Arsenite	No added Na Arsenite	+ Na Arsenite	No added Na Arsenite	+ Na Arsenite
CAT	3.31 ± 0.09	0.54 ± 0.05[b]	4.76 ± 0.04[a]	1.22 ± 0.08[c]	8.27 ± 0.23[a,d]	2.89 ± 0.11[c,d]	7.05 ± 0.09[a,d,e]	2.54 ± 0.01[c,d,e]	5.26 ± 0.06[a,d,e]	1.98 ± 0.04[c,d,e]
SOD	0.97 ± 0.05	0.17 ± 0.01[b]	1.36 ± 0.04[a]	0.32 ± 0.02[c]	2.71 ± 0.23[a,d]	0.73 ± 0.01[c,d]	2.22 ± 0.10[a,d,e]	0.54 ± 0.01[c,d,e]	1.65 ± 0.06[a,d,e]	0.43 ± 0.03[c,d,e]
GR	20.34 ± 0.21	7.82 ± 0.10[b]	24.45 ± 0.04[a]	14.89 ± 0.20[c]	32.34 ± 0.44[a,d]	20.25 ± 0.11[c,d]	30.55 ± 0.10[a,d,e]	19.82 ± 0.45[c,d,e]	27.66 ± 0.23[a,d,e]	16.87 ± 0.13[c,d,e]
GPx	0.66 ± 0.01	0.10 ± 0.01[b]	1.00 ± 0.02[a]	0.21 ± 0.02[c]	1.68 ± 0.03[a,d]	0.56 ± 0.01[c,d]	1.50 ± 0.02[a,d,e]	0.42 ± 0.01[c,d,e]	1.20 ± 0.03[a,d,e]	0.31 ± 0.01[c,d,e]

Values are mean ± SEM (n = 10 rats per diet group)
[a] Comparison between control group and other groups in normal case (p<0.05)
[b] Comparison between normal control and Na arsenite treated control group (p<0.05)
[c] Comparison between Na arsenite treated control group and other groups in Na arsenite treated group (p<0.05)
[d] Comparison between RBO group and MCFA-rich RBO treated groups both in normal and Na arsenite treated group (p<0.05)
[e] Comparison between caprylic acid rich RBO group and other MCFA-rich RBO treated groups both in normal and Na arsenite treated group (p<0.05)

Table 4 Antioxidant activity (U/min/mg protein) of brain tissue.

Antioxidant enzymes	Normal Control		Rice bran oil		Caprylic acid-enriched rice bran oil		Capric acid-enriched rice bran oil		Lauric acid-enriched rice bran oil	
	No added Na Arsenite	+ Na Arsenite	No added Na Arsenite	+ Na Arsenite	No added Na Arsenite	+ Na Arsenite	No added Na Arsenite	+ Na Arsenite	No added Na Arsenite	+ Na Arsenite
CAT	2.31 ± 0.13	0.54 ± 0.05[b]	3.40 ± 0.04[a]	1.02 ± 0.18[c]	7.23 ± 0.02[a,d]	2.89 ± 0.01[c,d]	6.05 ± 0.10[a,d,e]	2.23 ± 0.05[c,d,e]	4.15 ± 0.13[a,d,e]	1.34 ± 0.14[c,d,e]
SOD	0.92 ± 0.02	0.14 ± 0.01[b]	1.22 ± 0.09[a]	0.19 ± 0.02[c]	2.02 ± 0.23[a,d]	0.45 ± 0.01[c,d]	1.78 ± 0.12[a,d,e]	0.34 ± 0.01[c,d,e]	1.35 ± 0.03[a,d,e]	0.21 ± 0.01[c,d,e]
GR	18.12 ± 0.34	5.92 ± 0.13[b]	22.15 ± 0.14[a]	9.87 ± 0.09[c]	32.34 ± 0.12[a,d]	15.00 ± 0.22[c,d]	30.15 ± 0.15[a,d,e]	13.10 ± 0.25[c,d,e]	26.11 ± 0.34[a,d,e]	11.99 ± 0.13[c,d,e]
GPx	0.60 ± 0.01	0.09 ± 0.01[b]	0.79 ± 0.02[a]	0.19 ± 0.02[c]	1.50 ± 0.03[a,d]	0.40 ± 0.01[c,d]	1.32 ± 0.02[a,d,e]	0.32 ± 0.01[c,d,e]	1.19 ± 0.03[a,d,e]	0.26 ± 0.01[c,d,e]

Values are mean ± SEM (n=10 rats per diet group)
[a] Comparison between control group and other groups in normal case (p<0.05)
[b] Comparison between normal control and Na arsenite treated control group (p<0.05)
[c] Comparison between Na arsenite treated control group and other groups in Na arsenite treated group (p<0.05)
[d] Comparison between RBO group and MCFA-rich RBO treated groups both in normal and Na arsenite treated group (p<0.05)
[e] Comparison between caprylic acid rich RBO group and other MCFA-rich RBO treated groups both in normal and Na arsenite treated group (p<0.05)

plus sodium arsenite compared with those that received the native RBO plus sodium arsenite (Table 2). Among the three MCFA-enriched RBOs caprylic acid showed highest ameliorative effect while lauric acid showed the least effect.

3.3 Antioxidant enzyme activities

The same pattern of effect of the diets was seen on antioxidant enzyme activities in both liver and brain (Tables 3 and 4). In the absence of added sodium arsenite, antioxidant enzyme activities were much higher ($p < 0.05$) in the liver and brain of rats fed the MCFA-enriched RBO com-

pared with those fed the native RBO (Tables 3 and 4). Adding sodium arsenite to the diet decreased the activities of all enzymes, although the effect was not significant in all cases. However, all enzyme activities were higher in liver and brain when rats received the MCFA-enriched RBO plus sodium arsenite compared with those that received the native RBO plus sodium arsenite (Tables 3 and 4). The highest enzyme activities were always seen in the group receiving the caprylic acid-enriched RBO. Interestingly, enzyme activities were often higher in tissues of rats fed the caprylic acid-enriched RBO plus sodium arsenite than in those fed the native RBO.

3.4 Changes in antioxidant enzyme activities of erythrocyte membrane

The same pattern of effect of the diets was seen on antioxidant enzyme activities in erythrocyte membrane (Table 5). Adding sodium arsenite to the diet decreased the activities of all enzymes, although the effect was not significant in all cases. However, all enzyme activities were higher in erythrocyte membrane when rats received the MCFA-enriched RBO and groundnut oil plus sodium arsenite compared with those compared the control groundnut oil plus sodium arsenite (Table 5). The highest enzyme activities were always seen in the group receiving caprylic acid rich RBO in higher dose.

3.5 Lipid peroxidation

The same pattern of effect of the diets was seen on MDA concentrations in liver, brain and plasma (Table 6). In the absence of added sodium arsenite, MDA concentrations were much lower in tissues and plasma of rats fed the MCFA enriched RBO compared with those fed the native RBO (Table 6). Adding sodium arsenite to the diet increased MDA concentrations. However, MDA concentrations were lower when rats received the MCFA enriched RBO plus sodium arsenite compared with those that received the native RBO plus sodium arsenite (Table 5). The lowest MDA concentrations were always seen in the group receiving the caprylic acid enriched RBO plus sodium arsenite.

4 Discussion

The purpose of the study was to determine the effect of

Table 5 Antioxidant activity (U/min/mg protein) of erythrocyte membrane.

Antioxidant enzymes	Normal Control		Rice bran oil		Caprylic acid-enriched rice bran oil		Capric acid-enriched rice bran oil		Lauric acid-enriched rice bran oil	
	No added Na Arsenite	+ Na Arsenite	No added Na Arsenite	+ Na Arsenite	No added Na Arsenite	+ Na Arsenite	No added Na Arsenite	+ Na Arsenite	No added Na Arsenite	+ Na Arsenite
CAT	3.31 ± 0.20	1.14 ± 0.15[b]	5.40 ± 0.20[a]	2.02 ± 0.23[c]	9.11 ± 0.25[a,d]	4.19 ± 0.09[c,d]	7.05 ± 0.18[a,d,e]	3.33 ± 0.05[c,d,e]	6.09 ± 0.14[a,d,e]	2.20 ± 0.34[c,d,e]
SOD	1.56 ± 0.05	0.67 ± 0.04[b]	2.30 ± 0.10[a]	1.02 ± 0.02[c]	3.00 ± 0.20[a,d]	1.56 ± 0.02[c,d]	2.75 ± 0.10[a,d,e]	1.30 ± 0.01[c,d,e]	2.52 ± 0.11[a,d,e]	1.10 ± 0.01[c,d,e]
GSH	22.12 ± 1.02	8.45 ± 0.38[b]	29.10 ± 0.56[a]	11.23 ± 0.19[c]	38.01 ± 0.56[a,d]	16.23 ± 0.22[c,d]	33.15 ± 0.20[a,d,e]	14.00 ± 0.16[c,d,e]	31.05 ± 0.04[a,d,e]	12.33 ± 1.13[c,d,e]
GPx	0.90 ± 0.01	0.19 ± 0.02[b]	1.15 ± 0.02[a]	0.30 ± 0.01[c]	1.86 ± 0.03[a,d]	0.45 ± 0.01[c,d]	1.54 ± 0.01[a,d,e]	0.40 ± 0.01[c,d,e]	1.30 ± 0.02[a,d,e]	0.32 ± 0.01[c,d,e]

Values are mean ± SEM (n=10 rats per diet group)

[a] Comparison between control group and other groups in normal case ($p<0.05$)

[b] Comparison between normal control and Na arsenite treated control group ($p<0.05$)

[c] Comparison between Na arsenite treated control group and other groups in Na arsenite treated group ($p<0.05$)

[d] Comparison between RBO group and MCFA-rich RBO treated groups both in normal and Na arsenite treated group ($p<0.05$)

[e] Comparison between caprylic acid rich RBO group and other MCFA-rich RBO treated groups both in normal and Na arsenite treated group ($p<0.05$)

Table 6 Lipid peroxidation (nmoleMDA/min/mg protein) of liver and brain homogenates and plasma peroxidation.

Peroxidation	Normal Control		Rice bran oil		Caprylic acid-enriched rice bran oil		Capric acid-enriched rice bran oil		Lauric acid-enriched rice bran oil	
	No added Na Arsenite	+ Na Arsenite	No added Na Arsenite	+ Na Arsenite	No added Na Arsenite	+ Na Arsenite	No added Na Arsenite	+ Na Arsenite	No added Na Arsenite	+ Na Arsenite
Liver lipid	25.06 ± 0.23	41.19 ± 1.00[b]	20.70 ± 0.61[a]	28.88 ± 0.20[c]	10.13 ± 0.08[a,d]	17.11 ± 0.12[c,d]	13.18 ± 0.28[a,d,e]	18.42 ± 0.19[c,d,e]	15.42 ± 0.09[a,d,e]	20.70 ± 0.11[c,d,e]
Brain lipid	16.89 ± 0.05	23.58 ± 0.13[b]	12.71 ± 0.20[a]	19.05 ± 0.18[c]	5.33 ± 0.09[a,d]	14.31 ± 0.15[c,d]	6.97 ± 0.22[a,d,e]	16.71 ± 0.12[c,d,e]	8.83 ± 0.31[a,d,e]	17.88 ± 0.10[c,d,e]
Plasma	19.83 ± 0.11	28.33 ± 0.32[b]	17.29 ± 0.21[a]	25.24 ± 0.31[c]	5.96 ± 0.20[a,d]	12.42 ± 0.18[c,d]	8.98 ± 0.16[a,d,e]	14.83 ± 0.23[c,d,e]	12.25 ± 0.15[a,d,e]	0.32 ± 0.17[c,d,e]

Values are mean ± SEM (n=10 rats per diet group)

[a] Comparison between control group and other groups in normal case ($p<0.05$)

[b] Comparison between normal control and Na arsenite treated control group ($p<0.05$)

[c] Comparison between Na arsenite treated control group and other groups in Na arsenite treated group ($p<0.05$)

[d] Comparison between RBO group and MCFA-rich RBO treated groups both in normal and Na arsenite treated group ($p<0.05$)

[e] Comparison between caprylic acid rich RBO group and other MCFA-rich RBO treated groups both in normal and Na arsenite treated group ($p<0.05$)

medium chain fatty acid rich rice bran oil on oxidative stress generated by sodium arsenite in comparison to native rice bran oil. Arsenite is known to enhance the microsomal oxidation capacity of liver cells. This study shows significant decrease in antioxidant enzymes activity by administration of arsenic poisoning in the form of sodium arsenite. Such alteration of oxidative stress markers is suggested to be due overuse failure of the antioxidant defense system secondary to reactive oxygen species production, as evidenced by the study of Ramanathan *et al*.[11].

The activities of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase form the first line of defense against reactive oxygen species[30]. With the administration of sodium arsenite there was a decrease in the activities of the enzymes which proved the generation of oxidative stress. In the present study, the antioxidant enzyme activities were increased with the administration of MCFA rich rice bran oil both in normal and arsenite treated cases. Restoration of activities of these enzymes in the liver and brain tissues with MCFAs may be due to increased intracellular concentration of the non-enzymatic antioxidant GR, whose level was decreased in those tissues by administration of sodium arsenite. GR is one of the body's most important endogeneous antioxidants responsible for free radical scavenging in all cell types. RBO and MCFA rich RBO increased the level of GR in brain and liver tissues.

The present study was also designed to examine the levels of erythrocyte antioxidant enzyme activities in normal and arsenite treated groups. Several enzymes have evolved in aerobic cells to overcome the damaging effects of reactive oxygen species (ROS). They are significantly used to maintain the redox balance during oxidative stress and are collectively called as endogenous antioxidative enzymes. Superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) are the main endogenous enzymatic defense systems of all aerobic cells[17]. They give protection by directly scavenging superoxide radicals and hydrogen peroxide, converting them to less reactive species[31]. SOD catalyzes the dismutation of superoxide radical ($\cdot O_2$) to hydrogen peroxide (H_2O_2). Although H_2O_2 is not a radical, it is rapidly converted by fenton reaction into $\cdot$OH radical which is very reactive. Among various antioxidant mechanisms in the body, SOD is thought to be one of the major enzymes that protect cells from ROS. Glutathione peroxidase (GPx) neutralizes hydrogen peroxide by taking hydrogens from two GR molecules resulting in two H_2O and one GSSG. The enzyme glutathione reductase then regenerates GSH from GSSG with NADPH as a source of hydrogen. Another important part of the enzymatic defense system is catalase. CAT is one of the most active catalysts produced by nature. CAT is largely, but not exclusively, localized in peroxisomes, wherein many H_2O_2 producing enzymes reside. Thus CAT, which exhibits a high Km for H_2O_2, can act upon H_2O_2 produced before it diffuses to other parts of the cell. CAT is a tetrameric heme containing enzyme that is found in all aerobic organisms. Because of its wide distribution, evolutionary conservation and capacity to rapidly degrade hydrogen peroxide, it has been proposed that CAT plays an important role in systems which have evolved to allow organisms to live in aerobic environments. Therefore activity of CAT is one of the important biomarker of oxidative stress. Results showed that antioxidant enzyme activities in erythrocyte membrane were lowest in arsenite treated group due to excessive production of ROS. MCFA rich RBOs increased the enzyme activities thus lowering the oxidative stress induced by sodium arsenite. Caprylic acid rich RBO produces the highest effect against arsenic poisoning. MCFA rich RBOs produced greater effect than RBO.

We also evaluated lipid peroxidation by measuring hepatic levels of malonaldehyde (MDA). In agreement with other studies, we observed a significant increase in MDA levels in tissues of arsenic poisoning rats when compared to normal control rats. Both RBO and MCFA rich RBOs produced less increase in lipid peroxidation which indicates that they help in improving antioxidant potential of tissues. Moreover caprylic acid rich RBO produced the best results. RBO produced decreased lipid peroxidation due to its oryzanol content[32]. MCT rich RBO further decreased the lipid peroxidation by reducing the content of PUFA in RBO.

5 Conclusion

Thus it may be possible that in the in vivo conditions these MCFA rich RBO may have reduced the formation of hydro-peroxides by lowering the formation of free radicals. Therefore it appears that inclusion of MCFA in RBO in diet improves antioxygenic potential and protect against oxidative stress to a reasonable extent.

Acknowledgement

Authors would like to acknowledge University Grants Commission for providing the research fellowship and the research grant Dr. D.S. Kothari Postdoctoral Fellowship.

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