The hypolipidemic action of a diet supplemented with \( \text{p, p'}- \text{methoxyl-diphenyl diselenide} \) is not directly related to its antioxidant property.
The hypolipidemic action of a diet supplemented with \( p,p' \)-methoxyl-diphenyl diselenide is not directly related to its antioxidant property.

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Running title: (OMePhSe)\(_2\) diet attenuated lipid alterations in rats

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Abstract

The present study investigated if a \( p,p' \)-methoxyl-diphenyl diselenide \((\text{MeOPhSe})_2\)-supplemented diet causes toxicity in rats. A second aim of this study was to determine if a 10 ppm \((\text{MeOPhSe})_2\)-supplemented diet has hypolipidemic effect on Triton WR-1339-induced hyperlipidemia in rats. In order to rule out the antioxidant property of \((\text{MeOPhSe})_2\) in its hypolipidemic action, parameters of oxidative stress were carried out. Wistar rats were fed with 3, 10 or 30 ppm of \((\text{MeOPhSe})_2\)-supplemented diet for 30 days. None of \((\text{MeOPhSe})_2\)-supplemented diets caused alteration in general parameters of toxicity and lipid profile of rats. The hypolipidemic effect of 10 ppm of \((\text{MeOPhSe})_2\)-supplemented diet on rats treated with Triton WR-1339 (400 mg/kg, intraperitoneal) was investigated. The \((\text{MeOPhSe})_2\)-supplemented diet partially protected against the levels of total cholesterol (TC) and non-HDL-C and reduced the atherogenic index (AI) increased by Triton WR-1339 in rats. A positive correlation between TC and triglyceride levels \((r=0.679)\) and non HDL-C levels \((r=0.929)\) and AI \((r=0.889)\) was demonstrated. Triton WR-1339 altered parameters of oxidative stress in livers of rats but \((\text{MeOPhSe})_2\)-supplemented diet did not protect against these alterations. The results demonstrated that the hypolipidemic action of \((\text{MeOPhSe})_2\)-supplemented diet is not directly related to its antioxidant property and devoid of systemic toxicity in rats at the parameters analyzed.

Keywords: Hyperlipidemia; Selenium; Cholesterol.
1. Introduction

Hyperlipidemia is a general term for elevated concentrations of any or all lipids in the plasma. It represents one important and recognized risk factor for atherosclerosis and coronary heart disease (CHD) (Descamps et al. 2003). Hyperlipidemias can be divided into hypercholesterolemia, hypertriglyceridemia, and combined hyperlipidemia. Generally, the risk of atherosclerosis raises as the ratio of total cholesterol (TC) to high density lipoprotein cholesterol (HDL-C) increases (Kaur and Bansal 2009).

A logical strategy to prevent or treat atherosclerosis and reduce the incidence of CHD events is to target the hyperlipidemia by diet and/or lipid-lowering drugs. Some studies suggested the prospective role of selenium (Se) in cardiovascular disorders and in the management of plasma cholesterol concentration (Lee et al. 2003; Suadicani et al. 1992). In this way, the interest in the biochemistry, pharmacology and toxicology of organoselenium compounds has increased from the 80s due to a variety of organoselenium compounds that have biological activity (Gupta and Porter 2002; Nogueira and Rocha 2010). Moreover, the potential role of new synthetic organoselenium compounds as therapeutic or toxic agents has been the focus of many studies (Nogueira et al. 2004). In fact, researchers have found persuasive evidence for the potential atheroprotective action of organoselenium compounds (de Oliveira et al. 2013; Hort et al. 2011; Straliotto et al. 2013a; Straliotto et al. 2013b).

Our research group has demonstrated pharmacological properties of p,p'-methoxyl-diphenyl diselenide (MeOPhSe)$_2$, an organoselenium compound, in different experimental models (Pinto et al. 2008; Pinton et al. 2013; Prigol et al. 2009; Wilhelm et al. 2009). Thus, the present study was designed to investigate if different concentrations of (MeOPhSe)$_2$-supplemented diet causes toxicity in rats. A second aim of this study was to determine if a 10 ppm (MeOPhSe)$_2$-supplemented diet has
hypolipidemic effect on Triton WR-1339-induced acute hyperlipidemia in rats. The use of Triton-induced hyperlipidemia has been suggested as an approach to screen for or to differentiate the mechanism of action of hyperlipidemic drugs (Harnafi et al. 2007; Schurr et al. 1972). In order to rule out the antioxidant property of (MeOPhSe)$_2$ in its hypolipidemic action, parameters of oxidative stress were carried out.

2. Materials and Methods

2.1 Animals

Experiments were conducted using adult female Wistar rats (250-300 g). Animals were maintained at 22-25°C with free access to water and food, under a 12:12 h light/dark cycle with lights on at 7:00 a.m. All manipulations were carried out between 08.00 a.m. and 04.00 p.m. All experiments were performed on separate groups of animals and each animal was used only once in each test. Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources (#23081.007005/2010-96), the Federal University of Santa Maria, Brazil. All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments.

2.2 Drugs

$p,p'$-Methoxyl-diphenyl diselenide, (MeOPhSe)$_2$ (Figure 1), was synthesized according to a previously published method (Paulmier 1986). Analysis of the $^1$H NMR and $^{13}$C NMR spectra showed that the compound obtained presented analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of (MeOPhSe)$_2$ (99.9%) was determined by GC-MS. All other chemicals were of analytical grade and obtained from standard commercial suppliers.
2.3 Dietary supplementation

Animals were fed with 50 g/per animal/day standard diet chow or standard chow supplemented with \((\text{MeOPhSe})_2\). The standard diet was pulverized with ethyl alcohol, whereas the supplemented diet was pulverized with \((\text{MeOPhSe})_2\) dissolved in ethyl alcohol \((1 \text{ mg/10 ml})\). The standard and supplemented diets were kept at room temperature for 3 h to evaporate the alcohol and then kept at 4°C by not more than 1 week (Pinton et al. 2013).

2.4 Experimental design

This study was divided into two experimental protocols. In the protocol I, rats received 3, 10 or 30 ppm, which provided approximately 3, 10 or 30 µg selenium/g of diet/per day \((\approx 0.18/0.3/1.8 \text{ mg/kg body weight})\) of \((\text{MeOPhSe})_2\)-supplemented diet for 30 days. The general parameters of toxicity were evaluated and a concentration of 10 ppm, which has pharmacological potential (Pinton et al. 2013), was chosen to carry out the protocol II. In the protocol II, the effect of 10 ppm of \((\text{MeOPhSe})_2\)-supplemented diet for 30 days against acute hyperlipidemia induced by Triton WR-1339 injection was investigated.

2.4.1 Protocol I – General toxicity

Animals were randomly divided into four experimental groups and were fed for 30 days with four different chows: a standard diet chow (control group, \(n = 9\)) and a standard chow supplemented with 3 ppm \((n = 8)\), 10 ppm \((n = 8)\) or 30 ppm \((n = 8)\) of \((\text{MeOPhSe})_2\). The body weight gain, the consumption of food and water of animals were monitored throughout treatment. At the end of treatment, rats were food-deprived for one night. Heparinized blood was collected by cardiac puncture in anaesthetized animals (isoflurane), samples with hemolysis were discarded. Plasma was separated by centrifugation \((1486 \times g)\) for 10 min and used to determine aspartate aminotransferase.
(AST) and alanine aminotransferase (ALT) activities. The levels of urea, creatinine and the lipid profile were also determined.

2.4.2 Protocol II - Hypolipidemic effect of (MeOPhSe)$_2$-supplemented diet

Animals were randomly divided into two groups, which were fed with a standard diet chow ($n=14$) or a standard chow supplemented with 10 ppm of (MeOPhSe)$_2$ ($n=14$). After 30 days, animals were subdivided and received a single injection of Triton WR-1339 (400 mg/kg, intraperitoneally, i.p. $n=6$) (da Rocha et al. 2009) or vehicle (saline $n=8$).

Rats were starved overnight after Triton WR-1339 treatment. At 18 h after Triton WR-1339 injection, plasma was obtained as above described (item 2.4.1) and used to determine the lipid profile, samples with hemolysis were discarded. The livers of animals were removed and the samples were homogenized in 50 mM Tris–HCl, pH 7.4 (1/10, w/v), centrifuged at 1486 × g for 10 min. The low-speed supernatant ($S_1$) was separated and used for oxidative stress assays.

2.4.2.1 Oxidative stress

In order to rule out the antioxidant property of (MeOPhSe)$_2$ in its hypolipidemic action, parameters of oxidative stress were determined in the rat liver.

Reactive oxygen species (ROS)

To estimate the ROS production, $S_1$ was incubated with 10 µl 2’7’-dichlorofluorescein diacetate (DCFH-DA; 1 mM) at room temperature for 30 min. The RS levels were determined by a spectrofluorimetric method, using a DCFH-DA assay (Loetchutinat et al. 2005) DCFH-DA is a nonfluorescent compound that easily crosses cell membranes and is cleaved by cellular esterases. DCFH formed is rapidly oxidized in the presence of ROS to its highly fluorescent derivative dichlorofluorescein (DCF).
The DCF fluorescence intensity emission was recorded at 520 nm (with 480 nm excitation) 30 min after the addition of DCFH-DA to the medium. ROS levels were expressed as nmol DCF/mg protein/30 min.

Glutathione Peroxidase (GPx) activity

GPx activity was assayed spectrophotometrically according to method previously described (Wendel 1981), through the GSH/NADPH/GR system, by the dismutation of H$_2$O$_2$ at 340 nm. S$_1$ was added to the GSH/NADPH/GR system, and the enzymatic reaction was initiated by adding H$_2$O$_2$. In this assay, the enzyme activity is indirectly measured by means of NADPH decay. H$_2$O$_2$ is decomposed, generating GSSG from GSH. GSSG regenerated back to GSH by GR appears in the assay media at the expense of NADPH. The enzymatic activity was expressed as nmol NADPH/min/mg protein.

Superoxide dismutase (SOD) activity

SOD activity was assayed spectrophotometrically according to method previously described (Misra and Fridovich 1972). This method is based on the capacity of SOD in inhibiting autoxidation of epinephrine to epinechrome. The color reaction was measured at 480 nm. At the test day, S$_1$ was diluted 1:10 (v/v) for determination of SOD activity. Aliquots of S$_1$ were added in a 50 mM Na$_2$CO$_3$ buffer pH 10.3 and the enzymatic reaction was initiated by adding epinephrine. One unit of enzyme was defined as the amount of enzyme required to inhibit the rate of epinephrine autoxidation by 50% at 26°C. The enzymatic activity was expressed as U/mg protein.

δ-Aminolevulinic dehydratase (δ-ALA-D) activity

δ-ALA-D activity is sensitive to oxidative stress (Rocha et al. 2012). δ-ALA-D activity was assayed according to method previously described (Sassa 1982) by measuring the rate of product porphobilinogen (PBG) formation except that 100 mM sodium phosphate buffer pH 6.8 and 2.4 mM δ-ALA were used. An aliquot of 200 µl of
S1 tissue was incubated at 37 °C for 1h. The reaction product was determined using modified Ehrlich’s reagent at 555 nm. The enzymatic activity was expressed as nmol PBG/mg protein/h.

2.4.2.2 Biochemical analysis

Lipid profile

Plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides were determined by enzymatic colorimetric methods using commercial kits (Labtest Diagnostica, Minas Gerais, Brazil), as follows respectively.

Cholesteryl esters are hydrolyzed by cholesterol esterases to yield free cholesterol and fatty acids. The free cholesterol is oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. Phenol and 4-aminoantipyrine are oxidized yielding quinoneimine chromogen which can be measured at 500 nm. The red color intensity resulted from the end point reaction is proportional to the cholesterol concentration in the sample.

Very low density lipoprotein (VLDL), low density lipoprotein (LDL) and chylomicrons are precipitate with the mixture of phosphotungstate acid and magnesium chloride. After centrifugation, the cholesterol bounded to the HDL-C was determined in the supernatant.

Lipoprotein lipase hydrolyzes triglycerides yielding glycerol, which is converted by the glycerol kinase action to glycerol-3-phosphate. This is oxidized to dihydroxyacetone and hydrogen peroxide in the presence of glycerolphosphate oxidase. Afterwards, a couple-reaction occurs involving hydrogen peroxide, 4-aminoantipyrine and 4-chlorophenol catalyzed by peroxidase, yielding a quinoneimine with a maximum
absorbance at 505nm. The red color intensity produced is proportional to triglycerides concentration in the sample.

Because the Friedewald formula may not be considered accurate to estimate VLDL-C levels in hypercholesterolemic conditions, it cannot be used to calculate LDL-C. Thus, we combined VLDL-C and LDL-C levels and presented them as non-high-density lipoprotein cholesterol (non-HDL-C). Non-HDL-C values were obtained by TC (mg/dl) - HDL-C (mg/dl) (Friedewald et al. 1972). Atherogenic index (AI) was calculated as TC (mg/dl)/ HDL-C (mg/dl) (Friedewald et al. 1972). Plasma lipid levels were expressed as mg/dl.

**Hepatic profile**

Plasma AST, ALT activities were used as biochemical markers for early acute hepatic damage according to the method of Reitman and Frankel (Reitman and Frankel 1957) and were determined by commercial kits (LABTEST; Diagnostica S.A., Minas Gerais, Brazil).

AST catalyzes specifically the transfer of the amino group of aspartic acid to a ketoglutarate yielding glutamate and oxaloacetate. The oxaloacetate is reduced to malate by the enzyme malate dehydrogenase, and NADH is oxidized to NAD. The absorbance reduction at 340nm, as a consequence of NADH oxidation, is determined spectrophotometrically and is directly proportional to the AST activity in the sample.

ALT catalyzes the transfer of the amino group from alanine to ketoglutarate, yielding glutamate and pyruvate. The Pyruvate is reduced to lactate by the action of the lactate dehydrogenase, which oxidizes NADH to NAD. The reduction of absorbance at 340 nm due to oxidation of NADH, is spectrophotometrically monitored, and is proportional to the ALT activity in the sample. Enzyme activities were expressed as U/l.

**Renal profile**
Renal function, urea (Mackay and Mackay 1927) and creatinine levels (Jaffe 1986), was determined by using commercial kits (LABTEST, Diagnostica S.A., Minas Gerais, Brazil). Ammonia reacts with 2-ketoglutarate and NADH in a reaction catalyzed by glutamate dehydrogenase, promoting oxidation of NADH to NAD. The consequent reduction of absorbance measured at 340 nm is proportional to the urea concentration in the sample.

Creatinine reacts to alkaline picrate yielding a red complex. The amount of the color measured at 510 nm is proportional to creatinine concentration in the sample. Urea and creatinine levels were expressed as U/l.

2.4.4.5 Protein Quantification

Protein concentration of $S_1$ was measured according to methods previously described (Bradford 1976) using bovine serum albumin as a standard.

2.5 Statistical analysis

All experimental results are given as the mean (s) ± S.E.M. Comparisons between experimental and control groups were performed by one-way (for general toxicity data) or two-way analysis (supplemented diet × triton) of variance (ANOVA) followed by the Duncan’s test for post hoc comparison when appropriate. A value of $p<0.05$ was considered to be significant. The main effects are presented only when interactions were not significant. Pearson’s correlation coefficient was used for the estimation of correlation between parameters analyzed. For the correlation analysis, results from all animal groups were used.

3. Results
3.1 General toxicity

General parameters of toxicity are shown in Table 1. Three, 10 or 30 ppm of (MeOPhSe)$_2$-supplemented diet administered to rats for 30 days did not cause alteration in the body weight gain ($F_{3,28}=0.44; p>0.05$), the food consumption ($F_{3,28}=0.02; p>0.05$) and water intake ($F_{3,28}=1.18; p>0.05$).

(MeOPhSe)$_2$ supplemented in diets did not alter TC ($F_{3,27}=1.27; p>0.05$), HDL-C ($F_{3,26}=0.70; p>0.05$), non-HDL-C ($F_{3,26}=1.46; p>0.05$) and triglyceride levels ($F_{3,28}=0.39; p>0.05$) (Table 1).

The one-way analysis of data did not reveal differences in activities of ALT ($F_{3,28}=0.57; p>0.05$) and AST ($F_{3,28}=0.99; p>0.05$) in all (MeOPhSe)$_2$-supplemented diets (Table 1).

Urea ($F_{3,28}=1.23; p>0.05$) and creatinine ($F_{3,28}=1.23; p>0.05$) levels were not altered in plasma of rats for all concentrations of (MeOPhSe)$_2$-supplemented diets (Table 1).

3.2 Hypolipidemic property of 10 ppm (MeOPhSe)$_2$-supplemented diet

3.2.1 Oxidative stress

The two-way ANOVA of ROS levels data demonstrated a significant main effect of Triton WR-1339 ($F_{1,16}=6.62; p=0.0020$). Triton WR-1339 increased ROS levels and (MeOPhSe)$_2$-supplemented diet did not protect against this increase (Table 2).

The two-way ANOVA for GPx and SOD activities revealed no significant differences among groups (Table 2).

The two-way ANOVA of $\delta$-ALA-D activity data demonstrated a significant main effect of Triton WR-1339 ($F_{1,18}=7.13; p=0.015$). Triton WR-1339 inhibited $\delta$-ALA-D activity.
activity when compared to the control group and (MeOPhSe)$_2$-supplemented diet did not protect against this inhibition (Table 2).

3.2.2 Biochemical analysis

The two-way ANOVA of plasma triglyceride levels data demonstrated a significant main effect of Triton WR-1339 ($F_{1,23}=478.10; p<0.0000$). Triton WR-1339 significantly increased plasma triglyceride levels when compared to the control group and (MeOPhSe)$_2$-supplemented diet did not protect against this increase (Figure 2A).

The two-way analysis of variance of TC data yielded a significant main effect of Triton WR-1339 ($F_{1,23}=88.61; p<0.0000$) and (MeOPhSe)$_2$ ($F_{1,23}=5.49; p<0.0284$). Triton WR-1339 increased TC levels when compared to the control group. The (MeOPhSe)$_2$-supplemented diet protected partially against the increase in TC levels caused by Triton WR-1339 in rats (Fig 2B).

The two-way analysis of variance of non-HDL-C levels yielded a significant main effect of Triton WR-1339 ($F_{1,22}=157.05; p<0.0000$) and (MeOPhSe)$_2$ ($F_{1,22}=4.45; p<0.0465$). Triton WR-1339 increased plasma non-HDL-C levels in rats when compared to the control group. The (MeOPhSe)$_2$-supplemented diet partially protected against the increase in the levels of non-HDL-C caused by Triton WR-1339 injection in rats (Figure 2C).

The two-way analysis of variance of HDL-C data did not reveal differences in plasma of rats from all experimental groups (data not shown).

The two-way ANOVA of AI data demonstrated a significant WR-1339 × (MeOPhSe)$_2$ interaction ($F_{1,23}=9.86; p<0.0459$). Post-hoc comparisons showed that Triton WR-1339 increased AI in rats when compared to the control group. The (MeOPhSe)$_2$-supplemented diet was partially effective in preventing against the increase of AI induced by Triton WR-1339 administration (Fig 3).
3.2.3 Correlation analysis between total cholesterol levels and cholesterol fractions or atherogenic index

The Pearson’s correlation analysis revealed a positive correlation between plasma TC and triglyceride levels ($r=0.679$, $p=0.0001$, Figure 4A) and non HDL-C levels ($r=0.929$, $p=0.0001$, Figure 4B) and AI ($r=0.889$, $p=0.0001$, Figure 4C).

3.2.4 Correlation analysis between lipid profile and oxidative damage marker

The Pearson’s coefficient showed that there was no correlation between plasma TC and RS ($r=0.272$, $p=0.2457$); triglyceride levels and RS ($r=0.130$, $p=0.5838$); non HDL-C levels and RS ($r=0.248$, $p=0.291$) and HDL and RS ($r=0.196$, $p=0.407$).

4. Discussion

The results of the present study demonstrated that dietary supplementation with different concentrations of (MeOPhSe)$_2$ for 30 days neither cause general toxicity nor altered basal lipid levels in rats. The results demonstrated that the hypolipidemic action of 10 ppm (MeOPhSe)$_2$-supplemented diet is not directly related to its antioxidant property and devoid of systemic toxicity in rats at the parameters analyzed.

Dietary supplementation with 3, 10 and 30 ppm of (MeOPhSe)$_2$ for 30 days to rats did not alter food consumption, water intake, the body weight gain and other parameters indicative of general toxicity in rats. The markers of hepatic (AST and ALT activities) and renal damage (urea and creatinine levels) were also not altered by (MeOPhSe)$_2$-supplemented diets. Although urea and creatinine levels are used as markers of renal damage, these levels only are elevated when the renal damage is more severe (around 50% of renal function impaired), therefore, the clearance of creatinine would be a more sensitive marker of renal damage. Given that the clearance of creatinine was not determined in this experimental protocol, we assume this point as a
drawback of this study. The fact that AST and ALT activities are not specific markers of liver damage lead us to assume this as another drawback of this study.

The dietary supplementation with different concentrations of \((\text{MeOPhSe})_2\) did not change plasma lipid profile (TC, HDL-C, non-HDL-C and triglycerides). Altogether, these results point out the low toxicity of \((\text{MeOPhSe})_2\)-supplemented diet to rats. These results are in agreement with Barbosa and collaborators (Barbosa et al. 2008), who demonstrated that 10 ppm of diphenyl diselenide-supplemented diet caused no signs of toxicity in rats.

The second protocol of this study was designed to investigate whether hyperlipidemic state in adult rats could be attenuated by a previous \((\text{MeOPhSe})_2\)-supplemented diet. This way, from experiments of general toxicity, the concentration of 10 ppm, which has pharmacological potential (Pinton et al. 2013), was chosen to accomplish the hypolipidemic effects of \((\text{MeOPhSe})_2\)-supplemented diet in rats treated with Triton WR-1339. The levels of TC, non-HDL-C and triglycerides as well as the AI were increased 18 h after a single Triton WR-1339 injection to rats. These results are in accordance with those reported by others (Prigol et al. 2009; Rodrigues et al. 1992; Srinivasan and Chandrasekhara 1993). Regarding HDL-C levels, some authors reported that Triton WR-1339 decreased HDL-C levels in mice (da Rocha et al. 2009; Oh et al. 2006) while other authors did not demonstrate any alteration in HDL-C levels Adeneye et al. (2010); (Prigol et al. 2009) in rodents. In this study, no change was found in HDL-C levels of rats after Triton WR-1339 treatment.

The hypolipidemic property of \((\text{MeOPhSe})_2\)-supplemented diet was demonstrated by the reduction in TC and non-HDL-C increased by Triton WR-1339 in rats. Although the triglyceride levels were not reduced by \((\text{MeOPhSe})_2\)-supplemented diet, the Pearson’s coefficient revealed a positive correlation between triglyceride and
TC levels. Additionally, non-HDL-C levels increased so did the TC levels, showing also a positive Pearson’s correlation.

Triton WR-1339 is known to induce hyperlipidemia in two phases: phase I is believed to be due to increased hepatic cholesterol biosynthesis through interference of Triton WR-1339 in tissue uptake of plasma lipids (Holmes 1964; Tamasi et al. 1968), while phase II involves interference of Triton WR-1339 with cholesterol excretion and metabolism (Garattini et al. 1959). Therefore, the reduction in the plasma levels of TC and non-HDL-C by (MeOPhSe)$_2$ dietary supplementation, at 18 h after Triton WR-1339 administration, suggests that (MeOPhSe)$_2$ could be mediating its hypolipidemic action through the interference with hepatic cholesterol biosynthesis rather than through cholesterol metabolism and excretion.

The reduction of serum cholesterol concentration may be accompanied by a decrease in cholesterol secretion from the liver to serum resulting from the suppression of hepatic cholesterol biosynthesis, which is regulated by the enzyme 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA reductase). In line with this, a previous study from our research group demonstrated that diphenyl diselenide, a parent compound of (MeOPhSe)$_2$, reduced hepatic HMG-CoA reductase expression (da Rocha et al. 2013). Additionally, Nassir and collaborators (Nassir et al. 1997) reported that Se deficiency led to increased HMG-CoA reductase activity in rats resulting in increased endogenous cholesterol synthesis.

The hypothesis of hypolipidemic action of (MeOPhSe)$_2$-supplemented diet is reinforced by the decrease in the AI in a Triton WR-1339 model. The AI (TC/HDL-C ratio) is considered the most predictive, among the risk factors, for atherosclerosis. Thus, the AI is a strong and reliable indicator of whether or not cholesterol was deposited into tissues or it was metabolized and excreted (Hertog et al. 1993; Kaur and
Bansal 2009). A positive Pearson’s correlation between AI and TC further supports this idea. The mechanism of the hypolipidemic action of a (MeOPhSe)$_2$-supplemented diet is poorly understood at the present but it is possible that (MeOPhSe)$_2$ acts as an inhibitor of hepatic HMG-CoA reductase expression in a similar way to diphenyl diselenide.

Triton WR-1339 increased reactive oxygen species and inhibited δ-ALA-D activity in the rat liver but the (MeOPhSe)$_2$-supplemented diet had no effect in parameters of oxidative stress. In contrast to the other studies (Bao et al. 2012; Straliotto et al. 2013a; Vijayakumar and Nalini 2006) the experimental findings demonstrated here suggest that oxidative stress did not play an important role in the (MeOPhSe)$_2$ hypolipidemic action. The results demonstrated here are in accordance with those reported by da Rocha et al 2009 (da Rocha et al. 2009) with the same hyperlipidemic induction protocol.

Rather than a real discrepancy, it is likely that the hypolipidemic action of (MeOPhSe)$_2$ can be attributed to other mechanisms of action. Additionally, it was demonstrated that diphenyl diselenide a structural analogue of (MeOPhSe)$_2$ has hypolipidemic activity by modulating the expression of some proteins involved in cholesterol metabolism. (da Rocha et al. 2013) Besides, the divergent results obtained by us and other (Straliotto et al. 2013a) can be attributed to the eletronic effects of the methoxyl group that become the selenium atom less exposed even as major differences in the protocol used.

The results demonstrated that 3, 10 or 30 ppm of (MeOPhSe)$_2$-supplemented diet did not cause general toxicity in rats at the parameters analyzed. Dietary supplementation with 10 ppm of (MeOPhSe)$_2$ was effective in lowering lipid levels in plasma of rats treated with Triton WR-1339. Thus, we propose further studies with
(MeOPhSe)$_2$-supplemented in the diet to elucidate if it could be a nutraceutical strategy. The antioxidant property of (MeOPhSe)$_2$ is not related to its hypolipidemic action in Triton WR-1339 model. Further studies have to be performed to fully understand the mechanisms of action by which (MeOPhSe)$_2$-supplemented diet acts.

**Conflict of interest:**

No conflict of interest to be declared.

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References


Tables

Table 1- Parameters of general toxicity in rats exposed to different concentration of (MeOPhSe)$_2$ supplemented diet for 30 days.

Table 2- Parameters of oxidative stress in (MeOPhSe)$_2$ and Triton WR-1339 treated rats.
Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups [ppm of (MeOPhSe)$_2$]</th>
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<tr>
<td></td>
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<tr>
<td>Weight gain$^a$</td>
<td>35.75 ± 5.53</td>
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<tr>
<td>Food intake$^b$</td>
<td>19.77 ± 0.52</td>
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<tr>
<td>Water intake$^c$</td>
<td>32.84 ± 2.11</td>
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<tr>
<td>Total cholesterol$^d$</td>
<td>144.57 ± 16.75</td>
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<tr>
<td>HDL cholesterol$^d$</td>
<td>47.41 ± 11.39</td>
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<tr>
<td>Non-HDL cholesterol$^d$</td>
<td>96.84 ± 12.76</td>
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<tr>
<td>Triglycerides$^d$</td>
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<tr>
<td>AST$^e$</td>
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<tr>
<td>ALT$^e$</td>
<td>46.64 ± 4.43</td>
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<tr>
<td>Urea$^e$</td>
<td>34.92 ± 2.21</td>
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<tr>
<td>Creatinine$^e$</td>
<td>0.67 ± 0.04</td>
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</table>

Data are reported as means ± S.E.M. of 7-8 animals per group. Data are expressed as $^a$g/30 days; $^b$g/day; $^c$ml/day; $^d$mg/dl; $^e$U/l.

Table 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>ROS$^a$</th>
<th>GPx$^b$</th>
<th>SOD$^c$</th>
<th>δ-ALA-D$^d$</th>
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<td>Control</td>
<td>141.26 ± 30.98</td>
<td>204.62 ±10.76</td>
<td>53.23 ± 3.11</td>
<td>10.33 ± 0.97</td>
</tr>
<tr>
<td>Triton WR-1339</td>
<td>209.34 ±20.13*</td>
<td>191.61 ± 8.97</td>
<td>49.99 ± 1.69</td>
<td>8.65 ± 0.62*</td>
</tr>
<tr>
<td>(MeOPhSe)$_2$</td>
<td>124.69 ± 20.33</td>
<td>208.46 ± 24.79</td>
<td>51.26 ± 4.59</td>
<td>11.42 ± 1.39</td>
</tr>
<tr>
<td>(MeOPhSe)$_2$ + Triton WR-1339</td>
<td>168.56 ±16.03</td>
<td>178.56 ± 6.10</td>
<td>50.25 ± 2.73</td>
<td>8.51 ± 0.34</td>
</tr>
</tbody>
</table>

Data are reported as means ± S.E.M. of 4-6 animals per group. Data are expressed as $^a$nmol DCF/mg protein/30 min.; $^b$nmol NADPH/min/mg protein; $^c$U/mg protein; $^d$nmol PBG/mg protein/h. *Denotes $p<0.05$ as compared to the control group.
Figure Legends

Figure. 1 - Chemical structure of \(p,p'\)-methoxyl-diphenyl diselenide \([(\text{MeOPhSe})_2]\).

Figure. 2 - Effects of \((\text{MeOPhSe})_2\)-supplemented diet and Triton WR-1339 on plasma lipid profile in rats: A) Triglycerides; B) Total cholesterol; C) non-HDL-Cholesterol. Data are reported as means ± S.E.M. (\(n=6-8\)). *Denotes \(p<0.05\) as compared to the respective control group and \(^\#p<0.05\) as compared to the Triton WR-1339-induced hyperlipidemia group.

Figure. 3 - Effects of \((\text{MeOPhSe})_2\)-supplemented diet and Triton WR-1339 on the atherogenic index (AI) in rats. Data are reported as means ± S.E.M. (\(n=6-8\)). *Denotes \(p<0.05\) as compared to the control group and \(^\#p<0.05\) as compared to the Triton WR-1339-induced hyperlipidemia group.

Figure. 4 - Correlations between total cholesterol levels and cholesterol fractions or atherogenic index in \((\text{MeOPhSe})_2\)-supplemented diet rats. (A) Positive correlation between total cholesterol and triglyceride levels. (B) Positive correlation between total cholesterol levels and non HDL-C levels. (C) Positive correlation between total cholesterol levels and atherogenic index (Pearson’s correlation coefficient). Data are individual values for each rat of all groups.
Figures

Figure 1:
Figure 2:

A

Triglycerides (mg/dl)

Control

(OMePhSe)₂₂

Triton

(OMePhSe)₂₂ + Triton

B

Total cholesterol (mg/dl)

Control

(OMePhSe)₂₂

Triton

(OMePhSe)₂₂ + Triton
The diagram shows the changes in non-HDL-C (mg/dl) levels across different conditions. The control group has the lowest non-HDL-C levels, followed by the (OMePhSe)₂ and Triton groups, which have significantly higher levels as indicated by the asterisks. The group treated with both (OMePhSe)₂ and Triton has the highest non-HDL-C levels, as indicated by the double asterisks.
Figure 3:

![Bar chart showing atherogenic index for different conditions]

- Control
- (OmePh-Se)$_2$
- Triton
- (OmePh-Se)$_2$ + Triton

Figure 4:

![Scatter plot showing relationship between triglyceride and total cholesterol]

Triglyceride (mg/dl) vs. Total cholesterol (mg/dl)