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Glutathione peroxidase mimicry of diphenyl diselenide: Plausible contribution of proteins' thiols

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Author contributions

E.M., Ale conceptualized, designed the research and carried out data analysis. Material preparation, administration and data collection were performed by S.O., Asuelimen. Supervision was done by O., Otitoju. The first draft of the manuscript was written by E.M, Ale and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare no conflicts of interest.

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Peer review information

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Abbreviations

GPx, Glutathione peroxidase; DPDS, Diphenyl diselenide; δ-ALAD, delta-aminolevulinic acid dehydratase; GSH, reduced glutathione; TBA, Thiobarbituric Acid; ROS, reactive oxygen species; TCA, trichloroacetic acid; TBA, Thiobarbituric Acid; H_20, Hydrogen peroxide; TBARS, Thiobarbituric Acid Reactive Species; SDS, Sodium Dodecyl Sulphate; MDA, Malondialdehyde.

Citation

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Abstract

Organoseleniums are a class of compounds attracting attention across the globe owing to their Glutathione peroxidase (GPx) mimicry, which confers on them a strong antioxidant activity. Diphenyl diselenide (DPDS) is an Organoselenium whose GPx mimetic property has been suggested to rely on the oxidation of non-protein or protein thiols critical to the activities of some sulfhydryl enzymes. This study, therefore investigated the GPx mimic/antioxidant property of DPDS as well as the role of thiols of two key sulfhydryl enzymes, cerebral Na^+/K^+-ATPase (sodium pump) and hepatic delta-aminolevulinic acid dehydratase (δ-ALAD) in the GPx mimicry of DPDS. Albino Wistar rats were euthanized, and the liver and brain were removed and used to assay for the effect of DPDS on lipid peroxidation induced by two prooxidants [Fe^{2+}(10 μM) and H_2O_2, (1 mM)] as well as the activities of the sulfhydryl enzymes. The results revealed that DPDS profoundly ($P < 0.05$) counteracted Fe^{2+} and H_2O_2-induced lipid peroxidation in the rats' hepatic and cerebral tissues. Furthermore, the results of assay systems for lipid peroxidation and sodium pump revealed that DPDS inhibited Na^+/K^+-ATPase and lipid peroxidation in the brain tissue homogenates in the same reaction system. A similar result was obtained in the assay system for lipid peroxidation and hepatic δ-ALAD as DPDS simultaneously inhibited the enzyme's activity and lipid peroxidation. This suggests that the GPx mimetic property of DPDS may be linked to the enzymes' loss of activity, which further validates the suggestions that the enzymes' inhibition, as well as the antioxidant action of DPDS, rely on the oxidation of critical thiols of the enzymes. However, the GPx mimicry of DPDS should be investigated in the presence of thiol-blocking or oxidizing agents in biological systems in order to further ascertain the role of protein thiols.

Keywords: Organoseleniums; diphenyl diselenide; Glutathione peroxidase; antioxidant; thiols; delta-aminolevulinic acid dehydratase; Na^+/K^+-ATPase

Introduction

Organoselenium chemistry has attracted great interest over the years for the reason that many Organoselenium compounds exhibit antioxidant properties [1, 2], and selenium based compounds have been considered in the efforts to prevent and manage degenerative diseases mediated by oxidative stress [3, 2]. Generally, the antioxidant activity of Organoselenium has been attributed to their GPx-like property [4, 5]. The native glutathione peroxidase is an important selenoenzyme that reduces peroxides (ROOH, H_2O_2 and peroxynitrite) using reduced glutathione (GSH) as a reductant [4].

Diphenyl diselenide (DPDS) is an Organoselenium with GPx-like activity that carries out its antioxidant activity by reducing reactive oxygen species (ROS) in three mechanistic steps (Figure 1) in which selenol (the resting state) is oxidized by ROS to selenenic acid. The active selenol is then formed from the reduction of the selenenic acid by glutathione (GSH) through a selenenyl sulfide intermediate [6].

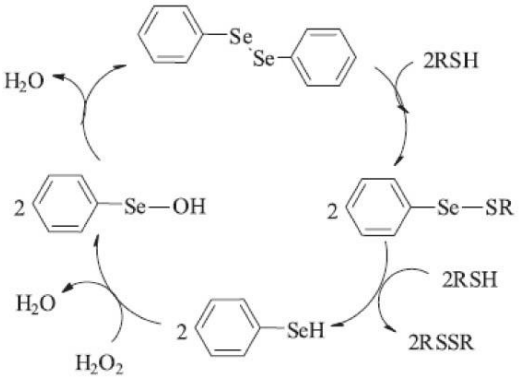

Figure 1 Catalytic mechanism of action of DPDS [6]

There are evidences suggesting the possible involvement of both proteins and non-proteins thiols in the glutathione peroxidase mimicry of DPDS [7]. These evidence are linked to the fact that DPDS inhibited sulphydryl enzymes, including Na^+/K^+-ATPase (sodium pump) and lactate dehydrogenase, at a concentration far lower than its effective pharmacological concentration under *in vitro* conditions [5, 8–12]. Consequently, herein, we evaluated the possible contribution of thiols on the sodium pump and δ-ALAD in the glutathione peroxidase mimicry of DPDS.

Materials and methods

Chemicals

Diphenyl diselenide, trichloroacetic acid (TCA), Thiobarbituric Acid (TBA), Tris-HCl, Hydrogen peroxide (H_20_2), Acetate buffer pH 3.4, Dimethyl sulfoxide (DMSO), Potassium chloride, Sodium chloride and Magnesium chloride were gotten from Sigma Chemical Co. USA. Other chemicals were purchased from standard suppliers.

Experimental animals

Healthy male albino rats weighing 200-250 g were obtained from the VOM Local Government Area of Jos, Plateau State, Nigeria. The rats were kept in the animal house of the Biochemistry Department, Federal University Wukari, Taraba State, at ambient temperature. Food and water were made accessible, and animals were handled following the guideline of the Care and Use of Experimental Animal Committee with the approval number FUW/FPAS/22/010.

Preparation of tissue homogenate

Cervical dislocation method was adopted in sacrificing the animals, followed by the removal and homogenization of brain and liver in 50 mM Tris–HCl buffer (pH 7.4). The supernatants used for assays were then obtained by centrifuging the homogenates at 4000 rpm for 10 minutes.

Thiobarbituric Acid Reactive Species (TBARS) assay

The modified method of [13] was used to measure TBARS by incubating 100 µl of homogenate for 1 hour at 37 ℃ in the presence of DPDS (Diphenyl diselenide) (final concentrations range of 0-160 µM), in a reaction system containing 50 mM Tris-HCl buffer (pH 7.4), 100 µl of tissue homogenate with pro-oxidants [$FeSO_4$ (10 µM) or H_2O_2 (1 mM)]. 200 µl of 8.1% SDS (Sodium Dodecyl Sulphate) was added to develop the color reaction. 500 µl of pH 3.4 acetate buffer and 500 µl of 0.8% TBA were added, and the mixture was incubated for 30 minutes at 100 ℃. TBARS formed was quantified at 532 nm in a UV-spectrophotometer

Incubation systems for TBARS and sodium pump assays

TBARS and Na^+/K^+-ATPase activity assays were carried out in a system consisting of 125 mM NaCl, 3 mM MgCl, 20 mM KCl, 50 mM pH 7.4 Tris-HCl, pro-oxidants [$FeSO_4$ (10 µM) or H_2O_2 (1 mM)] and DPDS (final concentrations range of 0-160 µM) in a final volume of 160 µl. ATP was added to 3.0 mM final concentration to initiate the reaction. The same condition was adopted for the controls with 0.1 mM ouabain added, and the system was incubated at 37 ℃ for 30 minutes.

Sodium pump assay. Cerebral Na^+/K^+-ATPase activity was assayed as described under "Incubation systems for TBARS and sodium pump assays" section. However, 5% TCA was added to stop the reaction after the 30 min incubation. Inorganic phosphate released (Pi) was quantified according to [14]. The Na^+/K^+-ATPase activity was gotten as the difference between assay with ouabain and without ouabain. All experiments were replicated three times, and enzyme activity was expressed as mole number of released phosphate (Pi) $min^{-1}mg\ protein^{-1}$.

TBARS assay in a reaction system for sodium pump. TBARS was assayed as described under "Incubation systems for TBARS and sodium pump assays" section. However, TBARS formed was quantified following the method of Okhawa et al. (1979) after the 30 min incubation, except that the color reaction buffer has a pH of 3.4. The color reaction was developed by the addition of 200 µl of 8.1% SDS. 500 µl of pH 3.4 acetate buffer and 500 µl of 0.8% TBA were added to the mixture, which was incubated for 30 minutes at 100 ℃, and TBARS formed was quantified at 532 nm in a UV-spectrophotometer

Incubation systems for TBARS and delta-aminolevulinic acid dehydratase assays

TBARS and δ-ALAD activity was assayed in a reaction system that contained 1 M phosphate buffer, pH 6.8, pro-oxidant [$FeSO_4$ (10 µM) or H_2O_2 (1 mM)], and DPDS (0-160 µM final concentrations) in 500 µl final volume. Delta-aminolevulinic acid was added to 2.4 mM final concentration to initiate the reaction. The system was then incubated at 37 ℃ for 1 hour, after which δ-ALAD activity and TBARS produced were assayed.

Delta-aminolevulinic acid dehydratase assay. Hepatic δ-ALAD activity was assayed as described under "Incubation systems for TBARS and delta-aminolevulinic acid dehydratase assays" section. However, 250 µl of 10% trichloroacetic acid containing 10 mM $HgCl_2$ was added to stop the reaction after the 1 hours incubation. Then, the tubes were all centrifuged at 4000 rpm for 7 minutes, an Aliquot of 300 (µl) was collected, and 200 (µl) of distill H_2O (µl) was added. The porphobilinogen formation rate was measured according to the modified method of [15], and the product was determined at 555 nm using modified Ehrlich's reagent.

TBARS assay in a reaction system for δ-ALAD. Hepatic TBARS was measured as described under "Incubation systems for TBARS and delta-aminolevulinic acid dehydratase assays" section. However, TBARS formed was quantified following the method of Okhawa et al. (1979) after the 30 min incubation, except that the color reaction buffer has a pH of 3.4. 200 µl of 8.1% SDS was added to develop the color reaction, after which 500 µl of pH 3.4 acetate buffer and 500 µl of 0.8% TBA was added. The system was incubated for 30 minutes at

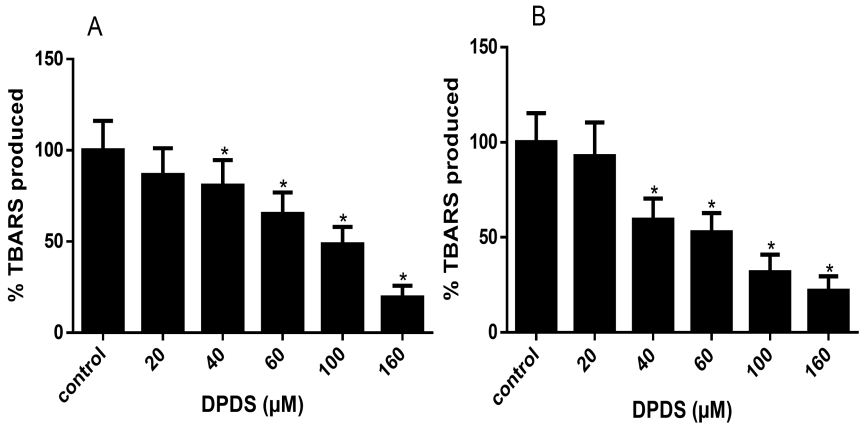

Figure 2 Effects of DPDS on Fe^{2+}-induced lipid peroxidation in rat brain (panel A) and liver (panel B) homogenates. Data depict mean ± SEM of three independent experiments carried out. * Represent significant difference from control at $P < 0.05$.

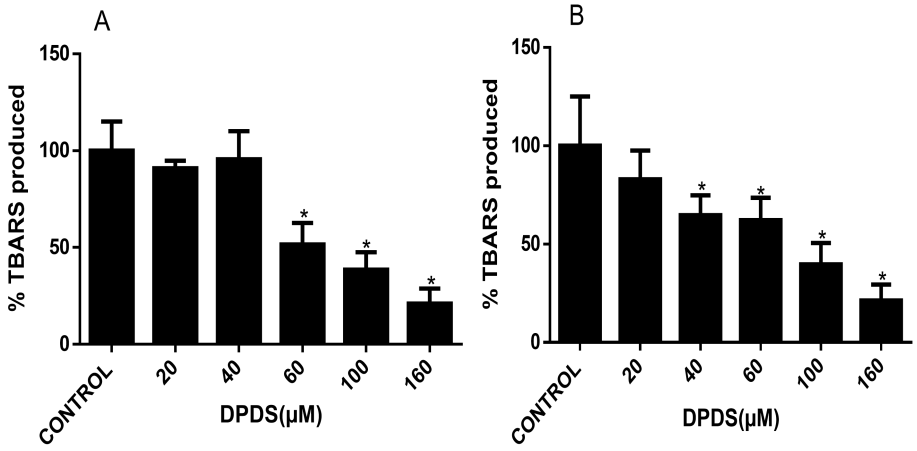

Figure 3 Effects of DPDS on H_2O_2-induced lipid peroxidation in rat brain (panel A) and liver (panel B) homogenates. Data depict mean ± SEM of three independent experiments carried out. * Represent significant difference from control at $P < 0.05$.

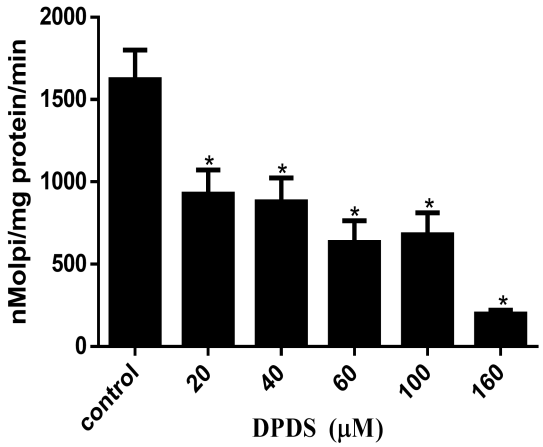

Figure 4 Effects of DPDS on basal Na^+/K^+-ATPase activity in rat brain homogenate. Data depict mean ± SEM of three independent experiments carried out. * Represent significant difference from control at $P < 0.05$.

100 ℃, and TBARS formed was quantified at 532 nm in a UV-visible Spectrophotometer.

Statistical analysi

All values were presented as mean ± SEM. Data were analyzed appropriately using analysis of variance (ANOVA) and Duncan's multiple-range tests as appropriate.

Results

Effect of DPDS on Fe^{2+}-induced lipid peroxidation in rat brain and liver

As shown in Figure 2, DPDS profoundly ($P < 0.05$) inhibited lipid peroxidation induced in rat cerebral and hepatic tissue homogenates (Figure 2, panel A and B, respectively) by Fe^{2+} in a concentration-dependent manner.

Effect of DPDS on H_20_2-induced lipid peroxidation in rat brain and liver

Also, Figure 3 shows that DPDS inhibited H_20_2-induced lipid peroxidation in rat brain and liver (Figure 3, panels A and B, respectively) with increasing concentration.

Effects of DPDS on Basal Na^+/K^+-ATPase activity in rat brain homogenates

Figure 4 reveals that DPDS profoundly inhibited basal Na^+/K^+-ATPase activity, and this effect was markedly different ($P < 0.05$) when compared with the control.

Effects of DPDS on lipid peroxidation and $Na^+/K+$-ATPase activity in Fe^{2+}-treated rat brain homogenate

Figure 5 shows that DPDS markedly inhibited both lipid peroxidation (Figure 5 panel B) and Na^+/K^+-ATPase activity (Figure 5 panel A) in a Fe^{2+}treated brain homogenate.

Effects of DPDS on lipid peroxidation and Na^+/K^+-ATPase activity in H_20_2-treated rat brain homogenate

Figure 6 shows that DPDS markedly inhibited both lipid peroxidation (Figure 6 panel B) and Na^+/K^+-ATPase activity (Figure 6 panel A) in an H_20_2-treated brain homogenate.

Effects of DPDS on hepatic basal delta-aminolevulinic acid dehydratase activity

Figure 7 shows that DPDS profoundly inhibited delta-aminolevulinic acid dehydratase activity in a concentration-dependent manner.

Effects of DPDS on delta-aminolevulinic acid dehydratase activity and lipid peroxidation in Fe^{2+}-treated liver homogenates

As depicted in Figure 8, DPDS concomitantly inhibited lipid peroxidation (Figure 8 panel B) and delta-aminolevulinic acid dehydratase activity (Figure 8 panel A) in the same reaction system.

Effects of DPDS on delta-aminolevulinic acid dehydratase activity and lipid peroxidation in H_20_2-treated liver homogenates

In a similar manner, DPDS inhibited both lipid peroxidation (Figure 9 panel B) and delta-aminolevulinic acid dehydratase activity (Figure 9 panel A) with increasing concentration and these effects were markedly different ($P < 0.05$) when compared with the control.

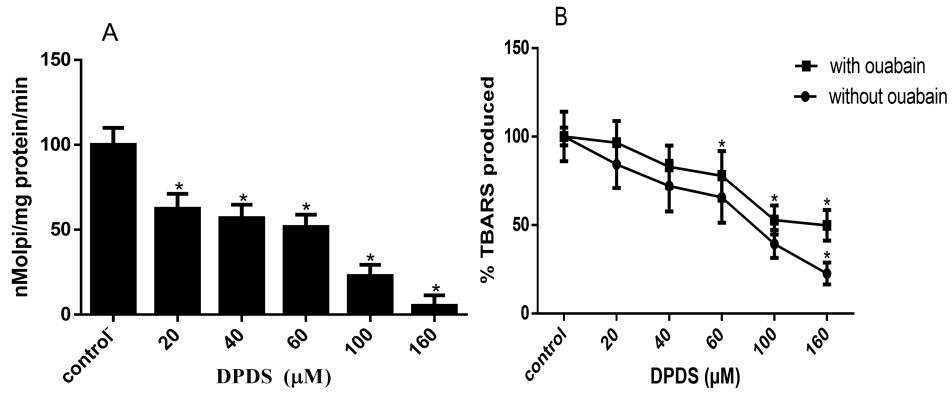

Figure 5 Effects of DPDS on Na^+/K^+-ATPase activity (panel A) and lipid peroxidation (panel B) in Fe^{2+}-treated rat brain homogenate. Data depict mean ± SEM of three independent experiments carried out. * Represent significant difference from control at $P < 0.05$.

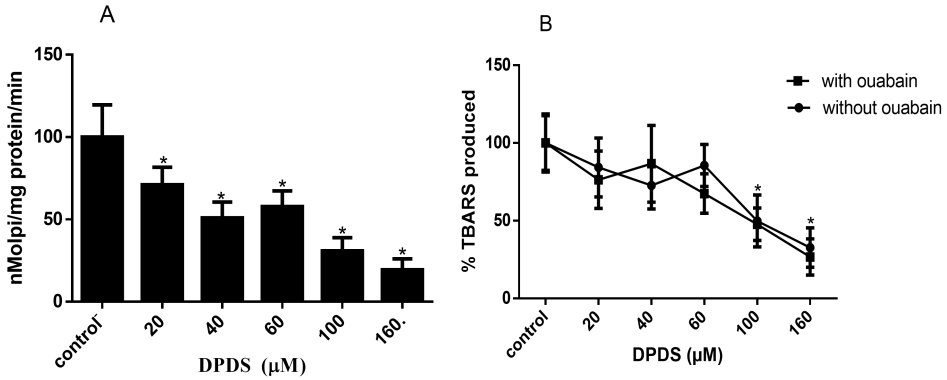

Figure 6 Effects of DPDS on Na^+/K^+-ATPase activity (panel A) and lipid peroxidation (panels B) in H_2O_2-treated rat brain homogenate. Data depict mean ± SEM of three independent experiments carried out. * Represent significant difference from control at $P < 0.05$.

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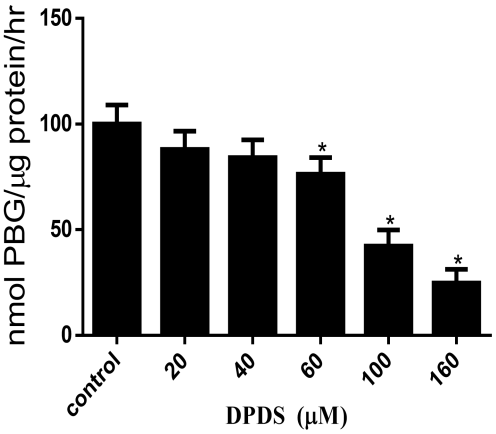

Figure 7 Effects of DPDS on basal delta-aminolevulinic acid dehydratase activity in rat liver homogenate. Data depict mean ± SEM of three independent experiments carried out. * Represent significant difference from control at $P < 0.05$.

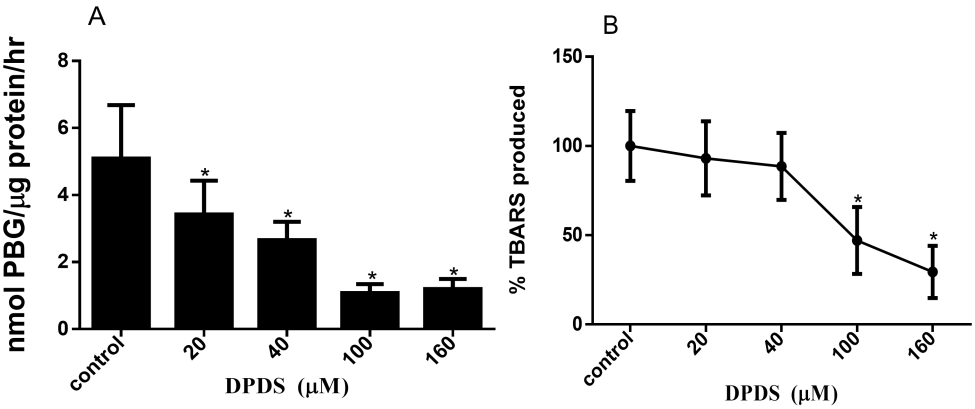

Figure 8 Effects of DPDS on delta-aminolevulinic acid dehydratase activity (panel A) and lipid peroxidation(panel B) in Fe^{2+}-treated liver homogenate. Data are presented as mean ± SEM of two independent experiments carried out. * Represent significant difference from control at $P < 0.05$.

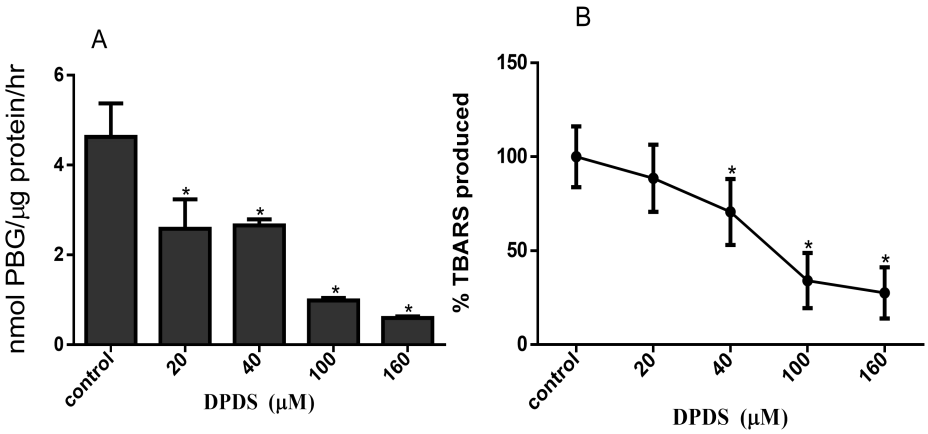

Figure 9 Effects of DPDS on delta-aminolevulinic acid dehydratase activity (panel A) and lipid peroxidation (panel B) in H_2O_2-treated liver homogenate. Data are presented as mean ± SEM of two independent experiments carried out. * Represent significant difference from control at $P < 0.05$.

Discussion

Several reports proposed Organoselenium compounds as potential drugs against free radicals formation and harmful effects of ROS sequel to myriad of tissues or cellular injuries in various parts of the mammalian systems [5, 16, 17]. In fact, the pharmacological action of diphenyl diselenide and ebselen has been linked mainly to their antioxidant actions [18, 19]. Diphenyl diselenide is an

Organoselenium of great interest owing to its numerous pharmacological properties, including antioxidant, neuroprotective, antinociceptive, and antidepressant-like actions [9, 20–22]. Researchers have hypothesized that proteins' thiols are crucial to the antioxidant action/GPx mimic of DPDS in biological systems [9]. Hence, we investigated the probable involvement of either free thiols or protein thiols in the glutathione peroxidase mimicry of DPDS in biological systems *in vitro*.

Malondialdehyde (MDA) is one of the products of lipid peroxidation mostly used as a lipid peroxidation biomarker [23, 24]. MDA forms adducts that constitute a damage to biomolecules upon reaction with cell and tissue proteins or DNA [25, 26].

The antioxidant action of DPDS was evaluated in the presence of two prooxidants (Fe^{2+} and H_20_2). One of the most potent neuro- and hepatotoxic agents is the transition metal Fe^{2+}. The mechanism of Fe^{2+}-induced lipid peroxidation entails a reaction between Fe^{2+} and O_2 or H_2O_2 to form reactive oxygen species [27, 28]. As shown in Figure 2, Fe^{2+} evoked an increase in lipid peroxidation in the cerebral tissues (Figure 2, panel A) and hepatic (Figure 2, panel B) tissues, but DPDS counteracted the formation of TBARS with increasing concentration. H_2O_2 has been reported to be involved in free radical formation in biological systems, leading to tissue injury [29, 30] through the Fenton reaction. The results in Figure 3 reveal that H_2O_2 provoked lipid peroxidation adduct formation in the rat cerebral (Figure 3, panel A) and hepatic (Figure 3, panel B) tissues. Conversely, DPDS counteracted the adduct formation with increasing concentration. The results of TBARS assays suggest that DPDS possesses GPx mimetic property by inhibiting or abolishing the harmful effect of lipid peroxidation exhibited by Fe^{2+} and H_20_2 via oxidation of available thiols in the reaction medium.

In the second phase of this research, two sulphydryl enzymes, Na^+/K^+-ATPase and δ-ALAD, were employed to examine the likely role of proteins' thiols in the *in vitro* GPx mimicry of DPDS since these enzymes contain thiols in their amino acid residues critical to their activities.

Na^+/K^+-ATPase is a transmembrane protein found in the cells of the brain and neurons. It is a critical enzyme that maintains the ionic gradient of the neuron by facilitating the active transport of Na^+ and K^+ in the central nervous system (CNS). This enzyme has been documented to possess sulphydryl groups essential for its function, and it has been reported that agents such as iron, mercury and selenium compounds that oxidize thiol often inhibit the activity of this enzyme [5, 10, 11, 31, 32]. Sequel to this observation, we assayed the effect of DPDS on cerebral Na^+/K^+-ATPase activity and observed that DPDS inhibited basal enzyme's activity in concentration-increasing order (Figure 4). We further employed an assay system that simultaneously evaluates lipid peroxidation and Na^+/K^+-ATPase activity in the cerebral tissue of the rat. Our results in Figures 5 and 6 reveal that DPDS simultaneously inhibited Na^+/K^+-ATPase activity (panel A) and lipid peroxidation (panel B) when the brain tissue homogenate was treated with Fe^{2+} (Figure 5) or H_2O_2 (Figure 6). This suggests that DPDS antioxidant action could be linked to the enzyme's loss of activity. This agrees with the report of [7] that enzyme's inhibition was mediated by the oxidation of thiols critical to its activity.

δ-ALAD is an enzyme that is inhibited by several thiol oxidizing agents [33–36]. This is an important enzyme in the heme synthetic pathway that catalyzes the synthesis of porphobilinogen [37]. Figure 7 shows that DPDS inhibited the basal activity of δ-ALAD in concentration based manner. In a similar model to the pump, we employed a reaction system that simultaneously assayed for lipid peroxidation as well as the activity of hepatic δ-ALAD, and the results revealed that DPDS inhibited both δ-ALAD activity (panel A) and lipid peroxidation (panel B) in a concentration-dependent manner in Fe^{2+}-treated (Figure 8) or H_2O_2-treated (Figure 9) liver homogenates. These agree with the report of [5] and [8, 9] that DPDS inhibited sulphydryl enzymes such as Na^+/K^+-ATPase and lactate dehydrogenase which further validates the suggestions that the enzymes' inhibition, as well as the antioxidant action of DPDS rely on the oxidation of essential thiols of the enzymes.

Conclusion

This study further validates the GPx mimicry of DPDS and also reveals that DPDS is a potent antioxidant against lipid peroxidation induced by Fe^{2+} and H_20_2. It also suggests that thiols of sulphydryl proteins/enzymes are essential for the GPx mimetic activity of DPDS *in vitro*.

Recommendation

Further research is necessary to investigate the GPx mimicry of DPDS in the presence of thiol-blocking or oxidizing agents in biological systems *in vitro* in order to establish the role of protein thiols.

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