

A NON GLUTATHIONE DEPENDENT MECHANISM FOR DIPHENYL DISELENIDE IN ITS PROTECTION AGAINST OXIDATIVE ASSAULTS ON CEREBRAL LIPIDS

T. Ogunmoyole^{1*}, I.J. Kade² and J.B.T. Rocha³

^{1*}Department of Medical Biochemistry, College of Medicine, Ekiti State University, P.M.B 5363, Ado Ekiti, Ekiti State, Nigeria

²Department of Biochemistry, Federal University of Technology, P. M. B. 704, Akure, Ondo State, Nigeria

³Postgraduate Programme in Biochemical Toxicology, Centre of Natural and Exact Sciences, Federal University of Santa Maria, RS, Brazil

*Corresponding author's e-mail and phone: ogunmoyoledayo@yahoo.com, +2348062741702

Abstract

Literatures have reported that the pharmacological mechanism of diphenyl diselenide (DPDS) is strictly dependent on its ability to mimic the endogenous antioxidant enzyme, glutathione peroxidase (GPx), utilizing reduced glutathione (GSH) to deactivate hydroperoxides. However, in the present study, we investigated the strict dependence of DPDS on GSH utilization via its GPx mimetic ability by monitoring the level oxidative damage in cerebral lipids subjected to oxidative assaults induced by Iron (II), Sodium nitroprusside (SNP), Hydrogen peroxide (H₂O₂) and Sodium oxalate. Results showed that DPDS markedly inhibited the peroxidation of cerebral lipids both in the presence and absence of GSH irrespective of the prooxidant employed for oxidative damage, suggesting that DPDS may utilize dual pharmacological mechanisms to combat oxidative assaults on biological macromolecules. We speculate that in vitro, DPDS preferentially utilize GPx mimic to prevent radical assaults on cerebral lipids when GSH is available. However, in the absence of GSH, DPDS possibly switch to a possible non-glutathione

dependent mechanism to inhibit peroxidation of cerebral lipids. From the foregoing, we conclude that DPDS could switch between two mechanisms (GSH-dependent and non GSH dependent) both with similar but potent antioxidant efficacy.

Keywords: Reduced glutathione, Diphenyldiselenide, GPx mimetic, Mechanism, lipids, antioxidant.

{**Citation:** T. Ogunmoyole, I.J. Kade, J.B.T. Rocha. A non glutathione dependent mechanism for diphenyl diselenide in its protection against oxidative assaults on cerebral lipids. American Journal of Research Communication, 2015, 3(9): 137-169} www.usa-journals.com, ISSN: 2325-4076.

1.0 Introduction

The interest in organoselenium chemistry and biochemistry has increased in the last three decades mainly due to the fact that several organoselenium compounds possess antioxidant property (Andersson *et al.*, 1994), which has been attributed to their glutathione-peroxidase-like activity (Wilson *et al.*, 1989; Nogueira *et al.*, 2004). Yet, despite several study and excellent reviews on the pharmacological potentials of organoselenium compounds (Nogueira *et al.*, 2004; Mugesh *et al.*, 2001; Barbosa *et al.*, 2006; Kade *et al.*, 2009a & b) the precise mechanism(s) by which they elicit their observed pharmaco-effect is far from being understood. The complexity of the mechanisms that may be involved in the observed antioxidant action of these class of compounds becomes more intricately complex as *in vitro* data does not always correlate with *in vivo* results. The first example of organoselenium compound was ebselen (Muller *et al.*, 1984; Daiber *et al.*, 2000), and this compound has been extensively studied in reason of its antioxidant

and anti-inflammatory properties (Saito *et al.*, 1998; Yamaguchi *et al.*, 1998). Considering the pharmacological properties of ebselen, we have been studying the pharmacological properties of another organoselenium compound, diphenyl diselenide (PhSe)₂. Diphenyl diselenide (DPDS) is a member of the diorganyl diselenide that have shown promise against a number of degenerative diseases (Mugesh *et al.*, 2001; Nogueira *et al.*, 2004). Recently, Ogunmoyole *et al.* (2009) and Kade *et al.* (2008; 2009a & b) have shown that the precise mechanism of action of DPDS *in vitro* involves its utilization of the ubiquitous neuropeptide glutathione (GSH) to reduce hydroperoxides and studies have shown that DPDS also exhibits thiol-peroxidase-like activity which is about two times that of ebselen (Meotti *et al.*, 2004; Wilson *et al.*, 1989). Consequently, *in vitro* reduction of hydroperoxides is associated with concomitant decrease in the level of GSH in the reaction medium. Conversely, *in vivo* data shows that while DPDS is efficacious in management of degenerative diseases in which free radical have been implicated in their etiology, such *in vivo* pharmaco-effect exhibited by DPDS is associated with an increase in the amount of GSH in various organs of animals (Kade *et al.*, 2008). This observation has become an enigma to unravel with respect to the mechanism of action of DPDS vis-à-vis glutathione utilization. Recently, as part of our contribution to the unraveling of this puzzle surrounding the seemingly antagonistic antioxidant mechanism of DPDS, we examined the glutathione-peroxidase mimic and other possible antioxidant mechanisms of DPDS in altered acid-base balance using *in vitro* model (Ogunmoyole *et al.*, 2009). We concluded that glutathione peroxidase mimic activity of DPDS shifts depending on the pH of the medium and therefore, we can partly associate the sparing effect of endogenous glutathione by DPDS action to a possible distorted pH homeostasis in cases of ketoacidosis that is associated with hyperglycemia. However, arising from that work is the puzzling finding that in cases of induced lipid

peroxidation in *in vitro* models, DPDS exerted significant inhibitory effect on cerebral lipid peroxidation irrespective of the pH. Hence, we concluded that under *in vivo* conditions, there may be a sequential biotransformation of DPDS, with each intermediate product utilizing diverse, but potent antioxidant mechanism which would apparently exclude glutathione peroxidase mimic that require the utilization of endogenous glutathione. It is good to note at this point that studies on the glutathione peroxidase mimic of DPDS have been conducted in a tissue free system. In the present study however, we further investigate the evidence of a non- strict glutathione peroxidase mimetic-like dependence mechanism exhibited by diphenyl diselenide in its protection against oxidative assaults on cerebral lipids. Our intention is to observe if DPDS may be utilizing at least two mechanisms to effect its antioxidant action in tissues intentionally assaulted by free radicals. We speculate that these mechanisms which are GSH dependent on GSH independent may be mutually exclusive. Our finding may thus provide another insight into the seemingly dual antagonistic mechanisms employed by DPDS in its pharmacological action.

2.0. Materials and methods

2.1. Chemicals

Reduced glutathione (GSH), 5, 5 - dithio-bis-(2-nitrobenzoic acid) (DTNB), and thiobarbituric acid (TBA) were obtained from Sigma (St. Louis, MO). DPDS was synthesized according to literature methods (Paulmier, 1986). Analysis of ¹H NMR and ¹³C NMR spectra showed analytical and spectroscopic data in full agreement with their assigned structures. The chemical purity of DPDS (99.9%) was determined by GC/HPLC. All other chemicals were of analytical grade and were obtained from standard commercial suppliers.

2.2. Animals

Male adult Wistar rats (200–250 g) from our own breeding colony were used. Animals were kept in separate animal cages, on a 12-h light:12-h dark cycle, at a room temperature of 22–24 °C, and with free access to food and water. The animals were used according to standard guidelines of the Committee on Care and Use of Experimental Animal Resources.

2.3. Tissue preparation

Rats were decapitated under mild ether anesthesia and the brain was rapidly removed, placed on ice and weighed. Tissues were immediately homogenized in cold 50mM Tris–HCl, pH 7.4 (1/10, w/v). The homogenate was centrifuged for 10 min at 4000×g to yield a pellet that was discarded and a low-speed supernatant (S1).

2.4. Thiobarbituric acid reactive species (TBARS) assay

An aliquot of 100 µl of S1 was incubated for 1 h at 37 °C in the presence of DPDS with and without prooxidants (iron, sodium nitroprusside, hydrogen peroxide and sodium oxalate). Productions of TBARS were determined as described by method of Ohkawa *et al.* (1979) excepting that the buffer of color reaction have a pH of 3.4. The color reaction was developed by adding 300 µl 8.1% SDS to S1, followed by sequential addition of 500 µl acetic acid/HCl (pH 3.4) and 500 µl 0.8% of thiobarbituric acid (TBA). This mixture was incubated at 95°C for 1 h. TBARS produced were measured at 532 nm and expressed as µM MDA/g brain/hour.

2.5. Incubation conditions possible GPx-mimetic and TBARS assay

The catalytic effects of DPDS on the utilization of GSH in its inhibition of TBARS production in tissues assaulted with prooxidant were determined as described below. Generally, 100 µl of S1,

DPDS (1–100 μ M) were incubated in a medium containing GSH (20 mM) with and without prooxidants (iron, sodium nitroprusside, H₂O₂, and sodium oxalate) for 1 hour. At 0, 30 and 60 min, aliquots of the reaction mixture (200 μ l) were checked for the amount of GSH and TBARS production. In order to better explore the utilization of GSH by DPDS to inhibit prooxidant-induced TBARS production, four separate incubation conditions were carried out. In the first condition, GSH was incubated alone (condition I). In the second case, GSH and DPDS were incubated (condition II). In the third condition, GSH, and prooxidant were incubated (condition III). In the fourth condition, GSH, DPDS and prooxidants were incubated (condition IV). For various conditions set up above, two set of the same experimental conditions were run concurrently, one for the assay of TBARS and the other for the estimation of residual glutathione present in the reaction media. TBARS determination was carried as described earlier (Ohkawa, 1979). Residual glutathione was carried out as described below (section 2.6).

2.6. Determination of residual GSH in tissue homogenate

GSH levels were estimated using Ellman's reagent after deproteinization with TCA (5% in 1 mmol/EDTA) following the method of Ellman (1959).

2.7. Statistical analysis

All values obtained were expressed as mean \pm SEM. The data were analyzed by appropriate ANOVA followed by Duncan's multiple range tests where appropriate and this is indicated in the text of results. The differences were considered significant when $p < 0.05$. All assays were carried out four independent times to ensure reproducibility.

3.0. Results

3.1. DPDS vs GSH consumption vs TBARS production vs prooxidants

Although we have reported the effect of 100 μM DPDS in the present report, it is noteworthy that other concentrations (10, 20, 40, and 80 μM) were also tested (results not shown). The trend in the data obtained is similar to what we are presenting for 100 μM . This is irrespective of whether we assayed for TBARS or glutathione level in the reaction media.

3.1.1. DPDS vs GSH consumption vs TBARS production vs Fe^{2+}

Figure 1a shows that DPDS was able to exert significant concentration dependent inhibitory effect on iron induced lipid peroxidation in the low-speed supernatant from brain homogenate. Apparently, the inhibitory effect of DPDS on the iron induced lipid peroxidation is significant at concentration $\geq 40 \mu\text{M}$. Considering Figure 1b we observe the results obtained when the tissues were incubated as described in the methodology (section 2.5). From the graph, it is apparent that GSH alone did not alter the level of TBARS produced in brain homogenate when incubated up till 1 hour at 37°C (condition I). However, in the presence of GSH, DPDS caused a time dependent decrease in the level of basal TBARS produced in the cerebral tissues (condition II). Furthermore, incubation of cerebral tissue with Fe^{2+} caused a significant increase in the level of aldehydic compounds produced in the brain lipids (Condition III). Finally, the addition of DPDS in the presence of GSH caused a significant decrease in the amount of TBARS produced under Fe^{2+} oxidative assaults (condition IV). Figure 1c shows the level of glutathione in the reaction medium as peroxidation of lipid/its inhibition by DPDS progresses under the various conditions described above. Estimation of the amount of GSH shows that the relative amount of glutathione is in the order condition I > condition III > condition II > condition IV. Significant differences were tested at $p < 0.05$.

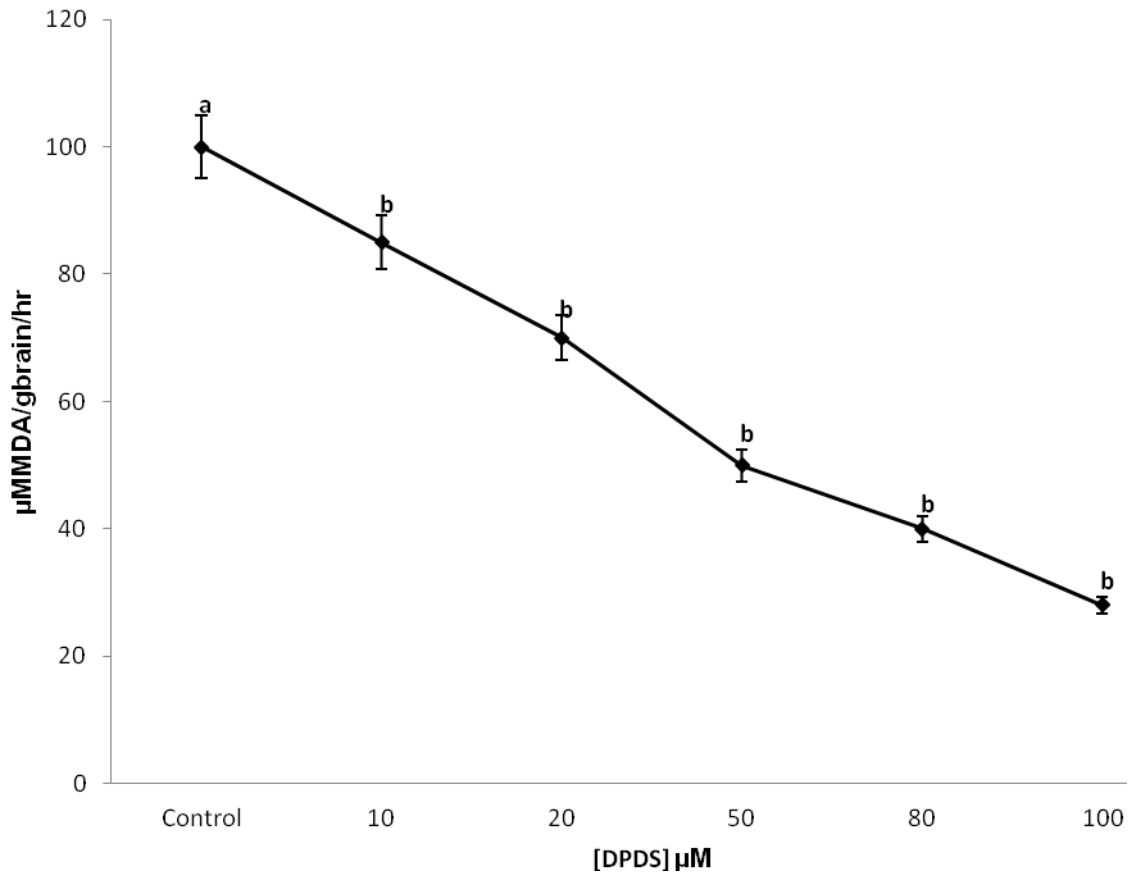


Fig 1a: Inhibitory effect of DPDS on iron-induced cerebral lipid peroxidation in the absence of GSH. Data show means \pm SEM values averages from 4 independent biological replicates. ‘a’ indicate control at $p < 0.05$, while ‘b’ indicates a significant difference from the control.

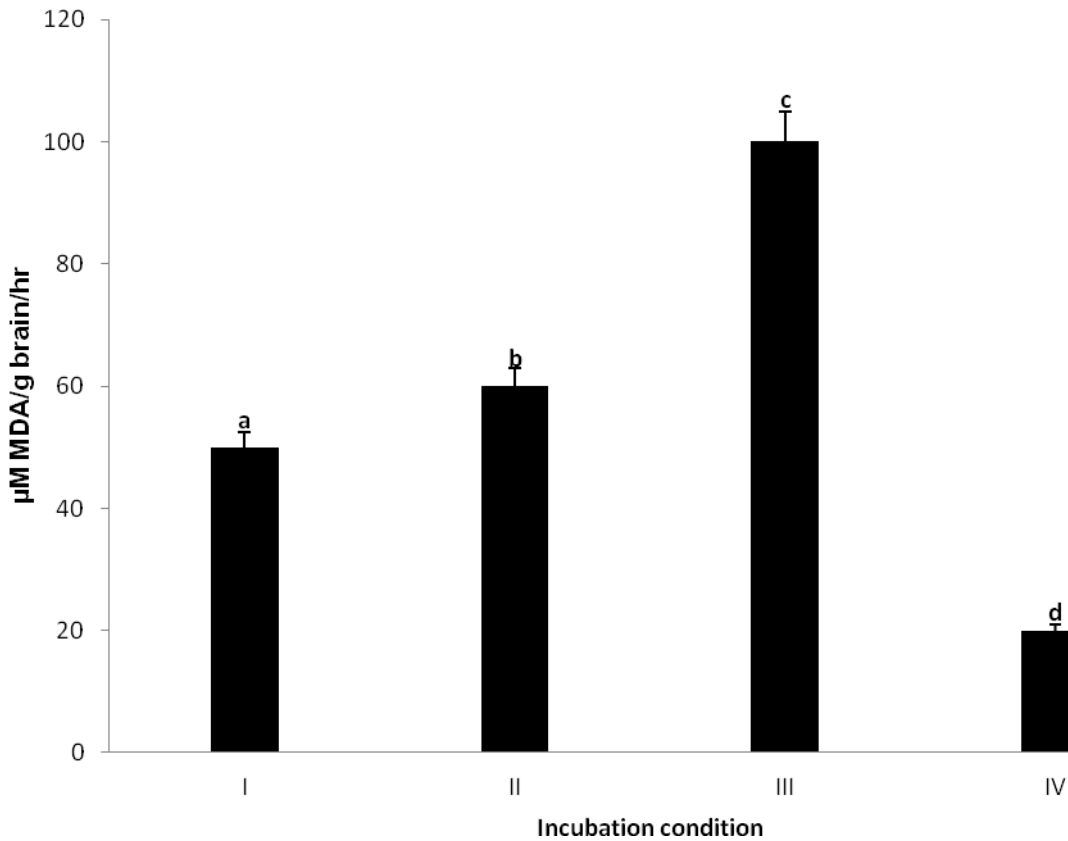


Fig 1b: Inhibitory effect of DPDS on iron-induced cerebral lipid peroxidation under various incubation conditions. I (GSH only), II (GSH and DPDS only), III (GSH and iron (II) only) and IV (GSH, DPDS and iron (II) only). Data show means \pm SEM values averages from 4 independent biological replicates. 'b', 'c' and 'd' indicate a significant difference from control 'a' at $p < 0.05$.

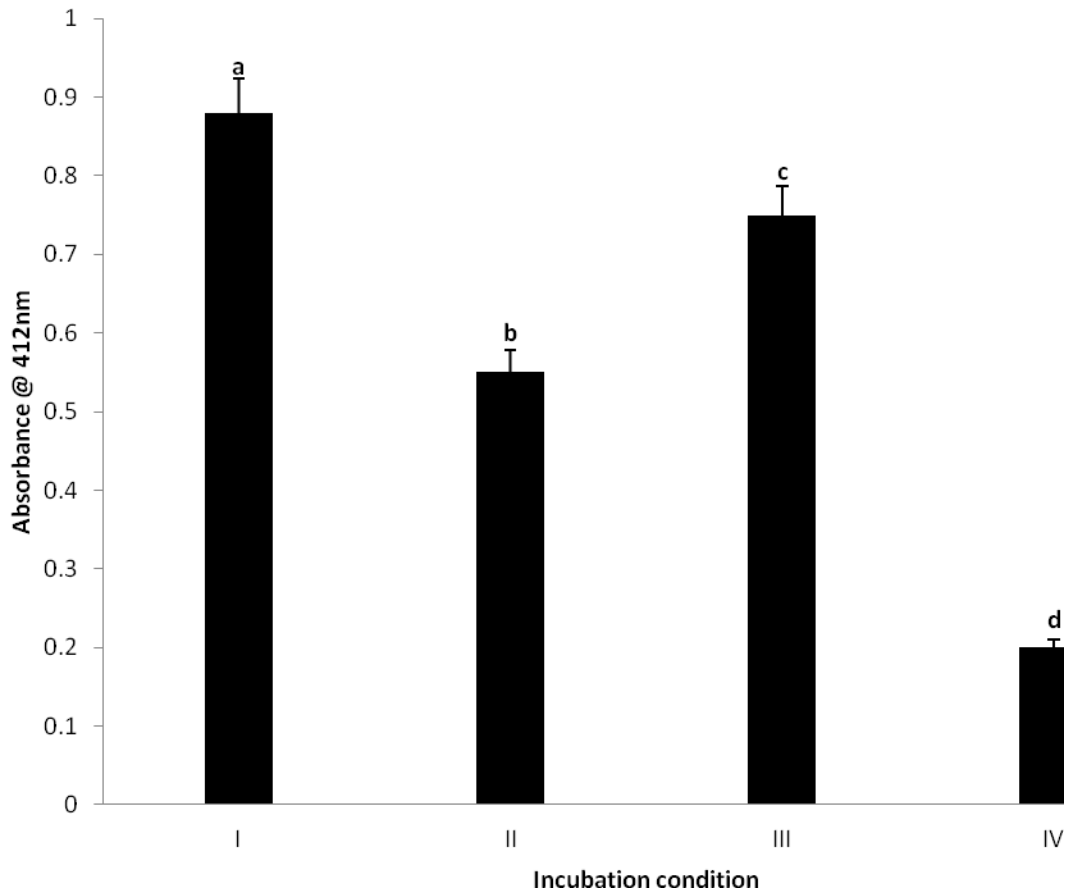


Fig. 1c: Residual GSH in an iron-induced cerebral lipid peroxidation. I (GSH only), II (GSH and DPDS only), III (GSH and iron (II) only) and IV (GSH, DPDS and iron (II) only). Data show means \pm SEM values averages from 4 independent biological replicates. 'a' indicates control at $p < 0.05$. While 'b', 'c' and 'd' indicate a significant difference from the control.

3.1.2. DPDS vs GSH consumption vs TBARS production vs SNP

The pattern of result obtained when sodium nitroprusside was used as a prooxidant to induce cerebral lipid peroxidation is presented in Figure 2a. Here, it is equally clear that DPDS is able to counteract the concerted peroxidation caused by the photodegradation products of SNP. In addition, we observed that the inhibitory pattern shows concentration dependence. Furthermore, in Figure 2b, the graph shows the results obtained when incubation was done as described in section 2.5. Here we also observe that while GSH did not cause any increase per se in the level of TBARS (condition I), SNP induced a potent oxidative effect on the production of aldehydic products from cerebral lipids (condition III). On the other hand, DPDS (condition II) or in a reaction medium containing SNP (condition IV) caused a significant inhibitory effect on the level of TBARS generated in the cerebral tissues. Similarly, Figure 6b shows the level of glutathione in the reaction medium as peroxidation of lipid/its inhibition by DPDS progresses under the various conditions described above. Estimation of the amount of GSH shows that the relative amount of glutathione is in the order condition I > condition III > condition II > condition IV. Significant differences were tested at $p < 0.05$.

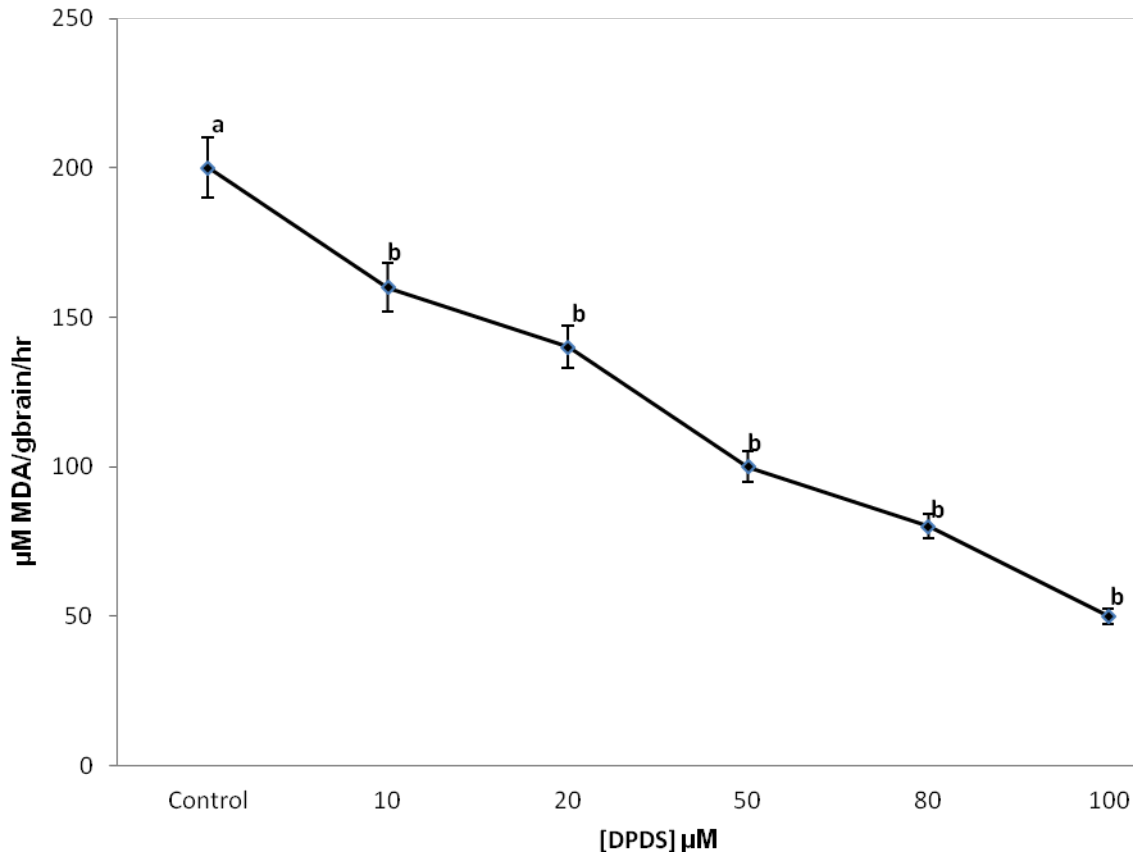


Fig. 2a: Inhibitory effect of DPDS on SNP-induced cerebral lipid peroxidation in the absence of GSH. Data show means \pm SEM values averages from 4 independent biological replicates. 'a' indicates control at $p < 0.05$, while 'b' indicate a significant difference from the control.

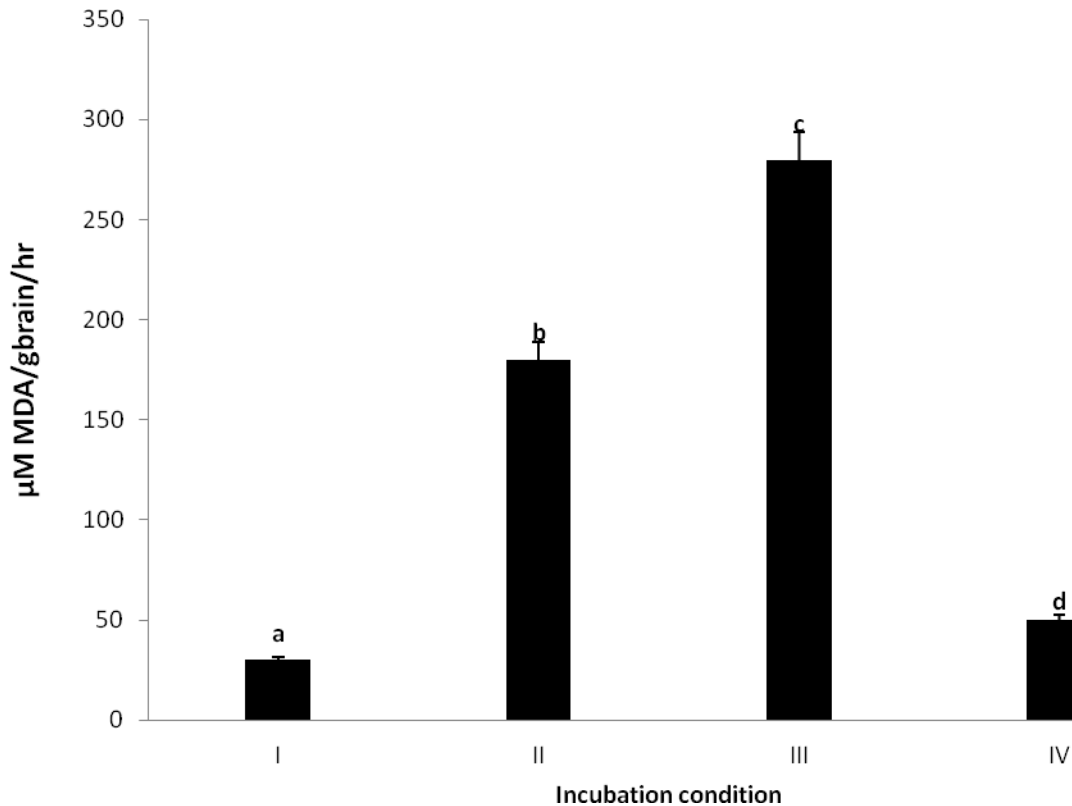


Fig. 2b. Inhibitory effect of DPDS on SNP - induced cerebral lipid peroxidation under various incubation conditions. I (GSH only), II (GSH and DPDS only), III (GSH and SNP only) and IV (GSH, DPDS and SNP). Data show means \pm SEM values averages from 4 independent biological replicates. 'b', 'c' and 'd' indicate a significant difference from control 'a' at $p < 0.05$.

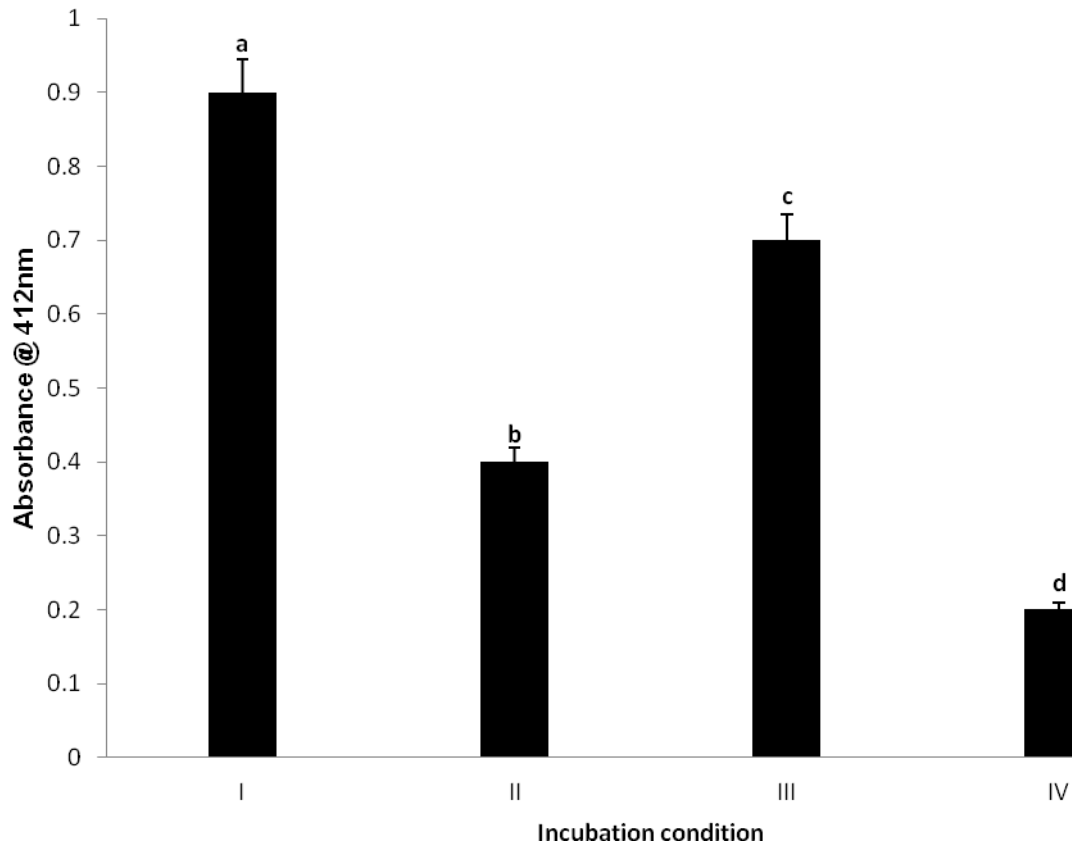


Fig 2c: Residual GSH in a SNP-induced cerebral lipid peroxidation under various conditions. I (GSH only), II (GSH and DPDS only), III (GSH and SNP only) and IV (GSH, DPDS and SNP only). Data show means \pm SEM values averages from 4 independent biological replicates. 'a' indicates control at $p < 0.05$, while 'b' and 'c' indicate a significant difference from the control.

3.1.3. DPDS vs GSH consumption vs TBARS production vs H_2O_2

Figure 3a shows that DPDS exhibit potent inhibitory effect on lipid peroxidation induced in brain tissues when $\cdot OH$ was the oxidant. Apparently, as in the case of Fe^{2+} and SNP, the inhibitory

effect of DPDS on lipid peroxidation when H_2O_2 was the oxidant is concentration dependent. In Figure 3b we also observe that the presence of GSH alone did not have any significant effect on the reduction of basal TBARS (condition I). However, in the presence of GSH, H_2O_2 caused an increase in the amount of lipid peroxidation products generated in the brain tissues assaulted by this prooxidant (condition III). Similar to our results when iron and SNP were used as prooxidant, DPDS alone (condition II), or in a combination of DPDS and H_2O_2 , DPDS significantly counteract the prooxidative effect of $\cdot OH$ against cerebral lipids. Figure 3c shows that there is a concomitant consumption of GSH when DPDS acts as an antioxidant whether on basal or prooxidant induced lipid damage. Estimation of the amount of GSH shows that the relative consumption of GSH in the various conditions is in the order condition IV > condition II > condition III > condition I. Significant differences were tested at $p < 0.05$.

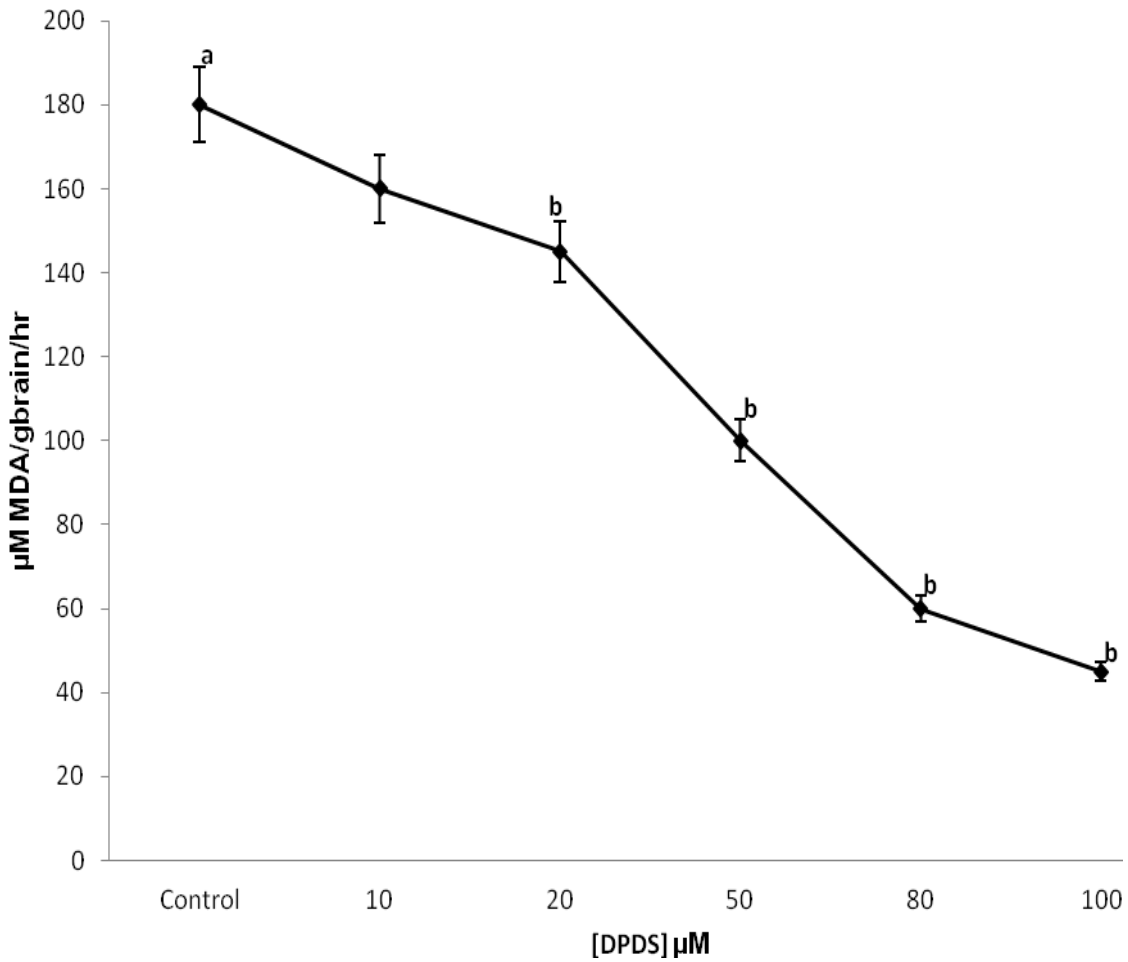


Fig. 3a: Inhibitory effect of DPDS on H_2O_2 -induced cerebral lipid peroxidation in the absence of GSH .Data show means \pm SEM values averages from 4 independent biological replicates. ‘a’ indicate control at $p < 0.05$, while ‘b’ indicates a significant difference from the control.

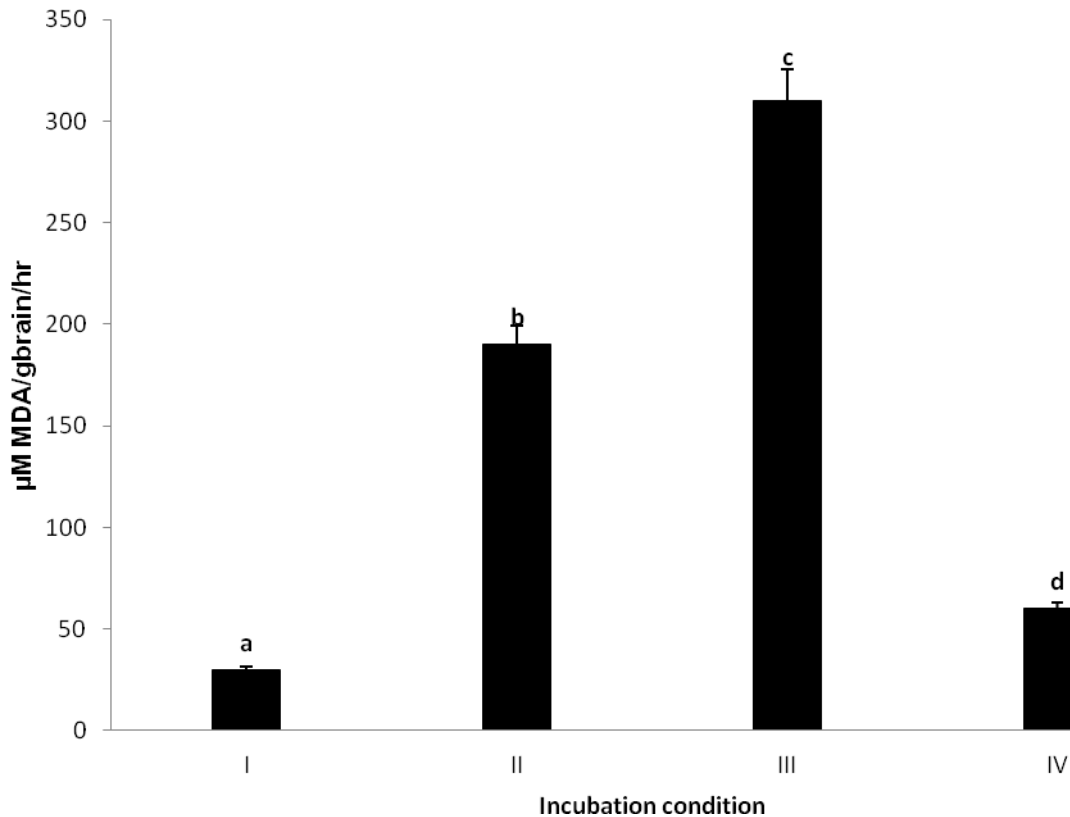


Fig 3b: Inhibitory effect of DPDS on H₂O₂-induced cerebral lipid peroxidation under various incubation conditions. I (GSH only), II (GSH and DPDS only), III (GSH and H₂O₂ only) and IV (GSH, DPDS and H₂O₂). Data show means ± SEM values averages from 4 independent biological replicates. ‘a’ indicates the control, while ‘b’, ‘c’ and ‘d’ indicate a significant difference from control at $p < 0.05$.

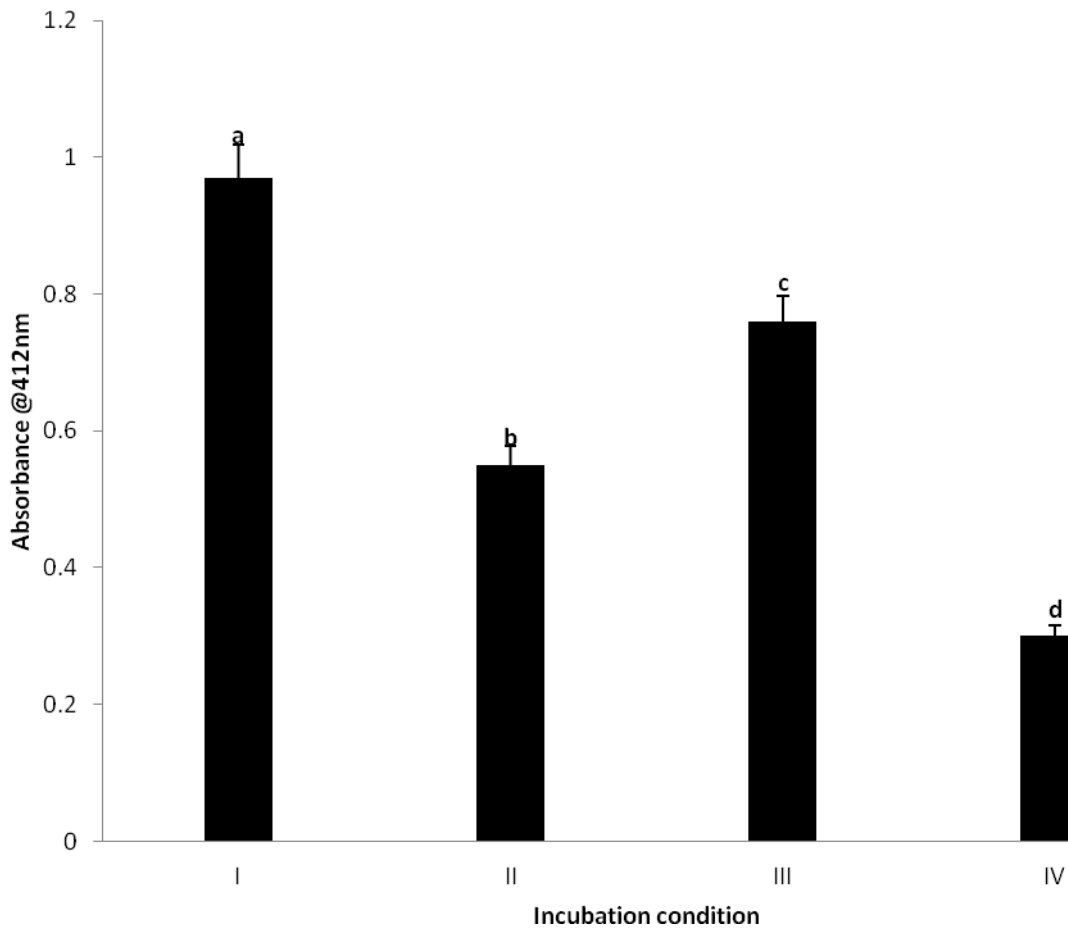


Fig 3b: Inhibitory effect of DPDS on H₂O₂-induced cerebral lipid peroxidation under various incubation conditions. I (GSH only), II (GSH and DPDS only), III (GSH and H₂O₂ only) and IV (GSH, DPDS and H₂O₂). Data show means \pm SEM values averages from 4 independent biological replicates. 'a' indicates the control, while 'b', 'c' and 'd' indicate a significant difference from control at $p < 0.05$.

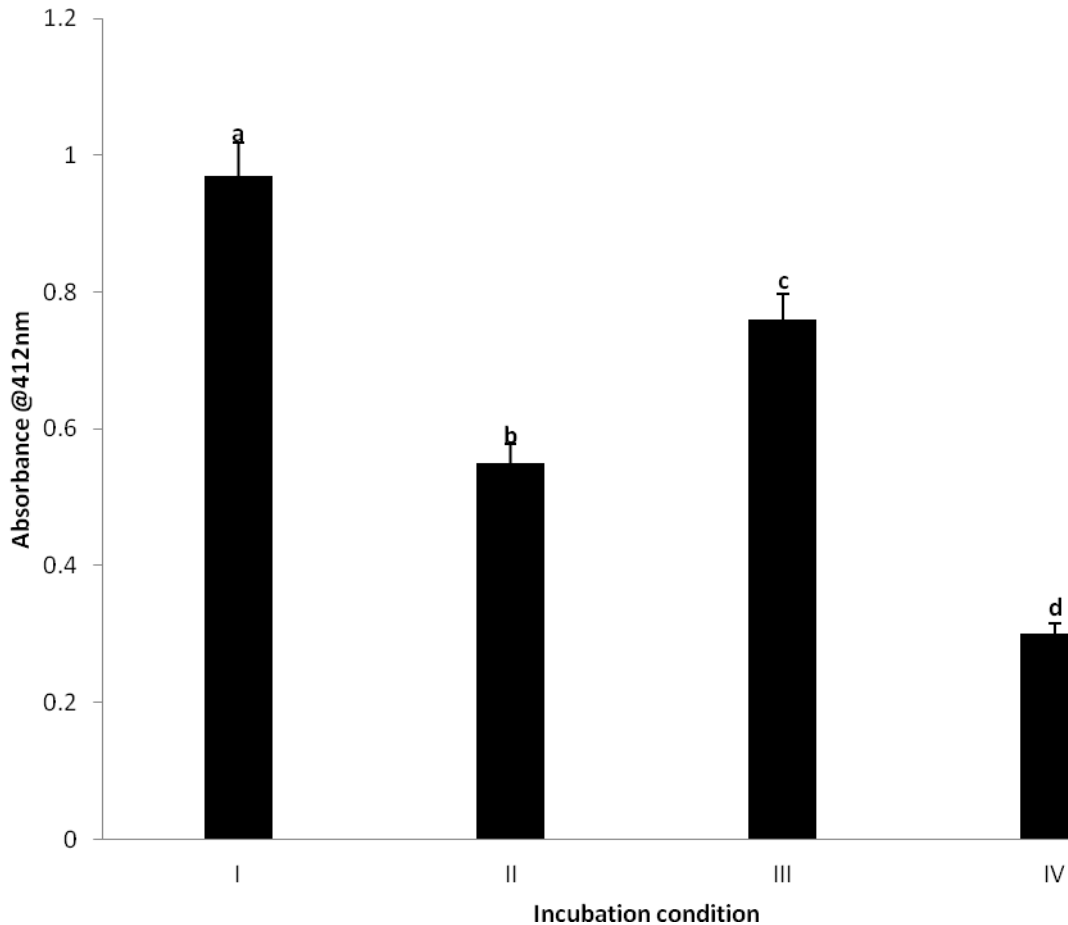


Fig 3c: Residual GSH in a H₂O₂-induced cerebral lipid peroxidation. I (GSH only), II (GSH and DPDS only), III (GSH and H₂O₂ only) and IV (GSH, DPDS H₂O₂ only). Data show means ± SEM values averages from 4 independent biological replicates. ‘a’ indicates control at $p < 0.05$, while ‘b’ and ‘c’ indicate a significant difference from the control.

3.1.4. DPDS vs GSH consumption vs TBARS production vs oxalate salts

Sodium oxalate induces oxidation of cerebral lipids as shown in Figure 4a. However, DPDS exerted considerable inhibitory effect on the prooxidative effect of sodium oxalate in a concentration dependent manner. Apparently, In Figure 4b, the pattern of results obtained is somewhat similar to the other prooxidants tested. As observed earlier GSH did not alter per se the peroxidation of lipids in the brain (condition I), while sodium oxalate induced lipid cerebral peroxidation as observed in condition III. As earlier observed, DPDS caused a decrease in basal TBARS level (condition II) and also under sodium oxalate assault, DPDS counteracted the peroxidation of cerebral lipids (condition IV). In the same vein, Figure 4c shows that there is a relationship between GSH utilization and the antioxidant action of DPDS. Estimation of the amount of GSH shows that the utilization of GSH in the various conditions is in the order condition IV > condition II > condition III > condition I. Significant differences were tested at $p < 0.05$.

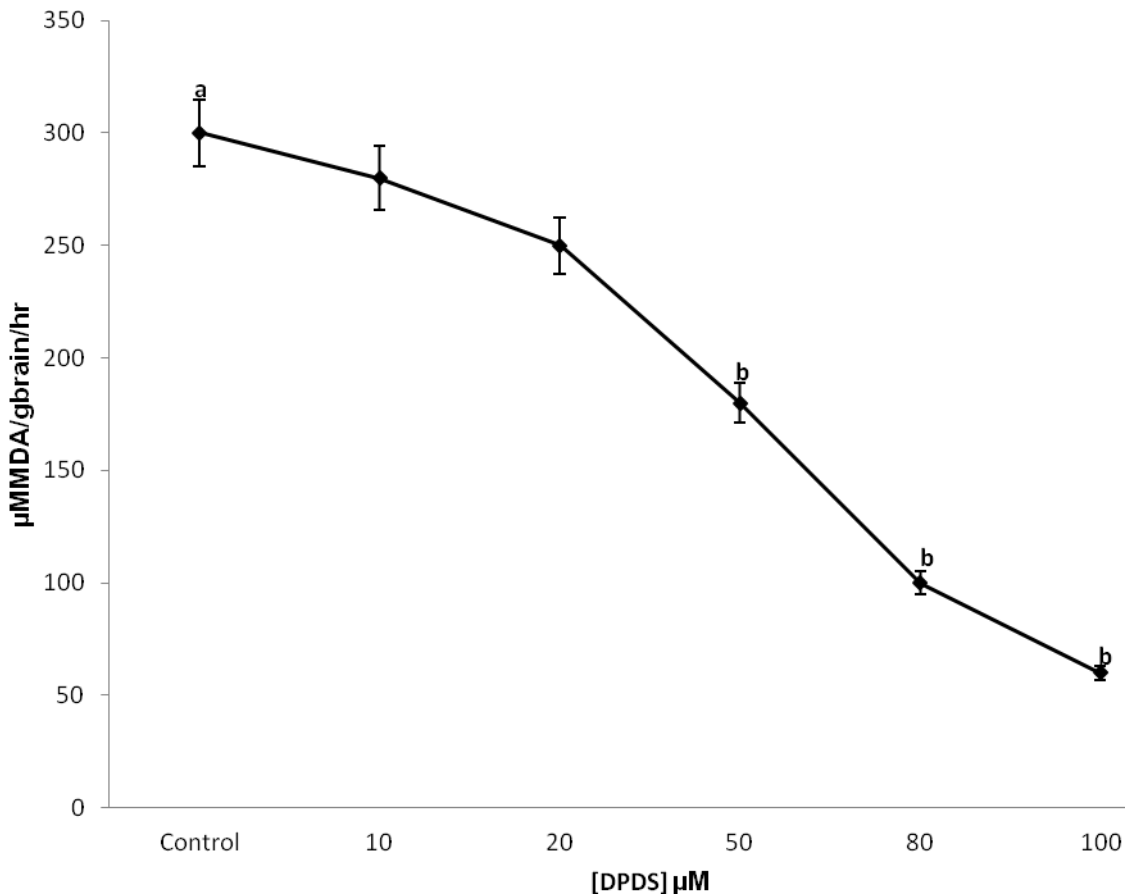


Fig 4a: Inhibitory effect of DPDS on sodium oxalate-induced cerebral lipid peroxidation in the absence of GSH. Data show means \pm SEM values averages from 4 independent biological replicates. 'a' indicates control at $p < 0.05$, while 'b' indicates a significant difference from the control.

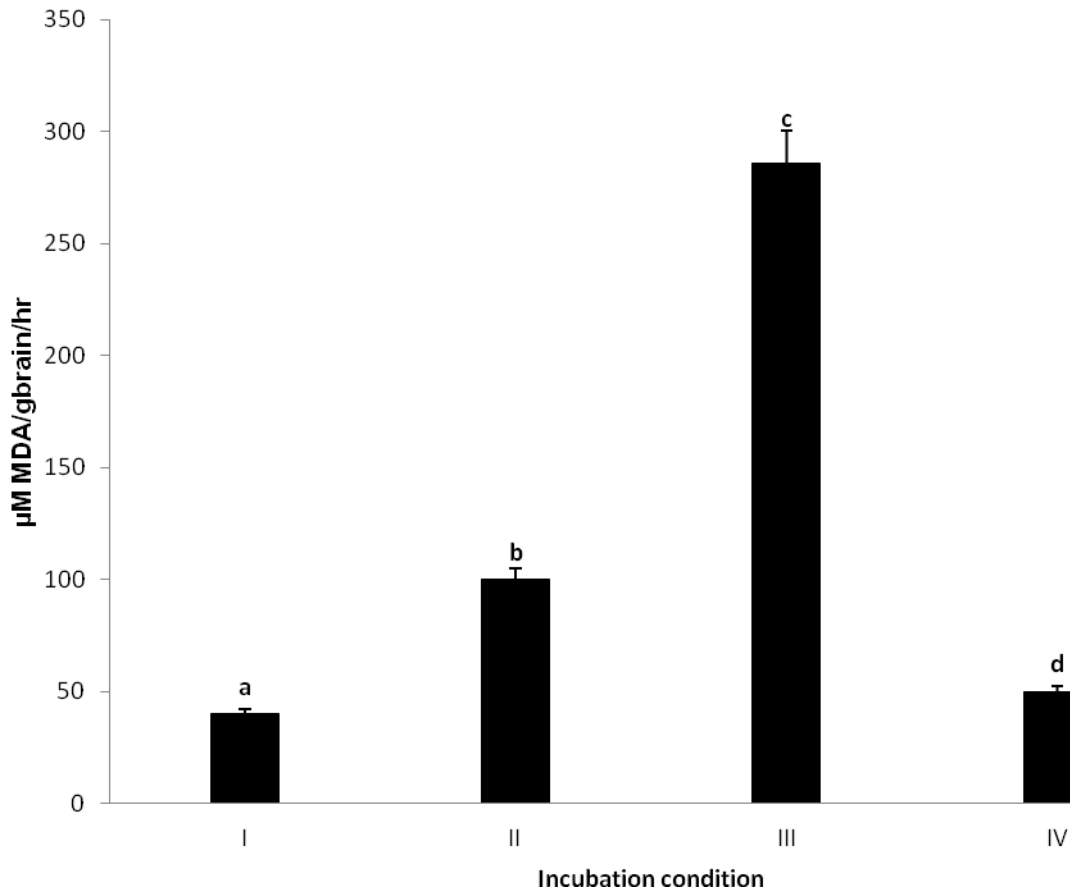


Fig.4b: Inhibitory effect of DPDS on oxalate- induced cerebral lipid peroxidation under various incubation conditions. I (GSH only), II (GSH and DPDS only), III (GSH and oxalate only) and IV (GSH, DPDS and oxalate). Data show means \pm SEM values averages from 4 independent biological replicates. 'a' indicates the control while 'b', 'c' and 'd' indicate a significant difference from control at $p < 0.05$.

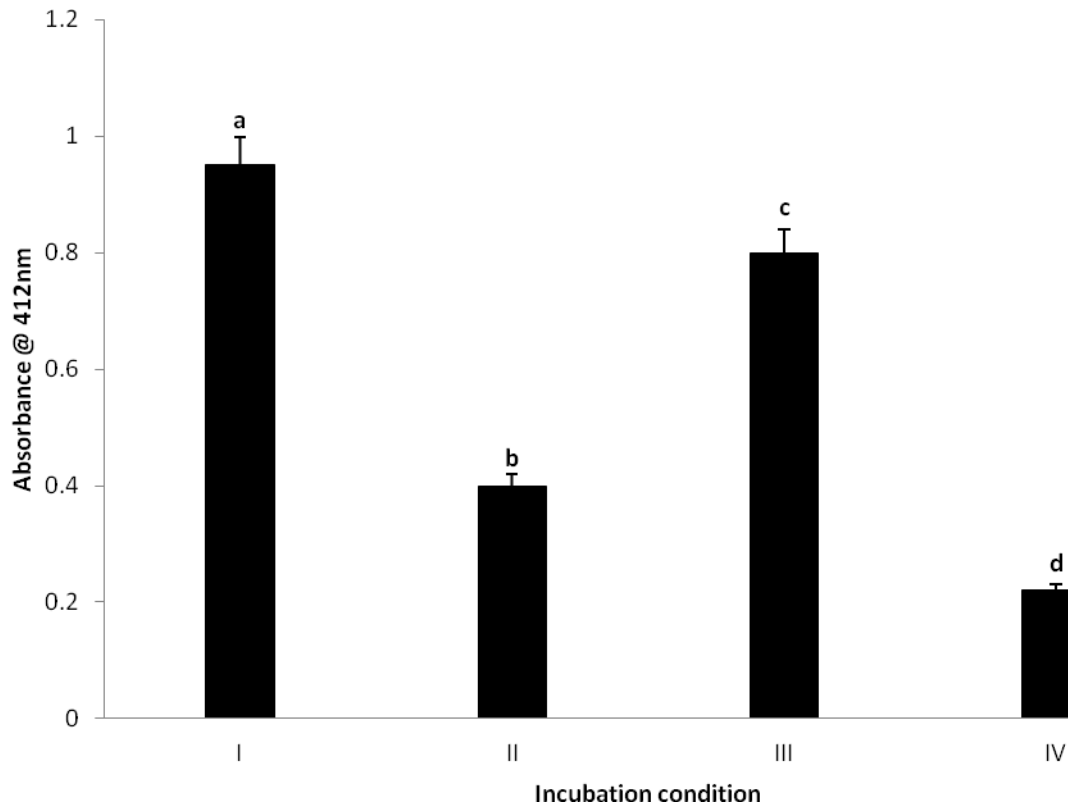


Fig 4c: Residual GSH in an oxalate -induced cerebral lipid peroxidation. I (GSH only), II (GSH and DPDS only), III (GSH and oxalate only) and IV (GSH, DPDS and oxalate). Data show means \pm SEM values averages from 4 independent biological replicates ‘a’ indicates control at $p < 0.05$, while ‘b’, ‘c’ and ‘d’ indicate a significant difference from the control.

4.0 DISCUSSION

Since the overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been considered to be involved in the pathogenesis of various neurodegenerative diseases

(Coyle and Pultfarker, 1993; Kanterewicz *et al.*, 1998; Woltjer *et al.*, 2005) as well as in the aging process (Tolmasoff *et al.*, 1980; Sohal and Allen, 1990; Finkel and Holbrook, 2000), the search for new compounds with antioxidant properties and their potential utilization in antioxidant therapies have been stimulated. Of particular importance, seleno-organic compounds, which display thiol-peroxidase activity, have been experimentally studied as potential protective molecules against brain oxidative stress (Nogueira *et al.*, 2004). Diphenyl diselenide (PhSe)₂ (Paulmier, 1986), whose catalytic activity involves the reduction of peroxides at the expense of thiol compounds, represent important molecules whose protective properties against experimental oxidative stress have been reported (Dawson *et al.*, 1995; Takasago *et al.*, 1997; Porciuncula *et al.*, 2001; Rossato *et al.*, 2002a & b; Farina *et al.*, 2003; Moretto *et al.*, 2005; Mugesh *et al.*, 2001; Santos *et al.*, 2005 a & b; Posser *et al.*, 2006; Borges *et al.*, 2006).

Although we have noted that DPDS have seemingly antagonist mechanisms under *in vitro* and *in vivo* conditions with regards to utilization of the ubiquitous antioxidant tripeptide GSH (Kade *et al.*, 2008; Kade *et al.*, 2009a; Barbosa *et al.*, 1991; Nogueira *et al.*, 2004) little attention is given to unravel this pharmacological enigma exhibited by DPDS. This becomes obvious considering the volume of information that is available describing the toxicity of this promising antioxidant compound (Nogueira *et al.*, 2004). We took an initial stride recently to unravel the overwhelming mystery associated with *in-vitro* and *in vivo* antioxidant potential of DPDS in relation to GSH dynamics (Ogunmoyole *et al.*, 2009). In this study, we further expanded our scope of study to observe the interaction between glutathione and DPDS pharmacology in *in vitro* models of animal tissue homogenate. Again, it is good to state that most work that have been done to evaluate the glutathione peroxidase mimetic antioxidant property of DPDS has been carried out in *in vitro* models that is devoid of tissue homogenates (Kade *et al.*, 2008). In

the present study, we want to clarify the existence of a strict dependence of DPDS on GSH to counteract various free radicals that are potentially toxic to cerebral lipids. In order for the readers to better appreciate our argument, we need to state that in the present report, principally three sets of experimental design were carried out independently on brain homogenate. These studies are: 1) effect of DPDS on prooxidant-induced lipid peroxidation in brain homogenate; 2) effect of combination of DPDS and GSH on prooxidant-induced lipid peroxidation in brain homogenate and 3) determination of the amount of residual GSH that is left in situation 2. In Figure 1a, it is apparent that iron caused peroxidation of cerebral lipids and that DPDS caused a concentration dependent inhibitory effect on the peroxidation processes caused by iron. This information is not new considering our earlier reports (Kade *et al.*, 2008; Kade *et al.*, 2009a; Kade *et al.*, 2009b). In these reports on the protective effect of DPDS on iron-induced cerebral lipid damage, we comprehensively gave the detailed possible mechanism of action of iron based on the findings of other authors and we gave plausible explanation as to the possible mechanism employed by DPDS to protect against the radical assault of Fe^{2+} (Kade *et al.*, 2008). It is noteworthy, that the basal GSH levels in these tissues were examined and were generally too low to give a brilliant colour with DTNB (data not shown). However, in Figure 1b, we observe that iron stimulated cerebral lipid peroxidation (bar III) and that DPDS significantly abolished this effect (bar IV). A comparison of the corresponding amount of residual GSH left in each case shows that while GSH level was not diminished in the presence of Fe^{2+} [Figure 1c (bar III)], the introduction of DPDS caused a marked decrease in the amount of residual GSH [Figure 1c (bar IV)]. This may suggest that the prevention of TBARS production by DPDS is associated with the concomitant utilization of GSH. This is further confirmed by the fact that even in the absence of a radical assault, Figure 1b (bar II), DPDS also caused a marked decrease in the level of GSH to

effect a reduction in basal TBARS production [compare Figure 1b (bar II) and Figure 1c (bar II)]. Overall, comparing Figure 1a (DPDS action without GSH), Figure 1b (bar II and IV) (DPDS action with GSH) and Figure 1c (bar II and IV), it is apparent that DPDS action may be strictly dependent on the utilization of GSH, thereby confirming the classical hypothesis of its classical glutathione peroxidase mimetic antioxidant mechanism as described in scheme 1. On the other hand, the fact that DPDS exerted its pharmacological effect in the absence of GSH (Figure 1a), shows that DPDS may not strictly require GSH to effect its action. In a way, we may observed that during in vivo conditions, DPDS antioxidant mechanism may not be GPx dependent and this hypothesis may explain in part the sparing effect of endogenous GSH by DPDS treatment.

To further expand our scope of argument, we employed other prooxidants such as SNP. The prooxidant action of SNP has been well reported (Bates *et al.*, 1991) and we have also reported on the possible mechanism employed by DPDS to counteract the oxidative damage elicited by SNP. The interesting observation is that DPDS counteracted SNP assault on cerebral lipid in a fashion similar to that of iron and in fact, there is strong evidence to establish the fact that in the presence and absence of GSH, DPDS counteracted the oxidative assault of the photodegradation products of SNP (compare Figure 2a, b and c).

Furthermore, a model often used to demonstrate the GPx mimetic activity of DPDS often utilizes H_2O_2 . Although H_2O_2 is not a radical, but it can ultimately generate the destructive $\cdot OH$ radical which can in effect, exert deleterious effect on brain lipids. Therefore, it is logical to test whether the observed GSH consumption-dependent mechanism of DPDS action on the decomposition of hydrogen peroxide will exactly reproduce in tissue systems. Our result shows that in conformity with the earlier proposed GPx-mimetic activity of DPDS, this organoselenium mediated its antioxidant action with the concomitant utilization of GSH in brain tissues (compare Figures 3b

and 3c). However, one striking observation is that there is a consumption of GSH in the absence of hydrogen peroxide by DPDS (compare Figure 3b bar II and Figure 3c bar II). Earlier, in our lab, while comparing the GPx mimetic property of two organoselenium (DPDS and dicholesteroyl diselenide, DCDS) compounds, we observed that both DPDS and DCDS did not consume GSH in the absence of hydrogen peroxide; but now in brain tissue, there is a consumption of GSH in the absence of hydrogen peroxide. One rational explanation is that endogenous prooxidants such as transition metals like iron (possibly present in the brain tissue) may contribute to this phenomenon. In the absence of GSH, Figure 3a shows that DPDS can mediate its antioxidant activity and that the efficacy/potency of its action is independent on the availability GSH.

In this study, we equally employed the use of another prooxidant, sodium oxalate. Both *in vivo* and *in vitro* studies have revealed that the mechanism of induction of lipid peroxidation by oxalate may involve the inhibition of catalase activity as well as the combined effect of Fe^{2+} (Selvan and Bijikurien, 1987). The precise mechanism employed by DPDS to counteract the peroxidation of lipids as observed in Figure 4 may not be fully understood. From the data available in the present study, it is obvious that DPDS action may involve two mechanisms to inhibit the adverse effect of sodium oxalate in mammalian brain. There is increasing evidence that the mammalian brain may be exceptionally vulnerable to oxidative stress through oxygen radical attack which possibly contribute to cerebral ischemic injury by promoting membrane lipid peroxidation and oxidative damage to DNA and proteins (Yamaguchi *et al.*, 1998). Due to the delicate nature of the cerebral and neuronal processes, it is highly imperative that the mechanisms of drugs that are potential candidate in the management of stresses in the brain be well understood. DPDS from the foregoing is a strong candidate against a variety of free radicals

employing diverse mechanisms to cause an assault or injury on brain lipids. However, the fact that DPDS treatment can potentiate the level of GSH in the brain, while desirable can be worrisome, since experimental evidence in our lab shows that GSH can inhibit the activity of sodium pump (unpublished data). Hence, a good understanding of the mechanism of its antioxidant action in vivo is necessary for its application in clinical trials in the future. However, in the present study, in vitro evidences suggest that DPDS may use some mechanisms different from its generally believed antioxidant mechanism. This calls for a concerted experimental design to unravel this possible mechanism. In our lab, we are expanding to scope of this study using in vitro models to study the reactivity of DPDS especially vis-à-vis its switch from its glutathione peroxidase mimetic activity.

Acknowledgements

The authors are grateful for the unflinching support of Prof. JBT Rocha, Brazil. Also the work was partly funded by the DTLC, granted to Biochemistry Department, Federal University of Technology, Akure, Nigeria.

References

- [1] Andersson, C.M., Hallberg, A., Linden, M., 1994. Antioxidant activity of some diarylselenides in biological systems. *Free Radic. Biol Med* 16, 17–28.
- [2] Borges, L.P., Nogueira, C.W., Panatieri, R.B., Rocha, J.B.T., Zeni, G., 2006. Acute liver damage induced by 2-nitropropane in rats: effect of diphenyl diselenide on antioxidant defenses. *Chem. Biol. Interact.* 160, 99–107.

- [3] Bates, J.N., Baker, M.T., Guerra, R., Harrison, D.G., 1991. Nitric oxide generation from nitroprusside by vascular tissue. Evidence that reduction of the nitroprusside anion and cyanide loss are required. *Biochem Pharmacol.* 42, S157- S165.
- [4] Barbosa, N.B.V., Rocha, J.B.T., Wondracek, D.C., Perotoni, J., Zeni, G., Nogueira, C.W., 2006. Diphenyl diselenide reduces temporarily hyperglycemia: possible relationship with oxidative stress. *Chem. Biol. Interact.* 163, 230–238.
- [5] Coyle, J.T., Puttfarcken, P., 1993. Oxidative stress, glutamate, and neurodegenerative disorders. *Science* 262, 689–695.
- [6] Daiber, A., Zou, M.H., Bachschmid, M., Ullrich, V., 2000. Ebselen as a peroxynitrite scavenger in vitro and ex vivo. *Biochem Pharmacol.* 59, 153–60.
- [7] Dawson, D.A., Masayasu, H., Graham, D.I., Macrae, I.M., 1995. The neuroprotective efficacy of ebselen (a glutathione peroxidase mimic) on brain damage induced by transient focal cerebral ischaemia in the rat. *Neurosci. Lett.* 185, 65–69.
- [8] Ellman, G.L., 1959. Tissue sulfhydryl groups, *Arch. Biochem. Biophys.* 82, 70–77.
- [9] Farina, M., Frizzo, M.E., Soares, F.A., Schwalm, F.D., Dietrich, M.O., Zeni, G., Rocha, J.B.T., Souza, D.O., 2003. Ebselen protects against methylmercury-induced inhibition of glutamate uptake by cortical slices from adult mice, *Toxicol. Lett.* 144, 351–357.
- [10] Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of ageing. *Nature* 408, 239–247.
- [11] Kade, I.J., Paixao, M.W., Rodrigues, O.E.D., Barbosa, N.B.V., Braga, A.L., Avila, D.S., Nogueira, C.W., Rocha, J.B.T., 2008. Comparative studies on the effect of a dicholesteroyl

diselenide and diphenyl diselenide on the activities of Na^+/K^+ ATPase and δ -aminolevulinic acid dehydratase in the rat brain. *Neurochem Res* 33, 167-178.

[12] Kade, I.J., Paixão, M.W., Rodrigues, O.E.D., Ibukun, E.O., Braga, A.L., Zeni, G., Nogueira, C.W., Rocha, J.B.T., 2009a. Studies on the antioxidant effect and interaction of diphenyldiselenide and dicholesteroyl diselenide with hepatic d-aminolevulinic acid dehydratase and isoforms of lactate dehydrogenase. *Toxicol. in Vitro* 23, 14–20.

[13] Kade, I.J., Nogueira, C.W., Rocha, J.B.T., 2009b. Diphenyl diselenide and streptozotocin did not alter cerebral glutamatergic and cholinergic systems but modulate antioxidant status and sodium pump in diabetic rats, *Brain Res.* 1284, 202–211.

[14] Kanterewicz, B.I., Knapp, L.T., Klann, E., 1998. Stimulation of p42 and p44 mitogen-activated protein kinases by reactive oxygen species and nitric oxide in hippocampus. *J. Neurochem.* 70, 1009–1016.

[15] Meott, F.C., Stangherlin, E.C., Zeni, G., Nogueira, C.W., Rocha, J.B.T., 2004. Protective role of aryl and alkyl diselenides on lipid peroxidation. *Environ Res* 94, 276–82.

[16] Moretto, M.B., Funchal, C., Santos, A.Q., Gottfried, C., Boff, B., Zeni, G., Pureur, R.P., Souza, D.O., Wofchuk, S., Rocha, J.B.T., 2005. Ebselen protects glutamate uptake inhibition caused by methyl mercury but does not by Hg^{2+} , *Toxicol.* 214, 57–66.

[17] Muges, G., Du Mont, W.W., Sies, H., 2001. Chemistry of biologically important synthetic organoselenium compounds. *Chem. Rev.* 101, 2125–2179.

- [18] Muller, A., Cadenas, E., Graf, P., Sies, H., 1984. A novel biologically active seleno-organic compound. I. Glutathione peroxidase-like activity in vitro and antioxidant capacity of PZ 51 (Ebselen). *Biochem Pharmacol.* 33, 3235 – 9.
- [19] Nogueira, C.W., Zeni, G., Rocha, J.B.T., 2004. Organoselenium and organotellurium compounds: toxicology and pharmacology. *Chem. Rev.* 104, 6255–85.
- [20] Ogunmoyole, T., Rocha, J.B.T., Okoronkwo, A.E., Kade, I.J., 2009. Altered pH homeostasis modulates the glutathione peroxidase mimics and other antioxidant properties of diphenyl diselenide. *Chem. Biol. Interac.* 182, 106–111.
- [21] Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 95, 351–358.
- [22] Paulmier, C., 1986. Synthesis and properties of selenides, in: J.E. Baldwin (Ed.), *Selenium reagents and Intermediates. Organic Synthesis*, Pergamon Press, Oxford, pp. 84–116.
- [23] Porciuncula, L.O., Rocha, J.B.T., Boeck, C.R., Vendite, D., Souza, D.O., 2001. Ebselen prevents excitotoxicity provoked by glutamate in rat cerebellar granule neurons. *Neurosci. Lett.* 299, 217–220.
- [24] Posser, T., Moretto, M.B., Dafre, A.L., Farina, M., Rocha, J.B.T., Nogueira, G. Zeni, C.W., Ferreira Jdos, S., Leal, R.B., Franco, J.L., 2006. Antioxidant effect of diphenyl diselenide against sodium nitroprusside (SNP) induced lipid peroxidation in human platelets and erythrocyte membranes: an in vitro evaluation. *Chem. Biol. Interact.* 164, 126–135.

- [25] Rossato, J.I., Ketzer, L.A., Centuriao, F.B., Silva, S.J., Ludtke, D.S., Zeni, G., Braga, A.L., Rubin, M.A., Rocha, J.B.T., 2002b. Antioxidant properties of new chalcogenides against lipid peroxidation in rat brain. *Neurochem. Res.* 27, 297–303.
- [26] Rossato, J.I., Zeni, G., Mello, C.F., Rubin, M.A., Rocha, J.B.T., 2002a. Ebselen blocks the quinolinic acid-induced production of thiobarbituric acid reactive species but does not prevent the behavioral alterations produced by intra-striatal quinolinic acid administration in the rat. *Neurosci. Lett.* 318, 137–140.
- [27] Saito, I., Asano, T., Sano, K., 1998. Neuroprotective effect of an antioxidant, ebselen, in patients with delayed neurological deficits after aneurismal subarachnoid hemorrhage. *Neurosurgery* 42, 269–77.
- [28] Santos, F.W., Zeni, G., Rocha, J.B.T., do Nascimento, P.C., Marques, M.S., Nogueira, C.W., 2005b. Efficacy of 2, 3-dimercapto-1-propanesulfonic acid (DMPS) and diphenyl diselenide on cadmium-induced testicular damage in mice. *Food Chem. Toxicol.* 43, 1723–1730.
- [29] Santos, F.W., Zeni, G., Rocha, J.B.T., Weis, S.N., Fachineto, J.M., Favero, A.M., Nogueira, C.W., 2005a. Diphenyl diselenide reverses cadmium-induced oxidative damage on mice tissues. *Chem. Biol. Interact.* 151, 159–165.
- [30] Sohal, R.S., Allen, R.G., 1990. Oxidative stress as a causal factor in differentiation and aging: a unifying hypothesis. *Exp. Gerontol.* 25, 499–522.
- [31] Selvan R., and BijiKurien, 1987. Induction of lipid peroxidation by oxalate in experimental rat urolithiasis. *J. Biosci.* 12, 367-373

- [32] Takasago, T., Peters, E.E., Graham, D.I., Masayasu, H., Macrae, I.M., 1997. Neuroprotective efficacy of ebselen, an anti-oxidant with anti-inflammatory actions, in a rodent model of permanent middle cerebral artery occlusion. *Br. J. Pharmacol.* 122, 1251–1256.
- [33] Tolmasoff, J.M., Ono, T., Cutler, R.G., 1980. Superoxide dismutase: correlation with life-span and specific metabolic rate in primate species. *Proc. Natl. Acad. Sci. USA.* 77, 2777–2781.
- [34] Wilson, S.R., Zucker, P.A., Huang, R.R.C., Spector, A., 1989. Development of synthetic compounds with glutathione peroxidase activity, *J. Am. Chem Soc.* 111, 5936–9.
- [35] Woltjer, R.L., Nghiem, W., Maezawa, I., Milatovic, D., Vaisar, T., Montine, K.S., Montine, T.J., 2005. Role of glutathione in intracellular amyloid-alpha precursor protein/carboxy-terminal fragment aggregation and associated cytotoxicity. *J. Neurochem.* 93, 1047–1056.
- [36] Yamaguchi, T., Sano, K., Takakura, K., 1998. Ebselen in acute ischemic stroke: a placebo-controlled, double-blind clinical trial. *Stroke* 29, 12–7.