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Comparative study of the antioxidant properties of an organic and inorganic selenium compound

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ABSTRACT

Compounds of selenium have been associated with potent antioxidant properties. Hence, the present study is aimed at comparing the antioxidant properties of two selenium compounds - diphenyl diselenide (DPDS) and selenium dioxide (SeO_2). This was done by measuring their free radical scavenging ability, ferric reducing and Fe (II) chelating properties. Moreover their inhibitory effect against prooxidant - induced lipid peroxidation was also determined in the brain and liver homogenate of rat in a simulated hyperglycemia model in vitro. Their effect on lipid peroxidation was further tested by pre-incubating and post incubating the selenium compounds with hepatic tissues in the presence of the prooxidants. Results showed that none of the two compounds scavenged DPPH radicals and chelate Fe (II). Meanwhile, when pre – incubated, DPDS markedly reduced Fe^{3+}, and demonstrated potent and concentration dependent inhibitory effect against lipid peroxidation regardless of the prooxidant causing oxidative assault. Furthermore, the potent inhibitory effect of DPDS against lipid peroxidation was not altered in the presence of high glucose concentration but was lost on post incubation. On the other hand, selenium dioxide did not show any significant activity in all antioxidant parameters determined. From the foregoing, DPDS is a potent antioxidant while selenium dioxide is not. This observation may explain why organic selenium compounds are better antioxidants than their inorganic counterparts. Hence, research efforts should be tailored towards the discovery and characterization of organic selenium compounds that could be exploited as panacea to free radical menace.

KEYWORDS

Selenium dioxide;
Diphenyl diselenide;
Hyperglycemia;
Organic;
Incubation.

INTRODUCTION

Interest in selenium (Se) has escalated in the past two decades and the reason is not farfetched. Se is a trace micronutrient having important benefits for higher animals, and particularly for mammals due to its catalytic role in a variety of enzymes that contain selenocysteine residues as part of their active site[i]. Its deficiency has

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been linked to epileptic seizures and may even contribute to Parkinson's disease. The biological importance of selenium and its inorganic forms led to the development of pharmacologically active organoselenium (OS) compounds with low toxicity, since the selenium atom was not delivered to the intracellular selenium pool[2,3]. Meanwhile, reports have shown that selenium-containing organic compounds are generally more potent antioxidants than classical antioxidants and this fact serves as the basis for an increased interest in the rational design of synthetic organoselenium compounds[1,4]. Specifically, Diphenyl diselenide (DPDS) has been shown to exhibit antiulcerogenic, anti inflammatory and antidiabetic properties among others. On the other hand, inorganic selenium has also been shown to exhibit a number of potentially beneficial effects against the development of several degenerative diseases[5] including diabetes mellitus[6]. In fact, literature data have indicated that inorganic selenium compounds can attenuate cytotoxic effects of hyperglycemia via its insulin-mimetic and anti-glycating properties[7] as well as exhibit protective effects against cardiovascular disease, modulation of platelet aggregation and protection against toxic heavy metals and lipid peroxidation. However, literatures seem scanty on the antioxidant properties of selenium dioxide. Hence, there is need to investigate its possible antioxidant properties and compare same with an organoselenium compound (DPDS).

Meanwhile, free radical overproduction has been implicated in the etiology of a variety of acute and chronic degenerative diseases[8-10]. However, clinically effective drugs for the treatment of these diseases are rare. Consequently, continued efforts geared towards the development and biological testing of new antioxidant compounds for the treatment of these degenerative disorders have increased considerably in recent times[11-14]. Hence, there is the need to compare the antioxidant activity of both organic (DPDS) and inorganic (selenium dioxide) compounds of selenium with a view to identifying which of them would exhibit greater antioxidant properties that could be exploited for therapeutic purposes. Keeping the above views in mind, the present study sought to compare the antioxidant properties of selenium dioxide and DPDS.

MATERIALS AND METHODS

Chemicals

Selenium dioxide and thiobarbituric acid (TBA) were obtained from Sigma (St. Louis, MO). DPDS was synthesized according to literature methods[15]. Analysis of ^{1}HNMR and ^{13}C 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.

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^{0}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, Federal University of Technology, Akure.

Reducing property

The ability of Selenium dioxide (SeO_2) and DPDS to reduce $FeCl_3$ solution were determined as described by[16]. 250µl of Selenium dioxide and DPDS (10 - 100µM) was mixed with 250µl 200mM Sodium phosphate buffer (pH 6.6) and 250µl of 1% (w/v) Potassium ferrocyanide. The mixture was incubated at 50^{0}C for 20 min. Thereafter 250µl, (10% v/v) Perchloric acid (PCA) was added and subsequently centrifuged at 650 rpm for 10 min. 250µl of the supernatant was mixed with equal volume of water and 100µl of 0.1% (w/v) Ferric chloride. The absorbance was later measured at 700 nm, a higher absorbance indicates a higher reducing power.

Free radical scavenging ability

Free radical scavenging ability of Selenium dioxide and DPDS against DPPH (2, 2 –diphenyl -1-picrylhydrazyl) radical was determined according to[17]. Exactly 600µl of SeO_2/DPDS (10-100µM) was mixed with 600µl 0.4mM of DPPH in methanol. The mixture was left in the dark for 30 min before measuring the absorbance at 516 nm.

Fe^{2+} chelating assay

Fe^{2+} chelating property of $Se0_2$ and DPDS was determined using a modified method of[18]. Freshly prepared 500µmol/L $FeSO_4$ (150µl) was added to a reaction mixture containing 168µl of 0.1 mol/l Tris-HCl (pH 7.4), 218µl saline and $Se0_2$/DPDS (10 - 100µM) separately. The reaction mixture was incubated for 5 min, before the addition of 13µL of 0.25% (w/v) 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510nm. The Fe (II) chelating ability was subsequently calculated with respect to the sample blank (which contains all the reagents without samples).

Hydroxyl radical scavenging property (Deoxyribose degradation)

Hydroxyl radical scavenging activity of Selenium dioxide and DPDS was assessed by the method of[19]. Deoxyribose is degraded by hydroxyl radicals with the release of thiobarbituric acid (TBA) reactive substances. Deoxyribose (3mM) was incubated at 37^0C for 30 min with 50mM potassium phosphate (pH 7.4) along with ferrous sulphate (0.1mM) and/or H_2O_2 (1mM) to induce deoxyribose degradation. Thereafter, selenium dioxide and DPDS (10-100µM) were added separately. After incubation, 0.4 ml of TBA (0.8% (w/v) and 0.8 ml of TCA 2.8% were added, and the resulting mixture was heated for 20 min at 100^0C, allowed to cool and absorbance measured at 532 nm.

Tissue preparation

Rats were decapitated by mild ether anesthesia liver was rapidly removed, placed on ice and weighed. Tissues were immediately homogenized in cold 50 mM Tris–HCl, pH 7.4 (1/10, w/v). The homogenate was centrifuged for 10 min at 4,000g to yield a pellet that was discarded and a low-speed supernatant.

Thiobarbituric acid reactive species (TBARS) assay

100µl aliquots of supernatant were incubated for 1h at 37^0C with either Selenium dioxide or DPDS (10-100µM) with and without the prooxidants; iron (final concentration (10µM) and sodium nitroprusside (SNP) (final concentration 20µM). Productions of TBARS were determined as described by[20] except that the buffer of colored reaction had a pH of 3.4. The color reaction was developed by adding 300 µl 8.1% SDS to the reaction mixture, 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 95^0C for 1 h. TBARS produced were measured at 532 nm. To better explore the antioxidant effects of the two compounds, each was then post incubated (added to the reaction mixture after the first 1hour incubation at 37^0C). Finally, another parallel assay was carried as earlier stated except that there was a high glucose concentration (10mM) in the assay mixture.

Statistical analysis

All values obtained were expressed as mean ± 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 $p<0.05$.

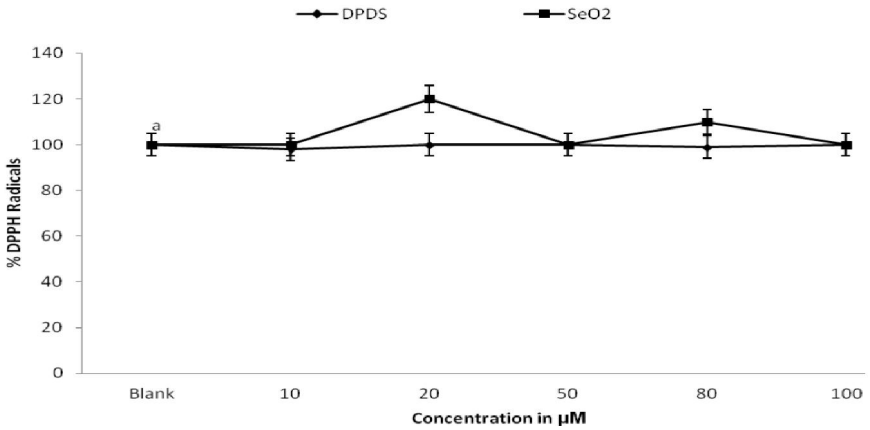

Figure 1: Free radical scavenging property of DPDS and SeO_2. Data show means ± SEM values averages of 4 independent biological replicates performed in triplicate. 'a' indicates the control at $P < 0.05$.

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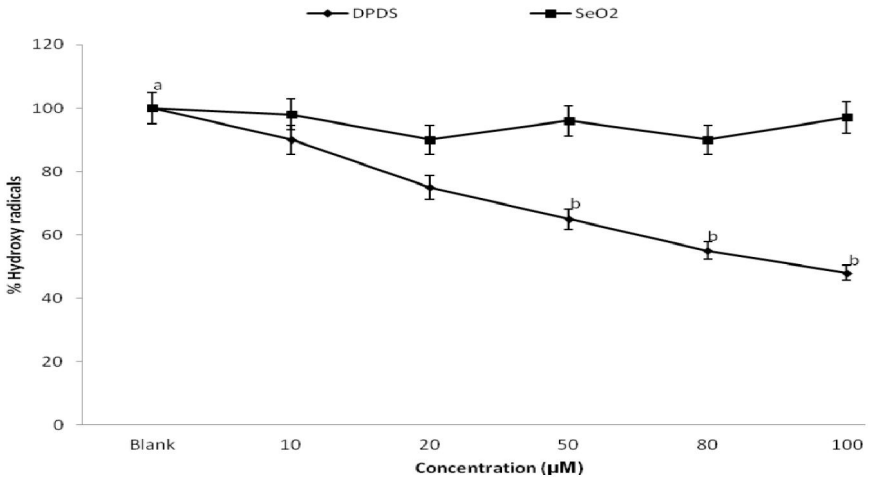

Figure 2 : Hydroxyl radical scavenging property of DPDS and SeO_2. Data represents means ± SEM values averages of 4 independent biological replicates performed in triplicate. 'a' indicates the control while 'b' represents significant difference from 'a' at P < 0.05.

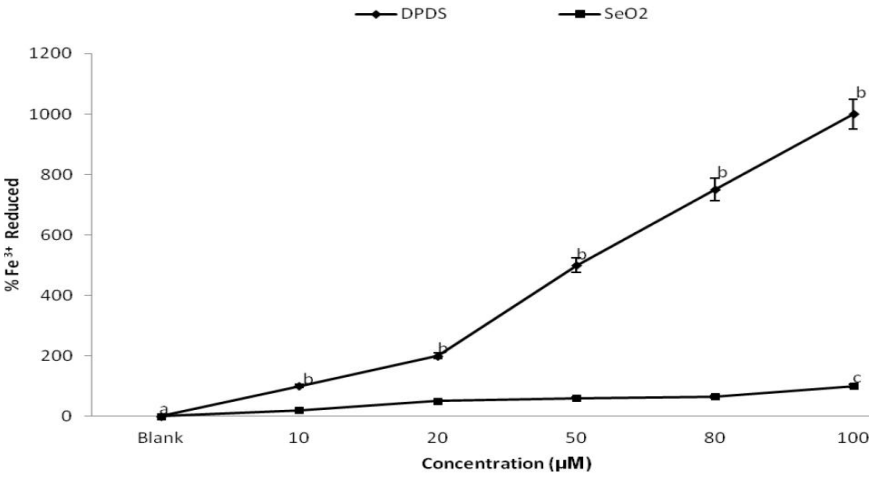

Figure 3 : Ferric Reducing Property of DPDS and SeO_2. Data show means ± SEM values averages of 4 independent biological replicates performed in triplicate. 'a' indicates the control while 'b' and 'c' represent significant difference from 'a' at P < 0.05.

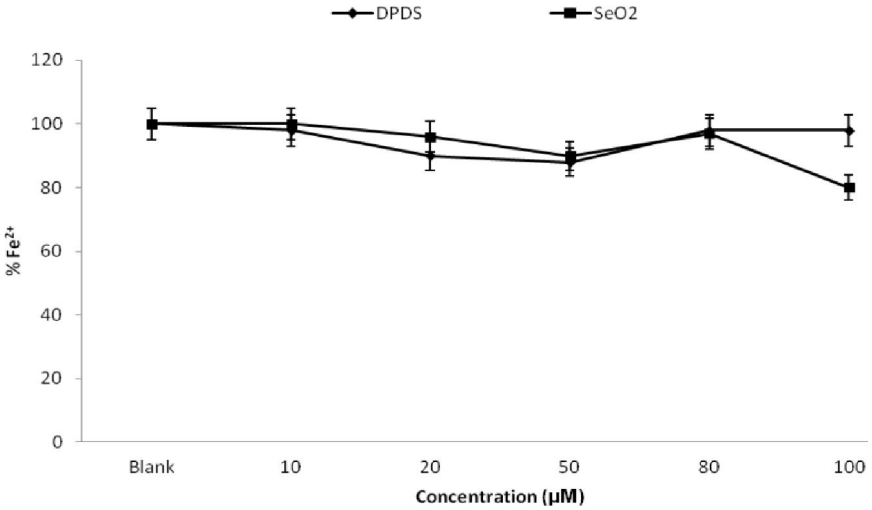

Figure 4 : Iron (II) – chelating property of DPDS and SeO_2. Data show means ± SEM values averages of 4 independent biological replicates performed in triplicate. 'a' indicates the control at P < 0.05.

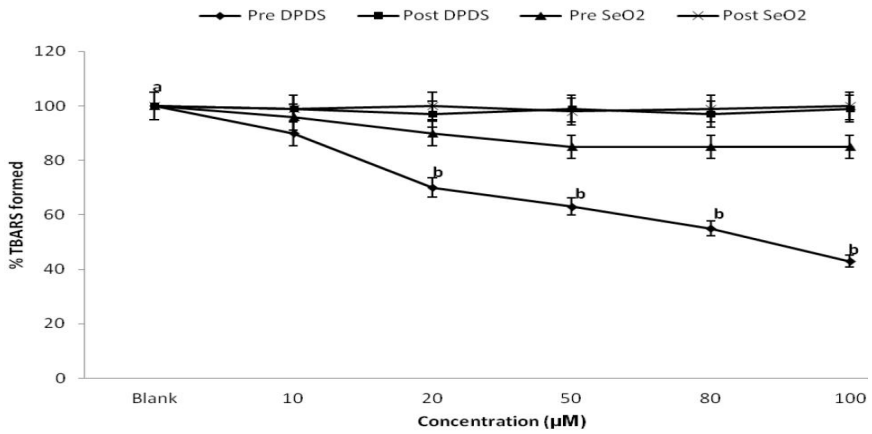

Figure 5a : Inhibitory effects of DPDS and SeO_2 on Fe (II) - induced hepatic lipid peroxidation when pre-incubated (pre DPDS/Pre SeO_2) or Post incubated (post DPDS/post SeO_2) with tissue homogenate with tissue homogenate. Data show means ± SEM values averages of 4 independent biological replicates performed in triplicate. 'a' indicates the control while 'b' represents significant difference from 'a' at P < 0.05.

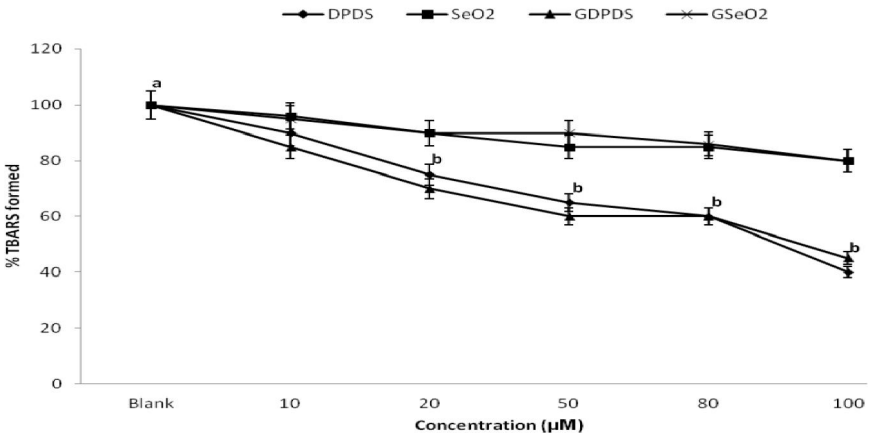

Figure 5b : Inhibitory effects of DPDS and SeO_2 on Fe (II) - induced hepatic lipid peroxidation in the presence (GDPDS/ $GSeO_2$) and absence (DPDS/SeO_2) of high glucose concentration (10mM). Data show means ± SEM values averages of 4 independent biological replicates performed in triplicate. 'a' indicates the control while 'b' represents significant difference from 'a' at P < 0.05.

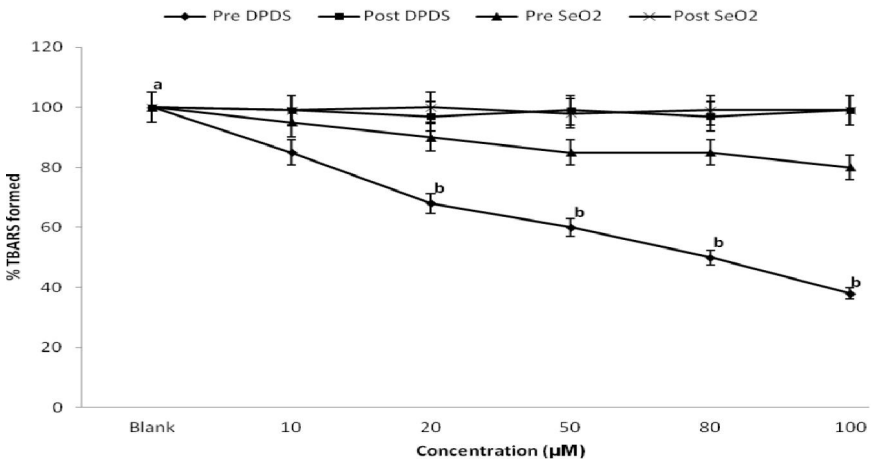

Figure 5c : Inhibitory effects of DPDS and SeO_2 on SNP - induced hepatic lipid peroxidation when pre-incubated (Pre DPDS/ Pre SeO_2) or Post incubated (Post DPDS/post SeO_2) with tissue homogenate. Data show means ± SEM values averages of 4 independent biological replicates performed in triplicate. 'a' indicates the control while 'b' represents significant difference from 'a' at P < 0.05.

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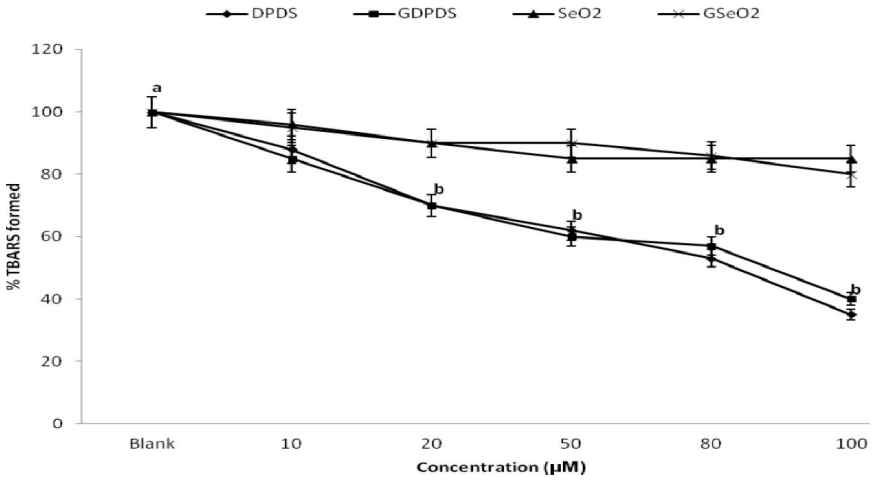

Figure 5d : Inhibitory effects of DPDS and SeO_2 on SNP - induced hepatic lipid peroxidation in the presence (GDPDS/ $GSeO_2$) and absence (DPDS/SeO_2) of high glucose concentration (10mM). Data show means ± SEM values averages of 4 independent biological replicates performed in triplicate. 'a' indicates the control while 'b' represents significant difference from 'a' at P < 0.05.

RESULTS & DISCUSSION

Since the overproduction free radicals have been implicated in the etiology of diseases, research efforts have been directed at the discovery of agents that could help in the management of degenerative diseases[21,22]. Interestingly, selenium has been known as potent antioxidant as it plays a critical role in the physiological system. However, since, selenium occurs both in organic and inorganic forms, it is pertinent to compare their antioxidant property and provide logical reason for any difference in activity between the compounds.

Free radical scavenging activity has been adopted as an index of antioxidant strength of agents. DPPH, an unstable diamagnetic magnetic molecule becomes stable following the addition of antioxidant, changing from its purple colour to golden yellow which can be visually observed and read spectrophotometrically. However, the addition of DPDS and SeO_2 to DPPH solution did not cause any bleaching of its deep purple colour showing that the mechanism involved in the antioxidant properties of the two compounds did not involve free radical scavenging (Figure 1). This could suggest a number of things. Since, the mechanism involved in the bleaching of the purple colour is the donation of proton from the antioxidant agent to the unstable DPPH radical which would eventually result in its stability which is visually noticeable as a discoloration. It shows that both compounds lack the ability to donate protons to the free radical hence their inability to scavenge DPPH radicals. However, the fact that both compounds did not scavenge DPPH radicals does not imply that they are not potent antioxidants. Hence, other antioxidant parameters were also employed to determine their individual antioxidant capacity. Hydroxyl radicals are produced from an interaction between hydrogen peroxide and iron (II) via Fenton reaction. These radicals are highly deleterious and could be detrimental when they attack critical macromolecules. For instance, when deoxyribose- a component of DNA is incubated in the presence of Fe (II) and peroxide, it undergoes degradation producing spoiled products of oxidation. Hence, the ability of agents/substances to protect against these radicals has been used as a measure of its antioxidant strength. Unfortunately, neither DPDS nor SeO_2 scavenged hydroxyl radical (Figure 2), an indication that the mechanism of antioxidant activity of both compounds does not involve hydroxyl radical scavenging. Despite the poor radical scavenging activity of both selenium compounds, they may still exhibit potent antioxidant activity via other known mechanisms. Hence, other antioxidant parameters were investigated.

Reducing power is considered a defense mechanism which is related to the ability of the antioxidant agents to transfer electron or hydrogen atom to oxidants or free radicals. The reducing power of the two selenium compounds was evaluated based on their ability to reduce Fe^{3+} to Fe^{2+}. Interestingly, DPDS demonstrated marked, concentration dependent ferric reducing power even at the least concentration of DPDS tested (Figure

3). On the other hand, SeO_2 showed an insignificant ferric reducing effect except at the highest concentration used. This observation could imply that the potent antioxidant property of that has been reported may be intricately linked to its ferric reducing effect. Consequently, the antihyperglycemic, anti inflammatory, and anti carcinogenic properties of DPDS that has been reported may be partly due to its potent reducing power. While, the poor reducing power as compared to DPDS may suggest that organic selenium compounds may be better antioxidants than inorganic probably due to their organic moiety which is not found with their inorganic counterparts.

The ability of agents to chelate and deactivate transition metals is generally regarded as an antioxidant mechanism to prevent oxidative assault on biological macromolecules such as lipids, proteins and nucleic acids. The result of the Fe (II) chelating ability of DPDS is presented in Figure 4. It could be observed that, in similarity with the result obtained for radical scavenging, both DPDS and SeO_2 do not have any significant transition metal (in this case Fe) chelating ability presumably due to a similar reason.

Furthermore, antioxidants can act by preventing oxidative assault to polyunsaturated lipid which serves as pivot of membrane integrity. Since free radical assaults, if kept unchecked, would result to diseases[23,24] antioxidants could be assessed *in vitro* by their ability to offer protective shields to lipids intentionally assaulted with prooxidants such as Fe^{2+}, SNP and H_2O_2.

Meanwhile, the use of Fe^{2+} as prooxidant is due to the fact it can catalyze one-electron transfer reactions that generate reactive oxygen species (ROS), such as the reactive OH radical. Interestingly, DPDS was able to inhibit TBARS formation in hepatic lipids placed under Fe^{2+} assault. This observation may be tightly linked to its potent reducing power observed in Figure 3.0. Since, Fe^{2+} must be oxidized before eliciting its proxidative reaction, and DPDS is potent reductant, it must have engaged its reductive ability against the oxidative effect of Fe^{2+} thereby shielding hepatic lipids from free radical attack consequently preventing TBARS formation. On the other hand, other hand, SeO_2 could not inhibit TBARS formation even at the highest concentration tested. From the foregoing, it is apparently obvious that organic

Moreover, sodium nitroprusside (SNP) have been reported to elicit cytotoxic effect through the release of nitric oxide (NO) (via a photo-catalytic reaction process)[25,26], which has been implicated in the pathophysiology of strokes, traumas, seizures and Alzheimer's, and Parkinson's diseases[27,29]. After the release of NO, the iron moiety may react with SNP, which could lead to the formation of highly reactive oxygen species, such as hydroxyl radicals via the Fenton reaction[30]. DPDS exhibited a significant inhibitory effect against the formation of TBARS in hepatic lipid placed under oxidative assault of SNP. Meanwhile, SeO_2 did not prevent TBARS formation at all concentrations tested indicating that DPDS is a better, more potent antioxidant than 'selenium dioxide. Worthy of note is the fact that even in the presence of high glucose concentration (hyperglycemic condition), its inhibitory effect was not distorted (Figure 5b & d). This observation may imply that the DPDS could act as antidiabetic agent since its antioxidant activity is not perturbed by extremely high glucose concentration. This may partly explain its earlier reported antihyperglycemic property[31]. It is pertinent to mention that apart from the reductive power of DPDS which may be responsible for its inhibitory effect against lipid peroxidation, reports have shown that it could mimic the endogenous antioxidant enzyme, glutathione peroxidase (GPx) via its GPx mimetic ability. Hence, its potent inhibitory effects against TBARS formation may not exclude its GPx mimetic antioxidant mechanism. Meanwhile, when DPDS was post-incubated (added to the assay mixture after the first incubation at 37°C/1 hour after the addition of prooxidant) its inhibitory effect was lost (Figure 5a & c). The reason for this observation is not farfetched. Lipid peroxidation process must have been completed after the first round of incubation with prooxidant; hence, the addition of DPDS cannot reverse the process since it cannot react with the already formed aldehydic products of lipid peroxidation.

From the foregoing, DPDS is a potent antioxidant while selenium dioxide is not. Moreover, organic selenium compounds may be considered better antioxidants than the inorganic forms. Although, further work is needed to establish this speculation, earlier reports have not only shown that synthetic

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organoselenium compounds are better antioxidant than the classical antioxidants already known, but that they are relatively non toxic when compared to inorganic selenium compounds. This may explain in part, why DPDS exhibited more potent antioxidant activity than SeO_2 in all antioxidant indices determined. Hence, more efforts should be tailored towards exploiting organoselenium compounds for therapeutic purposes while exploring the selenium world for the discovery of inorganic selenium compounds that would exhibit similar if not better antioxidant potency than the organoselenium compounds.

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