Biochemical Mechanism of Diphenyl Diselenide on *Escherichia coli:* a Possible Involvement on Sulphydryl-containing protein *in vitro and in vivo study*

Adetuyi Aanuoluwapo, Bello Maryam, Ayeni Tolulope
Departament of Biochemistry, Federal University of Technology Akure,
Akure, Ondo State, Nigeria
*Corresponding Author: adetuyiar@futa.edu.ng

***Abstract*—The biochemical mechanisms underlying the antibacterial action of DPDSe was elucidated through, the *in-vitro* sensitivity of *Escherichia coli* (*E. coli*) and *in-vivo* assays. The minimum inhibitory concentration and minimum bactericidal concentrations [MIC and MBC respectively] of DPDSe on the growth of the bacterium as well as the effect of various mono-[cysteine (CYS), and reduced glutathione (GSH)] and dithiols [dithiothreitol] and thiol modifiers; on the antibacterial action of DPDSe were carried out. Albino rat were infected with *E. coli* and treated with DPDSe, The activities of delta aminolevulinic acid dehydratase (ALA-D) and Na+/K+-ATPase were also tested. The results indicated that DPDSe exerted antibacterial action against *E. coli* with MIC and MBC of 100 µM and 1000 µM respectively. Mono and dithiols (1Mm and 2Mm) which co-incubated with DPDSe profoundly prevented and reversed the inhibitory effect of DPDSe on the growth of *E.coli*. *In-vivo* results show how DPDSe was able to reverse the infection effect on the sulfhydryl enzymes. Apparently, the non-selective target of DPDSe is in accordance with the thiols modifiers on *E.coli*. Thus, this organoselenium would be a potent candidate in the management of antibiotic-resistant strains of *E.coli*.**

Keywords—Diphenyl Diselenide; Escherichia coli; Organoselenium; Thiols.

I. INTRODUCTION

The abusive and indiscriminate use of antimicrobial drugs over the years is the principal factor responsible for the appearance of bacterial resistance [1, 2]. This reason imposes severe impediments on therapeutic choices, suggesting a threat to public health. Consequently, considering the galactic development of antibiotic-resistant strain of bacterial, there is need to discover novel antimicrobial agents with new modes of action.

In the last recent couple of decades, investigation of selenium chemistry and biochemistry has expanded because of the fact that elemental selenium is fundamental to human, as it is involved in various redox equilibrium and redox processes in living systems [3, 4]. However, the ingestion of dietary sources of selenium, such as selenomethionine, selenocysteine and inorganic selenium is resisted in terms of the toxicity [5]. Hence, a number of synthetic organoselenium derivatives have been developed [6, 7] and reported as pharmacological agents. In fact, antioxidant, enzyme inhibitors, neuroprotective, anti-tumor, anti-infective, immunomodulators and antifungal activities have been demonstrated for organoselenium derivatives [8, 9, 10].

Diphenyl diselenide (DPDSe) is a new organoselenium compound with promising biological properties and uses which have been reported for remedial purposes [11, 12]. It has been proven to be an antioxidant compound in rat brain homogenate *in-vitro* [6]. Conversely, DPDSe at high doses has been reported as a pro-oxidant compound [12]. The pro-oxidant activity of DPDSe could decrease non-enzymatic and enzymatic cell resistances and cause cell damage by the production of Reactive Oxygen Species (ROS) [14]. ROS, such as superoxide anion (O_2^-), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2), are usually produced during aerobic metabolism [15] which are involved in diverse cellular processes of cell death [7].

Deserving attention is the toxicity of the organoselenium, which originates from the fact that it does not exhibit strict fidelity toward Reduced Glutathione (GSH) for their Glutathione Peroxidase (GPx) mimicry. It is a known fact that Diphenyl diselenide can utilize a wide variety of thiols, this thereby possess adverse effect on some proteins (enzymes) that have cysteine residue(s) at their catalytic site. Thus, Diphenyl diselenide can lead to loss of biological functions and possibly the death of an organism [16]. In light of these foundations, this study revealed the biochemical mechanism of diphenyl diselenide on *Escherichia coli,* a possible involvement on Sulphydryl-containing protein in *in-vitro* and *in-vivo.*

II. MATERIALS AND METHODS

- Chemicals

Diphenyl diselenide, iodoacetamide, dithiothreitol (DTT), diamide (DA), reduced glutathione (GSH), cysteine, were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals, which

were of analytical grade, were obtained from standard commercial suppliers.

- Microoganism

Clinical isolates of *Escherichia coli* (*E.coli*) was obtained from Patient positive to *E.coli* infection at Ekiti State University Teaching Hospita, Ado-Ekiti, Nigeria. The identity was confirmed at the Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria.

- Animals

Male adult Wistar rats (120–150 g) were used for the entire experiments. Animals were housed in separate plastic cages on natural light (about 12 hours) and dark (about 12 hours) cycle at a room temperature 25-350C. The animals were allowed free access to food and water. The animals were used according to the standard guidelines of the committee on care and use of experimental animal resources.

- Escherichia coli induction

Albino rats were orograstically dosed with Escherichia coli cells previously diluted in sterile distilled water. Control rats received an equivalent amount of sterile distilled water. Infection state was checked 72 h after induction with Escherichia coli. Animals weight, temperature and stool samples were taken and used to determine infection levels.

- Treatment

The animals were divided randomly into the following groups six rat per group: (group 1) control; (group 2) DPDSe; (group 3) Escherichia coli infected and (group 4) infected + DPDSe. Groups 2 and 4 were administered DPDSe by gavage at a dose of 10 mg/kg (once/day) for 7 days after the establishment infection. The organoselenium compound was pre-dissolved in soya bean oil. Control rats were also administered soya bean oil. At the end of the experimental period, infected rats and the corresponding control animals were fasted 12 h prior to euthanasia.

- Preparation of Culture Media

Mullerhilton broth (3.8 g) was dissolved in 100 ml of water through heating and was sterilized in an autoclave at 121^0C for 15 min which was then used for the culture of *E.coli* within 24 h [17].

A. Agar Diffusion Test to Determine Susceptibility of E.coli to Diphenyl Diselenide

The susceptibility of *E.coli* to Diphenyl diselenide was investigated using the well variant of the agar diffusion method described by [18]. With the aid of a sterile 6mm cork borer, 4 equally spaced holes were bored in the agar plate. The agar plugs were discarded using a sterile needle. One hundred microlitres (100 µL) of Diphenyl diselenide was then introduced into each of the 3 wells while the last well was filled with an equal volume of ethanol to serve as control. The plates were incubated for 24 hours at 37°C in an upright position. They were then examined for zones of inhibition which indicate the degree of susceptibility or resistance of the test organism to the antibacterial agent. The test was carried out in duplicates and the average of three (3) readings were taken as the zone of inhibition in each case. Inhibition zones were measured with the aid of a ruler in millimeters (mm).

- Determination of Minimum Inhibitory Concentration (MIC)

MIC is the lowest concentration of a specific antimicrobial needed to prevent the growth of a given organism in-vitro [20] Various concentrations of the DPDSe was prepared in increasing order (10 µM, 100 µM, 1000 µM). 100 µl of each DPDSe concentration was introduced into each agar diffusion respectively. The plates were incubated for 18- 24 hours and examined for visible zone of inhibition. The DPDSe concentration at which no visible growth was observed when compared with the controls was regarded as the MIC.

- Determination of Minimum Bactericidal Concentration (MBC)

MBC is the lowest concentration of a specific antimicrobial that kills 99.9% of cells of a given bacterial strain [19]. MBC was determined by assaying for live organisms in the plate from the MIC tests which showed no visible zone of inhibition. A loopful of inoculum from the MIC plate was streaked on fresh nutrient agar plates without DPDSe incorporated into them. The plates were incubated at 37°C for 24 hours after which they were observed for growth. Absence of growth indicated a bactericidal effect of the concentration which is the MBC.

B. Thiol Oxidation Assay (GSH, DTT and CYS assay)

Free -SH (thiols) groups was determined following [18] agar well diffusion method [18]. The rate of individual thiol [dithiothreitol (DTT), glutathione (GSH) and cysteine (CYS)] oxidation was determined in the presence of DPDSe (1000 µM), Iodoacetamide (5 mM) and Diamide (5-10 mM), varying concentration of individual thiols (1-2 mM) and one hundred microlitres (100 µL) of each concentration were added to the wells under various condition. The rate of thiol oxidation was evaluated by measuring the zone of inhibition according to [18].

- DPDSe interaction with thiols under two conditions of incubation

Condition One: The co-incubation of Diphenyl diselenide (DPDSe) with individual thiol within 24 and 48 hours.

Condition Two: Diphenyl diselenide (DPDSe) was incubated with *E. coli* first within 24 hours, thereafter individual thiols were added and incubated for further 24 hours to check the effect on the diameter of the zone of inhibition produced by DPDSe.

- Effect of co-incubation of thiols oxidizing agent with individual thiol under three different conditions**.**

Condition One: The co-incubation of Iodoacetamide (IA) with individual thiol within 24 and 48 hours.

Condition Two: Individual thiols (CYS, GSH and DTT) were incubated first within 24 hours, thereafter Iodoacetamide (IA) was added and incubated for 48 hours.

Condition Three: Iodoacetamide (IA) was incubated first within 24 h, thereafter individual thiols were added and incubated for 48 hours to check the effect on the diameter of the zone of inhibition produced by DPDSe.

C. *Preparation of tissue homogenate*

Animals were anaesthetized with mild ether and euthanized by decapitation. Consequently, the liver, kidney and brain were removed, quickly placed on ice and washed in appropriate buffer solution containing protease inhibitors and homogenized (1:10 weight/volume) in cold 50 mM Tris–HCl buffer (pH 7.4) at 4ºC. The homogenates were centrifuged at 4,000 rpm for 10 minutes to yield a low-speed supernatant (S1). The homogenate was used for the antioxidant assay and enzymatic assay.

- Thiobarbituric acid reactive species (TBARS) assay

The level of thiobarbituric acid reactive species (TBARS) production in the brain, liver and kidney tissue homogenate of an infected rat and DPDSe was determined using the modified method of [20]. 200 µL of SI was introduced into the reaction medium containing Tris HCl buffer of pH 7.4. The colour reaction was developed by adding 200 µL of 8.1% Sodium Dodecyl Sulphate (SDS) to the reaction mixture containing SI, this was subsequently followed by the addition of 500 µL of acetate buffer, pH 3.4, and 500 µL of 0.8% TBA. This mixture was incubated at 100^0C for 30 minutes. TBARS produced was measured at 532 nm in UV-visible spectrophotometer.

- Determination of Reduced Glutathione (GSH) level

GSH level of the cerebral, renal and hepatic tissue homogenate of an infected rat and DPDSe was determined using the method of [21]. 200 µL of SI was incubated at room temperature in reaction medium containing 0.1 M phosphate buffer, pH 7.4. The absorption was read at 412nm after the reaction was terminated with DTNB (5'5' dithio-bis-2-nitrobenzoic acid).

- DPPH Free Radical Scavenging Ability

The free radical scavenging ability of the cerebral tissue against DPPH (1, 1-diphenyl-2 picrylhydrazyl) free radicals were evaluated as described earlier in our laboratory with slight modifications [22]. Briefly, brain tissues homogenates were mixed with 600 µL, 0.3 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 minutes and the absorbance of the resulting golden yellow products was measured at 516 nm.

- Delta-ALAD activity assay

The activities of hepatic delta aminolevulinic acid dehydratase (δ-ALAD) in the rat hepatic tissue homogenate after treatment with DPDSe was determined by measuring the rate of porphobilinogen formation using the modified method of [23]. The reaction medium containing 200 µL of SI was incubated at 370C for 1 hour. After the incubation, the reaction was stopped by the addition of 250 µL of 10% trichloroacetic acid containing 10 mM $HgCl_2$. The reaction product was determined using modified Erhlich's reagent at 555 nm.

- Na^+/K^+ -ATPase activity assay

The sodium-potassium pump (Na^+/K^+-ATPase) activity of the hepatic and renal tissue homogenate after treatment with DPDSe was carried out by measuring released inorganic phosphorus (Pi) by the method described by [24]. The reaction mixture for Mg^{2+}-dependent Na^+/K^+-ATPase activity assay contained 3 mM $MgCl_2$, 125 mM NaCl, 20 mM KCl, 50 mM Tris HCl buffer (pH 7.4) and 50 µL of brain homogenate in a final volume of 500µL. The reaction was initiated by addition of ATP to a final concentration of 3.0mM. Controls were carried out under the same conditions with the addition of 0.1 mM ouabain. The reaction was terminated after about 30 minutes with 250 µL of 10% Trichloroacetic acid containing 10 mM $HgCl_2$. The tubes were centrifuged and 500 µL of the supernatant was correspondingly transferred into new test tubes. Distilled water, ammonium molybdate and freshly prepared vitamin C were sequentially added to all tubes. Tubes were incubated in the dark for about 5-10 minutes. Absorbance was read at 650 nm and Na^+/K^+-ATPase activity was calculated by the difference between the two assays. Enzyme activity was expressed as number of moles of phosphate (Pi) released in min-1mg protein.

D. *Statistical analysis*

All values obtained were expressed as mean ± SEM. Data were analysed statistically by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test where appropriate. All differences with $p < 0.05$ were considered significant.

III. RESULTS AND DISCUSSION

The susceptibility of *E. coli* to diphenyl diselenide in the agar diffusion test (Figure 1) shows no zone of inhibition at low concentration (0-10 µM), while at high concentration (100-1000 µM) there was inhibitory effect. This implied that DPDSe is bacteriostatic at lower concentrations and bactericidal at higher concentrations. Smith *et al*. (1998) and Zhou *et al.* (2015) [25, 26] have suggested this behavior appears to be related to the presence of the outer cell membrane in *E.coli*, which gives greater protection, making them more difficult to be inhibited at lower concentrations. This is not a paradox, it is expected that with antimicrobial agents, as the concentration increases so also the zone of inhibition increases. The

zone of inhibition shows the potency of DPDSe as an antimicrobial agent.

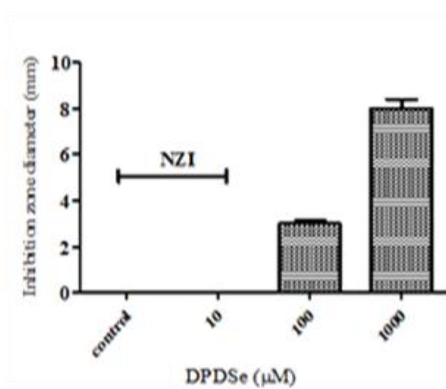

Fig. 1 diphenyl diselenide (dpdse) antimicrobial sensitivity screening on e.coli growth within 24 hours. Nzi represent no zone of inhibition.

Figure 2 result reveals the Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of DPDSe on *E. coli*. The MIC has been described as the lowest concentration of a specific antimicrobial needed to prevent the growth of a given organism, which was 100µM. MBC is the lowest concentration of a specific antimicrobial that kills 99.9% of cells of a given bacterial strain which was 1000µM [19].

Therefore, in order to explore the possible involvement of thiol oxidation as a component of antimicrobial action of DPDSe in *E. coli*, two monothiols- cysteine (CYS), glutathione (GSH) and one dithiol- dithiothreitol (DTT) were co-incubated separately with *E. coli* under two different conditions. In this regard, Figure 3 shows the result obtained when DPDSe was co-incubated with thiols CYS (panel A and B), GSH (panel C and D) and DTT (panel E and F) on the growth of *E. coli* within 24 and 48 hours respectively (condition one). Herein, DPDSe exhibited an inhibitory effect alone but interestingly, its co-incubation with thiols generated a significant reduction in the zone of inhibition.

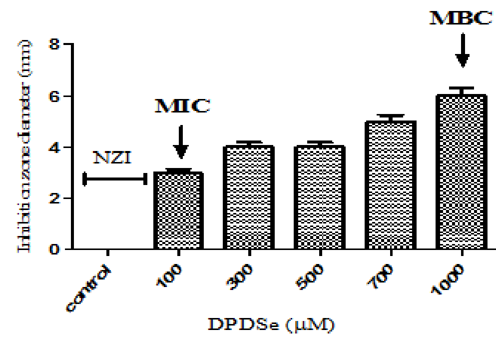

Fig. 2 The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of (DPDSe) Antimicrobial activity on E.coli growth within 24 hours. NZI represent No zone of inhibition.

Also, with time variation there was no significant change in the zone of inhibition. To further confirm the effect of the thiol on the action of DPDSe on the growth *E. coli*. Figure 4 (condition two) shows that the co-incubation of DPDSe with and without CYS (panel A and B), GSH (panel C and D) and DTT (panel E and F) on the diameter of the zone of inhibition produced by DPDSe on *E. coli* growth after 24 hour of incubation. The result revealed that DPDSe exhibited a marked inhibitory effect on the growth of *E. coli* within 24 hours of incubation, [Figure 4 (A, C and E)]. However, the individual thiols substantially reversed the inhibitory effect when incubated further for another 24 hours presented in Figure 4 (B, D and F). The reduction in the zone of inhibitions when co-incubated with thiols in the assay system buttressed the possible involvement of these thiols in the antibiotic mechanism of DPDSe and hence, it may be concluded that DPDSe mechanism of antimicrobial against E.coli is influenced negatively by thiols alteration.

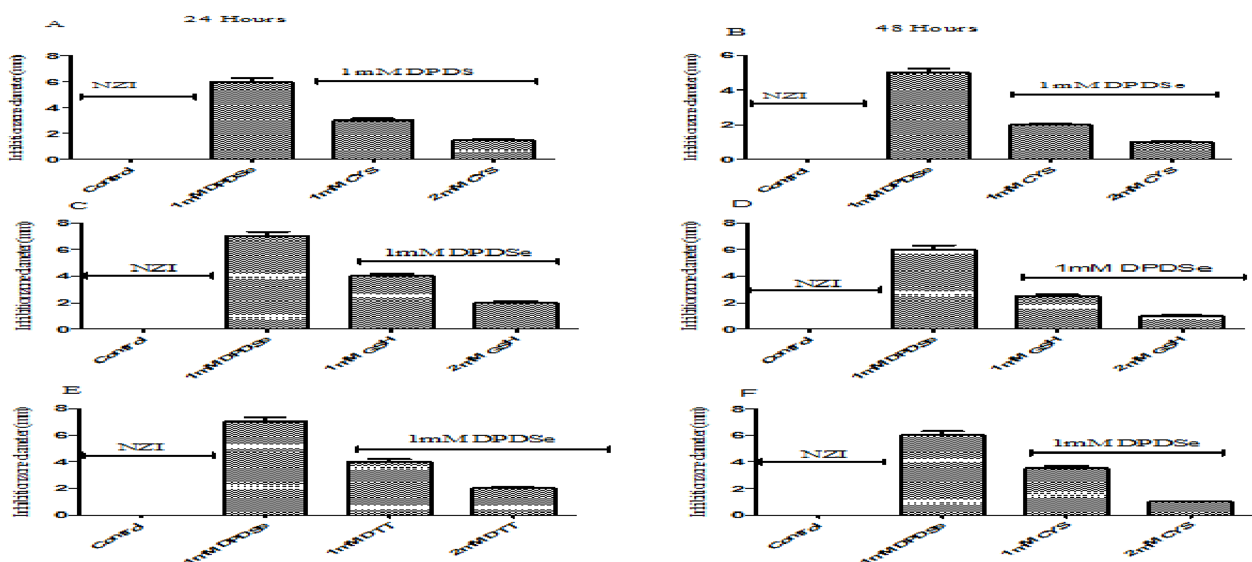

Fig
hou

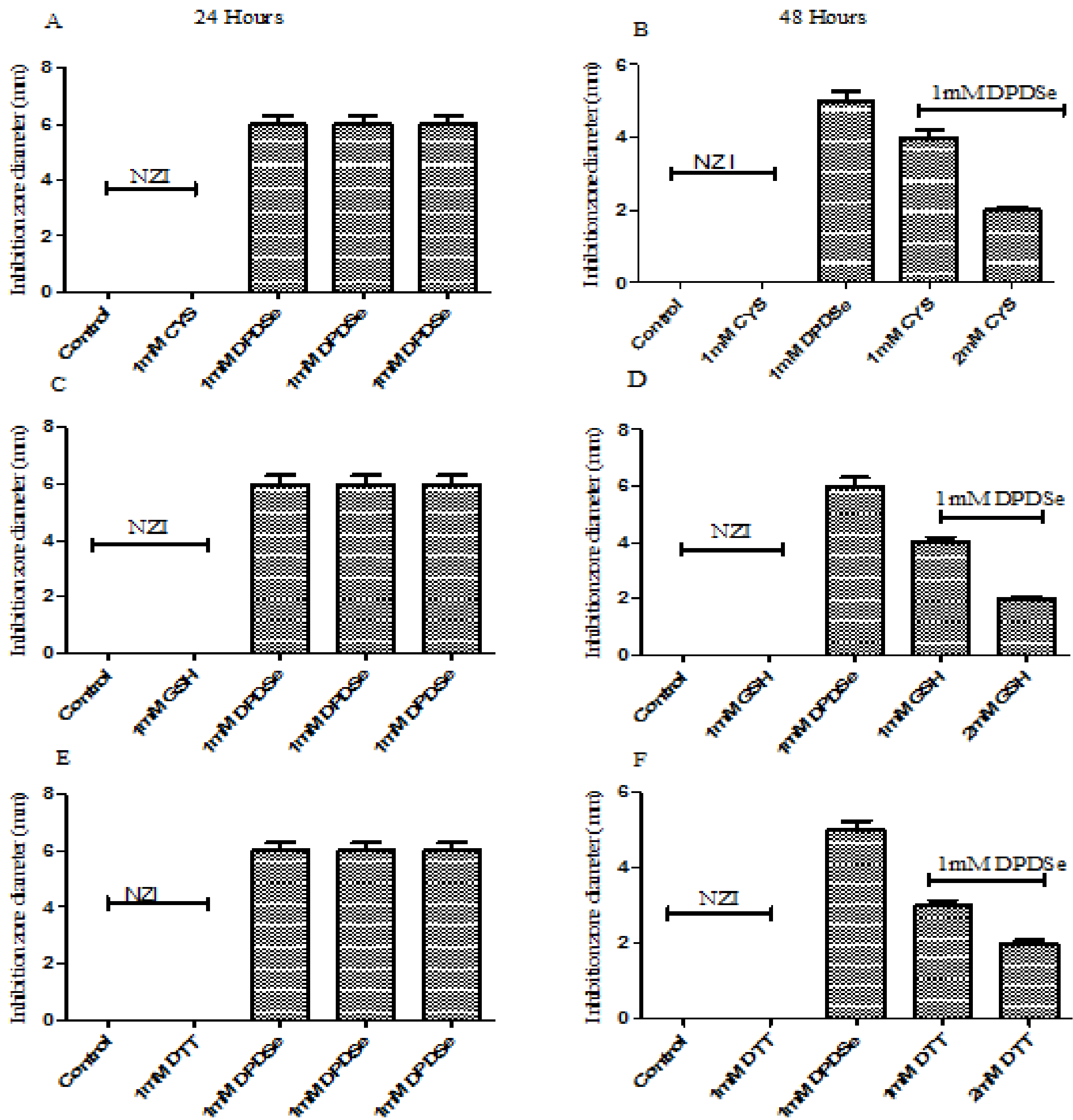

Fig. 4 The effect of co-incubation of DPDSe with and without CYS (panel A & B), GSH (panel C & D) and DTT (panel E & F) on the diameter of the zone of inhibition produced by DPDSe within 24 hours on E.coli (Condition two). NZI represent No zone of inhibition.

Kade *et al.*, 2013 [27] noted that in the absence of supplied exogenous thiols, ebselen (an organoselenium) attacks thiols of importance on biological proteins thus expressing its toxicity when it was added to the basal tissue homogenate, and the introduction of exogenous thiols only serve to relieve this inhibitory effect of ebselen on the proteins. Consequently, the observed reversal in the inhibitory effect of DPDSe on *E. coli* in this study indicates that the exogenous thiols were probably oxidized in an attempt to salvage critical sulphydryl proteins of the *E. coli.*

Additionally, in order to determine the proximity of the critical thiols of *E. coli* that is sensitive to oxidation by DPDSe, the sensitivity of *E. coli* to two thiols modifiers (Iodoacetamide, a thiol alkylating agent and Diamine a thiol crosslinking agent) were tested on the growth of the *E. coli* (Figure 5). The crosslinking agent Diamine (DA) (Panel A) and the alkylating agent Iodoacetamide (IA) (Panel B) exerted marked concentration-dependent inhibitory effects on the growth of *E. coli* at 10mM and 5mM respectively.

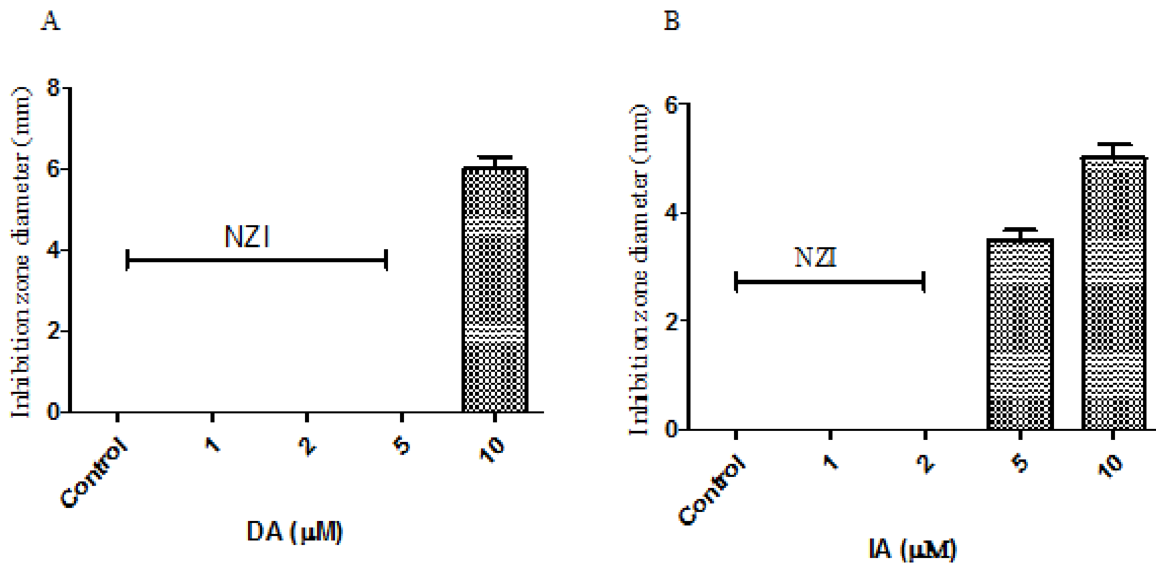

Fig. 5 Effect of thiol oxidizing agent- panel a crosslinker Diamine (DA) and panel B alkylating agent Iodoacetamide (IA) on E.coli growth. NZI represent No zone of inhibition

In order to further establish the possible involvement of thiols oxidation as a component mechanism of the thiol modifiers on the growth of *E. coli*, three different incubation conditions were tested.

Figure 6 shows the co-incubation of Iodoacetamide (IA) with thiols CYS (panel A and B), GSH (panel C and D) and DTT (panel E and F) on the growth of *E. coli* within 24 and 48 hours respectively (Condition one).

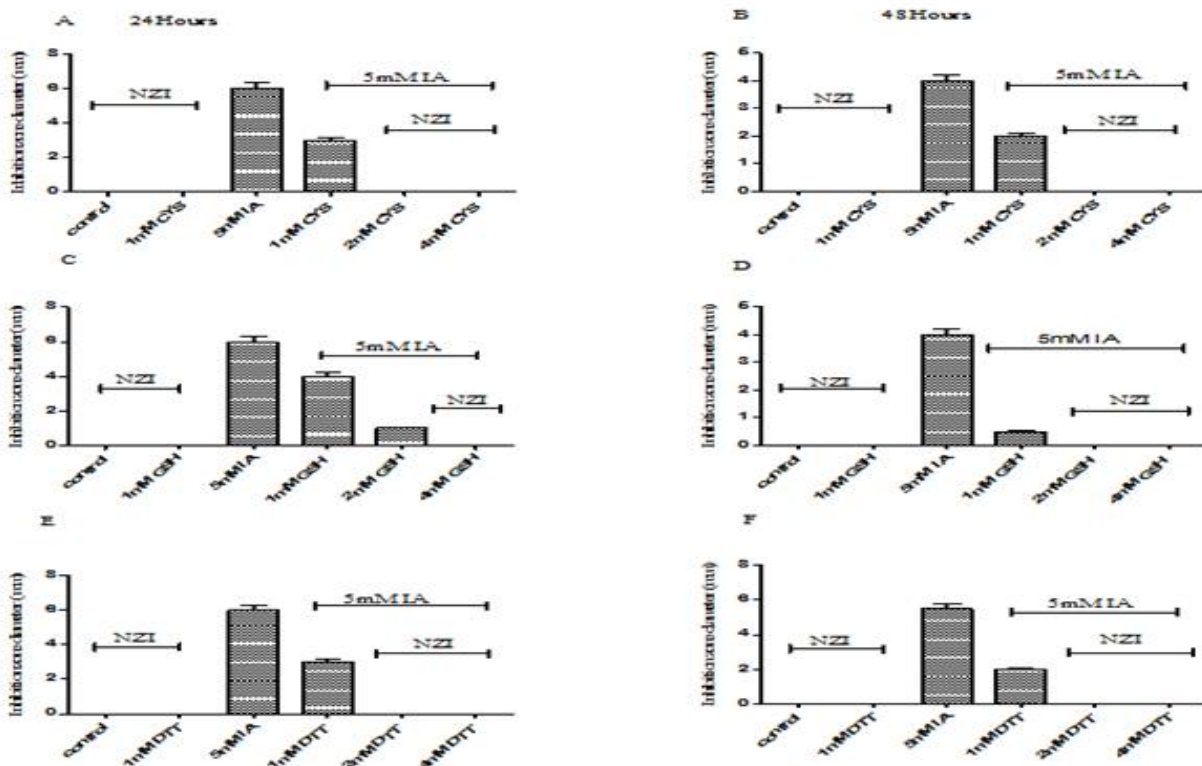

*Fig. 6 Effect of co-incubation of Iodoacetamide (IA) with thiols CYS (panel A & B), GSH (panel C & D) and DTT (panel E & F) on the growthof Escherichia coli within 24 and 48 hours respectively (**Condition one**). NZI represent No zone of inhibition*

.

Figure 7 shows co-incubation of thiols (CYS, GSH and DTT) with and without iodoacetamide within 24 and 48 hours of incubation (condition two). After 24 hours, individual thiol showed no zone of inhibition on *E. coli* growth. When compared with IA alone which exhibited a profound inhibitory effect on the growth of the organism. Figure 7 (panel A, B and C) and Figure 8 show that the co-incubation of IA with and without CYS (panel A and B), GSH (panel C and D) and DTT (panel E and F) on the diameter of the zone of inhibition produced by IA on *E. coli* after 24 hours of incubation (condition three).

Apparently, the three conditions reveal that IA exhibited an inhibitory effect alone. It was evident that the presence of the thiols alters the oxidation of the thiols that are possibly present in the cell membrane of the organism. This was due to significant reduction in the zone of inhibition, the higher the concentration of the thiols, the lower the inhibition which was produced by IA. In fact, Iodoacetamide and diamide have been reported to be a sulphydryl blocking agent, oxidizing thiols to disulfides [29].

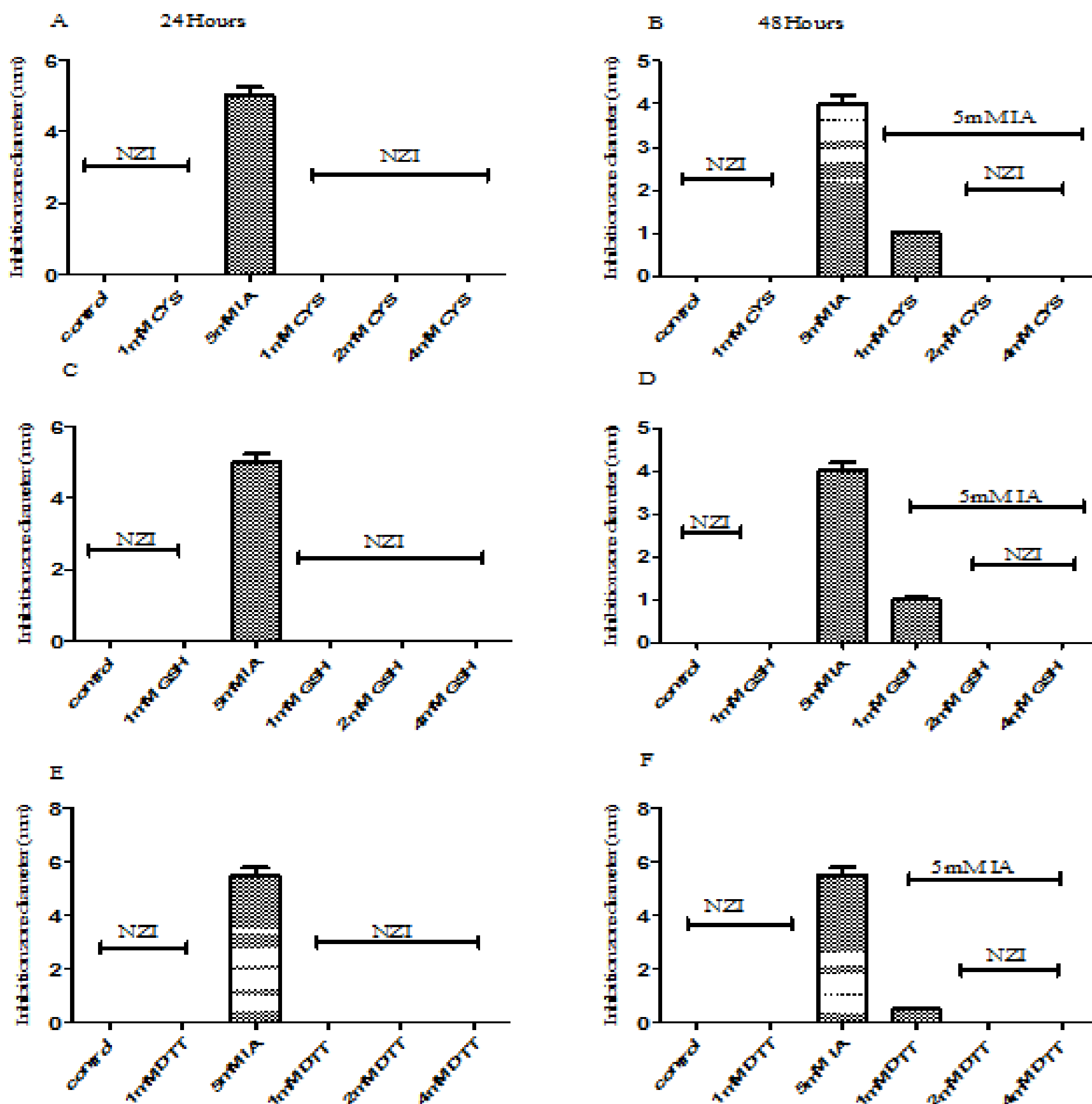

*Fig. 7 Effect of co-incubation of thiols CYS (panel A & B), GSH (panel C & D) and DTT (panel E & F) with and without iodoacetamide within 24 and 48 hours of incubation (**Condition Two**). NZI represent No zone of inhibition.*

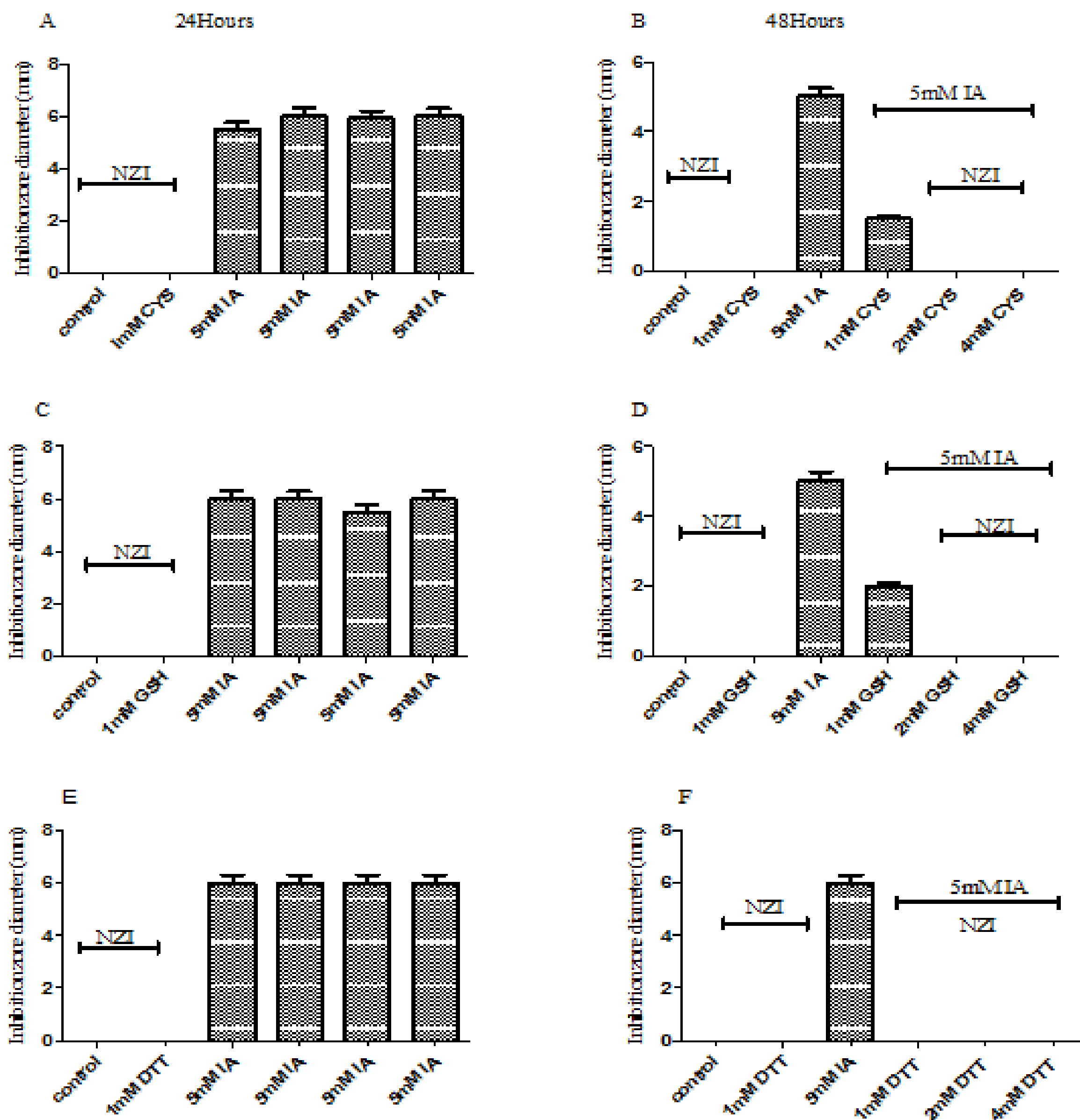

Fig. 8 Effect of thiols CYS (panel A & B), GSH (panel C & D) and DTT (panel E & F) on the diameter of the zone of inhibition produced by Iodoacetamide (Condition Three). NZI represent No zone of inhibition.

Lipid peroxidation (LPO) and its reactive products, such as malondialdehyde (MDA), can profoundly alter the structure and function of cell membrane and cellular metabolism, leading to cyto-toxicity [30]. In this study, Figure 9 shows lipid peroxidation occurrence in the liver, brain and kidney tissues of rats infected *E. coli* (panels A, B and C), However, DPDSe treated group had a significant decrease in the level of TBARS produced. These results supported the earlier observations that the major pharmacological potency of DPDSe could be strongly related to its antioxidative properties [9].

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule [31].

In Figure 10, it was observed that the group infected only appeared to have the highest level of DPPH free radicals unscavenged. However, DPDSe treatment significantly restored this effect by reducing the level of DPPH free radicals generated as a result of the infection.

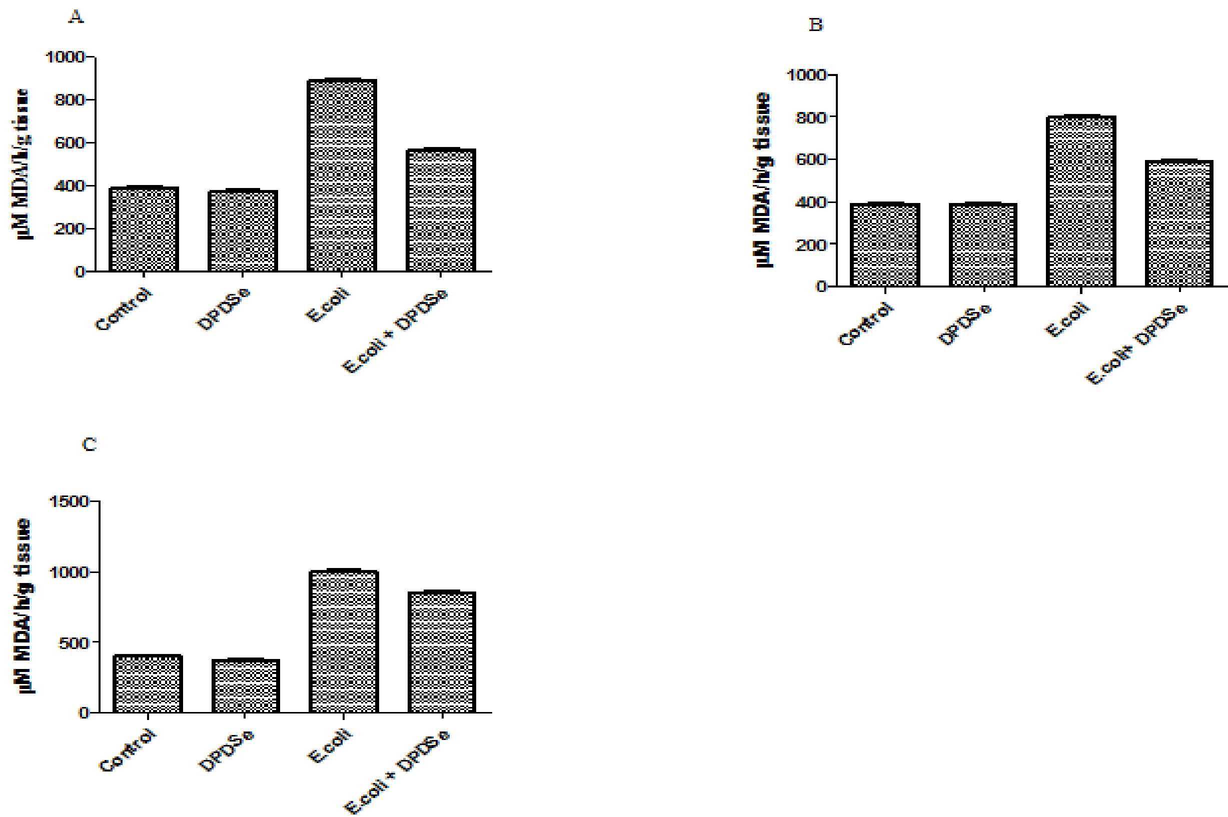

Fig. 9: Effect of DPDSe on lipid peroxidation in (Panel A) liver, (Panel B) brain and (Panel C) kidney homogenate tissue of rat infected with E.coli.

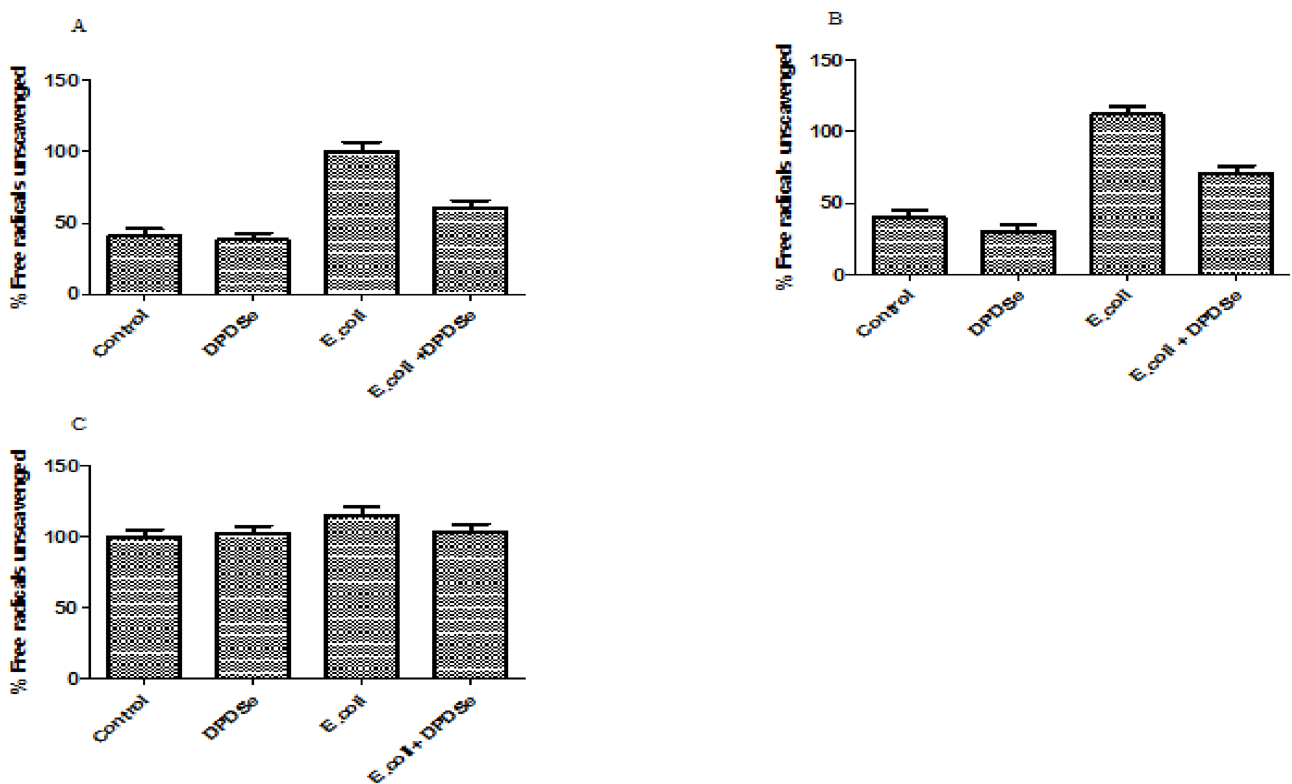

Fig. 10: Effect of DPDSe on DPPH free radical scavenging ability of liver (Panel A), brain (Panel B) and kidney (Panel C) tissues homogenates of rat infected with E. coli

.

With respect to non-enzymic antioxidants such as Reduced glutathione (GSH), levels were observed to be significantly decreased in the liver (Panel A), brain (Panel B) and kidney (Panel C) and DPDSe appeared to reverse the effect by elevating the GSH level (Figure 11). Recent evidences have shown that under in vivo conditions and even under various conditions of oxidative stress related disease models where the level of GSH is generally compromised, administration of organoselenium compounds generally elevated the physiological level of the tripeptide, GSH [32, 28]. In other to understand the antibiotic mechanism of action, the activities of some sulphydryl enzymes were evaluated in this study and these include δ-ALAD, and Na^{+}/K^{+}-ATPase.

Figure 12 panel A and B shows the activity of hepatic and renal δ-ALAD enzyme. It was observed that *E. coli* caused an inhibition in the activity of δ-ALAD and DPDSe was able to restore the activity of the enzyme inhibition caused by *E. coli.*

This result is in line with the report of [33] which suggested that DPDSe could improve status as well as relieve inhibition of thiol-containing enzymes such as hepatic and renal δ-ALAD *in-vivo*. Figure 13 shows the activity of the brain and kidney sodium-pump of infected rats *in-vivo*. As observed in this result, the activity of the enzyme was significant inhibited *E. coli* infected group. Hence, lipid peroxidation and free radicals consequently leads to inhibition of Na^{+}/K^{+}-ATPase activity in rat brain [17]. However, diphenyl diselenide significantly restored the activity of the enzyme in the infected rats. DPDSe prevented lipid peroxidation associated with free radical assault, thereby maintaining the structural integrity of the cell membrane, and as a result, abolishing the inhibition of Na+/K+-ATPase [17]. In any case, we can safely conclude that Diphenyl diselenide appear to be a promising intervention in the treatment of rat infected with *Escherichia coli* especially because this compound has a direct effect on the sulphydryl-containing enzymes and the antioxidant system of the rat.

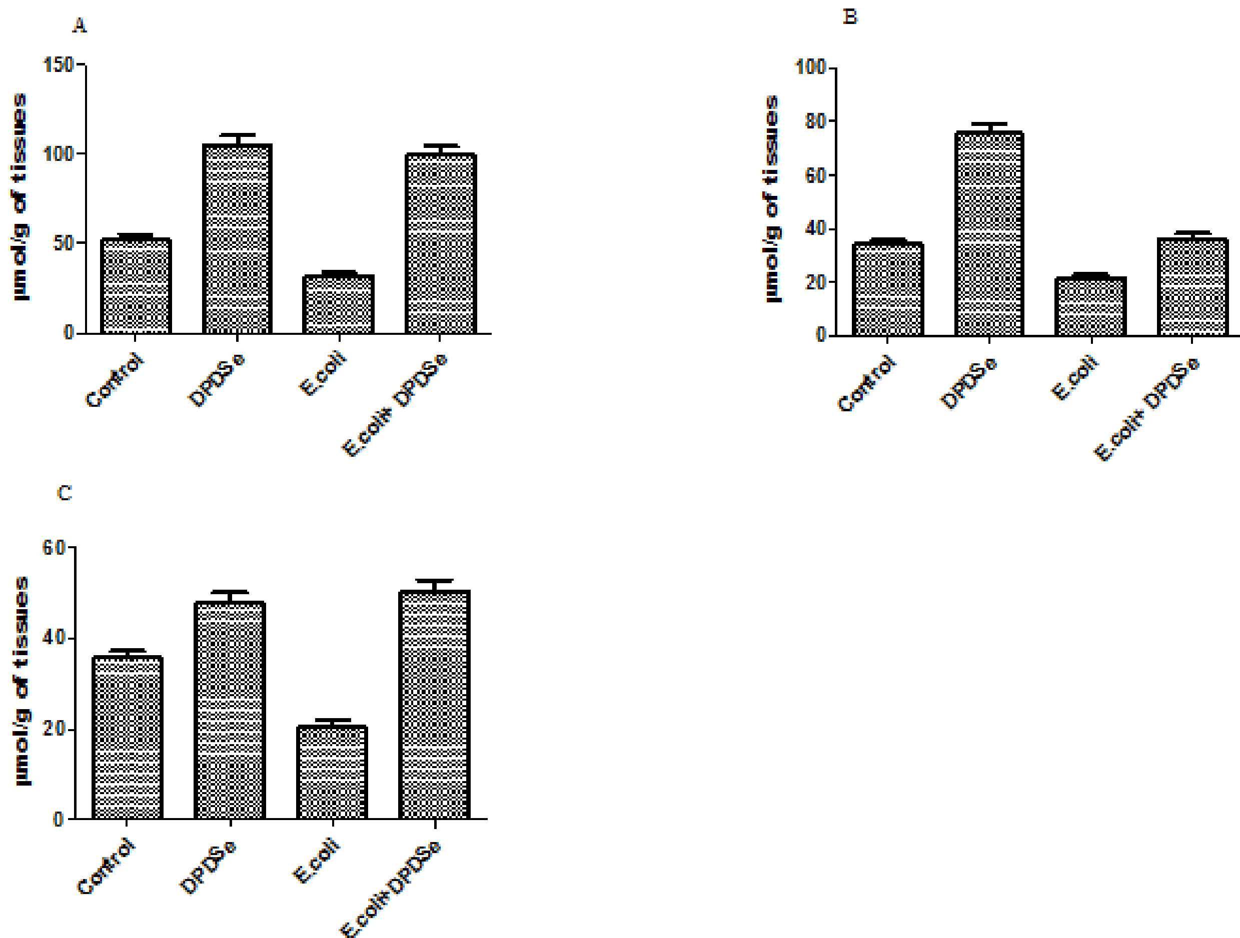

Fig. 11: Effect of DPDSe on GSH level of liver (Panel A), brain (Panel B) and kidney (Panel C) tissues homogenates of E. coli infected rat.

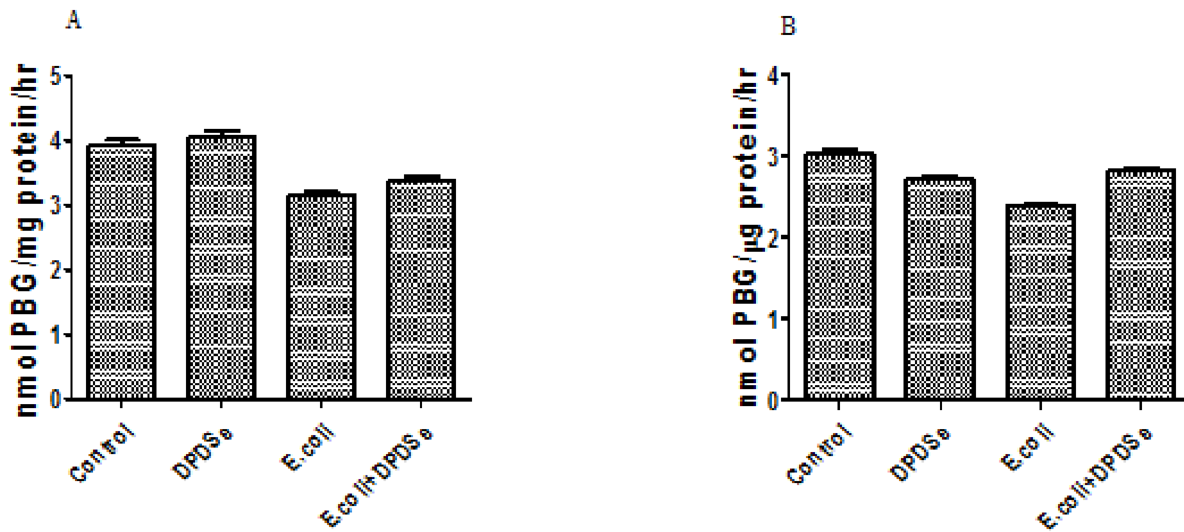

Fig. 12: Effect of DPDSe on liver (Panel A) and kidney (Panel B) delta aminolevulinate dehydratase activity of E. coli infected rats.

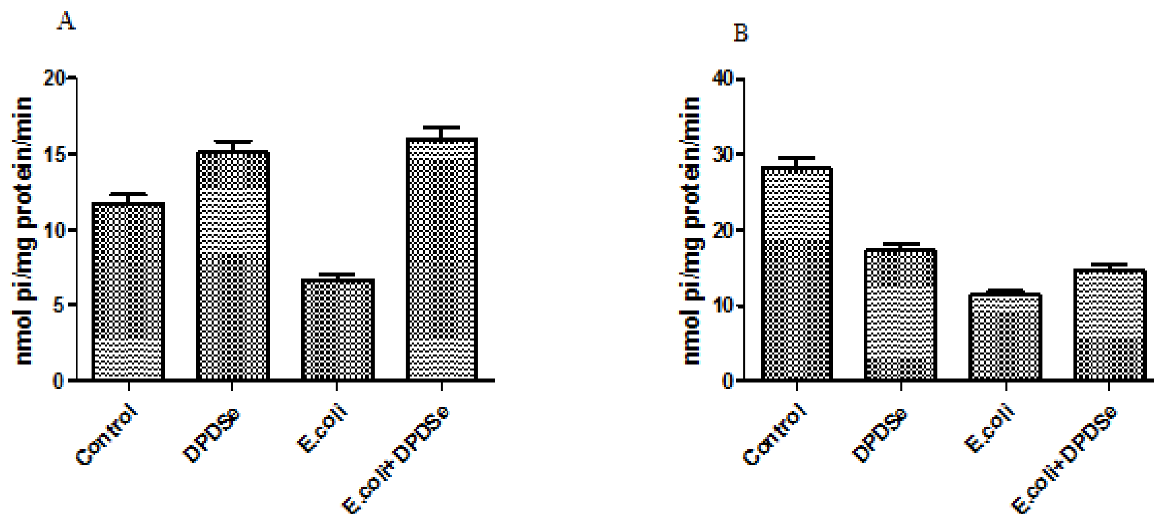

Fig. 13: Effect of DPDSe on the activity of cerebral (Panel A) and renal (Panel B) sodium-potassium pump of rat infected with E. coli.

CONCLUSION

In conclusion, within the limit of our present data and following the arguments aforementioned, it is obvious that DPDSe antibiotic activity on *E. coli* involve alteration of the critical thiols of *E. coli.* DPDSe could be very good candidate for the development of new Antibiotic against *E. coli* infections.

ACKNOWLEDGMENT

The authors are grateful to Prof M. K. Oladunmoye and Dr. I. J. Kade, Department of Microbiology and Biochemistry, The Federal University of Technology, Akure respectively, for their numerous assistances and guidance in the cause of the study.

REFERENCES

[1] A. Andremont, “The future control of bacterial resistance to antimicrobial agents,” American Journal of Infection Control, vol. 29, no. 4, pp. 256–258, 2001.

[2] C. Manyi-Loh, S. Mamphweli, E. Meyer, and A. Okoh, “Antibiotic Use in Agriculture and Its Consequential Resistance in Environmental Sources: Potential Public Health Implications,” Molecules, vol. 23, no. 4, p. 795, 2018.

[3] M. P. Rayman, “The importance of selenium to human health,” The Lancet, vol. 356, no. 9225, pp. 233–241, 2000.

[4] N. V. Barbosa, C. W. Nogueira, P. A. Nogara, A. F. D. Bem, M. Aschner, and J. B. T. Rocha, “Organoselenium compounds as mimics of selenoproteins and thiol modifier agents,” Metallomics, vol. 9, no. 12, pp. 1703–1734, 2017.

[5] S. T. Miorelli, R. M. Rosa, D. J. Moura, J. C. Rocha, L. A. C. Lobo, J. A. P. Henriques, and J. Saffi, “Antioxidant and anti-mutagenic effects of ebselen in yeast and in cultured mammalian V79 cells,” Mutagenesis, vol. 23, no. 2, pp. 93–99, Aug. 2008.

[6] D. Alves, J. S. D. Reis, C. Luchese, C. W. Nogueira, and G. Zeni, “ChemInform Abstract: Synthesis of 3-Alkynylselenophene Derivatives by a Copper-Free Sonogashira Cross-Coupling Reaction.,” ChemInform, vol. 39, no. 21, 2008.

[7] A. P. Pesarico, G. Sartori, C. F. D. Santos, J. S. Neto, V. Bortolotto, R. C. V. Santos, C. W. Nogueira, and M. Prigol, “2,2′-Dithienyl diselenide pro-oxidant activity accounts for antibacterial and antifungal activities,” Microbiological Research, vol. 168, no. 9, pp. 563–568, 2013.

[8] G. Mugesh, W.-W. D. Mont, and H. Sies, “Chemistry of Biologically Important Synthetic Organoselenium Compounds,” Chemical Reviews, vol. 101, no. 7, pp. 2125–2180, 2001.

[9] C. W. Nogueira, G. Zeni, and J. B. T. Rocha, “Organoselenium and Organotellurium Compounds: Toxicology and Pharmacology,” Chemical Reviews, vol. 104, no. 12, pp. 6255–6286, 2004.

[10] É. S. Loreto, S. H. Alves, J. M. Santurio, C. W. Nogueira, and G. Zeni, “Diphenyl diselenide in vitro and in vivo activity against the oomycete Pythium insidiosum,” Veterinary Microbiology, vol. 156, no. 1-2, pp. 222–226, 2012.

[11] A. S. D. Freitas, A. D. S. Prestes, C. Wagner, J. H. Sudati, D. Alves, L. O. Porciúncula, I. J. Kade, and J. B. T. Rocha, “Reduction of Diphenyl Diselenide and Analogs by Mammalian Thioredoxin Reductase Is Independent of Their Gluthathione Peroxidase-Like Activity: A Possible Novel Pathway for Their Antioxidant Activity,” Molecules, vol. 15, no. 11, pp. 7699–7714, 2010.

[12] I. A. Adedara, A. O. Abolaji, J. B. T. Rocha, and E. O. Farombi, “Diphenyl Diselenide Protects Against Mortality, Locomotor Deficits and Oxidative Stress in Drosophila melanogaster Model of Manganese-Induced Neurotoxicity,” Neurochemical Research, vol. 41, no. 6, pp. 1430–1438, 2016.

[13] P. M. Chagas, C. F. Bortolatto, E. A. Wilhelm, and C. W. Nogueira, “High doses of 2,2′-dithienyl diselenide cause systemic toxicity in rats: anin vitroandin vivostudy,” Journal of Applied Toxicology, vol. 33, no. 6, pp. 480–487, 2011.

[14] R. Rosa, R. Roesler, A. Braga, J. Saffi, and J. Henriques, “Pharmacology and toxicology of diphenyl diselenide in several biological models,” Brazilian Journal of Medical and Biological Research, vol. 40, no. 10, pp. 1287–1304, 2007.

[15] H. Wójtowicz, M. Chojnacka, J. Młochowski, J. Palus, L. Syper, D. Hudecova, M. Uher, E. Piasecki, and M. Rybka, “Functionalized alkyl and aryl diselenides as antimicrobial and antiviral agents: synthesis and properties,” Il Farmaco, vol. 58, no. 12, pp. 1235–1242, 2003.

[16] I. Kade, C. Nogueira, and J. Rocha, “Diphenyl diselenide and streptozotocin did not alter cerebral glutamatergic and cholinergic systems but modulate antioxidant status and sodium pump in diabetic rats,” Brain Research, vol. 1284, pp. 202–211, 2009.

[17] M. Idu. “Antimicrobial activity and phytochemistry of Khaya senegalensis root,” International Journal of Ayurvedic and Herb Medicine, vol. 2, no. 3, pp. 416-422, 2012.

[18] C. Valgas, S. M. D. Souza, E. F. A. Smânia, and A. S. Jr., “Screening methods to determine antibacterial activity of natural products,” Brazilian Journal of Microbiology, vol. 38, no. 2, pp. 369–380, 2007.

[19] E. Nester, D. Anderson, C. Roberts, and M. Nester, “Microbiology: A Human Perspective, 6th edition,” The McGraw-Hill Companies, Inc., New York. pp 480-481, 1979.

[20] H. Ohkawa, N. Ohishi, and K. Yagi, “Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction,” Analytical biochemistry, vol. 95, no. 2, pp. 351-358, 1979.

[21] G. L. Ellman, “Tissue sulfhydryl groups,” Archives of Biochemistry and Biophysics, vol. 82, no. 1, pp. 70–77, 1959.

[22] P. Omololu, J. Rocha, and I. Kade, “Attachment of rhamnosyl glucoside on quercetin confers potent iron-chelating ability on its antioxidant properties,” Experimental and Toxicologic Pathology, vol. 63, no. 3, pp. 249–255, 2011.

[23] S. Sassa, “Delta-Aminolevulinic Acid Dehydratase Assay,” Enzyme, vol. 28, no. 2-3, pp. 133–145, 1982.

[24] C. Fiske, and Y. Subbarow, “The colorimetric determination of phosphorus,” Journal of Biological Chemistry, vol. 66, no. 1, pp. 375–381, 1925.

[25] Smith-Palmer, Stewart, and Fyfe, “Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens,” Letters in Applied Microbiology, vol. 26, no. 2, pp. 118–122, 1998.

[26] G. Zhou, Q.-S. Shi, X.-M. Huang, and X.-B. Xie, “The Three Bacterial Lines of Defense against Antimicrobial Agents,” International Journal of Molecular Sciences, vol. 16, no. 9, pp. 21711–21733, Sep. 2015.

[27] I. Kade, B. Balogun, and J. Rocha, “In vitro glutathione peroxidase mimicry of ebselen is linked to its oxidation of critical thiols on key cerebral suphydryl proteins – A novel component of its GPx-mimic antioxidant mechanism emerging from its thiol-

modulated toxicology and pharmacology," Chemico-Biological Interactions, vol. 206, no. 1, pp. 27–36, 2013.

[28] C. Wolf, F. Hochgräfe, H. Kusch, D. Albrecht, M. Hecker, and S. Engelmann, "Proteomic analysis of antioxidant strategies of Staphylococcus aureus: Diverse responses to different oxidants," Proteomics, vol. 8, no. 15, pp. 3139–3153, 2008.

[29] M. Pooja, A. N. R. Pradeep, S. P. Hungund, C. Sagar, K. M. Ponnuvel, A. K. Awasthi, and K. Trivedy, "Oxidative stress and cytotoxicity elicited lipid peroxidation in hemocytes of Bombyx mori larva infested with dipteran parasitoid, Exorista bombycis," Acta Parasitologica, vol. 62, no. 4, Jan. 2017.

[30] R. L. Prior, X. Wu, and K. Schaich, "Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements," Journal of Agricultural and Food Chemistry, vol. 53, no. 10, pp. 4290–4302, 2005.

[31] T. Ogunmoyole, J. Rocha, A. Okoronkwo, and I. Kade, "Altered pH homeostasis modulates the glutathione peroxidase mimics and other antioxidant properties of diphenyl diselenide," Chemico-Biological Interactions, vol. 182, no. 2-3, pp. 106–111, 2009.

[32] I. J. Kade, M. W. Paixão, O. E. D. Rodrigues, N. B. V. Barbosa, A. L. Braga, D. S. Ávila, C. W. Nogueira, and J. B. T. Rocha, "Comparative Studies on Dicholesteroyl Diselenide and Diphenyl Diselenide as Antioxidant Agents and their Effect on the Activities of Na /K ATPase and δ-Aminolevulinic acid Dehydratase in the Rat Brain," Neurochemical Research, vol. 33, no. 1, pp. 167–178, 2007.