African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 7, Issue 4, 2024 (pp. 1-11)
www.abjournals.org

ACUTE ETHANOL INTOXICATION IN RATS IS EXPOSURE-TIME DEPENDENT, DIPHENYL DISELENIDE OFFERS A REMEDY.

Mulikat Adenike Adewole[a,b*], Ige Joseph Kade[b], Akeem Olalekan Lawal[b], Olusola Elekofehinti[b], and Damilola Mafolayomi Ige[b].

[a]Department of Biochemistry, University of Medical sciences, Ondo, Ondo State, Nigeria.

[b]Department of Biochemistry, Federal University of Technology, Akure, Ondo State, Nigeria.

[*]Corresponding Author's Email: madewole@unimed.edu.ng; Tel.: 2348066246146

Cite this article:

Adewole, M. A., Kade, I. J., Lawal, A. O., Elekofehinti, O., Ige, D. A. (2024), Acute Ethanol Intoxication in Rats Exposure-Time Dependent, Diphenyl Diselenide Offers a Remedy. African Journal of Biology and Medical Research 7(4), 1-11. DOI: 10.52589/AJBMR-9BMKGOLK

Manuscript History

Received: 2 Jul 2024

Accepted: 6 Sep 2024

Published: 20 Sep 2024

ABSTRACT: *Acute ethanol intoxication causes a lot of pathologies which have been linked to oxidative stress, despite the attending toxicity of ethanol, its acute exposure is sometimes necessitated. Although, a lot of researchers have investigated acute ethanol intoxication with possible management regimens especially the use of compounds with antioxidant properties. However, less information is available on the time-dependent effects of ethanol on the biochemical indicators of oxidative stress and the possible interactive effects that diphenyl diselenide (DPDSe) may have on it in liver tissues. Hence, this study sought to investigate the time-dependent effects of ethanol and the possible influence DPDSe may have on intoxication at different times.10mg/kg DPDSe was administered orally to white Albino rats 30 minutes before and after oral administration of 13ml/kg of 28% ethanol solution, the reaction was terminated at 1hr, 3hrs, 6hrs and 12hrs after administration of ethanol for different groups. Thereafter, the oxidative stress indicators such as lipid peroxidation, total thiol, and non-protein thiol as well as activities of enzymes that are stress-responsive such as δ-aminolevulinic acid dehydratase (ALAD), 5'-nucleotidase and nucleoside triphosphate diphosphohydrolase (NTPDase) were evaluated. The results of lipid peroxidation, and thiol level show that ethanol-induced stress is more pronounced at times 3hrs and 6hrs of exposure while the protective effect of diphenyl diselenide on the tissues is maximum at 6 hrs after exposure to ethanol. The time-dependence intoxication effect of ethanol on the liver and the time-dependent ameliorative effect of DPDSe show that in the search for more therapeutic agents against acute intoxication of ethanol, time of exposure to ethanol is a valid factor that is to be considered.*

KEYWORDS: Ethanol intoxication, time-dependent, oxidative stress, diphenyl diselenide.

Article DOI: 10.52589/AJBMR-9BMKGOLK
DOI URL: https://doi.org/10.52589/AJBMR-9BMKGOLK

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 7, Issue 4, 2024 (pp. 1-11)

www.abjournals.org

INTRODUCTION

Intoxication by ethanol has been investigated by many researchers using various models to unravel how it affects different cells and organs with a view of finding agents that could have ameliorative effects on it (Boby *et al*., 2021, Carton et al., 2019, Wang et al., 2013). Some of the previous work that has been done confirmed that oxidative stress could be one of the mechanisms of intoxication by ethanol (Hernendez *et al*., 2016, Jegal et al., 2023, Vucevic *et al*., 2013, Flora et al., 2013). Oxidative stress is the phenomenon that describes stress that is induced by the imbalance in the antioxidant and oxidant levels in the cells in favour of the oxidants. These resultant oxidants cause damage to cells and this may result in various pathologies in the cell depending on which part of the body is affected (Carton et al., 2019). The stress occurs either by depleting the antioxidant system in the cell or by producing the oxidant at an exorbitant rate than what exogenous antioxidant can cope with, thereby accumulating in the blood to generate free radicals which can initiate a chain reaction leading to the generation of more radicals and reactive oxygen species (ROS).

Aside from the research into mechanisms of ethanol intoxication, factors that affect the severity of intoxication have also been investigated which include the age of the animal (Vetreno et al., 2023), dosage and concentration of ethanol taken (Carton et al., 2019), time of exposure (Ibrahim et al., 2014) and interaction with other chemical compounds (Souli *et al*., 2013) The effect of time of exposure to ethanol intoxication on various organs is another area that has attracted the interest of researchers (Ibrahim et al., 2014) and their results show that intoxication in the liver and heart is through oxidative stress and that it depends on the time of exposure to ethanol.

In search of the solution to alcohol intoxication, several antioxidant compounds have been used to ameliorate the effect (Luczaj and Skrzydlewa,2004, Reiter *et al*., 1999, Jegal *et al*., 2023). Diphenyl diselenide is an organoselenium compound with proven antioxidant properties which have been used in the management of many pathologies that are linked with oxidative stress (Wang et al., 2022, Petrinilho *et al*.,2016, Quispe *et al*., 2019).

Although many antioxidant compounds with ameliorative effects on ethanol intoxication have been studied, less information is available in the literature as to the effects of these antioxidant compounds on ethanol intoxication at various hours of exposure to ethanol, this research sought to investigate the effect of ethanol on hepatic and neuronal cells at different hours (1hr, 3hrs, 6hrs, 12hrs) after a single oral administration of 8g/kg ethanol and the interactive effect of diphenyl diselenide on the intoxication at the various hours of exposure studied using rat model.

Article DOI: 10.52589/AJBMR-9BMKGOLK
DOI URL: https://doi.org/10.52589/AJBMR-9BMKGOLK

MATERIALS AND METHODS

Chemicals

Diphenyl diselenide, trichloroacetic acid (TCA), Dithiothreitol (DTT), Tris salt, dithio-bis-(2-nitrobenzoic acid) DTNB, ethanol and other chemicals were gotten from Sigma Chemical Co. USA. Other chemicals were purchased from standard suppliers.

Experimental animals

Male adult Wistar rats (120-150 g) were purchased, acclimatised at the Animal House of the Department of Biochemistry, The Federal University of Technology, Akure, Nigeria, for 2 weeks and used in the entire experiment according to standard guidelines of the Committee on Care and Use of Experimental Animal Resources.

Experimental Design (*in vivo*)

The study of DPDSe influence on acute intoxication by ethanol after 1 hr, 3 hrs, 6hrs and 12 hours of treatment was carried out. The rats were divided into five groups (n=6) for each hour treatment and allowed to acclimatise for two weeks, after which group 1 (Control) was administered distilled water, group 2 was treated with 10 mg/kg DPDSe only, group 3 induced with a single oral dose of 8 g/kg ethanol only, group 4 was pretreated with 10 mg/kg DPDSe before induction with a single oral dose of 10ml/kg of 28% ethanol solution, group 5 was treated with 10mg/kg DPDSe after 30 minutes of a single oral dose of 8g/kg ethanol, the experiment was terminated at different hours (1 hr, 3 hrs, 6 hrs, 6 hrs) after the treatment with ethanol.

Lipid peroxidation assay

Lipid peroxidation was measured as thiobarbituric acid reactive substances (TBARS). TBARS were determined in tissue homogenates as previously described (Ohkawa et al., 1979; Rossato et al., 2002). MDA values were determined using the absorbance coefficient (1.56 · 105 /cm/mmol) of the MDA–TBA complex at 532 nm.

Thiol oxidation

The thiol oxidation was determined in the presence of 50 mM Tris HCl pH 7.4 in both proteinised and deproteinized samples. The rate of thiol oxidation was evaluated by measuring the disappearance of the SH group. The free SH-group was determined by Ellman, (1959).

5’-Nucleotidase and NTPDase-like activities.

The 5’-Nucleotidase activity was determined in a reaction medium essentially as described by Heymann, *et al*. (1984). The reaction was initiated by the addition of AMP and ATP to a final concentration of 2.0 mM for both enzymes (NTPDase and 5’-Nucleotidase) respectively. The assays were stopped by the addition of 250 µl of 10% trichloroacetic acid (TCA). Inorganic phosphate was measured by the method of Fiske and Subbarow (1925). Control experiments were carried out to correct for non-enzymatic hydrolysis of the nucleotides. All samples were run in duplicate and Enzyme-specific activities are reported as nmol P_i released/min/mg of protein.

Article DOI: 10.52589/AJBMR-9BMKGOLK
DOI URL: https://doi.org/10.52589/AJBMR-9BMKGOLK

Statistical analysis

The results of replicate readings were pooled and expressed as mean ± standard deviation (S.D). One-way Analysis of Variance (ANOVA) was used to analyse the results followed by Turkey's post hoc test, with levels of significance accepted at $p < 0.05$. All statistical analyses were carried out using the software Graph pad PRISM (V.5.0).

RESULTS

INFLUENCE OF ETHANOL AND DIPHENYL DISELENIDE ON BIOCHEMICAL INDICES OF OXIDATION STRESS.

Influence of DPDSe on ethanol-induced lipid peroxidation

Fig. 3.1.1 shows that ethanol intoxication causes an increase in the production of thiobarbituric acid reactive species (TBARS) at 3hrs and 6hrs and that pre-DPDSe treatment markedly ($p<0.05$) reverses the ethanol-induced decrease in the at these hours while post-treatment markedly increase TBARS formed at all the hours studied.

Influence of DPDSe on ethanol-induced reduction in total protein thiol at different times after exposure.

Fig. 3.1.2 shows that ethanol decreases the total thiol level with an increase in time after exposure. Both pre-and post-DPDSe treatment markedly mitigates ethanol-induced reduction in total thiol level with a decreased effect at 12 hrs after exposure.

Influence of DPDSe on ethanol-induced reduction in non-protein thiol

Fig. 3.1.3 shows that ethanol intoxication causes an increase in non-protein thiol at 1hr and a reduction in non-protein thiol at 3hrs and 6hrs after exposure and that both pre- and post-DPDSe treatments markedly reverse the ethanol-induced decrease in the *in vivo* thiol level.

Influence of DPDSe on ethanol-induced modulation in ALA-D activity

Fig. 3.1.4 shows that ethanol intoxication causes a reduction in ALA-D activity and that both pre- and post-DPDSe treatments markedly decrease the activity of the enzyme mostly at times 6 hrs after exposure to ethanol.

Influence of DPDSe on ethanol-induced modulation of NTPDase activity

Fig. 3.1.5 shows that ethanol intoxication causes an elevation in NTPDase activity at 1hr, 3hrs,6hrs and 12hrs, it also shows that both pre-and post-DPDSe treatments markedly increase the activity of the enzyme at different hours studied except at 6hrs.

Influence of DPDSe on ethanol-induced modulation of Nucleotidase activity

Fig. 3.1.5 shows that ethanol intoxication causes elevation in Nucleotidase activity at 1 hr, 3hrs, 6hrs and 12hrs, it also shows that both pre- and post-DPDSe treatments markedly increase the activity of the enzyme at different hours studied except at 6hrs after exposure.

Article DOI: 10.52589/AJBMR-9BMKGOLK
DOI URL: https://doi.org/10.52589/AJBMR-9BMKGOLK

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 7, Issue 4, 2024 (pp. 1-11)
www.abjournals.org

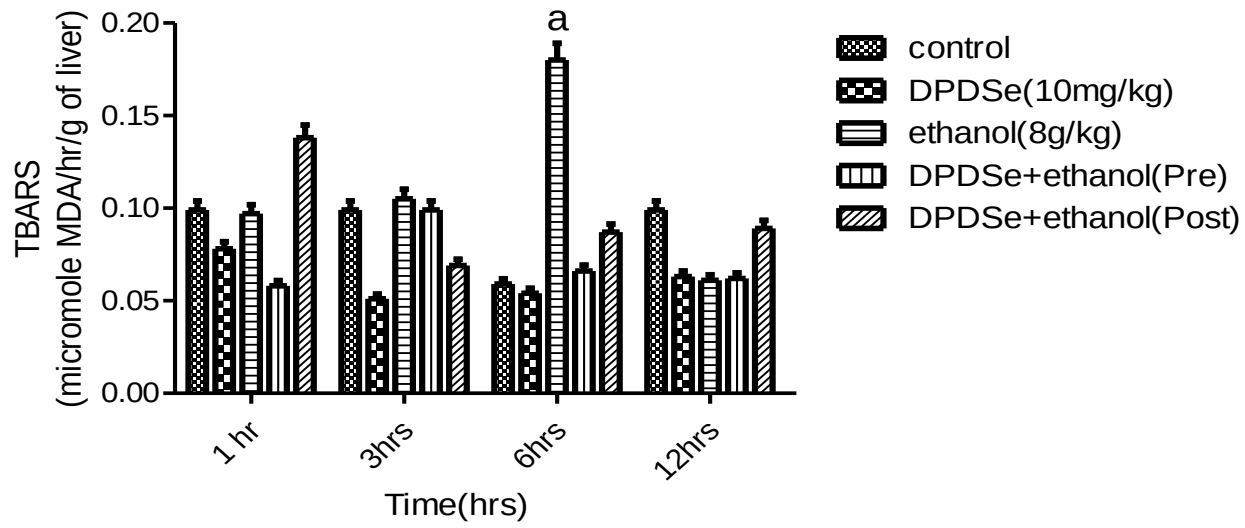

Fig.3.1.1: Influence of DPDSe pre- and post-treatment on TBARS level in the liver of acute ethanol-intoxicated male Wistar rats at different times after exposure. [a]p<0.05 significant difference compared to control and ethanol at each hour of treatment.

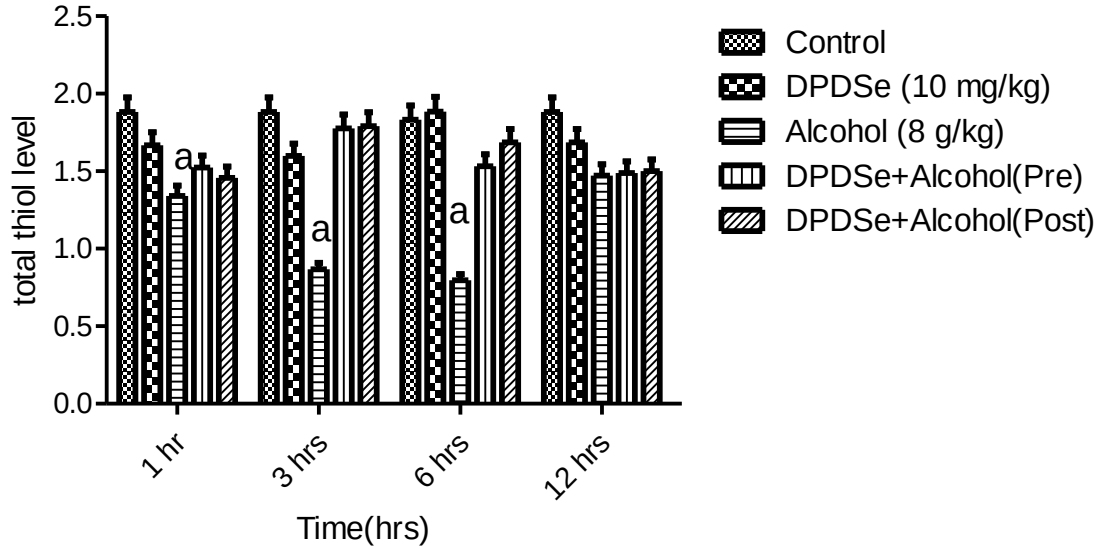

Fig.3.1.2: Influence of DPDSe pre- and post-treatment on total thiol level in the liver of acute ethanol-intoxicated male Wistar rats at different times after exposure. [a]p<0.05 significant difference compared to control and ethanol at each hour of treatment.

Article DOI: 10.52589/AJBMR-9BMKGOLK
DOI URL: https://doi.org/10.52589/AJBMR-9BMKGOLK

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 7, Issue 4, 2024 (pp. 1-11)
www.abjournals.org

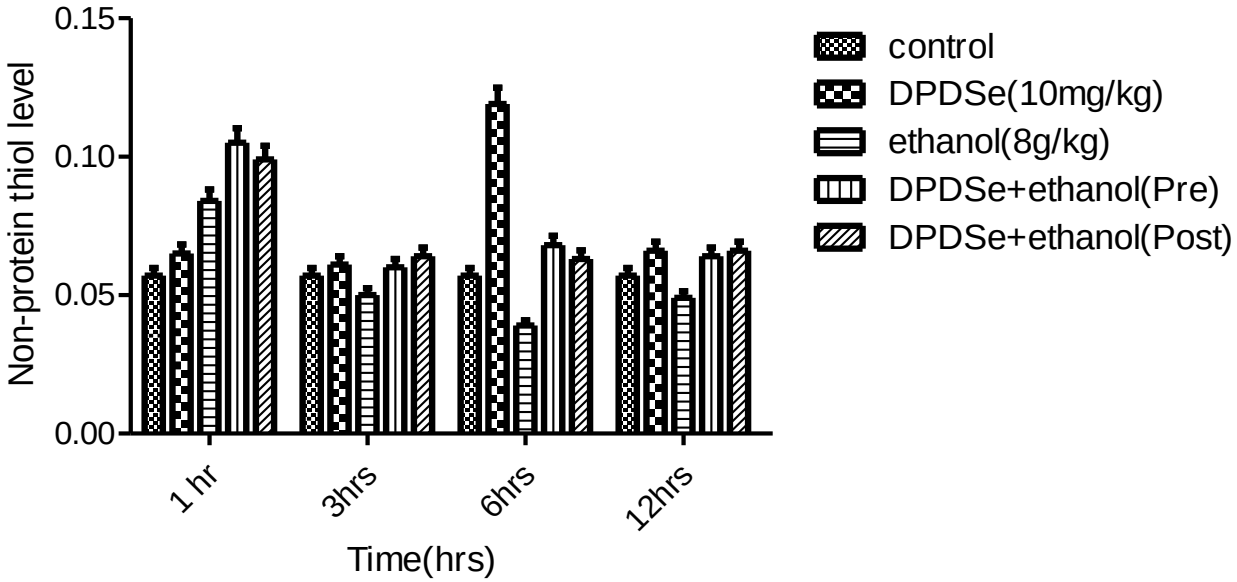

Fig.3.1.3: Influence of DPDSe pre- and post-treatment on non-protein thiol level in the liver of acute ethanol-intoxicated male Wistar rats at different hours after exposure.

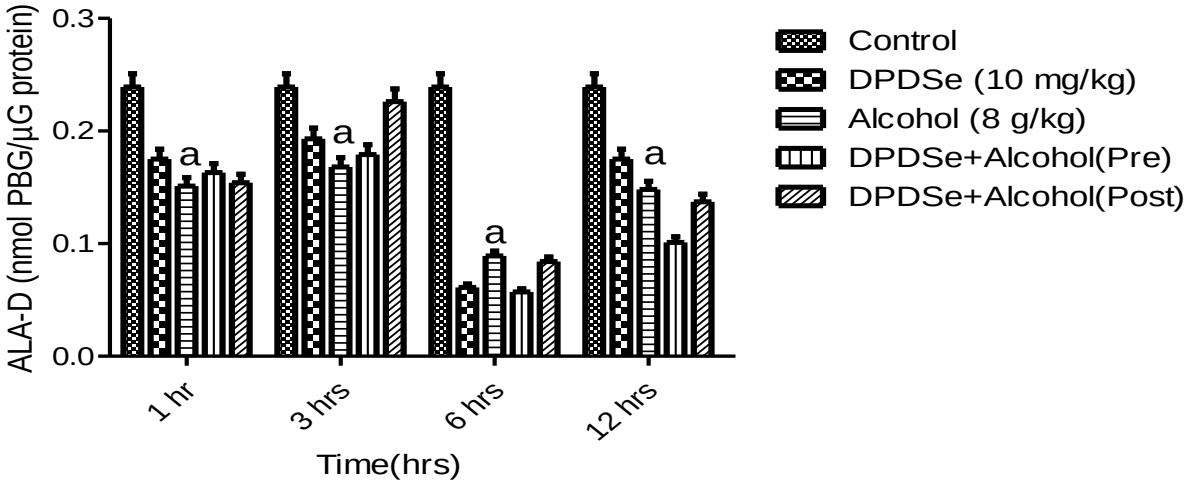

Fig.3.1.4: Influence of DPDSe pre- and post-treatment on the activity of ALA-D in the liver of acute ethanol-intoxicated male Wistar rats at different times after exposure. $^{a}p<0.05$ significant difference compared to control and ethanol at each hour of treatment.

Article DOI: 10.52589/AJBMR-9BMKGOLK
DOI URL: https://doi.org/10.52589/AJBMR-9BMKGOLK

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 7, Issue 4, 2024 (pp. 1-11)
www.abjournals.org

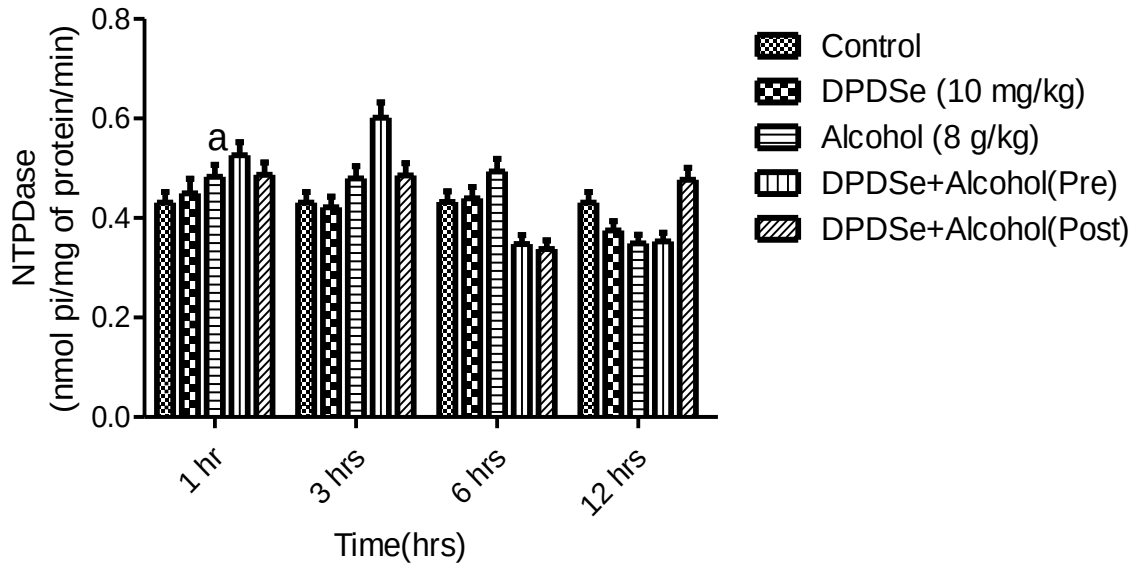

Fig.3.1.5: Influence of DPDSe pre- and post-treatment on the activity of NTPDase in the liver of acute ethanol-intoxicated male Wistar rats at different hours after exposure. $^{a}p<0.05$ significant difference compared to control and ethanol at each hour of treatment

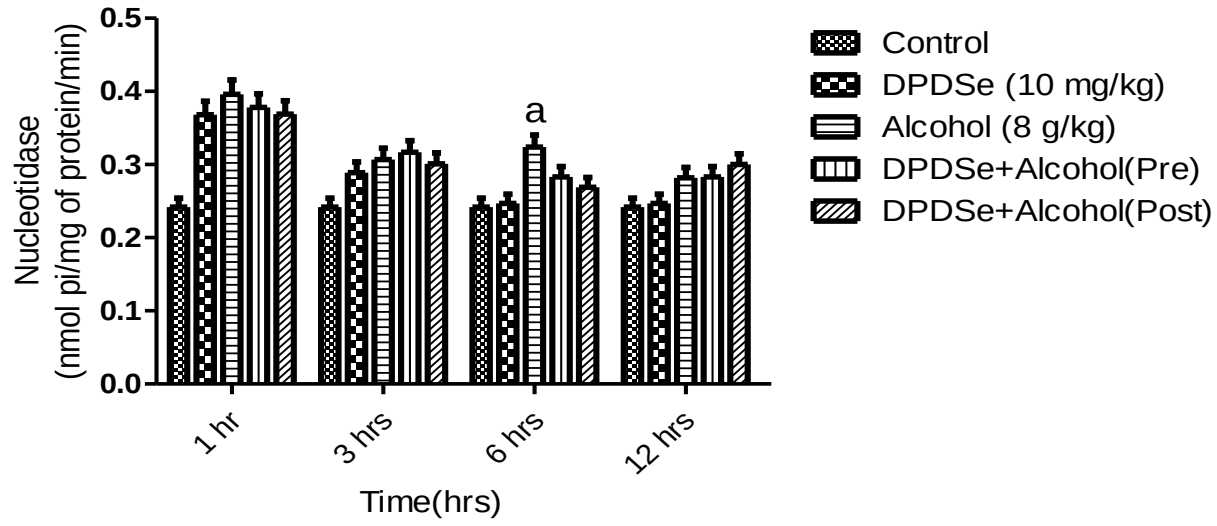

Fig.3.1.6: Influence of DPDSe pre- and post-treatment on the activity of 5'-Nucleotidase in the liver of acute ethanol-intoxicated male Wistar rats at different hours after exposure. $^{a}p<0.05$ significant difference compared to control and ethanol at each hour of treatment.

Article DOI: 10.52589/AJBMR-9BMKGOLK
DOI URL: https://doi.org/10.52589/AJBMR-9BMKGOLK

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 7, Issue 4, 2024 (pp. 1-11)

www.abjournals.org

DISCUSSION

Most of the earlier reports on acute ethanol intoxication have shown that ethanol intoxication occurs through oxidative stress (Tsermpini *et al*., 2022), this is partly due to its metabolism course through alcohol and acetaldehyde dehydrogenase which includes more generation of ROS by enhanced activity of respiratory chain. (Gonthier and Barret., 1991, Nordmann *et al*., 1992, Wu and Cedarbaum, 2003). One of the methods of evaluating oxidative damage resulting from the overproduction of ROS is measuring the antioxidant capacity of the system before and after exposure to pro-oxidants. One of the classes of endogenous antioxidant system that help in scavenging free radicals is thiol which may be non-protein thiol (often glutathione) or total protein thiol, thiols in all their forms serve a protective role against oxidative stress. The present study shows that the non-protein thiol level of ethanol-intoxicated animals increased at 1hr after administration and decreased after 3hrs and 6hrs of administration. This suggests that at 1hr, there is a release of more non-protein thiol as part of the initial defence mechanism put up against the massive production of ROS induced by ethanol intoxication, however, as more of the oxidants are being produced with progression in time, the thiol level reduces and the reduction is maximum at 6hrs. However, pre- and post-treatment with DPDSe mitigates the effect on thiol at all these different hours. This same pattern of result was obtained in the total protein thiol (TPT) assay, except that the TPT level was not high at time 1hr as was observed in the NPT assay.

Following the depletion of the endogenous shield of antioxidants in the cells, cells are left vulnerable to oxidative damage of different macromolecules in the cell body which includes lipids, proteins, carbohydrates and DNA which may result in various pathologies depending on the severity of the damage. Lipid peroxidation is one of the measures of oxidative damage to lipids in the biological system, it occurs when the ROS interact with lipids within cell membranes to form lipid radicals which react with other susceptible molecules in a chain reaction that causes cell damage. Evaluation of TBARS is one of the measures of lipid peroxidation, this is because malondialdehyde (MDA), a byproduct of lipid peroxidation usually reacts with TBARS to form a compound that is easily read spectrophotometrically at 532nm. In this study, administration of ethanol did not lead to an increase in the production of TBARS at 1hr and 12hrs, however, more TBARS was produced at 3hrs and 6 hrs after exposure with more effect at 6hrs, pre- and post-treatment with DPDSe lowers the oxidative damage mostly at time 3hrs and 6hrs after exposure to ethanol, this result corroborates the result obtained in the thiol assay which means at hours of exposure (3hrs and 6hrs) where the thiol level is lowest is when there is more oxidative damage to lipids in the form of TBARS-MDA adduct. This confirms the earlier reports that oxidative stress is one of the mechanisms of intoxication of ethanol (Metro *et al*., 2022, Vucevic *et al*., 2013) and that the time of exposure also has a role to play in the extent of the intoxication.

Since endogenous thiol forms an integral part of the antioxidant capacity of the cell and the ROS have been found to almost always decrease this protective molecule, it is then imperative to investigate the possible effects of ethanol intoxication on enzymes which are sulphydryl proteins. δ-aminolevulinc acid dehydratase (ALA-D). Delta-aminolevulinic acid dehydratase (ALA-D) is an enzyme sensitive to oxidative stress, and its activity is often used as a marker for oxidative damage, particularly in the context of heavy metal toxicity and oxidative insults (La-Llave-León *et al*., 2017) The reduction in ALA-D activity observed following ethanol intoxication is consistent with the idea that ROS can inhibit enzyme activity by modifying thiol groups essential for enzymatic function (Checa *et al*., 2020). However, the reduction in ALA-

Article DOI: 10.52589/AJBMR-9BMKGOLK
DOI URL: https://doi.org/10.52589/AJBMR-9BMKGOLK

D activity following DPDSe treatment also suggests that while DPDSe can mitigate oxidative damage, it may also interact with the enzyme's active sites, potentially modulating its activity. The elevation in NTPDase and Nucleotidase activities observed in this study following ethanol intoxication suggests an adaptive response to maintain nucleotide homeostasis under oxidative stress conditions. NTPDases and Nucleotidases play critical roles in the regulation of extracellular nucleotide levels, which are important for cellular signaling and energy metabolism. The increase in these activities could be a compensatory mechanism to counteract the disrupted nucleotide balance caused by ethanol-induced oxidative stress.

REFERENCES

[1] Boby N, Lee EB, Abbas MA, Park NH, Lee SP, Ali MS, Lee SJ, Park SC (2021), Ethanol-Induced Hepatotoxicity and Alcohol Metabolism Regulation by GABA-Enriched Fermented *Smilax china* Root Extract in Rats, Foods 10(10):2381. doi: 10.3390/foods10102381. PMID: 34681429; PMCID: PMC8535858.

[2] Carton L, Auger F, Kyheng M, Pétrault M, Durieux N, Allorge D, Cottencin O, Jardri R, Bordet R and Rolland B. (2019), Dose-dependent metabolite changes after ethanol intoxication in rat prefrontal cortex using in vivo magnetic resonance spectroscopy. Scientific Reports 9. https://doi.org/10.1038/s41598-019-47187-4

[3] Checa, J., & Aran, J. M. (2020). Reactive Oxygen Species: Drivers of Physiological and Pathological Processes. *Journal of Inflammation Research, 13*, 1057-1073. https://doi.org/10.2147/JIR.S275595

[4] Ellman GL (1959), Tissue sulphydryl groups, Archives of Biochemistry and Biophysics 82:70–77, https://doi.org/10.1016/0003-9861(59)90090-6.

[5] Fiske CH, SubbaRow Y (1925), The colorimetric determination of phosphorus, Journal of Biological Chemistry 66 (1925)375- 381.

[6] Flora SJ, Gautam P and Kushwaha P (2012), Lead and Ethanol Co-Exposure Lead to Blood Oxidative Stress and Subsequent Neuronal Apoptosis in Rats, Alcohol and Alcoholism, 47(2), 92-101. https://doi.org/10.1093/alcalc/agr152

[7] Gonthier B, Jeunet A, Barret L (1991), Electron spin resonance study of free radicals produced from ethanol and acetaldehyde after exposure to a fentom system or to brain and liver microsomes. Alcohol 8:369–375; 1991.

[8] Hernández JA, López-Sánchez RC, Rendón-Ramírez A (2016). Lipids and Oxidative Stress Associated with Ethanol-Induced Neurological Damage, Oxidative Medicine and Cellular Longevity 2016:1543809. doi: 10.1155/2016/1543809PMID: 26949445; PMCID: PMC4753689.

[9] Heymann D, Reddington M, Kreutzberg GW (1984) Subcellular localization of 5'-nucleotidase in rat brain, *Journal of Neurochemistry* 43:263-273

[10] Ibrahim BM, Fan M and Abdel-Rahman AA. (2014). Oxidative Stress and Autonomic Dysregulation Contribute to the Acute Time-Dependent Myocardial Depressant Effect of Ethanol in Conscious Female Rats. Alcoholism: Clinical and Experimental Research, 38(5), 1205–1215. doi:10.1111/acer.12363

[11] Jegal KH, Park HR, Choi BR, Kim JK, Ku SK (2023), Synergistic Protective Effect of Fermented Schizandrae Fructus Pomace and Hoveniae Semen cum Fructus Extracts Mixture in the Ethanol-Induced Hepatotoxicity, *Antioxidants 12*: 1602. https://doi.org/10.3390/antiox12081602

Article DOI: 10.52589/AJBMR-9BMKGOLK
DOI URL: https://doi.org/10.52589/AJBMR-9BMKGOLK

[12] La-Llave-León, O., Méndez-Hernández, E. M., Castellanos-Juárez, F. X., Esquivel-Rodríguez, E., Vázquez-Alaniz, F., Sandoval-Carrillo, A., García-Vargas, G., Duarte-Sustaita, J., Candelas-Rangel, J. L., & Salas-Pacheco, J. M. (2017). Association between Blood Lead Levels and Delta-Aminolevulinic Acid Dehydratase in Pregnant Women. *International Journal of Environmental Research and Public Health*, *14*(4). https://doi.org/10.3390/ijerph14040432

[13] Łuczaj W and Skrzydlewska, E (2004), Antioxidant properties of black tea in alcohol intoxication, Food and Chemical Toxicology, 42(12), 2045–2051. doi:10.1016/j.fct.2004.08.009

[14] Metro D, Corallo F, Fedele F, Buda M, Manasseri L, Buono VL, Quartarone A, Bonanno L (2022), Effects of Alcohol Consumption on Oxidative Stress in a Sample of Patients Recruited in a Dietary Center in a Southern University Hospital: A Retrospective Study, Medicina (Kaunas). 58(11):1670. doi: 10.3390/medicina58111670. PMID: 36422209; PMCID: PMC9698473.

[15] Nordmann R, Ribiere C, Rouach H, Implication of free radical mechanisms in ethanol induced cellular injury. Free Radical.Biology and Medicine 12:219–240; 1992.

[16] Okhawa H, Ohishi H, Yagi K, (1979), Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction, Analytical Biochemistry 95:351-358.

[17] Petronilho F, Michels M, Danielski LG, Goldim MP, Florentino D, Vieira A, Rocha, J. B. T. D. (2016), Diphenyl diselenide attenuates oxidative stress and inflammatory parameters in ulcerative colitis: A comparison with ebselen, Pathology - Research and Practice, 212(9), 755–760. doi:10.1016/j.prp.2016.04.012

[18] Quispe RL, Jaramillo ML, Galant LS, Engel D, Dafre AL, Radi R and Farina M. (2019), Diphenyl diselenide protects neuronal cells against oxidative stress and mitochondrial dysfunction: Involvement of the glutathione-dependent antioxidant system. Redox Biology, 20, 118-129. https://doi.org/10.1016/j.redox.2018.09.014

[19] Rossato JI, Ketzer LA, Centuriao FB, Silva SJN, Ludtke DS, Zeni G, Braga AL, Rubin MA, Rocha JBT (2002), Antioxidant properties of new chalcogenides against lipid peroxidation in rat brain. Neurochemical Research 27:297–303

[20] Reiter HG, Tan RJ., KimD and Cabrera JD. (1999), Inhibitory effect of melatonin on products of lipid peroxidation resulting from chronic ethanol administration, Alcohol and Alcoholism, 34(6): 842-850. https://doi.org/10.1093/alcalc/34.6.842

[21] Souli A, Sebai H, Chehimi L, Rtibi K, Tounsi H, Boubaker S, Sakly M, El-Benna J and Amri M. (2013), Hepatoprotective effect of carob against acute ethanol-induced oxidative stress in rat, Toxicology and Industrial Health. https://doi.org/10.1177/0748233713475506

[22] Teixeira FB, Santana S, Bezerra FR, Carvalho SD, Fontes-Júnior EA, Prediger RD, Crespo-López ME, Ferraz Maia CS and Lima RR (2014), Chronic Ethanol Exposure during Adolescence in Rats Induces Motor Impairments and Cerebral Cortex Damage Associated with Oxidative Stress. *PLOS ONE*, *9*(6) e101074. https://doi.org/10.1371/journal.pone.0101074

[23] Tsermpini EE, Plemenitaš Ilješ, A, Dolžan V (2022), Alcohol-Induced Oxidative Stress and the Role of Antioxidants in Alcohol Use Disorder: A Systematic Review. *Antioxidants 11*:1374. https://doi.org/10.3390/antiox11071374

Article DOI: 10.52589/AJBMR-9BMKGOLK
DOI URL: https://doi.org/10.52589/AJBMR-9BMKGOLK

[24] Vetreno RP, Campbell J and Crews FT (2023), A multicomponent ethanol response battery across a cumulative dose ethanol challenge reveals diminished adolescent rat ethanol responsivity relative to adults. Advances in Drug and Alcohol Research. 3:11888. doi: 10.3389/adar. 11888

[25] Vučević D, Mladenović D, Ninković M. (2013), Influence of aging on ethanol-induced oxidative stress in digestive tract of rats, Human & Experimental Toxicology. 32(7):698-705. doi:10.1177/0960327112467045

[26] Wang J., Du H., Jiang L., Ma X., de Graaf RA., Behar KL and Mason G F. (2013), Oxidation of ethanol in the rat brain and effects associated with chronic ethanol exposure, Proceedings of the National Academy of Sciences, 110(35): 14444–14449. doi:10.1073/pnas.1306011110

[27] Wang X., Huan Y., Liu S., Li C., Cao H., Lei L., Liu Q., Ji W., Sun S., Huang K., Zhou J. and Shen Z. (2022), Diphenyl Diselenide Alleviates Tert-Butyl Hydrogen Peroxide-Induced Oxidative Stress and Lipopolysaccharide-Induced Inflammation in Rat Glomerular Mesangial Cell, International Journal of Molecular Sciences *23*(19). https://doi.org/10.3390/ijms23191121

[28] Wu D, Cederbaum AI (2003) Alcohol, oxidative stress, and free radical damage. Alcohol Research and Health. 27(4):277-84. PMID: 15540798; PMCID: PMC6668865.

Article DOI: 10.52589/AJBMR-9BMKGOLK
DOI URL: https://doi.org/10.52589/AJBMR-9BMKGOLK