Impact of Diphenyl Diselenide on Acute Ethanol-Induced Disruption of Antioxidant Defense and Inflammatory Gene Expression in Rats

ABSTRACT

Perturbation in antioxidant status which often leads to inflammation of liver cells and alteration in activities of purinergic enzymes are common outcomes of ethanol intoxication,this makes any compound that is able to prevent or repair the redox imbalances induced by alcohol intoxication a potential therapeutic measure. Hence, this study aims to assess the effect of diphenyl diselenide

(DPDSe) on thiol redox system, inflammatory genes and activities of purinergic enzymes in ethanol intoxicated male Wistar rats. 10mg/kg DPDSe was administered orally 30 minutes before and after a single dose of 13 ml/kg (28% ethanol solution) was given to the rats. Thereafter, the antioxidant status, activities of purinergic enzymes and expression of redox-sensitive genes in the rats were evaluated. It was observed that ethanol evoked high production of lipid peroxidation, increased Nucleotidase and NTPDase activities with concomitant decrease in thiol level, it also causes the downregulation of Nrf2 and upregulation of (NF-kB), however, treatment with DPDSe caused a reversal of these effects on the biochemical and molecular parameters evaluated. The reversal of ethanol-induced changes in biochemical parameters, expression of genes linked to inflammation and antioxidant status in the liver of acute ethanol intoxicated rats by DPDSe suggests that it has high prospect as a suitable therapeutic agent for hepatotoxicity linked with acute ethanol intoxication.

INTRODUCTION

Ethanol intoxication has complex effects on living cells. To explore the molecular events associated with ethanol intoxication, several studies have focused on the role of redox-sensitive genes, such as Nrf2 and NF-kB. These genes have been shown to be modulated by ethanol, contributing to its inflammatory effects [26, 25]. Another mechanism by which ethanol induces toxicity is through the alteration of enzymes in the purinergic pathway. Enzymes such as 5'-nucleotidase and nucleoside triphosphate diphosphohydrolases(NTPDase), which are critical indicators of liver disease, are significantly affected by ethanol intoxication [19]. Research has documented that ethanol increases the activity of these enzymes in the platelet cell membrane, as they are sensitive to redox imbalances, with heightened activity observed during acute ethanol intoxication [5].

Although endogenous antioxidant systems provide some protection against oxidative damage, the excessive production of free radicals can overwhelm these systems, necessitating external sources of antioxidants. Exogenous antioxidants, such as flavonoids found in plants, have been shown to mitigate the effects of ethanol intoxication [6, 14]. Specifically, studies have demonstrated the protective effects of quercetin and green tea extracts on diseases linked to ethanol intoxication by reducing lipid oxidative damage and enhancing antioxidant enzyme activity [30, 29, 14]. These findings confirm that oxidative stress is a key mechanism of ethanol

intoxication [4, 27], and the protective effects of phytochemicals are primarily due to their antioxidant capabilities. However, the use of plant-derived antioxidants poses challenges, including the potential for oxidation in the presence of oxygen and contamination with metals, which can turn them into pro-oxidants [16].

To address these issues, synthetic antioxidants have been explored and have shown effectiveness in reducing oxidative stress-related pathologies [3, 9]. For example, research into the antioxidative properties of organoselenium compounds has gained traction, as glutathione peroxidase (GPx), a potent endogenous enzymatic antioxidant, relies on selenocysteine at its active site for activity [23]. Diphenyl diselenide, one of the most studied organoselenium compounds, has demonstrated significant antioxidant properties [12, 17] and has been used to alleviate diseases related to oxidative stress [11, 12]. Studies have confirmed its ability to mimic glutathione peroxidase activity [21, 13], helping to reduce hydrogen peroxide and lipid peroxides to water and corresponding alcohols, similar to the action of endogenous GPx.

While diphenyl diselenide has been applied in the management of various oxidative stress-related pathologies, its effects on acute ethanol intoxication in male Wistar rats are not well understood. Therefore, this study aims to investigate the potential influence of diphenyl diselenide on acute ethanol intoxication in the liver of male Wistar rats.

2.0 MATERIALS AND METHODS

2.1 Chemicals

Diphenyl diselenide, trichloroacetic acid (TCA), Dithiothretol (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.

2.2 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.

2.3 Estimation of ethanol influence on thiol oxidation induced by DPDSe *in vitro.*

The influence of ethanol on the *in vitro* activity of diphenyl diselenide (DPDSe) induced thiol oxidation was carried out by measuring the formation of 2-nitro-5-thiobenzoic acid (TNB) at 412 nm.

2.4 Experimental Design (*in vivo*)

The study of DPDSe influence on acute intoxication by ethanol after 6 hours of treatment was carried out. The rats were divided into four groups (n=6) and allowed to acclimatize 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, group 4 was pretreated with 10 mg/kg DPDSe before induction with a single oral dose of 10ml/kg of 28% ethanol solution, after 30 minutes of pretreatment with DPDSe, the experiment was terminated 6 hours after the treatment with ethanol.

2.5 Lipid peroxidation assay

Lipid peroxidation was measured as thiobarbituric acid reactive substances (TBARS). TBARS were determined in tissue homogenates as previously describ*ed* [18,22]. MDA values were determined using the absorbance coefficient (1.56 · 105 /cm/mmol) of the MDA–TBA complex at 532 nm.

2.6 Thiol oxidation

The thiol oxidation was determined in the presence of 50 mM Tris HCl pH 7.4 in both proteinized and deproteinized samples. The rate of thiol oxidation was evaluated by measuring the disappearance of SH-group. The free SH-group was determined by [7].

2.7 5’-Nucleotidase and NTPDase like activities.

The 5’-Nucleotidase activity was determined in a reaction medium essentially as described by [10]. 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 [8]. Control experiments were carried 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.

2.8 Gene expression analysis using real-time quantitative polymerase reaction

TRI Reagent (Zymo Research, USA) was used to extract total RNA from the tissues. 1 mg of RNA was used to make cDNA in a three-step reverse transcriptase reaction with the ProtoScript II First Strand cDNA Synthesis Kit (BioLabs, New England) at 65 °C for 5 min, 42 °C for 1 h, and 80 °C for 5 min. Table 1 lists the rat cDNA primers (InqabaBiotec, Hatfield, SA) that were utilized for PCR. Solis BioDyne Reverse Transcriptase RT qPCR System and FIREPol® Master Mix (BioLabs, New England) were used to perform real-time quantitative PCR (RT-qPCR) according to the manufacturer's instructions. The following were the PCR conditions: 95 °C for 3 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. The relative quantity of cDNA was measured using the comparative cycle threshold (DDCT) method. The relative expression level of each gene was normalized using the β-actin gene.

2.9 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 analyze 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).

TABLE 1

Primer sequence for Real - time quantitative PCR

S/N	Gene	Sequence
1	β-actin	Forward: CTCCCTGGAGAAGAGCTATGA
		Reverse: AGGAAGGAAGGCTGGAAGA
2	NFKB	Forward: AGACATCCTTCCGCAAACTC
		Reverse: TAGGTCCATCCTGCCCATAA
3	Nrf2	Forward: ACGTGATGAGGATGGGAAAC

Reverse: TATCTGGCTTCTTGCTCTTGG

3.0. RESULTS

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

3.1.1 Influence of DPDSe on ethanol-induced reduction in total thiol level *in vivo*

Fig. 1 shows that ethanol intoxication causes reduction in total thiol level and that both pre-and post-DPDSe treatment markedly ($p<0.05$) reverse the ethanol- induced decrease in the *in vivo* thiol level.

3.1.2Influence of DPDSe on ethanol-induced lipid peroxidation

Fig. 2 shows that DPDSe pre-treatment markedly mitigates ethanol induced lipid peroxidation in ethanol intoxicated rats while post- treatment has no significant effect on lipid peroxidation induced by ethanol intoxication.

3.1.3Influence of DPDSe on ethanol-induced reduction in non-protein thiol

Fig. 3 shows that ethanol intoxication causes reduction in non-protein thiol and that both pre- and post- DPDSe treatments markedly reverse the ethanol- induced decrease in the *in vivo* thiol level.

3.1.4Influence of DPDSe on ethanol-induced elevated NTPDase activity

Fig. 4 shows that ethanol intoxication causes elevation in NTPDase activity and that both pre- and post- DPDSe treatments markedly decreases the activity of the enzyme.

3.1.5Influence of DPDSe on ethanol-induced elevated Nucleotidase activity

Fig. 5 shows that ethanol intoxication causes elevation in Nucleotidase activity and that both pre- and post-DPDSe treatments markedly decrease the activity of the enzyme.

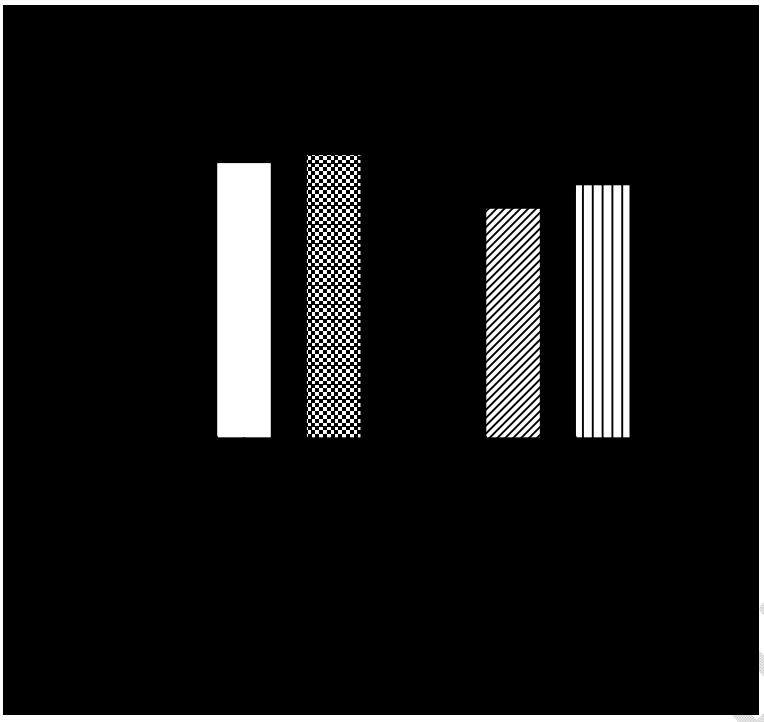


Fig.1Influence ofDPDSe pre- and post- treatment on total thiol level in liver of acute ethanol-intoxicated male Wistar rats. [a]p<0.0001 significant difference compared to control [b]p<0.0001 significant difference compared to DPDSe [C]p<0.0001 significant difference compared to ethanol.

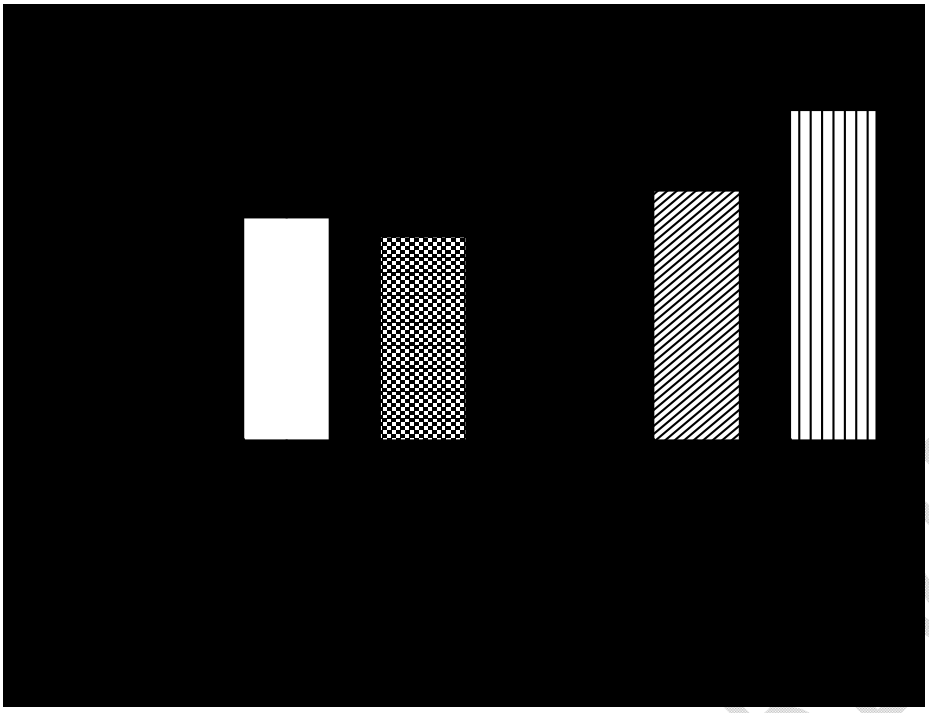


Fig.2Influence ofDPDSe pre- and post- treatment on lipid peroxidation in liver of acute ethanol-intoxicated male Wistar rats. [a]p<0.0001 significant difference compared to control [b]p<0.0001 significant difference compared to DPDSe [C]p<0.0001 significant difference compared to ethanol.

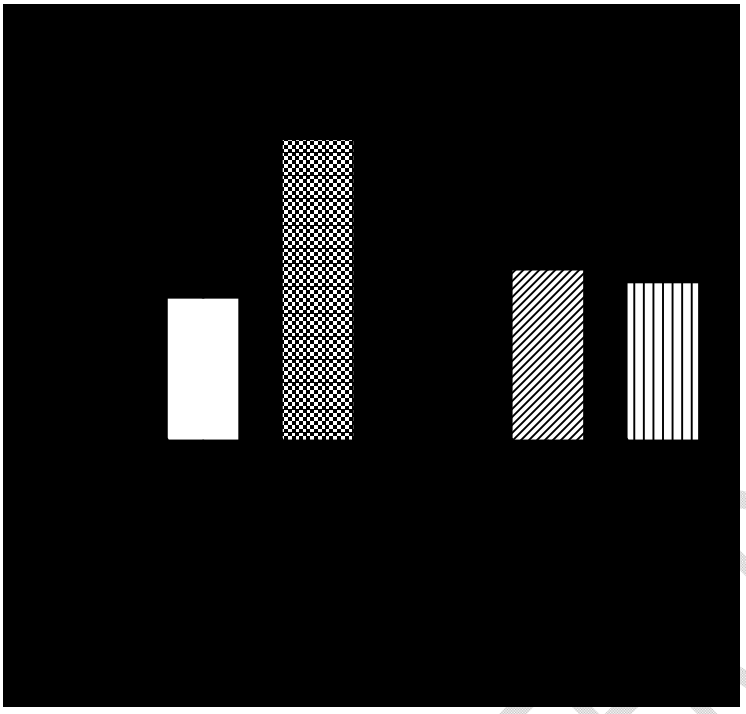


Fig.3Influence ofDPDSe pre- and post- treatment on non-protein thiol level in liver of acute ethanol-intoxicated male Wistar rats. [a]p<0.0001 significant difference compared to control [b]p<0.0001 significant difference compared to DPDSe [C]p<0.0001 significant difference compared to ethanol.

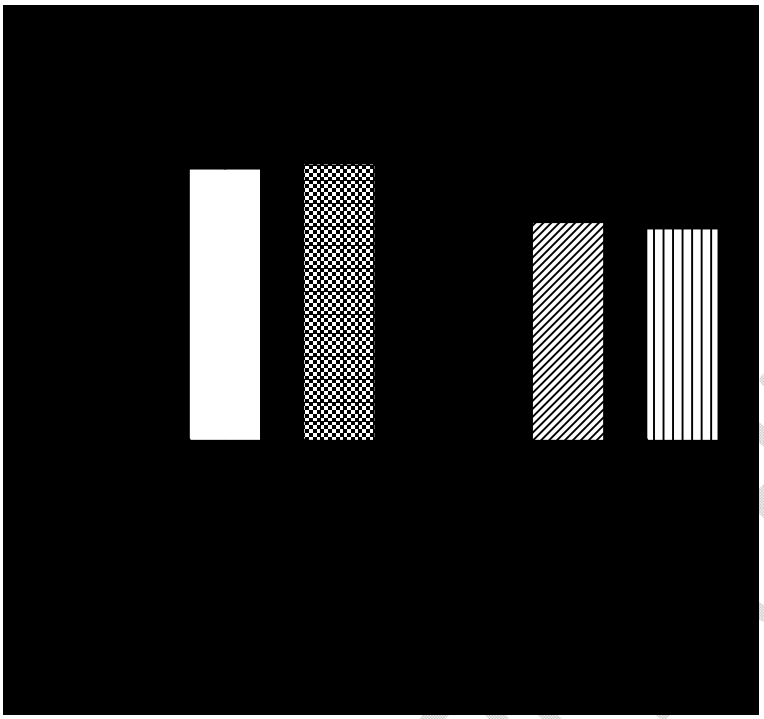


Fig.4Influence ofDPDSe pre- and post- treatment on activity of NTPDase in liver of acute ethanol-intoxicated male Wistar rats. [a]p<0.0001 significant difference compared to control [b]p<0.0001 significant difference compared to DPDSe [C]p<0.0001 significant difference compared to ethanol.

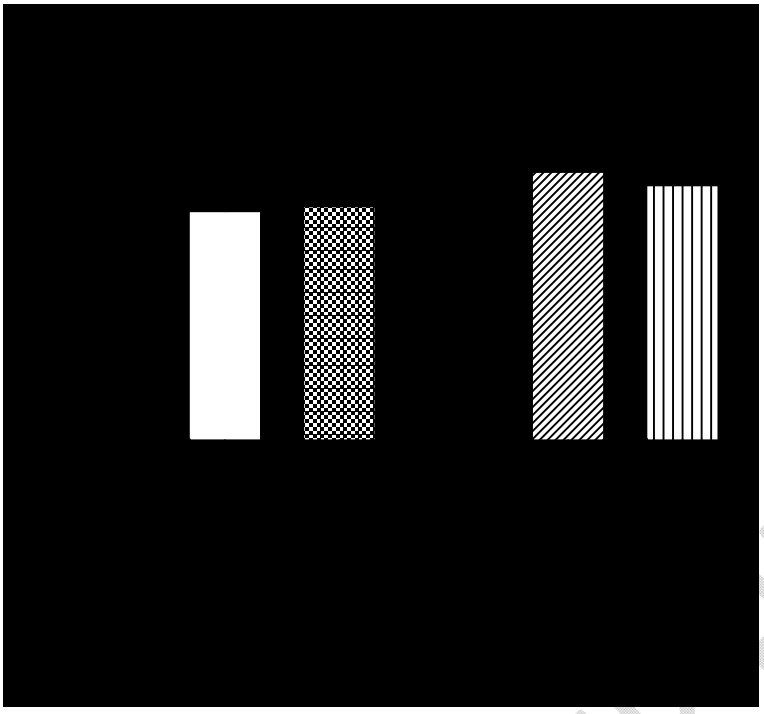


Fig.5Influence ofDPDSe pre- and post- treatment on the activity of Nucleotidase in liver of acute ethanol-intoxicated male Wistar rats. [a]p<0.0001 significant difference compared to control [b]p<0.0001 significant difference compared to DPDSe [C]p<0.0001 significant difference compared to ethanol.

3.2 INFLUENCE OF DIPHENYL DISELENIDE AND ALCOHOL ON EXPRESSION OF ANTIOXIDANT AND PRO-INFLAMMATORY GENES.

3.2.1Influence of DPDSe on ethanol-induced downregulation of Nrf2

Fig. 6 shows that ethanol intoxication causes a downregulation in the expression of Nrf2 and that post treatment of the animals with DPDSe markedly upregulate the expression of the gene while the DPDSe pretreatment do not have any stimulatory effect on the expression of the gene.

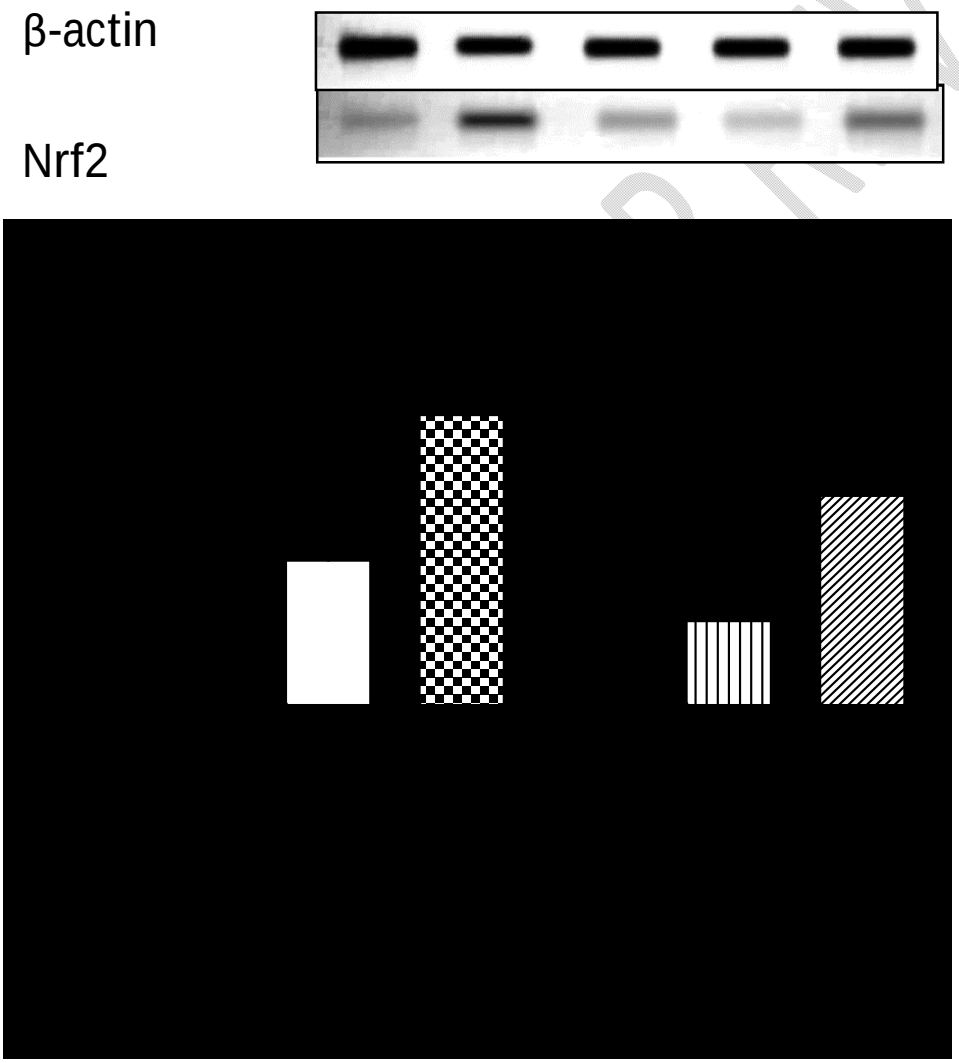
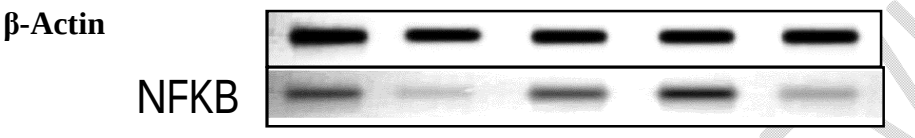


Fig. 6 Influence ofDPDSe pre- and post- treatment on Nrf2 in liver of acute ethanol-intoxicated male Wistar rats. [a]p<0.0001 significant difference compared to control [b]p<0.0001 significant difference compared to DPDSe [C]p<0.0001 significant difference compared to ethanol.



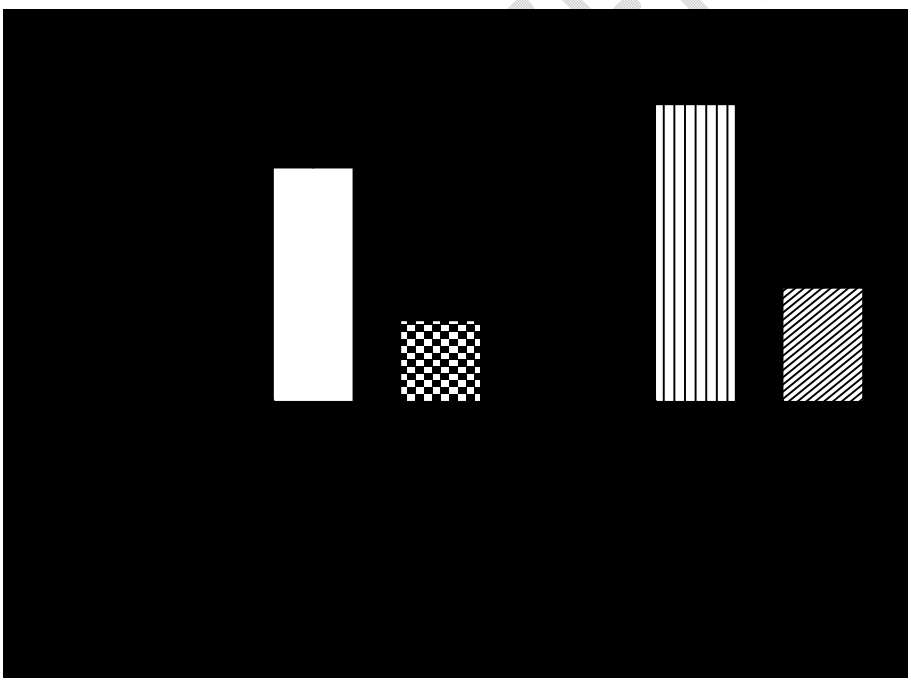


Fig. 7Influence ofDPDSe pre- and post- treatment on NFKB in liver of acute ethanol-intoxicated male Wistar rats. [a]p<0.0001 significant difference compared to control

[b]p<0.0001 significant difference compared to DPDSe [C]p<0.0001 significant difference compared to ethanol.

DISCUSSION

Redox imbalance is a hallmark of ethanol intoxication in the liver of male Wistar rats, and diphenyl diselenide (DPDSe) has been shown to improve antioxidant status, reverse altered activities of purinergic enzymes, and modulate the expression of redox-sensitive genes. When the redox balance shifts in favor of oxidants, macromolecules such as lipids, proteins, and DNA are vulnerable to oxidative damage, which is a key indicator of oxidative stress [1, 24]. Lipid oxidative damage occurs when reactive oxygen species (ROS) abstract hydrogen from polyunsaturated fatty acids (PUFAs), leading to lipid peroxidation and subsequent cell membrane damage. This process releases malondialdehyde (MDA) and other reactive aldehydes, which form adducts with thiobarbituric acid (TBA). The MDA-TBA adduct can be measured spectrophotometrically at 532 nm to quantify the extent of lipid peroxidation. In this study, elevated thiobarbituric acid reactive substances (TBARS) were observed in ethanol-treated animals, which were significantly reduced by DPDSe treatment. The high TBARS levels in the ethanol group indicate that lipid biomolecules were oxidatively damaged, while the reduction in TBARS in the DPDSe-treated group suggests that DPDSe either enhanced antioxidant defense or repaired oxidative damage by donating hydrogen to stabilize PUFAs.

In addition to measuring oxidative stress through lipid peroxidation and antioxidant capacity, assessing the activities of redox-responsive enzymes offers further insight. 5’-nucleotidase (5NT) and nucleoside triphosphate diphosphohydrolases (NTPDases) are crucial enzymes involved in nucleotide metabolism and are known to respond to oxidative stress. Their dysregulation can lead to various pathological conditions, making them targets for therapeutic interventions. Increased activities of these enzymes have been linked to ethanol-induced oxidative stress, with serum 5NT levels being a marker of hepatobiliary disease [19]. Consistent with previous studies, this research demonstrated increased activities of 5’-nucleotidase and NTPDase in the liver of ethanol-intoxicated rats, likely due to elevated levels of NADH driving ATP production and, in turn, increasing purinergic enzyme activity. However, DPDSe treatment reduced the activities of these enzymes.

To investigate the mechanism by which DPDSe mitigates oxidative damage, the antioxidant capacity of untreated ethanol-intoxicated animals was compared with pre- and post-DPDSe-treated animals by measuring reduced glutathione (GSH) levels. Since over 90% of non-protein thiols in rats are GSH [20], the observed decrease in thiol levels in ethanol-treated animals suggests that thiols were consumed in stabilizing ethanol-induced free radicals. This finding aligns with previous studies showing that thiol oxidation is a key event in ethanol intoxication [28]. Both pre- and post-DPDSe treatments elevated thiol levels, indicating that DPDSe either prevented thiol depletion or repaired oxidative damage by halting lipid peroxidation, thus sparing thiol groups. This confirms that ethanol intoxication is linked to oxidative stress and supports the antioxidant and hepatoprotective potential of DPDSe in mitigating ethanol-induced liver damage.

Many endogenous antioxidants are gene products, and the protective effects of DPDSe against ethanol-induced oxidative stress may also occur at the molecular level. To explore this, the expression of the antioxidant gene Nrf2 was evaluated. Nrf2 is crucial in regulating antioxidant defenses and responding to oxidative insults [15]. In ethanol-intoxicated rats, Nrf2 expression was downregulated, indicating that ethanol impairs the cell's endogenous defense system at the molecular level, consistent with previous reports [2]. However, DPDSe treatment restored Nrf2 expression, showing that DPDSe can modulate cellular defense systems compromised by ethanol intoxication.

To further understand the molecular effects of ethanol on inflammation, the expression of nuclear factor kappa B (NF-kB), a key component of the inflammatory pathway, was assessed. Ethanol intoxication upregulated NF-kB expression, while post-treatment with DPDSe reduced its expression, highlighting the anti-inflammatory potential of DPDSe.

Overall, this study demonstrates that DPDSe significantly reduces oxidative stress markers such as TBARS, downregulates NF-kB expression, increases total and non-protein thiol levels, and upregulates Nrf2 expression in the liver of ethanol-intoxicated rats. These findings confirm DPDSe's potent antioxidant and anti-inflammatory properties, effectively reversing ethanol-induced oxidative damage.

Conclusion

Acute ethanol exposure remains a challenge due to its well-documented toxicity, despite its known health benefits. This study reveals several toxic mechanisms of ethanol, including thiol oxidation, lipid peroxidation, downregulation of the antioxidant gene Nrf2, and upregulation of inflammatory pathways via NF-kB. Diphenyl diselenide (DPDSe) has emerged as a promising agent for mitigating these toxic effects. It effectively reverses ethanol-induced oxidative stress by modulating redox-sensitive transcription factors such as Nrf2 and NF-kB, reducing oxidative stress markers like TBARS, and enhancing antioxidant capacity by preserving non-protein and total thiol levels. Additionally, DPDSe ameliorates the effects on purinergic enzymes, further supporting its potential as a therapeutic agent in managing the oxidative damage and inflammation associated with acute ethanol intoxication.

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