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The potential anti-inflammatory and anti-apoptotic influence of hydrogen sulfide on xenoestrogen induced-toxicity in rats

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ABSTRACT

Hydrogen sulphide (H_2S) is a gas transmitter that plays a vital role in a variety of physiological and pathological processes. Xenoestrogen (XE) a regularly used manufacturing chemical, has been related to endocrine disruptor. The purpose of this study was to investigate the effects of H_2S donor (sodium hydrogen sulfide [NaHS]) on xenoestrogen (XE)-induced toxicity in rats. Twenty-four adult female rats were divided into three groups (8 rats /group): group I: control group, group II: rats were intraperitoneally (IP) injected with XE (150 mg/kg body weight/ day) dissolved in corn oil for 4 weeks, group III: rats were IP injected with H_2S donor NaHS intraperitoneally (IP) at daily dose of 5 mg/kg body weight/day dissolved in deionized water for 6 weeks and were injected with XE as group II. Biomarkers including lactate dehydrogenase (LDH), Interleukin 6 (IL- 6), inducible nitric oxide synthase (iNOS), hypoxia-inducible factor 1 alpha (HIF 1α), caspase-3 were determined and lymphocytes %. The obtained results revealed that; in group 3, a significant decrease in serum LDH, lymphocytes %, iNOS, with marked decrease in serum Caspase-3 when compared to group II. These results suggests that H_2S might ameliorate XE induced-toxicity *via* its anti-inflammatory and anti-apoptotic effects.

1. INTRODUCTION

Endocrine disrupters are defined as an external chemical that alters the activities of the endocrine system and generates undesirable consequences in an exposed organism (Vandenberg et al., 2010). Xenoestrogens (environmental estrogens) are substances that have endocrine disrupting effects and activate estrogen receptors. These chemicals exhibit estrogen-like effects but differ in structure (Hoekstra and Simoneau, 2013). They might be artificial or natural chemical substances. Some commonly used industrial ingredients in synthetic XE include bisphenol-A (BPA), an estrogenic substance that can interact with human estrogen receptors (ER). Even at low concentrations, BPA has an endocrine disrupting effect. BPA is a chemical that is used in container linings and plastics such as infant bottles (Jahanshahi et al., 2022). BPA has been linked to premature sexual maturation, changed behavior, and effects on the prostate and mammary glands. XE has been linked to cardiovascular illness, diabetes, and male sexual dysfunction among employees who have been exposed to it. XE may pollute the environment in substantial levels by leaching from items and as industrial byproducts (Niu et al., 2012).

Hydrogen sulphide (H2S) is a gas transmitter that is involved in a variety of physiological and pathological activities. The third gas transmitter in mammals is hydrogen sulphide (H_2S), which is created by three enzymes: cystathionine-synthase (CBS), cystathionine-lyase (CSE), and 3-mercaptopyruvate sulphur transferase (3-MST) (Guan et al., 2018). H_2S has powerful anti-oxidant, anti-inflammatory, and other physiological regulatory effects and is a key biological element in the response to damage in many organs such as the lungs, liver, heart, and adrenal gland (Murphy et al., 2019). Endogenous H_2S regulates critical respiratory tract physiological activities such as airway tone, pulmonary circulation, cell proliferation, and apoptosis, as well as organ fibrosis, oxidative stress, and inflammation. An intact H_2S-generating system appears to have an essential disease-prevention function (Zhang et al., 2016).

The aim of the present study is to investigate the harmful complications of xenoestrogen mediated inflammatory response, as well as the antioxidant and anti-inflammatory effects of hydrogen sulfide on xenoestrogen (XE)-induced toxicity in rats

2. MATERIAL AND METHODS

2.1. Approval Ethics

The experimental protocol was carried out according to ARRIVE guidelines (serial: 18–2019) as approved by the Animal Ethical Committees of Benha University with an ethical approval number (BUFVTM05-01–22).

2.2. Chemicals

Xenoestrogen (Cat # 80–05-7), NaHS (Cat # 161527) and all other chemicals and reagents were obtained from Sigma-Aldrich Chemical Co., St. Louis, MO, USA.

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2.3. Experimental animals

Twenty-four 5 weeks-old Wister female rats (150±20 g) were obtained from the National Centre for Radiation Research and Technology (NCCRT), Cairo, Egypt. Rats were kept in a pathogen-free environment with specialized air conditioning, a 12:12 daylight/darkness cycle, and unrestricted access to food and drink.

2.4. Experimental design

After a week of acclimatization, all rats were randomly divided into three equal groups (8 rats/group) as follows: Group I (Control): untreated rats, group II (Xenoestrogen, XE): rats received xenoestrogen (150 mg/kg body weight/day) intraperitoneally (IP), dissolved in corn oil, for 4 weeks (Yamaguchi et al., 2016), group III (XE + H_2S): rats were treated with H_2S donor NaHS intraperitoneally (IP) at a daily dose of (5 mg/kg body weight/ day) dissolved in deionized water for 6 weeks (Xuan et al., 2012) and were injected with XE as group II for 4 weeks.

2.5. Sampling

At the end of the experiment, the rats were decapitated and blood samples for serum separation and left to clot. Then, centrifuged at 3000 rpm for 15 min at 4 ℃, and all sera were collected and stored at − 20 ℃ for subsequence biochemical analysis.

2.6. Biochemical analysis

Determination of serum IL-6 by IL-6 ELISA Kit (Cat NO.: E-UNEL-R0030) (Zhao et al., 2023). Caspases -3 was done by ELISA kits (Cat. NO.: ab39401) (Lai et al., 2022). Serum iNOS levels was done using ELISA kit (Elabscience, USA) (Cat.No.: E-EL-R0367) following the manufacturer's instructions. The activity of serum lactate dehydrogenase (LDH) was measured via the method of **(Dito, 1979)**. HIF1-αwas estimated using ELISA commercial kits (Abcam, Cambridge, MA, United States (Cat. NO.: ab275103) (Abd El-Fattah et al., 2022). Lymphocytes counts were determined using a hemocytometer. Lymphocytes % was calculated as their ratio×100 divided by the total number of cells in blood sample.

2.7. Statistical analysis

The statistical analysis was performed using SPSS 20 software package (Analytical Software, USA), one-way ANOVA and the Bonferroni multiple comparison tests. All parameters are expressed as the mean±SE. The criterion for significance was set at $p<0.05$.

3. RESULTS

3.1. Effect of H_2S administration on blood lymphocytes % and serum INOS in XE induced -toxicity

As presented in table 1, the exposure of rats to XE resulted in a significant increase in the levels of Lymphocytes and INOS when compared to the control group. On the other hand, significant decreases in Lymphocytes and INOS levels were observed in rats treated with XE+H_2S as compared to XE group.

Table (1) Effect of H_2S administration on blood lymphocytes % and serum INOS in XE induced -toxicity in female rats (Mean± SE) (n=8).

Exp. groups	lymphocytes %	INOS(IU/ml)
Group I: Control	0.36 ± 0.01^{b}	3.1 ± 0.4^{b}
Group II: (XE)	9.7 ± 0.5^{ac}	11.9 ± 0.5^{ac}
Group III:(XE+H2S)	1.9 ± 0.3^{b}	6.1 ± 0.6^{b}

Values with different superscripts within the same column differed significantly at (P≤0.05).

3.2. Effect of H_2S administration on serum caspase 3 and HIF-1α in XE induced -toxicity.

As shown in table 2, the exposure of rats to XE resulted in a significant increase in the levels of caspases 3 and HIF-α when compared to the control group. On the other hand, significant decreases in caspases 3 and HIF-α levels were observed in rats treated with XE+H_2S as compared to XE group.

Table (2) Effect of H_2S administration on serum caspase 3 and *HIF-1α* in XE induced -toxicity in female rats (Mean± SE) (n=8).

Experimental groups	Caspase 3 (pg/ml)	HIF- 1α (pg/ml)
Group I: Control	0.51 ± 0.02^{b}	2451 ± 200^{b}
Group II: (XE)	4.2 ± 0.2^{ac}	$5462\pm360^{\mathbf{ac}}$
Group III:(XE+H2S)	1.16 ± 0.17^{b}	$2780\pm250^{\mathbf{b}}$

Values with different superscripts within the same column differed significantly at (P≤0.05).

3.3. Effect of H2S administration on serum LDH and IL-6 in XE induced -toxicity

As shown in table 3, the exposure of rats to XE resulted in a significant increase in the levels of LDH and IL-6 when compared with the control group. On the other hand, significant decreases in the LDH and IL-6 levels were observed in rats treated with XE+H_2S as compared to XE group.

Table (3) Effect of H2S administration on serum LDH and IL-6 in XE induced -toxicity in female rats (Mean± SE) (n=8).

Experimental groups	LDH (IU/L)	IL-6 (Pg/ml)
Group I: Control	18.9 ± 3^{b}	2415 ± 200^{b}
Group II: (XE)	93.4 ± 5.3^{ac}	$5462\pm360^{\mathbf{ac}}$
Group III:(XE+H2S)	25.3 ± 1.8^{b}	$2780\pm250^{\mathbf{b}}$

Values with different superscripts within the same column differed significantly at (P≤0.05).

4- DISCUSSION

The current study established that H_2S had a pathophysiological role in the stress in the rat model. The obtained findings reveal a link between substantial inflammation in the XE group with the control and XE+NaHS groups in animal models. The current results show the relationship between the significant inflammation in animal model in XE group compared to the control and XE+NaHS groups. This finding is in agreement with previous studies that demonstrated decreased expression of H_2S-synthesizing enzymes in other animal models of stress- and non-stress-induced injury (Guan et al., 2018; Guan et al., 2020; Han et al., 2020). The imbalance between pro-oxidants and antioxidants causes various diseases (Samad et al., 2020). The present study indicates that XE induced oxidative stress in rats as proved by the significant increase in iNOS in comparison with control and rats received XE+NaHS. H_2S can limit ROS either by directly scavenging free radicals, thus regulating oxidative signaling pathways, or by suppressing the inflammatory response (Stein and Bailey, 2013).

The inducible nitric oxide synthase (iNOS) is scarcely present normally but can be expressed during immunological challenge and stress (Elbassuoni and Nazmy, 2018). The significant increase in iNOS levels with XE exposure observed in the current study can be explained by the significant increase of iNOS level that in turn increased NO production in rats exposed to XE in comparison with control and NaHS groups and this result is in agreement with previous studies (Saber et al., 2019). The overexpression of NO with stress results in formation of reactive nitrogen species that is considered to be one of the

leading causes of injury with stress exposure (Elbassuoni and Nazmy, 2018) and as found in the present study.

In the current research, there was a substantial increase in caspases 3level in XE rats compared to the control and NaHS groups. This elevation in serum caspase 3 may be linked to the development of inflammation by activating caspase 3 in death of cells (Predescu et al., 2017).

The caspase family plays a substantial role in mediating the process of apoptosis (Li et al., 2016). According to the present results, there was significantly less value of caspase 3 in NaHS + XE rats as compared to XE rats. This could be attributed to the demonstrated significant greater content of H_2S content and expression of H_2S-synthesizing enzymes that confirm the anti-apoptotic action of H_2S in this study that is in line with another study (Mendes et al., 2019). Similarly, NaHS conferred protection against injury in rats and inhibited lung apoptosis through attenuating the inflammatory response and suppressing oxidative stress (Xu et al., 2013).

Hypoxia-inducible factor 1 alpha (HIF 1α), a critical oxygen sensor that correlates with inflammation. HIF-1α plays an important role in a variety of pathophysiology. HIF-1α protein synthesis can be mediated by a variety of inflammation mediators (Guan et al., 2018). ROS generation caused the upregulation of NF-kB, which in turn led to HIF-1α mRNA induction (Chen et al., 2018) in agreement with the present study. In this study, XE-induced inflammation and cytotoxicity were manifested by increased serum LDH. Confirming that XE induces oxidative stress and inflammation. Injection of NaHS before XE caused a significant decrease in serum LDH which comes in in agreement with the results reported by Abdelrahman et al. (2015).

As well-known inflammatory cytokines IL-6 are involved in various types of inflammatory responses, and play key roles in the inflammatory process. The results showed that the value of serum Il-6 was significantly increased after XE treatment, and NaHS adding display marked decrease in serum IL-6 level. This data is consistent with previous reports (Lin et al., 2022) showed that H_2S exhibited an anti-inflammatory effect on various tissues and organs in mammals (Ali et al., 2018), vascular inflammation (Martelli et al., 2021), cardiovascular inflammation (Sikura et al., 2019; 2020), and airway inflammation (Bazhanov et al., 2017). However, Sağlam et al. (2017) showed that NaHS addition did not show any decrease in IL-6 levels.

5. CONCLUSION

In conclusion, the current study revealed that the H_2S donor (sodium hydrogen sulfide [NaHS]) had a protective effect against an injured rat. Pretreatment with NaHS reversed the effects of XE. This was substantiated by a significant reduction in tissue damage, which was associated by low levels of oxidative stress (iNOS), lower serum HIF-1andapoptotic marker caspase-3. Additionally, NaHS's protective impact may be due to its anti-inflammatory effects.

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