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HYDROGEN SULFIDE ATTENUATES ACONITINE AND BARIUM CHLORIDE INDUCED CARDIAC ARRHYTHMIAS IN RATS

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Abstract: Hydrogen sulfide (H_2S) is an endogenous gaseous messenger suggested to regulate cardiovascular functions. This study evaluates the possible protective effect of H_2S in aconitine and barium chloride ($BaCl_2$) models of arrhythmias in rats. The effects of sodium hydrosulphide (NaHS, i.v.) on electrocardiograph (ECG) patterns, biochemical cardiac markers (creatine kinase-MB isozyme and cardiac troponin I), cardiac histopathology and aconitine (30 µg/kg, i.v.) and $BaCl_2$ (15 mg/kg, i.v.) - induced arrhythmias were studied in rats. NaHS significantly decreased heart rate at doses of 3, 4, and 6, but not 0.8 and 1.2 mg/kg. Aconitine caused 100% ventricular tachycardia (VT), 80% ventricular fibrillation (VF), and 60% mortality after 26±5 sec. NaHS (0.8 mg/kg, i.v.) pretreatment significantly decreased the VT, VF and mortality to 62.5, 25, and 0% respectively and delayed the occurrence of VT by 349±2 sec. Similarly, $BaCl_2$ caused 75% VF and 37.5% mortality after 18 ± 8 sec. NaHS (0.8 mg/kg i.v.) pretreatment significantly decreased VF to 50% without affecting mortality rate. Moreover, NaHS (0.8 mg/kg, i.p., daily for 3 days) had no significant effects on ECG patterns, cardiac biomarkers or histopathology. Our results indicate that H_2S has a protective role against arrhythmias without affecting ECG patterns, cardiac biomarkers or histopathology.

Keywords: Hydrogen sulfide (H_2S), Arrhythmia, Aconitine, Barium chloride, Rat.

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INTRODUCTION

Arrhythmias are a life-threatening problem worldwide, carried by dysfunction of ion channel properties leading to abnormalities in impulse formation and conduction in the myocardium.

Hydrogen sulfide (H_2S) has been recently identified as an endogenously produced gaseous messenger that to regulate cardiovascular functions [1]. The production of H_2S in mammalian systems has been attributed to two key enzymes- the pyridoxal-5′-phosphate dependent enzymes- cystathionine β-synthase (CBS) and cystathionine γ-lyase (CGL or CSE). The distribution of CBS and/or CSE is tissue specific with CSE, but not CBS, is enriched in cardiovascular system [2].

H_2S plays important roles in different systems including the cardiovascular system [3]. H_2S has been shown to decrease heart contractility, left-ventricular pressure development and left ventricular end systolic pressure [4]. Additionally, H_2S produces a negative inotropic effect in the heart [5]. H_2S has been also shown to regulate vascular tone by opening ATP-sensitive potassium channel (K_{ATP}) or blocking L-type Ca^{2+}channels (LTCC) [6;7]. Moreover, it has been reported that H_2S protects the heart against ischemia reperfusion (I/R)-induced arrhythmias, cell injuries and death, contractile dysfunction, and myocardial infarction (MI) [8;9]. Since H_2S has effects on cardiac function, ion channels and vascular tissue, therefore we proposed that its properties may have a protective effect in arrhythmia. This study was undertaken to evaluate the possible protective effect of H_2S in aconitine and barium chloride models of arrhythmias in rats.

METHODS AND MATERIALS

Drugs and chemicals

Sodium hydrosulfide (NaHS), aconitine, barium chloride ($BaCl_2$), pentobarbital sodium and urethane were purchased from Sigma Aldrich chemical Co. (St. Louis, MO, USA).

Experimental animals

Male Sprague Dawley rats, weighing 200 ± 20 g, were purchased from “Egyptian Organization for Biological Products and Vaccines”, Giza, Egypt. The animal care and experiments described in this study comply with the ethical principles and guidelines for the care and use of laboratory animals adopted by the ‘‘Research Ethics Committee’’ of Faculty of Pharmacy, Mansoura University, Egypt which are in accordance with ‘‘Principles of Laboratory Animal Care’’ (NIH publication No. 85-23, revised 1985).

Experimental protocol

Protocol (1): Rats were allocated into 6 groups (8 rats, each). Group (1): Control group, rats receiving equivalent volume of normal saline. Group (2, 3, 4, 5, and 6): rats receiving NaHS (0.8, 1.2, 3, 4, and 6 mg/kg respectively), i.v. bolus injection in jugular vein.

Protocol (2): Rats were divided into 4 groups (8 rats, each) as following: Group (1): Control group, rats receiving normal saline. Group (2): Aconitine group, rat's receiving aconitine (30 µg/kg, i.v.). Group (3): (NaHS 0.8 mg/kg+ aconitine group), rats receiving NaHS (0.8 mg/kg, i.v.) 15 min before aconitine injection. Group (4): (NaHS 1.2 mg/kg+ aconitine group), rats receiving NaHS (1.2 mg/kg, i.v.) 15 min before aconitine injection. Both aconitine and NaHS were injected i.v. as bolous injection in jugular vein.

Protocol (3): Rats were divided into 3 groups (8 rats, each) as following: Group (1): Control group, rats receiving normal saline. Group (2): ($BaCl_2$ group), rat's receiving $BaCl_2$ (15 mg/kg, i.v.). Group (3): (NaHS 0.8 mg/kg+ $BaCl_2$ group), rats receiving NaHS (0.8 mg/kg, i.v.) 15 min before $BaCl_2$ injection. Both $BaCl_2$ and NaHS were injected i.v. as bolous injection in jugular vein.

Protocol (4): Rats were grouped into 2 groups (8 rats, each) as following: Group (1): control group, rats receiving normal saline. Group (2): Rats receiving NaHS (0.8 mg/kg, i.p) daily. After 3 days rats were anaesthetized with pentobarbital Sodium (35 mg/kg, i.p.) and **ECG** was recorded at 0 and end time. Blood samples were collected at 0 and end time from the retro-orbital venous plexus to obtain serum. Additionally hearts were excised immediately for histopathological examination at the end of experimental period.

Electrocardiogram (ECG)

Rats of protocol 1,2 and 3 were anesthetized with urethane (1.8 g/kg, i.p) [10], rats of protocol 4 were anaesthetized with pentobarbital Sodium (35 mg/kg, i.p.) [11] and electrocardiograms were recorded from standard lead II limb leads using a single channel ECG (Fukuda ME Kogyo Co. Ltd., Model: 501-B III, Tokyo, Japan). The electrocardiograph was standardized before each tracing to get: Sensitivity (2 mV pulse produces 20 mm height), with speed 50 mm/sec.

Biochemical parameters in serum:

A: <u>Determination of serum creatine kinase-MB isozyme (CK-MB) activity.</u>

CK-MB activity was determined according to the method of [12] using a commercial kit (Centronic GmbH, Germany). The method is based on measuring CK activity in the presence of an antibody to the CK-M monomer but not affect the activity of CK-B subunits. CK-MB activity was measured at wavelength 340 nm and expressed as a unit per liter (U/L),

B: Determination of serum cardiac troponin I (cTnI) concentration.

Cardiac troponin I (cTnI) concentration was determined according to the method of [13] using a solid-phase, enzyme-labeled chemiluminescent immunometric assay (Immulite 1000 Troponin I, Siemens Medical Solutions Diagnostics, California, USA). The solid phase is coated with monoclonal murine anti-troponin I antibody. The liquid phase consist of alkaline phosphatase conjugated to polyclonal goat anti-troponin I antibody (Immulite 1000 Troponin I, Siemens). cTnI concentrations was expressed as (ng/ml)

Histopathological examination:

At the end of the experiment, the heart was rapidly dissected out and washed immediately with saline and fixed in 10% buffered formalin. The fixed tissues were embedded in paraffin and serial sections (5 µm thick) were cut. Each section was stained with hematoxylin and eosin (H&E). The analyses were performed microscopically (Leica Imaging Systems, Cambridge, UK); the images were analyzed with a specific software (ImageQuant, Leica). The pathologist performing histopathological evaluation was blinded to the treatment assignment of different study groups.

STATISTICAL ANALYSIS

Data are expressed as mean ± standard error of the mean (SEM), where n= no. of rats. Statistical analysis was carried out using two-way ANOVA followed by *Bonferroni post hoc* tests, *paired t, or* chi-square test where appropriate. The level of significance was set at ($p < 0.05$). Statistical tests and graphs were performed with GraphPad Prism V 5.02 (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

Effect of NaHS on normal heart rate of rats:

Injection of NaHS at doses of 3 mg/kg, 4 mg/kg, and 6 mg/kg caused AV block and a significant ($p<0.05$, n=8) decrease in heart rate after NaHS at 0.5 by 58%, 50%, 67.5% and at 1 min. by 50.6%, 50.4%, 59.75% respectively as compared to control group (Figure 1). Conversely, NaHS at doses 0.8 and 1.2 mg/kg caused no significant change in the heart rate. Therefore doses of 0.8 and 1.2 mg/kg were selected for the other experiment.

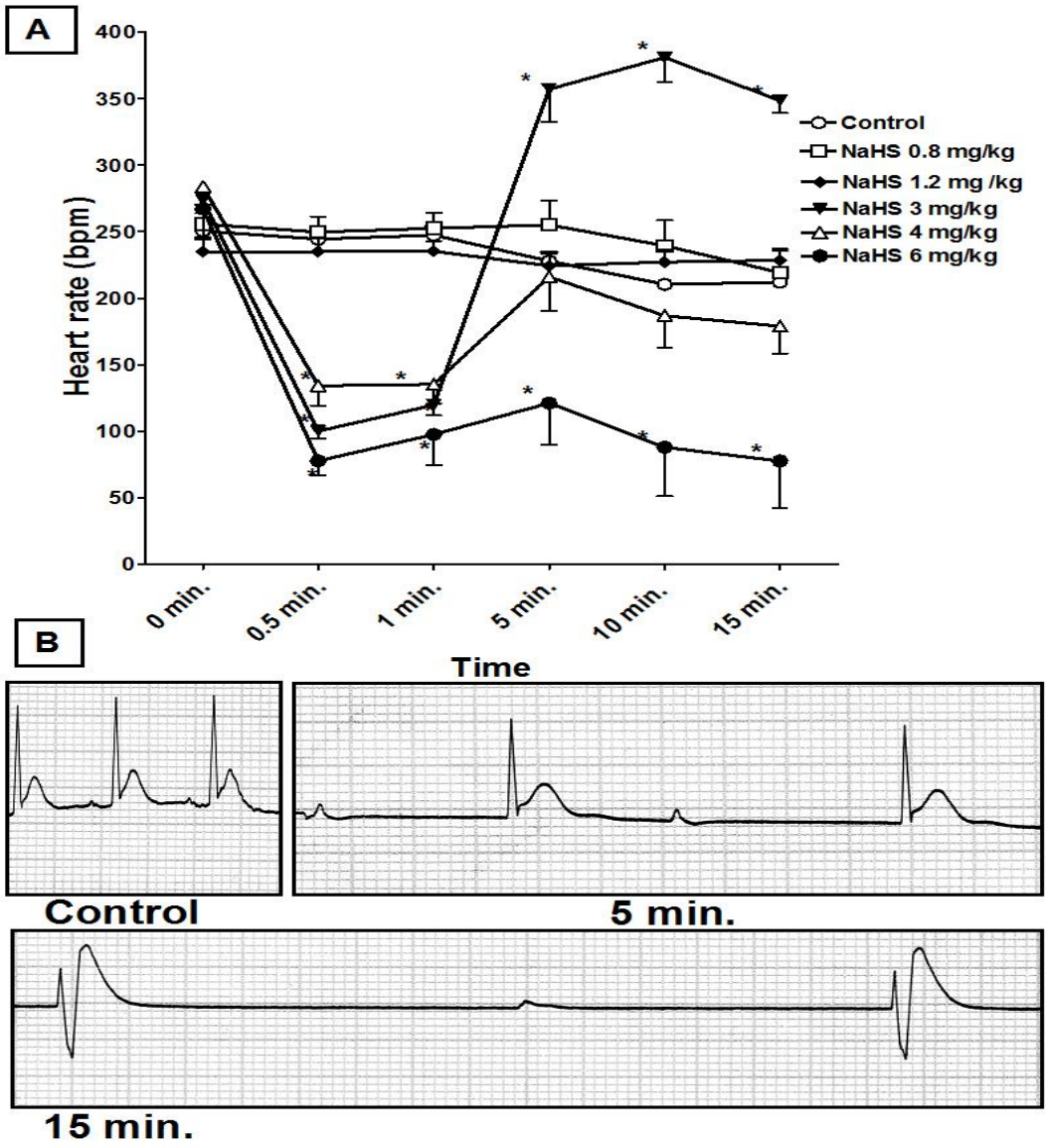

Figure (1): Effect of NaHS on normal heart rate of rats:

Rats were anesthetized with urethane (1.8 g/kg, i.p.) 10 min before injection of NaHS (0.8, 1.2, 3, 4 and 6 mg/kg, i.v.) in jugular vein. Electrocardiograms (ECGs) were recorded for 15 min. from standard lead II limb leads. A) Effect of NaHS on normal heart rate of rats; B) Representative tracing of ECG changes induced by large doses of NaHS.

Data are expressed as mean ± SEM; n=8. * $p<0.05$, significantly different from control group using two-way ANOVA, followed by Bonferroni *post hoc* test.

Effect of NaHS on aconitine-induced increase in heart rate of rats:

Aconitine i.v. injection caused a significant ($p<0.05$, n=8) increase in heart rate of rats at 10 sec, 30 sec, 1 min, 5 min, 10 min, 15 min by 78.5%, 95.7%, 139.3%, 155.6%, 182.4%, and 171.4% respectively when compared to control group (Figure 2). NaHS at 0.8 mg/kg and 1.2 mg/kg caused a significant decrease of aconitine induced tachycardia at 10 sec, 30 sec, 1 min, 5 min, 10 min, 15 min by 41.0%, 48.4%, 58.2%, 26.8%, 31.5%, and 30.6% respectively for 0.8 mg/kg , and 29.2%, 15.8%, 32.8%, 19.6%, 11.4% and 9.2% respectively for 1.2 mg/kg. The effect of NaHS 0.8 mg/kg on aconitine induced tachycardia was more significant than NaHS 1.2 mg/kg

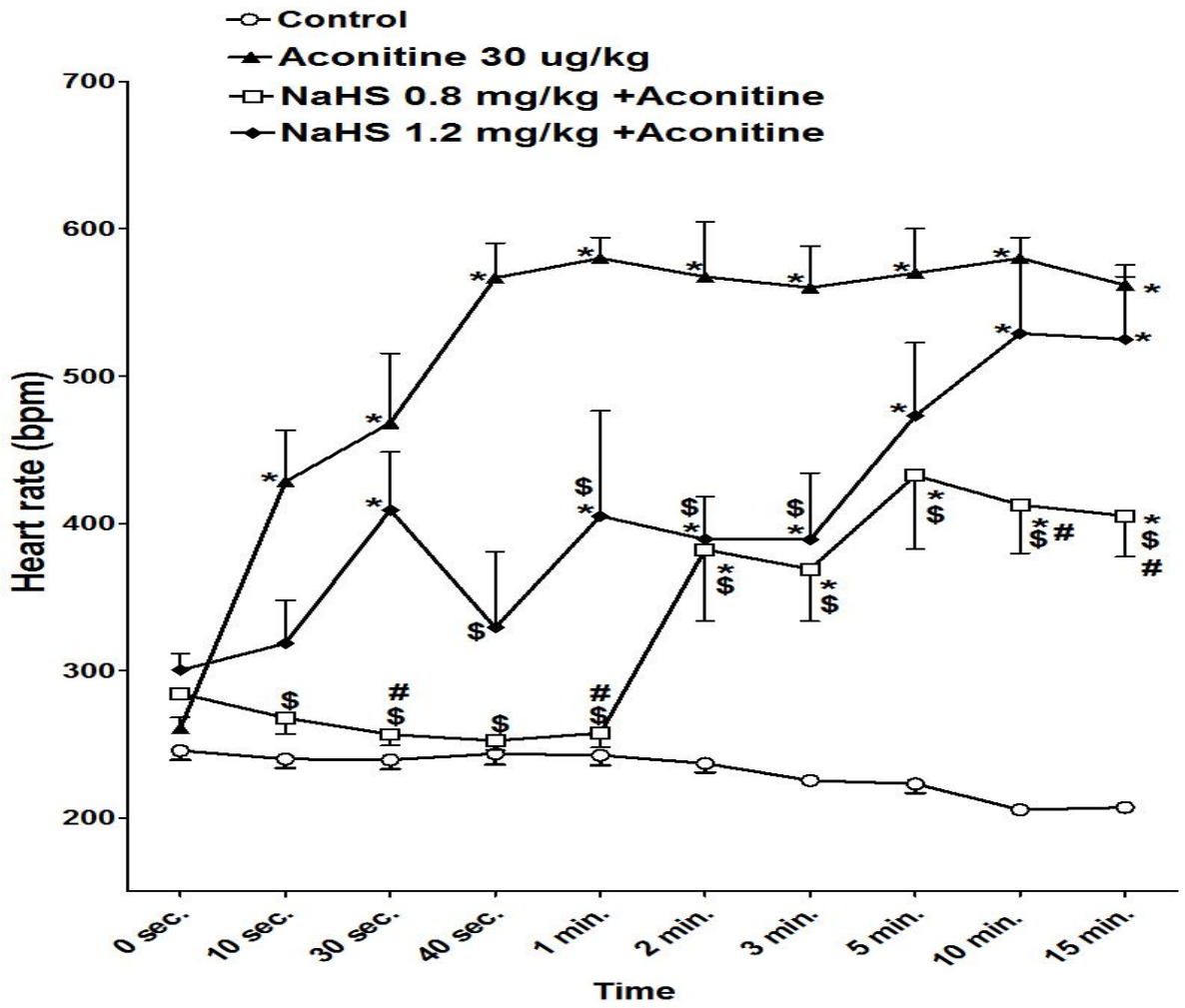

Figure (2): Effect of NaHS on aconitine-induced increase in heart rate of rats:

Rats were anesthetized with urethane (1.8 g/kg, i.p.) and aconitine (30 µg/kg, i.v.) bolus injection in jugular vein alone or after NaHS (0.8 and 1.2 mg/kg, i.v.). Electrocardiograms were recorded for 15 min. from standard lead II limb leads. Data are expressed as mean ± SEM; n=8.

*, $, # $p<0.05$, significantly different from control, aconitine, or NaHS (0.8 mg/kg) respectively using two-way ANOVA, followed by Bonferroni *post hoc* test.

Effect of NaHS on aconitine-induced ventricular tachycardia, ventricular fibrillation and death in rats:

After 26 ± 5 sec. of its i.v. injection, aconitine caused 100% ventricular tachycardia (VT) in rats, 80% ventricular fibrillation (VF), and 60% mortality (Figure 3). Administration of NaHS (0.8 mg/kg) i.v. as a bolus injection in jugular vein, 15 min. before aconitine injection significantly decreased the percentage of VT, VF and mortality to 62.5%., 25% , and 0% respectively. Additionally, NaHS delayed the occurrence of VT by 349 ± 2 sec.

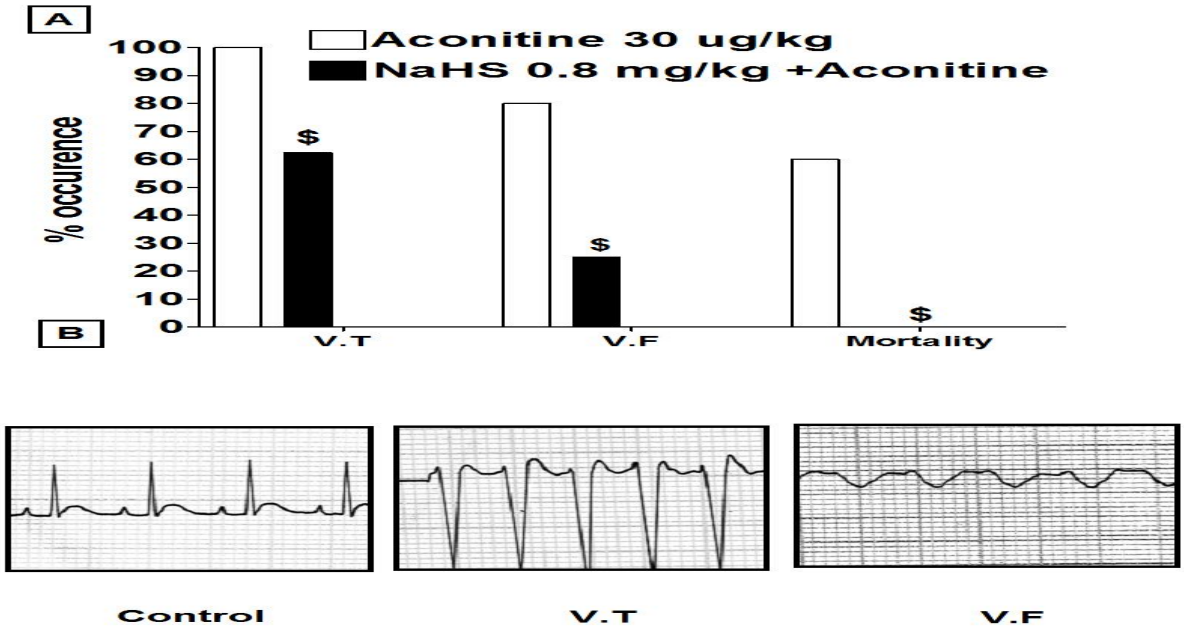

Figure (3): Effect of NaHS on aconitine-induced arrhythmia in rats.

Rats were anesthetized with urethane (1.8 g/kg, i.p.). Aconitine (30 µg/kg, i.v.) was injected as a bolus injection in jugular vein. Electrocardiograms (ECGs) were recorded from standard lead II limb leads. (A) The % occurrence of ventricular tachycardia (V.T), ventricular fibrillation (V.F) and mortality in aconitine-induced arrhythmia in rats; (B) Representative tracing of ECGs for aconitine-induced arrhythmia in rat, n=8. $ $p<0.05$, significantly different from aconitine group using Chi-square test.

Effect of NaHS on $BaCl_2$-induced ventricular fibrillation and death in rats:

After 18 ± 8 sec. of its i.v. injection, $BaCl_2$ caused 75% VF in rats and 37.5% mortality (Figure 4). Administration of NaHS (0.8 mg/kg) i.v. as a bolus injection in jugular vein, 15 min. before $BaCl_2$

injection significantly decreased the % of VF to 50%. Additionally, NaHS decreased the % of death to 12.5% and delayed the occurrence of death by 30 sec.

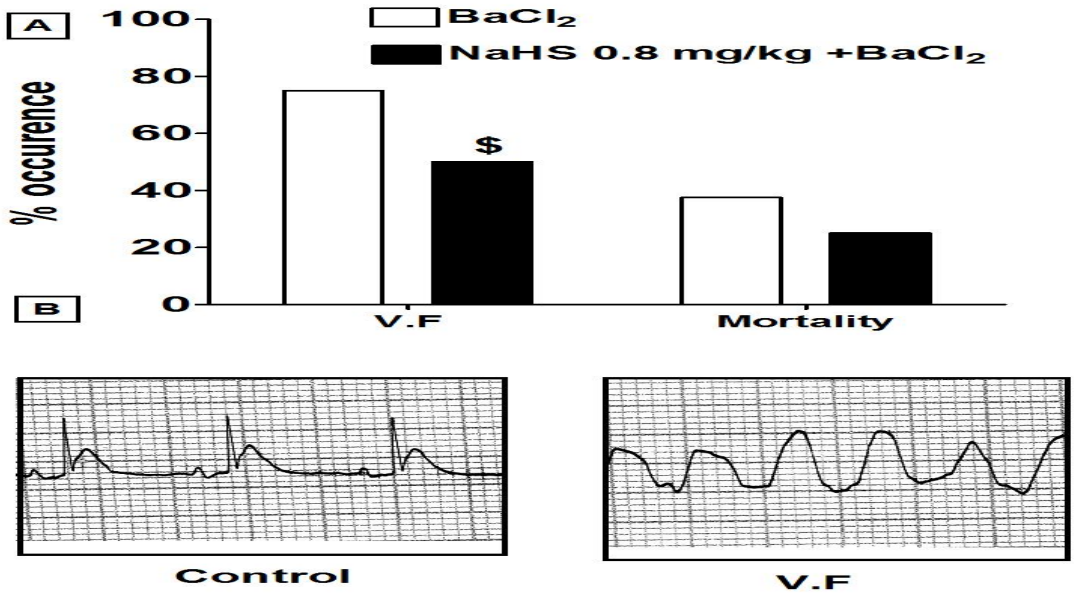

Figure (4): Effect of NaHS on $BaCl_2$-induced arrhythmia on rats.

Rats were anesthetized with urethane (1.8 g/kg, i.p.) and $BaCl_2$ (15 mg/kg, i.v.) was injected as a bolus injection in jugular vein. Electrocardiograms were recorded from standard lead II limb leads. (A) The % occurrence of ventricular fibrillation (V.F) and mortality in $BaCl_2$-induced arrhythmia in rats; (B) Representative tracing of ECGs for $BaCl_2$-induced arrhythmia in rats, n=8.

$^{\$}$ $p<0.05$, significantly different from $BaCl_2$ group using Chi-square test.

Effect of administration NaHS (0.8 mg/kg, i.p., daily for 3 days) on ECG patterns.

To evaluate direct effect of NaHS on ECG pattern, NaHS administrated for 3 days and ECG changes were measured. Rats treated with NaHS 0.8 mg/kg, i.p daily, for 3 days did not show any significant change in heart rate, ST segment elevation and QT interval, R amplitude, PR interval as shown in Table 1.

Effect of administration NaHS (0.8 mg/kg, i.p), for 3 days on heart markers.

Biochemical markers for heart dysfunction (CK-MB and cTnI) were evaluated to test any detrimental effect of NaHS. Rats treated with NaHS (0.8 mg/kg, i.p.), for 3 days did not show any significant change in serum CK-MB and cTnI (Table 1).

Table (1): Effect of NaHS (0.8 mg/kg, i.p. daily for 3 days) on ECG patterns and heart markers.

	Before	After
Heart rate (beats/min.)	421.7 ± 25.21	434.53 ± 36.2
R amplitude (mV)	0.96 ± 0.128	1.02 ± 0.24
ST segment elevation (mV)	0.2 ± 0.00	0.267 ± 0.033
PR interval (msec.)	44 ± 0.122	42 ± 0.1
QT interval (msec.)	64 ± 0.1225	60 ± 0.05
cTnI (ng/ml)	0.02 ± 0.00	0.02 ± 0.00
CK-MB (U/L)	338.66 ± 32.5	405.5 ± 29.8

***Effect of administration NaHS (0.8 mg/kg, i.p), for 3 days on histopathological examination*.**

As a further confirmation for any effect of NaHS on heart tissue, we evaluated histopathological change induced by NaHS. Rats treated with NaHS (0.8 mg/kg, i.p., daily for 3 days) showed normal cardiac muscle without any pathological changes. (Figure 5.)

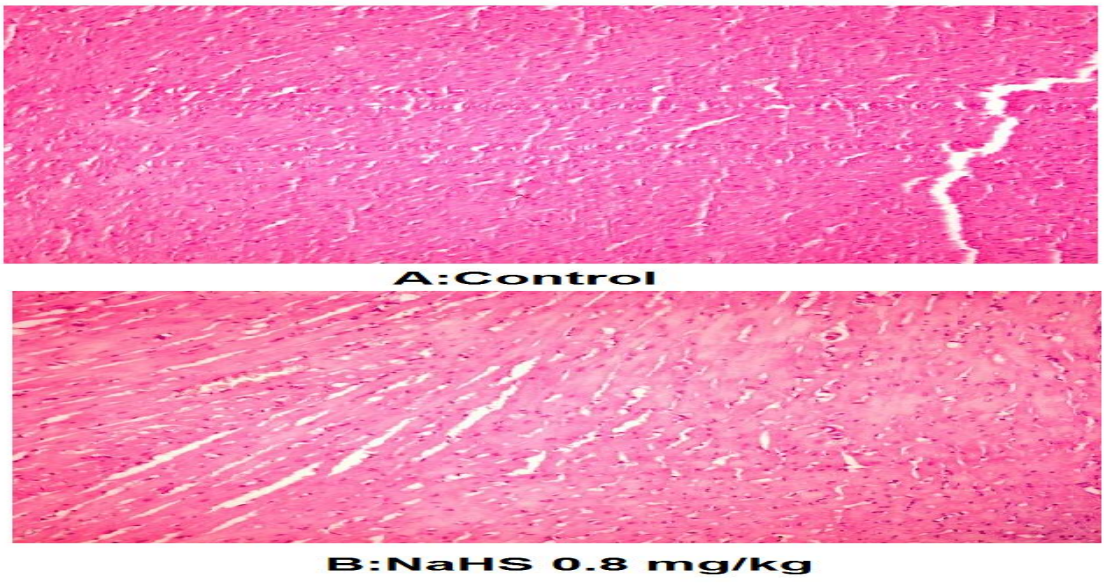

Figure (5): Effect of NaHS on histopathological changes in rat myocardial tissue.

Rats were administrated NaHS (0.8 mg/kg, i.p.) daily for 3 days and hearts were collected for histopathological examination. (A): Normal control heart; (B): NaHS (0.8 mg/kg) treated hearts, both showing normal myocardium [H and E stain, X10].

DISCUSSION

This study is the first study to show that H_2S attenuated aconitine and $BaCl_2$-induced ventricular tachycardia, fibrillation and mortality. Additionally it had no detrimental effect on ECG patterns, cardiac markers or histopathology.

H_2S in our experiments decreased heart rate at doses above 1.2 mg/kg. These results agree with previous reports [5;14], but in another study H_2S administration did not alter heart rate [15]. This effect of H_2S may be attributed to its effect on ion channels. The first study about the action of H_2S on ion channels was from Zhao's study [6]. They found that the decreasing effect of H_2S on blood pressure was antagonized by blockade of K_{ATP} channels, and H_2S relaxed rat aorta *in vitro* through decreasing K_{ATP} channel currents [6]. Zhang and colleagues found that H_2S had a cardioprotective effect against ischemia-reperfusion injury and decreased arrhythmia score Through opening of K_{ATP} channel [16].

Additionally, H_2S has been shown to inhibit the chloride channels in a concentration-dependent manner [17]. Moreover, several studies showed that H_2S closed the L-type calcium channel (LTCC). Both in *vivo* and in *vitro* experiments proved that H_2S had a negative inotropic effect on rat heart [5], through direct inhibition of LTCC in cardiomyocytes [7]. These effects of H_2S on ion channels may explain its inhibitory effect on the heart rate presented in our study.

The negative chronotropic effect of H_2S could play a protective role in arrhythmia. In our study, we induced arrhythmia by using aconitine. Aconitine is an alkaloid of *Aconitum napellus* that is a potent cardiac arryhtmogenic agent. It produces atrial flutter and fibrillation upon application on the cat heart as well as VT and VF [18]. Also injection of aconitine in the lateral cerebral ventricle in conscious rabbits produce a biphasic effect: an initial phase of bradycardia and a second phase of cardiac acceleration terminated by VF[19;20]. The arrhythmogenic effect of aconitine is due to block of K^+ channels [21-23], and opening sodium (Na^+) channels leading to accumulation of intracellular Na^+ that eventually result in intracellular Ca^{2+} overload via a Na^+–Ca^{2+} exchange (NCX) mechanism [24].

This protective effect of H_2S was demonstrated in our study by the decrease of VT, VF and mortality rate induced by aconitine. This effect of H_2S may be explained by activation of K_{ATP} channel, blocking of chloride and Ca^{2+} channels, therefore, it may have an antagonist effect on arrhythmia. NaHS (0.8 mg /kg) produced more significant decrease in heart rate and for longer time than the higher dose of NaHS (1.2 mg/kg). This may be due to the therapeutic effect of

sulphide-mediated cardioprotection is bell-shaped, both in perfused hearts and in whole animals: raising the dose of sulphide above the optimal dose results in diminished therapeutic efficacy [3;15].

As a confirmation of H_2S antiarrhythmic effect, we tested its protective effect against $BaCl_2$ - induced arrhythmia. The effects of $BaCl_2$ on the heart are largely attributable to the decline in the outward diffusion of K^+ from the cell [25]. The $BaCl_2$ toxicity lead to prolongation of QT interval and VF, which is the leading cause of sudden cardiac death[26]. Since H_2S activates K_{ATP} channel, blocks chloride and Ca^{2+} channels, therefore, it may have an antagonistic effect on $BaCl_2$-induced arrhythmia. This protective effect of H_2S was demonstrated in our study by the decrease of VF and mortality rate induced by $BaCl_2$.

Additionally we tested the safety of H_2S to measure its direct effect on the heart. The NaHS administration for 3 days on ECG patterns, heart markers (CK-MB and cTnI) and histopathological examination was evaluated using the same protective dose we found against aconitine and $BaCl_2$-induced arrhythmias (0.8 mg/kg). We found that NaHS did not affect ECG patterns, heart markers or histopathological examination. These results confirm that NaHS at its antiarrhythmic dose in our model is safe.

H_2S represent a promising new pathway for treatment of arrhythmias; however, more investigations are required to elucidate its precise mechanism of action.

In conclusion, H_2S has a protective effect against aconitine and $BaCl_2$ induced arrhythmias as elucidated by decreasing VT, VF and mortality, without affecting ECG patterns, cardiac markers or histopathology in rats.

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CONFLICT OF INTEREST:

None

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