Introduction

The gastro-protective activity of sodium hydrosulfide (NaHS) well established against different gastric irritants such as ischemia-reperfusion injury, non-steroidal anti-inflammatory drugs (Wallace, 2012) and water-immersion restraint stress (Magierowski et al., 2015). Studies have been shown that NaHS through a variety of mechanisms including inhibition of the acid output in response to distention and histamine, increasing the mucosal nitric oxide level (Mard et al., 2018).
promoting the production of mucous secretion and increasing the mRNA expression level of prostaglandin E2 receptors (Mard et al., 2017) contributed in mucosal defense barrier. It has been shown that the gastric mucosa in diabetic rats has a more susceptibility to gastric irritants (Takeuchi et al., 1994; Tashima et al., 1998). A study showed that the prevalence of gastric erosions and gastric ulcers in diabetic patients is more than 45% (Boehme et al., 2007). Moreover, according to twenty-four pH studies, the prevalence of asymptomatic reflux in insulin-dependent diabetic patients were 28% higher than in healthy population (Lluch et al., 1999). There are many reasons for decreased ability of gastric mucosa against irritants such as inability of the gastric mucosa to deliver the enough amount of calcitonin-gene related peptide, impairment of gastric mucosal blood flow and decrement the efficiency of antioxidant defense system in the gastric mucosal layer (Goldin et al., 1997; Tashima et al., 1998). Therefore, the present study was designed to evaluate the gastro-protective effect of NaHS against indomethacin-induced gastric lesions in diabetic rats.

Materials and methods

Animals
Male Wistar rats weighing 150-200 g were supplied from the animal house of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Animals were fed on conventional diets and tap water. They were maintained under standard conditions of humidity, temperature (22±2 °C) and light/dark cycle (12:12 h). Animals were fasted 24 h before the experiments.

Animal grouping and procedures
In the first set of experiments, to evaluate the protective effect of NaHS on indomethacin-induced gastric lesions, four diabetic groups of rats were used. They were diabetic control group and 3 NaHS-treated groups (6 in each group). The control rats were given an intraperitoneally (ip) injection of normal saline (2 ml/kg) and NaHS-treated groups received a single ip injection of NaHS (Sigma, USA) at doses 320, 640 or 1280 µg/kg. Thirty min after administration of normal saline or NaHS, all groups of animals received indomethacin (40 mg/kg, orally) to induce gastric lesions (Kianbakht and Mozaffari, 2009). Five hour after the administration of indomethacin, animals sacrificed under a high dose of anesthetics (100 mg/kg ketamine + 20 mg/kg xylasine, ip, Alfasan Co.Woerden-Holland), their stomachs rapidly removed, opened along the greater curvature, rinsed with physiologic saline and pinned out on ice-cooled saline.

Evaluation of effects of NaHS on gastric mucus production
To evaluate the mucus production, gastric mucus was gently scraped using a glass slide and the obtained mucus weighed with an accurate digital device.

Evaluation of NaHS effects on gastric acid secretion
To determine the antisecretory effect of NaHS, 4 separate groups of fasted rats (n=5 in each group) were subjected to a pylorus ligation procedure as described by Shay et al. (1945). The control and 3 NaHS-treated groups received a single ip injection of saline or NaHS at doses of 320, 640 or 1280 µg/kg, respectively, 30 min prior to pylorus ligation. Five hours after pylorus ligation, animals were killed by an overdose of anesthetics (ketamine+xylasine), their stomachs removed and the entire gastric contents were transferred into centrifuge tubes for measuring pH using a digital pH meter (iSTEK, South Korea).

Induction of diabetes
The fasted rats were received a single ip injection of alloxan monohydrate (Sigma, USA) at 130 mg/kg. After 72 h, the level of fasting blood glucose was checked using a glucometer (Elegance, Frankenberg, Germany). Rat with fasting blood glucose higher than 250 mg/dl was considered as diabetic. Two weeks later, animals with fasting blood sugar more than 250 mg/dl subjected to induce the gastric ulcer (Mard et al., 2016; Qiu et al., 2008).

Histopathological evaluation
For histological evaluation, stomachs from control and treated animals were fixed in 10% formalin, dehydrated in grade ethanol and embedded in paraffin. Thereafter, sections of tissue were cut at 5 µm using a microtome, stained with hematoxylin and eosin and assessed under an Olympus microscope.
Gastroprotective effect of NaHS in diabetic rats

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Statistical analysis
Data are shown as mean±SEM. Statistical analysis was performed by one-way ANOVA and followed by post hoc Tukey’s Test. Significance was set at a P<0.05 level.

Results

Effect of NaHS on indomethacin-induced morphological and histological alteration of the gastric mucosa in diabetic rats
As shown in Figure 1, oral administration of indomethacin caused multiple mucosal erosions, exfoliation and necrosis of superficial cells, and hemorrhages in the mucosal layer in the gastric mucosa (Fig. 1A). The microscopic and macroscopic data showed that the lowest studied dose of NaHS (320 µg/kg) could not prevent the gastric mucosa against indomethacin-induced gastric lesions (Fig. 1B). Pretreatment with NaHS at doses 640 and 1280 µg/kg effectively improved the histopathological changes (Figs. 1C and D). Figure 2 shows that the total area of gastric lesions in NaHS-treated rats were lower than in control group. The area of gastric lesions in groups received NaHS at doses 640 and 1280 µg/kg was significantly lower than in control rats (P<0.05 and P<0.01, respectively)

Effect of NaHS pretreatment on the mucus production of gastric wall in indomethacin-induced gastric ulcer rats
As shown in Figure 3, pretreatment with NaHS increased the rate of mucus production in indomethacin-induced gastric lesion rats. The weight

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Fig. 2. The effect of sodium hydrosulfide on indomethacin-induced gastric ulcer in diabetic rats. NaHS at doses 640 and 1280 µg/kg decreased the total area of gastric ulcer. C: diabetic control rats; D+NaHS-(320), (640) or (1280): diabetic rats pretreated with NaHS at 320, 640 or 1280 µg/kg. *P<0.05 and **P<0.01 versus diabetic control group.

Fig. 3. The effect of sodium hydrosulfide on the production of gastric wall mucus. NaHS at doses of 640 and 1280 µg/kg increased the rate of mucus secretion by the gastric mucosa. C: diabetic control rats; D+NaHS-(320), (640) or (1280): diabetic rats pretreated with NaHS at 320, 640 or 1280 µg/kg. *P<0.05 and ***P<0.001 versus diabetic control group.
of mucus in NaHS-treated diabetic rats received NaHS at doses 640 or 1280 µg/kg was significantly higher than in control rats \(P<0.001\) and \(P<0.05\), respectively).

**Effect of NaHS on the profile of gastric secretion induced by pylorus-ligated rats**

As shown in Figure 4, the total acid content of the gastric effluents significantly decreased by NaHS at doses 320 and 1280 µg/kg \(P<0.05\) at both cases).

**Discussion**

The results of the present study showed that a single administration of NaHS in diabetic rats: 1. decreased the total area of gastric lesions induced by indomethacin; 2. decreased the gastric acid output and 3. increased the rate of mucus production in the gastric wall.

Our results showed that NaHS at 640 and 1280 µg/kg had a stimulatory effect on the production of gastric wall mucus in diabetic rats. It is a general fact that prostaglandins \(E_2\) and \(F_{2\alpha}\) increase the rate of mucous production/release in gastric mucosal layer. A study showed that exogenous \(H_2S\) by increasing the production of prostaglandin \(E_2\) protected the gastric mucosa against water-immersion restraint stress in rats (Magierowski et al., 2015). Another recently published research showed that NaHS up-regulated the mRNA expressions of prostaglandin \(E_2\) receptors in male Wistar rats in response to mucosal acidification by 100 mM hydrochloric acid and distention-induced gastric acid secretion (Mard et al., 2017). These finding together showed that NaHS by increasing the levels of prostaglandin \(E_2\) and/or up-regulating its receptors, increased the production of gastric wall mucous in diabetic rats received NaHS. Moreover, these finding was in agreement with the previous report as shown by authors NaHS at dose 80 µg/kg increased this rate in response to distention-induced acid output in nondiabetic rats (Mard et al., 2017). As shown in the current results, NaHS at 320 µg/kg failed to stimulate the mucus secretion in diabetic rats. This finding implied that the needed dose of NaHS to increase the rate of mucus secretion in diabetic rats is more than in the nondiabetic ones. However, the results showed that this effect was not dose-dependent because the highest response was not observed in rats received the highest dose of NaHS. As shown in the present results, NaHS at 640 µg/kg had the strongest stimulatory effect on the mucus secretion in diabetic rats. This finding seems to be a secondary/compensatory response to lower potency of this dose to inhibit the acid output compared to the rats received NaHS at 1280 µg/kg. Moreover, the current results showed that NaHS at 1280 µg/kg effectively decreased the acid output while this effect was not observed in rats received it at 640 µg/kg. Therefore, comparing antiulcer effect of these two effective doses (640 or 1280 µg/kg) showed that
inhibiting the acid output plus stimulating the production of gastric wall mucus as occurred in response to NaHS at 1280 µg/kg had a stronger antiulcer effect.

The antacid result of NaHS at 320 µg/kg was not enough to inhibit/decrease the inducing of gastric lesions by indomethacin in diabetic rats. This result showed that the endogenous production of H$_2$S plays an important protective role against ulceration induced by indomethacin. Non-steroidal anti-inflammatory drugs through inhibiting cyclooxygenases (1 and 2) decrease the production of prostaglandins, increase the rate of gastric acid output and at the same time decrease the rate of mucus secretion (Musumba et al., 2009). As mentioned above, indomethacin by increasing the gastric acid output, induces gastric ulceration. Therefore, multiplying the gastric acid output in diabetic rats due to decreased output of mucosal H$_2$S in one hand and administration of indomethacin in the other hand, dominated the antacid effect of NaHS at 320 µg/kg.

**Conclusion**

The results of the present study showed that NaHS effectively decreased the gastric mucosal ulceration induced by indomethacin in diabetic rats through decreasing the gastric acid output and increasing the rate of mucus secretion in the gastric wall of diabetic rats.

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**Conflict of interest**

The authors declare that they have no conflict of interest

**References**


