

Original Article

# Sodium hydrosulfide: A new potential candidate for treating delayed gastric emptying in diabetes

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## Abstract

**Introduction:** Sodium hydrosulfide (NaHS) has shown to enhance the gastric emptying rate in normal rats but till now its effect on gastric emptying of food stuffs in diabetic rats was not investigated. Therefore, this study designed to determine the role of an oral administration of NaHS on gastric emptying rate (GER) of glucose, albumin and olive oil in gastroparetic and normal rats.

**Methods:** To evaluate the effect of NaHS on the gastric emptying of glucose, albumin and olive oil in normal rats, thirty-six normal rats randomly assigned in six experimental groups (6 per group). Three groups of rats considered as control. They received albumin, glucose or olive oil orally. Three other normal groups considered as NaHS-treated animals. These groups received NaHS (320 µg/kg, orally) 30 min prior to food stuffs. To investigate the effect of NaHS on the gastric emptying of food stuffs in diabetic rats, the same protocols carried out. Thirty min after intragastric administration of food stuffs, animals received acetaminophen (as a marker for gastric emptying rate).

**Results:** The results showed that in normal and gastroparetic rats, an oral administration of NaHS accelerated gastric emptying of glucose, albumin and olive oil. The increased gastric emptying of glucose, albumin and olive oil in NaHS-pretreated gastroparetic rats was 89.9, 92.3 and 60% respectively more than in corresponding's controls.

**Conclusion:** The results showed that orally administration of NaHS can increase the gastric emptying of glucose, albumin and olive oil in diabetic rats.

## Keywords:

Sodium hydrosulfide;  
Gastric emptying rate;  
Gastroparesis;  
Diabetic rat

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## Introduction

Delayed gastric emptying of food without mechanical obstruction is called gastroparesis. This gastric neuromuscular disorder caused by a relatively wide range of diseases such as diabetes mellitus, Parkinson, scleroderma and etc. Gastroparesis in

diabetic patients leads to uncomfortable gastrointestinal symptoms including abdominal pain, epigastric fullness, early satiety, nausea and vomiting (Koch and Calles-Escandon, 2015). Forty to fifty percent of diabetic patients suffer from gastroparesis (Horowitz et al., 1991).

Prokinetic agents such as metoclopramide and new generation of ghrelin and motilin agonist's receptors

through promoting the gastrointestinal motility relieve the symptoms of gastroparesis (De smet et al., 2009). The gastro-protective activity of the third gas-transmitter, hydrogen sulfide ( $H_2S$ ), is well established. The literatures have been shown that  $H_2S$  through different mechanisms such as stimulating the gastric mucous and bicarbonate secretion, increasing the gastric mucosal blood flow, decreasing the rate of gastric acid secretion and etc. protects the gastric mucosa against endogenous and exogenous irritants and maintains mucosal integrity (Wallace, 2012; Mard et al. 2014). Recently, it has been demonstrated that  $H_2S$  in addition to regulating the gastric secretions, promotes the gastric motility. Animal studies have shown that a single intraperitoneally administration of exogenous hydrogen sulfide (sodium hydrosulfide, NaHS), enhances the gastric emptying rate in rat (Mard et al., 2016) and mice (Medeiros et al., 2012). The recent effect is mainly mediated through exciting the spontaneous contraction of gastric wall muscles and relaxing the pyloric sphincter as shown by mechanistic *in vitro* studies (Mard et al., 2016; Medeiros et al., 2012).

As far as we know, the effect of NaHS on gastric emptying of food stuffs was not investigated. Therefore, this study designed to determine the role of NaHS on gastric emptying of glucose, albumin and olive oil in gastroparetic and normal rats.

## Materials and methods

### Chemicals

Alloxan monohydrate, NaHS (as a  $H_2S$  donor), sodium metaperiodate and phenol were purchased from Sigma chemical Co. (St. Louis, MO, USA). Acetaminophen was gifted by Jalinous Pharmaceutical CO, Iran. Ketamine and xylazine were purchased from Alfasan Co. (Woerden, Holland).

### Animals

Male Wistar rats (150-200 g) were purchased from the Animal House of Ahvaz Jundishapur University of Medical Sciences. The animals were fed on conventional diet and had free access to tap water. They were maintained under standard conditions of humidity, temperature ( $22\pm 2^\circ C$ ) and 12 h light/dark cycle. The animals were deprived of food but not

water overnight before all interventions of study including evaluating gastric emptying rate and induction of diabetes. All experiments were carried out in accordance with the regulations set by Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS. REC.1395-122).

### Induction of gastroparesis

To induce diabetes gastroparesis, overnight fasted rats were received a single intraperitoneally injection of alloxan monohydrate at 175 mg/kg (Qiu et al., 2008). After 72 h, blood glucose was checked by glucometer (Elegance, Germany). Rat with fasting blood glucose higher than 250 mg/dl was considered as diabetic. Two weeks later, animals with fasting blood sugar higher than 250 mg/dl considered as gastroparetic.

### Evaluation of the gastric emptying rate (GER)

To determine GER, animals received a single orally administration of acetaminophen at 100mg/kg, 60 min prior to scarifying by an overdose of anesthetics (Srinivas, 2015). Blood samples were withdrawn from the right ventricle and after coagulating, centrifuged at 3000 rpm for 10 min. From the obtained serum, 200  $\mu$ l was mixed with 500  $\mu$ l ethyl acetate and 100 mg NaCl. Then, the mixture was vortexed for 30 sec. Five min later, 200  $\mu$ l of supernatant was collected and mixed with 100  $\mu$ l HCl (6 N) and then incubated in boiling water for 10 min. After cooling, to generate a colored complex, 3 ml of prepared reagent [a combination of 2,5-dimethylphenol (82 mg/l), sodium metaperiodate (32 mg/l) and KOH (0.4 M)] was added to prepared sample and mixed. Thirty min later, the optical density was measured at 635 nm (Qiu et al., 2008). A standard curve of acetaminophen was constructed over the concentration range of 0-5 mg/ml (nine concentrations) and used for measurement of serum level of acetaminophen. To calculate the effect of NaHS on the GER, serum level of acetaminophen in normal groups was considered as 100% and other groups were compared with the corresponding controls.

### Animal grouping and experimental procedures

In the first set of experiments, to find the optimal time for inducing diabetic gastroparesis, eighteen rats (6 normal and 12 alloxan-treated rats) were used and assigned in three experimental groups (n=6 in each

group). They were normal control (N-C) and two alloxan-induced diabetic rats (D-15 and D-30 groups). In D-15 group, 15 days after inducing diabetes, the GER measured while in D-30 group, 30 days after inducing diabetes, the GER evaluated. Following that the GER in D-15 and D-30 groups was compared to normal control to find the optimal time for inducing gastroparesis.

In the second set of experiments, to evaluate the effect of NaHS on the gastric emptying of food markers in normal rats, thirty-six normal rats randomly assigned in six experimental groups ( $n=6$  per group). Three groups of rats considered as control. They received albumin as a protein (500 mg/kg) (Jansen et al., 1994), glucose as a carbohydrate (500 mg/kg) (Schirra et al., 1996) or olive oil as a lipid (1 ml/ 200 g of body weight) (Damgaard et al. 2013), orally. Three other normal groups considered as NaHS-treated animals. These groups received NaHS (320  $\mu\text{g}/\text{kg}$ , orally) (Mard et al., 2016) 30 min prior to albumin, glucose or olive oil. Glucose and albumin are dissolved in distilled water and the rats received the same volume (1 ml/ 200 g of body weight) of glucose, albumin solutions or olive oil (SABROSO, Rafael Salgado; Spain).

In the third set of experiments, to investigate the effect of NaHS on the gastric emptying of albumin, glucose or olive oil in diabetic rats, thirty-six alloxan-induced diabetic rats randomly assigned in six experimental groups ( $n=6$  per group). The animal grouping and protocols to test the prokinetic effect of NaHS in diabetic rats were the same as performed in normal groups. One hour after intra-gastric administration of albumin, glucose or olive oil, animals received acetaminophen (as a marker for gastric emptying rate).

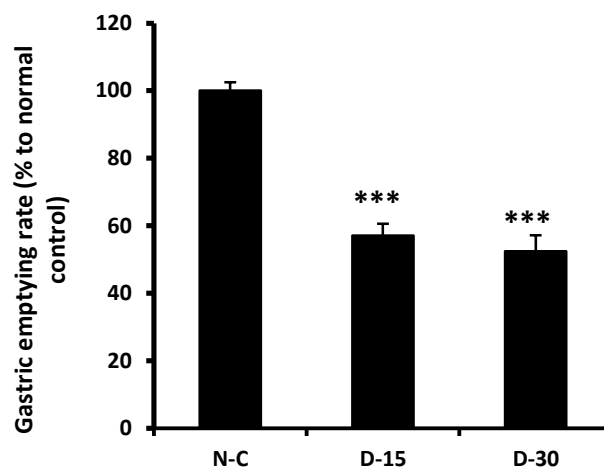
### Statistical analysis

Data are shown as mean $\pm$ SEM. Statistical analysis was performed by one-way, two-way ANOVA and followed by post hoc Turkey's test. Significance was set at a  $P<0.05$  level.

## Results

### Effect of alloxan administration on gastric emptying rate

As illustrated in Figure 1, the gastric emptying rate in diabetic rats, 15 and 30 days after inducing diabetes



**Fig.1.** Effect of alloxan monohydrate on the gastric emptying rate (GER), 15 and 30 days after inducing diabetes. The results showed that the rate of gastric emptying 15 days (D-15) and 30 days (D-30) after inducing diabetes was significantly lower than in normal control (N-C) rats ( $***P<0.001$ , in both cases). Data are expressed as mean $\pm$ SEM.

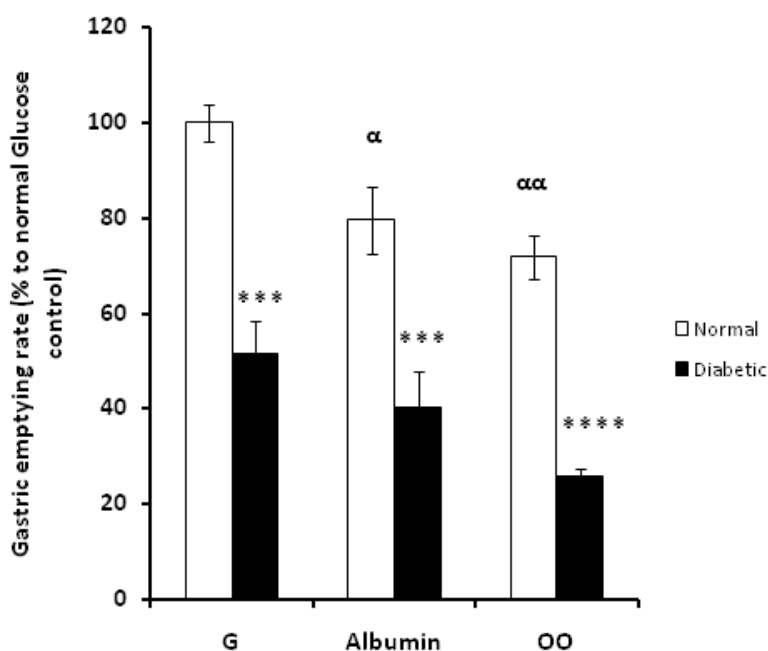
significantly decreased compared to normal control rats ( $P<0.001$  in both cases). The gastric emptying rate, 15 days after inducing diabetes was approximately equal to 30 days and there was not any significant difference between two groups.

### Effect of alloxan monohydrate on the gastric emptying of glucose, albumin and olive oil

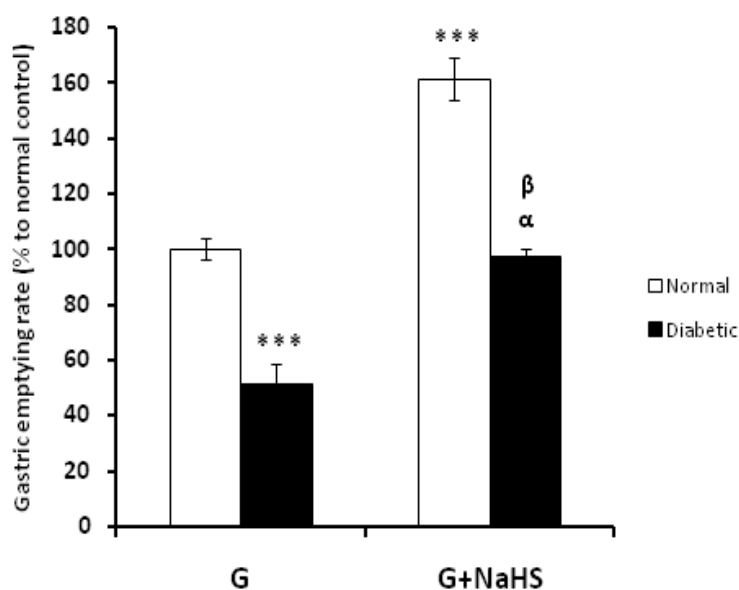
As shown in Figure 2, 15 days after inducing diabetes, the gastric emptying of glucose, albumin and olive oil in gastroparetic rats significantly decreased compared to the corresponding's normal controls ( $P<0.001$ ,  $P<0.001$  and  $P<0.0001$  respectively). The rate of gastric emptying of glucose, albumin and olive oil in diabetic rats was 48.72, 48.7 and 64.3% respectively, less than in normal corresponding's groups.

### Effect of NaHS on the gastric emptying of glucose, albumin and olive oil in normal and gastroparetic rats

As demonstrated in Figures 3-5, in normal rats, an oral administration of NaHS significantly increased the gastric emptying of glucose, albumin and olive oil compared with corresponding's normal controls ( $P<0.001$ ,  $P<0.001$ , and  $P<0.01$  respectively). This increased rate in NaHS-pretreated normal rats was 61.53, 83.87 and 42.85% for glucose, albumin and olive oil, respectively. Figures 3-5 also shows that



**Fig.2.** Effect of alloxan monohydrate on the gastric emptying of glucose, albumin and olive oil 15 days after inducing diabetes. The results showed that the gastric emptying of all foods in gastroparetic rats significantly decreased compared to corresponding's normal controls. In diabetic rats, the decreased rate of gastric emptying of olive oil was higher than in glucose and albumin. G: glucose; OO: olive oil. \*\*\* $P < 0.001$  and \*\*\*\* $P < 0.0001$  significant difference compared with the corresponding's normal controls.  $^{\alpha}P < 0.05$  and  $^{\alpha\alpha}P < 0.01$  significant difference compared with normal rats received glucose. The GER in normal rats received glucose considered as 100%. Data are expressed as mean $\pm$ SEM.



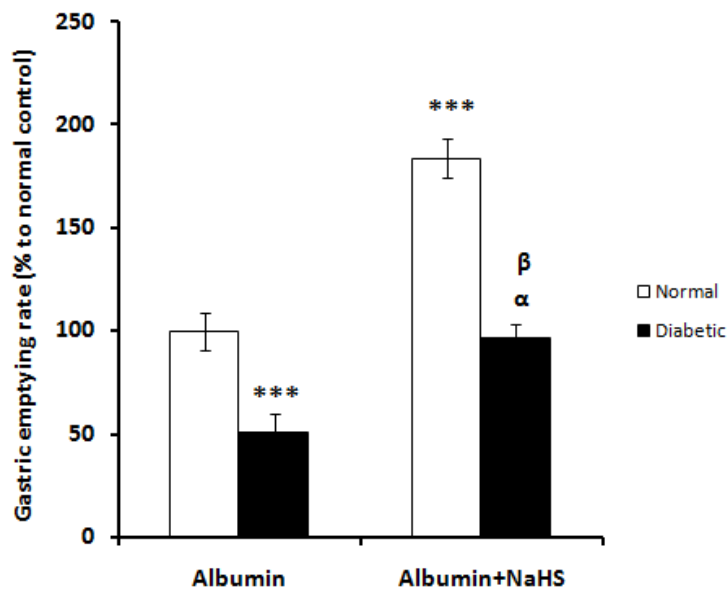
**Fig.3.** Effect of NaHS on the gastric emptying of glucose in normal and gastroparetic rats. An oral administration of NaHS significantly increased the gastric emptying of glucose (G) in normal and diabetic rats. \*\*\* $P < 0.001$  significant difference versus normal control rats;  $^{\alpha}P < 0.001$  significant increase compared with gastroparetic control group;  $^{\beta}P < 0.001$  significant decrease compared with normal NaHS-treated animals; Data are expressed as mean $\pm$ SEM.

oral administration of NaHS significantly increased the gastric emptying of glucose, albumin and olive oil in gastroparetic rats ( $P < 0.0001$ ,  $P < 0.0001$ , and  $P < 0.001$  respectively). The increased gastric emptying of glucose, albumin and olive oil in NaHS-pretreated gastroparetic rats was 89.9, 92.3 and 60%

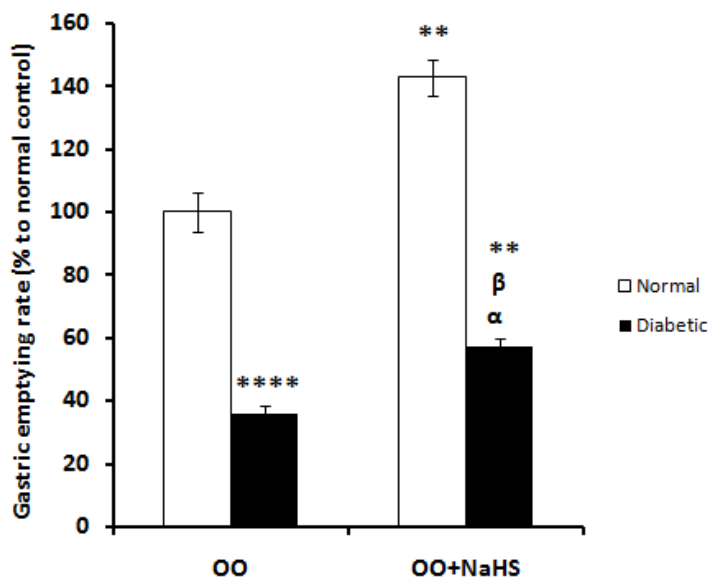
respectively.

## Discussion

The results demonstrated that two and four weeks after inducing diabetes, the GER in diabetic rats was



**Fig.4.** Effect of NaHS on the gastric emptying of albumin in normal and gastroparetic rats. An oral administration of NaHS significantly increased the gastric emptying of albumin in normal and diabetic rats. \*\*\* $P < 0.001$  significant difference versus normal control rats;  $^{\alpha}P < 0.001$  significant increase compared with gastroparetic control group;  $^{\beta}P < 0.001$  significant decrease compared with normal NaHS-treated animals; Data are expressed as mean±SEM.



**Fig.5.** Effect of NaHS on the gastric emptying of olive oil in normal and gastroparetic rats. An oral administration of NaHS significantly increased the gastric emptying of olive oil (OO) in normal and diabetic rats. \*\* $P < 0.01$  and \*\*\*\* $P < 0.0001$  significant difference versus normal control rats;  $^{\alpha}P < 0.001$  significant increase compared with gastroparetic control group;  $^{\beta}P < 0.001$  significant decrease compared with normal NaHS-treated animals; Data are expressed as mean±SEM.

significantly decreased compared to normal rats. In both normal and gastroparetic rats, an oral administration of NaHS accelerated gastric emptying rate of glucose, albumin and olive oil.

The gastric emptying of lipids is slower than carbohydrates and proteins (Barrett, 2006). As expected, our results also showed that the gastric emptying of olive oil in normal rats was 28.21% lower

than glucose. Therefore, using these groups of foods for evaluating the stimulatory effect of NaHS on gastric emptying in the present study was reasonable.

The reported time period for inducing diabetic-induced gastroparesis in literatures is different between 2 to 52 weeks (Yamano et al., 1997). The results of the present study showed that a single



intraperitoneally injection of alloxan monohydrate significantly decreased the gastric emptying rate in rats, 15 and 30 days after inducing diabetes. The current findings demonstrated that the decreased rate of gastric emptying was approximately equal 15 and 30 days after inducing diabetes. Therefore, in the present study, 15 days after inducing diabetes, animals tested for evaluating the effect of NaHS on gastric emptying rate of glucose, albumin, and olive oil.

The present results showed that NaHS at 320 mg/kg increased gastric emptying of albumin, glucose and olive oil in both normal and gastroparetic rats. A previous report has been shown that NaHS enhanced the GER of liquids in normal mice (Medeiros et al., 2012). In agreement with above findings in a newly published study, it has been shown that a single orally administration of NaHS at 150  $\mu$ mol/kg increased the gastric emptying rate in normal mice (Lucetti et al., 2017). Recently, the excitatory effect of endogenous and exogenous H<sub>2</sub>S on gastric emptying of normal saline in normal and diabetic rats has been reported (Mard et al., 2016). These results together showed that NaHS in addition to liquids is able to enhance the gastric emptying of carbohydrate (glucose), protein (albumin) and lipid (olive oil).

As shown in Figures 3 and 4, oral administration of NaHS in gastroparetic rats, increased the gastric emptying of glucose and albumin to normal levels. These findings concluded NaHS is an effective molecule to improve the delayed gastric emptying in gastroparesis. However, NaHS effectively enhanced (60% increase) the gastric emptying of olive oil but could not revert it to normal level (Fig. 5). This result may be due to much more decrease of gastric emptying of olive oil compared to gastric emptying of glucose and albumin in gastroparetic rats (64.3% decrease versus 48.72 and 48.7%).

According to the aim of study, NaHS has been administered through different routes such as intraperitoneally (Mard et al., 2015), intravenous (Mard et al., 2012) or intragastric (Yonezawa et al., 2007). To investigate the prokinetic effect of NaHS on gastric emptying of liquids in mice and rats, it administered intraperitoneally in two earlier studies (Mard et al., 2016; Medeiros et al., 2012). In the present study, NaHS administered orally and the results showed that NaHS was still effective. Route of administration of a drug is important and can be

affect its acceptability. Certainly, the easiest, cheapest, convenient, safest and most common route of administration of a drug is orally. Sodium hydrosulfide in aqueous solution such as gastric juice releases hydrogen sulfide (Nagai et al., 2004). Because of its nature (to be a gas molecule and high solubility in lipophilic solvent), H<sub>2</sub>S easily passes the cell membranes (Fiorucci et al., 2006) and maybe begin its effect directly and rapidly by absorbing through gastric mucosa before leaving the stomach when it consumed orally. As mentioned earlier, the gastro-protective activities of hydrogen sulfide is also well established (Wallace, 2012). A research also showed that a new made H<sub>2</sub>S-releasing NSAID (ATB-346) had not the gastric side effect of other NSAIDs such as naproxen (Fomenko et al., 2014). Therefore, the release of hydrogen sulfide in the stomach in addition to mucosal protection, enhance the gastric emptying without making the disadvantages of oral route.

What is the clinical implication of the present results? As mentioned in introduction, NaHS through modulating the gastric secretions acts as a gastro-protective agent. The present results also showed that this molecule improved gastric emptying rate of glucose, albumin and olive oil both in normal and abnormal situations. Therefore, these effects of NaHS implied that it is a safe and effective agent to alleviate gastric symptoms in diabetic gastroparesis.

## Conclusion

The results suggest that orally administration of NaHS can increase the gastric emptying rate of glucose, albumin and olive oil in diabetic rats. This prokinetic effect of NaHS can be considered as a new approach to improve the GER in gastroparetic patients.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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