Research Article

The preventive effect of sodium hydrogen sulfide against L-arginine induced acute pancreatitis in male albino rats

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Abstract
The role of H_2S in inflammation is still controversial. Some studies have shown pro-inflammatory effects of H_2S in various models of inflammation. While, others reported that H_2S has anti-inflammatory effects. So, the aim of the present study is to evaluate the effect of H_2S against acute pancreatitis induced by l-arginine. **Methods and materials:** Rats were randomly classified into control group, acute pancreatic (AP) group in which rats were given 2 doses of 250 mg/100 g of L-arginine, H_2S donor; sodium hydrosulphide (NaHS) + AP group (NaHS + AP) in which rats were i.p injected with NaHS at a dose of 10 mg/kg 1 h before induction of AP and H_2S blocker; DL- Propargylglycine (PAG) + AP group (PAG + AP) in which rats were i.p injected with PAG at a dose of 50 mg/kg 1 h before induction of AP. Serum pancreatic amylase and lipase levels were measured and pancreatic histopathology was done for confirming the induction of AP. **Results:** AP induced by L-arginine resulted in significant increase in serum levels of pancreatic amylase and lipase in comparison with control group. In NaHS + AP group, serum pancreatic amylase and lipase levels were decreased in comparison with AP group. However, in PAG + AP, serum pancreatic amylase and lipase levels were increased in comparison with AP group. **Conclusions**: The obtained data suggested that H_2S has anti-inflammatory effect in case of AP induced by L-arginine.
Keywords: L-arginine, acute pancreatitis, Propargylglycine

Introduction
Acute pancreatitis (AP) is a localized inflammation of the pancreas that often leads to local and systemic complications. The mortality rate in patients with AP is approximately 5%. Mortality in AP is ascribed to multiple organ failure that may be triggered by the associated systemic inflammatory response. AP is characterized by interstitial edema, cell vacuole accumulation and inflammatory infiltrate of macrophages and neutrophils together with necrosis of the pancreatic tissue (Pérez et al., 2015).

Hydrogen sulfide (H_2S) is a gaseous mediator that plays an important role in a wide range of physiological and pathological processes. H_2S has been extensively studied for its various roles in cardiovascular and neurological disorders. However, the role of H_2S in inflammation is still controversial. Also, other studies found that the effect of H_2S in case of inflammation is dose dependent (Whiteman and Winyard, 2011).

Materials and Methods
Animals:
The present study was conducted on 40 adult male albino rats, after exclusion of the dead, weighing (250 –300 gm.). They were housed in wire mesh cages at room temperature with natural light/dark cycle. They were left for two weeks after arrival from the supplier for acclimatization. Rats were fed a standard diet of commercial rat chow and tap water *adlibitum* through the time of the study (Sadek and Khattab, 2016).
Rats were randomly classified into the following groups

1. **Control group.**
   In which each rat was i.p. injected with saline as a vehicle.
2. **AP group.**
   In which rats were given 2 doses of 250 mg/100 g of L-arginine (Titan biotech ltd, India) dissolved in saline

(1 gm./4 ml) which was_administered i.p at a 1 hour interval. Rats were sacrificed by decapitation 24 hours after last L-arginine administration **(**Yılmaz et al., 2016).

3. **NaHS + AP:** In which rats were i.p injected with NaHS at a dose of 10 mg/kg (gfschemicals, USA) which was given 1 h before induction of AP (Rao et al., 2015).
4. **PAG+ AP group (PAG + AP):** In which rats were i.p injected with PAG (chemimpex , USA) at a dose of 50 mg/kg which was given 1 h before induction of AP (Rao et al., 2015).

Blood sample collection and storage:
At the end of all experiments, all rats were sacrificed by decapitation and blood samples from jugular vein were obtained. Blood samples were collected in tubes and left to clot at room temperature then centrifuged at 3000 rpm for 15 min in a cooling centrifuge (Hettich centrifuge). The supernatant serum was then withdrawn into labeled eppendorf tubes and stored at -20°C till the time of assay of:

- Serum pancreatic amylase.
- Serum pancreatic lipase.

Removal and storage of pancreas: (Asmat et al., 2016).
Pancreases taken from mice of different groups were fixed in 10% buffered formalin. Processing of pancreatic tissues was done for hematoxylin and eosin (H&E) staining following the standard techniques. The slides were examined by a pathologist blinded to the sources of the pancreatic tissues for pancreatic histopathology for confirming the induction of AP.

Results

Data presented in table (1), show that serum amylase and lipase levels were significantly increased in AP group as compared to control (6.98% and 50.37% respectively). Pretreatment with NaHS resulted in significant decrease in these parameters in comparison with AP group (-6.34% and -27% respectively). While, administration of PAG resulted in significant increase in serum amylase and lipase levels in PAG + AP group in comparison with AP group (5.43% and 35.1% respectively). Also, administration of PAG resulted in significant increase in serum amylase and lipase levels in PAG + AP group in comparison with NaHS + AP group (12.57% and 85.1% respectively). This is illustrated in figures (1, 2).

Table (1): **Shows the changes in the measured parameters in AP group treated with either NaHS or PAG:**

Groups / Parameters	Control	AP	NaHS + AP	PAG + AP
-Serum amylase (IU/l). -% change from control -% change from AP -% change from NaHS + AP	1032 ±1.15[c]	1104 ± 1.80 [b] 6.98%	1034±0.4733 [c] .19% - 6.34 %	1164 ± 1.412 [a] 12.8% 5.43% 12.57%
-Serum lipase (U/l). -% change from control -% change from AP -% change from NaHS	40.46±0.70[c]	60.84 ± 1.03 [b] 50.37%	44.41± 0.685 [c] 9.76% -27%	82.19± 1.074 [a] 1.031% 35.1% 85.1%

Means in the same horizontal rows with different superscripts [a,b and c] are significantly different ($P \leq 0.05$).
AP (acute pancreatitis), NaHS +AP (sodium hydrosulfide+ acute pancreatitis), PAG+AP (DL-propargylglycin + acute pancreatitis) .
Data are expressed as M ± S.E.M of 10 rats in each group.

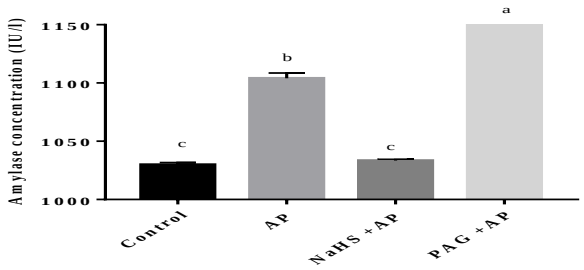

Figure (1): Shows the changes in serum amylase level in AP group treated with either NaHS or PAG:

[a, b and c] Means that columns with different superscripts are significantly different ($P \leq 0.05$).
AP (acute pancreatitis), NaHS +AP (Sodium hydrosulfide+ acute pancreatitis), PAG+AP (DL- propargylglycin + acute pancreatitis) .
Data are expressed as M ± S.E.M of 10 rats in each group.

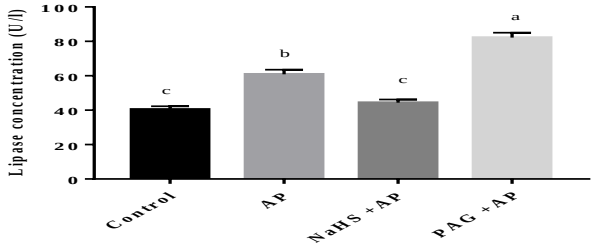

Figure (2): Shows the changes in serum lipase level in in AP group treated with either NaHS or PAG:

[a, b and c] Means that columns with different superscripts are significantly different ($P \leq 0.05$)
AP (acute pancreatitis), NaHS +AP (Sodium hydrosulfide+ acute pancreatitis), PAG+AP (DL- propargylglycin + acute pancreatitis) .
Data are expressed as M ± S.E.M of 10 rats in each group.

Pancreatic histopathology
Acute pancreatitis was scored using Schmidt criteria of grading system based on the leukocytic infiltration, acinar cell necrosis, edema and hemorrhage (Schmidt et al, 1992 and Chen et al., 2015)

Table (2): **Shows the quantitative grading score for pancreatitis:**

Quantitative grading score for pancreatitis	**0**	**1**	**2**	**3**
Interstitial edema	None	Interlobular	Lobule involved	Isolated island like acinar cells
Leukocyte infiltration	none	<20%	20–50%	>50%
Acinar cell necrosis	none	<5%	5–20%	>20%
Hemorrhage	none	1–2 points	3–5 points	>20%

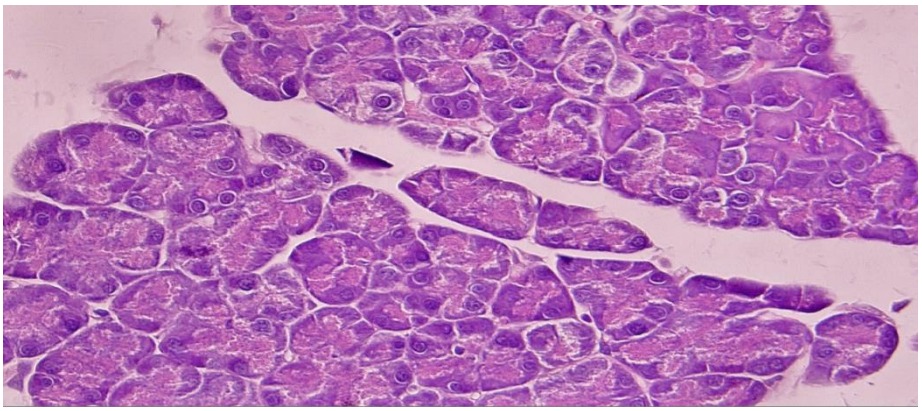

Figure 3: shows pancreatic histopathology in control group:

Normal appearance of pancreatic acini with no pathologic changes (grade 0)

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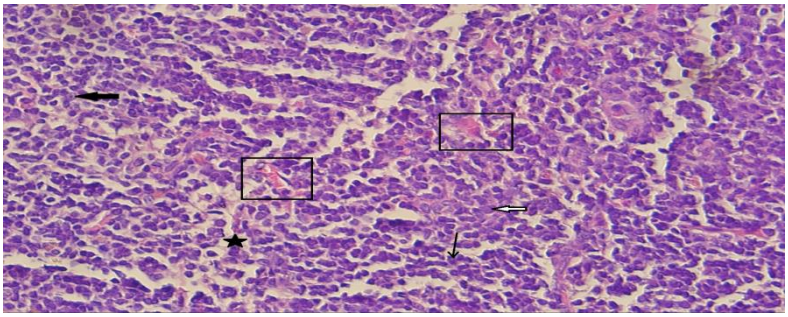

Figure 4: shows pancreatic histopathology in AP group:

This figure shows picture of AP in the form of disruption of the pancreatic architecture, significant interstitial edema marked by star, acinar cell necrosis (>20%) marked by white arrow, extensive inflammatory cell infiltration mainly neutrophils (>50%) marked by arrow, acinar cells show intracytoplasmic vacuolization marked by bold arrow (around 20%), some collagen bundles and few areas of hemorrhage marked by square (severe pancreatitis). Pancreatic islet cells remained unaffected (grade 1).

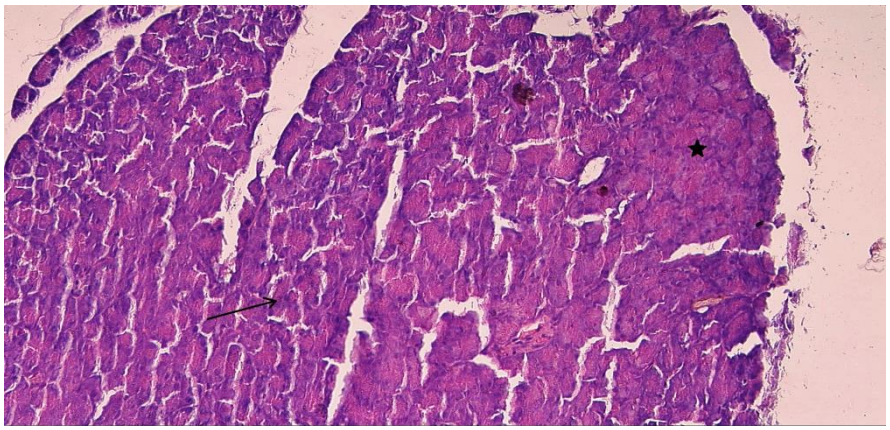

Figure 5: shows pancreatic histopathology in NaHS + AP group:

This figure shows minimal leukocyte infiltration of the pancreas (<20%) marked by arrow and mild focal interstitial edema marked by bold star. No other pathologic changes (insignificant inflammation near normal) (grade 1).

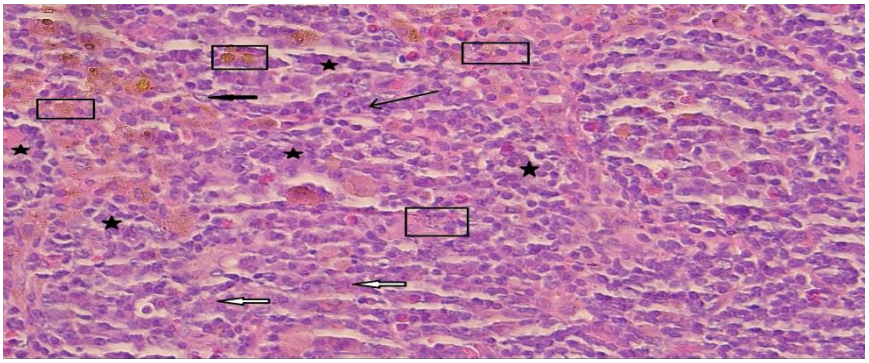

Figure 6: shows pancreatic histopathology in PAG + AP group:

This figure shows marked disruption of the pancreatic architecture, presence of isolated island like acinar cells, significant interstitial edema marked by star, acinar cell intracytoplasmic vacuolization marked by bold arrow, and necrosis (>20%) marked by white arrow, extensive inflammatory cell infiltration (>50%) mainly neutrophils marked by arrow, some collagen bundles and extensive areas of hemorrhage marked by the square (severe pancreatitis). Pancreatic islet cells remained unaffected (grade 3).

Discussion

Acute pancreatitis is one of the most common diseases of the gastrointestinal tract with wide clinical variation, ranging from a mild to a severe necrotizing inflammation (Akyazi et al., 2013).

Acute pancreatitis develops after a cascade of multiple pathways leading to activation of trypsinogen which in turn activates other digestive enzymes. This causes a surge in the oxidative stress leading to generation of free radical from oxidation of lipid and proteins which disrupts the pancreatic membrane. In both experimental and clinical studies, it has been seen that oxidative stress plays a central role in the pathogenesis of AP (Manohar et al., 2017).

Acute pancreatitis can be induced by several methods. One of these methods is intraperitoneal injections of L-arginine. L-arginine induced AP is a good-established model that induces pancreatitis with similar presentation to that in humans. So, L-arginine was selected for induction of AP in this study.

In this study serum amylase and lipase levels were measured as a diagnostic markers for estimation of AP as reported previously by Kaur et al., 2016 who found that these enzymes are highly specific and highly sensitive for AP so they are sufficient for diagnosis of AP.

The result of the present study demonstrated that administration of L-arginine produces AP at a dose 2.5g / Kg in the form of increase in the serum levels of pancreatic enzymes (amylase and lipase). In addition, disruption of the pancreatic architecture, significant interstitial edema, acinar cell necrosis, extensive inflammatory cell infiltration mainly neutrophils and few areas of hemorrhage which revealed by pancreatic histopathology which are in a line with Yılmaz et al., 2016.

Hydrogen sulfide is a gaseous mediator which plays an important role in a wide range of physiological and pathological processes. It is a small molecular weight liposoluble gas which permeates through the cell membrane freely to exert important physiological functions (Cutillas et al., 2015).

H_2S is generated enzymatically through the reverse trans-sulfuration pathway by the activity of 3 enzymes which include cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3MST). CBS and 3-MST are present in the CNS. While, CSE presents in the periphery

incuding liver, stomach and pancreas. D-propargylglycin (PAG) blocks H_2S synthesis through inhibition of CSE (Lioret et al., 2015).

The results of this study demonstrated that H_2S has potent anti-inflammatory and anti-oxidant properties. This was evidenced by the obtained data which revealed a significant decrease in serum levels of pancreatic enzymes amylase and lipase levels in NaHS treated group to the level of control group in comparison to the AP group. It was also evidenced by pancreatic histopathology that showed minimal leukocyte infiltration of the pancreas and mild focal interstitial edema.

Rao et al., (2015) reported that AP is an inflammatory condition of the pancreas that is characterized by elevated pancreatic enzymes and various inflammatory cytokines in the blood. He also showed the anti-inflammatory role of H_2S in L- arginine induced AP and showed that NaHS successfully reduced the serum level of pancreatic amylase.

In PAG treated group, there were significant increase in serum levels of pancreatic enzymes amylase and lipase levels in comparison to the AP group. Also, the pancreatic histopathology showed disruption of the pancreatic architecture, significant interstitial edema, extensive inflammatory cell infiltration mainly neutrophils and extensive areas of hemorrhage and these are in agreement with Rao et al., 2015.

The protective effect of H_2S in AP is mediated through several mechanisms. They include inhibiting phosphatidyl-inositol 3-kinase (PI3K), neutrophil activation, reduction of the leucocyte infiltration, the NO production and free radicals (Greabu et al., 2016).

Módis et al., (2014) reported that H_2S reduces the inflammation in AP by inhibiting PI3K which leads to protein kinase B (AKT) dephosphorylation. Thus, there is no degradation of I-kappa B kinase (IκB) from nuclear factor kappa B (NF-κB) with subsequent inactivation of NF-κB. It also increases neutrophil activation which is associated with a negative modulation of pro-inflammatory factors including IL-6 and IL-8 and a positive modulation of the anti-inflammatory factor IL- 10. Thus, the role of H_2S in AP involves the suppression of IκB degradation, NF-κB transcription and pro-inflammatory gene expression.

The anti-inflammatory effect of H_2S is also explained by reduction of the leucocyte infiltration either by local vasoconstriction or inhibition of the intercellular adhesion molecule (ICAM-1). In addition, it reduces IL-1β production in the pancreas either by inhibition of both p38 mitogen activated protein kinase (p38 MAPK) phosphorrylation and its translocation from the cytosol to the nucleus of the p65 subunit of NF-kB or by decreasing the leucocyte migration to the pancreatic tissue (Rao et al., 2015).

In conclusion, administration of H_2S is protective in case of AP as evidenced by the obtained marked improvement of pancreatic histopathology. In addition, administration of H_2S donor; NaHS led to significant decrease in pancreatic enzymes; amylase and lipase.

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