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Comparing the preventive effect of sodium hydrosulfide, leptin, and curcumin against L-arginine induced acute pancreatitis in rats: role of corticosterone and inducible nitric oxide synthase

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Objectives. Acute pancreatitis (AP) is a life-threatening condition. Using antioxidants in AP is insufficient and conflicting. Therefore, this study compared the effect of hydrogen sulfide (H2S) donor, sodium hydrosulfide (NaHS), leptin or curcumin pretreatment on AP induced by L-arginine.

Methods. Forty adult male rats were used and classified into: 1) control; 2) AP group [each rat was intraperitoneally (i.p.) injected with 2 doses of L-arginine of 250 mg/100 g body weight (b.w.) with an interval of 1 h]; 3) NaHS+AP group (each rat was i.p. injected with 10 mg/kg b.w. of NaHS 1 h before induction of AP); 4) leptin+AP group (each rat was pretreated with 10 μg/kg b.w. of leptin 30 min before induction of AP; and 5) curcumin+AP group (in which rats were i.p. injected with 150 mg/kg b.w. of curcumin 30 min before induction of AP). Serum amylase, lipase, nitric oxide (NO), tumor necrosis factor alpha (TNF-α), and corticosterone (CORT) levels were assayed. In addition, pancreatic tissues were obtained for histopathological examination and malondialdehyde (MDA), total antioxidant capacity (TAC), and inducible nitric oxide synthase (iNOS) levels were measured.

Results. All AP treated groups showed significant decrease in serum levels of pancreatic enzymes, NO, and TNF-α, and pancreatic MDA and iNOS levels, while TAC levels were significantly increased. NaHS caused more limitation of inflammation than leptin and curcumin by affecting iNOS. Leptin was more potent than curcumin due to the stimulatory effect of leptin on glucocorticoid release to counteract inflammation.

Conclusions. NaHS was more effective in AP amelioration than the leptin and curcumin.

Key words: acute pancreatitis, L-arginine, NaHS, leptin, curcumin, iNOS, corticosterone

Acute pancreatitis is a localized inflammation of the pancreas, which ranges from a mild self-limiting disease that requires no more than supportive measures to severe disease with life-threatening complications, such as lung injury and renal failure (Perez et al. 2015). Incidence of AP is increasing from 13 to 45/100 000. About 30% of AP patients will develop severe acute pancreatitis (SAP) that is characterized by severe attacks, including pancreatic necrosis, intestinal barrier dysfunction, and bacterial translocation, leading to multiple organ dysfunction. The mortality rate in patients with SAP is approximately 15–30% (Yan et al. 2018). The pathophysiology of AP is not obvious, although intracellular activation of the digestive enzymes in acinar cells has been accepted as the starting point. Free oxygen radicals released from the injured cells and cytokines from the leukocytes play a major role in the progression of AP and multi-organ failure. Autodigestion and failure of microcirculation in pancreas are the main

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mechanisms in the pathophysiology of pancreatitis (Saglam et al. 2017).

L-arginine-induced AP is one of the most common animal models. It is used to study the biochemical and histological alterations of AP. L-arginine can produce AP in rats by various mechanisms, such as oxygen and nitrogen free radicals generation that distorts the cellular membranes of zymogen granules, thus releasing the digestive enzymes, cellular proteins, and increased levels of inflammatory mediators (El-Ashmawy et al. 2018).

The clinical trials using antioxidants in AP are insufficient, showing limited effectiveness and conflicting. Therefore, further studies are needed in order to design more efficient therapeutic strategies based on antioxidants together with the direct anti-inflammatory therapies (Perez et al. 2015; Yan et al. 2018).

Hydrogen sulfide (H_2S) is a gaseous mediator that plays an important role in the physiological and pathological processes (Wu et al. 2015). However, the role of H_2S in inflammation is still controversial. Some studies have shown pro-inflammatory effects of H_2S (Tamizhselvi et al. 2007; Wang et al. 2013), while others have reported that H_2S has anti-inflammatory effects (Mard et al. 2012; Wallace et al. 2015; Ali et al. 2018). Moreover, other studies have found that the effect of H_2S in case of inflammation is dose dependent (Sidhapuriwala et al. 2009; Whiteman and Winyard 2011; Saglam et al. 2017).

Leptin is one of the adipokines, which regulates body weight and energy expenditure. It also affects gastric and pancreatic secretions, insulin release, protects the gastric mucosa against noxious agents, and influences the inflammatory process in the pancreas by removing noxious factors and attenuating the inflammatory process (Jaworek and Konturek 2014).

Curcumin is a yellow-colored substance derived from turmeric (curcuma longa). Studies conducted on curcumin for the last few decades have demonstrated that it has antioxidant, anti-inflammatory, anti-fibrotic, anti-apoptotic, and anti-cancerogenic features. However, studies evaluating the effects of curcumin in AP are still considered novel (Gulcubuk et al. 2013; Ma et al. 2017).

Therefore, the present study was done to compare the effect of pretreatment with either H_2S donor, sodium hydrosulfide (NaHS), leptin or curcumin on AP induced by L-arginine by determining the levels of pancreatic enzymes (amylase and lipase), oxidative stress parameters [malondialdehyde (MDA), nitric oxide (NO), inducible nitric oxide synthase (iNOS), and total antioxidant capacity (TAC)], inflammatory marker [tumor necrosis factor alpha (TNF-α)] and corticosterone (CORT), in addition to assessment of pancreatic histopathological changes.

Materials and methods

Animals. The present study was conducted on adult male Wistar albino rats (n=40) weighing 280±20 g. They were kept under standard laboratory conditions with natural light/dark cycle. They were after arrival from the supplier left for two weeks for acclimatization. Rats were fed a standard diet of commercial rat chow and tap water *ad libitum* through the time of the study (Sadek and Khattab 2017). The animal care and experiments protocol described in this study were complied with "Research Ethics Committee" Faculty of Medicine, Minia University, Egypt, which are in accordance with the NIH Guide for Care and Use of Laboratory Animals (U.S. Department of Health and Human Services, NIH 1992).

Rats (8/group) were randomly classified into the following groups: Control group – each rat was intraperitoneally (i.p.) injected with saline as a vehicle. 24 h later, the rats were sacrificed by decapitation; AP group – each rat was given 2 doses of L-arginine (250 mg/100 g body weight, b.w.) i.p. with an interval of 1 h, the rats were sacrificed by decapitation 24 h after last L-arginine administration (Yilmaz et al. 2016); NaHS+AP group – each rat was i.p. injected with NaHS at a dose of 10 mg/kg b.w., which was given 1 h before induction of AP (Rao et al. 2015), the rats were sacrificed by decapitation 24 h later; Leptin+AP group – each rat was i.p. pretreated with leptin at a dose of 10 μg/kg b.w. (Gultekin et al. 2007) 30 min before induction of AP (Motawi et al. 2008), the rats were sacrificed by decapitation 24 h later; Curcumin+AP group – rats were i.p. injected with 150 mg/kg b.w. of curcumin 30 min before induction of AP (Yu et al. 2018), the rats were sacrificed by decapitation 24 h later.

Chemicals. All the chemicals used in this study were purchased from Sigma Aldrich (St. Louis, MO, USA). They were freshly dissolved in physiological saline on the day of the experiment.

Blood sample collection and storage. Blood samples were obtained, left to clot at room temperature and then centrifuged at 4 000 rpm for 15 min. The clear supernatant sera were withdrawn into labeled Eppendorf tubes and stored at –20 °C till the time of chemical assay of pancreatic enzymes (amylase and lipase), NO, TNF-α and CORT.

Chemical assays. Serum α-amylase was measured using commercially available kit (Biodiagnostic,

Egypt). Serum level of lipase was estimated using commercially available kit (Spectrum, Egypt). Measurement of serum NO was carried out using colorimetric assay kit (Promega, USA). Estimation of serum TNF-α was done by using ELISA kit (Sigma Aldrich, USA) following manufacturer's instructions. Measurement of CORT was based on extraction of free 11-hydroxycorticosteroids from serum, mainly cortisol and CORT, by methylene chloride followed by their condensation with an acidic fluorescence reagent. The induced fluorescence was measured on a spectrofluorometer at 510 nm after excitation at 450 nm (Mattingly 1962).

Chemical analysis of pancreas tissue. The abdomen of each rat was opened and the pancreas was removed. Pancreatic specimens were obtained and stored at –80 °C for determining levels of MDA, a marker of lipid peroxidation, and TAC by the commercial colorimetric kits (Biodiagnostic, Egypt). Estimation of iNOS was also done using ELISA kit (Sigma Aldrich, USA) according to the manufacturer's instructions.

Pancreatic histopathology. The remaining pancreatic specimens 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 confirming the induction of AP. AP was scored using Schmidt criteria of grading system based on the leukocytic infiltration, acinar cell necrosis, edema and hemorrhage (Chen et al. 2015; Schmidt et al. 1992) (Table 1).

Statistical analysis of data. Data were expressed in the form of mean ± standard error of the mean (mean ± SEM). They were considered statistically significant values if the probability value (p value) ≤0.05. Statistical analysis of data was carried out by one-way analysis of variance (ANOVA). GraphPad Prism software version 6 was used for all statistical analyses.

Results

Changes in serum amylase, lipase, NO and TNF-α levels. The results clearly showed that administration of L-arginine to rats in AP group caused significant increase in serum amylase, lipase, NO and TNF-α levels in comparison with control group. While, pretreatment with either NaHS, leptin or curcumin resulted in significant decrease in the levels of these parameters in comparison with AP group. Comparing the effect of different treatments, administration of NaHS led to significant decrease in serum levels of amylase, lipase, NO and TNF-α in NaHS+AP group in comparison with both leptin and curcumin treated groups (Table 2).

Changes in serum corticosterone level. Our results showed that administration of L-arginine in AP group caused significant increase in serum CORT

Table 1
Quantitative grading score for acute pancreatitis

Parameter \ Score	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%

Table 2
Changes in serum parameters levels in different experimental groups

Parameter	Control	AP	NaHS+AP	Leptin+AP	Curcumin+AP
Amylase (IU/l)	1019±4.97^{d}	1564±36.59^{a}	1027±6.13^{d}	1201±41.64^{c}	1334±40.26^{b}
Lipase (IU/l)	40.46±0.70^{d}	60.84±1.03^{a}	44.41±0.69^{d}	50.83±0.94^{c}	56.23±1.10^{b}
NO (μmol/l)	8.10±0.27^{d}	14.77±0.68^{a}	8.46±0.23^{d}	10.34±0.31^{c}	12.25±0.52^{b}
TNF-α (pg/ml)	102.50±0.55^{c}	141.40±1.67^{a}	107.10±0.55^{c}	126.40±1.05^{b}	129.10±0.85^{b}
CORT (μg/l)	48.30±1.27^{c}	55.10±1.26^{b}	50.31±0.90^{c}	60.56±1.34^{a}	50.63±1.53^{c}

Data are expressed as mean ± S.E.M. of 8 rats in each group. Means in the same horizontal row with different superscripts ($^{a, b, c, d}$) are significantly different (p≤0.05). Abbreviations: AP – acute pancreatitis; NaHS+AP group – sodium hydrosulfide + acute pancreatitis; NO – nitric oxide; TNF-α – tumor necrosis factor-alpha; CORT – corticosterone.

level compared to control group. However, pretreatment of AP rats with leptin resulted in significant increase in CORT level as compared to AP group with and without treatment with either NaHS or curcumin (Table 2).

Pancreas oxidative state. The obtained data demonstrated that pancreas MDA and iNOS levels were significantly increased while TAC level was significantly decreased in AP group as compared to control. While, administration of either NaHS, leptin or curcumin resulted in significant decrease in pancreas MDA and iNOS levels, and increase in TAC level as compared to AP group. Comparing the effect of different treatments, NaHS pretreatment proved to cause significant decrease in pancreas MDA and iNOS levels, and increase in TAC level compared to both leptin and curcumin treated groups (Table 3).

Pancreas histopathology results. Microscopic examination of pancreas sections of control group showed normal appearance of pancreatic acini with no pathologic changes. However, in AP group pancreatic histopathology revealed picture of severe pancreatitis in the form of disruption of the pancreatic architecture, significant interstitial edema, acinar cell necrosis (>20%), extensive inflammatory cell infiltration mainly neutrophils (>50%). Acinar cells showed intracytoplasmic vacuolization (around 20%), some collagen bundles and few areas of hemorrhage (severe pancreatitis). Pancreatic islet cells remained unaffected. In NaHS+AP group, minimal leukocyte infiltration of the pancreas (<20%) and mild focal interstitial edema were detected. No other pathologic changes were found (insignificant inflammation near normal). In leptin+AP group, pancreatic histopathology showed minimal disruption of the pancreatic architecture, mild interlobular edema, scattered acinar cell necrosis (<5%), mild neutrophil infiltration (<20%). No areas of hemorrhage or fibrosis were detected (mild pancreatitis). Pancreatic islet cells remained unaffected. In curcumin+AP group, there were moderate lobular architecture disruption with lobular edema, acinar cell necrosis (<20%), some acinar cells showed intracytoplasmic vacuolization (around 20%) and moderate amount of neutrophilic infiltration of the pancreas (about 30%). Minimal fibrosis and areas of hemorrhage (<5%) were detected (moderate pancreatitis). Pancreatic islet cells remained unaffected (Figures 1–5).

Discussion

Acute pancreatitis is one of the most common diseases of the gastrointestinal tract. In both experimental and clinical studies, oxidative stress and inflammation play a central role in the pathogenesis of AP (Manohar et al. 2017; Yu et al. 2018). 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.

L-arginine produces AP by several ways. L-arginine is metabolized by two pathways, which include the NO-dependent and the NO-independent pathways. In the NO-dependent pathway, L-arginine at high dose is metabolized by nitric oxide synthase (NOS) to produce L-citrulline and larger amount of NO, which is the key mediator in cell injury in AP and induces nitrostative and oxidative stress. Furthermore, in the NO-independent pathway, L-arginine is hydrolyzed by arginase to urea and L-ornithine which induce severe AP (Foster 2014; Kui et al. 2014). Moreover, L-arginine may also stimulate the induction of AP through increased production of cytokines, such as TNF-α and IL-6 (Aziz et al. 2017).

The results of the present study demonstrated that administration of L-arginine to rats produced severe AP, which was confirmed with histopathological examination. In addition, marked increase in the serum levels of pancreatic enzymes, NO, CORT and TNF-α, and pancreatic MDA and iNOS levels were detected what is in a line with previous studies (Ucmak et al. 2016; Yilmaz et al. 2016; Aziz et al. 2017).

The increase in pancreatic MDA and serum TNF-α, and decrease in pancreatic TAC levels in L-arginine-

Table 3
Pancreatic MDA, iNOS and TAC levels in different experimental groups

Parameter	Control	AP	NaHS+AP	Leptin+AP	Curcumin+AP
MDA (nmol/g)	24.85 ± 1.07^{c}	41.50 ± 0.79^{a}	27.20 ± 1.23^{c}	34.90 ± 0.58^{b}	36.92 ± 0.58^{b}
iNOS (pg/mg)	129.20 ± 1.64^{d}	161.30 ± 1.76^{a}	130.50 ± 2.22^{d}	139.30 ± 1.71^{c}	149.80 ± 2.10^{b}
TAC (μmol/g)	11.27 ± 0.51^{a}	2.91 ± 0.33^{d}	8.65 ± 0.43^{b}	6.55 ± 0.40^{c}	4.98 ± 0.41^{c}

Data are expressed as mean ± S.E.M. of 8 rats in each group. Means in the same horizontal row with different superscripts ($^{a, b, c, d}$) are significantly different ($p\leq0.05$). Abbreviations: AP – acute pancreatitis; NaHS+AP – sodium hydrosulfide+acute pancreatitis; MDA – malondialdehyde; iNOS – inducible nitric oxide synthase; TAC – total antioxidant capacity.

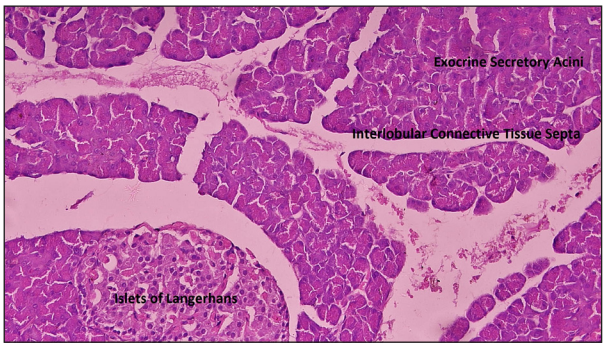

Figure 1. Pancreas histopathology of control group. Representative (H&E) stained pancreatic sections using (original magnification was 200×) from control group showing normal appearance of pancreatic acini with no pathologic changes. Abbreviations: H&E – hematoxylin and eosin.

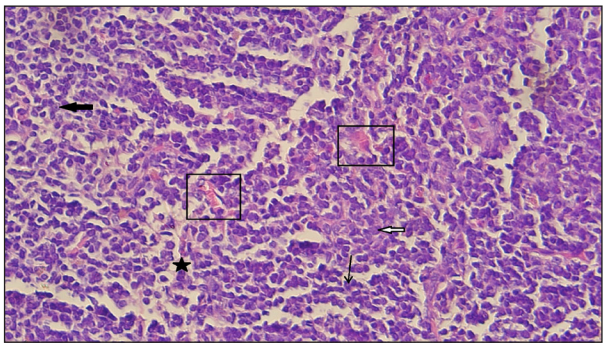

Figure 2. Pancreas histopathology of AP group. Representative (H&E) stained section of pancreas obtained from L-arginine-treated mice (AP) (original magnification was 400×) showing picture of severe pancreatitis in the form of disruption of the pancreatic architecture, significant interstitial edema 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. Abbreviations: AP – acute pancreatitis; H&E – hematoxylin and eosin.

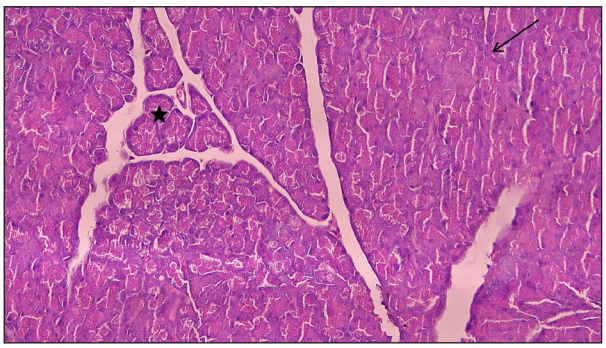

Figure 3. Pancreas histopathology of NaHS+AP group. Representative (H&E) stained pancreatic sections using (original magnification was 200×) from NaHS+AP group showing minimal leukocyte infiltration of the pancreas (<20%) marked by arrow and mild focal interstitial edema marked by bold star. No other pathologic changes (insignificant inflammation near normal). Abbreviations: AP – acute pancreatitis; NaHS – sodium hydrosulfide; H&E – hematoxylin and eosin.

treated group in our study can be due to enhancement of oxidation of cellular membrane biomolecules including polyunsaturated fatty acids by L-arginine. This could result in formation of MDA, which can activate nuclear factor-kappa B (NF-κB) with a subsequent up regulation of various inflammatory cytokines, which has been found by Yenicerioglu et al. (2013). Hasan et al. (2015) have also reported that the excess ROS could attack lipids and proteins in the membranes of zymogen granules in pancreas which led to infiltration of great amount of digestive enzymes into pancreatic tissues with subsequent autodigestion and elevation of pancreatic enzymes that is in accordance with our study results. Furthermore, Mirmalek et al. (2016) have shown that activation of granulocytes and other proinflammatory mediators is regulated by TNF-α, which activates intracellular protease (trypsinogen) and thus cellular necrosis.

According to the present study results, there was significant increase in serum NO level that was accompanied with significant increase in pancreatic iNOS level in L-arginine-treated rats as compared to other groups. NO is an important inflammatory mediator that is crucial for the early evolution of L-arginine pancreatitis. An elevated level of NO could be ascribed to induction of iNOS that was evoked by inflammatory cytokines. NO reacts with superoxide anion to form peroxynitrite that can react directly with lipid, protein and DNA resulting in cellular dysfunction and tissue injury what has been demonstrated in many studies (Biradar and Veeresh 2013; Al-Malki 2015; Hasan et al. 2015; Aziz et al. 2017).

The present study and other previous research data have demonstrated that serum CORT level was increased in human and experimental animals with AP (Abe et al. 1995; Muller et al. 2006; Cao and Liu 2013). In our study, the increase of serum CORT in L-arginine-treated rats can probably be a response to stress. Moreover, glucocorticoids are an important factor for acinar cell survival by decreasing their sensitivity to apoptosis during AP by inhibiting cytokine production, which is in agreement with Muller et al. (2006).

There is much controversy about the effect of H_2S, leptin and curcumin in inflammatory condi-

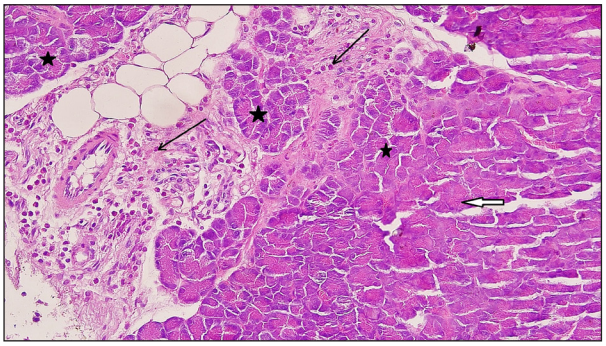

Figure 4. Pancreas histopathology of leptin+AP group. Representative (H&E) stained pancreatic sections (original magnification was 200×) from Leptin+AP group showing minimal disruption of the pancreatic architecture, mild interlobular edema marked by star, scattered acinar cell necrosis (<5%) marked by white arrow, mild neutrophil infiltration (<20%) marked by arrow. No areas of hemorrhage or fibrosis were detected (mild pancreatitis). Pancreatic islet cells remained unaffected. Abbreviations: AP – acute pancreatitis; H&E – hematoxylin and eosin.

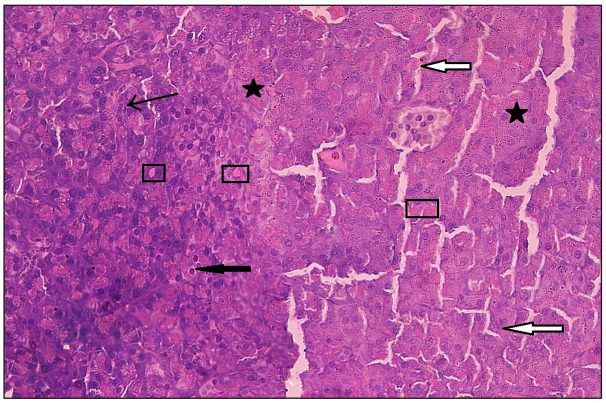

Figure 5. Pancreas histopathology of curcumin+AP group. Representative (H&E) stained pancreatic sections using (original magnification was 200x) from Curcumin+AP group showing moderate lobular architecture disruption with lobular edema marked by star, acinar cell necrosis (<20%) marked by white arrow, some acinar cells show intracytoplasmic vacuolization marked by bold arrow (around 20%), moderate amount of neutrophilic infiltration of the pancreas (about 30%) marked by arrow. Minimal fibrosis and areas of hemorrhage marked by square (<5%) was detected (moderate pancreatitis). Pancreatic islet cells remained unaffected. Abbreviations: AP – acute pancreatitis; H&E – hematoxylin and eosin.

tions. Some studies have shown that they may have pro-inflammatory effects (Ang et al. 2013; Singh et al. 2014; Pham and Mathis 2017), while, others have proved the anti-inflammatory effects of them (Qi et al. 2017; Ali et al. 2018; Hernandez et al. 2018).

The results of this study demonstrated the anti-inflammatory and anti-oxidant properties of H_2S donor, NaHS. This was evidenced by histopathological assessment and the significant decrease in serum levels of pancreatic enzymes, NO and TNF-α and pancreatic MDA and iNOS levels with increase in pancreatic TAC levels in AP group treated with NaHS as compared to AP group.

The significant decrease in pancreas MDA and increase in TAC levels in AP group treated with NaHS as compared to AP group in this study is in line with previous studies (Olas and Kontek 2015; Hu et al. 2016; Zhang et al. 2016).

Nuclear factor (erythroid-derived 2)-like 2, also known as nuclear factor-erythroid 2 (NF-E2) related factor 2 (Nrf2), is a transcription factor that regulates a wide variety of genes expression. Nrf2 is found mostly in the cytoplasm as an inactive complex with Kelch-like ECH-associated protein 1 (Keap1). Under oxidative stressed conditions, Keap1 undergoes ubiquitination and promotes Nrf2 translocation to the nucleus, in which Nrf2 binds to promoters containing the antioxidant response element (ARE) sequence and inducing ARE-dependent gene expression. H_2S can S-sulfhydrate Keap1 at cysteine-151, which causes a conformational change in Keap1 and thereby leads to Nrf2 dissociation from Keap1. The activated Nrf2 finally translocates to nucleus and promotes antioxidant gene transcription (Xie et al. 2016). Furthermore, Corsello et al. (2018) have reported that H_2S has been shown to exert antioxidant effects through several mechanisms including direct quenching of ROS, modulation of cellular levels of reduced glutathione (GSH) and thioredoxin (Trx-1), or increased expression of antioxidant enzymes (AOE), such as superoxide dismutase (SOD) catalase and glutathione peroxidase (GPx) by activating the transcription factor Nrf2.

According to our results, administration of NaHS to L-arginine-treated rats led to a significant decrease in both pancreatic iNOS and serum NO levels and this is in agreement with the data of Ekundi-Valentim et al. (2010) who found that H_2S reduced iNOS protein expression and NO production by macrophages.

Several studies have discussed the interaction of H_2S with NO in various systems. They have reported that exogenous H_2S could suppress iNOS activity and expression, reduce NO content and exert an anti-inflammatory effect. Therefore, iNOS expression in rats was positively correlated with the severity of injury (Yang et al. 2017).

The key role of TNF-α as a pro-inflammatory mediator has been implicated in the pathogenesis of AP. In addition, there is direct evidence between blocking the action of TNF-α and delay in the onset

of AP (Rashidian et al. 2018). This is in accordance with our results where the anti-inflammatory effect of NaHS was confirmed by the significant decrease in TNF-α in AP group treated with NaHS as compared to AP group. This can be explained by inhibition of NF-κB activation, which is associated with an array of diseases, such as AP (Rao et al. 2015; Xie et al. 2016).

In this study, there was a significant decrease in the serum levels of pancreatic enzymes, NO, TNF-α, and pancreatic MDA and iNOS levels with increase in pancreatic TAC level in AP group treated with leptin in comparison with AP group. But these results were still higher than control group. This was accompanied with improvement of pancreatic histopathological changes which is in agreement with the data of Ghantous et al. (2015). This can be explained by inhibition of pancreatic exocrine secretion through direct effect on pancreatic acini with subsequent reduction of intrapancreatic enzyme activity and attenuation of pancreatic damage in the course of AP, increase in the anti-inflammatory mediators with decrease in the release of pro-inflammatory cytokines, glucocorticoids release, decrease in inducible NO and increase in antioxidant level (Demirci et al. 2012; Zhou et al. 2016).

The protective effects of leptin in our study may be mediated by its effect on serum corticosterone and pancreatic iNOS levels, which is in agreement with the data of Landgraf et al. (2014) who have found that leptin pretreatment through increasing corticosterone level and decreasing iNOS expression, which is responsible for the excess production of NO and subsequently chemokine expression, could eradicate lung inflammation induced by LPS.

Landgraf et al. (2014) have reported that the response to acute inflammation is regulated by the immune and endocrine systems. There is a regulatory loop between the hypothalamus-pituitary-adrenal axis (HPA) axis and the circulating leptin. A positive correlation between leptin and corticosterone was observed. The observed increase in serum corticosterone may have led to the down regulation of lung injury because corticosterone inhibits the action of various inflammatory mediators and reduces the synthesis of chemokines and cytokines and the activation of leukocytes. By sharing structural and functional similarities with cytokines, such as TNF-α, leptin is classified as a type I cytokine, which could activate the HPA and increase the corticosterone levels. That is in accordance with the data our study.

Curcumin is a natural anti-inflammatory agent that has been used for treating medical conditions for many years (Fadus et al. 2017). In the present study, there was a significant decrease in the serum levels of pancreatic enzymes, NO and TNF-α, and pancreatic MDA and iNOS levels in AP group treated with curcumin in comparison with AP group. But these results were still higher in comparison with control group. In addition, this was accompanied with improvement in pancreatic histopathological changes, which is in agreement with other studies (Di Savoia 2014; Rashidian et al. 2018; Yu et al. 2018).

Curcumin has a low level of toxicity and adverse reactions. In many studies, it has been proved that anti-inflammatory is an important function and has been widely used in therapeutic study of diabetes, cardiovascular diseases, and autoimmune diseases (Panda et al. 2017; Qin et al. 2017). In the hepatic ischemia/reperfusion (I/R) injury, curcumin significantly inhibited cell apoptosis and decreased the levels of TNF-a, IL-1β, and IL-6 *via* blocking the NF-κB signaling pathway (Wang et al. 2017). In neuroinflammation, curcumin could suppress overexpression of inflammatory mediators *via* inhibiting the NF-κB pathway (Rahimifard et al. 2017). This suggested that NF-κB signaling pathway may be an important mechanism underlying the anti-inflammatory effect of curcumin (Zhang and Zeng 2019).

Yu et al. (2018) have reported that pretreatment with curcumin dramatically inhibited the nuclear translocation of NF-kB and thus suppressed expression levels of IL-6 and TNF-α in case of AP. The activation of c-Jun NH2-terminal protein kinase (JNK) was also inhibited, leading to impaired NF-kB inflammatory signaling suppression. In addition, Rashidian et al. (2018) have mentioned that the protective effects of curcumin on experimental AP occurred through inhibition of various transcription factors such as NF-κB and inflammatory cytokines such as TNF-α, IL-1β and IL-6. Wu et al. (2017) have found that the antioxidant related pathways may be activated by curcumin. They also have shown that the curcumin significantly increased the expression of heme oxygenase-1 (HO-1) and Nrf2 and suggested that curcumin treatment protected from oxidative stress by activating regulating these two proteins which led to lowering the levels of MDA and ROS.

The detected decrease in serum NO level in AP group treated with curcumin in our study may be due to inhibition of induction of iNOS, which resulted in decrease in NO level, which is in agreement with Liu et al. (2017) who have demonstrated that the iNOS/NO signaling pathway was suppressed with curcumin treatment indicating that the preventive effect of curcumin could be mediated by its ability to suppress the transcription of iNOS and attenuate the downstream signaling pathway of iNOS/NO. iNOS

rather than eNOS is the major NOS that is responsible for the dramatic increase in the production of NO and activation of the signaling pathway. They have reported that inhibition of iNOS by curcumin could be explained by its ability to reduce the transcription ability of iNOS promoter.

In the present study, NaHS was more effective in AP amelioration than leptin and curcumin. This can be interpreted by the detected significant decrease in the level of pancreatic iNOS enzyme, which resulted in decreasing NO level and peroxynitrite anion production with subsequent decrease in the pancreatic MDA and serum TNF-α levels and increase in TAC level in AP group pretreated with NaHS as compared to leptin and curcumin treated groups. Moreover, leptin was more potent in decreasing the serum levels of pancreatic enzymes and NO than curcumin. These findings may be due to the stimulatory effect of leptin on glucocorticoid release, which could result in decreasing the activity of iNOS enzyme.

Conclusions

Administration of L-arginine to rats in our study resulted in severe AP, which was evidenced by disruption of the pancreatic architecture, pancreatic oxidative stress, and inflammation. NaHS was efficient to counteract the inflammatory changes produced by L-arginine and bring them back to insignificant level from control group by its ability to scavenge free radicals, suppress inflammation, and decrease pancreatic iNOS. While the administration of either leptin or curcumin partially corrected the inflammatory changes, their effect couldn't reach the control group level. Thus, NaHS caused more limitation of inflammation than leptin and curcumin by affecting iNOS. On the other side, leptin was more potent in controlling the inflammatory changes than curcumin due to the stimulatory effect of leptin on glucocorticoid release to counteract inflammation in addition to its effect on iNOS level.

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