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AMELIORATIVE EFFECTS OF WATERY EXTRACTS OF *BOSWELIA SERRATA* AND *SYZYGIUM AROMATICUM* ON L-ARGININE INDUCED ACUTE PANCREATITIS IN RATS

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ABSTRACT

The following study was conducted to evaluate the possible ameliorative effects of *Boswellia serrata* (frankincense) and *Syzygium aromaticum* (clove) extracts on L-arginine induced acute pancreatitis (AP) in rats. Sixty adult male rats were divided into 6 groups (10 rats /group). Control group, *Boswellia serrata* (1000 mg/kg), *Syzygium aromaticum* (1000 mg/kg), L-arginine (500mg/100g), L-arginine + *Boswellia serrata*, and L-arginine + *Syzygium aromaticum*. Pancreatic weight, amylase, insulin, IL-6, MDA, NO, GSH, GPx, GR, SOD and catalase were estimated and histological analysis was examined for pancreas. Induction of AP by L-arginine resulted in significant elevation of pancreatic edema and serum amylase level ($P<0.05$) as well as elevation of IL-6, GPx, MDA and NO, whereas, it caused significant reduction in insulin, GSH, GR, SOD and catalase ($P<0.05$). The treatment with *Boswellia serrata* and *Syzygium aromaticum* significantly ($P<0.05$) restored the levels of insulin, GSH, GPx, SOD and catalase and decreased amylase, lipid peroxide levels (MDA) and NO induced by L-arginine compared to the vehicle. Moreover, histopathological analysis further confirmed that administration of selected extracts relatively prevented pancreatic acinar cell damage compared to those animals received L-arginine alone. These findings pointed out the ameliorative role of *Boswellia serrata* and *Syzygium aromaticum* against acute pancreatitis induced by high doses of L-arginine.

KEYWORDS: *L-arginine, pancreatitis, Boswellia serrata, Syzygium aromaticum.*

INTRODUCTION

Acute pancreatitis (AP) is an acute inflammatory disease and one of the most common gastrointestinal disorders requiring acute hospitalization worldwide, with a reported annual incidence of 13 to 45 cases per 100000 patients. [1] It is characterized by various degrees of acinar cell damage with concomitant local and systemic inflammation, mediated by inflammatory cytokines and chemokines. [2] The most common symptoms of AP are acute abdominal pain which may be accompanied by nausea, vomiting, and increased serum concentrations of amylase and lipase. [3] Although the clinical symptoms of AP often resolve completely, about 20% of cases are severe and can lead to serious complications and even death.[4] Additionally, recurrent bouts of AP lead to fibrosis/ damage and chronic pancreatitis, a risk factor for the development of pancreatic cancer. [5]

AP is a disease with heterogeneous etiologies and an obscure pathogenesis. Gallstones, alcohol, hypercalcemia, hyperlipidemia, malnutrition, abdominal trauma, drugs or toxins, infections, and structural abnormalities as pancreas divisum are the well-known causes of acute pancreatitis. However, about 10-30% of cases are still categorized as idiopathic. [1, 6, 7]

Large dose (500 mg/100 g b.wt.) of intraperitoneal (IP) injection L- arginine is known to induce necrotizing pancreatitis in rats and it was found that such dose can selectively induce pancreatic acinar cell damage without morphological changes in the islets of Langerhans (8 and 9). Moreover, this single dose (500 mg/100 g b.wt.) of L-arginine causes a significant mortality in rats. While, the use of double dose (2X250 mg/100 g b.wt.) at 1 hour intervals causes AP without mortality. [10]

Many therapies and medical management is aimed to control the signs and symptoms of AP, using steroids, analgesics and anti-inflammatory agents. The use of synthetic treatment has various kinds of problems like sensitive skin reaction, intolerance and parts of these compounds are expensive and not reliable. [11] There is need to explore potential antioxidant and anti-inflammatory agents available from natural sources, which are cost- effective and has several save advantages. *Boswellia serrata*, Indian frankincense, has been used in Ayurvedic systems of medicine against a number of inflammatory diseases, including osteoarthritis, chronic colitis, ulcerative colitis, Crohn's disease, and bronchial asthma. [12] Its main pharmacologically active ingredients are α and β boswellic acid and other pentacyclic triterpenic acids. [13] Acetyl-11-keto-β- boswellic acid (AKBA), the active compound isolated from this plant possess inhibitory activities against experimental ileitis. [14] Experimental

colitis.[15] nociception. [16] Inflammation and atherogenesis.[17] *Syzygium aromaticum* (clove) is one of the most valuable spices that has been used for centuries as food preservative and for many medicinal purposes.[18]. In Ayurveda as well as Iranian traditional medicinal formulations; clove oil is used as nervous stimulant and cognitive enhancer. [19] Phenolic compound, eugenol is reported to be the major chemical constituent present in the clove oil, which is also responsible for its biological activities. The oil also contains eugenol acetate, β-caryophyllene, chavicol, humulenes in lesser amounts. [20] Eugenol is reported for its wide range of pharmacological properties including analgesic, anti-oxidant, anti-inflammatory, anti-allergic, anti-carcinogenic and anti-mutagenic activities. [21, 22]

The protective effect of *Boswellia serrata* (frankincense) and *Syzygium aromaticum* (clove) on experimental AP hasn't been investigated to date. Therefore, the principle goal of the current study was to evaluate the potential role of aqueous extract *Boswellia serrata* (frankincense) and *Syzygium aromaticum* (clove) against L-arginine induced acute pancreatitis in rats.

MATERIALS AND METHODS

Chemicals: L- arginine (Sigma Aldrish Co. PVT Ltd, USA), the drug powder was prepared as a solution by dissolving in 0.9 % saline to final concentration of 500 mg/100g and the PH was adjusted to 7 with 5N HCl. (11).

Plants: Boswelia serrata (frankincense) and *Syzygium aromaticum* (clove) were obtained from the Egyptian Herbal Market, Cairo, Egypt. The watery extract of the plants was performed according to the method described by Ravi et al. [23]

Animals and experimental design

Sixty adult male rats weighing 100-150 g were obtained from the holding company for biological products and vaccines (VACSERA) Cairo, Egypt and acclimated for one week in a specific pathogen free (SPF) barrier area where the temperature (25±1) and humidity (55%). Rats were controlled constantly with a 12 h light/dark cycle at the Laboratory of Physiology, Faculty of Science, Helwan University, Cairo, Egypt. The animals were maintained on standard laboratory diet and water *ad libitum*. After the acclimatization period, the rats in the current study were divided into 6 groups (10 rats /group) and the experiment was performed after 12 h of fasting. Group (1) received two intraperitoneal injections of saline at 1-h intervals and served as control group. Group (2) received daily oral administration of

Boswelia serrata watery extract (1000 mg/kg b.wt.) for 7 days. [24] Group (3) received daily oral administration of *Syzygium aromaticum* (clove) watery extract (1000 mg/kg b.wt.) for 7 days. [25] Group (4), (5) and (6) received two intraperitoneal injections of L-arginine (500 mg/100 g b.wt.) at 1-h intervals and each injection containing 50% of the dose as a model of acute pancreatitis (10). After one hour of the last injection, group (4) was received daily oral administration of saline for 7 days and served as (AP induction group) while group (5) was treated with daily oral administration of *Boswelia serrata* watery extract (1000 mg/kg b.wt.) for 7 days and group (6) was treated with daily oral administration of *Syzygium aromaticum* (clove) watery extract (1000 mg/kg b.wt.) for 7 days. At the end of the experimental period, rats were weighed, suddenly decapitated and blood samples were collected. Serum was then separated and stored at -70 ^{o}C for biochemical analyses. Pancreas was dissected, trimmed of fats and weighed then divided into 2 pieces; one piece was stored at – 70 ^{o}C for biochemical studies and the second was preserved in formalin saline (10%) for histological investigation.

Serum analyses

Serum amylase was measured by using commercial kit (Asan Phamaceutical Co., Ltd., Seoul, Korea). Insulin and interleukin-6 (IL-6) activities were assayed by ELISA technique using assay kit purchased from R&D Systems Inc, Minneapolis, USA according to the instructions provided. Lipid peroxidation (MDA) was determined according to the method of Ohkawa et al. [26] The assay of nitrite/nitrate (NO) level in serum was done according to the method of Berkels et al. [27] Serum glutathione (GSH) was determined by the methods of Ellman. [28] The activities of glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD) were determined using commercially available kits from Cayman chemical, Ann Arbor, Michigan, USA according to the manufacturer instructions.

Pancreatic analyses

Lipid peroxidation (MDA), nitrite/nitrate (NO), glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD) were determined in pancreas homogenate according to the methods mentioned above.

Histological Investigation

After fixation of the other piece of pancreas tissues of rats in the different studied groups in formalin saline (10%) for 24 hours, these pieces were washed in tap water then subjected to serial dilutions of alcohol (methyl, ethyl and absolute ethyl) for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in hot air oven for twenty four hours.

Paraffin bees wax tissue blocks were prepared for sectioning at 4 µm by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stain and examined through the electric light microscope. [29]

Statistical analysis

The experiment was set up with a completely randomized design. Data were presented as means ± S.E for the indicated number of independently performed experiments using the Statistical Package for the Social Sciences (SPSS 17.0 for Windows). The statistical significances within parameters were evaluated by one-way and multiple analysis of variation (ANOVA), where significant differences at $P < 0.05$.

RESULTS

The present study was conducted to evaluate therapeutic effects of *Boswellia serrata* and *Syzygium aromaticum* (clove) on the toxicity produced by L-arginine induced acute pancreatitis (AP) in adult male albino rats model. As shown in table (1) the treatment with *Boswellia serrata* and *Syzygium aromaticum* (clove) in normal animals showed non-significant change in pancreas weight/body weight as compared to control group. On the other hand, the induction of AP causes significant increase in this ratio recording (1 mg/g) as compared to control group (0.21 mg/g). However, the treatment with B.S and clove after the induction of AP resulted in significant decrease in the pancreatic weight recording 0.25 mg/g and 0.23 mg/g respectively as compared to AP group.

Table (2) recorded a significant decline ($p<0.05$) in serum insulin of AP model recording (3.87±0.77 µU/mL) as compared to control group (15.65±1.34 µU/mL). However, the induction of AP followed by administration of *Boswellia serrata* and *Syzygium aromaticum* (clove) for 7 days resulted in significant increase in serum insulin level as compared to AP group while it still significantly ($p<0.05$) reduced as compared to control group. However, there was a significant increase in the level of serum amylase in AP group (4467.27±274.25 U/L) comparing with the control group. Administration of clove (1000mg/kg) for 7 days showed significant decrease in serum amylase recording 287405±488.24 U/L, as well as the treatment of AP animals with *Boswellia serrata* also causes significant decrease in serum amylase level as compared to AP model while these levels still significantly increased as compared to control group.

Table 1: Effect of oral administration of B.S. and clove watery extracts (1000 mg/kg b.wt) for 7 days on pancreas weight in L-arginine induced acute pancreatitis in rats.

Groups	Pancreas weight/body weight (mg/g)
Control	0.21±0.01
***Boswellia serrata* (B.S.)**	0.21±0.03
Clove	0.22±0.005
Acute pancreatitis (AP)	1.0±0.03 [a]
AP + B.S.	0.25±0.02 [b]
AP + Clove	0.23±0.01 [b]

Data are expressed as means ± standard error (SE) for 8 animals/ group.

a: Significance change at P < 0.05 in comparison with control group.

b: Significance change at P < 0.05 in comparison with acute pancreatitis (AP) induced group.

Table 2: Effect of oral administration of *Boswellia serrata* (B.S.) and clove watery extracts (1000 mg/kg b.wt.) for 7 days on serum insulin and amylase in L-arginine induced acute pancreatitis in rats.

Groups	Insulin (µU/mL)	Amylase (U/L)
Control	15.65±1.34	1894.87±268
***Boswellia serrata* (B.S.)**	15.98±1.55	1987.99±301.65
Clove	14.94±2.04	2014.01±309.95
AP	3.87±0.77 [a]	4467.27±274.25 [a]
AP + B.S.	7.98±0.98 [ab]	2684.14±457.46 [ab]
AP + Clove	7.05 ±0.9 [ab]	2874.5±488.24 [ab]

Data are expressed as means ± standard error (SE) for 8 animals/ group.

a: Significance change at P < 0.05 in comparison with control group.

b: Significance change at P < 0.05 in comparison with acute pancreatitis (AP) induced group.

Table (2) recorded a significant decline ($p<0.05$) in metabolic hormone (insulin) in serum of AP model recording (3.87±0.77 µU/mL) as compared to control group (15.65±1.34 µU/mL). On the other hand, the induction of AP followed by administration of studied natural extracts for 7 days resulted in significant increase in serum insulin level as compared to AP group while it still significantly ($p<0.05$) reduced as compared to control group. However, there was a significant increase in the level of serum amylase in AP group (4467.27±274.25 U/L) comparing with the control group. Administration of clove (100mg/kg) for 7 days showed significant decrease in serum amylase recording 287405±488.24 U/L, as well as the treatment of AP animals with *Boswelia serrata* also causes significant decrease in serum amylase level as compared to AP model while these levels still significantly increased as compared to control group.

As shown in table (3) induction of AP resulted in significant raise in interleukins-6 (IL-6) as compared to normal control group. Treatment of AP model with clove and B.S at dose level of 1000mg/kg showed significant decrease in the level of IL-6 comparing with AP model.

Table 3: Effect of oral administration of *Boswellia serrata* (B.S.) and clove watery extracts (1000 mg/kg b.wt.) for 7 days on serum interleukin-6 (IL-6) activity in L-arginine induced acute pancreatitis in rats.

Groups	IL-6 (pg/ml)
Control	50.67±7.68
***Boswellia serrata* (B.S.)**	51.71±7.88
Clove	52.34±8.08
AP	89.82±10.32 [a]
AP + B.S.	66.84±8.82 [ab]
AP + Clove	71.5±8.45 [ab]

Data are expressed as means ± standard error (SE) for 8 animals/ group.

a: Significance change at P < 0.05 in comparison with control group.

b: Significance change at P < 0.05 in comparison with acute pancreatitis (AP) induced group.

The results obtained in Table (4) revealed that, the MDA and NO level in serum of L-arginine induced AP group were significantly ($p<0.05$) elevated as compared control group. The treatments with selected extracts (B.S and clove) were significantly reduced the increased in MDA and NO levels induced by L-arginine as compared to AP model.

Table 4: Effect of oral administration of *Boswellia serrata* (B.S.) and clove watery extracts (1000 mg/kg b.wt.) for 7 days on serum lipid peroxidation (MDA) and nitrite/nitrate (NO) levels in L-arginine induced acute pancreatitis in rats.

Groups	MDA (nmol/ml)	Nitrite/Nitrate(µmol/L)
Control	13.58±1.25	55.37±4.67
***Boswellia serrata* (B.S.)**	11.97±2.11	58.37±6.41
Clove	14.05±1.11	47.31±11.34
AP	28.72±4.38 [a]	81.92±6.37 [a]
AP + B.S.	17.68±2.11 [ab]	60.08±5.87 [b]
AP + Clove	21.04±1.57 [ab]	64.18±6.46 [ab]

Data are expressed as means ± standard error (SE) for 8 animals/ group.

a: Significance change at P < 0.05 in comparison with control group.

b: Significance change at P < 0.05 in comparison with acute pancreatitis (AP) induced group.

Table (5) illustrated the effects of L-arginine injection (500mg/100g) on serum enzymatic level of GSH, GR, CAT and SOD which resulted in significant decrease in their levels (0.47± 0.14, 0.65± 0.05, 0.49± 0.05 and 0.88± 0.04) respectively at ($p<0.05$). However, the daily

oral administration of clove and B.S for 7 days in animals injected with 500mg/100g L-arginine resulted in significant amelioration in the enzymatic activity of GPx, GR, CAT and SOD in serum as comparing to AP group. On the other hand the induction of acute pancreatitis caused significant increase in GPx level in serum as compared to control group, whereas, the treatment of rats with B.S and Clove after being treated with L-arginine resulted in decline in the serum level of GPx ($p<0.05$).

Table 5: Effect of oral administration of *Boswellia serrata* (B.S.) and clove watery extracts (1000 mg/kg b.wt) for 7 days on serum glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD) activities in L-arginine induced acute pancreatitis in rats.

Groups	GSH(mmol/ml)	GPx (IU/l)	GR(µmol/ml)	CAT(IU/l)	SOD(IU/l)
Control	0.71±0.15	0.26±0.11	1.14±0.23	0.88±0.11	1.33±0.2
***Boswellia serrata* (B.S.)**	0.68±0.13	0.24±0.055	0.97±0.1	0.9±0.09	1.39±0.22
Clove	0.76±0.17	0.28±0.09	1.25±0.2	0.84±0.13	1.28±0.23
AP	0.47±0.14 [a]	0.57 ± 0.09 [a]	0.65±0.05 [a]	0.49±0.05 [a]	0.88±0.02 [a]
AP+ B.S.	0.65±0.11 [b]	0.34 ± 0.11 [b]	0.89±0.11 [ab]	0.75±0.09 [ab]	0.94±0.11 [ab]
AP+ Clove	0.59±0.1	0.38 ± 0.12 [ab]	0.84±0.16 [ab]	0.86±0.17 [b]	1.11±0.09 [ab]

Data are expressed as means ± standard error (SE) for 8 animals/ group.

a: Significance change at $P < 0.05$ in comparison with control group.

b: Significance change at $P < 0.05$ in comparison with acute pancreatitis (AP) induced group.

Table 6: Effect of oral administration of *Boswellia serrata* (B.S.) and clove watery extracts (1000 mg/kg b.wt.) for 7 days on pancreas lipid peroxidation (MDA) and nitrite/nitrate (NO) levels in L-arginine induced acute pancreatitis in rats.

Groups	MDA (nmol/g tissue)	NO (µmol/g tissue)
Control	100.76±12.02	114.61±10.55
***Boswellia serrata* (B.S.).**	102.34±13.02	117.89±16.72
Clove	96.31±9.37	119.33±5.22
AP	128.91±21.34 [a]	147.32±13.08 [a]
AP+ B.S.	105.31±5.21 [b]	118.71±12.57 [b]
AP+ Clove	111.66±13.25 [ab]	122.18±12.44 [ab]

Data are expressed as means ± standard error (SE) for 8 animals/ group.

a: Significance change at $P < 0.05$ in comparison with control group.

b: Significance change at $P < 0.05$ in comparison with acute pancreatitis (AP) induced group.

The data illustrated in Table (6) showed a significant increase in Tissue MDA and NO level in AP group as compared to control group and also showed significant decrease in the activity of these enzymes in pancreatic tissue of the animals treated with selected natural extract (B.S and clove) after induction of acute pancreatitis for 7 days as compared to AP

group. The induction of AP in adult male albino rats as a result of i.p injection with L-arginine 500mg/100g resulted in significant decrease in pancreatic GSH, GPx, GR, CAT and SOD as compared to control group. However, the oral administration with 1000mg/kg daily for 7 days in AP induced animals resulted in significant increase in the tissue levels of GSH, GPx, GR, CAT and SOD as compared to AP model (table 7).

Table 7: Effect of oral administration of *Boswellia serrata* (B.S.) and clove watery extracts (1000 mg/kg b.wt.) for 7 days on pancreas glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD) activities in L-arginine induced acute pancreatitis in rats.

Groups	GSH (mmol/g tissue)	GPx (IU/g tissue)	GR (µmol/g tissue)	CAT (IU/g tissue)	SOD (IU/g tissue)
Control	1.28±0.12	17.19±2.55	0.78±0.08	1.47±0.09	9.37±1.08
B.S	1.19±0.08	19.88±1.24	0.8±0.1	1.56±0.27	9.29±1.31
Clove	1.31±0.09	18.12±1.09	0.71±0.06	1.41±0.14	9.14±1.1
AP	0.74±0.11 [a]	10.65±2.3 [a]	0.43±0.03 [a]	1.01±0.13 [a]	6.74±1.51 [a]
AP + B.S.	1.17±0.07 [b]	16.72±1.14 [b]	0.7±0.06 [b]	1.42±0.1 [b]	9.08±1 [b]
AP + Clove	0.91±0.14 [ab]	15.25±0.97 [b]	0.64±0.04 [b]	1.38±0.16 [b]	8.43±1.89 [ab]

Data are expressed as means ± standard error (SE) for 8 animals/ group.

a: Significance change at $P < 0.05$ in comparison with control group.

b: Significance change at $P < 0.05$ in comparison with acute pancreatitis (AP) induced group.

Histopatological results

Histological studies revealed that pancreas sections from control rats showed normal histological structure of the island of Langerhans cells and the acini (Fig 1A). Animals treated with *Boswellia serrata* and clove exhibited normal histological structure, indicating the non-toxic effect of *Boswellia serrata* and clove (Fig 1B&C). In contrast, the pancreas sections of rats injected with L-arginine induced acute pancreatitis showed atrophy in the islets of Langerhans cells associated with coagulative necrosis in the epithelial cells lining the exocrine acini (Fig 1D&E). Also, sever congestion in the interlobular stromal blood vessels was noted (Fig 1F). Microscopic investigation of pancreas tissue section of acute pancreatitis-induced rats treated with *Bowellia serrata* for 7 days showed normal histological structure in the islands of Langerhans with massive number of inflammatory cells infiltration in between the acini and lobules and congestion in the blood vessels (Fig 1G) and the investigation of pancreas tissue section of acute pancreatitis-induced rats treated with clove showed mild atrophy in the islands of Langerhans (Figs 1H).

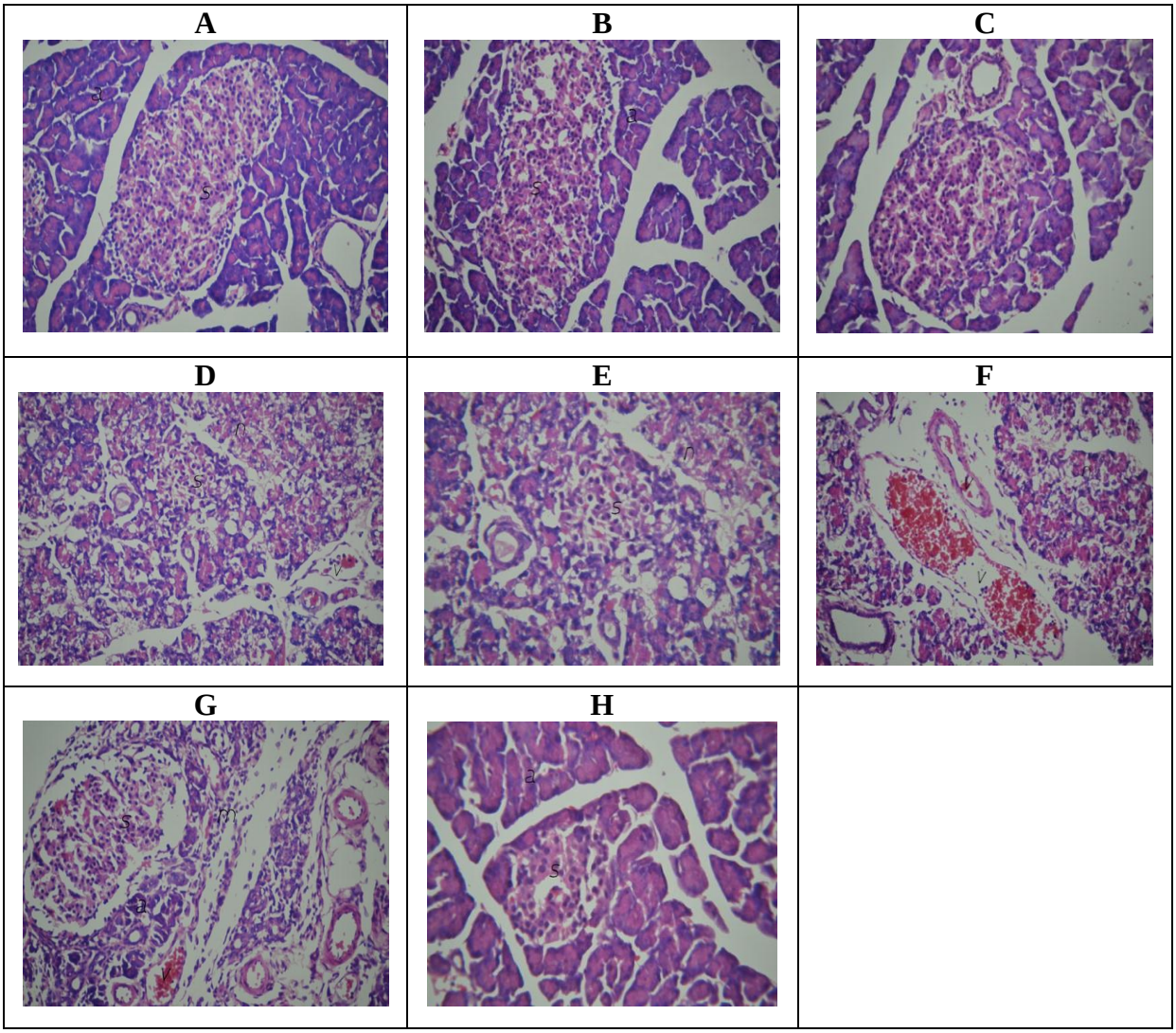

Fig. 1: Histopathology of pancreas showed normal histological structure of the island of Langerhans cells and the acini of control, *Boswellia serrata* and clove groups respectively (A-C) (H &E X 40). (D) Micrograph of acute pancreatitis induced group showed atrophy in the islands of Langerhans cells associated with coagulative necrosis in the epithelial cells lining the exocrine acini (H &E X 40). (E) Magnified part of (D) to identify the atrophy in islands of Langerhans (H &E X64). (F) Micrograph acute pancreatitis induced group showed sever congestion in the interlobular stromal blood vessels (H &E X40). (G) Micrograph of pancreas section from acute pancreatitis induced group treated with *Boswellia serrata* for 7 days showed normal histological structure in the islands of Langerhans with massive number of inflammatory cells infiltration in between the acini and lobules and congestion in the blood vessels. (H&E X 40). (H) Micrograph of pancreas section from acute pancreatitis induced group treated with clove for 7 days showed mild atrophy in the islands of Langerhans (H &E X40).

DISCUSSION

The present results demonstrated that, the treatment with B.S. and clove (1000mg/kg) each, efficiently reduced the severity of L-arginine induced acute pancreatitis (AP) in rats. Similar to the previous reports.[30, 31] in the present study injection of L-arginine significantly developed the AP characterized by raised level of serum amylase and acinar cell necrosis. In most clinical studies, pancreatic enzyme (amylase and lipase) release was found to be a good diagnostic parameter in AP. They usually rise within 4-8 hours. [11] This rise in amylase levels may be due to the production of hydrolytic enzymes in AP which hydrolyzes phospholipids to liberate arachidonic acid and lysophospholipids and the latter has a cytotoxic function, causing acinar cell necrosis. [32] The destruction in the acinar cells resulted in elevation of serum pancreatic enzymes especially amylase and lipase levels. [33] Treatment with B.S and clove decreased the serum amylase level, indicates the ameliorative effects of both extracts on the acinar cells of pancreas. *In vitro* studies on *Boswellia serrata* (B. S) reported that boswellic acids were found to inhibit the synthesis of pro-inflammatory enzyme, Arachidonate 5-lipoxygenase (A5-LO) including 5-hydroxyeicosatetraenoic acid (5-HETE) and leukotriene B4 (LTB-4), which cause inflammation to various organs and tissues. [34] Other anti-inflammatory constituents of B.S, such as quercetin, also block this enzyme, but they do so in a more general fashion, as an antioxidant, whereas boswellic acids seem to be specific inhibitor of 5-LO. [35] In the same manner, Raghavenra et al. [36] reported that aqueous extract of clove and also its active principle eugenol significantly inhibits 5-LO enzyme activity in human polymorphneuclear leukocytes (PMNL) cells.

Most of the previous studies confirmed that the induction of acute pancreatitis resulted in no changes in the serum insulin level. Surprisingly, the serum insulin level in the present study was significantly declined in acute pancreatitis experimental model. This may be due to the progressive inflammation and destruction of pancreatic parenchyma, exocrine atrophy and endocrine insufficiency leading to diabetes in the presence of severe AP leading to chronic pancreatitis. This may lead to destruction of pancreatic β-cells mass and numbers resulted in endocrine insufficiency. [37] In the present study, the treatment with B.S and clove extract resulted in significant increase in insulin level which indicated their ameliorative effects on the endocrine of the pancreas. As mentioned above, the severity AP is caused by acinar cell damage. As a result there is an overproduction of inflammatory mediators and free oxygen radicals. Tissue macrophages are the main source of proinflammatory and anti-inflammatory cytokines that attract neutrophils and more macrophages, and induce the production of

proteases, elastases, and phospholipases. These enzymes, as well as free oxygen radicals cause tissue damage, mainly vascular endothelial necrosis which leads to circulatory stasis. The increase of proinflammatory and decrease of anti-inflammatory cytokines are crucial factors in the progression of inflammation of severe acute pancreatitis. [38] The largest studies have focused on the role of TNF-alpha, IL-1, IL-6, and IL-10. Most of these studies have shown that the levels of proinflammatory cytokines (TNF-alpha and IL-6) are higher in severe forms of AP. The systemic manifestations of severe acute pancreatitis are not only caused by local inflammatory processes, but also by an excessive production and systemic spreading of inflammatory mediators. [39] In view of the aforementioned studies, our study provides further support to these findings by elevation of inflammatory cytokines (IL-6) in serum of AP animals. This elevation in serum IL-6 cytokines was restored by treatment by B.S. and clove extracts in AP model group.

Similarly, the oxygen free radicals could damage extracellular tissue by degrading hyaluronic acid and collagen in the intercellular matrix and directly attack biological membrane through the peroxidation of structurally and functionally important lipids. Furthermore, they could denature enzymes and other important proteins, and damage nucleic acid. In addition, they could indirectly trigger the accumulation of polymorphonulclear (PMN) leukocyte in the tissue. [40] L-arginine is metabolized and hydrolysed in the body in the presence of nitric oxide synthase (NOS) to produce nitric oxide (NO), L-ornithin and urea. Most studies reported that NO is aggravates pancreatic oxidative stress and damage. Several studies demonstrated elevation in MDA which attributed to accumulation of free radicles proposed to be generated from L-arginine. These free radicles initiate lipid peroxidation of the membrane bound polyunsaturated fatty acids, leading to impairment of the membrane functional and structural integrity. [41] This was in agreement with our results which recorded significant increase in serum MDA and NO in AP model group.

Free radical scavenging enzymes such as catalase, superoxide dismutase, glutathione peroxidase and glutathione-S-transferase are the first line cellular defence against oxidative injury. The equilibrium between these enzymes is an important process for the effective removal of oxidative stress in intracellular organelles. Glutathione plays an important role in the regulation of variety of cell function and in cell protection from oxidative injury. The results obtained in the present study revealed that there was increased in the severity of pancreatitis of AP group caused decreased in GSH level in serum with increased in serum

lipid peroxidation products. The reduction in GSH level was similar to the results obtained by Luthen *et al*. [42] Significant changes in GSH level may be the result of massive attack of ROS produced by L-arginine [11] and the participation of GSH in enzymatic elimination of oxidative stress product. Similarly, the present study recorded a significant increase in serum GPx in animal model of AP. A decrease in GSH level with increase in GPx activity may be due to the enhance activity of GPx in elimination of ROS using GSH as a substrate. [38]

Jaladi *et al.* [43] observed significant increase in SOD and catalase level indicating an oxidative stress caused by L-arginine may up regulate the activity of antioxidant enzymes to facilitate rapid removal of accumulated ROS during 24 h of L-arginine injection. A significant decrease in SOD and catalase levels after 72 h suggested increased levels of ROS as the disease progressed. Increased ROS may attach the active site of antioxidant enzyme and make them to lose their function so, ROS levels get increased and antioxidant enzyme levels get decreased and this will explain our present results. The study performed by Afsar et al. [44] reveals that, the extract from B. S. contains high amounts of total phenolics and total flavonoids and it exhibited strong reducing power and antioxidant activity and antiinflamatory activity. Also, boswellic acid is characterized by pentacyclic triterpenes containing antioxidant properties such as antiinflamatory, antiatherosclerotic and antihyperlipidimic. These active ingredients act as free radicle scavengers, and sometimes as metal chelators, acting as the initiation step in the propagation of antioxidative process. [45] The extract of *Synzygium aromaticum* has been reported to possess phenolic compound and flavanoids which exhibit lipid peroxidation, antioxidant and free radical scavenging properties. [46] The clove has the ability to decrease oxidative stress in the diapetic rats. [23] Likewise, Atawodi *et al.* [47] deduced that clove has triggered the secretion of antioxidant enzymes in enhanced level which in turn stopped the oxidative damage caused by free radicles. In the present study, changes in serum and pancreatic MDA, NO, GSH, GPx, Catalase and SOD levels in animals that received L-arginine induced AP have been restored by treatment with B.S and clove extracts, these findings suggested that the treatment was significantly attenuated the lipid peroxidation, free radicles and improved the intracellular and extracellular antioxidant defenses. The extent of damage of pancreas tissue in AP correlates with the level of free radicles generation. In accordance with previous studies. [41] In the present study hitopathological assessments revealed that the induction of pancreatitis resulted in damage to pancreas cells. Treatment with B.S and clove extracts ameliorated histological changes in pancreas.

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