

## Preventive Effect of Three Pomegranate (*Punica granatum* L.) Seeds Fractions on Cerulein-Induced Acute Pancreatitis in Mice

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### ABSTRACT

**Background:** Acute pancreatitis (AP) refers to afflicted inflammation of pancreas with unfavorable adverse effects and developed multiple organ failures. Unfortunately, there is not a certain therapeutic method for this disease. Oxidative stress has a serious role in the pathogenesis of AP. Thus, decreasing of oxidative stress may prevent induction and progression of AP. *Punica granatum* L. has been extensively used in traditional medicine and possesses various active biological elements. Due to antioxidant and anti-inflammatory properties of pomegranate, it could be considered as a good candidate alternative medicine with beneficial effects on AP. In this study, we decided to study the protective effect of three fractions of pomegranate seeds on cerulein-induced AP.

**Methods:** AP was induced in male Syrian mice by five intraperitoneal (i.p.) injection of cerulein (50 µg/kg) with 1 h intervals. Treatments with pomegranate freeze-dried powder (PFDP) and hydroalcoholic pomegranate seeds extract (PSE) at doses of 125, 250, 500 mg/kg (i.p.) were started 30 min before pancreatitis induction. Pomegranate seed oil fraction (PSOF) was orally administered (50, 100, 200 µL/kg) and continued for 10 days. Pancreatic tissue was evaluated for histopathological parameters and pancreatic myeloperoxidase (MPO) activity as well as lipase and amylase levels were measured in plasma.

**Results:** The higher doses of three fractions (250 and 500 mg/kg for PFDP and PSE and doses of 100, 200 µL/kg for PSOF) significantly reduced amylase and lipase activity in serum (at least  $P < 0.01$ ), pancreatic MPO activity ( $P < 0.001$ ), edema, leukocyte infiltration and vacuolization in comparison to the control group ( $P < 0.05$ ).

**Conclusions:** These results propose that pomegranate seeds fractions can prevent and/or treat the AP.

**Keywords:** Acute pancreatitis, anti-inflammatory, antioxidant, preventive therapy, *Punica granatum* L., seeds

### INTRODUCTION

Acute pancreatitis (AP) is an inflammatory disorder of pancreas that is diagnosed by increased serum levels of digestive

enzymes such as amylase and lipase.<sup>[1]</sup> Prevalence of AP is estimated to be 4.9-35/100,000 persons and unfortunately about 30% of patients with severe pancreatitis will die.<sup>[2]</sup> AP has general symptoms such as nausea; vomiting and acute abdominal pain.<sup>[3]</sup> The main causes of death due to AP are cardiac insufficiency, respiratory, renal and hepatic failure and circulatory shock.<sup>[4]</sup> The major causes of AP are gallstone and alcohol abuse.<sup>[5]</sup> Other causes are hypertriglyceridemia, viral infection, bacterial disease, hypercalcemia and some drugs such as tetracycline, furosemide and estrogens.<sup>[2]</sup> The pathophysiology of this disease is still unknown and complex.<sup>[5]</sup> however; two main mechanisms have been suggested for its development: (a) The digestive enzymes of pancreas are released and start to destroy pancreatic tissue, which in consequence cause edema and necrosis.<sup>[4]</sup> (b) Chronic intoxication with some agents like alcohol may result in depletion in antioxidant capacity of pancreas tissue which causes free radicals of oxygen (O<sub>2</sub>) and nitrogen (N<sub>2</sub>) accumulation and lipid peroxidation of cell layers.<sup>[6]</sup> At present, a specific medical treatment or gold standard medication does not exist.<sup>[7]</sup> The main therapy of this disease is really supportive and consists controlling of pain with analgesic drugs, total parental nutrition and antibiotic usage for chemoprophylaxis of infections.<sup>[3,8]</sup>

Pomegranate (*Punica granatum* L.) is a small tree that belongs to punicacea family. This plant is indigenously grown in Iran, Afghanistan, India and China but, now is cultured in several regions such as American southwest, California and Mexico.<sup>[9]</sup> Pomegranate has been used in Iranian traditional medicine as vermifugal, recovery of wounds, relieving diarrhea and gastric inflammation.<sup>[10]</sup> The pomegranate seeds and peel have sugars, organic acids,<sup>[11]</sup> vitamins (especially ascorbic acid),<sup>[12]</sup> phenolic compounds including flavonoids (anthocynins, catechins, quercetin, leteulin, etc.), hydrolyzable tannins (punicalin, pedunculagine, punicalagin, gallic acid and ellagic acid) and alkaloids (pelletierines and its derivative).<sup>[9,13]</sup> The pomegranate extracts have antimicrobial activities against enteric pathogens such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Vibrio cholera*.<sup>[14,15]</sup> The anti-viral effect of pomegranate against herpes simplex and human immunodeficiency virus was proved.<sup>[16,17]</sup> Due

to existence of phytoestrogenic compounds, pomegranate has good potential for prophylaxis and treating menopausal complains and osteoporosis.<sup>[18]</sup> Main phenolic compounds of the pomegranate fruit or seeds such as ellagic acid, gallic acid and punicalagin have several preventive and therapeutic effects.<sup>[9]</sup> Gallic acid has been demonstrated to be chemopreventive for cancer therapy due to its cytoprotective and antioxidant activity, which prevent the deoxyribonucleic acid damage.<sup>[19,20]</sup> Punicalagin is the most dominant phenolic compound that has antioxidant activity in pomegranate juice.<sup>[21]</sup> It can inhibit oxygen free radicals and lipid peroxidation.<sup>[22]</sup> Punicalagin and punicalin significantly reduce production of nitric oxide and prostaglandin E2 (PGE2) so it acts as an anti-inflammatory compound.<sup>[23,24]</sup> Another effect of pomegranate polyphenols is prevention of atherosclerosis development and cardiovascular disease.<sup>[25,26]</sup> The anti-cancer effect of pomegranate fruit's ellagitannins and its hydrolyzed products especially ellagic acid and punicalagin on colon cancer cells and prostate cancer cells have been demonstrated.<sup>[27,28]</sup> Pomegranate seed oil contain 65-80% conjugated linolenic acids (CLnAs) mostly punicalic acid.<sup>[29]</sup> Punicalic acid has anti-inflammatory properties since it can inhibit synthesis of PGs, tumor necrosis factor (TNF- $\alpha$ ) and neutrophils myeloperoxidase activity.<sup>[30]</sup> Another study showed that CLnAs have anti-cancer activity relating to an effect on the tumor suppressor agent, protein tyrosine phosphatase receptor type- $\gamma$ .<sup>[31]</sup> In addition, pomegranate seed oil has a high amount of tocopherols which exert strong natural antioxidant activity.<sup>[32]</sup> Because of antioxidant and anti-inflammatory properties of pomegranate seeds and the principal role of inflammatory and oxidative stress in the pathogenesis of AP, this study was designed to investigate the effects of three pomegranate fractions on AP prevention and therapy.

## METHODS

### Plant material and extraction

#### Preparation of freeze-died powder and hydroalcoholic extract of pomegranate seeds

Pomegranate fruits were collected from Saveh, Iran in summer and were washed. Pomegranate seeds were separated. Then the seeds were divided

into three parts. The first part (200 g of seeds) was used for extracting juice. The juice of pomegranate was powdered by freeze-dried method. The second part (100 g of seeds) was used for preparation of hydroalcoholic pomegranate seeds extract (PSE). For this purpose percolation method was used. Briefly, the seeds were soaked in adequate volume of ethanol: water (70:30) and then was transferred to percolator apparatus for 48 h. Then the product was shaken and filtered. The semisolid extract was obtained by evaporating in a rotary evaporator and then this extract was freeze-dried under reduced pressure.<sup>[33]</sup>

#### **Preparation of pomegranate seed oil fraction**

The third part of pomegranate seeds was dried. The dried seeds were mill in a grinder and passed through a 40-mesh sieve. 50 g of milled powder was extracted with about 750 ml hexane using Soxhlet apparatus for 6 h. The extraction was repeated four times. The product was evaporated by a rotary evaporator at reduced pressure and was concentrated under nitrogen flow. The extract was stored in a dark place and at 2-8°C until the time of pharmacologic experiments.<sup>[34]</sup>

#### **Determination of total phenols**

The total phenols of pomegranate freeze-dried powder (PFDP) and PSE were determined via Folin-Ciocalteu micro-method by depicting the standard curve using gallic acid solutions (50, 100, 150, 250 and 500 mg/L) as reference agent.<sup>[35]</sup> A total volume of 20 µl of blank, standard and sample solutions were separately added into tubes and to each tube 1.58 mL of distilled water was added. Then 100 µL of Folin-Ciocalteu Reagent (Sigma, St. Louis, MO, USA) was added and mixed well. After 8 min, 300 µL of sodium carbonate solution 20% was added and mixed. The solutions were maintained at 40°C for 30 min and the absorbance of solutions was detected at 760 nm against the blank using ultra violet (UV)-Vis spectrophotometer.

#### **Animals**

Male Syrian mice weighting 25-30 g were procured from animal house of Isfahan School of Pharmacy and Pharmaceutical Sciences for this study. Animals were maintained in controlled conditions of temperature, humidity and light/dark cycles. Tap water and rodent chow pellets were free and available. Animals were fasted over the night (10-12 h) before initiation of the trial. All the experiments were approved by Ethics Committee

of Research Council, Isfahan University of Medical Sciences, Isfahan, Iran.

#### **Induction of AP**

For induction of AP, cerulein (50 µg/kg body weight) (Sigma, St. Louis, MO, USA) was injected intraperitoneally (i.p.) 5 times with 1 h intervals according to Mazzon *et al.* method.<sup>[36]</sup>

#### **Grouping**

Animals were randomly assigned into the following normal group and test groups (six mice in each).

Sham (normal) groups: Normal saline (5 ml/kg) was injected i.p. or administered orally (p.o.) to normal mice.

Control groups: Two groups of mice were separately pre-treated with normal saline i.p. or p.o. (5 ml/kg), 0.5 h before cerulein-induced AP.

PFDP groups: Three groups of mice were pre-treated with PFDP (125, 250, 500 mg/kg i.p.), 0.5 h before inducing AP.

PSE groups: Three groups of mice were pre-treated with PSE (125, 250, 500 mg/kg i.p.), 0.5 h before inducing AP.

PSOF groups: The last three groups of mice were pre-treated with PSOF (50, 100 and 200 µL/kg p.o.) once daily for 10 days, before AP induction. The last dose of PSOF was administered 0.5 h before pancreatitis induction.

All mice were sacrificed 6 h after the last administration of cerulein. Blood samples were taken by directed intracardiac puncture and kept at -70°C for biochemical analysis. The pancreas was quickly exploited and divided into two parts. A part of pancreas was fixed in formaldehyde (10%) for histological examination and a second part was frozen in liquid nitrogen and stored at -70°C for further analysis.

#### **Amylase and lipase activity measurement**

Determination of serum amylase and lipase activity was performed using amylase and lipase specific kits (Pars-Azmun Company, Tehran, Iran).<sup>[37]</sup>

#### **Measurement of myeloperoxidase (MPO) activity**

MPO activity is an index of polymorphonuclear leukocyte accumulation that was assayed according to the modified method of Bradley *et al.*<sup>[38]</sup> Tissue samples were homogenized in 1 mL of 50 mM phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide (Sigma-Aldrich). Then the samples were sonicated in an ice bath for 10 s while continuously



freeze-thawed for three cycles. After freeze-thawing, the suspension was sonicated for 10 s again. In this stage, the suspensions were centrifuged at 15000 rpm for 15 min at 4°C, then 0.1 mL of supernatant was removed and mixed with 2.9 mL of 50 mM phosphate buffer (pH 6.0) including O-dianisidine dihydrochloride (0.167 mg/mL) and 0.005% H<sub>2</sub>O<sub>2</sub>. Absorbance of this mixture was determined at 450 nm using UV-Vis spectrophotometer. MPO activity was reported in units (U)/g of wet tissue weight.

### Histological analysis

Tissue samples of each group were evaluated and semi-quantitatively expressed in terms of edema, leukocyte infiltration and vacuolization. Paraffin-embedded pancreas samples were cut out (5 µm), stained with hematoxylin and eosin (H and E) and analyzed by a pathologist unaware from experimental protocol. The histological grading of edema was categorized using a scale ranging from 0 to 3 (0 = no edema, 1 = interlobular edema, 2 = interlobular and moderate intralobular edema, 3 = interlobular and severe intralobular edema). Besides, Leukocyte infiltration was classified from 0 to 3 (0 = absent, 1 = scarce perivascular infiltration, 2 = moderate perivascular and scarce diffuse infiltration and 3 = abundant diffuse infiltration). Grading of vacuolization was based on the appropriate percentage of acinar cells involved 0 = absent, 1 ≤ 25%, 2 = 25-50% and 3 = more than 50% of acinar cells.<sup>[39]</sup>

### Statistical analysis

Biochemical results are represented as mean ± standard error of the mean. The statistical analysis was accomplished by one-way analysis of variance followed by Tukey's multiple comparison tests. Non-parametric data have presented as median and analyzed by Mann-Whitney U-test. The minimal level of significance was considered at  $P < 0.05$ .

## RESULTS

### Total phenol content

The absorbance of PFDP and PSE was measured via gallic acid standard curve and total phenol content as gallic acid equivalent (GAE) was determined. The total phenol content of PFDP and

PSE were  $62.2 \pm 2.8$  mgGAE/g and  $63.7 \pm 6.7$  mgGAE/g respectively.

### Effect of PFDP and PSE on the serum levels of amylase and lipase

Amylase and Lipase serum levels of mice treated with PFDP and PSE are reported in Figures 1 and 2, respectively. Our findings indicated that both of treatments in doses of 250 mg/kg and 500 mg/kg by i.p. injection caused significant reduce in amylase and lipase activity in mice with AP ( $P < 0.001$ ). The lower dose of both fractions (125 mg/kg, i.p.) couldn't meaningfully exert a decline in serum levels of amylase and lipase in cerulein treated mice.

### Effect of PSOF on the serum levels of amylase and lipase

As shown in Figures 3 and 4, results were obtained by oral administration of PSOF for continuous 10 days on serum level of amylase and lipase. Two greater doses of PSOF (100, 200 µL/kg) were effective enough to reduce serum levels of amylase and lipase activity in mice with AP (at least  $P < 0.01$ ), but this effect was not significant with a lower dose of 50 µL/kg.

### Effect of PFDP and PSE on pancreatic MPO activity

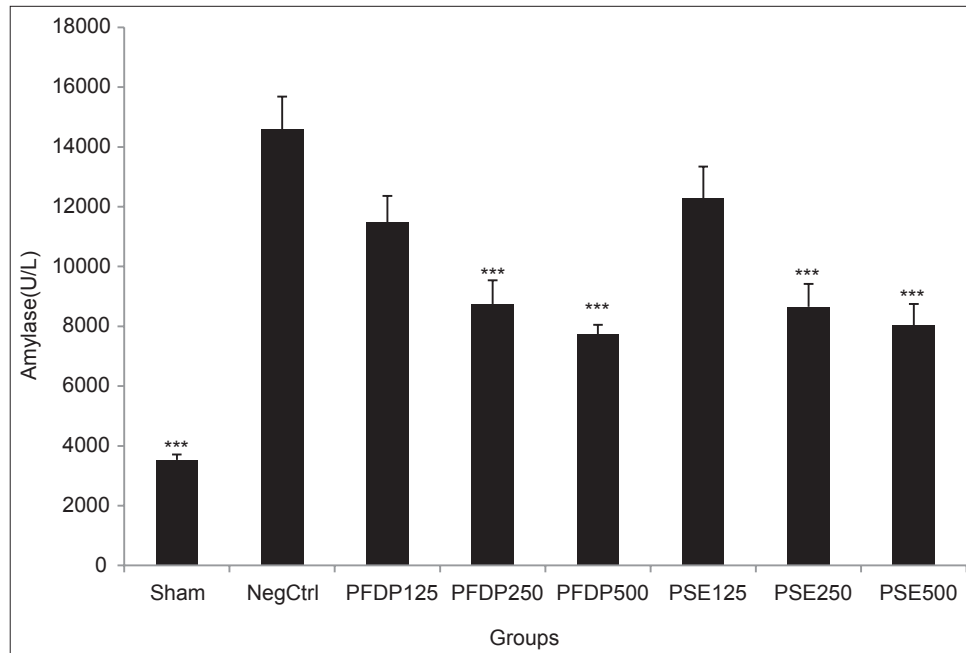
Increased MPO activity as a marker for leukocyte accumulation was previously observed in the pancreatic tissue following the cerulein administration.<sup>[40]</sup> Pre-treatment with PFDP and PSE in doses of 250 mg/kg and 500 mg/kg by i.p., decreased MPO activity in comparison to cerulein treated mice ( $P < 0.001$ ) [Figure 5]. The dose of 125 mg/kg was similarly not effective to decrease this parameter.

### Effect of PSOF on pancreatic MPO activity

Figure 6 shows that PSOF in doses of 100 µL/kg and 200 µL/kg by oral administration for 10 days diminished MPO activity in AP induced in mice ( $P < 0.001$ ).

### Effect of the PFDP, PSE and PSOF on the histological parameters

The pancreas tissue of normal saline treated mice didn't show any injuries. In the control group (pancreatitis treated with normal saline), prominent interlobular and severe intralobular



**Figure 1:** Effect of pomegranate freeze-dried powder and hydroalcoholic seeds extract on serum amylase level (U/L) of cerulien-induced acute pancreatitis in mice. Sham: normal mice treated with normal saline (5 mL/kg), negative control (NegCtrl) treated with normal saline (5 mL/kg), PFDP: pomegranate freeze-dried powder treated mice (125, 250, 500 mg/kg i.p.), PSE: pomegranate seeds extract treated mice (125, 250, 500 mg/kg i.p.). Data are shown as mean  $\pm$  standard error of mean of six animals in each group. \*\*\* $P < 0.001$  versus NegCtrl (one-way analysis of variance followed by Tukey)

edema, moderate perivascular and abundant diffuse inflammatory infiltration and vacuolization were found without necrosis or hemorrhagic injuries. Severity of edema, leukocyte infiltration and vacuolization meaningfully was declined compared to the control group ( $P < 0.05$ ) when the mice were treated with triple pomegranate fractions individually. The effective doses of PFDP and PSE were 250 mg/kg and 500 mg/kg respectively. PSOF was also effective at doses of 100  $\mu$ L/kg and 200  $\mu$ L/kg [Table 1, Figure 7].

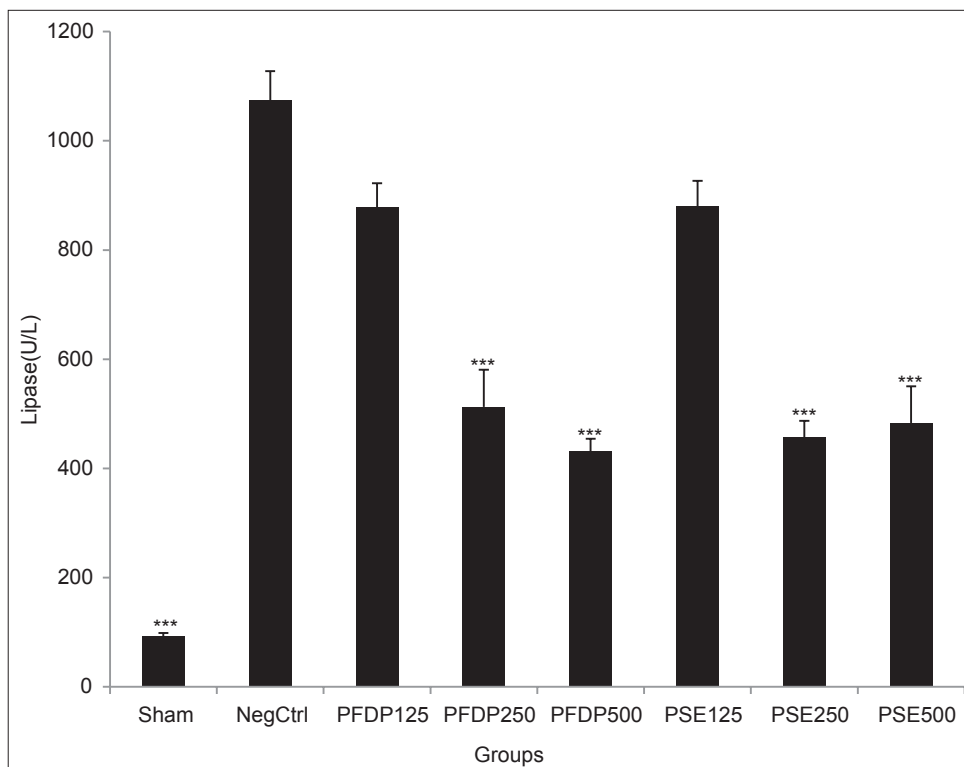
## DISCUSSION

In accordance to results of biochemical, immunological and histological evaluations, it could be stated that pomegranate seed is a good candidate for prevention of mild AP in mice. Our findings showed that PDFP and PSE and also, PSOF cause decreasing of amylase and lipase serum levels, MPO activity and improvement of histological parameters. I.p. injection of PDFP and PSE, at doses of 250 mg/kg and 500 mg/kg showed significant protective effect against AP compared with the control group, however smallest

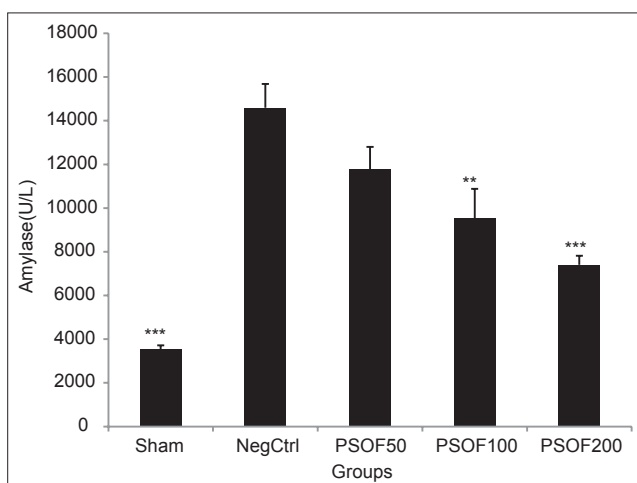
**Table 1:** Effect of three different fractions of pomegranate on pathological scores of pancreas in cerulein-induced acute pancreatitis in mice

Group	Route	Edema (0-3)	Leukocyte infiltration (0-3)	Vacuolization (0-3)
Sham	i.p./p.o.	0.0	0.0	0.0
NegCtrl	i.p./p.o.	2.0	2.0	2.0
PFDP125	i.p.	1.5	2.0	1.5
PFDP250	i.p.	1.0*	1.0*	0.5*
PFDP500	i.p.	1.0*	1.0*	0.5*
PSE125	i.p.	2.0	2.0	1.0
PSE250	i.p.	1.0*	1.0*	0.5*
PSE500	i.p.	0.5*	1.0*	0.5*
PSOF50	p.o.	2.0	2.0	1.0
PSOF100	p.o.	1.0*	1.0*	0.5*
PSOF200	p.o.	0.0*	1.0*	0.5*

Sham=Normal mice treated with normal saline (5 mL/kg), NegCtrl=Negative control treated with normal saline (5 mL/kg), PFDP=Pomegranate freeze-dried powder treated mice (125, 250, 500 mg/kg), PSE=Pomegranate seeds extract treated mice (125, 250, 500 mg/kg), PSOF=Pomegranate seed oil fraction treated mice (50, 100, 200  $\mu$ L/kg) for continuous 10 days. Data are shown as median of six animals in each group (Mann-Whitney *U* test). \* $P < 0.05$ =Significant difference compared to negative control group



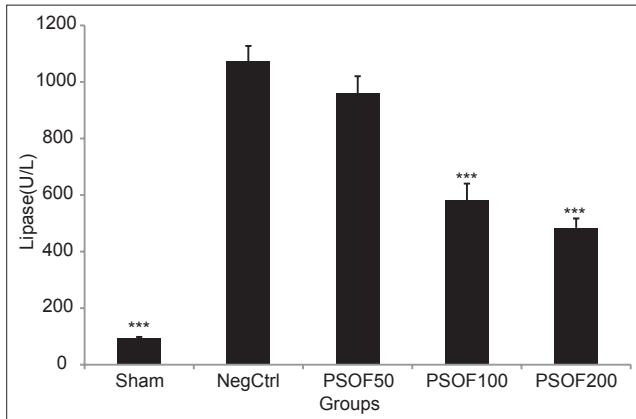
**Figure 2:** Effect of pomegranate freeze-dried powder and hydroalcoholic seeds extract on serum lipase level (U/L) of cerulien-induced acute pancreatitis in mice. Sham: normal mice treated with normal saline (5 mL/kg), negative control (NegCtrl) treated with normal saline (5 mL/kg), PFDP: pomegranate freeze-dried powder treated mice (125, 250, 500 mg/kg i.p.), PSE: pomegranate seeds extract treated mice (125, 250, 500 mg/kg i.p.). Data are shown as mean  $\pm$  standard error of mean of six animals in each group. \*\*\* $P < 0.001$  versus NegCtrl (one-way analysis of variance followed by Tukey)



**Figure 3:** Effect of pomegranate seed oil fraction on serum amylase level (U/L) of cerulien-induced acute pancreatitis in mice. Sham: normal mice treated with normal saline (5 mL/kg), negative control (NegCtrl) treated with normal saline (5 mL/kg), PSOF: pomegranate seed oil fraction treated mice (50, 100, 200  $\mu$ L/kg p.o.) for continuous 10 days. Data are shown as mean  $\pm$  standard error of mean of six animals in each group. \*\* $P < 0.01$  \*\*\* $P < 0.001$  versus NegCtrl (one-way analysis of variance followed by Tukey)

dose of both fractions (125 mg/kg i.p.) didn't have a meaningful effect on AP. Data of PSOF indicated that the doses of 100  $\mu$ L/kg and 200  $\mu$ L/kg which were administered orally for 10 days had significant effects on cerulein-induced acute pancreas inflammatory changes considering the biochemical and histological parameters. However, PSOF at a dose of 50  $\mu$ L/kg was unable to improve biochemical and histological indices of AP. Hence, it is assumed that the dose of each fraction is important for its effectiveness. It means higher doses have effective protection against AP while the smaller doses have negligible or non-significant effect. Although higher doses, didn't have meaningful difference with previous examined doses. These are in accordance with our previous report about *Cichorium intybus* L. extract<sup>[37]</sup> which represents that cerulein-induced pancreatitis could be appropriately protected by higher doses of applied extracts (100 mg/kg and 200 mg/kg, i.p.). Similarly lower doses (50 mg/kg, i.p.) of *C. intybus* L. extracts were statistically ineffective.

Cerulein, as an analog of cholecystokinin (CCK) acts as an agonist for CCK1 and CCK2 receptors which in turn activate Jak/Stat pathway, generates free oxygen radicals by inducing of

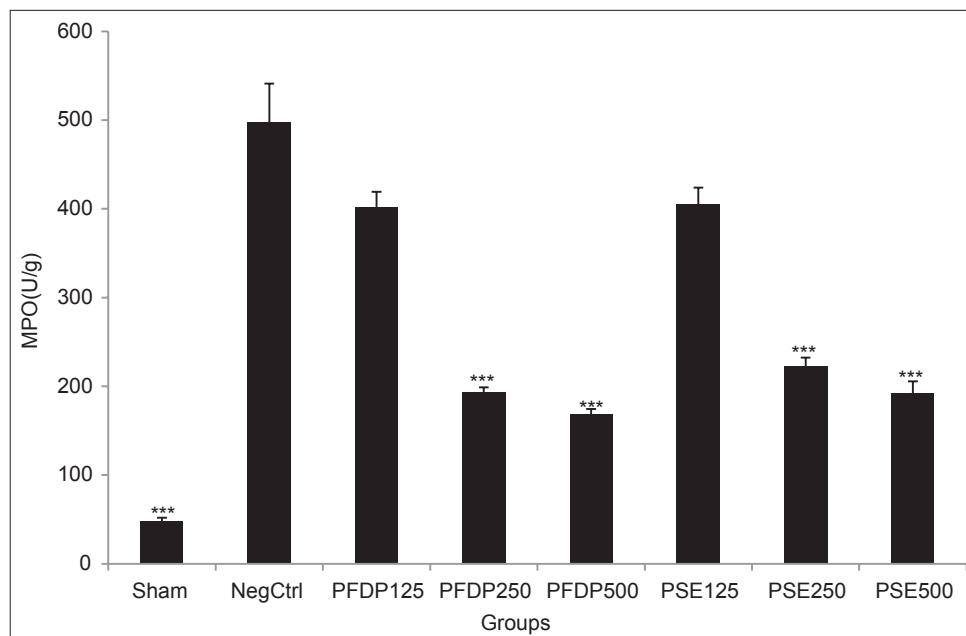


**Figure 4:** Effect of pomegranate seed oil fraction on serum lipase level (U/L) of cerulien-induced acute pancreatitis in mice. Sham: normal mice treated with normal saline (5 mL/Kkg), negative control (NegCtrl) treated with normal saline (5 mL/kg), PSOF: pomegranate seed oil fraction treated mice (50, 100, 200  $\mu$ L/kg p.o.) for continous 10 days. Data are shown as mean  $\pm$  standard error of mean of six animals in each group. \*\*\* $P < 0.001$  versus NegCtrl (one-way analysis of variance followed by Tukey)

oxidant-sensitive transcription factor (nuclear factor kappa-light-chain-enhancer of activated B cells NF- $\kappa$ B), increasing of cytokine expression and lipid peroxidation. In cerulein-induced pancreatitis, reactive oxygen species are produced which result in the collection of destructive oxygen by-products, lipid peroxidation, deficiency of scavenging enzymes such as superoxide dismutase, activated leukocyte and prostaglandin synthesis.<sup>[6,41]</sup> So it is plausible that each active compound which is able to scavenge free radicals and suppress NF- $\kappa$ B generation can diminish the inflammatory response and finally improve AP.<sup>[42,43]</sup>

*P. granatum* L. is rich in several bioactive compounds and has various biological advantages. Antibacterial, antiviral, antioxidant, anti-inflammatory, anti-carcinogenicity, cytoprotective activity and prevention of cardiovascular disease and osteoporosis are among the most cited properties of pomegranate.<sup>[14-28]</sup>

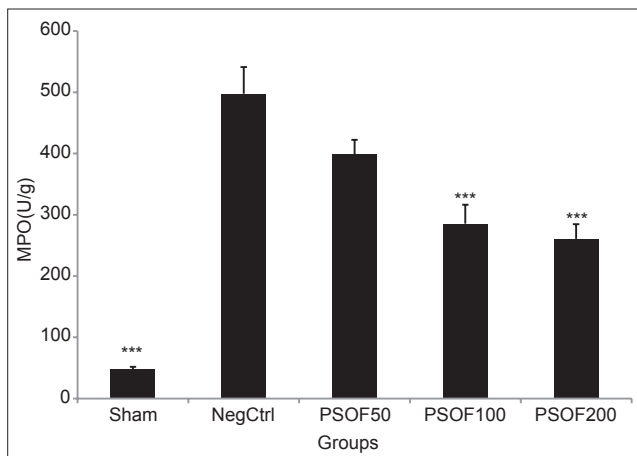
Protective effect of PFDP and PSE could be related to the presence of polyphenolic compounds and ellagitannins (especially ellagic acid and punicalagin). Antioxidant activity of pomegranate juice and fruit extract was demonstrated by Lansky and Newman.<sup>[44]</sup> A study done by Gil *et al.* also



**Figure 5:** Effect of pomegranate freeze-dried powder (PFDP) and hydroalcoholic pomegranate seeds extract (PSE) on pancreatic myeloperoxidase activity (U/g wet tissue) of cerulien-induced acute pancreatitis in mice. Sham: normal mice treated with normal saline (5 mL/kg), negative control (NegCtrl) treated with normal saline (5 mL/kg), treated mice (125, 250, 500 mg/kg i.p.), PSE treated mice (125, 250, 500 mg/kg i.p.). Data are shown as mean  $\pm$  standard error of mean of six animals in each group. \*\*\* $P < 0.001$  versus NegCtrl (one-way analysis of variance followed by Tukey)



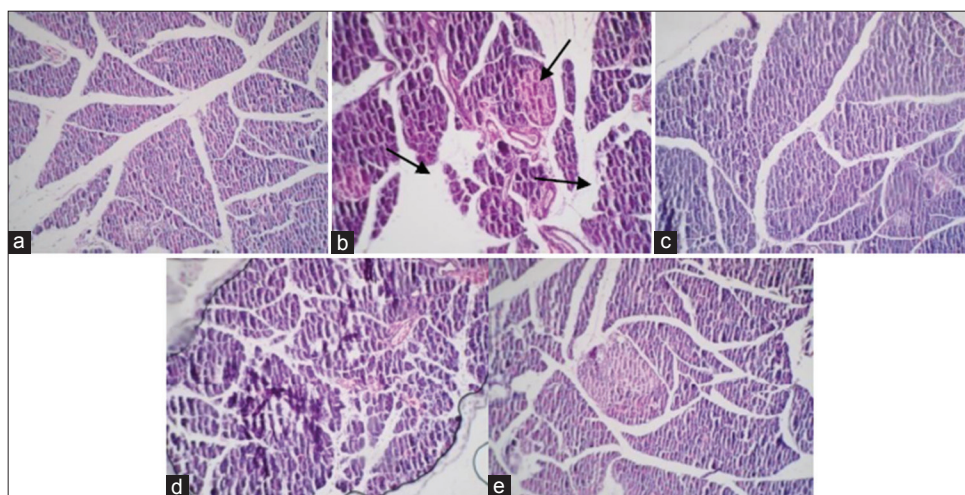
expressed antioxidant activity of punicalagin.<sup>[45]</sup> In another study that antioxidant capacity of 3 Chinese pomegranate cultivar juices were compared by Zhuang *et al.*, it was reported that pomegranate juice has the ability to scavenge oxidoradicals like 1,1-diphenyl-2-picryl hydrazine,  $\text{OH}^-$ ,  $\text{O}_2^-$ .<sup>[46]</sup> Anti-inflammatory effects of a pomegranate extract and its active metabolite urolithin-A was investigated



**Figure 6:** Effect of pomegranate seed oil fraction (PSOF) on pancreatic myeloperoxidase activity (U/g wet tissue) of cerulien-induced acute pancreatitis in mice. Sham: normal mice treated with normal saline (5 mL/kg), negative control (NegCtrl) treated with normal saline (5 mL/kg), PSOF treated mice (50, 100, 200  $\mu\text{L}/\text{kg}$  p.o.) for continuous 10 days. Data are shown as mean  $\pm$  standard error of mean of six animals in each group. \*\*\* $P < 0.001$  versus NegCtrl (one-way analysis of variance followed by Tukey)

in dextran sodium sulphate-induced colitis in male Fisherrats.<sup>[47]</sup> In this study, Larrosa *et al.* demonstrated that anti-inflammatory activity in rats with colitis could be due to the non-metabolized ellagitannine related fraction. They suggested that in healthy subjects, urolithin-A is probably the most active and responsible metabolite for anti-inflammatory effects of pomegranate juice. Moreover, it is possible that antioxidant and anti-inflammatory activity of pomegranate juice and seeds extract are related to the presence of flavonoids like leuteolin, quercetin, rutin and anthocynins (cyanidin 3-o-glucoside, delphinidine).<sup>[44,48]</sup> Cyanidin 3-o-glucoside for example, in a dose and time-dependent fashion attenuates PGE2 production, cyclooxygenase-2 expression and in-turn NF- $\kappa\text{B}$  and peroxisome proliferator-activated receptor- $\gamma$  activity in LNCaP human prostate cancer cells.<sup>[49]</sup> Delphinidine on the other hand, can inhibit cyclooxygenase-2 expression induced by TNF- $\alpha$  while both are potent inflammatory and tumorigenesis factors in most experimental models of colitis, AP and cancers.<sup>[50,51]</sup>

Our results also demonstrated that PSOF was similarly effective to protect against pancreatitis in greater applied doses. Significant effects of PSOF may be due to bioactive compounds like flavonoids with antioxidant and anti-inflammatory activities are present in seed oil fraction of pomegranate fruits. In one study carried out by Schubert *et al.*<sup>[52]</sup> antioxidant and eicosanoid enzyme activity inhibition of cold pressed seed oil of pomegranate was compared with



**Figure 7:** Microscopic illustration of pancreatic tissue in mice. (a) Normal tissue (intact pancreatic cells and acinar ducts) (b) Pancreatitis induced by cerulein (Inflammation, vacuolization, leukocyte infiltration and edema are visible) (c-e) Pancreatitis tissue treated with pomegranate freeze-dried powder (500 mg/kg), pomegranate seeds extract (500 mg/kg) and pomegranate seed oil fraction (200  $\mu\text{L}/\text{kg}$ ) respectively. H and E staining with  $\times 40$  magnification



butylated hydroxyanisole, green tea and red wine. They reported that seed oil extract was significantly stronger than red wine while it was close to butylated hydroxyanisole and green tea as antioxidant and anti-inflammatory agent. Moreover, punicic acid as the most abundant component of pomegranate seed oil<sup>[29,53]</sup> possesses enough potential for prevention and suppression of inflammatory mediators. This idea was confirmed by Boussetta T and his co-operator who investigated the effects of punicic acid, a CLnA derivative, on the experimental model of colitis induced by trinitrobenzene sulfonic acid in rat.<sup>[30]</sup> They demonstrated that punicic acid exerted a protection against experimental colitis by inhibiting TNF- $\alpha$  expression and TNF- $\alpha$  induced neutrophil hyperactivation in the rat colon. Alfa-tocopherol as another active component of PSOF has strong antioxidant capacity which might share some responsibility for protective effects of PSOF in AP.<sup>[32]</sup> Taken together, three pomegranate fractions used in this study was potent enough to protect against experimental model of AP induced by cerulein in mice. This property, as expected and expressed previously could be attributed to many active ingredients are present in different parts of this miracle fruit including seeds. Several bioactivity and biological mechanisms of actions have been investigated and demonstrated for them however, more mechanistic experiments are needed to identify the exact mechanisms are involved in this experiment.

## CONCLUSIONS

This is the first study to investigate the pomegranate seeds fractions for pancreatitis. Our findings demonstrated that three fractions of pomegranate seeds (freeze-died powder, hydroalcoholic extract and oil fraction) can improve AP *in vivo* by reducing biochemical and histological disease indices. This acceptable potency which is basically due to several active compounds exist in this plant propose that this dietary compound may provide a novel alternative therapeutic or at least preventive strategy in inflammatory disease conditions such as pancreatitis.

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