Protective effect of *Fragaria ananassa* and *Vaccinium corymbosum* fruit extracts against L-arginine induced acute pancreatitis in rats

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**ABSTRACT**

The study was aimed to evaluate the protective effects of alcoholic extracts of *Strawberry* and *Blueberry* fruits [AESF and AEBF] in acute pancreatitis in rats. Treatment groups received AESF and AEBF at doses of 200 and 400 mg/kg for 7 days with prior injections of L-arginine on 5th day. Biochemical parameters were estimated in serum and pancreatic tissue samples. Histopathological studies and DNA fragmentation assay were carried out in isolated pancreatic tissue. The results of the study indicated that treatment of AESF and AEBF exhibited a significant dose dependent protective effect. Upon the treatment, anti-oxidant enzymes were significantly (*p<0.05) increased. Biochemical results were correlated with the histopathological findings. In addition, the DNA fragmentation assay showed an intact DNA in pancreatic cells of treated groups. In conclusion, berry fruit extracts exerted a potential protective effect against L-arginine induced damage in rat pancreas, at least in part, due to its antioxidant properties.

**Key words:** Amylase, *Blueberry*, L-Arginine, Lipase, Oxygen free radicals, Pancreatitis, *Strawberry*.

**INTRODUCTION**

Acute pancreatitis (AP) is a critical self limiting gastrointestinal condition with wide clinical variation. Although 80% of the cases are mild 20% may lead to severe necrotizing pancreatitis causing high mortality rates in spite of the availability of advanced treatment modalities (Kui et al., 2015). The incidence of acute pancreatitis is increasing by 13 to 45 cases per 100,000 persons (Yadav et al., 2013). Many etiological factors have been derived for the occurrence of the disease of which alcohol and biliary tract abnormalities are the most common. The risk of AP ranges from 2 to 5 % among patients who are chronic alcoholics (Lankisch et al., 2002). In 10% of the cases, the cause is unknown and may be secondary to microlithiasis of gall bladder. Although many pathogenic mechanisms have been derived; auto digestion, generation of oxygen free radical and lipid peroxidation is broadly accepted theory leading to rapid activation of inflammatory responses at the site of activation with the involvement of systemic organs (Abdin et al., 2010). The systemic complications are implied by the activation of inflammatory cytokines like TNF α, IL-6 which are macrophage derived factors involved in the progression of the disease (Czako et al., 1998). It has been suggested that trypsinogen, play a key role in the progression of severe acute pancreatitis. The balance of trypsinogen conversion to trypsin is mediated by a negative feedback loop and excessive activation of trypsinogen adds to the disturbance of the homeostasis leading to severe acute pancreatitis (Ning et al., 2013). Lack of conventional therapy opens a novel approach for the use of antioxidants obtained from many resources for the development of new drugs.

Phytochemicals from plant origin are responsible for antioxidant property and are principally contributed by phenolics, anthocyanins and flavonoid compounds (Wang et al., 2000). The consumption of fruits has been associated with decreased incidence of diseases. Berry fruits have been widely described for their antioxidant activity (Jaime Guerrero et al., 2010). Wang et al., (2000) has described notable antioxidant property of extracts of *Blueberry, Raspberry* and *Strawberry* against chemically generated superoxide radical species (Wang et al., 2000). *Strawberry* fruit has been recommended in prevention of obesity, dermatitis, skin rejuvenation, and well documented evidence is available for its *in-vivo* and *in-vitro* antioxidant property (Wang et al., 2000; Scalzo et al., 2005; Tulipani et al., 2009). It has been reported that fruit and leaves of *Strawberry*...
showed a decrease in the incidence of cancer, obesity, infections and other chronic pathological conditions like cardiovascular diseases, diabetes and oxidative stress (Abdulaleezeez et al., 2014; Zhu et al., 2015).

Anthocyanins are the naturally occurring plant pigments which are a subgroup of flavonoids and render color to fruits and flowers. It is hypothesized that anthocyanins execute free radical scavenging activities in alleviating oxidative stress and cellular damage (Canuto et al., 2016). Blueberry is rich of anthocyanin content and exhibits the highest recorded antioxidant activities among the fruits in-vitro (Prior et al., 1998). It was regarded as brain berry and is used for age related neuronal /behavioral dysfunction and further demonstrated the inhibition of tumorigenesis in wide variety of cancers (Shukitt-Hale et al., 2012; Johnson et al., 2013). Both berries are available commercially as fresh and frozen fruits, jams, jellies, yoghurt, etc. and contain the presence of phenols and anthocyanins. Consumption of these as dietary supplementation with benefits in regard with health make them the most exciting nutraceuticals being researched (Sinha et al., 2013). Despite the fact that the exact mechanisms behind the beneficial effects are not well understood, usage of basic amino acids for induction of APhas been reported. Many reports support the administration of excessive dosage of L-arginine induced acute pancreatitis is mediated by free radical generation without the damage to islet of langerhans with high reproducibility (Mizunuma et al., 1984). Considering the potentiality of antioxidant property of berry fruit extracts and fewer studies conducted in this area, study was planned to evaluate the protective effect of Blueberry and Strawberry fruit extracts against L-arginine induced acute pancreatitis.

**MATERIALS AND METHODS**

L-Arginine powder is obtained from Sigma Aldrich Private Ltd. (USA). L-Arginine was prepared as a 20% solution in normal saline. Amylase and CRP kits were purchased from Akray healthcare Private Ltd, Mumbai (India). Lipase kit was purchased from Aggape Diagnostics Ltd, Kerala (India). DNA Isolation kit was procured from Bioartis Private Ltd, Hyderabad (India). Other chemicals and reagents used in this work were of analytical grade and purchased from local chemical agencies.

**Sample extraction:** Fresh fruits of Strawberry and Blueberry were purchased from the local market. They were sorted for uniform size, washed, dried and blended. The blended fruits were subjected to extraction with 95% ethanol for 24 hrs on an orbital shaker. The extracts were filtered, and the filtrate was evaporated. The resultant extracts were stored in cool temperature and were resuspended in normal saline just before the experiment. The fruits purchased were authenticated by Department of Botany, Osmania University by comparison of the taxonomical features with that of the specimens [Voucher numbers: 0927 (Strawberry), 1172 (Blueberry)].

**Determination of total phenolic content:** The total phenolic content of the extracts was determined using modified colorimetric Folin Ciocalteu estimation. In brief, 0.5 ml of known concentration of the extract was taken in the test tube and 2.5 ml of Folin Ciocalteu reagent dissolved in water was added. The solution is incubated at room temperature for 5 min. Now add 2.5 ml of 7.5% of sodium bicarbonate solution and the mixture is diluted with 3 ml of deionized water. After 45 min, the reaction mixture absorbance is checked at 765 nm using a spectrophotometer. The measurement is compared to a standard curve of Gallic acid and the content of phenolics is expressed as Gallic acid equivalents per 100 g (Dewanto et al., 2002).

**Determination of total flavonoidal content:** The flavonoidal content was determined by taking 1 ml of known concentration of the extract and 1 ml of 2% aluminium chloride in methanol solution was added. The mixture is diluted further with 3 ml of deionized water and was incubated at room temperature for 1 hr. After the incubation the reaction mixture absorbance was checked at 415 nm using a spectrophotometer. The measurement is compared to a standard curve of Rutin and the content of flavonoids is expressed as rutin equivalents per 100 g (Jia et al., 1999).

**Determination of anthocyanin content:** The anthocyanin content is determined by pH differential method. Briefly, 1 ml of the known concentration of the extract is taken in a 10 ml of volumetric flask for preparing two dilutions of the sample. One solution is adjusted with KCl buffer pH 1.0 and the other with Sodium acetate buffer pH 4.5. Both the solutions were incubated for 15 min at room temperature. The absorbance of the mixture was measured at 510 nm and 700 nm against distilled water blank (Boyles et al., 1993; Liu et al., 2002). All the measurements should be made between 15 min to 1 hr. The anthocyanin content is calculated as follows

\[
\text{Total monomeric anthocyanins (mg/100 g of extract) = } \frac{A^* \times MW^*}{1000 / (\epsilon^*C)}
\]

Where, \(A\) is absorbance \((A_{310} - A_{700})^*\) pH 1.0 - \((A_{315} - A_{300})\) pH 4.5;

\(MW\) is molecular weight for cyanidin 3-glucoside 449.2;

\(\epsilon\) is the molar absorptivity of cyanidin 3-glucoside 26 900;

and \(C\) is the concentration of the buffer in milligrams per milliliter.

Anthocyanin content was expressed as milligrams of cyanidin 3-glucoside equivalents per 100 g of extracts.

Male albino rats (200-250 gm) were selected for the study. They were acclimatized for 1 week before starting of the experiment. Animals were maintained at temperature of 22 ± 2°C with 12 h light and 12 h dark cycle. They were fed with standard pellet diet with free access to water ad libitum. All the experiments were approved by Institutional Animal ethics committee of SSJ College of Pharmacy.
Table 1: Experimental design.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TREATMENT</th>
<th>DOSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal Control</td>
<td>Normal Saline</td>
</tr>
<tr>
<td>Group II</td>
<td>Disease Control</td>
<td>L-arginine 20%</td>
</tr>
<tr>
<td>Group III</td>
<td>AESF</td>
<td>Alcoholic extract of</td>
</tr>
<tr>
<td>Group IV</td>
<td>AESF</td>
<td>Strawberry fruit</td>
</tr>
<tr>
<td>Group V</td>
<td>AEBF</td>
<td>Alcoholic extract of</td>
</tr>
<tr>
<td>Group VI</td>
<td>AEBF</td>
<td>Blueberry fruit</td>
</tr>
</tbody>
</table>

AP group received single dose of 2.5g/kg b.w. 20% L-arginine solution for induction of acute pancreatitis. Control and treatment groups were administered with normal saline and AESF and AEBF respectively per oral for 7 days. On day 5 of the study, treatment group rats were administered with a single dose of 2.5g/kg b.w. 20% L-arginine solution 1 hr after the administration of the extracts.

At the end of the study, blood samples were withdrawn from retro-orbital plexus. The collected blood was centrifuged; serum was separated and stored at -20°C till they were used for the analysis of serum amylase, lipase, C-reactive protein (CRP), catalase, SOD. The abdomen of the rats was dissected, pancreas was isolated, washed in normal saline and a part of it was fixed in 10% formalin. The formalin fixed tissue was subjected for histopathological analysis with haematoxylin and eosin staining under light microscope.

The other part of the tissue was immediately kept in PBS at 4°C. The tissues were homogenized, and supernatant is separated and used for the estimation of tissue malondialdehyde (MDA), tissue nitrite levels, tissue glutathione, pancreatic MPO (myeloperoxidase) and total content of DNA and RNA.

Serum amylase: Serum amylase levels (U/L) were measured using commercial kits obtained from Arkay healthcare private limited. Briefly, 20µl of the serum was mixed with 1000µl of amylase mono reagent and the absorbance was read at 405nm after 60 sec.

Serum lipase: Briefly, 20µl of the serum was mixed with 1000µl of reagent 1 at 37°C and incubated it for 1-5 min. 250µl of reagent 2 was added and, incubated it for another 2 min and absorbance is read at 580nm.

Serum CRP: The quantitative agglutination was observed with coarse, fine and smooth agglutination among the serum samples which were treated with one drop of CRP Latex reagent.

Superoxide dismutase: The estimation was done by erythrocyte sedimentation and photo oxidation method. Briefly, the packed red cells were taken and an equal quantity of Cold water and chloroform / ethanol mixture (15:1 ratio) were added. The mixture was centrifuged at 2000 rpm for 20 min. 0.1ml of supernatant was separated and to it 0.88ml of riboflavin, 60 µL of O-dianisidine was added and the absorbance was measured at 460nm (Misra et al., 1977).

Catalase: The estimation of catalase was done according to Beers and Sizer (1952) with slight modifications. To 0.1 ml of serum, add 2.5ml of phosphate buffer and incubated at 25°C for 30 min. After transferring into a cuvette, the absorbance was measured at 240 nm, 650 µl of hydrogen peroxide solution was added to initiate the reaction. The change in absorbance was measured for 3 min (Beers et al., 1952).

Vitamin C: Vitamin C levels were estimated using method developed by Omaye et al. (1979). To 0.5ml of plasma, 1.5ml of 6% TCA was added and centrifuged (3500 rpm=20 min). To 0.5 ml of supernatant 0.5 ml of DPNH reagent (2% DNP and 4% thiourea in 9 N H2SO4) was added and developed color was read at 530 nm after 30min (Omaye et al., 1979).

Tissue glutathione: A part of tissue was homogenated in cool 0.1M phosphate buffer. Equal volumes of 20% TCA and 1mM EDTA were added to it. It was allowed to stand for 5 min and was centrifuged for 10 min at 2000rpm. 200µL of the supernatant was separated and 1.5ml of DTNB reagent was added. The absorbance was measured at 412nm (Sedlak et al., 1968).

Tissue Nitrite levels: Total nitrite levels were determined as a measure of nitric oxide with the use of Griess reagent. The pancreatic tissue was homogenized in 50 mmol/L potassium phosphate buffers (pH 7.8) and centrifuged at 11000X g for 15 min at 4°C. One hundred micro liters of the supernatant was mixed with 100µL Griess reagent [0.1% N-(1-naphthyl) ethylenediamide dihydrochloride, 1% sulphanilamide in 5% phosphoric acid], and after 10 min, the absorbance was measured at 540 µm. The standard curve was obtained by using sodium nitrite. The results were calculated from a standard curve by using sodium nitrite and expressed as micromoles of nitrite (Green et al., 1982).

Pancreatic MPO: Pancreatic myeloperoxidase (MPO) activity was assayed spectrophotometrically according to method described by Bradley et al., (1982). This method is based on kinetic measurement of the formation rate of the yellowish-orange product of the oxidation of O-dianisidine.
with MPO in the presence of hydrogen peroxide (\(\text{H}_2\text{O}_2\)) at 460 nm. One unit of MPO was defined as that degrading 1 mmol of \(\text{H}_2\text{O}_2\) per minute at 25°C. A molar extinction coefficient of 1.3x104 M cm⁻¹ of oxidized 0-dianisidine was used for the calculation. MPO activity was expressed as Units/mg of proteins (Haqqani et al., 1999).

**Pancreatic MDA:** Four milliliters of reaction mixture containing 0.4 ml of the tissue homogenate, 1.5 ml of 0.8 % TBA, 1.5 ml of acetic acid (20 %, pH 3.5) and distilled water was placed for 1h in a boiling water bath at 95°C for duration of 1h. The reaction mixture was then cooled, and 1 ml of distilled water was added. 5 ml of butanol; pyridine mixture (15:1) was added to the reaction tube, mixed well and centrifuged at 3000 RPM for 10 min. Absorbance of the clear supernatant was measured at 532 nm against butanol; pyridine mixture. The MDA was calculated with the help of a standard graph prepared by using different concentrations (1-10nmol) of 1’1’3’3’-tetramethoxypropane in 1ml distilled water and is expressed as nmol of MDA/mg protein (Ohkawa et al., 1979)

**DNA Fragmentation assay:** A Part of the pancreatic tissue is subjected for DNA isolation by using the commercial kit by Bioartis Private Limited. The isolated DNA is further solubilized in alcohol and subjected for Agar gel electrophoresis to detect the fragmentation levels (Basnakian et al., 1994).

**Statistical analysis:** All the results obtained were represented in Mean ± SEM and were subjected to one-way ANOVA followed by Dunnet ‘t’ test with a significance levels of p<0.001. Data analysis was carried out using Graph Pad Prism tool (Version 5).

**RESULTS AND DISCUSSION**

Total phenolic content, total flavonoidal content and total anthocyanin content were found to be high in alcoholic extracts of *Blueberry* in comparison to *Strawberry* extract. Results were shown in Table 2.

Amylase and Lipase levels are two significant biomarkers of acute pancreatitis. L-argine increased the levels of amylase and lipase indicating the induction of pancreatitis when compared to control group of rats. Rats treated with berry extracts significantly (p<0.001) reduced the levels of Lipase and amylase when compared to L-arginine group of rats in a dose dependent manner. *Blueberry* extracts was shown to be more effective in reducing the levels and *Strawberry* extracts had moderate effect (Table 3).

Serum CRP represents the damage and inflammation in the pancreatic tissue. CRP levels were increased in rats with L-arginine and reduced significantly (p<0.001) in treatment animals when compared to L-arginine group of rats in a dose dependent manner. *Blueberry* extracts was found to have more effective in reducing the levels and *Strawberry* extracts had moderate effect (Table 3).

Pancreatic MPO, nitrate and MDA levels indicate the lipid peroxidation and reactive oxygen species formation. High levels were seen in rats which received a single high dose of L-argiine when compared to that of control group of rats. Following treatment of *Blueberry* and *Strawberry* extracts, the levels of MDA and MPO reduced significantly (p<0.001) in a dose dependent manner when compared to L-arginine group of rats (Table 3).

SOD, catalase, Vitamin C and reduced glutathione levels designate the antioxidant status in the tissues. Alcoholic extracts of *Blueberry* and *Strawberry* remarkably (p<0.001) improved the levels in a dose dependent manner in contrast to rats treated with L-arginine where the levels were very low (Table 3).

The histopathological changes in pancreatic tissue were presented from Fig 1(a) to 1(g). Control group showed a normal pattern of acinar cells as in Fig 1(a), L-arginine treated pancreatic tissue depicted large area of necrosis and hemorrhage with fat accumulation [Fig 1(b) and (c)]. Berry extract treated groups defined a protective effect by maintaining the acinar cell structure with less lymphocytic filtration and normal pancreatic acinar cells with islets [Fig 1(d) to 1(g)].

DNA Fragmentation assay was performed to assess the amount of apoptosis. The isolated pancreatic tissue was subjected for isolation of DNA and its fragmentation was performed by using agarose gel electrophoresis. Rats treated with L-arginine showed a smeared pattern demonstrating the fragmentation of DNA leading to apoptosis of the tissue. Whereas the rats pretreated with berry extracts retained the DNA denoting the protecting effect of the extracts (Fig 2).

The results of the study indicate a protective effect of fruit extracts of *Strawberry* and *Blueberry* on L-arginine induced acute pancreatitis in rats in a dose dependent manner.

L-arginine induced acute pancreatitis is a well established noninvasive highly reproducible model of acute pancreatitis that produces dose dependent acinar necrosis.

**Table 2:** Total Phenolic content, Total Flavonoidal content and total Anthocyanin content in alcoholic extracts of *Strawberry* and *Blueberry* fruits.

<table>
<thead>
<tr>
<th>Phytochemical contents</th>
<th>Alcoholic extracts of <em>Strawberry</em></th>
<th>Alcoholic extracts of <em>Blueberry</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Phenolic content (mg of Gallic acid equivalent/g)</td>
<td>2.89 ± 0.19</td>
<td>9.34 ± 0.23</td>
</tr>
<tr>
<td>Total Flavonoidal Content (mg of Rutin equivalent/g)</td>
<td>7.23 ± 0.11</td>
<td>36.78 ± 1.01</td>
</tr>
<tr>
<td>Total Anthocyanin Content(mg of Cyanidin-3-glucoside equivalent/g)</td>
<td>1.34 ± 0.20</td>
<td>25.34 ± 1.12</td>
</tr>
</tbody>
</table>
L-arginine administration in large doses produced a characteristic laboratory change in 24h (Hegyi et al., 2004).

Serum amylase is an important diagnostic marker for acute pancreatitis. It usually rises within 4-8 h of the initial attack of L-arginine and peaks at 24 h. Our study results implied that, rats treated with L-arginine showed a significant raise of serum amylase levels with acinar cell necrosis after 24 h of administration which is clearly evident with the a larger area of necrosis and hemorrhage in the histological reports. The oral administration of the Blueberry and Strawberry fruit extracts reduced the activity of the enzyme significantly could represent its protective effect on acute pancreatitis. The protective effect is also reflected in the histological reports which showed diminutive

<table>
<thead>
<tr>
<th>Serum Enzyme</th>
<th>Normal control</th>
<th>Disease Control</th>
<th>FA – 200mg/kg</th>
<th>FA – 400mg/kg</th>
<th>VC – 200mg/kg</th>
<th>VC – 400mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase</td>
<td>14.17 ± 0.116*</td>
<td>198 ± 0.9444*</td>
<td>164 ± 0.8165*</td>
<td>156.5 ± 0.7638*</td>
<td>103.8 ± 2.386*</td>
<td></td>
</tr>
<tr>
<td>Lipase</td>
<td>16.17 ± 0.9804*</td>
<td>56.83 ± 0.7032*</td>
<td>41.67 ± 0.9545*</td>
<td>46.5 ± 1.118*</td>
<td>37.5 ± 0.5627*</td>
<td></td>
</tr>
<tr>
<td>SOD</td>
<td>18.5 ± 0.4282*</td>
<td>26 ± 0.5164*</td>
<td>38.33 ± 0.3333*</td>
<td>23 ± 0.3651*</td>
<td>40.5 ± 0.5*</td>
<td></td>
</tr>
<tr>
<td>Catalase</td>
<td>48.18 ± 0.3056*</td>
<td>33.27 ± 0.3383*</td>
<td>42.95 ± 0.3304*</td>
<td>41.43 ± 0.1174*</td>
<td>50.77 ± 0.3694*</td>
<td></td>
</tr>
<tr>
<td>Gpx</td>
<td>2236 ± 0.9574*</td>
<td>2195 ± 2.486*</td>
<td>2391 ± 0.8819*</td>
<td>2410 ± 1.77*</td>
<td>2901 ± 2.548*</td>
<td></td>
</tr>
<tr>
<td>SOD</td>
<td>14.89 ± 0.3381*</td>
<td>21.35 ± 0.5771*</td>
<td>154.2 ± 0.8497*</td>
<td>196.4 ± 1.472*</td>
<td>174.8 ± 0.714*</td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>11.08 ± 0.212*</td>
<td>35.0 ± 0.1522*</td>
<td>25.01 ± 0.1202*</td>
<td>20.24 ± 0.191*</td>
<td>16.19 ± 0.0878*</td>
<td></td>
</tr>
<tr>
<td>MPO</td>
<td>486.8 ± 2.358*</td>
<td>1675 ± 3.307*</td>
<td>1179 ± 1.522*</td>
<td>1328 ± 1.476*</td>
<td>1014 ± 2.04*</td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>402 ± 0.0548*</td>
<td>15.8 ± 0.2546*</td>
<td>13.89 ± 0.1744*</td>
<td>17.38 ± 0.144*</td>
<td>11.42 ± 0.202*</td>
<td></td>
</tr>
<tr>
<td>LDH</td>
<td>23.4 ± 0.5379*</td>
<td>92.63 ± 0.3875*</td>
<td>73.82 ± 0.6348*</td>
<td>71.92 ± 0.3619*</td>
<td>65.05 ± 0.5334*</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed in Mean ± SEM (n=6); *, # p<0.001 when compared to Normal Control and Disease control group respectively. FA stands for Fragaria annanassa and VC stands for Vaccinium corymbosum; Units of all the parameters as follows. Serum amylase- U/L; lipase levels - U/L; Superoxide dismutase - (U/ mg protein); Catalase- (µ moles of H2O2 metabolized / mg protein/ min; Glutathione-S-transferase (nmoles of CDNB conjugated/ mg protein/ min); Vitamin C- mg/dL; MDA: nmol/ gm tissue; Nitrite- µM; CRP- mg/L; MPO- U/ gm tissue; LDH- U/L.

Fig 1: Histopathological studies of isolated pancreatic tissue in all the groups. The protective effects of AESF and AEBF, formalin fixed paraffin embedded sections of Pancreas stained with hematoxylin and eosin. (a) Photomicrograph of the normal pancreas. (b) Rats induced with acute pancreatitis by administration of L arginine. Sections show extensive acinar cell damage (c) Pancreas of rats induced with pancreatitis; sections show fatty necrosis. (d) and (e) protective effect of alcoholic extract of Strawberry. Sections show partial acinar cell damage and leukocyte infiltration. (f) and (g) protective effect of alcoholic extract of Blueberry. Sections show normal acinar cells as that of normal pancreas.
accumulation of inflammatory cells with no evidence of necrosis or hemorrhage.

Serum lipase levels is more reliable diagnostic marker than amylase since it remains elevated for a longer period of time and its sensitivity is increased during acute pancreatitis (Yadav et al., 2002). The elevation of serum lipase levels contribute to the activation of pancreatic enzymes within the acinar cells and their entry in to the circulation (Abdel-Gawad et al., 2015). The results this study report that L-arginine has caused the elevation of Lipase levels and is consistent with the previous reports (Biradar et al., 2013). The administration of the fruit extracts revert back the elevated lipase levels to normal. The lipase inhibitory activity of fruit extracts of Strawberry is consistent with the previous reports (McDougall et al., 2009) and decreased lipase with the treatment of fruit extracts of Strawberry probably attributed to the synergistic interactions of proanthocyanidins and ellagitannins (McDougal et al., 2005).

Similarly, the significant reduction of the lipase levels with administration of Blueberry is in accordance with the previous reports (Wei et al., 2011) and may be due to the presence of Cyanidin 3-O-glycoside.

Glutathione, Catalase and SOD form a “mutual supportive team” in the antioxidant defense mechanisms in order to eradicate or detoxify the formed new free radicals. SOD generates H₂O₂, which is provided as a substrate for Catalase and glutathione further destroying it (Szabolcs et al., 2006). Therefore, these enzymes can be termed as the first line antioxidant defense mechanisms (Ayaz et al., 2016; Divya et al., 2018). In case of acute pancreatitis, the lack of these enzymes set off the free radical generation leading to organ damage. In the present study, the L-arginine treated rats showed very low profile of SOD, Catalase and reduced Glutathione owing to increased oxidative stress in the pancreas. Further Vitamin C, a potent antioxidant known for its chain breaking effect on lipid peroxidation is an essential nutrient, since it cannot be synthesized in our body. Its abundant availability in extracellular fluid prevents any cellular damage (Bendich et al., 1986). Concurrent administration of berry extracts has improved the defense mechanisms due to the presence of polyphenolics, ellagitannins and anthocyanins which is in accordance with the previous reports where administration of Strawberry and Blueberry fruit have elevated the levels of SOD, Catalase and reduced glutathione. Not only that, but they also contain vitamin C and glutathione as their chief constituents which constitute towards their high antioxidant status (Wang et al., 2000).

The chief byproducts of membrane lipid peroxidation are MDA and MPO. The levels of MDA have been reported to correlate with the levels of damage in acute pancreatitis in early stages. High levels of MDA increased the vascular permeability thereby triggering a cascade of cytokines and systemic inflammatory responses which in turn follow organ damage. In the present study, L-arginine induced rats showed elevated levels of MDA which is in accordance with the previous reports stating that these free radicals are important mediators of pancreatic tissue damage (Czako et al., 1998). Pretreatment of berry extracts reversed these conditions due to their high antioxidant capacity which is in agreement with the previous reports, where Strawberry and Blueberry fruits were recognized for their antioxidant activity because of the presence of polyphenolic compounds, anthocyanins (Mandave et al., 2013). MPO another byproduct of lipid peroxidation and a marker of local leukocyte sequestration was significantly elevated in rats with L-arginine which is in agreement with the previous results (Ibrahim et al., 2015; Poch et al., 1999) and the treatment with berry phenolics have reversed the conditions which is seen in the histopathological studies where the infiltration of leukocyte is less when compared to the L-arginine treated group.

Serum LDH is associated with the severity of acute pancreatitis. LDH enzyme is a cytoplasmic enzyme that is widely expressed in tissues and is functional when there is lack of oxygen supply (Cui et al., 2017). Inflammatory events lead to oxygen deprivation which activates the enzyme. In the present study, the elevated LDH levels was observed in L-arginine group of rats and berry extract groups showed a dose dependent reduction confirming the defensive effect against pancreatitis.

C-reactive protein is a significant biomarker of inflammation. The hepatic production of CRP was increased
in any type of inflammation and is used as a prognostic factor in severe acute pancreatitis (Frossard et al., 2001) (Lina et al., 2014). The levels of CRP increased in L-arginine treated groups which are in agreement with the histological data. Further the decreased levels of CRP in berry extract groups represented the protective aspects in controlling the inflammatory events. Both Strawberry and Blueberry fruits have been reported for their anti-inflammatory properties (Gasparrini et al., 2017; Torri et al., 2007).

Nitrogen reactive species also play a significant role in the inflammatory events produced by oxidative stress. Inducible NO aggravates pancreatic oxidative stress (Dabrowski et al., 1994) while endothelium derived NO ameliorates the pancreatic dysfunction (Takacs et al., 2002). However, the present study reported an increase in the NO levels in rats with L-arginine signifying induction of iNOS in pancreas indicating pancreatic edema and acinar cell damage. However, the levels were significantly reduced the NO levels in berry extract treated groups due to its radical scavenging properties. This could probably be explained by the radical scavenging properties of berry extracts.

L-arginine induced acute pancreatitis is possibly due to inhibition of polyamine synthesis that inhibit nucleic acid and protein synthesis (Mervi et al., 2006). Since protein metabolism is most active in pancreatic acinar cells, it is likely that acinar cells are the first target of an arginine overdose, resulting in degradation or necrosis. In our study, L-arginine treated rats showed a decreased level of total protein, describing the inhibition of nucleic acid synthesis which is depicted as fragmented DNA. DNA fragmentation occurs as a process of apoptosis, a programmed cell death phenomenon (Noorjahan et al., 2018). It is initiated by mitochondrial damage presumed consequence of increased intracellular calcium uptake. The high calcium loads in mitochondria induce drastic responses in culminating necrosis. It also has a significant role in generation of reactive oxygen species thereby circuitous loss of DNA integrity (Wen et al., 2016). DNA Fragmentation is considered as an indirect marker of mitochondrial injury. It has been reported that L-arginine induces apoptosis in acute pancreatitis which is in accordance with our study (Kang et al., 2014). In our study, berry extracts were remarkably attenuated DNA damage observed in L-arginine disease control group. The result is in consistent with previous report wherein the consumption of wild Blueberry drink intervention for 6 weeks significantly reduced the levels of oxidized DNA bases and increased the resistance to oxidatively induced DNA damage (Riso et al., 2013).

Berry phenolics are best known for their ability to act as antioxidants, but the biological activities exerted by berry phytochemicals in vivo extend beyond antioxidation. In fact, a large and growing body of evidence shows that berry are “poorly bioavailable” due to their relatively “low levels” in human circulation. However, berry phenolics are extensively metabolized and also further converted by colonic microflora into related molecules. These compounds accumulate in target tissues, and contribute significantly to the biological effects that have been observed for berry fruits (McDougall et al., 2009).

CONCLUSION
The data of the present study suggest that Berry fruit extracts exerted a potential protective effect against L-arginine induced damage in rat pancreas, at least in part, due to its antioxidant properties.

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