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ORIGINAL ARTICLE

# *Morinda Citrifolia* (Noni) Fruit Protects the Exocrine Pancreatic Dysfunction Against L-Arginine Induced Acute Pancreatitis in Rats

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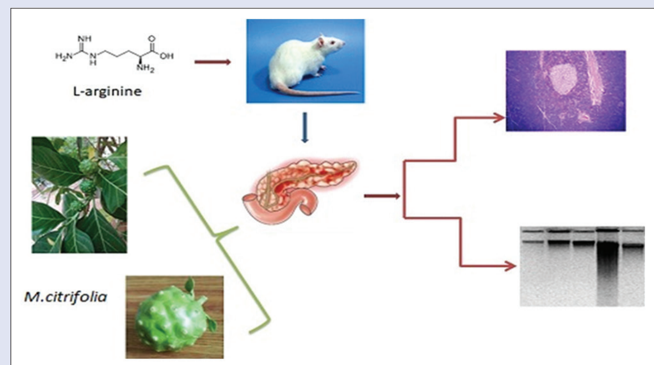
## ABSTRACT

**Background and Objective:** *Morinda citrifolia* (MC) commonly known as Noni is being used for many ailments and is considered as wellness drink. It is traditionally used for anti-inflammatory, anti-aging, and immunostimulant properties. The present study has been initiated to investigate the protective effects of MC fruit extract (MCFE) on L-arginine-induced acute pancreatitis (AP) in rats. **Materials and Methods:** Male Sprague-Dawley rats were randomly divided into groups of control, disease control, positive control, and treatment groups. AP is induced by the administration of a single dose of L-arginine (2 × 2.5 g/kg, intraperitoneally, 1 h apart). Positive control received melatonin (10 mg/kg); treatment groups received 200 mg/kg and 400 mg/kg MCFE 6 days before administration of L-arginine. After 12 h of induction, the serum samples were analyzed for biomarker enzymes such as amylase, lipase, C-reactive protein, superoxide dismutase, glutathione, catalase, tissue nitrate, lactate dehydrogenase, and myeloperoxidase. Histopathological studies and deoxyribonucleic acid (DNA) fragmentation assay were performed from the isolated pancreatic tissue. **Results:** MCFE administration showed a dose-dependent significant ($P < 0.001$) protective effect by improving the levels of antioxidant enzymes and reducing the elevated levels of amylase and lipase. The acinar cell damage was limited in histopathological findings and an intact DNA when compared to disease control. **Conclusion:** MCFE administration showed a protective effect against AP in rats, and it may be due to the attenuation of oxidative stress. Further investigation for the exact molecular mechanism is needed.

**Key words:** Amylase, arginine, lipase, *Morinda citrifolia*, oxygen free radicals, pancreatitis

## SUMMARY

- Noni juice demonstrated a protective effect against L-arginine induced acute pancreatitis which was in accordance with the positive control Melatonin. The protective effect is observed to be due to the presence of active constituents such as desacetylasperulosidic acid, 6-α-hydroxyadoxoside, 6-β-7-β-epoxy 8-episplendoside, americanin A which showed the antioxidant effects. The exploration of molecular level mechanism may lead to the development of essential therapeutic targets in acute pancreatitis.




**Abbreviations used:** AP: Acute Pancreatitis; MCFE: *Morinda citrifolia* fruit extract; DNA: Deoxyribonucleic acid; GI: Gastrointestinal; ROS: Reactive oxygen species; CRP: C-reactive protein; Kcl: Potassium chloride; UPLC: Ultrahigh-pressure liquid chromatography; LC-MS/MS: Liquid chromatography–mass spectrometry; OECD: Organization of economic cooperation and development; $LD_{50}$: Lethal dose 50; SOD: Superoxide dismutase; $H_2O_2$: Hydrogen peroxide; TCA: Trichloroacetic acid; DNPH: Di nitrophenylhydrazine; EDTA: Ethylenediaminetetraacetic acid; TBA: Thiobarbituric acid; MDA: Malondialdehyde; LDH: Lactate dehydrogenase; β-NADH: β-Nicotinamide adenine dinucleotide; SDS page: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ANOVA: Analysis of variance; iNOS: Inducible nitric oxide

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## INTRODUCTION

Acute pancreatitis (AP), a self-limiting disease is one of the most frequent diseases of pancreas and the most common cause for hospital admission among the Gastrointestinal diseases in many countries. AP is regarded as one of the leading acute diseases worldwide with increasing evidence of age-standardized rates over the past decades. Although it is self-limiting, up to 20% of the patients may encounter mild edematous to severe necrotizing form.[1] Pathogenesis involves the activation of intracellular pancreatic zymogen which triggers systemic and local inflammatory response by releasing mediators from macrophages and neutrophils, which eventually lead to multiorgan dysfunction.[2] One of the pivotal mechanisms of AP is based on the involvement of reactive oxygen species (ROS), which provoke the development of pancreatitis through

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degranulation of zymogen, migration of macrophages, tissue necrosis, and increase in serum amylase and lipase activity. Overproduction of ROS in AP and impaired neutralization ability by scavengers lead to accumulation in pancreatic tissue thereby the development of pancreatitis.[3]

*Morinda citrifolia* (MC) known as Noni or Indian mulberry is a well-known folk medicine used for many decades.[4,5] It belongs to the family of *Rubiaceae* and traditionally used for antimicrobial,[3,6] anticancer,[7] anti-inflammatory,[4,8,9] anti-aging,[10] and immunostimulant activities.[11] MC has been reported to have antioxidant,[6,12] vasodilatory,[6] antitumor,[13] anti-inflammatory, and angiotensin-converting enzyme inhibition activities.[14] All the parts of the plant possess several pharmacological activities. *In vitro* studies suggest that the plant has angiogenesis inhibition, cyclooxygenase-1, and 2 inhibitory and antioxidant effects. Many phytochemicals such as anthraquinones, iridoid and flavonol glycosides, and volatile compounds are isolated from fruit juice.[4,5] The aim of the present study is to evaluate its protective effect on L-arginine induced pancreatitis in rats.

## MATERIALS AND METHODS

### Methodology

#### *Chemicals*

L-arginine was purchased from Sigma chemicals Pvt. Ltd. Amylase estimation kit was purchased from Arkray Healthcare Pvt. Ltd. Lipase estimation kit was purchased from Agappe Diagnostics Ltd. C Reactive protein (CRP) estimation kit from Arkray Healthcare Pvt. Ltd. Deoxyribonucleic acid (DNA) isolation kit was obtained from Bioartis Pvt. Ltd. All other chemicals used in the study are of analytical and high-performance liquid chromatography grade and were purchased from SD fine Pvt. Ltd.

### Plant material

Fresh mature fruits of MC were collected from the plant in the campus premises of Sri Indu Institute of Pharmacy, from May to June 2017. The fruit material was authenticated by taxonomist, Department of Botany, S V University, Tirupati, with voucher number 1015. They were fermented in sealed containers for 2 months at room temperature to exudate the juice from the fruits. After 2 months the fermented juice is centrifuged to remove the debris, and the supernatant is dried under vacuum in rotary evaporator to obtain dry mass extract. The resultant dried powder is stored at 4°C until use.[15]

### Determination of total flavonoid, anthocyanins, and phenolic content

The total flavonoid content was determined as per the method described by Zhishen *et al.* (1999). Briefly, 1 ml of known concentration of the extract was mixed with 1 ml of 2% aluminum chloride in methanol solution and diluted with deionized water. This mixture was incubated at room temperature for 1 h and checked for absorbance against standard Rutin at 415 nm. The values are expressed as rutin equivalents per 100 g.[16]

The total anthocyanin content was determined by pH differential method. Briefly, 1 ml of the known concentration of the extract is taken in 10 ml volumetric flask for preparing two dilutions of the sample. One solution is adjusted with potassium chloride 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 h. The anthocyanin content is calculated as follows:

Total monomeric anthocyanins (mg/100 g of extract) = $A \times MW \times 1000/(€\times C)$

Where, A is absorbance $(A_{510}-A_{700})$ ‘pH 1.0-$(A_{515}-A_{700})$ pH 4.5;

- MW is molecular weight for cyanidin 3-glucoside) 449.2
- € 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.[17,18]

The quantity of the total phenolic content was carried out as described by Dewanto *et al.* using modified colorimetric Folin Ciocalteu estimation. In brief, 0.5 ml of known concentration of the extract was mixed with 2.5 ml of Folin-Ciocalteu reagent dissolved in water and incubated at room temperature for 5 min. To the mixture, 2.5 ml of 7.5% of sodium bicarbonate solution and 3 ml of deionized water was added and kept aside for 45 min after which it was checked for absorbance at 765 nm against standard gallic acid. The content of phenolics is expressed as Gallic acid equivalents per 100 g.[19]

### Detection of chemical composition

High-resolution masses of metabolites were measured after ultrahigh-pressure liquid chromatography (UPLC) separation. Chromatographic separation was performed on Acquity H-Class UPLC system (Waters, Milford, MA, USA) with a conditioned autosampler, using an Acquity BEH $C_{18}$ column (100 mm × 2.1 mm i. d., 1.7 μm particle size) (Waters, Milford, MA, USA). The column temperature was maintained at 45°C. The mobile phase consisting of water with 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) was pumped at a flow rate of 0.40 mL/min. The gradient elution program was as follows: 0 min, 2%B; 1.80 min, 2%B; 4.60 min, 10%B; 9.00 min, 80%B; 10.00 min, 2%B. Equilibration time was 2.80 min, and the injection volume was 2.0 μL. Liquid chromatography–mass spectrometry (LC-MS/MS) mode was applied to analyze the samples in ESI-Q-Tof-MS negative mode. Eluted compounds were detected from 30 to m/z 1200 using Xevo G2-XS QTOF mass spectrometer (Waters, Manchester, UK), which was connected to electrospray ionization interface with negative ion mode using the following instrument settings, capillary voltage, 2.0KV; sample cone, 40V; source temperature, 120°C; desolvation temperature 350°C; cone gas flow rate 50 L/h; desolvation gas ($N_2$) flow rate 850 L/h. All analyses were performed using the lock spray, which ensured accuracy and reproducibility. Leucine-Enkephalin (5 ng/mL) was used as lock mass generating a reference ion in negative mode at m/z 556.2771 and introduced by a lock spray at 10 μL/min for accurate mass acquisition. Data acquisition was achieved using MassLynx v 4.1. Acquiring data in this manner provided the collection of information of intact precursor ions.

### Animals

Male Sprague-Dawley rats of 150–200 g body weight were selected for the study. Animals were housed in polypropylene cages at a temperature of 25°C ± 2°C. They were provided with standard pellet diet with water *ad libitum*. They were acclimatized for 1 week before the start of the experiment. All the studies were approved from Institutional Ethics committee with reference number 1448/PO/Re/S/11/CPCSEA/07/2016.

### Acute toxicity studies

The dried powder is dissolved in normal saline and subjected to acute toxicity studies by fixed dose method according to the organization of economic cooperation and development guideline 420.[20] The lethal dose 50 ($LD_{50}$) was found to be above 2000 mg/kg and $1/5^{th}$ and $1/10^{th}$ dose of $LD_{50}$, i.e., 200 and 400 mg/kg body weight is used for the study.

## Study protocol

Animals were randomly divided into five groups of six animals each. Group I as control group received normal saline for 7 days. Group II as disease control group received a single-dose of 2.5 g/kg of body weight of 20% of L-arginine Hcl in 0.5% normal saline on day 5 of the study. Group III as positive control received melatonin at a dose of 10 mg/kg intraperitoneally, *Group IV and V* as treatment groups (MC fruit extract [MCFE–I] and MCFE-II group) received 200 and 400 mg/kg body weight of MCFE orally for 7 days. On day 5 of the study Group III, IV, and V received a single dose of 2.5 g/kg of body weight of 20% of L-arginine Hcl in 0.5% normal saline 1 h after the administration of melatonin and the extracts.[21]

At the end of the study, blood samples were collected from all the groups from retro-orbital plexus under light ether anesthesia. The serum from the collected blood samples was separated and subjected to the estimation of enzyme biomarkers. After the blood sampling, all the animals were sacrificed, pancreas were isolated, weighed and was subjected to tissue enzyme estimations, DNA fragmentation assay, and the remaining part was fixed in 10% formalin and subjected to histopathological studies using hematoxylin and eosin staining.

## Estimation of pancreatic biomarkers

Serum amylase and lipase are considered as two important biomarkers for pancreatitis. Serum amylase levels were measured with the help of kit from Arkray Healthcare Pvt. Ltd. Serum lipase levels were also estimated by the commercial kit purchased from Agappe Diagnostics Ltd. Serum CRP is considered as the parameter of choice to assess the severity of the disease. It was estimated using the commercial kit manufactured by Arkray Healthcare Pvt. Ltd. All the procedures were followed according to the leaflet provided with the kits.

## Estimation of antioxidant biomarkers

Serum superoxide dismutase (SOD) levels were measured by erythrocyte sedimentation and photo-oxidation method. The packed erythrocytes were used in the estimation, and to it, 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. 0.1 ml of supernatant was separated and to it 0.88 ml of riboflavin, 60 μL of O-dianisidine was added, and the absorbance was measured at 460 nm.[22]

Catalase was estimated by the rate of decomposition of hydrogen peroxide ($H_2O_2$). To 0.1 ml of serum, 2.5 ml of phosphate buffer was added and incubated at 25°C for 30 min. 650 μl of $H_2O_2$ solution was added to initiate the reaction. After transferring into a cuvette, the absorbance was measured at 240 nm. The change in absorbance was measured for 3 min.[23]

Vitamin C levels were estimated according to Omaye *et al*. In brief, to 0.5 ml of plasma, 1.5 ml of 6% trichloroacetic acid (TCA) was added and centrifuged (3500 rpm for 20 min). To 0.5 ml of supernatant 0.5 ml of Di nitrophenylhydrazine (DNPH) reagent (2% DNPH and 4% thiourea in 9 N $H_2SO_4$) was added and the developed color was read at 530 nm after 30 min.[24]

## Estimation of tissue biomarkers

The isolated pancreatic tissue was homogenated in cool 0.1M Phosphate buffer. To which equal volumes of 20% TCA and 1 mM ethylenediaminetetraacetic acid (EDTA) were added and kept at room temperature for 5 min. It was then centrifuged at 2000 rpm for 10 min. The supernatant was separated and to it 1.5 ml of DTNB reagent was added and checked for absorbance at 412 nm.[25]

Total nitrite levels were determined as a measure of nitric oxide with the use of Griess reagent. The nitrate levels were estimated, according to Green *et al.* (1982) with slight modifications. The pancreatic tissue was homogenized in 50 mmol/L potassium phosphate buffer (pH 7.8) and centrifuged at 11,000×g for 15 min at 4°C. One hundred microliters of the supernatant was mixed with 100 μL Griess reagent (0.1% N-[1-naphthyl] ethylenediamine dihydrochloride, 1% sulfanilamide in 5% phosphoric acid) and after 10 min, the absorbance was measured at 540 nm. The standard curve was obtained. The results were calculated from a standard curve using sodium nitrite and expressed as micromoles of nitrate/nitrite.[26]

Pancreatic myeloperoxidase (MPO) activity was assayed spectrophotometrically according to the method described by Haqqani *et al.* (1982). This method is based on kinetic measurement of the yellowish-orange product formed by the oxidation of o-dianisidine with MPO in the presence of $H_2O_2$ at 460 nm. One unit of MPO was defined as that degrading 1 μmol of $H_2O_2$ per min at 25°C. A molar extinction coefficient of 1.3 × 104 M/cm of oxidized 0-dianisidine was used for the calculation. MPO activity was expressed as units/mg of proteins.[27]

Four milliliters of reaction mixture containing 0.4 ml of the tissue homogenate, 1.5 ml of 0.8% thiobarbituric acid, 1.5 ml of acetic acid (20%, pH 3.5) and distilled water was kept in a boiling water bath at 95°C for 1 h. The reaction mixture was then cooled. To it 1 ml of distilled water and 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 malondialdehyde (MDA) was calculated with the help of a standard graph prepared using different concentrations (1–10 nmol) of 1'1'3'3'-Tetramethoxypropane in 1 ml distilled water and is expressed as nmol of MDA/mg protein.[28]

Lactate dehydrogenase (LDH) was estimated by the previously described method.[29] To 0.1 ml of tissue homogenate or serum, 3 ml of LDH reagent (2.8 ml of 0.13 mM β-Nicotinamide adenine dinucleotide and 0.1 ml of 34 mM sodium pyruvate) was added and incubated at 37°C for 5 min. After incubation, the absorbance was checked at 340 nm for every min for 3 min. ΔA activity was measured and represented as U/L.

## Estimation of apoptotic biomarker

### *Deoxyribonucleic acid fragmentation assay*

A characteristic mark of apoptosis is the fragmentation of DNA by endonucleases. For the assay of DNA fragmentation, the DNA of the pancreatic tissue is isolated using the commercial kit by Bioartis Pvt. Ltd. Briefly, about 50 mg of the pancreatic tissue was excised and transferred to the lysis solution containing 6M guanidine hydrochloride, 20 mM EDTA, 10 mM Tris-Cl and 2% Triton X-100 and lysed in a hand-held homogenizer. Proteinase K (10 mg/ml) was added to the lysate and incubated for 2–3 h at 55°C. After incubation, the solution is centrifuged at 10,000 rpm for 10 min. To the supernatant equal volume of ethanol (96%–100%) was added and slowly mixed and centrifuged at 10,000 rpm for 5 min. The DNA pellet thus isolated was then washed 2–3 times in 500 μl of 70% ethanol. DNA pellet is air dried and resuspended in TE buffer (pH-8.0) and 1 mM EDTA. The resuspended DNA is loaded on the sodium dodecyl sulfate page gel electrophoresis for analysis.[30]

## Statistical analysis

All the results obtained were represented in mean ± standard error of mean (SEM) and were subjected to one-way analysis of variance followed by Dunnett '*t*' test with a significant levels of $P < 0.001$. Data analysis was carried out using Graph Pad Prism tool (Version 7) (Graphpad Software Inc., San Diego, California, USA).

# RESULTS

## Determination of phytoconstituents

Total phenolic content, total flavonoid content, and total anthocyanin content were found to be 9.01 ± 0.45, 37.12 ± 0.78, and 16.3 ± 0.45, respectively [Table 1].

## Detection of chemical constituents

The UPLC-MS/MS of MCFE showed the following peaks and after the analysis was done using Reaxys data. The results of the analysis include the confirmation of the following compounds according to their respective molecular weights. The compound Deacetylasperuloside (m/z 390.341), Citrifolinoside (m/z 561.14), rutin (m/z 609.520), 6-α-hydroxyadoxoside (m/z 421.1346), 6-β-7-β-epoxy 8-episplendoside (m/z 421.367), Borreriagenin (m/z 215.217), citrifolinin (m/z 418.351), Scopoletin (m/z 191.11), Gentisic acid (m/z 152.12), Morindacin (m/z 214.217), p-Coumaric acid (m/z 163.16), Asperuloside (m/z 414.363), Quercetin (m/z 301.238), geniposidic acid (m/z 389.1083), and Yopaaoside C (m/z 412.133) [Figure 1].

## Estimation of pancreatic, antioxidant, and tissue markers

In the present study, a single dose of L-arginine was used to induce AP. The disease control group of rats showed a significant increase ($P < 0.001$) in the levels of amylase, lipase, CRP as well as tissue nitrite, MDA, MPO, and LDH levels indicating the inflammation of the pancreas. There was also a significant reduction ($P < 0.001$) in the antioxidant status of the rats which was observed as reduced levels of SOD, catalase, Vitamin C and tissue glutathione levels, revealing the involvement of oxidative stress. The effects were significant when compared with control and melatonin group of rats [Tables 2-4]. However, the contrast was seen in rats with melatonin administration, where there was a significant increase ($P < 0.001$) in the levels of SOD, Catalase, Vitamin C, Glutathione and reduction ($P < 0.001$) in the levels of amylase, lipase, CRP, Nitrite, MDA, LDH, and MPO levels. The treatment of MCFE significantly improved the protective effect against L-arginine by reducing the levels of amylase, lipase, CRP, MDA, MPO, LDH, and nitrite levels significantly ($P < 0.001$) when compared to disease control group of rats and improved the antioxidant levels [Tables 2-4].

**Table 1:** Total phenolic content, total flavonoid content and total anthocyanin content in *Morinda citrifolia* fruit extract

| Phytochemical contents | MCFE |
|---|---|
| Total phenolic content (mg of gallic acid equivalent/g) | 9.01±0.45 |
| Total flavonoid content (mg of rutin equivalent/g) | 37.12±0.78 |
| Total anthocyanin content (mg of cyanidin-3-glucoside equivalent/g) | 16.3±0.45 |

MCFE: *Morinda citrifolia* fruit extract

## Histopathological studies

The isolated pancreas was subjected to histopathological examination with the help of hematoxylin and eosin stain. Control and melatonin groups of rats showed a normal architecture of pancreas [Figure 2i]. L-arginine treatment showed interstitial edema with vacuolar degeneration and extensive damage to acinar cells [Figure 2ii and iii]. Treatment with melatonin and MCFE showed less interstitial edema with no vacuolar degeneration and maintenance of normal structural design [Figure 2iv-vi].

## Deoxyribonucleic acid fragmentation assay

DNA fragmentation assay was performed to assess the amount of apoptosis. Rats treated with L-arginine showed a smear pattern demonstrating the fragmentation of DNA, leading to apoptosis of the tissue [Figure 3d] when compared to the control group of rats with Intact DNA [Figure 3a]. Intactness of DNA was observed in rats pretreated with melatonin [Figure 3e] and MCFE indicating the shielding activity against degradation of DNA [Figure 3b and 3c].

# DISCUSSION

All the results of the present study revealed a protective effect of MCFE on L-arginine induced pancreatitis in a dose-dependent manner. The beneficial effect of MCFE is in harmony with positive control melatonin which is in agreement with previous reports, where melatonin administration ameliorates necrotizing pancreatitis and regenerates the pancreatic acini.[31] In the present study, L-arginine-induced AP is a reproducible well established non-invasive model that produces dose-dependent necrosis in the pancreatic acinar cells with characteristic

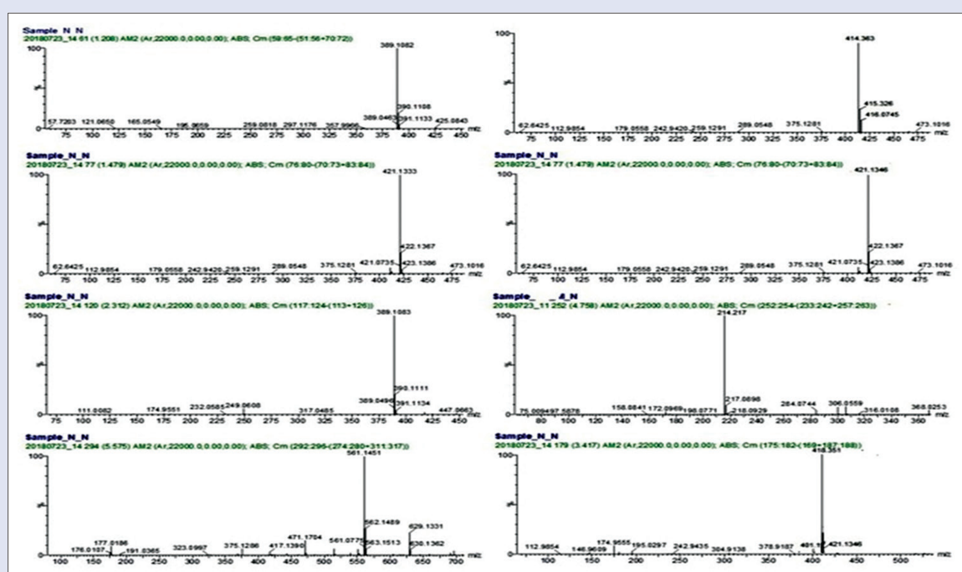

**Figure 1:** Liquid chromatography/mass spectrometry spectrum of alcoholic extract of *Morinda citrifolia*

changes within 24 h of administration.[32] Factors such as generation of free radicals, i.e., nitrosative and oxidative stress along with the liberation of inflammatory mediators contributed to the detrimental effects of pancreas by the administration of L-arginine.[33]

Serum amylase and lipase are two important diagnostic markers for interpretation of the severity of AP.[34] L-arginine administration elevated the levels of amylase and lipase representing the exocrine pancreatic damage which in accordance with the previous reports.[35] The treatment of MCFE or melatonin reduced the levels significantly eliciting their protective effect. MCFE protection has been observed to be in a dose-dependent manner and is in accord with the previous studies where the fruit extract inhibited *in vitro* pancreatic porcine lipase.[31,36] MCFE have been reported to inhibit alpha-amylase and alpha-glucosidase on uptake of glucose in HepG2 cells *in vitro* and the presence of flavonoids such as rutin, kaempferol, and quercetin contributed to the effect.[37]

Imbalance of oxidative and antioxidant defense potential has been noted with L-arginine administration.[32,38] In the present exploration, pancreatitis induced by L-arginine demonstrated an increase in the oxidative stress by increasing Which was observed as increased levels of MDA, MPO, and Nitrite due to peroxidation of membrane lipids and activation of NOS.[39] MCFE administration attenuated the reactive oxygen and nitrogen species by improving the levels of SOD, catalase, glutathione, and Vitamin C which is in accordance with the previous reports where the presence of constituents such as desacetylasperulosidic acid, 6-α-hydroxyadoxoside, 6-β-7-β-epoxy 8-episplendoside, americanin A contributed to the antioxidant effects and are the major constituents of the fruit as well as, MC have been reported for its high content of Vitamin C.[40,41] Further, this content is supported by the elution of the respective fragment peaks of the constituents in the present study by UPLC-MS/MS. The administration of Noni juice has reduced the plasma superoxide anion radical levels in smoking individuals by reducing the SOD levels in due course reduced MDA levels.[40] Loganin, an iridoid present in the fruit increased the expression of glutathione activity in rat mesangial cells, which may contribute to the increase in levels of glutathione in the present study.[42] Moreover, inducible nitric oxide synthase activation derives nitric oxide which is a pro-inflammatory mediator in the generation of pancreatic inflammation. Dussossoy *et al*. revealed that the administration of MCFE has inhibitory effects on nitric oxide and prostaglandin $E_2$ contributing to its anti-inflammatory effects.[43]

CRP and LDH have been well associated with progression of inflammation in many diseases and are accounted for the prognosis of the disease in AP.[37,44] The administration of L-arginine increased the levels of CRP and LDH signifying the infiltration of inflammatory mediators and oxygen deprivation. In contrast to this, MCFE and melatonin administration showed a beneficial effect by the reduction of CRP and LDH contributing its anti-inflammatory effects[42] where the administration of Noni juice reported to reduce the levels of CRP, triglycerides, low-density lipoprotein, and homocysteine in smoking patients.[45]

The current investigation studied the repair and protection of pancreatic tissue by examining the apoptosis of acinar cells. DNA fragmentation assay is a universal criterion for the measurement of apoptotic cell death.[46] The administration of L-arginine showed a smear pattern of DNA on the gel electrophoresis revealing the damage of pancreatic acini at the nuclear level. Reactive oxygen species oxidizes proteins to form cross linkage of DNA bases or cleavage of them which further trigger the cell to proceed towards apoptosis.[47] In addition to this, lipid peroxide derived DNA adducts was observed as a smear pattern in the gel picture [Figure 3] due to increased levels of MDA. In disparity with that the administration of Melatonin and MCFE markedly prevented the damage as shown by intact DNA in the gel picture [Figure 3] which is in harmony with the previous reports where, MC-induced DNA repair by increasing the expression of repair genes in cervical cancer cells and also reduced the formation of DNA adducts in smokers who have consumed Noni juice.[49-51] This content is in accordance with the present study.

Further, the UPLC-MS analysis of the MCFE revealed the presence of iridoid monoterpenoids such as asperuloside, deacetylasperuloside, citrifolinoside, 6-α-hydroxyadoxoside, 6-β-7-β-epoxy 8-episplendoside, Borreriagenin, Citrifolinin A, Morindacin, geniposidic acid, and

**Table 2:** Changes in the pancreatic biomarkers in rats treated with L-arginine and *Morinda citrifolia* fruit extract

| Groups | Amylase (IU/L) | Lipase (U/L) |
|---|---|---|
| Normal control | 14.17±0.116# | 16.17±0.9804# |
| Disease control | 330.5±3.233* | 83.17±1.138* |
| Melatonin (10 mg/kg) | 98.8±2.234*,# | 34.45±1.456*,# |
| MCFE-I (200 mg/kg) | 175.8±2.787*,# | 52.33±1.633*,# |
| MCFE-II (400 mg/kg) | 118.8±4.997*,# | 44.33±1.633*,# |

Values are expressed in mean±SEM (*n*=6); *,#*P*<0.001 when compared to normal control and disease control group, respectively. MCFE: *Morinda citrifolia* fruit extract; SEM: Standard error of mean

**Table 3:** Changes in the antioxidant biomarkers in rats treated with L-arginine and *Morinda citrifolia* fruit extract

| Groups | SOD (mg/protein/min) | Catalase (μM of $H_2O_2$/mg/protein/min) | Glutathione (mg/dl) | Vitamin C (mg/dl) |
|---|---|---|---|---|
| Normal control | 18.5±0.4282# | 48.18±0.3056# | 2236±0.9574# | 95.73±0.3509# |
| Disease control | 6.167±0.3073* | 22.7±0.2811* | 575.8±1.493* | 21.35±0.5771* |
| Melatonin (10 mg/kg) | 61.23±1.724#,* | 47.14±0.234# | 3124±3.549#,* | 212.34±0.6784*,# |
| MCFE-I (200 mg/kg) | 33.17±1.835*,# | 38.02±1.283*,# | 2291±4.502# | 165.4±1.33*,# |
| MCFE-II (400 mg/kg) | 50±1.095*,# | 45.33±1.54# | 3049±2.898*,# | 198.7±1.704*,# |

Values are expressed in mean±SEM (*n*=6); *,#*P*<0.001 when compared to normal control and disease control group, respectively. SOD: Serum superoxide dismutase; MCFE: *Morinda citrifolia* fruit extract; SEM: Standard error of mean

**Table 4:** Changes in the inflammatory biomarkers in rats treated with L-arginine and *Morinda citrifolia* fruit extract

| Groups | MDA (mM/dl/h) | Nitrate (μM/g) | CRP (μg/ml) | MPO (μM of peroxide/min) | LDH (U/L) |
|---|---|---|---|---|---|
| Normal control | 14.89±0.3381# | 11.08±0.212# | 486.8±2.358# | 4.032±0.05486# | 23.4±0.5379# |
| Disease control | 86.87±0.8747* | 35.05±0.1522* | 19,601±2.455* | 25.69±0.3327* | 127.5±0.3785* |
| Melatonin (10 mg/kg) | 35.56±0.9852*,# | 14.34±0.8765# | 697±4.345*,# | 6.45±0.4563# | 45.56±0.8976*,# |
| MCFE-I (200 mg/kg) | 39.05±0.4231*,# | 24.36±0.7204*,# | 1066±5.279*,# | 14.89±0.6695*,# | 82.05±1.7*,# |
| MCFE-II (400 mg/kg) | 31.88±0.5529*,# | 19.24±0.5925# | 991.5±3.507*,# | 10.44±0.8928# | 67.99±1.584*,# |

Values are expressed in mean±SEM (*n*=6); *,#*P*<0.001 when compared to normal control and disease control group, respectively. MDA: Malondialdehyde; CRP: C-Reactive protein; MPO: Myeloperoxidase; LDH: Lactate dehydrogenase; MCFE: *Morinda citrifolia* fruit extract; SEM: Standard error of mean

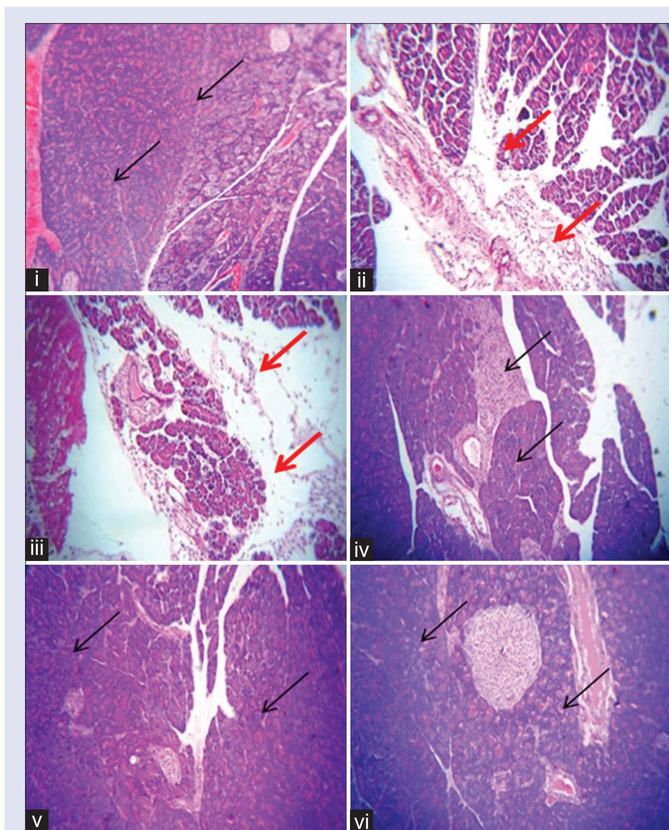


**Figure 2:** Histopathological findings of formalin-fixed paraffin embedded section of pancreas with hematoxylin and eosin.(i) Photomicrograph of the normal pancreas (ii and iii) L-arginine induced pancreas showing extensive acinar cell damage with interstitial edema, vacuolar degeneration. (iv) Melatonin-treated rat pancreas. Section shows normal echotexture. (v and vi) protective effect of *Morinda citrifolia* fruit extract. Sections show normal acinar cells as that of Normal pancreas

Yopaaoside C which is in accordance with the previously reported studies on fruit constituents.[52] In addition to the above, Akihisa *et al*. and Su *et al*. reported the anti-inflammatory, cancer chemopreventive and antioxidative effects of the above constituents, respectively,[53,54] advocating to their protective effect in controlling the progression of the disease by modifying inflammation, reducing the oxidative stress which in turn illustrates the normal histology of the pancreas treated with MCFE in the present study.

## CONCLUSION

The administration of MCFE has shown protective effects on L-arginine-induced pancreatitis. The enhancement owe to the anti-inflammatory and high antioxidative effects of MCFE. The protective effect is also in accordance with histopathological studies and intact DNA. In addition, there is a need to explore on its molecular level mechanism as well as its capability clinically for new supplementation of therapeutic targets in AP.

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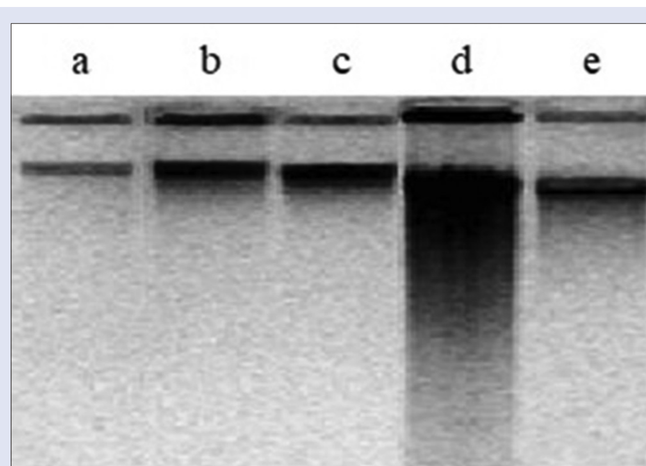


**Figure 3:** Gel picture of deoxyribonucleic acid fragmentation assay of the pancreatic tissue. (a) Group I; Normal pancreas. (b and c) Group IV, V: *Morinda citrifolia* fruit extract-treated pancreas showing intact deoxyribonucleic acid. (d) Group II; Disease control group showing fragmented deoxyribonucleic acid in the form of smear indicating extensive damage. (e) Group III: Melatonin treated pancreas showing intact deoxyribonucleic acid

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## Conflicts of interest

There are no conflicts of interest.

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