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Protective Effect of *Musa paradisiaca* Fruit Extract On L-Arginine Induced Acute Pancreatitis In Rats

Veena Gadicherla[1], Siva R Challa[2], Basaveswara Rao M V[3], Veena Rani I[4], Geetha Parvathi A[5]

[1*]Department of Pharmacology, Faculty of Pharmaceutical Sciences, Krishna University, Machilipatnam, Andhra Pradesh, India
[2]Department of Pharmacology, KVSR Siddhartha Institute of Pharmaceutical Sciences, Vijayawada, Krishna Dist., Andhra Pradesh, India
[3]Department of Chemistry, Faculty of Sciences, Krishna University, Machilipatnam, Andhra Pradesh, India
[4,5]Department of Pharmacology, SSJ College of Pharmacy, Vattinagulapally, Hyderabad, Telangana State, India
[*]Corresponding author: Email id: anugad@gmail.com

Abstract: ***Musa paradisiaca*** **often refereed as Banana is an ancient herbaceous flowering plant and the most earlier crop to be cultivated. It is one the important component of diet for all classes of people worldwide. All the parts of the plant is believed to have beneficial effects in different ailments and an excellent nutritional element. The present study was aimed to find the protective effect of** ***Musa paradisiacal*** **fruit extract on acute pancreatitis in rats.**

Male Sprague dawley rats were randomly divided in to 4 groups. Control group with normal saline; disease control group where acute pancreatitis was induced by using a single dose of L-arginine (2.5g/kg b.w); ***Musa paradisiacal*** **fruit extract were prophylactically administered before the induction of acute pancreatitis at a dose of 200 and 400 mg/kg b.w orally in normal saline for 7 days. At the end of the study, blood samples and isolated pancreas were subjected to different pancreatic, antioxidant and inflammatory biomarker analysis. The remaining tissue was subjected to histopathological studies and DNA fragmentation assay for assessing the damage and protection of the tissues.**

The results of the study revealed that prophylactic administration of the extracts reduced the amylase and lipase levels when compared to disease control group as well as improved the overall antioxidant status in a dose dependent manner. Further, the extracts also showed a protective effect against apoptosis.

In conclusion, the present study suggest that administration of ***Musa paradisiacal*** **fruit extract exhibited a protective effect on acute pancreatitis and further investigation is required for its molecular based mechanisms.**

Keywords**: *Musa paradisiacal*, acute pancreatitis, free radicals, apoptosis.**

I. INTRODUCTION:

Acute pancreatitis is a inflammatory disorder of exocrine pancreas with high mortality. It may be acute or chronic forms depending on the pathogenesis [1]. Acute pancreatitis can be self limiting form to necrotizing pancreatitis where the severity of the disease correlates with the degree of necrosis [2]. The pathogenesis of the disease is complicated and the most accepted theory is the auto digestion of the tissue due to the release of pancreatic digestive enzymes [3]. Another widely reported theory is the involvement of oxidative and nitrosative stress [4]. These free radicals have an important role in inflammation and systemic complications of pancreatitis. They damage the tissue cells by attacking the polyunsaturated fatty acids of the lipid membrane, structural and enzymatic proteins and DNA [4]. The treatment of acute

pancreatitis is limited to supportive and symptomatic therapies. Several antioxidants have been examined for their protective actions against acute pancreatitis [5-8]. Use of agents with antioxidant and anti-inflammatory features with high nutritional supplement might exercise a beneficial effect on acute pancreatitis.

Banana is an early cultivated plant and a major component of economy for various countries [9]. Banana belongs to the species *Musa paradisiaca* of the family *Musaceae* and offer many healthy benefits. They act as a rich source of Vitamins, potassium, calcium, phosphorus, magnesium etc [10]. All the parts of a banana plant possess certain medicinal properties, and thus have respective medicinal applications. The fruit of *Musa paradisiaca* is an exceptional fruit offering different forms of nutrition. It is an excellent source of potassium and a single banana is said to provide 23% of potassium on daily basis [10], [11]. The flowers are useful in bronchitis, dysentery, and ulcers, whereas cooked flowers are given to people with diabetes. The fruit and leaves of banana are useful in treating burns and wounds.]. The fruit has a mild laxative property where as the plant sap is an astringent which is used in cases of hysteria, epilepsy, leprosy, fevers, hemorrhages, dysentery and diarrhoea. The unripe fruits have been evaluated for its antihyperglycemic, antioxidant, wound healing, hypolipidemic, hair growth promoter, cardioprotective and against thyroid dysfunction [12], [13],[14],[15]. Banana is also considered to be a rich source of antioxidants [9]. Bioactive compounds in banana include, phenolic compounds, carotenoids, biogenic amines, phytosterols. Thus, the present study was designed to evaluate the beneficial effects of *M.paradiscia* fruit on L arginine induced pancreatitis in rats.

II. MATERIALS AND METHODS

A. Chemicals:

L-arginine was purchased from Sigma chemicals pvt ltd. Amylase estimation kit was purchased from Akray healthcare pvt ltd. Lipase estimation kit was purchased from Aggape diagnostics ltd. C Reative protein (CRP) estimation kit from Akray healthcare pvt ltd. DNA isolation kit was obtained from Bioartis pvt ltd. All other chemicals used in the study are of analytical grade and were purchased from SD fine pvt ltd.

B. Animals:

Male Sprague dawley rats were selected for the study. They were housed in clean polypropylene cages at 25 ± 5^{o}C with 12 hr light and dark cycle and acclimatized for 1 week before the start of the experiment. The animals were fed on standard pellet diet with water *ad libitum.* The study has been approved by Institutional animal ethics committee (1448/PO/Re/S/11/CPCSEA/07/2016).

C. Plant material:

The unripe fruits of *M.paradisica* were collected from the local market. The fruits were shade dried, powdered and stored till use. The dried powdered were subjected to soxhlation with ethanol for 72hrs. The resultant extract was evaporated to dryness and stored at 4^{o}C till use. The fruit material was authenticated by taxonomist, Department of Botany, S V University, Tirupati with voucher no 1003. The dried extract was re-suspended in normal saline and subjected for acute toxicity studies by fixed dose method of OECD guideline 420. The LD 50 was found to be more than 2000 mg/kg and 1/10th and 1/5th dose of LD50 was used in the study.

D. Study protocol:

Animals were randomly divided into groups consisting of 6 animals each. Group I considered as control group received normal saline orally throughout the study. Group II, disease control group received a single dose of 20% of L-arginine Hcl [2x2.5 g/kg, intraperitonially (i.p), 1 hour apart] in 0.5% normal saline on day 5 of the study. Group III served as positive control and administered Melatonin (10 mg/kg); Groups IV and V treatment groups received 200 and 400 mg/kg of *M.paradisica* fruit extract (MPFE) orally for 7 days. On day 5 the positive control and treatment groups received a single dose of 20% of L-arginine Hcl in 0.5% normal saline 1 hr after the administration of the extracts. At the end of the study on day 7, blood samples were collected from retro-orbital plexus for the enzyme estimations. The animals were sacrificed, pancreas were isolated and subjected for tissue enzyme estimation, histopathological study and DNA fragmentation assay.

E. Biochemical enzyme estimations:

Serum amylase, C-reactive protein (CRP) and lipase were estimated by the use of commercial kits from Akray healthcare pvt ltd and Aggape diagnostics ltd. respectively.

Briefly, serum amylase was estimated by mixing 20µl of serum with 1000µl of amylase mono reagent and the absorbance was read at 405nm after 60 seconds.

Serum lipase was measured by taking 20µl of the serum and to it 1000µl of reagent 1 was added and incubated it for 1-5 minutes at 37^{o}C. 250µl of reagent 2 was added and incubated it for another 2 minutes. The resultant solution was checked for absorbance at 580nm.

CRP was estimated quantitatively by preparing a series of dilutions of the test serum in normal saline (eg: 1:2, 1:4, 1:8 etc) and to which one drop of CRP latex reagent was added. The formation of agglutination on the glass slide was taken as the highest titre for CRP and represented as factor of 6 with units in micrograms /ml.

Antioxidant enzymes superoxide dismutase (SOD), was measured by reported method according to Misra and Fridovich, 1977 [16], Catalase estimated as per the method by Beers and Sizer, 1952 [17] and Vitamin C levels were estimated by method reported by Omaye et al., 1979 [18].

Tissue enzyme estimations were estimated by homogenizing a part of the tissue and their supernatants were used for the estimation of Glutathione, Lactate dehydrogenase (LDH), Myeloperoxidase (MPO), Malonylaldehyde (MDA) and nitrite levels as per the reported methods [19], [20], [21], [22], [23].

F. DNA fragmentation Assay:

The DNA fragmentation assay was performed on SDS page gel electrophoresis. Briefly, the DNA from the pancreatic tissue was isolated by using the kit by Bioartis pvt ltd. The isolated DNA pellet is air dried and resuspended in TE buffer [pH-8.0] and 1mM EDTA. The resuspended DNA is loaded on to the SDS Page Gel electrophoresis for analysis [24].

G. Histopathological studies:

Paraffin sections of 5 µm were cut and stained with hematoxylin and eosin and then assessed under dark field microscope for histopathological changes. Pancreatic damage was assessed for acinar cell degeneration, interstitial inflammation, edema, and hemorrhage.

H. Statistical analysis:

All the values are expressed as Mean ± SEM. Statistical Analysis was performed by one way ANOVA followed by Dunett's Multiple Comparison Test using Graph pad Prism 5 software with statistically significance up to $p < 0.001$.

III. RESULTS

To evaluate the protective effect, rats were pretreated with MPFE as described in the experimental design. L arginine was used for inducing acute pancreatitis and few hours after the induction, blood and tissue samples were collected and subjected for enzymatic analysis.

Rats in the disease control group showed an increase in the levels of serum amylase and lipase significantly [$p<0.001$] when compared to control and melatonin treated rats indicating the induction of pancreatitis [Table I]. The levels of SOD, Catalase, Vitamin C and glutathione in disease control rats were significantly lower than the control group of rats [Table II]. However, the levels of MDA, MPO, CRP, Nitrite levels were higher indicating the involvement of inflammation [Table III].

In contrast with the disease control group, Melatonin and MPFE treated rats showed a significant protection against damage to pancreas in dose dependent manner. The serum amylase and lipase levels were significantly [$p<0.001$] lower than the disease group of rats but a little higher than the control group [Table I]. The levels of SOD, Catalase, Glutathione and Vitamin C were higher in the MPFE treated rats indicating the antioxidant activity of MPFE and Melatonin [Table II]. The levels of MPO, MDA, CRP, Nitrite were significantly [$p<0.001$] reduced in MPFE treated rats dose dependently and the same was observed in melatonin treated rats indicating their anti-inflammatory effects [Table III.]. Although the levels of enzymes in MPFE treated rats differ significantly when compared to control group of rats.

In addition to this, Histopathological studies were carried out on the isolated pancreatic tissue by using hematoxylin and eosin stain. Pancreas of rats treated with L-arginine showed necrosis of the tissue with vacuolar degeneration and infiltration of leucocytes, whereas control, melatonin and MPFE treated rats showed a normal echotexture with retained cell membrane and no vacuolar degeneration [Fig. 1]. Further, DNA Fragmentation assay was carried out for detection of

apoptosis. DNA of L-arginine group of rats showed extensive damage which is depicted as a smear pattern in the gel picture. In disparity to it the control group, melatonin and MPFE treated rats had intact DNA when compared to disease control [Fig.2]

IV. DISCUSSION

The present study established the protective effect of MPFE on L-arginine induced acute pancreatitis in rats. Acute pancreatitis induced by L-arginine is a dose dependent induction model with hyperamylasemia and the histopathological findings are similar to Human disease [25]. After the initial attack of pancreatitis, within 4-8 hrs the amylase and lipase levels were increased swiftly reaching its peak at 24hours [25]. This observation is consistent with the present study where L-arginine administration increased the levels of amylase and Lipase. Melatonin and MPFE administration reduced the levels of amylase and lipase eliciting its protective effects. *M.paradisica* has been reported to inhibit serum α-amylase, lipase in diabetic rats and has been suggested that the presence of of polyphenolic compounds could attribute to its inhibitory effect [26], [27].

Free radical generation and depression of antioxidant defense have been reported to be central mediators of pancreatic tissue damage in acute pancreatitis [28]. The generated oxygen and nitrogen free radicals at the early onset of the disease, damage the mitochondria, activate nitric oxide synthase and polymorphonuclear leucocytes there by triggering various inflammatory markers. Thus, oxidative and nitrosative stress, mark as a significant role in the pancreatic damage and extra organ manifestations [29]. Further, the reactive oxygen species directly target the phospholipids and increase the levels of MDA and MPO which are the byproducts of lipid peroxidation. In the current investigation, L-arginine increased the levels of MDA, MPO, Nitrate, CRP and LDH levels and reduced the levels of SOD, Glutathione, Catalase and Vitamin C enlightening its role in stimulating the oxidative stress. Melatonin, in contrast have reduced the oxidative stress levels and improved the levels of antioxidant enzymes and is in accord with the previous reports [30]. MPFE administration reversed the conditions by improving the levels of SOD, Catalase, Glutathione and Vitamin C which form a mutual supportive team in eradicating the free radicals which was observed as reduced levels of MDA, MPO, Nitrate, CRP and LDH levels. The results are in consistent with the previous reports on *M.paradisica* fruit for its antioxidant and anti-inflammatory properties [31],[32]. The activities were suggested due to the presence of vitamin C, Kaempferol, Myrecetin, Quercetin and rutin flavonoids as their major constituents [33].

In addition to these estimations, the study also explored the protective effects on apoptotic damage of pancreas by using DNA fragmentation assay. The presumed consequence of apoptosis was damage to mitochondria due to increased calcium uptake triggering the apoptotic processes [34], [35]. This is visualized as a fragmented DNA with a smear pattern on the gel electrophoresis in rats treated with L-arginine. In contrast to this, pancreas of control and MPFE treated rats showed an intact DNA eliciting its protective effects. Further, the histopathological findings also suggest a positive role of MPFE in eradicating the inflammation and maintaining the normal echotexture and integrity of the pancreas when compared to L-arginine treated group of rats.

V. CONCLUSION

The present investigation concludes that MPFE administration has protective effects against L-arginine induced pancreatitis and it may be due to its antioxidative and anti-inflammatory effects. However, there is a need to explore in depth molecular mechanism for its ability as a new supplementation in acute pancreatitis.

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TABLE I: CHANGES IN THE LEVELS OF AMYLASE AND LIPASE IN RATS TREATED WITH L-ARGININE AND DIFFERENT DOSES OF THE MPFE EXTRACTS.

Serum Enzyme	Normal Control	Disease Control	Melatonin 10 mg/kg	MPFE 200 mg/kg	MPFE 400 mg/kg
Amylase (IU/L)	$14.17 \pm 0.116^{\#}$	$330.5 \pm 3.233^{*}$	$98.8 \pm 2.234^{*\#}$	$202.5 \pm 6.091^{*}$	$161.5 \pm 7.714^{*}$
Lipase (U/L)	$16.17 \pm 0.9804^{\#}$	$83.17 \pm 1.138^{*}$	$34.45 \pm 1.456^{*\#}$	$58.16 \pm 2.32^{*\#}$	$42.5 \pm 3.082^{*\#}$

Values are expressed in Mean ± SD (n=6); *, # p <0.001 when compared to Normal Control and Disease control group respectively.

TABLE II: CHANGES IN THE LEVELS OF ANTIOXIDANT ENZYMES IN RATS TREATED WITH L-ARGININE AND DIFFERENT DOSES OF THE MPFE EXTRACTS.

Antioxidant enzyme	Normal Control	Disease Control	Melatonin 10 mg/kg	MPFE 200 mg/kg	MPFE 400 mg/kg
SOD (mg/protein/min)	$18.5 \pm 0.4282^{\#}$	$6.167 \pm .3073^{*}$	$61.23 \pm 1.724^{\#*}$	$25.6 \pm 2.66^{*\#}$	$40.3 \pm 2.50^{*\#}$
Catalase (µM of H_2O_2/mg/protein /minute)	$48.18 \pm 0.3056^{\#}$	$22.7 \pm 0.2811^{*}$	$47.14 \pm 0.234^{\#}$	$36.16 \pm 1.17^{*}$	$41.83 \pm 2.04^{\#}$
Glutathione (mg/dl)	$2236 \pm 0.9574^{\#}$	$575.8 \pm 1.493^{*}$	$3124 \pm 3.549^{\#*}$	$2170.8 \pm 4.66^{\#}$	$2292.5 \pm 2.88^{\#}$
Vitamin C (mg/dl)	$95.73 \pm 0.3509^{\#}$	$21.35 \pm .5771^{*}$	$212.34 \pm 0.6784^{*\#}$	$181.66 \pm 0.876^{*\#}$	$228.8 \pm 1.812^{*\#}$

Values are expressed in Mean ± SD (n=6); *, # p<0.001 when compared to Normal Control and Disease control group respectively.

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TABLE III: CHANGES IN THE LEVELS OF TISSUE BIOMARKERS IN RATS TREATED WITH L-ARGININE AND DIFFERENT DOSES OF THE MPFE EXTRACTS.

Tissue marker	Normal Control	Disease Control	Melatonin 10 mg/kg	MPFE 200 mg/kg	MPFE 400 mg/kg
MDA (mM/dl/hr)	14.89± 0.3381$^{\#}$	86.87± 0.8747*	35.56 ± 0.9852$^{*\#}$	21.08±1.05$^{\#}$	14.53±0.99$^{\#}$
Nitrate (µM/g)	11.08 ± 0.212$^{\#}$	35.05 ± .1522*	14.34 ± 0.8765$^{\#}$	26.85±0.40$^{*\#}$	18.48±0.77$^{\#}$
CRP (µg/ml)	486.8± 2.358$^{\#}$	19601± 2.455*	697 ± 4.345$^{*\#}$	1596.6 ±4.92$^{*\#}$	1301±3.34$^{*\#}$
MPO (µM of peroxide/min)	4.032± 0.05486$^{\#}$	25.69± 0.3327*	6.45 ± 0.4563$^{\#}$	11.97 ± 0.2828$^{\#}$	7.902 ±0.2765$^{\#}$
LDH (U/L)	23.4± 0.5379$^{\#}$	127.5± 0.3785*	45.56 ± 0.8976$^{*\#}$	95.75±1.127$^{*\#}$	71.86±2.55$^{*\#}$

Values are expressed in Mean ± SD (n=6); *, $^{\#}$ p<0.001 when compared to Normal Control and Disease control group respectively.

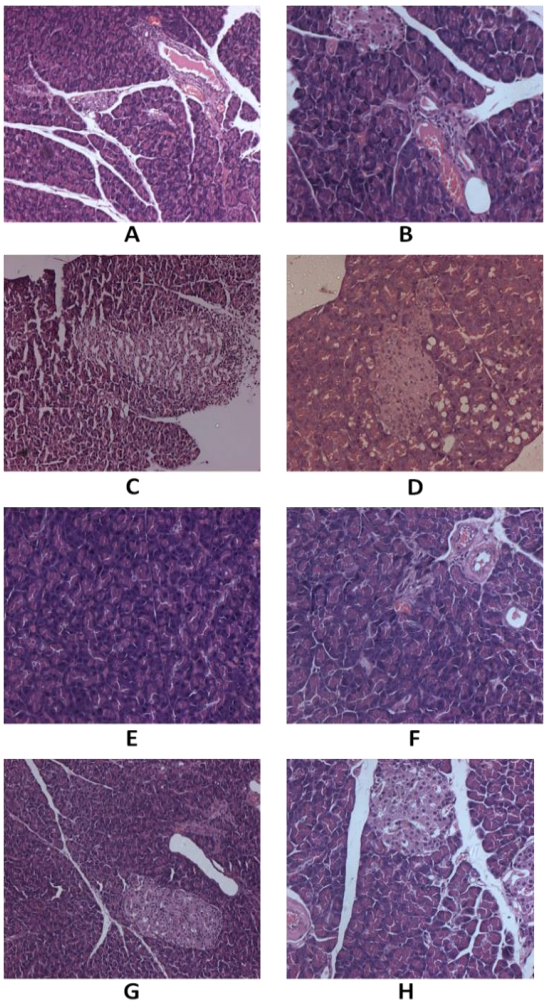

Fig 1: Histopathologgical changes in Pancreas of rats treated with L-arginine and MPFE extracts

Fig. 1. Histopathological findings of formalin fixed paraffin embedded section of pancreas with Hematoxylin and Eosin. (A) Photomicrograph of the normal Pancreas (B) L-arginine induced pancreas showing extensive acinar cell damage with interstitial edema, vacuolar degeneration. (C & D) protective effect of MPFE. Sections show normal acinar cells as that of Normal pancreas.

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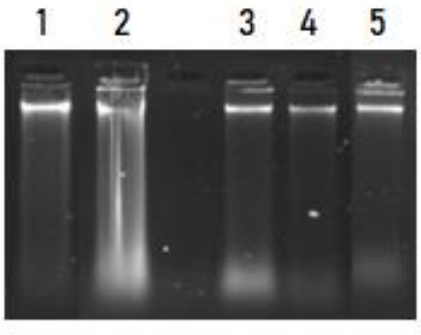

Fig 2: Gel picture of DNA fragmentation Assay

Figure 2: Gel picture of DNA fragmentation assay of the pancreatic tissue.

1 – Group I; Control group pancreas showing intact DNA

2 – Group II; Disease control pancreas treated with L-arginine showing fragmented DNA in the form of smear indicating extensive damage;

3, 4- Group IV & V; MPFE treated pancreas showing intact DNA eliciting its protective effect

5 – Group III; Melatonin treated pancreas showing intact DNA