



PHARMACOLOGICAL EVALUATION OF METHANOLIC EXTRACTION OF FRUITS OF *MOMORDICA DIOICA* ON L-ARGININE INDUCED PANCREATITIS IN RATS

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Abstract:

Background: Herbal drugs play an important role in health care, especially in developing countries. *Momordica (M.) dioica* (family Cucurbitaceae) is a climbing creeper and reported as an important medicinal herb since ancient times for various conditions. In India, fruits of *M. dioica* are used as a folk remedy for Diabetes mellitus. **Objective:** In this study, methanolic extract of *M. dioica* (MtEMD) fruits was investigated for its protective effects against L-Arginine (Arg)-induced pancreatitis in rats. **Methods:** Normal control group received vehicle orally (p.o), daily, low-dose and high dose test groups was treated with MtEMD daily (p.o) at 250 mg/kg and 500 mg/kg respectively. Standard group received 30mg/kg Methylprednisolone (MP), (p.o), daily. The treatment period was 21 days. Acute pancreatitis (AP) was induced on the 18th day of the treatment by two intraperitoneally (i.p.) injections of 250 mg/100 g body wt. of Arg as a 20% solution in 0.15 M saline at an interval of 1 h. Protective effects of MtEMD on Arg-induced pancreatitis in rats were determined for biochemical parameter of serum, various antioxidant enzymes, and histopathological damages in pancreas. **Results:** MtEMD-pretreated groups showed dose dependent significant ($p < 0.05$) reduction in pancreatic nitrate and lipid peroxidation (LPO) levels compared to disease group. MtEMD pretreatment also resulted a significant ($p < 0.05$) increase in pancreatic contents of protein, GSH concentration, and activity of superoxide dismutase (SOD) and catalase (CAT) with amelioration of histological changes of test groups as well as standard group. **Conclusion:** MtEMD pretreatment results in a protective effect comparable with that of MP pretreatment in AP rat model.

Keywords: *Momordica dioica*, Acute Pancreatitis, Arginine-Induced Pancreatitis.

INTRODUCTION

Pancreatic functions are essential for life. It secretes digestive enzymes and produces critical hormones to regulate glucose homeostasis.¹ Acute pancreatitis (AP) is an inflammatory disease of the pancreas that can be life-threatening. AP is associated with substantial morbidity and mortality; although most cases are mild, around 20-30% can be severe, associated with failure of single or multiple organs.^{2,3} Moreover, about 15-25% of patients with AP develop pancreatic necrosis, one of the most serious complications of AP, with a mortality rate of 20 to 30%.⁴ Additionally, the treatment of AP is essentially supportive and symptomatic; treatment options include fluids resuscitation, nutritional support, pain control, and treatment of the underlying cause.^{2,5} This demonstrates the importance of a healthy lifestyle and the need for protective options against AP.

Herbal drugs continue to play an important role in health care and drug development, especially in developing countries. Several modern drugs currently in use have been developed by researching medicinal plants. They also provide a source and food and nutrition. *Momordica dioica* is a perennial climbing creeper, reported as an important medicinal herb since ancient times to treat various conditions. In Indian folk medicine, fruits, leaves, and tuberous roots of *M. dioica* are used as a remedy for diabetes.^{6,7} Various studies have evaluated the pharmacological effects of this plant. Talukdar et al.⁸ reviewed various, previously reported, pharmacological activities of *M. dioica* such as antioxidant,⁹⁻¹² analgesic,^{13,14} nephroprotective,^{6,15,16} hepatoprotective,⁹ neuroprotective,¹⁷ antimalarial,¹⁸ anti-allergic,^{19,20} anti-ulcer,^{21,22} anticancer,²³ antimicrobial.^{24,25} This study aimed to investigate the protective effects of *M. dioica* fruits extract against L-Arginine (Arg)-induced pancreatitis in rats.

Arg-induced pancreatitis is one of the most commonly used animal models to study the biochemical and histological alterations of AP. Since the observations of Mizunuma et al.²⁶, Kishino et al.²⁷, and Tani et al.²⁸, this model has been used in different laboratories. The dose and frequency of administration have varied. The different doses and frequencies of administration of Arg in rat models evaluated by different researchers have been reviewed by Hegyi et al.²⁹ and summarized by Dawra et al.³⁰ The mechanism by which Arg causes pancreatitis is not fully known. Accumulating evidence suggests that generation of oxygen³¹⁻³⁴ and nitrogen³⁵ free radicals and increased levels of inflammatory mediators³⁶⁻³⁹ all have a key role in the development of AP.

The present study was conducted to investigate and compare the possible protective role of the methanolic extract of *M. dioica* (MtEMD) fruits versus methylprednisolone (MP) against Arg-induced AP in rats. MP, a commonly used steroid for symptomatic treatment of AP,⁴⁰ was used as a reference anti-inflammatory drug.⁴¹

MATERIALS AND METHODS

Plant Material and Extraction

The fruits of *M. dioica* used in this study were procured locally during June month, Bangalore, Karnataka, and were identified by Botanist Dr. Geetanjali department of botany, Sree Siddaganga College, Tumkur University (Reference No: 141/17-18). The fruits were chopped, shade dried, powdered, and sieved. Each 100 g powder was subjected to extraction with 1 liter methanol in a reflux condenser for 3 cycles, 7 hr. each, till the volume was reduced to half. The extract was filtered and evaporated to get constant weight.

Animals

30 Wistar Albino rats weighing 150-180 gm were maintained in standard laboratory conditions at room temperature (25±2 °C) with a 12-hr light/12-hr dark cycle free access to diet and water. The study protocol was duly approved by the Institutional Animal Ethics Committee at Karnataka College of Pharmacy, Bangalore (Reg No: 1564/PO/Re/S/11/CPCSEA).

Acute Oral Toxicity

Acute toxicity studies already exist.^{42,43} R. Singh et al.⁴⁴ evaluated the lethality of the aqueous extract of *M. dioica* to be above 3000 mg/kg. Jha DK et al.⁴⁵ recently examined the toxicity of Saponin isolate of MtEMD in rats and reported no mortality up to 5000 mg/kg body wt. In the present study we investigated the effect of 250 mg/kg and 500 mg/kg of MtEMD.

Induction of Acute Pancreatitis

AP was induced by two i.p. injections of 250 mg/100 g body wt.^{32-37,46,47} of Arg as a 20% solution in 0.15 M saline^{32,33,37,47} at an interval of 1 h, on the 18th day of the study; around 72 hrs. before sacrificing the animals, so peak histological changes could be observed.³⁰

Experimental Design

Animal were divided into five groups: normal control, disease control (AP group), standard (MP group), low dose test, and high dose test group. The experimental procedure is summarized in (Table 1).

N o.	Group	Treatment
I	Normal control group	Vehicle p.o. daily for 21 days.
II	Disease control group (AP group)	AP induction: Arg (2×250 mg/100 g) i.p. on 18 th day of the study.
III	Standard group (MP group)	Methylprednisolone (MP) 30mg/kg ⁴¹ p.o. daily for 21 days. + AP induction
IV	Low-dose test group	MtEMD 250 mg/kg p.o. for 21 days + AP induction
V	High-dose test group	MtEMD 500 mg/kg p.o. for 21 days + AP induction

Table 1: Experimental Protocol.

Sample Collection

At the end of the study rats were sacrificed under ether anesthesia. Blood was taken from the ventricle of the heart. Pancreas was isolated and divided into two portions; one portion was homogenized to be used for biochemical analysis; the other portion was preserved in 10% formaldehyde for histological examination.

Determination of serum α -amylase and lipase activities

Serum amylase and lipase activities were estimated with spectrophotometric techniques by semi auto analyzer using commercial kits.

Determination of pancreatic total protein

Total protein was determined in pancreatic tissue according to the method described by Lowry et al.⁴⁸ as modified by Hartree.⁴⁹

Determination of total nitrite levels (Pancreatic total nitrite)

Pancreatic total nitrite was assayed by colorimetric method using Griess reagent which was prepared by mixing (0.1%) N-(1-naphthyl)-ethylenediamine and (2%) sulfanilamide in a ratio of 1:1 v/v. The absorbance was measured at 540 nm and the results were expressed as nmol/mg protein.⁵⁰

Determination of lipid peroxidation (LPO)

The pancreatic tissue was homogenized in TCA and then used to estimate malondialdehyde (MDA). LPO was induced by adding 10 μ l, 400 mM ferric chloride and 10 μ l l-ascorbic acid (400 mM) to a mixture containing 0.3 ml tissue homogenate in 5 ml phosphate buffer saline (0.2 M, pH 7.4). After incubation for 1 hr. at 37°C, the reaction was stopped by adding 2 ml HCl (0.25 N) containing 1 ml TCA (15% w/v) and 0.5 ml TBA (0.375% w/v) boiled for 15 min, cooled, and centrifuged, and the absorbance was measured at 532 nm. The results were expressed as nmol MDA/g protein.^{51,52}

Determination of Reduced Glutathione (GSH)

GSH was determined utilizing Ellman reagent.^{53,54} Equal volumes of tissue homogenate and 10% trichloroacetic acid (TCA) were mixed and centrifuged at 750 g for 5 mins. 0.1 ml of supernatant was mixed with 1.7 ml of 0.1 M potassium phosphate buffer pH 8. Then 0.1 Ellman's reagent was added and the absorbance was measured at 412 nm against blank

Determination of superoxide dismutase (SOD)

100 μ L of EDTA (1 mM) and 20 μ L tissue supernatant were added to 2.78 ml sodium carbonate buffer (0.05 mM, pH 10.2), and then incubated at 30°C for 45 min. 100 μ L of adrenaline was to initiate the reaction. The change in absorbance was recorded at 480 nm for 3 min. Sucrose was used as a blank. The activity of SOD was expressed as U/mg of protein.⁵⁵

Determination of catalase (CAT)

100 μ L of supernatant was added to 1.9 mL phosphate buffered saline (pH 7.0). To this, 1 mL of H₂O₂ was added and the change in the absorbance was recorded at 240 nm for 3 min. The values were expressed as μ M/ H₂O₂/min/mg of protein.⁵⁶

Histopathological Analysis

Pancreatic tissues were collected and washed with the normal saline and placed in 10% neutral formalin for 24 hrs. The tissues were then dehydrated and cleared with ethanol and xylene, respectively. The samples were embedded in paraffin wax from which blocks were prepared. Sections of 5 μm were prepared a microtome. Sections were stained with hematoxylin and eosin (H&E) and examined using a light microscope.⁵⁷

Statistical analysis

The results are expressed as Mean \pm S.E.M. from n=6 rats in each group. Data were analyzed using statistical software Graph Pad Prism version 9. The significance of difference among the groups was assessed using one-way analysis of variance (ANOVA) followed by Tukey's test compared between Normal control (Untreated) Vs all groups $p < 0.05$ were considered significant.

RESULTS

Effect on Serum α -amylase and lipase activities

Disease group significantly raised serum α -amylase activity by 129.6% (372.7 ± 0.88 U/L) compared to control group (162.3 ± 0.42 U/L). On the contrary, pretreatment with *M. dioica* significantly reduced α -amylase activity by 21.7% (291.5 ± 0.76) for low dose, and by 31.9 % (253.6 ± 0.71) for high dose, compared to disease group. In addition, MP showed a significant reduction in α -amylase activity by 18.6% (303.2 ± 1.3) compared to disease group (Figure 1.a). Similar effect was observed with serum lipase activity. AP significantly raised lipase activity by 342.5% (285.4 ± 8.60 U/L) compared to control group (64.50 ± 2.49 U/L). *M. dioica* pretreatment, significantly reduced lipase activity by 33.6% (189.4 ± 2.97) for low dose, and by 56.1% (125.1 ± 4.03) for high dose, compared to disease group. MP showed a significant reduction in lipase activity by 43.2% (162.2 ± 3.09) compared to disease group (Figure 1.b).

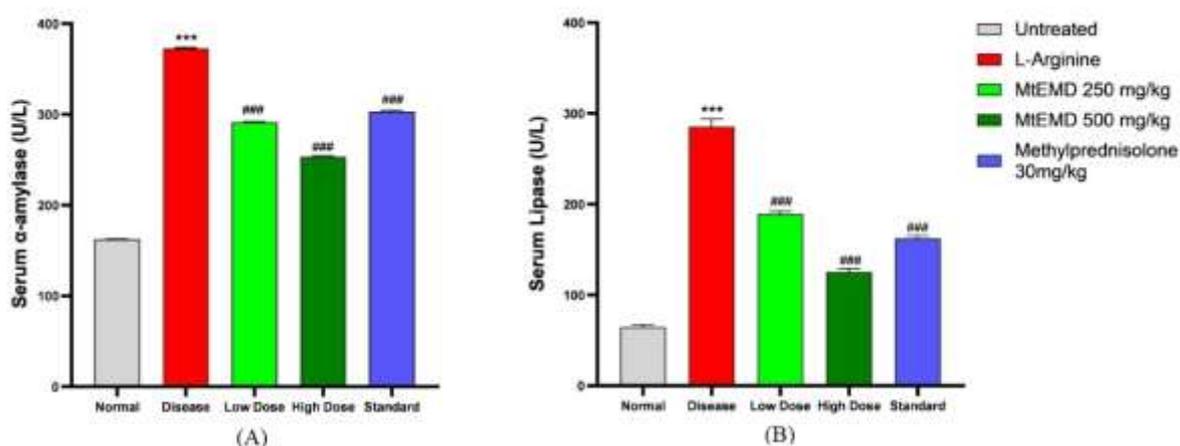


Figure 1: (a) Serum α -amylase activity in studied rat groups. (b) Serum lipase activity in studied rat groups.

*** Significant ($p < 0.05$) versus Normal control

significant ($p < 0.05$) versus disease control.

Effect on total pancreatic total protein

In AP group, pancreatic content of protein (24.94 ± 1.21 $\mu\text{g/ml}$ protein) was significantly reduced compared to normal group (52.58 ± 0.65 $\mu\text{g/ml}$ protein). Compared to disease group, all treatment groups showed significantly higher pancreatic total protein; 33.28 ± 0.87 $\mu\text{g/ml}$ protein for low-dose test group, 47.20 ± 1.39 $\mu\text{g/ml}$ protein for high-dose test group, and 50.43 ± 0.48 $\mu\text{g/ml}$ protein for standard group (Figure 2).

Effect on pancreatic total nitrate

Induction of pancreatitis resulted in a significant raise in nitrate levels (4.38 ± 0.12 nmol/mg protein) compared to control group (1.267 ± 0.08 nmol/mg protein). MP pretreated group showed significantly reduced levels (1.487 ± 0.06 nmol/mg protein) compared to disease group. Pretreatment with MtEMD dose-dependently reversed the change in nitrate levels; 1.747 ± 0.19 in low dose group and 1.398 ± 0.09 in high dose (Figure 3).

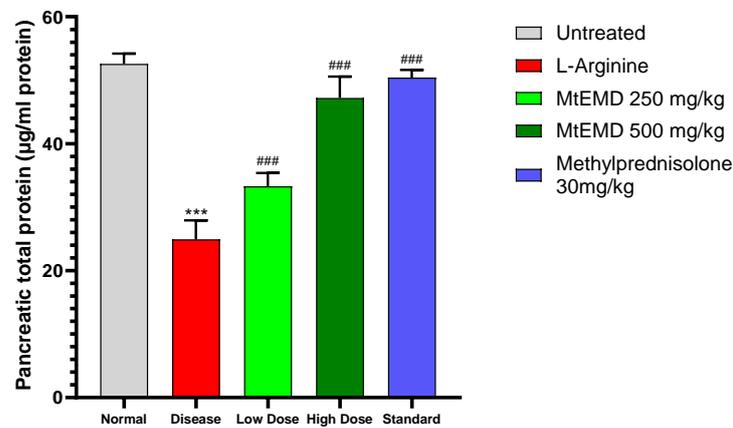


Figure 2: Pancreatic total protein in studied groups.
 *** Significant ($p < 0.05$) versus normal control.
 ### significant ($p < 0.05$) versus disease control.

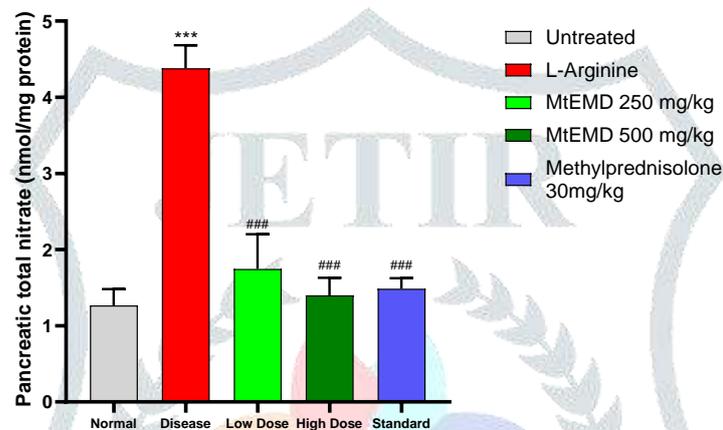


Figure 3: Pancreatic total nitrate in studied groups.
 *** Significant ($p < 0.05$) versus normal control.
 ### significant ($p < 0.05$) versus disease control.

Effect on lipid peroxidation (LPO)

The pancreatic tissue concentration of MDA was significantly elevated in disease group by 182.3% (3.07 ± 0.18 nmol MDA/g protein) compared to the control group (1.08 ± 0.065 nmol MDA/g protein). *M. dioica* pretreatment appeared to prevent the elevation in MDA activity showing significantly lower pancreatic tissue concentration of MDA compared to disease group; (1.39 ± 0.06 nmol MDA/g protein) in low dose group and (1.26 ± 0.03 nmol MDA/g protein) in high dose group. Similarly, MP pretreatment resulted in significantly lower levels of MDA (1.23 ± 0.048 nmol MDA/g protein) compared to disease group (Figure 4).

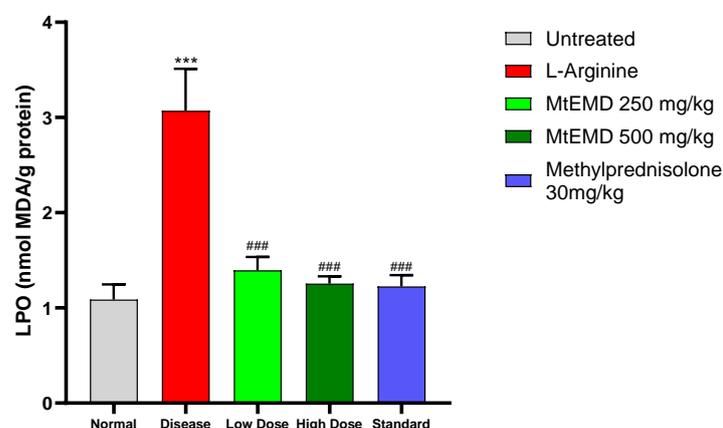


Figure 4: MDA concentration in pancreas tissue in studied rat groups.
 *** Significant ($p < 0.05$) versus normal control.
 ### significant ($p < 0.05$) versus disease control.

Effect on reduced glutathione (GSH)

L-arginine significantly decreased the pancreatic content of reduced glutathione ($1.039 \pm 0.049 \mu\text{mol/g tissue}$) by 63.2% compared to control group ($2.826 \pm 0.13 \mu\text{mol/g tissue}$). MP and low-dose MtEMD pretreated showed a similar effect. Both significantly increased the increased the pancreatic content of reduced glutathione by 45% (1.508 ± 0.033) and 56% (1.624 ± 0.029) respectively. Pretreatment with high dose of MtEMD showed a stronger effect, significantly increasing GSH by 109.6% (2.178 ± 0.06) compared to disease group (Table 2).

Effect on superoxide dismutase (SOD) activity

The disease group showed a significantly reduced activity of SOD ($30.01 \pm 0.73 \text{ U/mg}$), about 58% less, compared to the control group ($71.53 \pm 0.69 \text{ U/mg}$). The groups pretreated with *M. dioica* showed significantly increased SOD activity, in dose dependent-manner, compared to the disease group (Table 2). SOD activity in low-dose test group was 53% (45.93 ± 0.97) higher than that of the disease group. The high-dose test group showed a 113.8% (64.15 ± 0.85) higher activity compared to the disease group. MP pretreatment showed the highest effect, with SOD activity 152.5% (75.77 ± 0.10) higher than that of the AP group.

Effect on Catalase (CAT) activity

The activity of CAT in pancreas of AP group was significantly decreased ($6.01 \pm 0.71 \mu\text{M H}_2\text{O}_2/\text{min/mg of protein}$) compared to control group ($21.92 \pm 0.71 \mu\text{M H}_2\text{O}_2/\text{min/mg of protein}$). MP-pretreated group showed a significantly higher CAT activity ($19.54 \pm 0.61 \mu\text{M H}_2\text{O}_2/\text{min/mg of protein}$) than that of the disease group. MtEMD pretreatment significantly increased the enzyme activity in a dose-dependent fashion, 11.09 ± 0.95 in low-dose group and 18.36 ± 0.80 in high-dose group, as compared disease group diabetic rats (Table 2).

	GSH ($\mu\text{mol/g tissue}$)	SOD (U/mg)	CAT ($\mu\text{M H}_2\text{O}_2/\text{min/mg of protein}$)
Normal Control (Untreated)	2.826 ± 0.13	71.53 ± 0.69	21.92 ± 0.71
Disease Control (L-Arginine)	$1.039 \pm 0.049^{***}$	$30.01 \pm 0.73^{***}$	$6.01 \pm 0.71^{***}$
Low Dose (MtEMD 250 mg/kg)	$1.624 \pm 0.029^{###}$	$45.93 \pm 0.97^{###}$	$11.09 \pm 0.95^{###}$
High Dose (MtEMD 500 mg/kg)	$2.178 \pm 0.06^{###}$	$64.15 \pm 0.85^{###}$	$18.36 \pm 0.80^{###}$
STANDARD (METHYLPREDNISOLONE 30MG/KG)	$1.508 \pm 0.033^{###}$	$75.77 \pm 0.10^{###}$	$19.54 \pm 0.61^{###}$

Table 2: Effect of treatment on pancreatic MPO activity, GSH, and CAT concentrations in studied rat groups.

*** Significant ($p < 0.05$) versus normal control.

significant ($p < 0.05$) versus disease control.

Effect on Pancreatic Histopathology

Histological examination of pancreatic sections from control group showed the normal histological structure of pancreas, normal island of Langerhans with abundant basophilic cytoplasm. (Figure 5.a). On the contrary, sections from AP group showed atrophy of the beta cells; The beta cell cytoplasm was scanty and marked inflammatory cells infiltration were seen. (Figure 5.b). Pretreatment with low-dose MtEMD showed atrophied beta cells with scanty basophilic cytoplasm and no inflammatory cells were seen (Figure 5.c). Tissue collected from group pretreated with high-dose MtEMD showed normal beta cells with basophilic cytoplasm and no inflammatory cells (Figure 5.d). Moreover, pancreatic sections from MP group showed numerous beta cells with abundant basophilic cytoplasm and no inflammatory cells infiltration were seen (Figure 5.e).

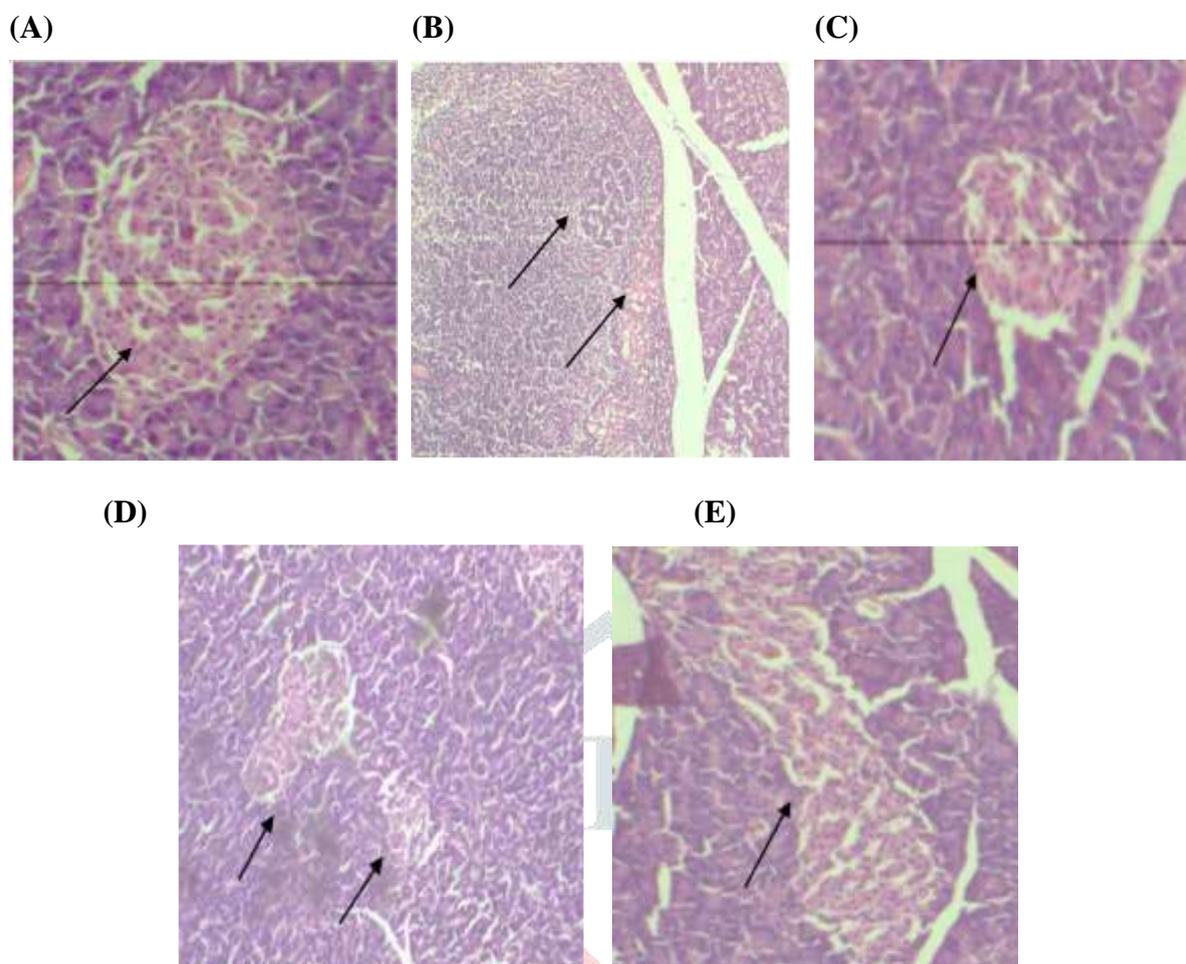


Figure 5: Histological Findings. (a) Control group normal showing histological structure of pancreas, normal island of Langerhans with abundant basophilic cytoplasm. (b) Disease group showing atrophy of the beta cells and marked inflammatory cells infiltration. (c) Low-dose test group showing atrophied beta cells with scanty basophilic cytoplasm and no inflammatory cells (d) High-dose test group showing normal beta cells and no inflammatory cells (e) Standard group showing numerous beta cells with abundant basophilic cytoplasm and no inflammatory infiltration

DISCUSSION

Acute pancreatitis (AP) is an inflammatory disease of the pancreas that can be life-threatening. The treatment of AP is essentially supportive and symptomatic; treatment options include fluids resuscitation, nutritional support, pain control, and treatment of the underlying cause. This study, was conducted to investigate methanolic extract of *M. dioica* (MtEMD) fruits for its protective effects against L-Arginine (Arg)-induced pancreatitis in rats.

In the present study, histological examination of pancreatic sections from animals in disease group, atrophy of the beta cells and marked inflammatory cells infiltration were seen. This may be attributed Arg which can induce oxidative stress that increases vascular permeability resulting in pancreatic edema and cellular damage.³⁰ On the contrary, pretreatment with MtEMD or MP significantly reduced pancreatic damage. The protective effects of MtEMD and MP were confirmed by histopathological findings; improvements in histopathological alteration of pancreatic cells were seen.

In the current study, AP group showed a significant increase in amylase and lipase activities, which are important biomarkers in the diagnosis of AP, whereas pretreatment with MtEMD and MP significantly reduced serum amylase and lipase levels.

In AP group, pancreatic content of protein was significantly reduced compared to normal group. Compared to disease group, MtEMD- and MP-treated groups showed significantly higher pancreatic total protein. Induction of pancreatitis also resulted in a significant raise in nitrate levels. MP pretreated group showed significantly reduced levels compared to disease group. Pretreatment with MtEMD dose-dependently reversed the change in nitrate levels.

Peroxidation of membrane lipids releases toxic byproducts such as MDA,⁵⁸ which in turn leads to systemic inflammatory response as a final consequence.⁵⁹ MDA is directly associated with tissue damage and organ failure in AP.⁶⁰ In our study, the pancreatic tissue concentration of MDA was significantly elevated in disease group. MP and *M. dioica* pretreatment appeared to prevent the elevation in MDA activity showing significantly lower pancreatic tissue concentration of MDA.

The antioxidant defense consists of SOD, CAT, and GSH.⁵⁹ Reduced antioxidants levels in pancreas is suggestive of oxidative stress at tissue as well as systemic levels in AP.⁶⁰ The present study revealed that AP induction with Arg significantly reduced pancreatic GSH concentration. GSH depletion is a characteristic of the initial phase of AP pathogenesis and assumed to allow the premature activation of digestive enzymes within pancreatic acinar cells triggering the inflammatory process.⁶¹ MtEMD- and MP-pretreated groups showed significantly elevated pancreatic GSH concentration compared to disease group. Similarly, the disease group show a significant decline in SOD and CAT levels, while MtEMD- and MP- pretreated groups showed and a significantly higher levels as compared to AP group. These results suggest that *M. dioica* indeed possess a potential pancreatic protective effect against acute pancreatitis.

CONCLUSION

The present study was carried out to investigate the protective effects of the methanolic extract of *M. dioica* fruits against L-Arginine -induced pancreatitis in rats. Based upon the results of the present study, we concluded that prior treatment with MtEMD provided significant and comparable protective effects to MP against Arg-induced AP.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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