Original article

Protective Effect of Nano-Moringa oleifera leaves Extract and/or Low Doses of γ- Irradiation on Acute Pancreatitis Induced in Rats Model

Gehad Farghly a,*, Neamat Hanafy b, Doaa gewily a, Mona El- Tonsy a, Nahed Farid a

a Zoology and Entomology Department, Faculty of Science AL-Azhar University (Girls)-Naser city-cairo.

bCell Biology& Histology National Center for Radiation Research and Technology Egyptian Atomic Energy Authority.

ARTICLE INFO

Received 28/05/2023
Revised 09/11/2023
Accepted 11/11/2023

Keywords

Nano-Moringa oleifera
Arg
Pancreatitis
IR

ABSTRACT

Advances in the field of nanotechnology and its applications to medicine and pharmaceuticals in phyto medicines are thought to have advantages over traditional medications and are regaining interest in current research. Yet, some studies have found that modest doses of -irradiation may activate the immune system. In this study, the impact of nano-Moringa oleifera (MO) leaves extract and/or low dosages of -irradiation on acute pancreatitis models produced in rats was studied. There were five groups of albino rats, each with ten rats. After one week of therapy, all animals were slaughtered. Acute pancreatitis resulted in a very substantial increase in lipase, amylase, Random Blood Sugar (RBS), and C-Reactive Protein (CRP) levels, as well as a considerable fall in insulin levels and Insulin growth factor 1. (IGF1). Histopathological examination of pancreatic tissue slices revealed a cluster of immune cells (lymphoid aggregation), haemorrhage, and necrotic acini. A significant reduction was observed after treatment with low doses of -irradiation and nano-Moringa leaves extract, either alone or combined, as compared to the positive control group (PC). On the contrary, normal tissue structure (normal islets of Langerhans and normal acini) was observed. In conclusion, nano-Moringa oleifera leaves extract and/or low doses of γ- irradiation has a therapeutic effect on acute pancreatitis model induced in rats.

Graphical abstract

* Corresponding author
E-mail address: GehadAllam.59@azhar.edu.eg; gehadfarghly88@gmail.com
DOI: 10.21608/IJTAR.2023.205193.1052

Special issue “Selected papers from the International Annual Conference on Basic and Applied Science (2nd ICBAS-2023)”
1. Introduction:

The pancreas' inflammatory state can range from moderate to severe, and is referred to as acute or chronic pancreatitis [1]. Pancreatitis is an inflammatory condition that produces perivascular infiltration and inflammation in the pancreas, culminating in lipid necrosis, polymorphonuclear leukocyte infiltration, haemorrhage, acinar cell necrosis, and tissue edema [2-3]. Phytopharmaceuticals are herbal medications whose effectiveness is attributed to one or more plant components or active elements. It can be used internally or externally to diagnose, treat, mitigate, or prevent any disease or ailment in humans or animals [4]. *Moringa oleifera*, Lam is a tree that grows abundantly in Asia and Africa's tropics and subtropics (Moringaceae). Asian village people have historically ingested its leaves [5]. *Moringa oleifera* includes numerous compounds that are of particular interest because of their therapeutic potential. Its leaves are rich in flavonoid pigments (kaempferol, rhamnose, kaempferitin, and isosoucirtrin) and antioxidants (ascorbic acid, flavonoids, phenols, and carotenoids) [6] and may help with anti-inflammatory activities [7]. Yet, there have been few reports of nano-*Moringa oleifera* leaves polyphenols extract improving and alleviating inflammation. Radiation damage is produced by oxidative stress, which is caused by an abundance of reactive oxygen species (ROS), which disrupts membrane lipids and leads to the generation of peroxide radicals. [8]. Many studies have found that modest doses of ionizing radiation from natural sources [9] or in professional radiation workers [10] can activate the immune system and enhance the activity of its effectors, a phenomenon known as "Radiation Hormesis." The purpose of this study was to find out if nano-*Moringa oleifera* leaves extract and/or modest doses of -irradiation may protect rats from an acute pancreatitis model.

2. Materials and Methods

Experimental animals

The experimental animals were fifty mature male albino rats weighing 180-200g obtained from the Egyptian Group Companies for Biological Items and Vaccines (Cairo-Helwan, Egypt). The rodents were housed in specifically built cages, 10 rats per cage, and given standard rodent pellets for one week prior to the experimental procedure. Throughout the trial, food and drink were available at all times during the experiment. The research ethics committee (REC) authorized this study procedure, which is planned and administered in compliance with the CIOMS and ICLAS International Guiding Standards for Biomedical Research Regarding Animals 2012. [11].

Radiation Facility

The gamma cell-40 irradiation was carried out by Egypt’s National Center for Radiation Research and Technology (NCRRT), Cairo. Atomic Energy of Canada Ltd manufactures the gamma cell-40, a cesium-1 irradiation device. According to Frey et al., [12] experimental animals were given 0.25 Gy x2 /week for two weeks at a dose rate of 0.423 Gy/min, computed using the NCRRT’s Dosimetry Department recommendations at the time of the experiment.

L-arginine monohydrochloride

L-arginine monohydrochloride (CAS No. 53308-83-1) was purchased from Sigma Chemical Company (Sigma, USA). Reagents with a grade of greater than 95% (pure).

Pancreatitis models (induction of pancreatitis)

L-arginine was given to the animals as a repeated intraperitoneal injection at a rate of 250 mg/rat twice at one-hour intervals, day after day for 10 days to produce acute pancreatitis [12-13]. L-arginine-HCl was diluted in saline and its pH was adjusted with NaOH to 7.4. Before each experiment, a new L-arginine solution was prepared [13].

Preparation of Nano-Moringa:

Plant material

The Egyptian Scientific Society of *Moringa* (ESSM), National Research Centre, Dokki, Giza, Egypt, provided the aqueous extract of *Moringa oleifera* leaves (MOL).

Preparation of *Moringa* leaves extract-loaded PLGA-PEG nanoparticles

According to Abd-Rabou et al. [14], the process producing nanoparticles was slightly modified. An initial emulsion was generated by dissolving 100 mg of PLGA polymer in 3 ml of chloroform to produce poly D, L-lactide-co-glycolide (PLGA) nanoparticles. An O/W emulsion was created in an aqueous polyvinyl alcohol (PVA) solution (12 ml, 2% w/v) using a microtip probe sonicator (VC 505, Vibracell Sonics, Newton, USA).

To obtain PLGA-PEG nano-void, three distinct nano-formulations of polyethylene glycol-blended PLGA (PLGA-PEG) were made with three different ratios of PLGA-PEG (1:2, 2:1, 1:1), then added to the watery PVA solution before emulsification with the PLGA polymer. The emulsion was agitated for eight hours to allow the organic solvent to evaporate. The following day, an excess of PVA was removed by ultracentrifugation at 50,602g at 4 °C for 20 minutes (Sorvall Ultraspeed Centrifuge, USA), followed by two washes with double distilled water.

*Moringa* leaf extract (ML)-encapsulated PLGA-PEG nanoparticles (MLn) were similarly created for medicinal applications by adding a certain concentration before emulsification.

Analysis of particle size and zeta potential

Photon correlation spectroscopy (PCS) was used to assess the particle size and zeta potential of the PLGA-PEG NPs using a Zeta Sizer (Nano ZS, Malvern Instruments, UK) and a red laser with a wavelength of λ=633 nm (He–Ne, 4.0 Mw). 1 mg of NPs was dissolved in 1 ml of water, which was then diluted 10 times with...
water and monitored for at least 120 seconds. Likewise, for zeta potential measurements, the samples were put in an electrophoretic cell with a 150 mV potential set. The temperature of the nano composites was kept constant at 25.00±1°C.

Transmission electron microscope (TEM)
Transmission electron microscope was employed to examine the particle morphology of the NPs (TEM, Philips CM-10, FEI Inc., and Hillsboro, OR, USA). After dropping 100 g/ml of the nano-suspensions into Formvar-coated copper grids, the samples were stained with 2% w/v uranyl (Electron Microscopy Services, Ft. Washington, PA). Digital Micrograph and Soft Imaging Viewer Software were utilized for image acquisition and analysis.

Experimental design
The adult male albino rats in the experiment were divided into five groups (n=10) using the following scheme:

G1 (negative control group) untreated normal animals. G2 (positive control group): animals were administered 7 intraperitoneal injections twice at 1h intervals with L-arginine (250mg/100g) to induce acute pancreatitis [15]. G3 (nano-moringa oleifera treated group): The positive control animals were given a daily dose of nano-moringa oleifera leaves extract (50 mg/kg/day) for 14 days. G4 (gamma-irradiated IR group): for two weeks, positive control animals were exposed to 0.25 Gy x2/week. G5 (nano-moringa oleifera + IR treated group): the positive control animals were treated with nano-moringa oleifera leaves extract (50 mg/Kg/day) daily for 14 days and were exposed to 0.25 Gy x2/week/2weeks.

To avoid excitation, all animals were sacrificed one week after treatment by exsanguination under light ether anesthesia after being gently removed from their cages. Blood samples were collected in sterile heparinized syringes and centrifuged at 3000 rpm for 10 minutes to separate the plasma for further biochemical analyses. The entire pancreas of all animals was excised as r separate the plasma for further biochemical analyses. The entire pancreas of all animals was excised as rapidly and rinsed with isotonic ice-cold saline before being fixed in 10% neutralized formalin and embedded in paraffin wax. Sections (5 m) were cut using a microtome, collected on glass slides with a polylime, and stained with hematoxylin and eosin stains (H & E) for histological investigation [19].

Statistical analysis
All statistical analyses were performed using the statistics package for Windows Version 15.0. (SPSS Software, Chicago, IL). The findings for continuous variables were provided as average standard error. One-way analysis of variance (ANOVA) was performed to compare the values and p values less than 0.05 were regarded as statistically significant.

3. Results
1- Characterization of nano-Moringa particles:
Measurements of particle size and zeta potential. By changing the PEG concentrations that coated the PLGA nanoparticles, three distinct nanocomposites of Moringa leaves (MLn) were created. The MLn (formation 1; F1) possesses nano-size (190.137± 6.5 nm; Figure. 1), nano-stability with zeta potential (-13.23±3.32 mV and polydispersity index (PDI)= 0.5± 0.02), and well-fitted association data (Figure 1, 2 and 3) as shown in table 1.

Examination of F3 nanoparticles by transmission electron microscopy (TEM) showed rounded particles containing a core and surrounding capsule.

B) TEM of F3 nanoparticles showed sizes ranging from 148.42 to 152.40 of selected particles.

2- The effect of nano-Moringa oleifera leaves extract and/or exposure to low doses of γ-radiation on rats treated with L-arginine (suffered from pancreatitis)
2-1- Biochemical results:
Table 2 showed the effect of nano-Moringa oleifera leaves extract and/or exposure to low doses of γ-radiation on plasma RBS, insulin levels, and insulin-like growth factor 1 (IGF1) levels.

Group 2 (PC) revealed a significant increase in RBS (232.67±3.35) in comparison with the control group (182.50±3.95). Meanwhile, a significant decrease in insulin (1.07±0.08) and IGF1(3.06±0.01) was observed after one week in all treated groups in comparison with the control group (9.17±0.35).

Treatment of experimental animals suffering from PC with γ-irradiation and nano- Moringa either alone or combined showed a remarkable decrease in RBS level (198.17±1.72, 198.63±3.58, 191.71±3.54 respectively) after one week when compared to PC group. On the contrast, insulin level (1.57±0.03, 1.67±0.03 and 1.71±0.07 respectively) and IGF1 level (8.20±0.03, 7.47±0.20 and 8.85±0.04 respectively) showed a
remarkable increase when compared to PC group. Table 3 showed the effect of nano-Moringa oleifera leaves extract and/or exposure to low doses of γ-radiation on the activity of pancreatic enzyme lipase and amylase activity and plasma C-reactive protein (CRP) levels after one week in all the experimental treatments. Group 2 (PC) had significantly higher levels of plasma lipase, amylase activity, and CRP (61.33±2.97, 44.43±0.54 and 36.74±2.36 respectively) activities as compared to the positive control level (54.95±1.08, 3525.67±150.21 and 94.82±2.32 respectively). Meanwhile, rats suffering from pancreatitis and treated with arginine (acute pancreatitis model) showed a remarkable decrease in plasma lipase (44.43±0.54 and 36.74±2.36 respectively) level compared to the positive control level (61.33±2.97, 3525.67±150.21 and 94.82±2.32 respectively). The acute pancreatitis model treated group showed normal structure following treatments with nano-Moringa and exposure to γ-radiation after one week of post-treatments (Figure 9 A&B).

Table (2): effect of nano-Moringa oleifera leaves extract and/or low doses of γ-radiation on RBS and insulin levels in adult male albino rats suffering from pancreatitis.

![Figure 1: size distribution of formula 1 F1](image1)

![Figure 2: size distribution of formula 2 F2](image2)

Each value represents the mean ± standard error (SE) of six values. Data with different superscripts are significantly different at P<0.05

G1: Control (C), G2: Positive control (PC), G3: Nano-Moringa (NM), G4: IR, and G5: Nano-Moringa (NM+IR). (a) Significant from control. (b) Significant from the positive control.

### Table (1): The effect of PLGA: PEG ratios on the particle size and zeta potential of MLn-based PLGA-PEG nanoparticles.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>PLGA</th>
<th>PEG</th>
<th>Nano-Size (nm)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1</td>
<td>2</td>
<td>190.13±6.5</td>
<td>0.5±0.02</td>
<td>-13.23±3.32</td>
</tr>
<tr>
<td>F2</td>
<td>2</td>
<td>1</td>
<td>341.99±15.4</td>
<td>0.6±0.03</td>
<td>-5.27±1.29</td>
</tr>
<tr>
<td>F3</td>
<td>1</td>
<td>1</td>
<td>141.77±14.5</td>
<td>0.05±0.01</td>
<td>-39.60±3.52</td>
</tr>
</tbody>
</table>

Notes: MLn; Moringa leaves extract nanoparticles, PLGA; poly D-Lactide-co-glycolide, PEG; polyethylene glycol, F1; formula 1 (ratio 1:2), F2; formula 2 (ratio 2:1), and F3; formula 3 (ratio 1:1), PDI; polydispersity index, S.E.; standard error.

After one week of therapy, sections of the pancreas of rats treated with arginine (acute pancreatitis model) revealed a clump of immune cells (lymphoid aggregation), bleeding, and necrotic acini (Figure 6 A&B).

However, rats suffering from pancreatitis and treated with nano-Moringa oleifera extract (Figure 7 A&B) or exposed to low doses of γ-radiation (Figure 8 A&B) showed a typical appearance of pancreatic tissue structure (normal secretory cells in both islets of Langerhans and acini) (Figure 8 A&B).
Critical care techniques, dietary support programs, fluid therapy, and mechanical ventilation, the fatality rate may approach 10-20%, [2-3]. The L-arginine model is one of the most commonly used experimental models of acute pancreatitis (AP) that matches the human form of the disease and is used to examine the various biochemical and histological changes that closely reflect the human phenotype in many aspects [20].

In 2012, the Chinese Ministry of Health Designated *M. oleifera* leaves as a fresh food supply. *M. oleifera* is additionally a famous traditional plant for treating irritation, diabetes, hypertension, anaemia, and other conditions [21-24]. Some flavonoid pigments, such as alkaloids, kaempferol, rhamnose, and isoorcetin, as well as antioxidant compounds, such as ascorbic acid, flavonoids, phenols, and carotenoids, occur naturally in *M. oleifera* [25] and may contribute to the anti-inflammatory activity of *M. oleifera*. Additionally, several active components of *M. oleifera* have already been demonstrated to alleviate some inflammatory process [26].

*Moringa* extract has a high antioxidant capacity, with 77% suppression of the generation of free radicals [27] and the damaging impact of reactive oxygen compounds, which may aid in the prevention of oxidative stress [27]. This is because plant extracts contain substantial amounts of flavonoids and phenolics. These plant chemicals have great medicinal applications since they are a class of natural antioxidants with significant therapeutic benefits on human health [28].

Low-dose IR stimulated a variety of signaling and stress identified by means that stimulate tissue defenses such as improved DNA repair processes, protein synthesis induction, improved detoxification of oxidative stress and antioxidant generation, cell survival/death passageway (cell death), endoplasmic reaction to stress, cytoprotective procedures such as autophagy, cell cycle control, protein unfolding, and improved immune reaction by inducing M2 polarization in macrophages which inhibits the adherence of mononuclear cells and granulocytes in the peripheral blood [29].

4. Discussion

The goal of this study was to determine the effect of *Moringa oleifera* leaves extract and/or low doses of γ-radiation on lipase, amylase, and CRP levels in adult male albino rats suffering from acute pancreatitis.
Radiation was also particularly helpful in treating a wide range of inflammatory illnesses, including asthma, arthritis, and immune system problems [30]. Our findings demonstrated that L-arginine injection caused acute pancreatitis, as evidenced by a significant rise in RBS plasma lipase, amylase activity, and CRP levels. In contrast, all treated groups showed a substantial drop in insulin and IGF1 after one week when contrasted with the control. Nevertheless, histological examination of the pancreas revealed a cluster of immune cells (lymphoid aggregation), haemorrhage, and necrotic acini after one week of all treatments.

The altitude of the pancreatic amylase and lipase activation is in accordance with that of Salem et al. [31] who revealed that the elevated levels of the pancreatic enzymes, principally amylase, and lipase, may be due to the generation of hydrolytic enzymes in acute pancreatitis which hydrolyses phospholipids to liberate arachidonic acid and lysophospholipids while the latter serves as a cytotoxic function, allowing acinar cells necrosis. Additionally, Wang et al. [32] showed that L-arginine selectively damages pancreatic acinar cells by producing amino acid imbalance, lowering polyamine, nucleic acid, and proteinase production, and resulting in excessive zymogen activation.

Yang et al. [33] discovered that pancreatic lipase interstitial leakage resulted in adipose lipolysis and an increase in unsaturated fatty acid contents. These toxic fatty acids induce an inflammatory storm and an oversupply of markers of inflammation, which can speed up disease progression and lead to multi-organ failure.

Acute pancreatitis is a pancreatic inflammatory disorder that affects both local and distant organs [34]. The elevation in TNF- and CRP levels in the acute pancreatitis group was comparable to that obtained by Al-Hashem [35], who discovered that toxic dosages of L-arginine promoted pancreatic tissue damage and raised pro-inflammatory mediators such as TNF- while decreasing the anti-inflammatory cytokine IL-10.

Acute pancreatitis can cause a systemic sickness that can lead to various organ malfunction and even death. TNF- and other inflammatory cytokines released during acute pancreatitis pathophysiology are considered to be linked to the occurrence of multiple organ failure [36].

Throughout the disease, a variety of pro- and anti-inflammatory cytokines produced by the pancreas as well as other sources impact systemic symptoms of acute pancreatitis [32]. Local recruitment and stimulation of inflammatory cells (neutrophils and macrophages) released from injured acinar cells results in the generation of proinflammatory cytokines tumour necrosis factor-alpha in acute pancreatitis (TNF-).
is released by activated pancreatic macrophages in response to local tissue injury, resulting in multiple organ failure [32] [37]. Moreover, Nieminen et al. [38] discovered that CRP levels in serum were related with the severity of acute pancreatitis and its outcome.

High-level glucose has been shown to be one of the reference markers for estimating the severity of acute pancreatitis in clinical practice [39]. In the group with acute pancreatitis caused by L-arginine, the results indicated a considerable increase in plasma glucose and a significant decline in insulin sensitivity when contrasted with the control group. According to Shoman and Nafeh [40], acute pancreatitis affects not only exocrine pancreatic function, as evidenced by significantly higher serum amylase and lipase levels, but also pancreatic endocrine function, as evidenced by decreased fasting plasma insulin (FPI) levels in association with hyperglycemia.

Moreover, severe pancreatitis caused collateral damage to several islets of Langerhans, notably insulin-secreting β-cells [41]. Abdelzaher et al. [42] also documented a decrease in insulin levels in rats given L-arginine, indicating Langerhans islet degeneration.

The islets of Langerhans are also vulnerable to an overabundance of cytokines during acute pancreatitis, which are cofactors in the development of hyperglycemia, which may increase the inflammatory response. Under stress, the intricate interaction of hormone and cytokine feed forward and feedback processes leads in increased hepatic glucoseogenesis and insulin resistance [43].

Stress hyperglycemia has been established in both experimental and clinical studies to cause intracellular glucose excess and abrupt glucotoxicity [44]. The intensity of acute pancreatitis, in addition to the function of the pancreatic insulin apparatus, alters glucose levels, which corresponds with the dynamics of toxemia indicators and liver failure. [45]. Nevertheless, an autoimmune impact on islet cells is feasible, as evidenced by morphological and functional alterations in cells [46].

The pancreas and liver are intimately connected in terms of anatomical location, physiological function, and hemodynamics. In pancreatic acinar cells, hyperglycemia combined with TNF- promotes the formation of reactive oxygen species (ROS) [47].

Excessive release of inflammatory factors increases microvessel density, promotes thrombosis and bleeding, and eventually increases acinar cell apoptosis and necrosis [32]. L-arginine causes necrotizing pancreatitis by preferentially damaging pancreatic acinar cells [33].

Inflammation and oxidative stress caused by hyperglycemia damage beta cells, resulting in secretory dysfunction and increased apoptosis [48]. Siriviriyakul et al. [49] The researchers detected a substantial increase in acinar cell death in L-Arg- stimulated acute pancreatitis compared to control mice.

Acute pancreatitis can develop to chronic pancreatitis or pancreatic fibrosis, which can lead to more difficult-to-cure pancreatic cancer. Treatment of acute pancreatitis and minimizing its severity, on the other hand, will avoid complications and progression to pancreatic cancer.

The treatment of experimental mice with PC with -irradiation and nano- Moringa, either alone or in combination, resulted in a significant reduction in RBS, plasma lipase, amylase activity, and CRP levels. In contrast, insulin and IGF1 levels increased significantly when compared to the PC group.

Yet, after one week of treatment, slices of pancreatic tissue displayed a typical appearance of tissue structure (typical islets of Langerhans and normal acini).

Anti-inflammatory activities of Moringa oleifera have been demonstrated. Various bioactive constituents discovered in M. oleifera extracts exhibited anti-inflammatory properties [50]. Moringa oleifera extracts have been shown in experiments to reduce the production of NO and proinflammatory mediators in LPS-stimulated macrophages [7] [51]. Moringa oleifera extracts efficiently reduce the production of inflammatory mediators. [22] [52].

Moringa leaf extracts are delivered into living cells via a PLGA nanocapsule labelled with PEG, which protects the nanoparticles from rapid phagocytosis and extends their longevity. The use of biodegradable and biocompatible polymers (PEG) with low cytotoxicity on the surface allows for the controlled and extended release of extracts that produce aberrant cell dying apoptosis [14].

According to Lau et al. [53], a minimal dosage of IR is necessary for life, recognising that the normal generation of ROS is sufficient to drive the defensive systems and provide a good health impact known as radiation hormesis.

Radiation therapy was also particularly successful in treating a wide range of inflammatory illnesses, including asthma, arthritis, and immune system problems [30].

Our findings indicated that LDR was related to a reduction in circulating levels of several inflammatory indicators, such as CRP concentrations. LDR (0.5-1.5 Gy) works on cells implicated in the inflammatory reaction (endothelial cells, polymorphonuclear leukocytes, and macrophages), generating anti-inflammatory effects, and may be beneficial by acting as a possible anti-inflammatory agent against the inflammatory mediators’ cascade [54].

Animal studies have shown that low-dose gamma irradiation can improve type II diabetes by raising insulin secretion and pancreatic superoxide dismutase activity [55]. Moreover, LDR has been shown to have the
capacity to alter the course of chronic renal failure in rats [56]. Additionally, earlier research has demonstrated that LDR dramatically reduces type 1 diabetes-induced kidney damage by reducing inflammation and oxidative stress [57].

5. Conclusion
Treatment of PC-affected experimental animals with γ-irradiation and/or nano-Moringa, either alone or in combination, resulted in a curative endeavour in oxidative stress defence exerted by L-arginine, which might be related to its antioxidant and anti-inflammatory action.

Acknowledgements
We appreciate the contribution of Prof. Dr. Ahmed Abd-Rabou Hormones Department, Medical Research Division and Prof. Dr. Aboelfetoh Abdalla Horticulture Technology Department, National Research Centre, Giza, Egypt for assistance in setting up preparation of nano-Moringa oleifera leaves extract.

References
223

International Journal of Theoretical and Applied Research, 2022, 2(2)


