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Research Article

Evaluation Of Protective Effect of Ethanolic Extract Of “*Nelumbo Nucifera*” Leaves On L-Arginine Induced Acute Pancreatitis in Wistar Rats

Surabhi M.*, Sri Suku J., Nayana Shaji, Anumol George

College of Pharmaceutical Sciences, Govt Medical College, Thiruvananthapuram., Kerala, India.

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ABSTRACT

Acute pancreatitis (AP) is a devastating pancreatic disease caused by pancreatic inflammation, which predominantly results in the loss and destruction of endocrine (islet) and exocrine (acinar) cells. It produces severe stomach pain, which radiates to the back. There is no FDA-approved medication for acute or chronic pancreatitis only the symptomatic treatment is available. Food habits include poor fibre intake, high fat intake, and addiction to alcohol or tobacco (snuffs or smoking). Numerous research suggests that using Chinese medicines may provide pharmacological benefits. Traditional Chinese medicine contains several phenolics, alkaloids, flavonoids, terpenes, and glycosides that have demonstrated promising preclinical result.. The future of phytomedicines lies in the bottleneck of pharmacokinetics, where many potential herbal medication candidates fail. To improve bioavailability,. The Phyto remedies has the potential to targeting the ROS mediated pathway and also alleviation of NF-kB mediated redox imbalance. Very few are established rest of all do not conclude yet. The current study assesses the preventive impact of *Nelumbo nucifera* leaves extract on an acute pancreatitis model of Wistar rats given L-arginine intraperitoneally for three days. The leaf was extracted using the Soxhlet apparatus, and the extract was dried and collected. Then, preliminary screening and invitro tests were performed. The study employed 24 male Wistar rats, divided into four groups. During the induction period, the animal is in severe pain. The leaf extract was administered in the low and high dose groups at doses of 500mg/kg and 750mg/kg respectively, whereas the diseased group received 2.5g/kg L-arginine i.p. Following treatment, the animals were humanely euthanized and blood samples were taken for serum enzyme and lipid profile analysis. Additionally, the tissue was obtained for gene expression studies and oxidative stress marker analyses. The pancreas was dissected for histological examination. According to the results of the foregoing assessments, the high dosage treated group displays substantial improvements, indicating that *Nelumbo nucifera* leaf extract may have a protective effect in dose-dependent manner.

***Corresponding Author:** Surabhi M.

Address: College of Pharmaceutical Sciences, Govt Medical College, Thiruvananthapuram., Kerala, India

Email ✉: surabhimundakkal01@gmail.com

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INTRODUCTION

Anti-inflammatory drugs are the only specific therapeutic options available for managing pancreatitis other than treating symptoms. Evidence has revealed that Phyto remedies can alleviate pancreatitis through a variety of pathways, such as reducing damage mediated by reactive oxygen species (ROS), modulating the NF-kB pathway, and having anti-inflammatory properties. Many alkaloids, flavonoids, and glycosides have been studied for their potential therapeutic uses in pancreatitis. A potential class of medicinal substances, phytoconstituents are widely accepted because of traditional values. A growing body of studies suggests that their protective impact is due to many molecular pathways. Numerous Phyto remedies have been tested in preclinical animal models of pancreatitis with promising results for treating pancreatic damage. A significant amount of phytochemicals influence the openly acting redox balance and target the inflammatory cascade, with nuclear factor kappa-light-chain-enhancer of activated B cells being the crucial molecule. Aside from many additional unique methods, they include the inhibition of apoptosis (by artemisinin and embelin), the inflammasome (by withaferin A), neutrophil rolling (by fucoidan), Ca^{+2} release (by coffee), and mitogen activated protein kinase (by guggulsterone). There are so many plant derived products like flavonoids, terpenes, alkaloids, glycosides, quinones and polyphenols have been used for the treatment of wide range of pancreatic conditions. Until now, a wide range of pancreatic conditions, including pancreatitis, have been treated with numerous Chinese herbal remedies. Numerous investigations were carried out to examine their efficacy in the identification, management, and deterrence of various medical disorders (both acute and chronic). Many drug development initiatives rely on leads with a botanical origin. Various preclinical models of pancreatitis are employed in current research to examine the underlying causes. The inability of one animal model to acquire a full disease compels

the researchers to make use of several animal models. The models can be roughly categorized into three groups according to the method of induction: (i) genetic models; (ii) chemically induced; and (iii) surgically induced. Pancreatic duct closure and retrograde bile acid infusion are the two basic surgical models; cerulein, L-arginine, dibutyltin dichloride, and choline-deficient diet-induced models are typically utilized in chemically induced models. Acute pancreatitis begins with modest discomfort and progresses to chronic severe organ dysfunction, which can occasionally result in acute pancreatic failure. Symptomatic therapy is part of the regimen. Drugs that address pain and inflammation and have a high (20–30%) fatality rate. Multiple organ failure, including respiratory and circulatory collapse, is the cause of the problems (McFadden, 1991).⁽¹⁾

ACUTE PANCREATITIS

Acute pancreatitis (AP) is a serious pancreatic illness that arises from inflammation of the pancreas, primarily resulting in the loss and destruction of the organ's endocrine (islet) and exocrine (acinar) cells. The illness condition might manifest in a variety of ways, each with a potential for severe consequences that could endanger life. The symptoms of AP might include nausea, vomiting, and, very rarely but extremely dangerously, gastrointestinal bleeding. It can also cause epigastric pain that radiates to the back. Multiple organ failure is frequently linked to acute pancreatitis and is caused by systemic inflammation brought on by an increase in oxidative stress, pancreatic necrosis, and local bleeding.⁽²⁾ It mainly characterised by oxidative stress and inflammation of pancreas. It fulfils two functions: Digestive juices are secreted into the small bowel to aid in food digestion and to counteract the production of stomach acid. Insulin is then released to control blood glucose levels. Three different cell types are found in the pancreatic ducts: 1) ductal cells lining the ducts secrete a watery fluid that carries the digestive enzymes into the intestine; 2) endocrine cells found in the islets of Langerhans secrete



hormones, including insulin. This area of the pancreas is the exocrine pancreas because ductal and acinar cells secrete into it. The inactive precursors of pancreatic digestive enzymes are produced and transported to the small bowel, where they undergo further enzymatic activities to become the active digestive enzymes needed to break down food. Pancreatitis is the result of early activation of pancreatic enzymes, which target the pancreas rather than aiding in food digestion. ⁽³⁾ The updated Atlanta classification divides the disease's severity into three categories: mild, moderately severe, and severe. According to this classification acute pancreatitis divides to two groups (1)First group characterised by pancreatic parenchymal and peripancreatic inflammation without necrosis, (2)The other category involves inflammation and necrosis. These levels are based on organ failure and both local and systemic complications. The severity of AP and its prognosis have been predicted using a variety of techniques, including imaging analysis, clinical assessment, and testing of several biochemical markers. ⁽⁴⁾ The onset of Acute Pancreatitis (AP) is attributed to the slower activation of digestive enzymes due to various etiological causes. It is a pathology marked by extensive inflammation and pancreatic tissues being digested by activated enzymes. Preventing inflammation or preventing its progression is the primary objective of AP treatment. Supportive therapy is used as main management therapy of AP. Nutritional assistance, anti-inflammatory medications, empirical antibiotic therapy, lowering pancreatic exocrine secretion, and fluid and electrolyte supplementation are all components of supportive care. Antioxidant and anti-inflammatory drugs for the underlying infection are being researched in addition to supportive care. ⁽⁵⁾ It's rapid inflammatory pancreatic disease that can cause anything from a minor soreness to a serious, potentially fatal condition. It is among the leading causes of hospitalizations in the US connected to gastrointestinal diseases. The pooled incidence of AP is about 34 instances per 100,000 people

worldwide, which raises serious concerns for public health. ⁽⁶⁾

MATERIALS AND METHODS

➤ PLANT COLLECTION AND AUTHENTICATION

Fresh nelumbo nucifera leaves were collected from Vellayani Lake, Thiruvananthapuram and was authenticated at Department of Botany, Karyavattom Campus, University of Kerala.

➤ PREPARATION OF PLANT EXTRACT

The leaves dried by shade condition for 20-25 days. Leaves were crushed to coarse powder using mechanical grinder and then sieved through sieve no:18. About 30g of coarse leaf powder was packed and loaded in Soxhlet apparatus. Defatting of leaf powder was done by extraction in petroleum ether (60-80°C). The marc after petroleum ether was dried and packed again in Soxhlet apparatus and then again extract with ethanol solvent. Then, extracts were filter and then the resultant extract was distilled to remove the solvent completely and dried in desiccator.

➤ PRELIMINARY PHYTOCHEMICAL SCREENING

The defatted hydroalcoholic extract of *Dracaena reflexa* leaves was subjected to quantitative test to identify various phytochemicals such as alkaloids, flavonoids, saponins, steroids and tannins.

➤ APPROVAL BY IAEC

The study protocol was authorized by Institutional Animal Ethical Committee of Govt. Medical College, Thiruvananthapuram convened on 30th January 2024 as per the permission no: 01/08/IAEC/2024/GMCT.

➤ ANIMAL SELECTION, MAINTENANCE, AND CARE

24 Male Wistar rats, weighing 200–300 grams, were purchased and housed in the Govt. Medical College's animal house in Thiruvananthapuram. Animals were housed in polypropylene cages with stainless steel lids with provisions for keeping food and drinking water ad libitum as per Committee for Control and Supervision of Experiments on Animals (CCSEA).

➤ EXPERIMENTAL DESIGN



Wistar rats were grouped in such a way that each group contain six animals (N=6). Acute pancreatitis was induced by administering 2.5g/Kg of L-arginine intraperitoneally for 3 days which is dissolved in Normal saline. Ethanolic extract of *Nelumbo nucifera* leaves was administered at doses of 500mg/kg (Low dose) and 750mg/kg (High dose) orally, ethanolic extract which is dissolved in distilled water. The control group received distilled water. The diseased group received 2.5g/Kg L-arginine only. After the administration of arginine, the animal exhibits some stretching and writhing movement. Writhing is the animal's response to severe pain, which includes stretching, rear leg extension, and abdominal contraction. A high dose of L-arginine produces acute pancreatic inflammation and significant discomfort. Following IP administration of L-arginine, water consumption increases during the next three hours, whereas food intake decreases. We perform the protective effect of extract hence the disease given first, received L-arginine intraperitoneally after 3,4 hour later administered the ethanolic extract of leaf. The drug treatment is considered as a preventive regimen and given concurrently along with the disease induction.

➤ EXAMINATION OF BODY WEIGHT CHANGES

The body weight changes were examined at first and third day.

➤ EXAMINATION OF GLUCOSE LEVEL CHANGES

Blood glucose was determined rats using a ONETOUCH Glucometer. The blood samples were collected through tail vein by puncturing with hypodermic needle. Blood glucose levels estimated on first and third day of experiment.

➤ BIOCHEMICAL INVESTIGATION OF BLOOD

i) Estimation of Amylase

Amylase synthesis and removal rates are typically balanced, as indicated by the serum amylase concentration. The serum amylase level rises quickly in the blood in the event of acute

pancreatitis, peaking at three to five times the upper limit of normal. Six hours after symptoms start, a ten-to twelve-hour half-life, and a three-to five-day duration of continuous elevation.⁽³⁸⁾

Procedure

The Amylase Kit (AGAPPE) was used to calculate the amylase activity. One milliliter of an enzyme reagent was combined with 25µL of plasma. After combining the contents, it incubated at 37°C for one minute. Over the course of three minutes, the change in absorbance per minute ($\Delta OD/min$) was observed. The contents were read at 405 nm on a SHIMADZU – UV 1900i UV-VIS spectrophotometer.⁽⁴¹⁾⁽⁴²⁾

Calculation

Calculate the average change in absorbance per minutes

Amylase Activity (U/L) = ($\Delta OD/min$) × K-factor,
K-factor - 3178

ii) Estimation of Lipase

Another pancreatic enzyme, serum lipase, is produced mostly by acinar cells and has a concentration 100 times higher than that of lipoprotein lipase, other hepatic isoforms, and endothelial lipase. During acute pancreatitis, high-level enzyme release is made possible by the permeability of serum lipase-producing cells being enhanced. Accordingly, the rise in serum lipase occurs three to six hours after the onset of symptoms, peaks in twenty-four hours, and continues for as long as two weeks. This allows lipase to have a wider diagnostic window than amylase. Furthermore, because the pancreas produces lipase primarily, it has a higher specificity than amylase.⁽³⁸⁾

Procedure

The Lipase Kit (Coral Clinical Systems, Catalogue No. 1102180025) was used to calculate the lipase activity. In a test tube, 0.04 ml of plasma was taken. After adding 1 millilitre of enzyme reagent, incubated at 37°C for 4 minutes. One milliliter of enzyme reagent and 0.04 milliliter of calibrator solution were added to the test tube labelled calibrator. Following incubation, a 5-minute delay was observed between the test and the calibrator's



initial and final absorbances. On a UV-VIS spectrophotometer (SHIMADZU – UV 1900i) set to 340 nm, the contents were read. The test and calibrator absorbance changes (ΔA) were computed.⁽⁴¹⁾⁽⁴²⁾

Calculation

Lipase Activity (U/L) = $\frac{\Delta AT}{\Delta AC} \times \text{conc. of Calibrator}$

Concentration of Calibrator - 282

iii) Estimation of C reactive Protein⁽⁴⁵⁾⁽⁴⁶⁾⁽⁴⁷⁾

C reactive protein is the most important marker for determining the severity of acute pancreatitis. It takes almost 72 hours for the serum level to peak following the onset of symptoms. C reactive protein has a sensitivity of 80–86% and specificity of 61–84 percent for identifying necrotizing pancreatitis

Calculation

CRP = $\frac{(A2-A1)\text{Sample}}{(A2-A1 \text{ calibrator})} \times \text{calibrator concentration}$

iv) Estimation of Triglyceride

Acute pancreatitis risk is highly correlated with elevated blood triglyceride levels, which are a form of fat. Triglyceride levels can cause pancreatitis when they rise abruptly, usually above 1,000 mg/dL. As triglycerides are broken down by lipases in the bloodstream, high triglycerides cause the release of free fatty acids. Pancreatic cells and capillaries can be harmed by too many free fatty acids, which can result in pancreatitis and inflammation.⁽⁴⁸⁾⁽⁴⁹⁾

Procedure

Prepare the Free Glycerol Reagent, as well as the Triglyceride Reagent. Pour 0.8 ml of the Free Glycerol Reagent into each cuvet. Pour 10 ml (0.01 ml) of water, Glycerol Standard, and sample into cuvettes labelled Blank, Standard, and Sample, accordingly. Mix by gently inverting. Incubate for 5 minutes at 37 °C. Incubate for 15 minutes at ambient temperature before moving to 30°C. Add 0.2 ml of dissolved Triglyceride Reagent to each cuvet, stir, and incubate at 37°C for 5 minutes. Measure and record the final absorbance (FA) of the Blank, Standard, and

Sample at 540 nm, using water as a reference. Determine the total triglyceride level in the sample.⁽⁵⁰⁾⁽⁵¹⁾

Calculation

Serum triglyceride = $(C \text{ std} \times A \text{ sa}) / A \text{ std}$

➤ SACRIFICE OF ANIMAL

By the end of the third day, animals were anesthetized using ketamine and xylene and blood was collected by cardiac puncture. After allowing blood to clot for 20 -30 min, serum was separated by centrifugation at 2000 rpm for 15 min at 4°C. Pancreas tissue was collected after confirming animal death and one part was homogenised in ice cold phosphate buffer and stored in aliquots at -80°C used for biochemical estimation And the other part was collected in 10% formalin to be further processed for histochemical examination.

➤ BIOCHEMICAL INVESTIGATION OF PANCREATIC TISSUE

• Preparation of tissue homogenate

After sacrificing pancreatic tissue were collected and washed with PBS (ph 7). The tissue was rinsed in ice cold PBS and homogenized in homogenizer for 5 minutes. Further centrifuged at 3000 rpm 15 minutes. The clear supernatant was used for biochemical estimations.

• Estimation of oxidative stress⁽⁵²⁾

Acute Pancreatitis shows higher oxidative stress level and its indicates disease intensity. Increased levels of lipid peroxidation products, Myeloperoxidase activity, Superoxide levels are seen in severe acute pancreatitis. And it correlates with tissue injury and organ dysfunction.

i) estimation of catalase activity⁽⁵³⁾

Catalase is an intracellular enzyme that is mainly located in cellular peroxisomes. Catalase is particularly important in case of limited glutathione availability and plays a role in the development of tolerance to cellular oxidative stress. Catalase levels in pancreatic tissue homogenate were determined correspondingly. The enzyme reaction was initiated by adding 1.0 ml of hydrogen peroxide solution to the 0.2 ml tissue homogenate that had been pipetted into the tube and combined with 1.2 ml of phosphate buffer



(0.05 M, pH 7.0). The blank was run concurrently with 1.0 ml of distilled water in place of hydrogen peroxide and the absorbance was immediately recorded at 240 nm for three minutes.⁽⁵⁶⁾

Catalase activity= $\Delta \text{Abs} \times \text{Total assay volume}$

$\Delta t \times \epsilon \times L \times \text{Enzyme sample volume}$

Abs is the change in absorbance

Δt is the time taken

ϵ is the extinction coefficient of enzyme $39.4 \text{ M}^{-1} \text{ cm}^{-1}$

L is the path length

ii) Malondialdehyde (MDA) estimation⁽⁵⁶⁾

End products of the cells peroxidation of polyunsaturated fatty acids is malondialdehyde. An increase in oxidative stress causes the overproduction of MDA has been widely used to assess lipid peroxidation and was measured. The homogenate was centrifuged in 10 minutes at 4600 nm. Since there was less than 0.2 milliliter of tissue homogenate in the samples, 0.8% aqueous TBA solution, 0.2 milliliters of 8.1% SDS, and 1.5 milliliters of 20% acetic acid solution (pH 3.5 adjusted with NaOH) were added. The mixture brought to 4.0 ml by adding distilled water, and it was then heated in an oil bath at 95°C for 60 minutes. After adding water and giving the mixture a good shake, add 1.0 ml water and 5.0 ml of an n-butanol and pyridine (15:1, v/v) mixture were added. Following the removal of the organic layer, the sample was centrifuged for 10 minutes at 4000 rpm in order to assess at 532 nm. as opposed to a blank. Tetra methoxy propane (TMP) was used as an external standard to reference the amount of lipid peroxide, and its level was expressed in nanomoles of MDA. The formula to calculate the MDA concentration was $C=A/b\epsilon$.

Where,

C = concentration of MDA in nmol/mg of protein

A = observed absorbance

B = pathlength in cm

ϵ = molar extinction coefficient ($1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$)

➤ ESTIMATION OF INTERLEUKIN 6

Interleukin is one of the key pro- inflammatory cytokines released early in responds to pancreatic

injury. It helps initiate and propagate the inflammatory cascade in acute pancreatitis. Elevated IL-6 in the bloodstream are linked to the intensity of the systemic inflammatory response. IL-6 contributes to the development of systemic complications such as multi organ dysfunction, which can occur in severe cases of acute pancreatitis.⁽³⁸⁾ After homogenizing the tissue sample 100 mg of tissue samples is taken in 1 ml of phosphate buffer, the homogenate in mortar and pestle. Then centrifuged(500rpm) for 10 minutes, and collected the supernatant. 100 μL of each was to the 96-well plate and incubated for 370 c for overnight. then the wells were drained and twice washed with PBS TWEEN. 200 μL of freshly prepared blocking buffer (0.2% gelatine in 0.05% Tween 20: Merck; Germany) in PBS was add and allow to incubate for one hour at room temperature. Following this, it was washed with phosphate buffer saline TWEEN. 100 μL of primary antibodies IL-6 was add and allow to leave for two hours at room temperature. Followed by incubation, it was wash with phosphate buffer saline TWEEN.

Calculation

Activity of Antibody = OD Value / Protein Concentration

➤ ESTIMATION OF TNF- α ⁽⁵⁷⁾⁽⁵⁸⁾⁽⁵⁹⁾

Its play important roles in responses of inflammation in acinar cell damage. In this assay, the quantitative sandwich enzyme immunoassay method is utilized. A microplate has been pre-coated with a monoclonal antibody that is specific for TNF alpha. Any TNF alpha that is present is bound by the immobilized antibody when standards and samples are pipetted into wells. The wells are filled with an enzyme-linked polyclonal antibody that is specific for TNF alpha after any unbound materials have been washed out. After washing the wells to get rid of any unbound antibody enzyme reagent, colour develops in the wells based on how much TNF alpha was bound in the first place. After homogenizing the tissue sample 100 mg of tissue samples is taken in 1 ml of phosphate buffer, the homogenate in mortar and



pestle. Then it centrifuged (5000 rpm) for 10 minutes, and collect the supernatant. 100µL of each was add to 96-well plate and incubate for 37°C overnight. Then the wells drained and twice wash phosphate buffer saline TWEEN. 200µl of freshly prepared blocking buffer (0.2% gelatine in 0.05% Tween 20: Merck; Germany) in PBS was add and allow to incubate for one hour at room temperature. Following this, it was twice wash with phosphate buffer saline TWEEN. 100µl of primary antibodies TNF-α was added and allowed to leave for two hours at room temperature. Followed by incubation, it was wash two times with phosphate buffer saline TWEEN.

Calculation

Activity of Antibody = OD Value / Protein Concentration

➤ ESTIMATION OF EXPRESSION OF NFκB (60)(61)(62)(63)

DNA fragments can be separated and seen using electrophoresis (agarose gel). Then exposed to electric field, then fragments sorted by charge, size and flow through the gel matrix (agarose). Applying potential across an electrolyte solution (buffer) creates the electric field. Agar dissolves when cooked in an aqueous buffer and forms into a gel when cooled. A 1.5% agarose gel was made with TE buffer and melt at 90°C in a hot water bath. Subsequently, the melted agarose was allow to 45°C (cool). After adding 6µl of 10 mg/ml ethidium bromide, the gel casting equipment with the gel comb was filled. The comb was taken out of the gel after it had set. The platform and gel tank were filled with the electrophoresis buffer.

➤ HISTOPATHOLOGICAL EVALUATION OF PANCREAS (64)(65)(66)(67)(68)

Histopathological analysis was conducted on the pancreas of 3-4 rats randomly chosen from each group. The pancreas were promptly fixed in 10% phosphate-buffered formaldehyde, embedded in paraffin, and 5 µm longitudinal sections were made. These sections were stained with hematoxylin and eosin (H&E).

➤ STATISTICAL ANALYSIS

Results are expressed as Mean ± SEM. Statistical analysis were performed by ANOVA multiple comparison test using Graphpad prism software version 10.3.

RESULTS

PERCENTAGE YIELD

The extract's percentage yield was 9.91% w/w.

PHYSICO CHEMICAL PROPERTIES OF EXTRACT

1. Colour of extract- Dark green
2. Consistency – Thick and Pasty
3. Distilled water-Soluble
4. 10% Sodium Hydroxide-Sparingly soluble
5. 10% Hydrochloric chloride- Sparingly soluble
6. Ethanol- Highly soluble
7. pH of extract-6.4

PRELIMINARY PHYTOCHEMICAL SCREENING

On phytochemical screening the ethanolic extract of *Nelumbo nucifera* leaves shows the presence of alkaloid, carbohydrates, glycosides, proteins, steroids and phenolic compounds.

IN VITRO EVALUATIONS

➤ DPPH SCAVENGING ACTIVITY

The percentage inhibition of DPPH free radical by ethanolic extract of leaf extract increased with increasing concentration of extract and showed a maximum at 200µg/ml. The value of standard ascorbic acid and extract are comparable. And the highest dose shows the maximum antioxidant activity. The IC₅₀ value of ascorbic acid was 44.6µg/ml and that of extract is 137.5µg/ml.

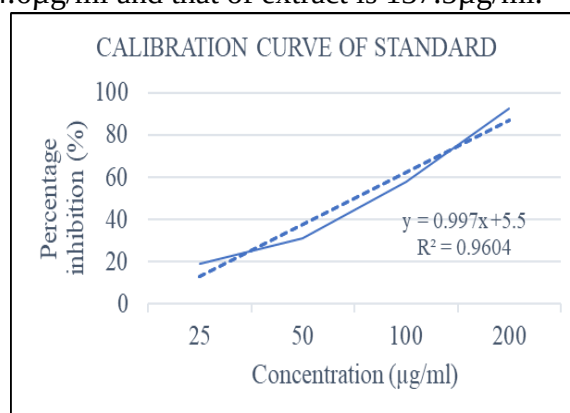


Fig 1: Calibration curve of Ascorbic acid

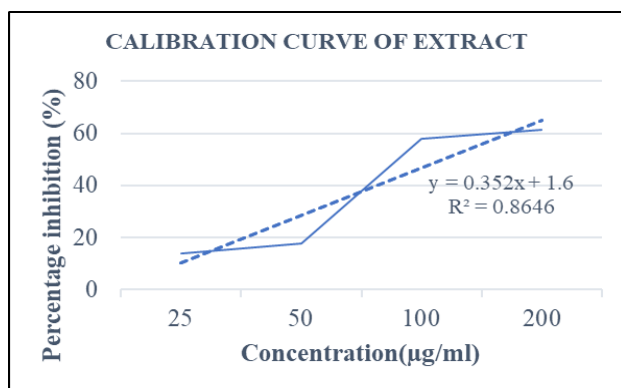


Fig 2: Calibration curve of extract

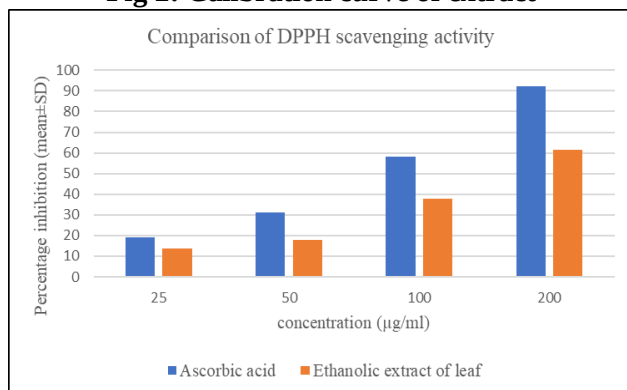


Fig 3: Comparison of DPPH assay

➤ **ASSAY FOR SCAVENGING FREE RADICALS WITH NITRIC OXIDE**

The leaf extract's percentage suppression of nitric oxide free radical was compared to ascorbic acid's. At a 200 µg/ml concentration, the extract exhibits a maximum percentage scavenging of 84%, while the ascorbic acid demonstrates a percentage inhibition of 93% at the same concentration. The highest dose displays the highest scavenging %. The extract's IC₅₀ value was found to be 152.9 µg/ml, while the standard ascorbic acid's was 48.9 µg/ml.

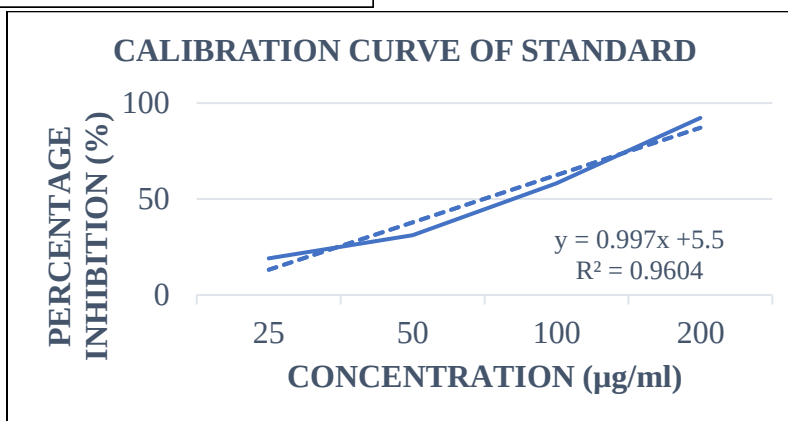


Fig 4 : Calibration curve of Ascorbic acid

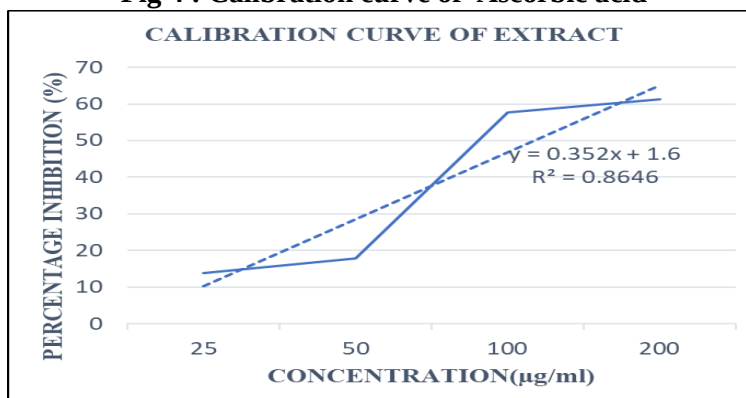


Fig 5 : Calibration curve of extract

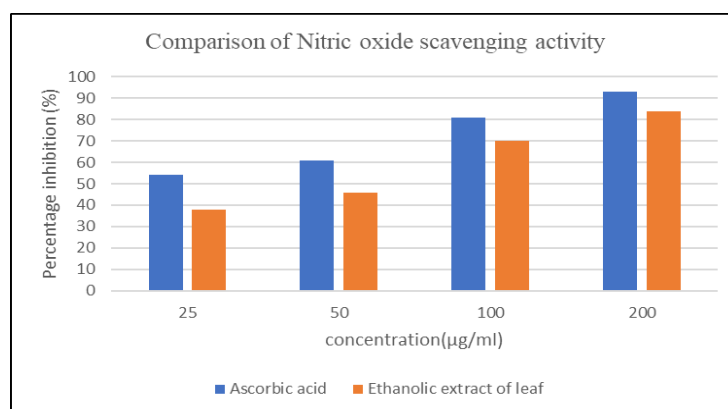


Fig 6 : Comparison of Nitric oxide scavenging assay

➤ **PROTEIN**

DENATURATION

INHIBITION ASSAY

Percentage inhibition of of Albumin denaturation by ethanolic extract was found to be 89% at a concentration of 200 µg/ml. And the standard

(Diclofenac sodium) was 98%.. And the IC₅₀ value of ethanol and standard was found to be 177.7µg/ml and 43.8µg/ml respectively.

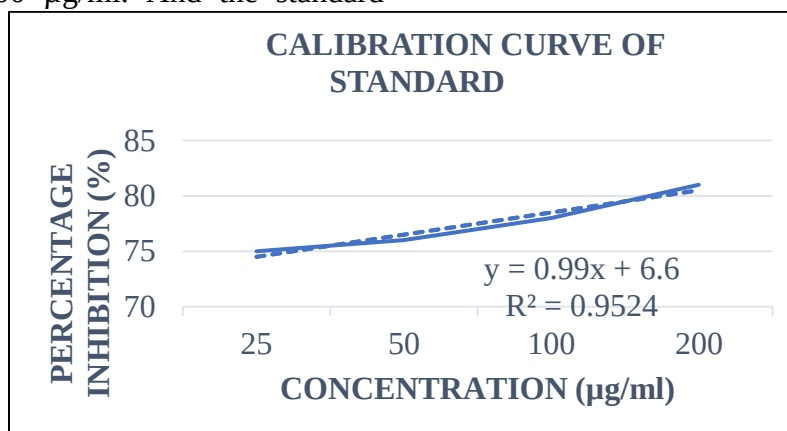


Fig 7: Calibration curve of Ascorbic acid

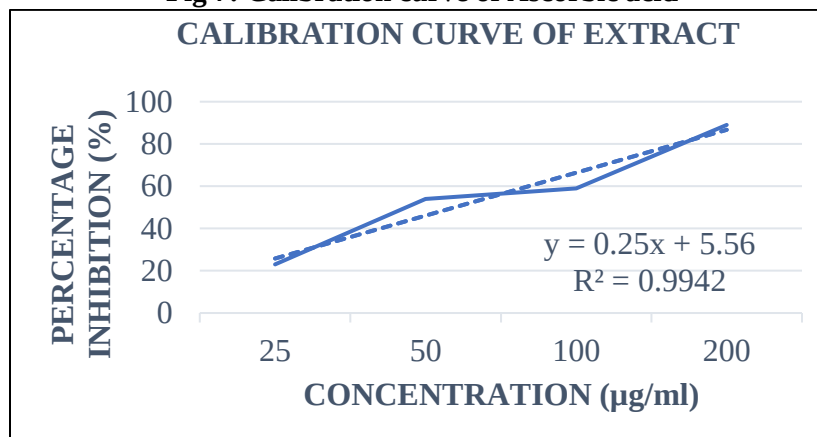


Fig 8: Calibration curve of extract

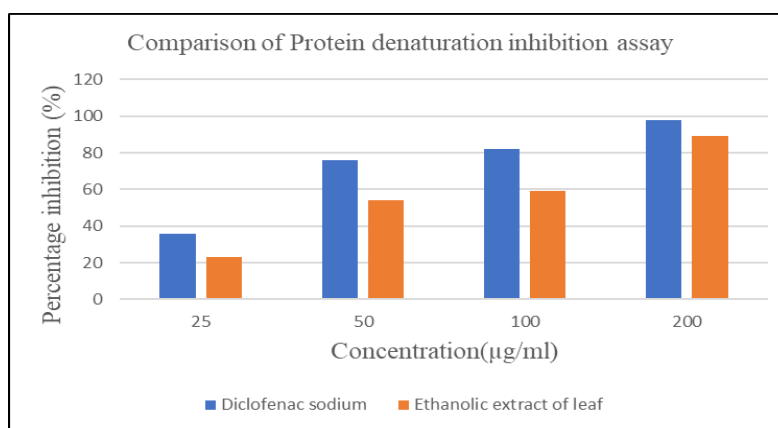


Fig 9: Comparison of Protein denaturation inhibition assay

IN VIVO EVALUATIONS

➤ EFFECT OF EXTRACT ON BODY WEIGHT

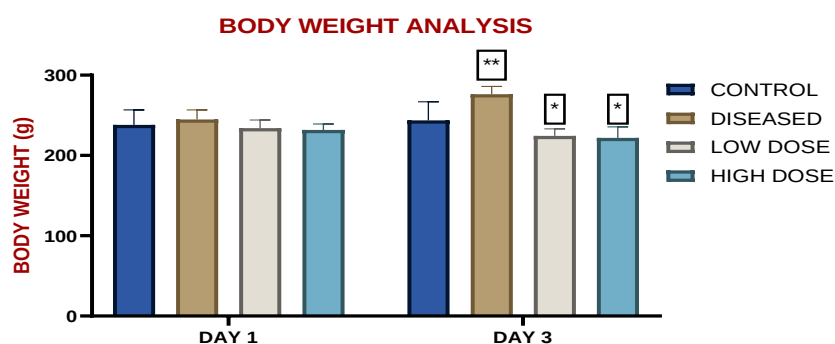


Fig 10: Comparison of body weight changes

Body weight changes in each group is noted during day 1, day 2 & day 3. Analysis was carried out by two way ANOVA followed Tukey analysis. Control group shows no differences in body weight throughout the study. In the high dose group and low dose show a significant difference with $p < 0.05$ And diseased group shows significant changes in body weight with $p < 0.001$ in first day and last day of treatment days.

When it comes to the animal's body weight, animals that get L-arginine likely to have higher levels of triglycerides and maybe impaired pancreatic functions. Increases in the body's lipid profile lead to a rise in body weight. Day 1 and Day 3 of the current study show that the diseased group's body weight increase. It suggests the pancreas may be performing improperly.

➤ EFFECT OF EXTRACT ON BLOOD GLUCOSE LEVEL

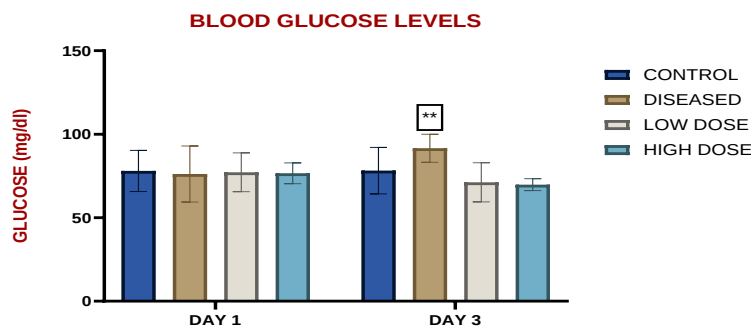


Fig 11 : Comparison of blood glucose changes

The blood glucose level effect is presented as MEAN $\pm$ SEM for a sample of six. Two-way ANOVA and Tukey Analysis were used for the statistical analysis. Minimal variations were noted in the low-dose and high-dose groups, but no discernible alterations were seen in the control group. But in the sick group, there was a statistically significant rise in blood glucose levels (P=0.01).

When pancreatic islet cells are damaged, as occurs in acute pancreatitis, blood glucose levels rise in the group that was given L-arginine. As a result, the pancreas is unable to regulate blood sugar. On days 1 and 3 of the trial, the blood glucose level of the sick group is elevated. This suggests a potential damage to the pancreatic islet cells.

➤ ESTIMATION OF EFFECT ON SERUM

ESTIMATION OF AMYLASE LEVEL

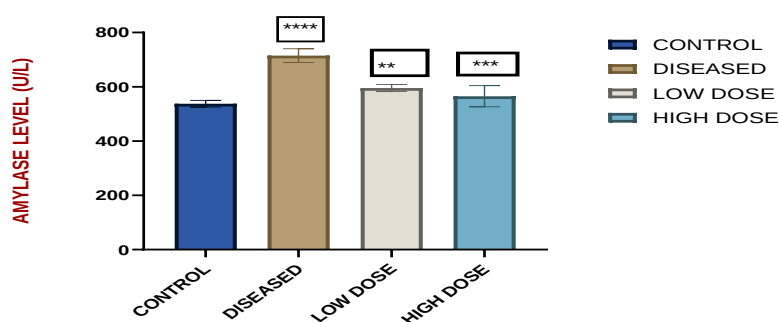


Fig 12: Estimation of serum Amylase level

Effect of serum amylase level expressed as MEAN $\pm$ SEM (n=4). Analysis was carried out by One- way ANOVA followed by Bonferroni

analysis. **** indicates p value <0.0001. *** indicates p value <0.001. ** indicates p value <0.01.

ESTIMATION OF LIPASE LEVEL

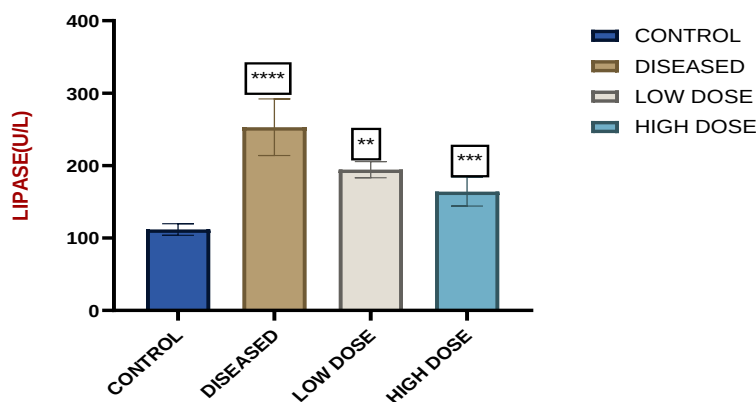


Fig 13: Estimation of serum Lipase level

Effect of lipase level expressed as MEAN $\pm$ SEM (n=4). Analysis was carried out by One- way ANOVA followed by Bonferroni analysis. ****

indicates p value <0.0001 and ** indicates p value <0.01

ESTIMATION OF C REACTIVE PROTEIN LEVEL

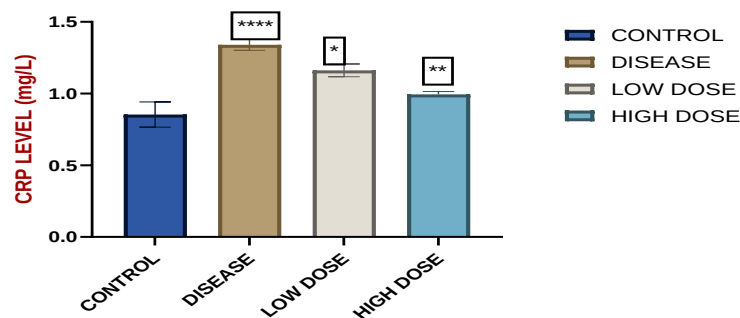


Fig 14 : Estimation of serum CRP level

Effect of c reactive protein expressed as MEAN $\pm$ SEM (n=4). Analysis is carried out by One- way ANOVA followed by Bonferroni analysis. **** indicates p value <0.0001 and *** indicates p value <0.001.

ESTIMATION OF TRIGLYCERIDE LEVELS

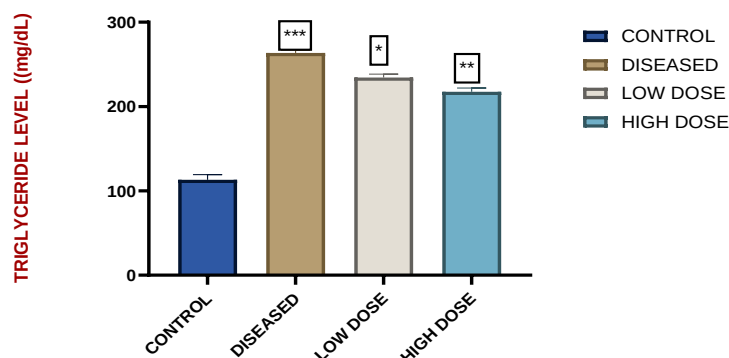


Fig 15 : Estimation of serum Triglyceride level

Effect of triglyceride expressed as MEAN $\pm$ SEM (n=4). Analysis is carried out by One- way ANOVA followed by Bonferroni analysis. **** indicates p value <0.0001 and *** indicates p value <0.001.

As per American College of Gastroenterology, serum lipase and amylase levels should be biochemically investigated for the treatment of acute pancreatitis. The pancreatic acinar cells in the acute pancreatitis model are destroyed, and early activation of digestive enzymes such as lipase and amylase increases, which results in auto-digestion of pancreatic tissue and inflammation. In this investigation, the treated groups exhibit lower levels of the enzymes than the diseased group, while the diseased group exhibits higher levels of lipase and amylase than

the control group. It demonstrates the extract's defensive characteristics.

The acute phase protein that the liver produces in reaction to inflammation is called CRP. Acute pancreatitis causes inflammation and the release of pro-inflammatory cytokines such as IL-6, which in turn promotes the liver's production of CRP, the measurement of which is used to determine the severity of pancreatitis. Serum CRP and triglyceride levels are investigated biochemically to evaluate the severity of the condition. Triglyceride levels rise as illness severity increases.

- **ESTIMATION OF EFFECT ON TISSUE HOMOGENATE**
- **ESTIMATION OF MALONDIALDEHYDE**

ESTIMATION OF MALONDIALDEHYDE

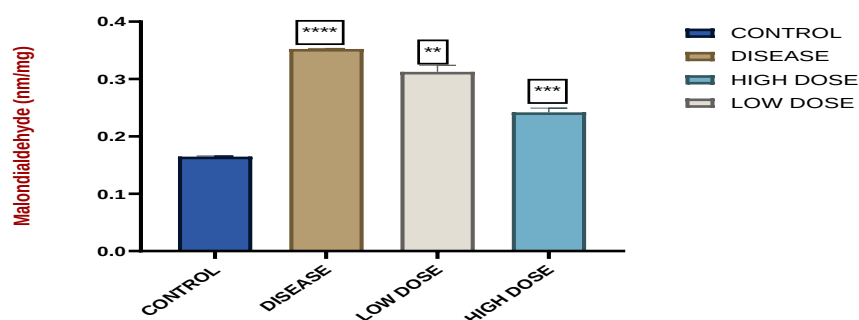


Fig 16: Estimation of MDA level

Effect of malondialdehyde expressed as MEAN $\pm$ SEM (n=4). Analysis is carried out by One- way ANOVA followed by Bonferroni analysis. **** indicates p value <0.0001 and ** indicates p value <0.01 .

Lipid peroxidation produces malondialdehyde, a sign of oxidative stress. Oxidative stress is one of the causes of acute pancreatitis. An elevated

amount of oxidative stress is shown by a rise in malondialdehyde in the pancreatitis model. It suggests the inflammation. In this study, the diseased group's malondialdehyde level increased suggesting inflammation, while the treated group's level decreased. This shows that the treated group's degree of inflammation has decreased.

- **ESTIMATION OF CATALASE**

ESTIMATION OF CATALASE ACTIVITY

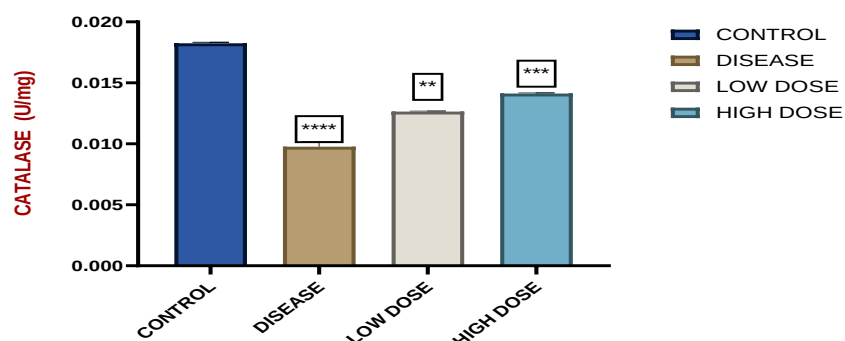


Fig 17: Estimation of Catalase level

Effect of catalase expressed as MEAN $\pm$ SEM (n=4). Analysis is carried out by One- way ANOVA followed by Bonferroni analysis. **** indicates p value <0.0001 . *** indicates p value <0.001 . ** indicates p value <0.01 .

When hydrogen peroxide is exposed to oxygen, an enzyme called catalase catalyzes the breakdown of the peroxide into water and oxygen. It guards against oxidative damage to cells. Oxidative stress is indicated by the lower catalase level. In the current study, the treated group's level of catalase

increased in comparison to the control group, whereas the diseased group's level decreased, which shows how extract protects cells from oxidative damage.

- **ESTIMATION OF TNF- α**

Effect of TNF- α expressed as MEAN $\pm$ SEM (n=3). Analysis is carried out by One- way ANOVA followed by Bonferroni analysis. **** indicates p value <0.0001 and *** indicates p value <0.001 .

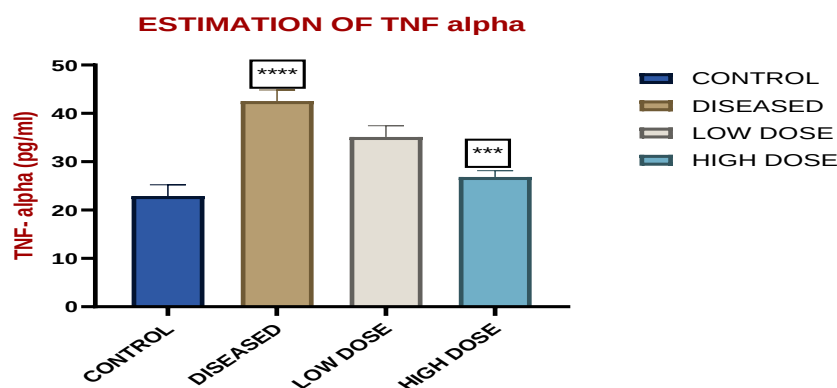


Fig 18: Estimation of TNF- α

• ESTIMATION OF IL-6

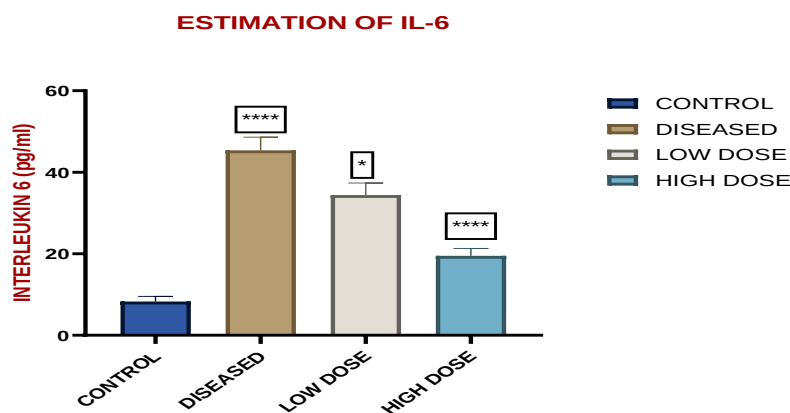


Fig 19: Estimation of IL-6

Effect of IL-6 expressed as MEAN $\pm$ SEM (n=3). Analysis is carried out by One- way ANOVA followed by Bonferroni analysis.**** indicates p value <0.0001 and * indicates p value <0.05. The two main members of the cytokine family that are expressed in inflammatory response syndrome are TNF-alpha and IL-6. Serum levels of TNF alpha and IL-6 rise in response to pancreatic damage, and this rise reflects the extent of pancreatic damage. Pro-inflammatory upregulation results from NFkB activation

triggered by TNF alpha stimulation of its MRNA and protein kinase C. TNF alpha and IL-6 both work in ways that are related to inflammation. According to the current study, the diseased group's elevated levels of TNF alpha and IL-6 reflect pancreatic inflammation, while the treatment group's decreased levels of these pro-inflammatory cytokines reveal the extract's protective impact

• NFkB GENE EXPRESSION

Gene expression study- NF KB1

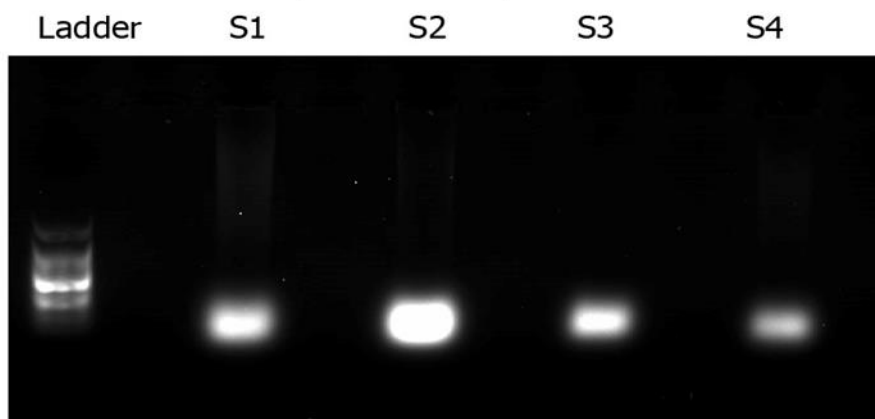


Fig 20: NF-kB Gene expression

S1=control S2= diseased group S3= Low dose group S4= High dose group

Transcription factor called NF-kB became active in the early stages of pancreatitis. This transcription factor modulates genes that govern numerous cellular processes, such as inflammation. The intensity of acute pancreatitis is correlated with the level of NFkB activation. Normally, it is inactive in the cytoplasm; however, when inflammation occurs, it becomes activated, translocate into the nucleus, and expresses itself fully. The gene that is fully expressed in the diseased group in the current study suggests inflammation, whereas the gene that is less expressed in the treated group demonstrates the extract's efficacy.

EX VIVO EVALUATIONS

➤ HISTOPATHOLOGICAL INVESTIGATION OF PANCREAS

A)CONTROL GROUP

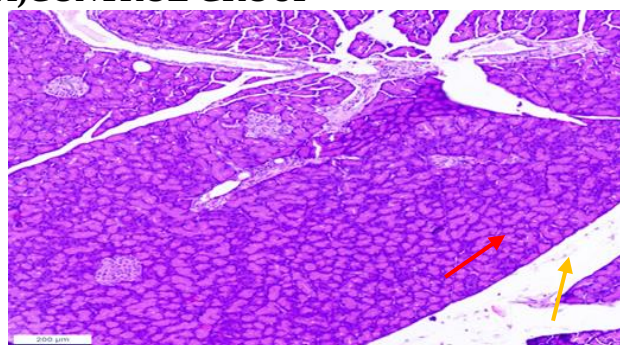


Fig 21: Control group

- Histopathological examination of H&E stained pancreas shows normal architecture of pancreatic cells. Black arrow represent pancreatic islet cells, red arrow indicates pancreatic duct and yellow arrow represents pancreatic acinar cells.

B) DISEASED GROUP

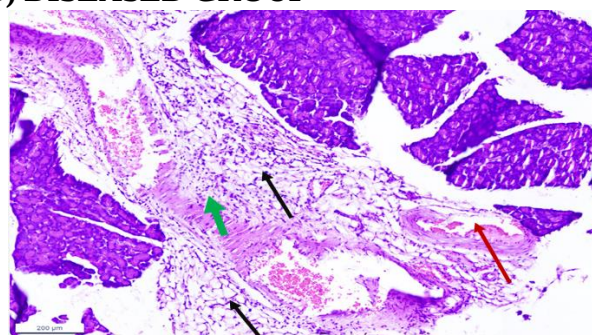


Fig 22: Diseased group

- Histopathological examination of H&E stained pancreas shows Marked degree of acinar atrophy (black arrow) and islets cells atrophy, Marked degree of inflammatory cells infiltrations (red arrow), predominantly lymphocytes (Green arrow) also in interstitial space and Loss of pancreatic duct architecture.

C)LOW DOSE TREATED GROUP

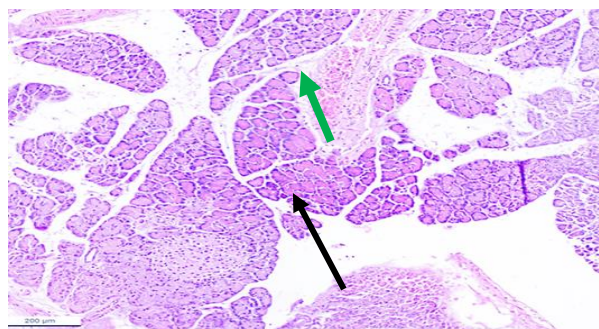


Fig 23: Low dose group

- Histopathological examination of H&E stained pancreas shows Moderate degree of acinar atrophy (black arrow) and moderate degree of inflammatory cells infiltrations, predominantly lymphocytes (Green arrow) also in interstitial space.

D) HIGH DOSE TREATED GROUP

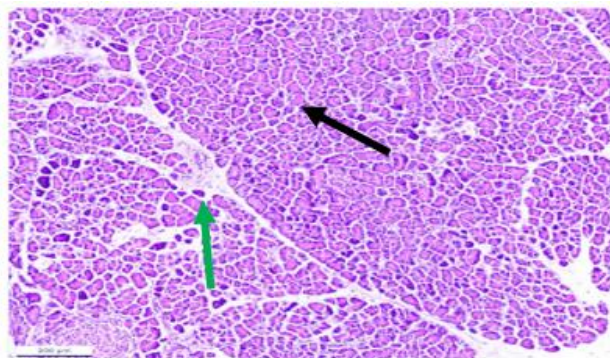


Fig 24: High dose group

- Histopathological examination of H&E stained pancreas show Minimal degree of acinar atrophy (black arrow), Mild degree of inflammatory cells infiltrations, predominantly lymphocytes (Green arrow) also in interstitial space And. Intact pancreatic duct architecture.

DISCUSSION

- Acute pancreatitis is an inflammatory condition that effects the exocrine pancreas. The regulator of the inflammatory responses in acute pancreatitis is NF-kB. It emphasizes that agents that control NF-kB are effective in the treatment of Acute pancreatitis. (Aybala et al 2023).
- Phyto remedies have shown a wide array of mechanisms behind alleviation of pancreatitis also which include alleviation of ROS

mediated damage and modulation of NF-kB mediated pathway.(Pratibha et al 2017)

- It has been shown that plant polyphenols such as flavonoids, have antioxidant and anti-inflammatory activity .(Aybala et al 2023).
- Oxidative stress is a major factor in acute pancreatitis formation and leading to multiple organ failure. Malondialdehyde, catalase are marker for estimation of oxidative stress. (Tosan Peter et al 2023).
- The plant extract decrease the lipid peroxidation and inflammation in this study supports previous reports on its anti inflammatory properties and analgesic activity.(Faisal Ahmed et al 2023).
- As per American College of Gastroenterology issued guideline for management of Acute pancreatitis includes the biochemical evidences of amylase and lipase level. Although the amylase and lipase level elevation specific markers for this disease.

CONCLUSION

To accelerate the therapeutic translation of Phyto remedies, focus on elucidating the molecular mechanism behind their potential pharmacological benefits. Herbal research should adhere to the same high standards as other medical research. The future of phytomedicines lies in the bottleneck of pharmacokinetics, where many potential herbal medication candidates fail. To improve bioavailability, delivery strategies such as spray drying, hot melt extrusion, nanocrystalline dispersions, self-emulsifying drug delivery, and nanoparticle formulation can be effective. The Phyto remedies has the potential to targeting the ROS mediated pathway and also alleviation of NFkB mediated redox imbalance. Very few are established rest of all do not conclude yet...

The purpose of this study was to determine if nelumbo nucifera protected rats from L-arginine-induced acute pancreatitis. In this investigation, twenty-four male Wistar rats were utilized, divided into four groups of six animals each, and given medication for three days. The pancreas is isolated and the animals are slaughtered after three

days. During the serum amylase estimation, the level of enzyme is decreased in the high dose treated group as compared to the diseased group which administered L-Arginine at 2.5g/kg dose. And the highest dose treated group shows the highly significant effect as compared to low dose treated group. In the evaluation of serum lipase, the enzyme is increased in the diseased group as compared to control group it indicates the pancreatitis and in the treatment group shows marked degree of decrease in the lipase level as compared to diseased group which indicates the reduction of the pancreatic inflammation. C-reactive protein is the enzyme which indicates the severity of acute pancreatitis disease hence the diseased group increases its level. In this study the treated group shows the decreased level of CRP level as compared to diseased group. Triglyceride is a marker for the severity of disease and this level also decreased in the treated group as compared to the diseased group. Also, the oxidative stress markers are noted the malondialdehyde level is decreased in the treatment group while catalase level increases. It indicates the inhibition capacity of oxidative stress by the extract. During the pro-inflammatory evaluation of TNF α and IL-6 shows a marked decrease in the treatment group which indicates the decrease in the inflammation. and the NF κ B gene expression also decreased in the treatment group. The gene expression in high dose treated group shows marked decrease in the expression which indicates the protective capacity of the extract and the high dose treated group shows marked degree in the gene expression. On histopathological examination the high dose treated group and the control group showing the similar morphological architecture of pancreatic acinar cells. In the diseased group shows the marked degree of acinar cell atrophy and inflammatory cells infiltrations are present and the normal architecture of pancreatic duct and acinar cells are damaged. Present study evaluated that the *Nelumbo nucifera* extract may have the protective effect in L-arginine induced Acute pancreatitis

model in Wistar Rats. Further studies are required to elucidate its mechanism at a better depth.

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CONFLICT OF INTEREST

All the authors have no conflict of interest..

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