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Screening of Natural Antioxidants by using L-Arginine induced acute Pancreatitis Model

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Abstract

Medicinal plants and their active constituents are traditionally used for herbal preparations and were proposed for their interesting antioxidant activities. Nearly all the medicinal plants are used for the therapeutic action and some of them are used in the investigation. Inflammation of pancreas of the exocrine part is called as acute pancreatitis. Inflammatory mediators and oxidative mediators are major factors for development of acute pancreatitis (AP). In the present study the protective effects of lawsone, myrcene, limonene, α-pinene and the underlying mechanisms in an experimental pancreatitis model. AP was induced in eleven groups of rats (n =6) by L-arginine (2x2.5 g/kg, intraperitoneal, 1 h apart) and 1 h later, they received a single oral dose of lawsone, myrcene, limonene, α- pinene, (100 and 200 mg/kg) respectively, vehicle (3% Tween 80) and methylprednisolone (30 mg/kg). A saline (0.9% NaCl) treated group served as a normal control. The efficacy was determined at 24 h by determination of serum levels of amylase, lipase and proinflammatory cytokines [tumor necrosis factor (TNF)-a, C-reactive proteins and interleukin (IL)], nitrate/nitrite levels, and the wet weight/body weight ratio. Lawsone, myrcene, limonene, α-pinene and methylprednisolone treatments significantly ($P < 0.05$) attenuated the L-arginine induced increases in pancreatic wet weight/body weight ratio, decreased the serum levels of amylase and lipase, TNF-a, IL-6 and significantly lowered pancreatic levels of TBARS, and nitrate/nitrite. The histoimmunological findings further proved the amelioration of pancreatic injury by lawsone, myrcene, limonene and α-pinene. Hence it proved anti-inflammatory and antioxidant agent property of lawsone, myrcene, limonene and α-pinene.

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Key words:
Acute pancreatitis, antioxidant, Inflammation, lawsone, methylprednisolone, L-arginine

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Introduction

Inflammation of pancreas of the exocrine part is called as acute pancreatitis. Inflammatory mediators and oxidative mediators are major factors for development of acute pancreatitis (AP). This may leads to complication as well as high mortality without treatment. The pathogenesis is not fully

understood, however the leukocyte activation, microcirculatory disturbances and oxidative stress are the major constituents of AP. This is characterized by activation of widespread inflammatory cell infiltration, leukocyte and digestive proteases. Further by releasing reactive oxygen, nitrogen species and various kinds of inflammatory mediators. Several factors are responsible for the AP, like alcohol, gallstones, hereditary pancreatitis, hypercalcemia, hyperlipidemia, malnutrition, abdominal trauma, penetrating ulcers, malignancy, drugs like steroids, sulfonamides, furosemide, thiazides, infections like mumps, coxsackie virus, mycoplasma pneumoniae, ascaris, clonorchis, and structural abnormalities like choledochocele and pancreas divisum. Repeated attacks of acute pancreatitis have the potential to develop into chronic pancreatitis or pancreatic cancer characterized by fibrosis and loss of acinar cell function [1, 2]. No specific treatment is available to treat AP. Many therapies and medical management is aimed to control the sign and symptoms of AP, using steroids, analgesics and anti-inflammatory agents. The use of the synthetic and semi-synthetic treatment has various kinds of drawbacks like photosensitivity skin reactions, intolerance and addiction. Apart from this these compounds are very expensive and not reliable. Hence, there is need of potential antioxidant & anti-inflammatory agent's available from natural sources, which are cost effective and have several advantages than the synthetic and semi synthetic compounds.

A naphthoquinone derivative and active constituent of henna (Lawsonia inermis L.) and it is used as hair dye. Lawsone had haematotoxic properties leads to stimulation of cell proliferation. Previous study has been reported for anti-oxidant, anti-inflammatory, anti-carcinogenic activity lawsone. The lawsone has haematotoxic properties, leading to stimulation of cell proliferation; represent a sufficient explanation for a weak induction of 'late micronuclei. As well as hepatic glutathione S-transferase and DT diaphorase specific activities were elevated above basal level.[3,4,5]. Alpha-pinene is one of the common monoterpenoids emitted from several aromatic plants and has growth-inhibitory activity. Previous study has been reported for antioxidant, anti-inflammatory, anti-carcinogenic activity for b-pinene [6, 7]. Myrcene is an olefinic natural organic compound. Previous study has been reported for its astringent and antibiotic properties and traditional uses in treatment insomnia, restlessness, nervous, tensions,irritability, charleyhorse. Also in modern medicine is antiseptic, antibacterial and antifungal and potentially giving them anticancer and uses for relaxation [8, 9]. D-limonene is a monocyclic monoterpene with a lemon-like odor and is a major constituent in several citrus oils. Because of its pleasant citrus fragrance, d-limonene is widely used as a flavor and fragrance additive in perfumes, soaps, foods, chewing gum, and beverages [10,11]. Hence in this study the non-toxic dose of lawsone, myrcene, limonene, α-pinene at non-toxic doses, which range from 100 to 200 mg/kg, were used to evaluate the potential effect to ameliorate pancreatic injury induced by L-arginine.

Materials and Methods:

Animals:

Sixty six male wistar rats obtained from Mahaveer Enterprises, Hyderabad and were maintained at a constant room temperature (23 ± 2^{O}C) with light-dark cycles of 12/12 h and free access to water and standard laboratory chow. The rats were randomly divided into eleven groups of six in each and experiments were performed after 12 h of fasting. Their body weights ranged between 180 and 200 g at the time of experimentation. Experimental protocols were approved by CPCSAE.

All the experimental procedures were carried out in accordance with committee for the purpose of control and supervision of experiments on animals

(CPCSEA) guidelines. The study was reviewed and approved by the Institutional Animal Ethics Committee (320/CPCSEA dated 03-01-2001), G.Pulla Reddy College of Pharmacy, Hyderabad-India.

Chemicals:

L-arginine (Sigma Aldrich Co Pvt Ltd, USA), lawsone, myrcene and b-pinene (Sigma Aldrich Co Pvt Ltd, Japan), limonene (Ayur Shanbagh, Banglore Karnataka), hexadecyltrimethylammonium bromide (HETAB) (Sigma Aldrich Co Pvt Ltd, Switzerland), odianisidinedihydrochloride, thiobarbituric acid (TBA) (Sigma Aldrich Co Pvt Ltd, Germany), griess reagent (Sigma Aldrich Co Pvt Ltd, Germany) and vanadium trichloride (Sigma Aldrich Co Pvt Ltd, USA) were purchased from sigma-aldrich chemical co. and all other chemicals and reagents were the highest commercial grade available.

L-arginine, powder: prepared as a solution by dissolving in 0.9% saline to a final concentration of 500 mg/ml and the pH was adjusted to 7 with 5 N HCl. Lawsone was prepared as a solution by dissolving in 3% tween 80 and 0.9% saline to a final concentration of 100 mg/ml, 200 mg/ml and the pH was adjusted to 7 with 0.1 N NaOH B-pinene was prepared as a solution by dissolving in 3% tween 80 and 0.9% saline to a final concentration of 100 mg/ml, 200 mg/ml and the pH was adjusted to 7 with 0.1 N NaOH. Limonene was prepared as a solution by dissolving in 3% tween 80 and 0.9% saline to a final concentration of 100 mg/ml, 200 mg/ml and the pH was adjusted to 7 with 0.1 N NaOH. Myrcene was prepared as a solution by dissolving in 3% tween 80 and 0.9% saline to a final concentration of 100 mg/ml, 200 mg/ml and the pH was adjusted to 7 with 0.1 N NaOH.

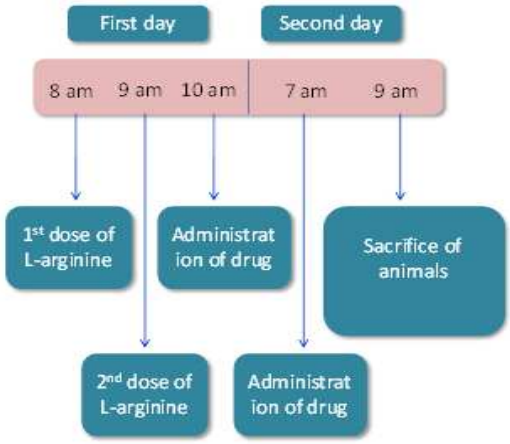
L-arginine-induced pancreatitis model

Acute pancreatitis was induced in eleven groups of rats (n =6) by two intraperitoneal (i.p) injections of Larginine (2.5 g/kg, 1 h apart). One hour following the last injection of L-arginine, the rats were treated orally as follows in table below:

Group	Dose
Group I (NC)	Receives saline orally
Group II (DC)	Receives two intraperitoneal injections of L-Arginine (2.5 g/kg,1 hr apart) and vehicle
Group III (Law 100)	Receives limonene 100 mg/kg/day/day, p.o. 1 hr after the last injection of L- arginine
Group IV (Law 200)	Receives limonene 200 mg/kg/day/day, p.o. 1 hr after the last injection of L- arginine
Group V (Pin 100)	Receives Lawsone 100 mg/kg/day/day, p.o. 1 hr after the last injection of L- arginine
Group VI (Pin 200)	Receives Lawsone 200 mg/kg/day/day, p.o. 1 hr after the last injection of L- arginine
Group VII (Lim 100)	Receives Myrcene 100 mg/kg/day/day, p.o. 1 hr after the last injection of L- arginine
Group VIII (Lim 200)	Receives Myrcene 200 mg/kg/day/day, p.o. 1 hr after the last injection of L- arginine
Group IX (Myr 100)	Receives Pinene 100 mg/kg/day/day, p.o. 1 hr after the last injection of L- arginine
Group X (Myr 200)	Receives Pinene 200 mg/kg/day/day, p.o. 1 hr after the last injection of L- arginine
Group XI (STD)	Receives methyl prednisolone 30 mg/kg/day, p.o. 1 hr after the last injection of L-Arginine

Twenty-four hours after the last injection of L-arginine or saline, a midline laparotomy was performed in rats under ether anesthesia and blood samples were collected from the inferior vena cava, the rats were then exsanguinated, the whole pancreas was quickly removed and stored at -70^{o}C until use. The pancreatic weight/bodyweight ratio was evaluated as an estimate of the degree of pancreatic edema (mg/g).

(a) Experimental design:



Macroscopic Evaluation:

Pancreas weight/body weight ratio:

The pancreas was removed immediately after the blood collection, trimmed free of fat and weighed. The pancreatic weight/body weight ratio (mg/g) was calculated for each animal, to estimate the level of pancreatic edema [12].

Serum analysis: For serum analysis, blood samples were centrifuged at 3000 ×g at 4°C for 10 minutes. The serum amylase and lipase were determined by routine colorimetric methods using the commercial kits for amylase (Rapid diagnostics), lipase (Accurex diagnostics), C-reactive protein and interleukin-á and expressed as U/dl [12]

Estimation of pancreatic total protein: Pancreatic total protein content was determined using the commercial kit for protein (Rapid diagnostics). Amount of protein in samples was expressed in g/dl [13].

Estimation of nitrate/nitrite levels

Nitrate/nitrite level estimation is a measure of nitric oxide produced. The pancreatic tissue was homogenised in 50 mmol/L potassium phosphate buffer (pH 7.8) & centrifuged at 11,000 g for 15 minutes at 4°C. 200 µl of supernatant was mixed with 200 µl saturated solution of vanadium trichloride in 1N HCl and immediately 200 µl Griess reagent (0.1% N- (1-naphthyl) ethylene diamidedihydrochloride, 1% sulfanilamide in 5% phosphoric acid) was added and mixed it, incubated at 37°C for 30 minutes and the absorbance was measured at 540 nm using auto analyser. The concentration was calculated from standard curve of sodium nitrite and expressed as micromoles of nitrate/nitrite [14, 15].

Estimation of malondialdehyde / thiobarbituric acid reactive substances (TBARS)

TBARS level in pancreatic tissue is a measure of lipid peroxidation. It was assayed using the principle and method described by Ohkawa et al. [16]. It depends on the fact that the MDA formed as a result of lipid oxidation react with TBA and produce TBARS ([TBA].2. MDA adduct) a pink chromogen. Briefly, 500 µl of 10% tissue homogenate in 0.15 mol/L KCl was mixed with 200 µl 8.1% SDS, and then incubated at room temperature for 5 min. The reaction mixture was heated at 95°C for 1 hr after the addition of 1.5 ml 20% acetic acid (PH 3.5) and 1.5 ml 0.8% thiobarbituric acid. After the mixture had cooled, 1ml distilled water and 5ml butanol/pyridine (15:1) solution were added under agitation using a vortex. This solution was centrifuged at 1000 × g for 15 min, and the resultant nbutanol layer was separated and the absorbance was measured at 532 nm against blank using Elico SL 164 spectrophotometer. The thiobarbituric acid reactive substance is calculated using 1.65×105 M-1Cm-1 as molar absorption coefficient and expressed as nmol/ml/mg protein. Tissue protein level was measured by colorimetric method using the commercial kit for protein (Rapid diagnostics).

Estimation of glutathione

Reduced GSH level was measured in pancreas using the method described by Sedlak and Lindasy [17]. The homogenate (in 0.1 M phosphate buffer, pH 7.4) was added with equal volume of 20 % trichloro acetic acid containing 1 mM EDTA to precipitate the tissue

proteins. The mixture was allowed to stand for 5 min prior to centrifugation for 10 min at 200 rpm. The supernatant (200 µl) was then transferred to a new set of test tubes and added 1.8 ml of the Ellman.s reagent (5, 5.-dithio bis-2-nitro benzoic acid (0.1 mM) was prepared in 0.3 M phosphate buffer with 1 % sodium citrate solution). Then all the test tubes make up the volume of 2 ml. After completion of the total reaction, solutions were measured at 412 nm against blank. The GSH concentration in the sample is calculated using the molar extinction coefficient of TNB (tri nitro benzoic acid) 14,150 M-1Cm-1 and expressed as µmol/l/g tissue.

Microscopic Evaluation:

1. Histopathological Evaluation:

Pancreas was removed immediately and part of it is fixed in 10% neutral buffered formalin and embedded in paraffin by standard methods. Paraffin sections of 5 µm were cut and stained with haematoxylin and eosin and then assessed under dark field microscope and examined blind by a morphologist for grading histopathological changes. Pancreatic damage was assessed & scored by grading acinar cell degeneration, interstitial inflammation, edema, and haemorrhage as described by schmidt's standards [18,19] with modification as follows: Grading for edema was scaled as 0: absent or rare; 1: edema in the interlobular space; 2: edema in the intralobular space; 3: isolated island shape of pancreatic acinus. Inflammation was scored as 0: absent; 1: mild; 2: moderate; 3: severe. Acinar cell necrosis was scored as 0: absent; 1: mild; 2: moderate; 3: severe. Parenchyma haemorrhage was scored as 0: absent; 1: mild; 2: moderate; 3: severe. The maximum score for acinar cell damage was 12.

Statistical analysis:

Statistical Analysis was performed by one way ANOVA followed by Bonferroni's Multiple Comparison Test as post hoc test using Graph pad Prism 5. Values of the measured parameters were presented as mean ± SEM. The difference was considered to be statistically significant when $p < 0.05$.

Result:

Serum biochemical parameters and pancreatic edema

As shown Table 02 and 03, Graph 9 and 10**,** induction of acute pancreatitis with L-arginine has significantly ($p < 0.001$) increased the serum amylase, lipase and pancreatic edema when compared with normal control group. Treatment with lawsone, myrcene, limonene, α-pinene (100 and 200 mg/kg) dose dependently decreased the serum amylase, lipase and pancreatic edema when compared with disease control. Similarly treatment with methyl prednisolone significantly decreased the serum amylase, lipase as well as pancreatic edema. Whereas, administration of lawsone, myrcene, limonene, α-pinene (200 mg/kg) prominently reversed the L-arginine evoked raise in amylase, lipase and edema and bring back to normal level.

Pancreatic total protein

As shown in Table 02 and 03, Graph 11, administration of L-arginine has significantly ($p < 0.001$) decreased the pancreatic total protein levels compared to normal control group. Treatment with lawsone, myrcene, limonene, α-pinene (100 and 200 mg/kg) dose dependently decreased the total protein compared to disease control group. However, treatment with lawsone (200 mg/kg) and methyl prednisolone (30 mg/kg) reversed the L-arginine evoked raise in pancreatic total protein and brings to normal level. Whereas, administration of both the doses of lawsone, myrcene, limonene, α-pinene (100 and 200 mg/kg) and methyl prednisolone (30 mg/kg) completely reversed the L-arginine evoked decrease in pancreatic total protein and brings to normal level.

Pancreatic MDA, nitrate/nitrite, GSH and antioxidant enzymes catalase:

As shown in Table 02 and 03, Graph 01 to 08, induction of pancreatitis resulted in a significant raise in MDA, nitrate/nitrite and catalase, and decline in GSH levels compared to normal control group. Treatment with lawsone, myrcene, limonene, α-pinene (100 and 200 mg/kg) dose dependently decreased the MDA, nitrate/nitrite and catalase and increased the GSH levels compared to disease control group and similar effect was observed with methyl prednisolone. Whereas, lawsone, myrcene, limonene, α-pinene (200 mg/kg) prominently reversed the L-Arginine evoked increase in pancreatic MDA, nitrate/nitrite and catalase levels.

Assessment of TNF, interleukins and C-reactive protein:

As shown in Table 04, 05 and 06, induction of pancreatitis resulted in a significant raise in TNF, interleukins and C- reactive protein compared to normal control group. Treatment with lawsone, myrcene, limonene, α-pinene (100 and 200 mg/kg) dose dependently decreased the TNF, interleukins and C- reactive protein compared to disease control group. Whereas, lawsone, myrcene, limonene, α-pinene (200 mg/kg) prominently reversed the L-Arginine evoked increase in pancreatic TNF, interleukins and C- reactive protein.

Table 2:

Parameter/Groups	N.C	D.C	STD	LW 100	LW 200	PIN 100	PIN 200
Pancreas weight	870.3±15.36	1015±19.0*	911.3±18.83α	843.3±46.33α	788.3±21.37α	862±83.67α	869±80.17α
Total body weight	187.5±4.889	192±3.578*	191.2± 3.60	188.7 ± 5.57	189 ± 3.68	188±18.85	174 ±18.7
Serum Amylase	2000±85.63	7667±349*	3317±110.8α	2733±140.6α	3167±187.4α	2933 ± 527.9α	3200 ± 46 α
Serum Lipase	191.7±4.014	566.7±30.84*	346.7±39.21α	236.7±14.06α	260±7.303α	233.3 ± 27.33α	250 ± 24.49α
Total Nitrate	11.87±1.372	16.07±1.462*	7.06±0.39α	10.13±1.31α	8.5±0.77α	0.85±0.796α	0.71±0.853α
Total Protein	0.73±0.032	0.355±0.037*	0.91±0.067α	0.73±0.02α	0.82±0.05α	0.73±0.02α	0.82±0.05α
Kidney GSH	0.47±0.011	0.284±0.04*	0.71±0.014α	0.51±0.017β	0.64±0.049α	0.10±0.049	0.10±0.061α
Liver GSH	0.48±0.010	0.284±0.04*	0.67±0.03α	0.48±0.03β	0.59±0.04α	0.10±0.049	0.08±0.060γ
Lung GSH	0.50±0.020	0.284±0.04*	0.67±0.03α	0.51±0.02γ	0.60±0.04β	0.08±0.039γ	0.10±0.047β
Pancreas GSH	0.48±0.011	0.284±0.04*	0.67±0.03α	0.52±0.02β	0.57±0.04α	0.10±0.049	0.08±0.060α
Kidney MDA	105.6±13.67	170.9±10.45*	135.2±16.42β	119.4±12.77α	127.1±12.16α	121.6±17.5α	129.4±13.55α
Liver MDA	105.6±13.67	170.9±10.45*	135.2±16.42γ	122.6±17.42α	132±22.09c	119.6±17.54α	126.9±16.51β
Lung MDA	14.95±0.66	17.09±1.04	9.82±1.04β	13.18±1.7α	11.5±0.66α	13.23±1.291α	14.24±1.62α

Table 3:

Parameter/Groups	N.C	D.C	STD	LIM 100	LIM 200	MY 100	MY 200
Pancreas weight	870.3±15.36	1015±19.0*	911.3±18.83α	843.3±46.33α	788.3±21.37α	862±83.67α	869±80.17α
Total body weight	187.5±4.889	192±3.578*	191.2± 3.60	188.7 ± 5.57	189 ± 3.68	188±18.85	174 ±18.7
Serum Amylase	2000±85.63	7667±349*	3317±110.8α	2600±469a	2967±388.2a	2733±206.6a	2933±163.3a
Serum Lipase	191.7±4.014	566.7±30.84*	346.7±39.21α	226.7±24.22a	260±25.3a	213.3±20.66a	256.7±19.66a
Total Nitrate	11.87±1.372	16.07±1.462*	7.06±0.39α	0.53±0.81a	0.35±0.86a	0.63±0.773a	0.53±0.82a
Total Protein	0.73±0.032	0.355±0.037*	0.91±0.067α	0.68±0.071a	0.88±0.075a	0.62±0.07a	0.89±0.073a
Kidney GSH	0.47±0.011	0.284±0.04*	0.71±0.014α	0.14±0.053c	0.13±0.045	0.10±0.046	0.17±0.049
Liver GSH	0.48±0.010	0.284±0.04*	0.67±0.03α	0.12±0.048	0.13±0.044	0.11±0.044	0.17±0.049
Lung GSH	0.50±0.020	0.284±0.04*	0.67±0.03α	0.10±0.049b	0.09±0.053a	0.084±0.046c	0.11±0.060a
Pancreas GSH	0.48±0.011	0.284±0.04*	0.67±0.03α	0.12±0.048	0.13±0.044	0.11±0.044	0.17±0.049
Kidney MDA	105.6±13.67	170.9±10.45*	135.2±16.42β	117.3±16.64a	132.3±15.42b	124.2±19.64a	127.2±16.82a
Liver MDA	105.6±13.67	170.9±10.45*	135.2±16.42γ	119.3±12.57a	129.1±25.19b	115.9±7.104a	134.4±20.8c
Lung MDA	14.95±0.66	17.09±1.04	9.82±1.04β	15.28±1.321b	15±1.132a	22.58±1.217a	12.24±1.107a

LW1: Lawsone 100, LW2: Lawsone 200, Pin1: pinene 100, Pin2: Pinene 200, Lim1: Limonene 100, Lim2: Limonene 200, My1: Myrcene 100, My2: Myrcene 200.
Data was analysed by one way ANOVA using Bonferronis multiple comaprision test and the results were as follows:
Disease control with normal control: * (P<0.0001)
Treated groups with disease control: a(P<0.0001); b(P<0.001); c(P<0.01)
Treated group with normal control ; α(P<0.0001); β(P<0.001); γ(P<0.01)

Table 4: IL-6 values

Animal number	N.C	D.C	LW 100	LW 200	PIN 100	PIN 200	LI 100	LI 200	MY 100	MY 200
1	28.6	91.2	63.4	34.1	69.3	38.4	59.3	35.3	64.4	31.8
2	24.3	85.6	67.2	29.8	64.4	27.4	46.1	32.5	67.2	36.8
3	32.6	96.3	58.6	37.4	63.5	44.6	51.5	26.7	56.3	29.6
4	34.5	94.1	64.8	36.2	65.6	33.1	64.6	42.2	65.6	25.4
5	26.1	87.4	61.2	32.5	70.2	31.4	56.2	35.4	69.2	34.4
6	29.7	88.3	59.3	31.4	68.4	37.2	58.3	33.1	63.7	28.2

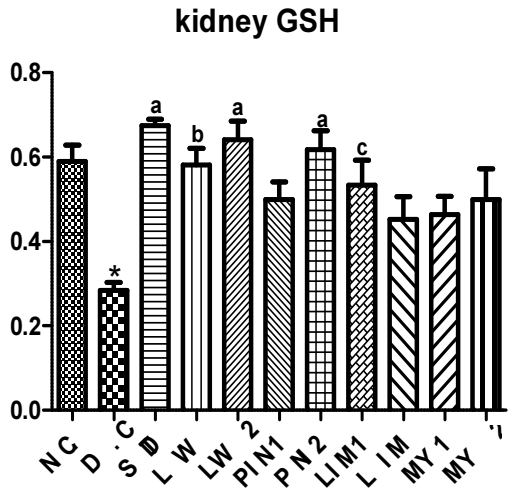
Table 5: CRP values

Animal number	N.C	D.C	LW 100	LW 200	PIN 100	PIN 200	LI 100	LI 200	MY 100	MY 200
1	405	16405	8642	551	7452	574	7621	498	7947	521
2	434	16530	8221	509	7556	621	7565	527	7681	547
3	426	15984	8427	527	7243	596	7643	531	7745	561
4	384	16840	8742	573	7621	554	7234	487	8011	492
5	412	16204	8921	564	7484	521	7095	457	7647	510
6	431	16456	8712	557	7261	511	7125	504	7421	489

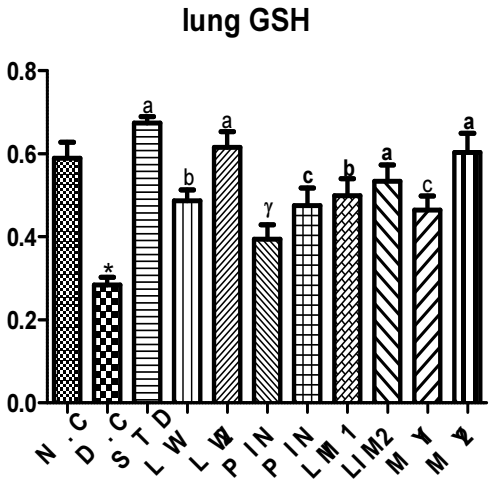
Table 5: Serum TNF-αvalues (pg/ml)

Animal number	N.C	D.C	LW 100	LW 200	PIN 100	PIN 200	LI 100	LI 200	MY 100	MY 200
1	18.3	26.2	23.4	14.1	29.3	18.4	21.3	15.3	24.4	13.8
2	14.2	28.3	22.2	19.8	24.4	17.4	26.1	12.5	27.2	16.8
3	22.6	26.3	18.6	17.4	23.5	14.6	21.5	23.7	16.3	19.6
4	24.5	14.1	24.8	16.2	15.6	23.1	14.6	17.2	25.6	21.4
5	16.8	37.4	21.2	22.5	20.2	13.4	26.2	15.4	19.2	14.4
6	19.6	25.6	19.3	21.4	18.4	17.2	18.3	18.1	23.7	18.2

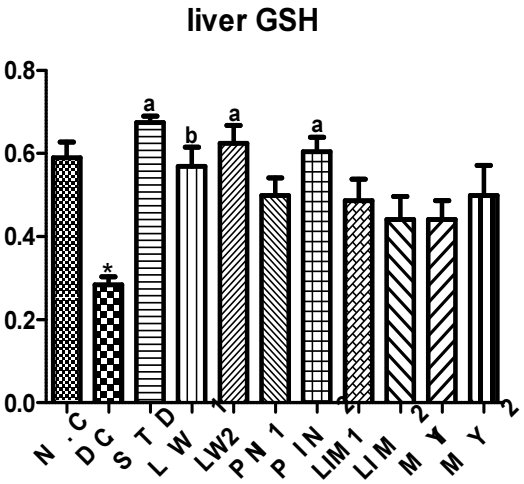
NC- Normal Control, DC- Disease Control, STD-Standard, LW 100- Lawsone 100 mg/kg, LW 200- Lawsone 200 mg/kg , PIN 100- B-pinene 100 mg/kg, PIN 200- B-pinene 200 mg/kg, LI 100-Limonene 100 mg/kg, LI 200-Limonene 200 mg/kg, MY 100-myrcene 100 mg/kg and MY 200-myrcene 200 mg/kg



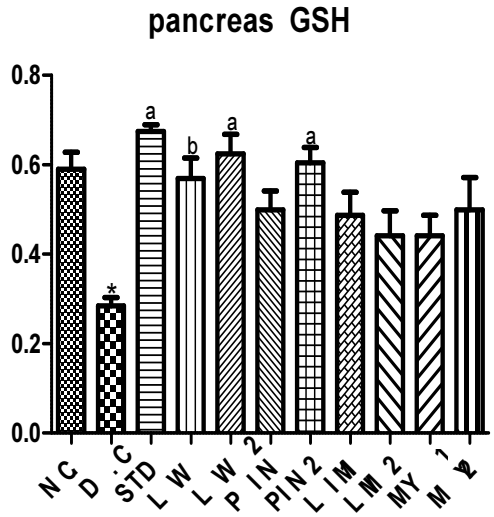
Graph 1



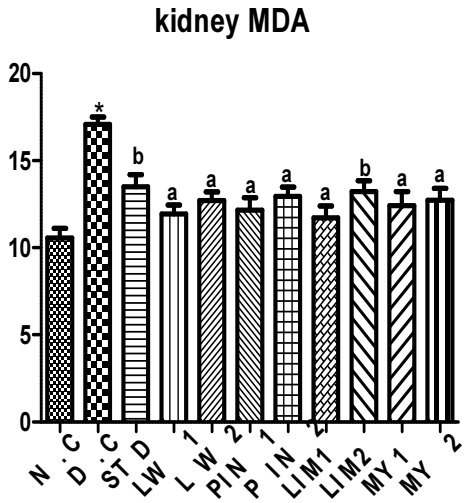
Graph 2



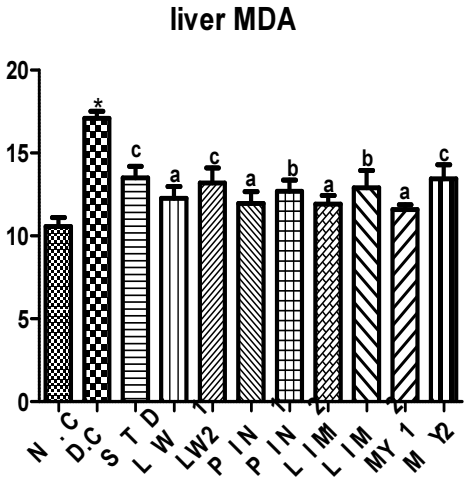
Graph 3



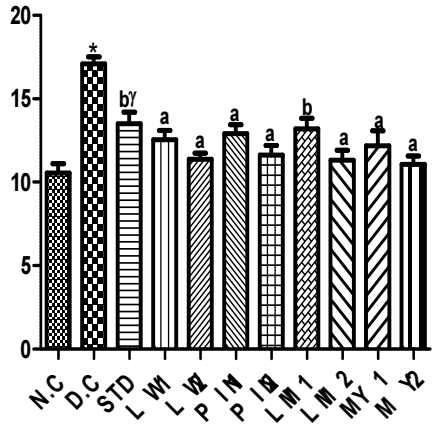
Graph 4



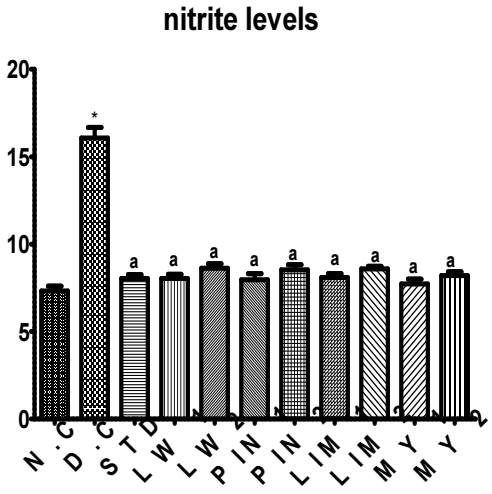
Graph 5



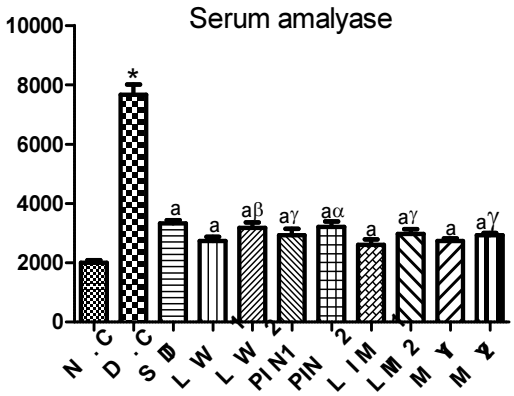
Graph 6



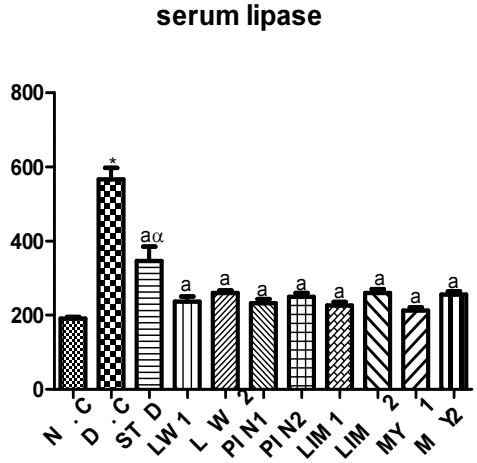
Graph 7



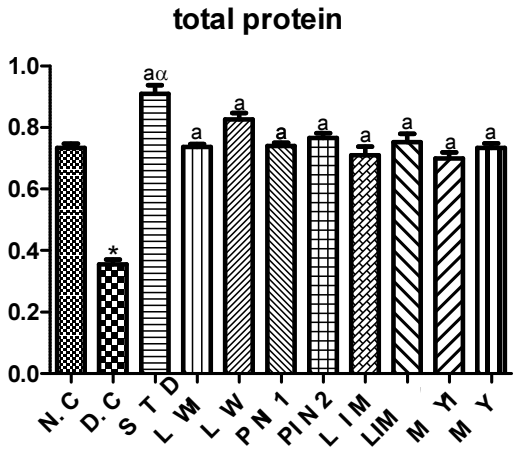
Graph 8



Graph 9

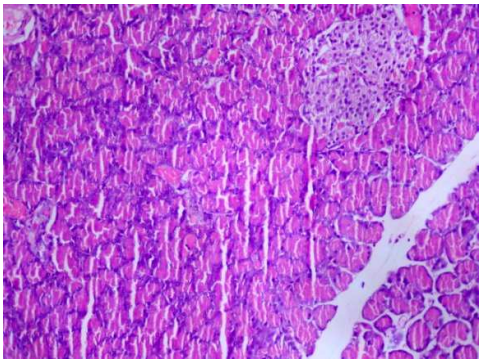


Graph 10

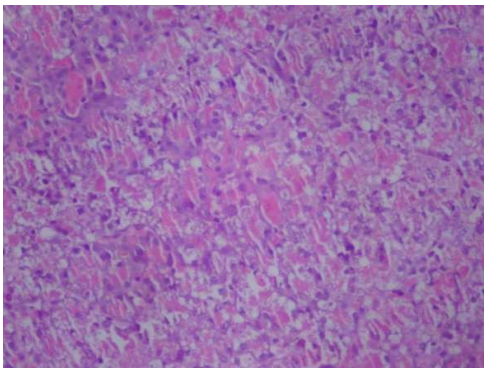


Graph 11

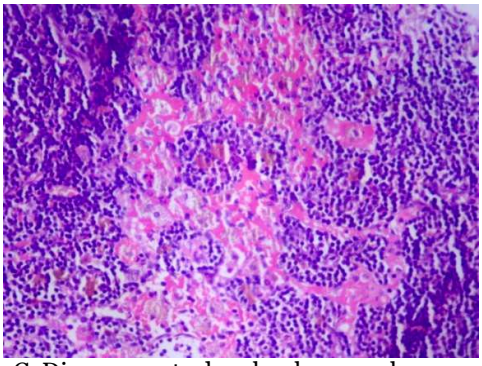
Histopathology:



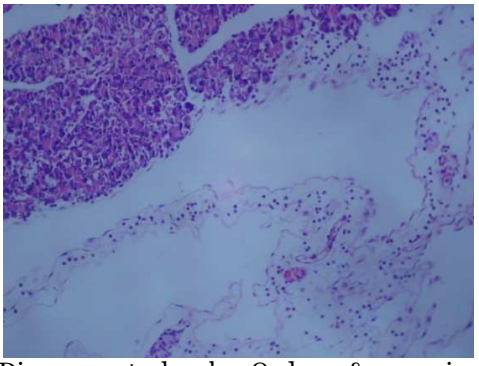
a. Normal Control



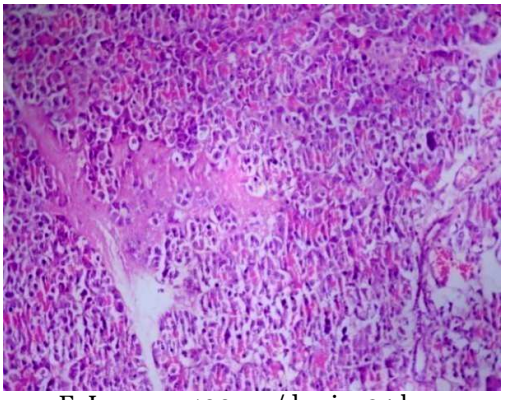
b. Standard Control



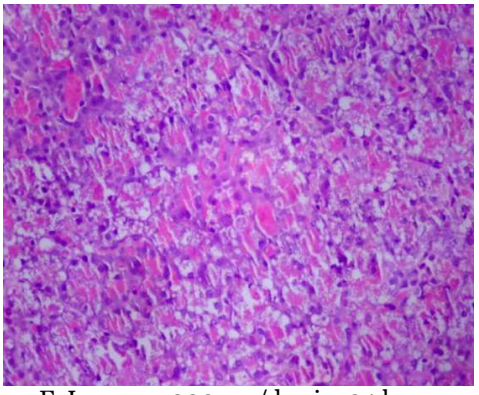
C. Disease control 24 hrs haemorrhage



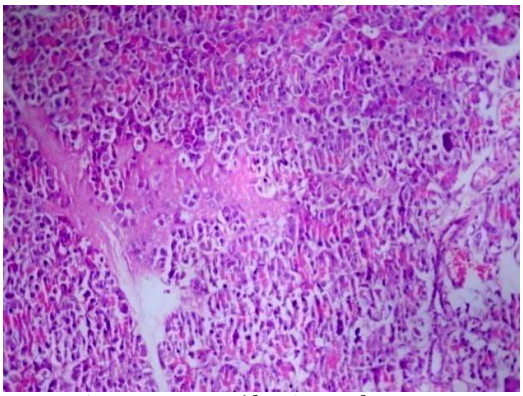
D. Disease control 24 hrs Oedema & necrosis



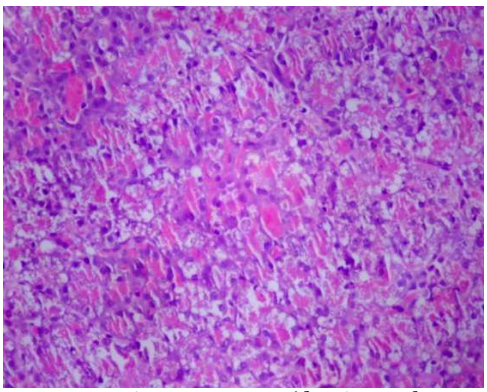
E. Lawsone 100 mg/ kg, ip, 24 hrs



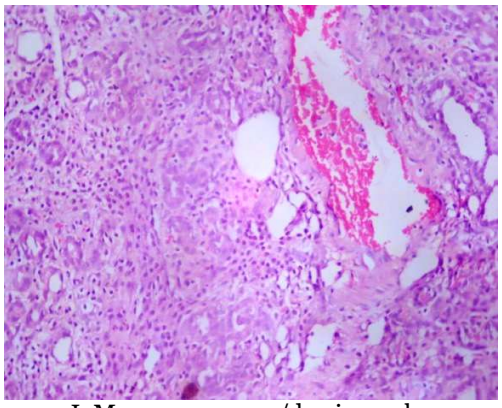
F. Lawsone 200 mg/ kg, ip, 24 hrs



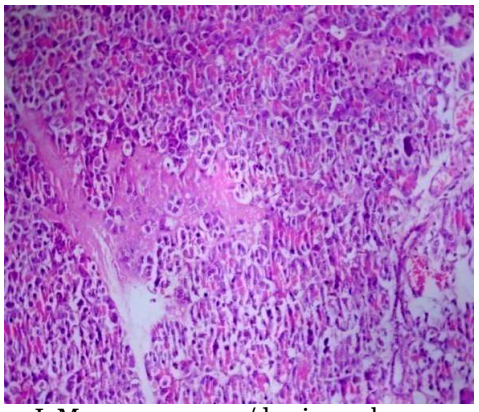
G. Pinene 100 mg/ kg, ip, 24 hrs



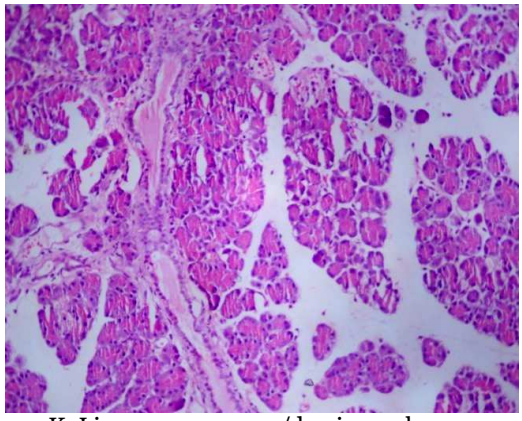
H. Pinene 200 mg/ kg, ip, 24 hrs



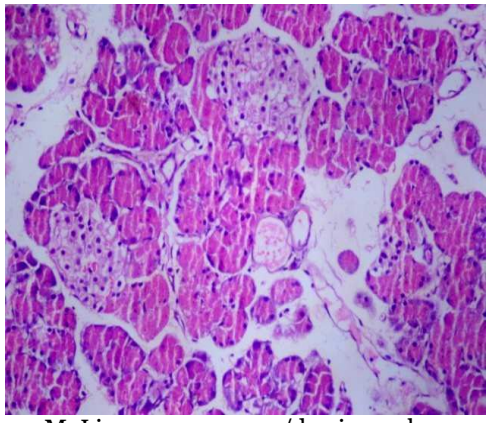
I. Myrcene 100 mg/ kg, ip, 24 hrs



J. Myrcene 200 mg/ kg, ip, 24 hrs



K. Limonene 100 mg/ kg, ip, 24 hrs



M. Limonene 100 mg/ kg, ip, 24 hrs

Pancreatic histology

As shown in Fig 1, histological examination of normal control group (saline treated) showed normal architecture and absence of edema, neutrophil infiltration, haemorrhage and necrosis. Whereas, pancreatic sections of disease control group showed extensive tissue damage characterized by acinar cell degeneration, necrosis, edema, mononuclear cell infiltration, haemorrhage and thus received significantly higher scores. Treatment with lawsone, myrcene, limonene, α-pinene (100 and 200 mg/kg) and 200 mg/kg) and methyl prednisolone (30 mg/kg) ameliorated the inflammation, edema and more significantly acinar cell degeneration and necrosis and protected the pancreas from L-arginine induced damage. On other hand, treatment with lawsone dose dependently decreased the total pathological scores compared to disease control group.

Discussion:

The present study demonstrated that treatment with lawsone, myrcene, limonene, α-pinene (200 mg/kg respectively) efficiently reduced the severity of L-arginine induced acute pancreatitis in rats. In consistent with previous reports [20, 21], in the present study administration of L-arginine significantly developed the acute pancreatitis characterized by raised levels of serum amylase, lipase and acinar cell necrosis.

Serum amylase and lipase levels are the important diagnostic markers for acute pancreatitis. They usually rise within 4-8 hours of the initial attack, peaks at 24 hours [20,21]. Similarly, in accordance with previous reports, in the present study induction of pancreatitis significantly increased the serum amylase and lipase levels at 24 hours. Treatment with lawsone, myrcene, limonene, α-pinene dose

dependently decreased the serum amylase and lipase levels, indicates protective effect of lawsone at early stage of the disease progression.

In consistent with previous reports [22, 23], in the present study induction of pancreatitis significantly increased the pancreatic total protein, MDA, nitrite, catalase and SOD and decreased the GSH levels.

MDA, a marker of lipid peroxidation was elevated in L-arginine treated rats. Lipid peroxidation is a process mediated by free radicals, which results in impairment of the membrane functional and structural integrity [24, 25] as a consequence of oxidative deterioration of polyunsaturated fatty acids of cell membrane. It could be attributed to the accumulation of free radicals proposed to be generated by L-arginine.

A few studies reported the fall in these enzyme levels at 24 hours [23]. Whereas others reported the raised levels of these enzymes [24].

The role of NO in the initiation and progression of acute pancreatitis remains controversial [22]. Some studies [26, 27, 28] reported that NO increase the pancreatic blood flow and/or secretion in response to endothelium derived NO and ameliorates the pancreatic dysfunction, whereas others [30,31] suggested that NO aggravates pancreatic oxidative stress and damage. In agreement with previous reports [31], in the present study significant increase in NO and pancreatic edema was observed in L-arginine received rats. Previous investigations [31] demonstrated that, administration of excess L-arginine could induce iNOS activity and increase the NO levels in pancreas. The raised levels of NO can increase vascular/micro capillary permeability and may contribute to the pancreatic edema and acinar cell damage [31].

Treatment with lawsone, myrcene, limonene, α-pinene significantly restored the pancreatic MDA, nitrite, edema, catalase, and GSH in L-arginine received rats. Passaglia [30] stated that acinar cells are the protein factory of the body. In acute pancreatitis, catabolism of proteins could increase up to 80%. Consequently, a sharp decline in protein content was observed in pancreas. In consistent with previous reports [23], pancreatic total protein content, a marker of the tissue damage was found to decrease in L-arginine received rats. Treatment with lawsone, myrcene, limonene, α-pinene significantly increased the total protein content.

It is well known that the extent of pancreatic tissue damage in acute pancreatitis correlates with the levels of inflammatory mediators (interleukin, TNF and CRP) and free radicals. In agreement with previous reports [19,20 31], in the present study, histopathological assessments revealed that, induction of pancreatitis resulted in pancreatic damage characterized by acinar cell necrosis, mono nuclear cell infiltration, edema and haemorrhage. Treatment with lawsone, myrcene, limonene, α-pinene protected the pancreas from L-arginine induced injury.

In conclusion, the present study suggests that treatment with lawsone, myrcene, limonene, α-pinene significantly ameliorated the severity of L-arginine induced pancreatitis by reducing the neutrophil infiltration and oxidative stress markers and this effect may be due to antioxidant and anti-inflammatory properties of the lawsone, myrcene, limonene, α-pinene.

Disclaimer

The views and opinions expressed in this article are those of the authors, and they do not reflect in any way those of the institutions to which they are affiliated with.

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