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L-citrulline modulates pancreatic enzyme activity and biochemical markers in adult female Wistar rats with metabolic syndrome

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Abstract: **Background**: Metabolic syndrome (MetS) induces profound pancreatic damage characterised by oxidative stress and chronic inflammation. **Objectives**: This study investigated the protective effects of L-citrulline (L-CIT) in the pancreas of a MetS animal model by evaluating oxidative stress responses, inflammatory cytokines, interferon gamma (IFN-γ), aldose reductase (AKR1B1), glucose kinase-3β, abdominal fat and body weight. **Methods**: The animals (Wistar rats; *Rattus norvegicus*) were divided into five groups (n = 6), three of which received MetS induction via a 20% fructose solution and received oral L-CIT at escalating doses (200, 400, or 800 mg/kg). Group 1 served as the normal control, while group 2 served as the negative control (20% fructose solution). Post-treatment, pancreas samples were collected for biochemical analysis to determine the dose-dependent effects of L-CIT. **Results**: Pancreatic oxidative stress was significantly reduced ($P< 0.05$) in the L-CIT-treated groups vs the MS-untreated group. TNF-α, IL-1β and IL-6 were significantly reduced ($P< 0.05$) with L-CIT treatment vs the MS-untreated group. However, IL-10 was significantly increased ($P < 0.05$) in the L-CIT group compared with the MS-untreated group. Pancreatic IFN-γ was significantly ($P< 0.05$) reduced in the L-CIT groups compared to the MS-untreated group, whereas the changes in pancreatic BDNF were not statistically significant. Both pancreatic AKR1B1 and GSK-3β were significantly reduced ($P< 0.05$) in the groups given L-CIT vs the MS-untreated group. Abdominal fat and body weight were significantly lower ($P< 0.05$) in the L-CIT groups than in the MS-untreated group. **Conclusion**: L-CIT holds promise as a therapeutic supplement for preserving pancreatic health in metabolic disease.

Keywords: Cytokines; Inflammation; L-citrulline; Metabolic syndrome; Oxidative stress; Pancreas

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INTRODUCTION

Metabolic syndrome (MetS) is linked to chronic inflammation and oxidative stress, which impair pancreatic function and enzyme activity (Monserrat-Mesquida *et al*., 2020). These biochemical disturbances, including altered glucose and lipid metabolism, immune dysregulation, endothelial dysfunction, and microbiota imbalance, connect MetS with diabesity, cardiovascular disease, and neurodegeneration (Martemucci *et al.,* 2023). Female Wistar rats are often used in MetS studies due to sex-specific metabolic and hormonal differences. Estrogen may enhance L-Citrulline's (LCIT) anti-inflammatory and metabolic effects, though further research is needed (Harding and Heaton, 2022).

Pancreatic enzymes are particularly affected in MetS. Aldose reductase (AR), upregulated under hyperglycemia, contributes to oxidative damage and apoptosis through redox imbalance (Hamaoka *et al*., 1993; Flores-Lopez *et al*., 2024). Glycogen synthase kinase 3 beta (GSK-3β), a regulator of glycogen synthase, impairs insulin signalling when overactive and has been implicated in inflammation (Pitasi *et al*., 2020). Deletion of GSK-3β in β-cells increases insulin levels and glucose tolerance, but its role in MetS remains unclear (Liu *et al*., 2010). Pro-inflammatory cytokines such as IL-1β, TNF-α and IFN-γ are known to induce β-cell apoptosis in diabetes, yet their activity in MetS is poorly understood (Cieslak *et al*., 2015; Abdel-Moneim *et al*., 2022). Adult female Wistar rats were selected because they are a well-characterized and widely accepted model for studying metabolic syndrome and also have fully developed metabolic and pancreatic systems. This animal model provides a biologically relevant and translationally meaningful framework for exploring the potential effects of L-citrulline on metabolic syndrome-related pancreatic dysfunction in humans.

LCIT, a non-essential amino acid from watermelon, shows promise as a therapeutic agent due to its antioxidant, anti-inflammatory and nitric oxide-enhancing properties (Volino-Souza *et al*., 2022). Unlike L-arginine, LCIT bypasses arginase metabolism, making it more effective for nitric oxide synthesis (Bahareh *et al*., 2018). Evidence suggests LCIT reduces oxidative stress, improves insulin sensitivity and modulates cytokines such as IL-6 and TNF-α (Abbaszadeh *et al*., 2021; Swentek *et al*., 2021). However, little is known about its effects on pancreatic inflammation and enzyme activity in MetS animal models.

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Addressing this gap may reveal new therapeutic strategies. This study, therefore, investigates LCIT's impact on pancreatic enzymes, cytokines and biochemical markers in female Wistar rats with MetS.

MATERIALS AND METHODS

All reagents were measured using a Changzhou Xingyun XY100C weighing machine (No. 1404273). The primary reagents used were D-fructose (Batch MCR-12863, Mumbai, India) and L-citrulline (NOW FOODS, USA). For biochemical analysis, a Vis Spectrophotometer S23A (Axiom UK) was utilised, along with commercial kits from Randox Laboratories (UK) and FineTest (Wuhan, China). The FineTest kits were specifically used to measure the levels of Rat GSK-3, TNF-alpha, IL-1 Beta, IL-10, IL-6, AKR1B1, GSK-3β, BDNF and INFγ.

Animal handling and experimental design

Thirty female Wistar rats (*Rattus norvegicus*) (aged 8–10 weeks, weighing 160–180 g) were obtained from the breeding facility at Ahmadu Bello University, Zaria and were confirmed to be healthy, immunocompetent, non-pregnant and disease-free at procurement. All animals were non-genetically modified and had not undergone any prior experimental procedures other than standard laboratory acclimatization to minimize stress. Animals were housed in a ventilated polycarbonate cage under a 12-hour light/dark cycle, lights on at 06:00, with unrestricted access to standard chow and water. Following a two-week acclimation period, rats were randomly allocated into five groups (n = 6 per group) using computer-generated randomisation (block randomisation, block size 4). Group allocation was concealed via coded identifiers maintained by an independent investigator. Investigators involved in animal handling were unaware of group allocation at the time of assignment. Due to the nature of the intervention, personnel administering L-citrulline were aware of group allocation but were not involved in outcome assessment or data analysis. All assessments of biochemical markers were conducted by investigators blinded to treatment groups, with samples coded before analysis. Data analysis was performed using coded group identifiers, and unblinding occurred only after completion of statistical analyses. A priori power analysis indicated that n = 6 per group provided > 99% power to detect a large effect size at α=0.05. All procedures were approved by the Ahmadu Bello University Animal Care and Use Committee (ABUCAUC/2024/082). Group 1 (normal control, N_CNT) received distilled water via oral gavage. Group 2 (metabolic syndrome untreated, MS-untreated) received 20% fructose solution *ad libitum* plus vehicle gavage. Groups 3 – 5 received 20% fructose solution *ad libitum* plus L-citrulline (L-CIT) at 200, 400, or 800 mg/kg/day, respectively, via oral gavage once daily at 09:00 for six weeks. Doses were selected based on prior literature (Danboyi *et al*., 2020) to evaluate dose-dependent effects. The primary outcomes were oxidative stress biomarkers (malondialdehyde, superoxide dismutase, catalase), inflammatory markers (TNF-α, IL-6, IL-1β) and enzymatic markers; secondary outcomes included waist circumference, body weight and metabolic parameters. Animals exhibiting >10% weight loss or gavage complications were excluded from analysis. Animals were monitored daily for signs of distress or adverse effects, including reduced food or water intake, significant weight loss, lethargy, abnormal posture, impaired mobility, ruffled fur, or signs of severe metabolic decompensation. A study protocol detailing the research question, experimental design, outcome measures and statistical analysis plan was prepared before commencement of the study. The protocol was not prospectively registered in a public registry.

Induction of metabolic syndrome

To induce MetS, a 20% fructose solution was prepared by dissolving 20 g of fructose in 100 mL of tap water. This solution was administered over six weeks and stored in foil-covered bottles to prevent microbial growth (Mamikutty *et al*., 2014).

Abdominal circumference, body weight and body length

To assess anthropometric parameters, body weight (BW) was measured at the beginning and end of the experiment. Abdominal (AC) was measured with a tape, while body length (BL) was recorded from nose to anus (Novelli *et al*., 2007). All measurements were taken under anaesthesia.

Collection of blood and tissue samples

Rats were fasted overnight before being anaesthetised with pentobarbital at a dose of 60 mg/kg. Following anaesthesia, blood was drawn via cardiac puncture and collected in plain tubes. These tubes were then centrifuged at 3000 g for 10 minutes to separate the serum for biochemical analysis. Concurrently, the liver was excised, rinsed with a cold phosphate buffer, and a portion was homogenised for subsequent biochemical analysis (Arumugam *et al*., 2023)

Estimations of oxidative stress biomarkers

To measure the level of pancreatic malondialdehyde (MDA), the protein concentration of the samples was determined, and the MDA level was then estimated at 532 nm, expressed as nmol/g of tissue (Ohkawa *et al*., 1979). The activity of superoxide dismutase (SOD) was determined by measuring its ability to inhibit the reduction of nitro blue tetrazolium (NBT) to blue-colored formazan by superoxide radicals. The estimation was carried out at 560 nm (Misra and Fridovich, 1972). Reduced glutathione (GSH) levels were measured using a commercial assay kit (E-BC-K030 Elabscience, China), while catalase (CAT) activity was determined using Claiborne's method (Claiborne, 1985). Protein concentrations were quantified using the Bradford assay (Bradford, 1976).

Assessment of pancreatic inflammatory and enzymatic biomarkers

Commercially available ELISA kits from FineTest (Wuhan, China) were used to assay tumour necrosis factor-alpha (ER1393), interleukin 1β (ER1094), IL-6 (ER0042), IL-10 (ER0033), interferon-γ (ER0012), brain-derived neurotrophic factor (ER0008), pancreatic aldose reductase (AKR1B1, ER1597) and glycogen synthase kinase-3 beta (GSK-3β, ER0060) according to the manufacturer's instructions.

Data analysis

All outcome variables were assessed to determine whether they met the assumptions required for parametric statistical testing. Data normality was evaluated using the Shapiro–Wilk test, while homogeneity of variances between groups was assessed using Levene's test. Visual inspection of histograms and Q–Q plots was also performed to support the results of formal tests. The data satisfied these assumptions, with no violations of normality. Statistical analysis was conducted using IBM SPSS version 27. All results are presented as the mean ± SEM. A one-way analysis of variance (ANOVA) was used to determine differences between groups, followed by Tukey's post hoc test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Malondialdehyde, superoxide dismutase, reduced glutathione and catalase

In Fig. 1a, pancreatic MDA was significantly raised ($P< 0.001$) in all the groups vs the normal control (N_CNT). However, in the groups given L-CIT, there was a significant ($P< 0.001$) dose-dependent decrease in pancreatic MDA levels compared with the MS-untreated group. Pancreatic SOD in Fig. 1b was significantly decreased ($P< 0.05$) in all the groups vs N_CNT. In the group given L-CIT at 800 mg/kg, SOD was significantly ($P< 0.001$) higher than in the MS-untreated group. There was a significant decrease in pancreatic GSH (Fig. 1c) across all groups except the group receiving the highest dose of LCIT, compared with N_CNT. However, in the groups given L-CIT at 400 and 800 mg/kg, it was significantly higher ($P<0.001$) than in the MS-untreated groups. Pancreatic CAT (Fig. 1d) was significantly reduced across all groups compared with N_CNT. A dose-dependent increase was observed in the L-CIT-treated groups compared with the MS-untreated group.

Tumour necrosis factor alpha, interleukin-1 beta, interleukin-6 and interleukin-10

In Fig. 2a, TNF-α was significantly higher ($P< 0.001$) in all the groups vs the N_CNT. In the L-CIT 400 and 800 mg/kg-treated groups, TNF-α was significantly reduced ($P< 0.001$) vs the MS-untreated and L-CIT 200 mg/kg groups. IL-1β in Fig. 2b was significantly higher ($P< 0.05$) in all the groups except L-CIT 800 mg/kg vs the N_CNT group. L-CIT at higher doses of 400 and 800 mg/kg reduced IL-1β vs the MS-untreated group. In Fig. 2c, IL-6 was significantly higher ($P<0.05$) in all the groups vs N_CNT. In the L-CIT-treated groups at 400 and 800 mg/kg, IL-6 was significantly lower than in the MS-untreated and L-CIT 200 mg/kg groups. In Fig. 2d, IL-10 was significantly reduced ($P<0.001$) in the groups vs N_CNT. Treatment with L-CIT significantly ($P<0.001$) increased IL-10 in the groups given L-CIT at 400 and 800 mg/kg vs the MS-untreated and L-CIT 200 mg/kg groups.

Interferon gamma, brain-derived neurotrophic factor, aldose reductase and glycogen synthase kinase 3-beta

In Fig. 3a, INF-γ was significantly ($P< 0.001$) higher in the MS-untreated and L-CIT 200 mg/kg groups vs N_CNT. In the groups receiving L-CIT at 400 and 800 mg/kg, INF-γ was significantly lower ($P< 0.05$) than in MS-untreated and L-CIT 200 mg/kg groups. No significant change in pancreatic BDNF was observed across all treated groups. However, there was a non-significant ($P> 0.05$) increase in BDNF (Fig. 3b) in all the L-CIT-treated groups compared to the MS-untreated group. In Fig. 3c and 3d, AKR1B1 and GSK-3β were significantly higher in the MS-untreated group and L-CIT 200 mg/kg vs N_CNT. However, in the groups given L-CIT at 400 and 800 mg/kg, both were significantly ($P< 0.001$) reduced vs MS-untreated and L-CIT 200 mg/kg.

Abdominal circumference, body length and percentage body weight increase

The abdominal body circumference (AC) is shown in Fig. 4a and was significantly higher ($P< 0.05$) in all the groups except L-CIT 800 mg/kg vs N_CNT. In the L-CIT 800 mg/kg, AC was significantly reduced compared to the MS-untreated, L-CIT 200 and 400 mg/kg groups. There was no statistically significant difference in body length ($P> 0.05$) in Fig. 4b. The percentage body weight increase was significantly higher ($P< 0.001$) in the MS-untreated group vs the N_CNT. In all the L-CIT-treated groups, this was significantly reduced vs the MS-untreated group.

DISCUSSION

Metabolic syndrome (MetS) often involves hyperglycemia, hyperlipidemia and insulin resistance, which overload pancreatic cells and increase mitochondrial activity, leading to excess reactive oxygen species (ROS) (Masenga *et al*., 2022). ROS attack polyunsaturated fatty acids (PUFAs) in cell membranes, initiating lipid peroxidation, as seen in the MS-untreated group of this study. Adipose tissue in MetS secretes pro-inflammatory cytokines such as TNF-α and IL-6, which further stimulate ROS production and worsen lipid peroxidation (Masenga *et al*., 2022). This aligns with the elevated TNF-α and IL-6 observed in the untreated group.

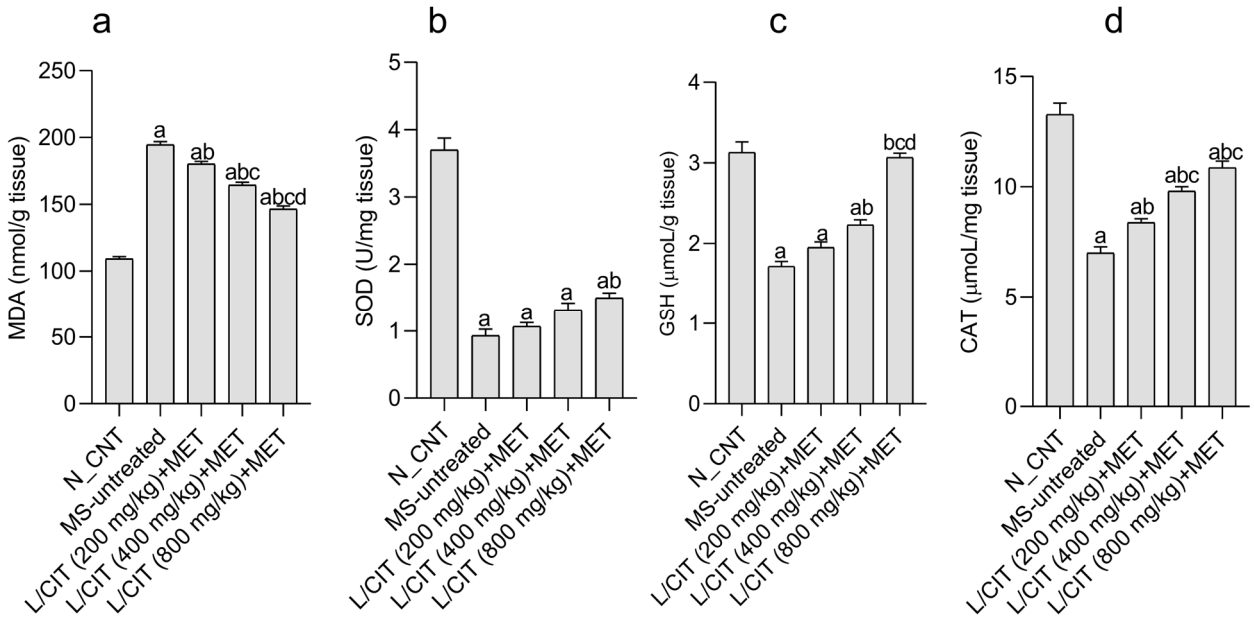

Fig. 1: (a) Pancreatic oxidative stress biomarkers: malondialdehyde; (b) superoxide dismutase; (c) reduced glutathione; (d) catalase. N_CNT = normal control, MS-untreated = negative control, L/CIT = L-citrulline, MET = metabolic syndrome. ANOVA followed by Tukey's post hoc test was conducted. Superscripts a, b, c and d indicate statistically significant difference (P< 0.05) compared with N_CNT, MS-untreated, L/CIT (200 mg/kg) + MET and L/CIT (400 mg/kg) + MET groups, respectively.

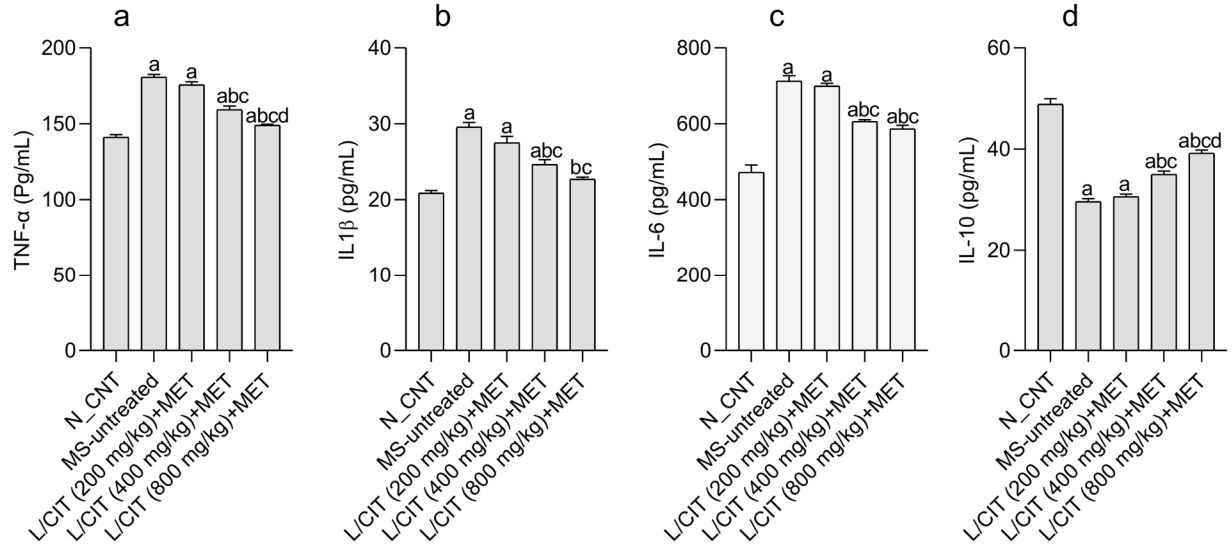

Fig. 2: Pancreatic inflammatory markers. (a) TNF-α; (b) IL-1β; (c) IL-6; (d) IL-10. N_CNT = normal control, MS-untreated = negative control, L/CIT = L-citrulline, MET = metabolic syndrome. ANOVA followed by Tukey's post hoc test was conducted. Superscripts a, b, c and d indicate statistically significant difference (P< 0.05) compared with N_CNT, MS-untreated, L/CIT (200 mg/kg) + MET and L/CIT (400 mg/kg) + MET groups, respectively.

In contrast, malondialdehyde (MDA) was significantly reduced in the L-CIT groups, accompanied by higher antioxidant enzyme activity (SOD, CAT and GSH). Increased nitric oxide (NO) from L-CIT may enhance antioxidant defences, lowering oxidative stress and protecting pancreatic tissue from ROS-induced lipid peroxidation. L-CIT appears to exert its protective effects in MetS through multiple interconnected pathways. One key mechanism is the nitric oxide NO-GSH axis. By enhancing NO availability, L-CIT improves vascular function and reduces oxidative stress. NO can modulate redox balance by influencing glutathione metabolism, maintaining GSH in its reduced form and preventing its oxidation to GSSG. This preserves cellular antioxidant capacity and protects pancreatic tissue from ROS-induced lipid peroxidation (Cabre *et al*., 2023).

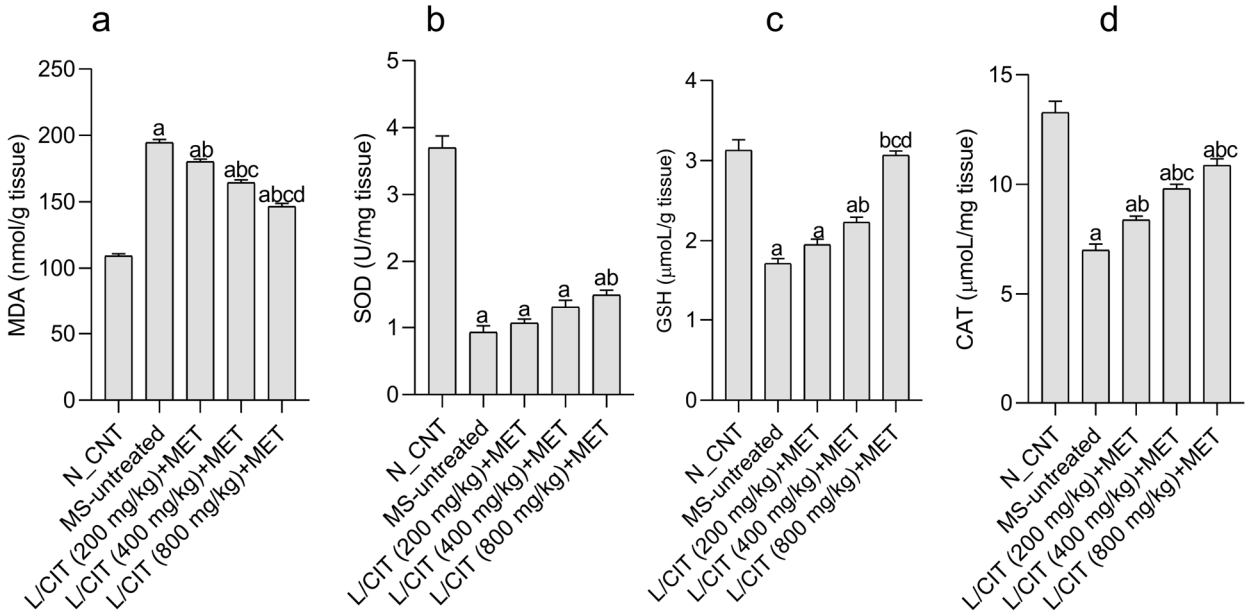

Fig. 1: (a) Pancreatic oxidative stress biomarkers; malondialdehyde; (b) superoxide dismutase; (c) reduced glutathione; (d) catalase. N_CNT = normal control, MS-untreated = negative control, L/CIT = L-citrulline, MET = metabolic syndrome. ANOVA followed by Tukey's post hoc test was conducted. Superscripts a, b, c and d indicate statistically significant difference (P< 0.05) compared with N_CNT, MS-untreated, L/CIT (200 mg/kg) + MET and L/CIT (400 mg/kg) + MET groups respectively.

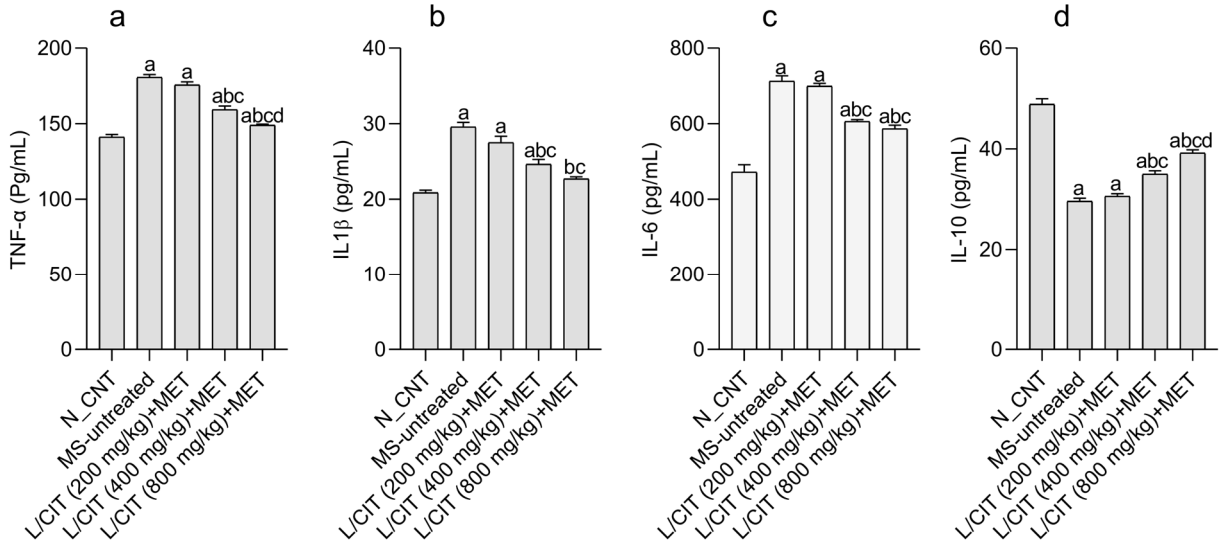

Fig. 2: Pancreatic inflammatory markers. (a) TNF-α; (b) IL-1β; (c) IL-6; (d) IL-10. N_CNT = normal control, MS-untreated = negative control, L/CIT = L-citrulline, MET = metabolic syndrome. ANOVA followed by Tukey's post hoc test was conducted. Superscripts a, b, c and d indicate statistically significant difference (P< 0.05) compared with N_CNT, MS-untreated, L/CIT (200 mg/kg) + MET and L/CIT (400 mg/kg) + MET groups, respectively.

In addition, L-CIT may activate the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, a master regulator of antioxidant defence. Nrf2 upregulates genes encoding enzymes such as SOD, CAT and glutathione peroxidase, strengthening the cellular response against oxidative stress. Reduced pro-inflammatory cytokines (TNF-α, IL-6, IL-1β, IFN-γ) observed in L-CIT-treated groups may further facilitate Nrf2 activation, since chronic inflammation often suppresses this pathway (Ngo and Duennwald, 2022). Together, these mechanisms suggest that L-CIT not only lowers oxidative stress directly but also reprograms pancreatic cells toward a more resilient state. By linking NO production to GSH preservation and Nrf2 signalling, L-CIT provides a coordinated defence against lipotoxicity, cytokine-driven inflammation and β-cell dysfunction in MetS.

L-CIT also reduces pro-inflammatory cytokines elevated in MetS, indirectly mitigating oxidative damage (Abbaszadeh *et al*., 2021). Thus, in this study, pancreatic lipid peroxidation was likely reduced through L-CIT's anti-inflammatory effects.

The reduced levels of antioxidant enzymes (SOD and CAT) in the MS-untreated group reflect ROS overproduction that overwhelms antioxidant defences. ROS also oxidises GSH to its inactive form (GSSG), explaining the lower GSH levels observed (Vaskova *et al*., 2023). Elevated cytokines such as TNF-α and IL-6 further suppress the expression of genes encoding antioxidant enzymes, weakening the defence system (Fu *et al*., 2025). Therefore, L-CIT's ability to restore pancreatic antioxidants may be linked to its inhibition of TNF-α and IL-6, which in turn upregulate antioxidant gene expression.

In this study, pancreatic TNF-α was significantly elevated in the MS-untreated group, reflecting the persistent low-grade inflammation characteristic of MetS. Visceral adipose tissue contributes to this inflammation by releasing cytokines such as TNF-α, which circulate systemically and affect organs, including the pancreas (Van Bilsen *et al*., 2019; Abdel-Moneim *et al*., 2024). TNF-α disrupts insulin signalling by interfering with insulin receptor substrates, promoting insulin resistance and further TNF-α production in pancreatic tissue (Castelli *et al*., 2024). Excess circulating lipids in MetS also accumulate in the pancreas, causing lipotoxicity, oxidative stress and increased TNF-α expression (Ertunc and Hotamisligil, 2016), consistent with findings in the untreated group.

In contrast, L-CIT administration significantly reduced pancreatic TNF-α. This reduction may be linked to enhanced antioxidant activity and decreased lipid peroxidation observed in the treated groups. Lower oxidative stress is associated with reduced inflammatory burden in pancreatic cells (Bagheripour *et al*., 2023). Increased nitric oxide (NO) from L-CIT can inhibit NF-κB, a key regulator of TNF-α expression, thereby indirectly suppressing TNF-α production. Although direct evidence that brain-derived neurotrophic factor (BDNF) reduces TNF-α is limited, studies in pancreatic adenocarcinoma suggest that increased BDNF alters immune cell profiles, thereby dampening pro-inflammatory signalling and TNF-α expression (Zhu *et al*., 2021). The elevated BDNF observed in L-CIT-treated groups may therefore contribute to reduced TNF-α. Additionally, L-CIT restores arginine availability, which supports T-cell function and reduces macrophage dysfunction, thereby improving immune resilience and reducing TNF-α (Breuillard *et al*., 2015). L-CIT can also activate AMP-activated protein kinase (AMPK), which reduces oxidative stress and inhibits NF-κB signalling. This leads to decreased IL-6 and TNF-α in metabolic tissues (Yang *et al*., 2024).

These findings align with Long *et al*. (2025), who reported decreased TNF-α following L-CIT administration, though their study focused on systemic rather than pancreatic inflammation.

In this study, pancreatic IL-10 was reduced in the MS-untreated group. MetS favours the production of pro-inflammatory cytokines such as TNF-α and IL-6, which suppress IL-10 synthesis in pancreatic cells (Fang *et al*., 2025). This likely explains the reduced IL-10 observed in the untreated group. By contrast, L-CIT administration increased pancreatic IL-10. This improvement may be linked to reduced free fatty acids, lowering stressors that impair IL-10 production. The larger abdominal circumference in the untreated group suggests greater adipose tissue deposits, which were reduced in the L-CIT groups.

Additionally, the decreased oxidative stress observed with L-CIT treatment may have contributed to improved IL-10 levels (Abbaszadeh *et al*., 2021; Bhol *et al*., 2024). Thus, L-CIT appears to enhance pancreatic IL-10 through its combined effects on adiposity, oxidative stress and inflammatory signalling.

In this study, pancreatic IL-6 was elevated in the MS-untreated group compared to controls. MetS promotes IL-6 production through systemic inflammation and metabolic stress, with excess lipids and glucose driving oxidative damage and NF-κB activation (Oguntibeju *et al*., 2019; Amer *et al*., 2025). Immune cell infiltration, including macrophages and NK cells, further contributes to IL-6 expression in pancreatic tissue (Amer *et al*., 2025).

Conversely, L-CIT treatment reduced pancreatic IL-6. This effect may be linked to lower free fatty acids, decreased abdominal adiposity and reduced oxidative stress, which together limit inflammatory signalling and immune cell infiltration. Mechanistically, this suppression may occur through inhibition of NF-κB activation, attenuation of MAPK/ERK signalling, activation of Nrf2 to enhance antioxidant enzyme expression, downregulation of the JAK/STAT3 pathway, stimulation of AMPK to improve lipid metabolism and inhibit NF-κB and increased nitric oxide bioavailability via eNOS activation, which improves vascular function and reduces immune cell adhesion, thereby collectively dampening IL-6 expression (Gao *et al*., 2022; Bhati *et al*., 2026).

In this study, pancreatic IL-1β was elevated in the MS-untreated group. MetS models show increased infiltration of M1 macrophages, which produce IL-1β in response to tissue damage and metabolic stress, amplifying inflammation (Ryu and Lee, 2024). IL-1β contributes to β-cell apoptosis, impaired insulin secretion and islet inflammation, often associated with fat deposition and reduced islet size (Boni-Schnetzler and Meier, 2019). The

reduced IL-1β in L-CIT groups may reflect lower macrophage infiltration, lower fat deposits and reduced pancreatic inflammation, consistent with TNF-α findings in this study.

Pancreatic BDNF was non-significantly increased in the MS-untreated group compared to controls. In MetS, BDNF may be upregulated under high-fat diet conditions to counter oxidative stress and inflammation (Yu Iu and Chan, 2022). Elevated insulin, leptin and glucose can also influence BDNF expression, with hyperinsulinemia and insulin resistance triggering compensatory upregulation (McFarlane, 2022). In L-CIT-treated groups, BDNF was reduced relative to untreated animals. While direct evidence of L-CIT lowering pancreatic BDNF is lacking, its anti-inflammatory and metabolic effects may indirectly reduce BDNF by lowering oxidative stress, inflammation and insulin resistance.

Pancreatic IFN-γ was elevated in the MS-untreated group, consistent with its role in driving inflammation, lipid accumulation and β-cell stress (Alspach *et al*., 2019; Chen *et al*., 2025). L-CIT reduced IFN-γ, likely through enhanced nitric oxide (NO), which suppresses pro-inflammatory signalling and limits immune cell infiltration.

Aldose reductase (AR) was also increased in the MS-untreated group. Hyperglycemia activates the polyol pathway, thereby increasing AR activity and depleting NADPH, thereby weakening antioxidant defences and fuelling oxidative stress (Zhu and Ding, 2024). Dyslipidemia further amplifies AR via endoplasmic reticulum stress (Mbara *et al*., 2024). L-CIT lowered AR, likely by reducing oxidative stress and metabolic imbalance. While AR is well studied in diabetic complications of the retina and kidney (Danila *et al*., 2024; Oates, 2010), its role in pancreatic dysfunction in MetS remains underexplored. This study highlights AR as a contributor to pancreatic damage in MetS.

In this study, pancreatic GSK-3β was elevated in the MS-untreated group, consistent with its role as a negative regulator of insulin signalling. Chronic insulin resistance and hyperglycemia increase GSK-3β activity, reducing insulin synthesis and secretion in β-cells and creating a feedback loop that further elevates its expression (Lemon *et al*., 2024). L-CIT significantly reduced GSK-3β, likely through suppression of pro-inflammatory cytokines (TNF-α, IL-6) and decreased fatty acids, which together limit lipotoxicity and inflammatory signalling cascades such as NF-κB and JNK.

Abdominal circumference (AC) was significantly higher in the MS-untreated group, reflecting visceral fat accumulation typical of MetS (Mustafa and Hasan, 2020). L-CIT treatment reduced AC, consistent with reports that it lowers triglycerides, decreases adipose lipid storage and improves leptin and adiponectin signalling (Joffin *et al*., 2015; Kudo *et al*., 2021).

Body length showed no significant changes across groups, but body weight was higher in MS-untreated animals. Insulin resistance promotes fat storage, particularly in visceral tissue, explaining this increase. L-CIT reduced body weight, possibly by enhancing hypothalamic proopiomelanocortin (POMC), which suppresses appetite and lowers food intake in obese and diabetic models (Kudo *et al*., 2017).

The translational relevance of these findings lies in their potential to inform clinical research and therapeutic strategies for MetS in human populations. The observed lipid-modulating, antioxidant and anti-inflammatory effects of L-CIT suggest that this amino acid could serve as an adjunctive therapy aimed at mitigating dyslipidemia, oxidative stress and cytokine imbalance, key drivers of cardiovascular risk in MetS. In human applications, L-CIT supplementation may be explored as a dietary or nutraceutical intervention to complement conventional pharmacological treatments such as statins, fibrates, or insulin sensitisers, particularly in patients with early-stage metabolic dysfunction. Moreover, the ability of L-CIT to enhance nitric oxide bioavailability, improve endothelial function and modulate inflammatory pathways highlights its promise in reducing vascular complications and long-term cardiovascular risk. Future clinical trials should therefore focus on dose optimisation, bioavailability and long-term safety in diverse populations. Collectively, these insights provide a rationale for integrating L-Citrulline into preventive and therapeutic frameworks for MetS, while emphasising the importance of rigorous human studies to validate its efficacy and mechanistic pathways.

CONCLUSION

In conclusion, L-CIT protected the pancreas in a MetS model by reducing inflammation, oxidative stress and systemic obesity, while specifically mitigating elevated levels of AR and GSK-3β. These findings highlight the potential of L-CIT as a therapeutic candidate for MetS, with implications for improving pancreatic health and reducing cardiometabolic risk in human populations. Nevertheless, the study's limitations must be recognised: the results are derived from an animal model, which may not fully replicate human physiology, and direct measurements of mechanistic endpoints such as protein phosphorylation were not included. Furthermore, the absence of long-term follow-up restricts conclusions about sustained efficacy and safety. Future clinical research should therefore focus on validating these effects in human subjects, optimising dosage and bioavailability and exploring molecular pathways more directly to establish L-CIT's translational relevance as a therapeutic agent for MetS.

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Authors' contributions
Adamu Imam Isa, Hanan Eissa and Mohamed-Nabih Abd-El Rahman: Conceived the research idea and designed the study; Nahid Ahmed Mohammed: Collected the data; Mohamed O'haj Mohamed: Performed data analysis. All authors discussed the results and wrote the manuscript.

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Data availability statement
All data generated or analysed during this study are included in this published article

Ethical approval
This research was conducted in compliance with international guidelines and following approval from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC/2024/082). This study was performed in adherence with the ARRIVE guidelines. See supplementary file for the ARRIVE checklist.

Conflict of interest
The authors declare there are no conflicts of interest related to this article.

Supplementary data
https://www.pjps.pk/uploads/2026/05/SUP1778927438.pdf

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