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PREVENTIVE EFFECT OF CILNIDIPINE ON CYCLOPHOSPHAMIDE-INDUCED LUNG TOXICITY IN *WISTAR* ALBINO RATS

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ABSTRACT

Cyclophosphamide (CP) is an alkylating cytotoxic agent prescribed across diverse malignancies and immune-mediated disorders, yet its bedside use is checked by collateral toxicity to non-tumour tissue, with the lung being particularly vulnerable to the action of its hepatic metabolite acrolein. Disturbance of redox equilibrium, loss of endogenous antioxidant reserves and amplified pro-inflammatory signalling drive alveolar injury, and the cytoprotective options currently available remain inadequate. The aim of the present work was to determine whether cilnidipine, a dual L/N-type calcium channel antagonist, can mitigate CP-induced pulmonary damage in Wistar albino rats.

Rats were assigned to five arms and dosed for 14 days; cilnidipine was given by mouth at 1 mg/kg or 2 mg/kg in conjunction with a single 200 mg/kg intraperitoneal dose of CP delivered on day 7. Endpoints comprised oxidative-stress indices (GSH, SOD, CAT, MDA), inflammatory mediators (TNF-α, IL-1β, NF-κB, Nrf2) and lung histoarchitecture. Cilnidipine, and most evidently at 2 mg/kg, replenished GSH and the antioxidant enzymes SOD and CAT, brought MDA down, suppressed TNF-α, IL-1β and NF-κB, upregulated Nrf2 and preserved alveolar structure on histology. The benefit observed is consistent with combined L- and N-type channel blockade together with the antioxidant and anti-inflammatory profile of the molecule. These findings position cilnidipine as a worthy candidate for further translational work in the context of CP-based chemotherapy.

Keywords: Cilnidipine; prevention; albino rats; cyclophosphamide.

INTRODUCTION

Cancer remains a leading global cause of mortality, with case load projected to rise sharply by 2050. It arises from deregulated, clonal cellular proliferation and is classified by tissue of origin or organ affected. Among cytotoxic agents in current use, cyclophosphamide (CP) shows efficacy across both solid and haematological tumours and in autoimmune indications, but its bedside value is limited by adverse effects on the lung, heart, brain, liver, bone marrow and gonads.[1,2] Hepatic CYP-mediated bioactivation of CP releases two distinct species: phosphoramide mustard, which delivers the tumour-killing alkylation, and acrolein, which is held responsible for most of the off-target organ injury through oxidative stress, antioxidant depletion, induction of cytokines including TNF-α and IL-6 and engagement of apoptotic cascades. The pulmonary compartment is especially vulnerable owing to progression toward fibrotic remodelling; cardiac injury arises through redox and inflammatory mechanisms; central nervous system effects (the so-called "chemo brain") follow acrolein passage across the blood-brain barrier; the liver displays enzymatic disturbance with concurrent inflammation and apoptosis; bone marrow shows myelosuppression secondary to disrupted haematopoietic signalling; and gonadal toxicity threatens fertility through oxidative reproductive injury. Cilnidipine, a fourth-generation calcium antagonist with combined L- and N-type activity, has emerged as a candidate cytoprotectant whose pharmacology reaches beyond blood-pressure control; the molecule dampens cytokine output, raises endogenous antioxidant capacity (including SOD and GPx), restrains calcium-driven cell death and has shown benefit in hypertensive, diabetic, nephropathic and neurodegenerative settings, providing a plausible mechanistic case for shielding tissues from CP-related multi-organ injury within integrative oncology regimens.[3,4,5]

The present study therefore tests whether cilnidipine pretreatment limits CP-induced lung injury in a Wistar rat model.

MATERIALS AND METHODS

Materials

Cilnidipine and cyclophosphamide were obtained from Sigma Aldrich (USA); cilnidipine was suspended in 0.5% CMC and cyclophosphamide in normal saline immediately before dosing. Female Wistar rats (150-200 g) were supplied by CAHF, Jamia Hamdard and maintained on Amrut Rat Feed (Nav Maharastra Chakan Oil Mills Ltd.) with water ad libitum[6.7].

Methodology

All in-vivo work was performed under IAEC approvals JH/1484/CPCSEA and JH/1610/CPCSEA (Jamia Hamdard) and followed CPCSEA guidelines.

Phase I – Experimental protocol

Table 1: Experimentation groups of animals

S. No.	Groups	Dose	Route & duration
1	Control	Normal saline	Oral, daily 14 days
2	Toxic	CP 200 mg/kg	i.p., once on 7th day
3	CIL + CP	CIL 1mg/kg + CP 200 mg/kg	p.o., daily 14 days + i.p once on 7th day
4	CIL + CP	CIL 2mg/kg + CP 200 mg/kg	p.o., daily 14 days + i.p once on 7th day
5	CIL *per se*	CIL 2mg/kg	p.o., daily 14 days
CP: Cyclophosphamide, **I.P:** Intraperitoneal, **p.o:** per os			

Methodology for in vivo study

Blood collection

On day 14, blood was drawn into sterile tubes and serum was separated by centrifugation for downstream assays[8,9].

Histopathology Study

Biochemical Estimation in Tissue (Lungs)

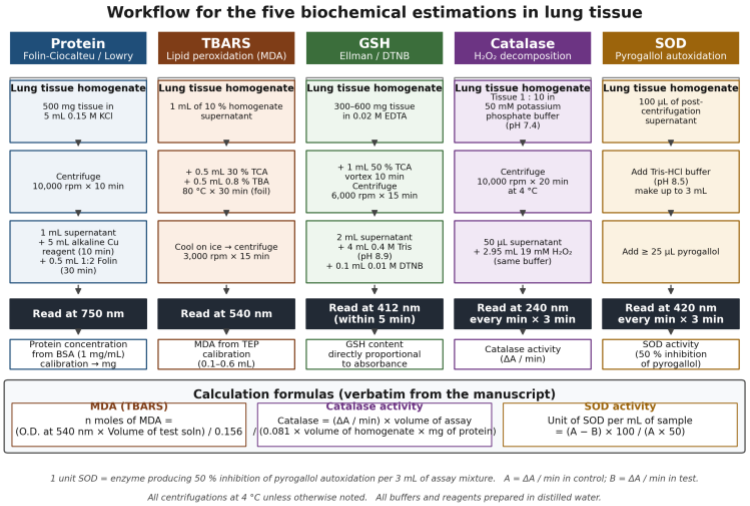

Fig. 1: Workflow used for the five biochemical estimations (protein, TBARS, GSH, catalase, SOD) carried out on lung tissue. Reagents, volumes, centrifugation parameters, reading wavelengths and result units are shown for each arm; calculation formulas are preserved at the foot of the figure.

Inflammatory parameters from Kits[10-14]

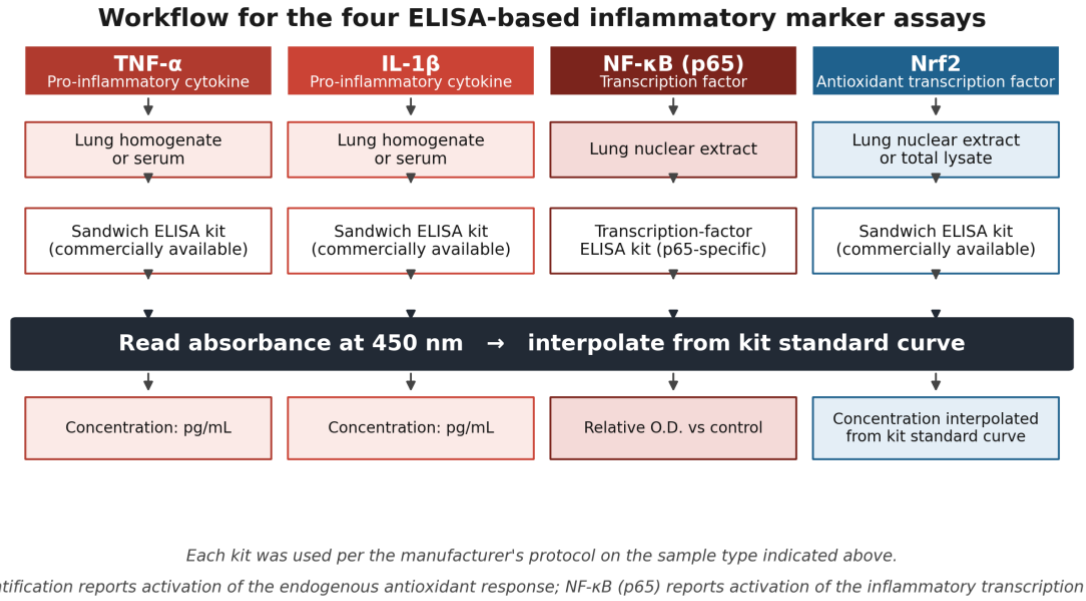

Fig. 2: Workflow used for the four ELISA-based inflammatory marker assays (TNF-α, IL-1β, NF-κB, Nrf2). Each marker was quantified in lung homogenate or nuclear extract using a commercially available sandwich or transcription-factor ELISA kit per the manufacturer's protocol, with absorbance read at 450 nm and concentrations interpolated from the kit standard curve.

Histopathology Study

Method: Eosin – Hematoxylin[15,16]

The right lung was processed into 3 µm paraffin sections, H&E stained, and read blinded[17,18].

Statistical Analysis

Group means (± SEM) were compared by one-way ANOVA with the Tukey-Kramer post hoc test; paired comparisons within an animal used Student's paired t-test. Significance was set at $p \leq 0.05$ and computations were run in Instat-2 (V2.04, GraphPad Software, San Diego, CA, USA)[19-21].

RESULTS

Body Weight

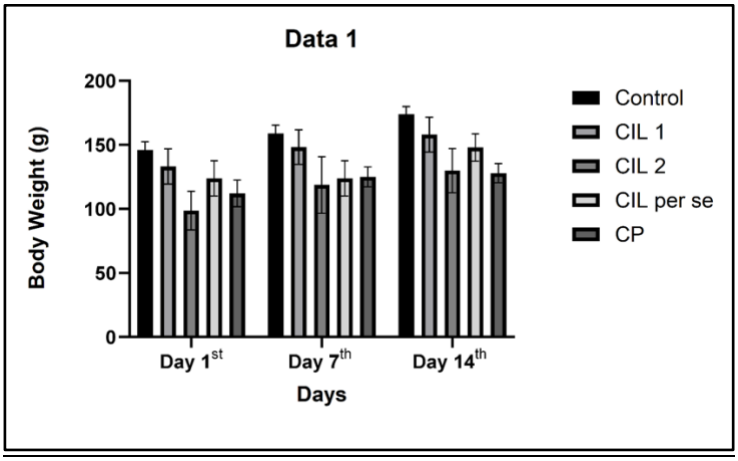

Fig. 3: Graph of Body weight

Body-weight trajectories over the 14-day window are summarised by group (Control, CIL 1, CIL 2, CIL per se and CP) in the figure. The control arm gained mass steadily from day 1 to day 14, reflecting normal growth, whereas the CP arm dropped sharply on day 1 and recovered only marginally by day 14, mirroring CP-driven toxicity and blunted growth.

Glutathione (GSH)

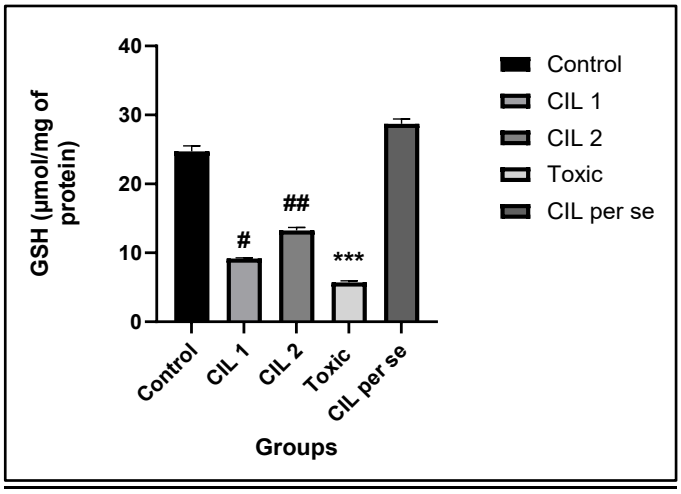

Fig 4: Effect of different treatments on tissue GSH of Different Groups. * Significantly different from the control group (p<0.001), # Significantly Different from Toxic groups.

Tissue GSH, a recognised marker of antioxidant status, was compared between the five groups (Control, CIL 1, CIL 2, CIL per se, CP). GSH stayed elevated in control animals, indicating preserved redox balance, whereas CP exposure produced a marked drop consistent with significant oxidative injury. Cilnidipine pretreatment recovered GSH in a dose-related manner: CIL 1 produced a moderate rebound

and CIL 2 approached control values, while CIL per se animals retained GSH at control level. Taken together, the data indicate that cilnidipine, particularly at 2 mg/kg, opposes CP-induced GSH depletion.

Superoxide Dismutase (SOD)

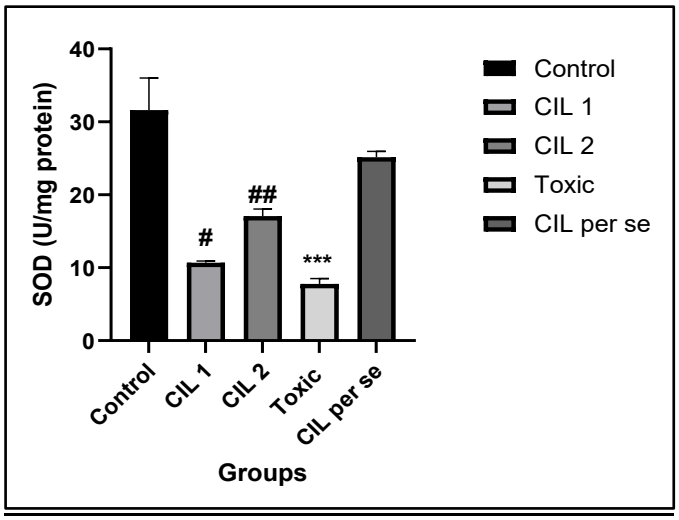

Fig 5: Effect of different treatments on tissue SOD of different groups. Asterisks denote significance against the control group (p<0.001); hash symbols denote significance against the toxic group.

CP exposure produced a substantial decline in SOD activity, indicating compromised oxidative defence, whereas the control arm retained elevated activity consistent with intact antioxidant function. Cilnidipine reversed this fall: the rise driven by CIL 2 exceeded that of CIL 1, reflecting a concentration-dependent benefit, while CIL per se animals matched the control level. Across the five groups (Control, CIL 1, CIL 2, CIL per se, CP), the data point to a restoration of antioxidant balance under oxidative stress.

Catalase (CAT)

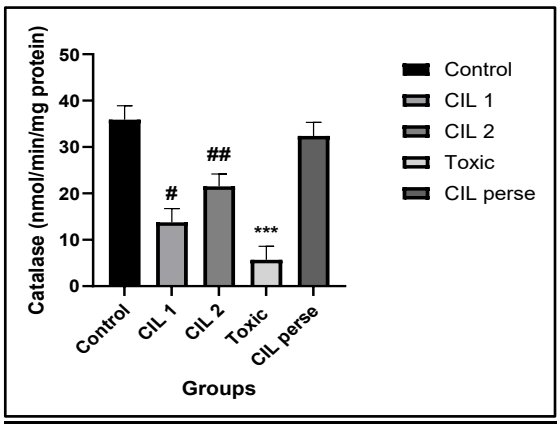

Fig 6: Effect of different treatments on tissue catalase of different groups. Significance versus control is shown by * (p<0.001) and versus the CP-treated arm by # (single) and ## (double).

Hepatic catalase activity, which detoxifies cellular hydrogen peroxide, was retained at high levels in control animals, indicating an undisturbed antioxidant status. The five groups compared were Control, CIL 1, CIL 2, CIL per se and CP. Catalase is a principal antioxidant enzyme responsible for the detoxification of cellular hydrogen peroxide.

Malondialdehyde (MDA)

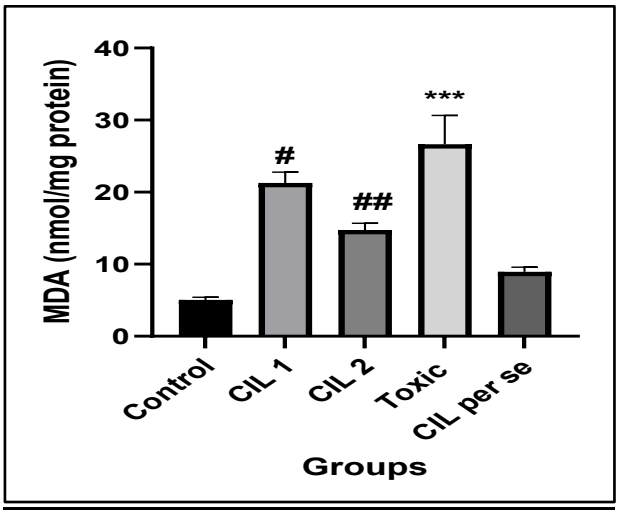

Fig 7: Effect of different treatments on tissue MDA of different groups. p<0.001 against control is marked *; differences against the toxic arm are marked # or ##.

MDA, a recognised marker of lipid peroxidation and oxidative stress, was profiled in liver tissue across the five groups (Control, CIL 1, CIL 2, CIL per se, CP). Baseline control values were low, reflecting minimal oxidative injury, whereas the CP arm recorded a sharp rise in MDA driven by heavy lipid peroxidation from CP metabolites. Both cilnidipine doses (CIL 1 and CIL 2) brought MDA down relative to CP, indicating a protective effect of cilnidipine against CP-induced oxidative liver damage.

Tumor Necrosis Factor-alpha (TNF-α)

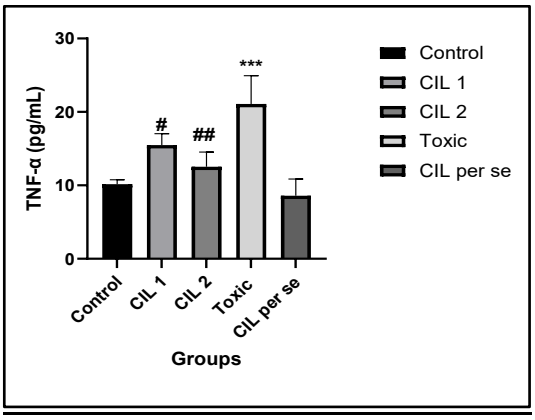

Fig 8: Effect of different treatments on tissue TNF-α of different groups. Symbols: * = p<0.001 vs control; # / ## = significance vs the toxic arm.

The toxic arm recorded a pronounced rise in TNF-α relative to control ($p < 0.001$), reflecting a strong inflammatory response. Cilnidipine brought TNF-α down: CIL 2 produced the greater reduction (##$p < 0.01$) and CIL 1 a smaller one (#$p < 0.05$), consistent with a dose-dependent anti-inflammatory action. TNF-α concentrations (pg/mL) for the treatment arms are presented in the bar graph.

Interleukin-1β (IL-1β

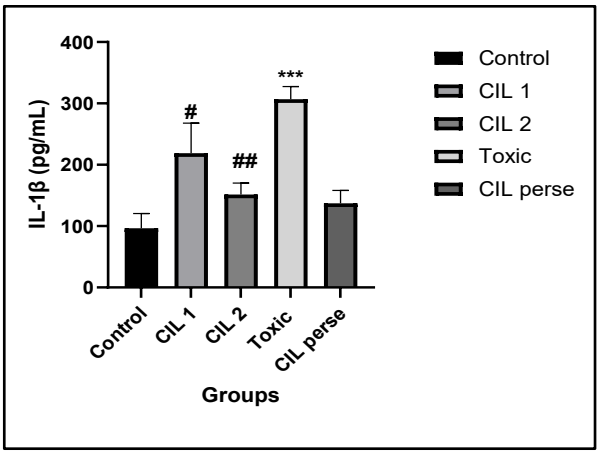

Fig 9: Effect of different treatments on tissue IL-1β of different groups. Asterisks denote significance against the control group ($p<0.001$); hash symbols denote significance against the toxic group.

IL-1β levels (pg/mL) across the experimental groups are depicted. Relative to control, the toxic group recorded a pronounced rise in IL-1β (***$p < 0.001$), reflecting a strong pro-inflammatory response following toxic insult. CIL 1 produced a moderate fall in IL-1β versus the toxic group (#$p < 0.05$), whereas CIL 2 produced a greater fall (##$p < 0.01$), indicating a dose-dependent anti-inflammatory effect.

Nuclear Factor Erythroid 2–Related Factor 2 (Nrf2)

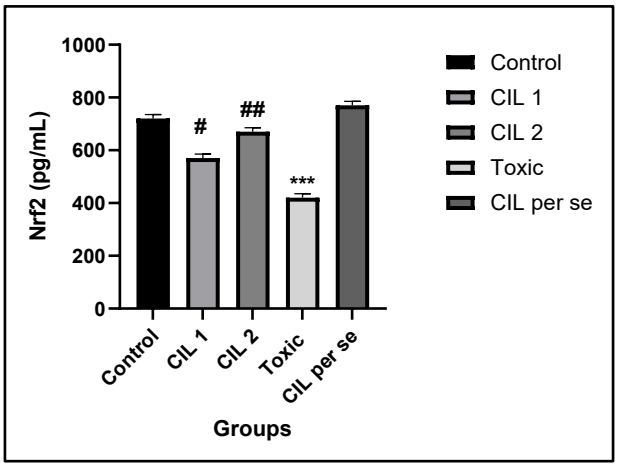

Fig 10: Effect of different treatments on tissue Nrf-2 of different groups. Significance versus control is shown by * ($p<0.001$) and versus the CP-treated arm by # (single) and ## (double).

Nrf2 expression (pg/mL) varied markedly between the experimental groups. Relative to control, the toxic arm showed a pronounced fall in Nrf2 (***p < 0.001), indicating oxidative stress and a compromised antioxidant defence. Both cilnidipine doses restored Nrf2, with CIL 2 producing a larger rise than CIL 1 (##p < 0.01 versus #p < 0.05), reflecting dose-dependent activation of the antioxidant pathway. Notably, the CIL per se arm recorded Nrf2 values that exceeded the control level,

Nuclear Factor kappa B (NF-κB)

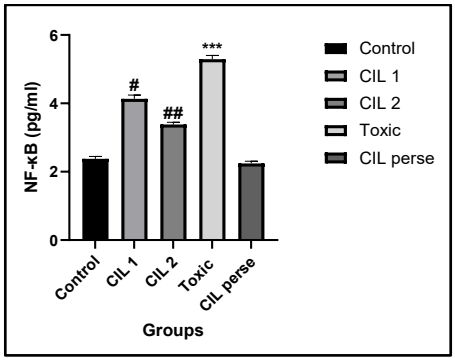

Fig 11: Effect of different treatments on tissue NF-κB of different groups. p<0.001 against control is marked *; differences against the toxic arm are marked # or ##.

Relative to control, the toxic arm displayed a pronounced rise in NF-κB (***p < 0.001), indicating intense inflammatory activation. Cilnidipine attenuated this response: CIL 1 produced a partial fall (#p < 0.05) and CIL 2 a larger fall (##p < 0.01), reflecting a dose-dependent anti-inflammatory action. CIL per se animals retained NF-κB at control-like values, indicating that cilnidipine alone does not provoke inflammatory signalling. NF-κB concentrations (pg/mL) varied between the experimental groups, with the data supporting attenuation of NF-κB activation by cilnidipine at the higher dose and a corresponding potential to limit inflammation following toxic insult.

Histopathology

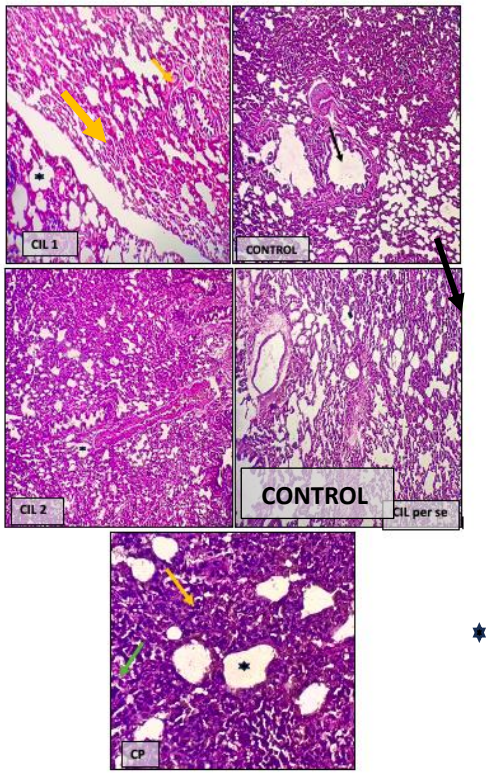

Fig. 12: Histopathology images of lungs tissues

Histological inspection of lung tissue revealed clear differences between the experimental groups. The control arm displayed an unremarkable pulmonary architecture with thin, intact interalveolar septa, no inflammatory infiltrate and well-preserved alveolar spaces, reflecting healthy lung morphology. By contrast, the CP-treated arm showed severe histopathological derangement including marked emphysema [★], conspicuous inflammatory cell infiltration [yellow arrows], arterial hypertrophy [green arrows] and thickening of the intra-alveolar septa [black arrows], indicating substantial pulmonary injury following CP administration.

Pulmonary histoarchitecture in the CIL per se arm, which received cilnidipine alone, was comparable to control with no overt pathological changes, indicating that cilnidipine on its own does not compromise pulmonary integrity.

The CIL 1 arm displayed moderate amelioration of the CP-induced damage, with partial attenuation of inflammatory infiltration and vascular changes, reflecting a protective but incomplete effect at this dose. In contrast, the CIL 2 arm preserved pulmonary architecture almost entirely, with minimal emphysematous change, negligible inflammatory infiltrate and intact alveolar and vascular structure, indicating robust protection by cilnidipine at the higher dose.

DISCUSSION

The present preclinical work is a focused evaluation of cilnidipine as a cytoprotective adjunct against the off-target injury produced by cyclophosphamide (CP), a widely deployed cytotoxic and

immunosuppressive agent. Despite its clinical value across a range of malignancies and autoimmune disorders, CP carries appreciable toxicity to vital organs, in particular the lung and the liver.

Wistar albino rats were allocated to five arms (control, toxic CP only, cilnidipine low dose plus CP, cilnidipine high dose plus CP and cilnidipine per se); CP was used to provoke lung and liver injury and cilnidipine was co-administered at two dose levels to interrogate its protective capacity. Biochemical readouts comprised TBARS (an index of MDA), GSH, SOD and CAT, alongside pro-inflammatory mediators determined by ELISA, with pulmonary histopathology used to characterise structural integrity and cellular injury.

The pattern of findings was internally consistent: CP exposure suppressed antioxidant enzymes, raised lipid peroxidation, elevated pro-inflammatory cytokines and produced overt histopathological injury, including alveolar thickening, congestion and inflammatory infiltrates. Co-administration of cilnidipine, and most evidently at 2 mg/kg, reversed these alterations: antioxidant capacity was restored, MDA and inflammatory markers were brought down, and pulmonary architecture was preserved, supporting a substantial cytoprotective role.

Taken in aggregate, the present data support an organoprotective activity for cilnidipine and motivate translational work to test its value in clinical oncology, where the molecule could plausibly improve patient quality of life and widen the therapeutic margin of indispensable but toxic agents such as cyclophosphamide.

Conclusion

The 2 mg/kg arm of cilnidipine pretreatment safeguarded Wistar rat lungs against the injury produced by a single 200 mg/kg dose of cyclophosphamide. Mechanistically the protection co-occurred with recovery of GSH, SOD and CAT activities, suppression of MDA, TNF-α, IL-1β and NF-κB, upregulation of Nrf2 and a near-complete preservation of alveolar architecture, consistent with combined L/N-type calcium-channel blockade complemented by antioxidant and anti-inflammatory effects. The findings support a potential role for cilnidipine as an adjunct within CP-based oncology regimens, where off-target organ injury continues to limit dose intensity. Controlled clinical studies are now warranted to verify these preclinical observations in patients.

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