Title of the project: Effect of fisetin in monocrotaline induced experimental pulmonary hypertension

Summary:

Background: PAH is a progressive and potentially fatal disease. The PI3K/Akt/mTOR pathway, is an important cellular signalling pathway which regulates the cell cycle. Cell viability is governed at the molecular level by a balance between proapoptotic and antiapoptotic signals mediated by a number of gene families, the most prominent being the Bcl-2 family. The expression of anti-apoptotic Bcl-xL is reported to be increased by the mediators of PAH. Fisetin having anti-oxidant activity, inhibits the release of various cytokines. Fistein also exerts systemic, coronary and pulmonary artery vasodilatation effects. Novelty: Various clinical studies reports that fisetin dramatically improved the treatment outcomes of the patients with cardiovascular complication. Additionally, fisetin supplementation suppressed the various inflammatory and oxidative stress pathways. However, the role of fisetin in pulmonary artery hypertension remains unknown. Therefore, it is worthwhile to study the role of this multi-targeted drug in PAH. Objectives:1.To evaluate the effect of different doses of fisetin in monocrotaline induced PAH in rats. 2.To find out the effect of fisetin in the expression and activity of PI3K/Akt/mTOR , Bcl- Xl, TNF-α and interleukins in experimental PH in rats. Methods: The model of PAH will be induced by single dose administration of monocrotaline 50 mg/kg s.c. injection. Fistein will be administered daily at a dose of 1.25 mg/kg, 2.5 mg/kg and 5 mg/kg for 3 weeks after one week of administration of followed by MCT. Expected outcomes: Fistein having pleotropic effect may be a novel agent for effective management of disease progression and severity. Thus, the use of fistein could be a multi-faceted treatment approach in ameliorating disease progression.

Keywords: pulmonary arterial hypertension, pulmonary arterial pressure, Fisetin, right ventricular systolic pressure

Abbreviations: PAH- pulmonary arterial hypertension, PAP- pulmonary arterial pressure, RVSP- right ventricular systolic pressure, SMC- smooth muscle cellsin rats.

Introduction: Pulmonary arterial hypertension (PAH) is a life-threatening condition marked by elevated pulmonary pressure, microvascular changes, and ventricular hypertrophy. Despite complex pathogenesis and limited therapies, PAH survival remains below 60%. Smooth muscle cell proliferation in pulmonary arteries drives vessel thickening, linked to PI3K/Akt/mTOR pathway. Balancing pro-apoptotic and anti-apoptotic signals influences cell viability via Bcl-2 family genes. Fisetin, found in plants, inhibits cytokine release, promotes apoptosis, and impacts vasodilation. Inflammatory cytokine activation contributes to PAH. Fisetin's cardiovascular benefits are supported by animal models. With evidence of PI3K/Akt/mTOR inhibition, investigating fistein's multi-targeted potential in PAH is promising for disease control.

Novelty:

Various clinical studies reports that fisetin dramatically improved the treatment outcomes of the patients with cardiovascular complications. Fisetin is known to possess the potential to supplement traditional treatments among patients with underlying cardiovascular complications and thereby extend the otherwise narrow therapeutic window and improve the treatment outcomes. Additionally, fisetin supplementation suppressed the various inflammatory and oxidative stress pathways. However, the role of fisetin has been evaluated in various cardiovascular diseases but its role in pulmonary artery hypertension remains unknown. Various pathways are known to be involved in the pathogenesis of PAH and fisetin has pleotropic effect which may be a novel agent for the effective management of disease progression and severity.

Objectives:

1. To evaluate the effect of different doses of fisetin alone in monocrotaline induced PAH in rats.

2. To evaluate the effect of different doses of fisetin in combination with the standard drug sildenafil.

3. To find out the effect of fisetin in the expression and activity of PI3K/Akt/mTOR , Bcl- Xl, TNF-α and interleukins in experimental PH in rats.

Methodology

The study employs a rat model to investigate the effects of Fisetin, both alone and in combination with Sildenafil, on pulmonary arterial hypertension (PAH). Hemodynamic measurements include right ventricular systolic pressure (RVSP), assessing PAH severity. Hearts are analyzed for right ventricular hypertrophy (RVH) indices like RV/(LV + S) and RV/BW. Tissues are collected for western blotting, histological assessment, and oxidative stress analysis. Lung tissue sections are stained for microscopic evaluation. Western blotting and RT-PCR techniques are used to quantify PI3K/Akt/mTOR protein and mRNA levels. Oxidative stress factors, including malondialdehyde (MDA) content and superoxide dismutase (SOD) and glutathione peroxidase (GSH) activity, are determined. ELISA assays measure TNF-α, IL-1β, IL-6, and BCL-xL levels. Fisetin's effects on PAH progression, inflammation, and oxidative stress are assessed through these methods. This comprehensive approach will shed light on Fisetin's potential to mitigate PAH-related pathophysiological changes and its synergistic effects with Sildenafil.

Statistical analysis

All experiments will be performed at least three times for each determination. Data will be expressed as the mean ± S.D. Differences in measured variables between groups will be determined by one-way ANOVA analysis of variance followed by the Student- Newman- Keul test for multiple comparisons. P value < 0.05 will be considered statistically significant. Statistical analysis will be performed with specific software.

Expected outcomes: Fistein having pleotropic effect may be a novel agent for effective management of disease progression and severity. Thus, the use of fistein could be a multi-faceted treatment approach in ameliorating disease progression.

Timelines

Duration	Activity
0-6 (months)	institutional animal ethics certificate and animal procurement
7-12 (months)	Procurement of chemicals and antibodies
13-18 (months)	Development of PAH model in rats and drug treatment
19-24 (months)	Molecular and biochemical evaluation
25-30 (months)	Results compilation and publication