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Original Article

Cardioprotective Effect of *Mikania micrantha* Kunth Leaf Extract in Isoproterenol-Induced Myocardial Injury in Experimental Animals

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ABSTRACT

Background: Myocardial Infarction (MI), driven by oxidative stress-induced cardiomyocyte injury, is the most predisposing disease, causing prevalent mortality globally, among the various cardiovascular diseases. Present study evaluated the cardioprotective potential of the methanolic extract of *Mikania micrantha* leaves (MME) in experimental animal model. **Materials and Methods:** The extract was evaluated for its phytochemical analysis, antioxidant and anti-inflammatory activities. Cardioprotection was assessed with Isoproterenol (ISO)-induced myocardial injury in Wistar rats via Heart Weight Index (HWI), serum biomarkers including Creatine Kinase-MB (CK-MB) and Lactate Dehydrogenase (LDH), tissue antioxidants (lipid peroxidation, nitric oxide), 2,3,5-Triphenyltetrazolium Chloride (TTC) staining, and histopathology. **Results:** The presence of bioactive constituents revealed 112.86±0.061 mg GAE/g phenolic and 234.22±0.081 mg QE/g flavonoid content in the extract. LC-MS/MS profiling further confirmed the presence of several polyphenolic compounds. *In vitro* antioxidant assays (DPPH and reducing power) and anti-inflammatory protein denaturation assays demonstrated promising effect. Acute toxicity testing endorsed the safety of MME up to 2000 mg/kg. *In vivo*, pretreatment with MME (200 and 400 mg/kg) significantly attenuated ISO-induced elevations in HWI, biochemical parameters, lipid peroxidation, and nitric oxide levels, while TTC staining and histological analysis further substantiated myocardial tissue preservation in treated groups. *M. micrantha* at 400 mg/kg demonstrated effects similar to the standard Enalapril. **Conclusion:** The findings suggest that the cardioprotective effects of *Mikania micrantha*, by means of its anti-inflammatory and antioxidant properties, highlighting its potential as a natural therapeutic agent for myocardial injury and supporting further investigation into its active constituents.

Keywords: Antioxidant Activity, Cardioprotection, Isoproterenol, *Mikania Micrantha*, Myocardial Infarction.

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INTRODUCTION

Globally, Cardiovascular Diseases (CVDs) are the primary contributors to death and their growing incidence causes a major concern for public health, especially in economically developing countries.[1] Myocardial Infarction (MI), the fatal form of cardiovascular disease, results from reduced blood flow to the heart, causing oxygen deprivation and subsequent myocardial damage through both necrosis and apoptosis. Because cardiomyocytes are terminally differentiated and lack regenerative capacity, it is crucial to prevent their injury and loss to provide an effective MI treatment.[2] Myocardial Infarction (MI) models have advanced cardiovascular research using experimental animals, with isoproterenol a synthetic sympathomimetic amine, commonly used to induce cardiac injury. Oxidative stress is promoted via the overproduction of Reactive Oxygen Species (ROS), resulting in cardiac tissue damage. Myocardial necrosis occurs through mechanisms such as increased oxygen demand, impaired oxygen utilization, calcium overload, and disrupted cellular metabolism.[3,4] Evidence suggests that consuming antioxidant-rich fruits, vegetables, notably, phytosterols exhibit diverse pharmacological effects, may help reduce the likelihood of developing cardiovascular diseases, owing to their cardioprotective effects driven by antioxidant, anti-inflammatory, anti-atherosclerotic properties.[5] The European Society of Cardiology also recommends plant sterols, alongside lifestyle modifications, as a strategy to mitigate serum cholesterol

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in dyslipidemia management, furthermore preventing coronary artery diseases and subsequent myocardial infarction.[6] *Mikania micrantha* is recognized as one of the most invasive plant species globally, resulting significant threats to agriculture and forestry in Asia and the Pacific by rapidly overgrowing and suppressing native vegetation. Despite its harmful ecological impact, its leaves are traditionally used for their antimicrobial, anti-inflammatory, analgesic, and ulcer-healing properties.[7,8] This unexplored chemodiversity may contain naturally occurring bioactive compounds and molecules linked to ecological interactions. However, its cardioprotective potential has not been thoroughly investigated. The objective of the present study is to evaluate the antioxidant profile and cardioprotective efficacy of methanolic extract of *Mikania micrantha* leaves in isoproterenol-induced rat model of myocardial injury.

MATERIALS AND METHODS

Collection, identification and extraction of plant

Fresh leaves were obtained in winter from Agarpara, West Bengal, and authenticated by the Botanical Survey of India as *Mikania micrantha* Kunth vide no. CNH/Tech.II/2023/33. Leaves were collected in winter, as cold stress increases protective compounds and enhances the plant's therapeutic potential.[9] Leaves were cleaned, followed by drying and powdering, then methanolic maceration for 72 hr. The extract was filtered, concentrated, labeled as MME, and stored for further studies.

Phytochemical Screening

Phytochemical analysis was conducted using standard methods to detect alkaloids, flavonoids, tannins, phenols, carbohydrates, glycosides, and terpenoids.[10] Using the Folin-Ciocalteu reagent, total phenolic content was quantified as per the protocol of Rizvi *et al*. Briefly, methanolic extract (1 mg/mL) of *M. micrantha* leaves was reacted with Folin-Ciocalteu reagent and 8% sodium carbonate, adjusted to 6 mL, incubated for 30 min maintained in darkness and absorbance was measured at 765 nm. Total phenolic content was represented as mg GAE/g extract using Gallic Acid (GAE) as standard.[11] Total Flavonoid Content (TFC) was assessed using the colorimetric method based on aluminum chloride. Extract (3 mL, 1 mg/mL) was mixed well with 5% sodium nitrite, 1 M sodium hydroxide and 10% aluminum chloride, finally adjusted to 6 mL, incubated for 30 min, and absorbance was recorded at 510 nm. TFC was represented as mg QE/g extract using Quercetin (QE) as the standard.[12]

In vitro Antioxidant and Anti-inflammatory Assays

Reducing Power Assay

The extract (50-1000 µg/mL) was combined with 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6), thereafter 2.5 mL of 1% potassium ferricyanide. The mixture was vortexed, and incubated at 50°C for 20 min followed by cooling. Additionaly 2.5 mL of 10% trichloroacetic acid was mixed with it and centrifuged at 3000 rpm for 10 min. The supernatant was separated and 0.5 mL of 0.1% ferric chloride and 2.5 mL of deionized water were added. Finally the absorbance was measured at 700 nm, using ascorbic acid as the standard.[13]

DPPH Free Radical Scavenging Assay

M. micrantha leaf extract was evaluated for its antioxidant activity using the 2,2-Diphenyl1-Picryhydrazyl (DPPH)assay. A 0.002% DPPH solution was prepared in methanol and 1 mL from it was mixed with 1 mL of extract of various concentrations (25-800 µg/mL). Control samples contained only methanol. Incubation of the mixtures in the dark at room temperature for 30 min was followed by absorbance measurement at 517 nm. Percent inhibition was determined, and the IC_{50} value was calculated. The experiment was repeated in triplicate.[13]

Protein Denaturation Assay

This method is widely used to study the anti-inflammatory activity of plant extract. In each test tube, 0.2 mL of egg albumin was combined with 2.8 mL of phosphate buffer and 2 mL of the extract at different concentrations. The mixtures were incubated at 37°C for 15 min, heated at 70°C for 5 min, and then cooled. Absorbance was measured at 660 nm, and the percentage inhibition of protein denaturation was calculated.[14]

LC-MS/MS Analysis

LC-ESI-MS/MS analysis was conducted using a Waters SYNAPT-XS HDMS system to identify and quantify polyphenolic metabolites.[15] Separation was performed on a C18 Waters ACQUITY BEH column (2.1×100 mm, 1.7 µm), with mobile phases comprising solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). A gradient elution was applied at a flow rate of 0.2 mL/min, with a total run time of 45 min and an injection volume of 5 µL. Mass spectrometric detection was carried out using Electrospray Ionization (ESI) in positive mode, utilizing Multiple Reaction Monitoring (MRM).

In vivo Study

Acute toxicity study

Animals were maintained as per CPCSEA guidelines, and experiments were conducted with approval from the Institutional Animal Ethics Committee, TAAB Biostudy Services (1938/PO/Rc/S/17/CPCSEA). Acute oral toxicity of methanolic extract of *M. micrantha* Kunth leaves was conducted as per OECD guideline no. 423. The animals were observed closely for the first 30 min, periodically during the first 24 hr, and daily for 14 days for mortality and clinical signs, thereafter up to one month.[16]

Isoproterenol-induced myocardial injury

Wistar rats (Male, 150±10 g) were used for this study and were randomly assigned to six groups as follows, with five animals in each group:

I.Healthy Control: Non-induced, untreated.II.Disease Control: Isoproterenol (ISO) induced, vehicle (distilled water) treated.III.Standard: ISO induced, Enalapril 10 mg/kg.IV.Test group (Low dose): ISO induced, *M. micrantha* extract (200 mg/kg).V.Test group (High dose): ISO induced, *M. micrantha* extract (400 mg/kg).VI.Test Control: Non-induced, *M. micrantha* extract only (400 mg/kg).Rats were orally administered the test compound and standard drug daily for 21 days. Induction of myocardial injury was done with Isoproterenol at 85 mg/kg subcutaneously on days 20 and 21, maintaining an interval of 24-hr in between.[3,17,18] After 48 hr, blood samples were collected from all the group of rats for biochemical analysis. Ahe animals were sacrificed by euthanasia and their hearts were excised and weighed.

Heart weight index evaluation & biochemical Analysis

Hearts were excised from each experimental group, rinsed with phosphate buffer to eliminate residual blood, blotted dry, and weighed.[4] The Heart Weight Index (HWI) is an important parameter for evaluating cardiac hypertrophy or edema in myocardial injury models. The heart weight to body weight ratio (g/100 g) was calculated using the formula:

$$\text{HWI \%} = [\text{Weight of heart (g)} / \text{Weight of rat (g)}] \times 100$$

Blood samples were collected for serum analysis of Creatine Kinase-MB (CK-MB) and Lactate Dehydrogenase (LDH) using standard biochemical assay kits.

2,3,5-Triphenyltetrazolium Chloride (TTC) staining

Ventricular sections of the heart were sliced into 2 mm thick segments and was incubated in a 1% TTC solution in phosphate-buffered saline at 37°C with intermittent shaking for 30 min. After incubation, the TTC solution was discarded, and the tissue was fixed in 10% formalin.[19] Images were captured using a high-resolution camera. TTC stained viable myocardium red, while infarcted or necrotic regions remained unstained.

Tissue antioxidant study

Homogenization of the cardiac tissue sections were done using 25 mM Phosphate Buffer (pH 7.4) to prepare a 10% w/v homogenate followed by centrifugation at 3000 rpm for 10 min. The supernatant was collected and stored at 4°C for subsequent antioxidant analyses. Protein concentration was quantified using the Bradford assay.[3] The homogenate was further used to assess Lipid Peroxidation (LPO) and Nitric Oxide (NO) generation by standard protocol. Briefly Lipid peroxidation in cardiac tissue was assessed using Thiobarbituric Acid Reactive Substances (TBARS). 0.5 mL of 10% tissue homogenate was mixed with 1 mL TBARS reagent, heated for 30 min, cooled, and centrifuged. Absorbance at 532 nm was measured, and MDA levels were quantified using a standard curve, normalized to tissue protein. Nitric Oxide (NO) levels were measured using the Griess assay by mixing 100 µL tissue homogenate with 500 µL Griess reagent, incubating for 30 min at room temperature, and measuring absorbance at 540 nm. NO levels were calculated from a sodium nitrite standard curve and normalized to protein content.[20]

Histological study

Tissue samples from hearts of different group of rats were fixed in 10% formalin, sectioned, stained with Hematoxylin and Eosin (H&E), and examined under a light microscope for histopathological analysis at 10x magnification.

Statistical analysis

The data are presented as mean±Standard Error of the Mean (SEM). Statistical analysis was performed with one-way ANOVA followed by a *post-hoc* Tukey test.

RESULTS

Phytochemical Analysis

The methanolic extract of *M. micrantha* leaves contains alkaloids, carbohydrates, glycosides, terpenoids, and significant amounts of phenolic and flavonoid compounds. These bioactive components may enhance its potential as a therapeutic agent for oxidative stress-related diseases. The extract has a high level of bioactive compounds, with a Total Phenolic Content (TPC) of 112.86±0.061 mg GAE/g and a Total Flavonoid Content (TFC) of 234.22±0.081 mg QE/g. The significant amount phenolic and flavonoid content of the extract substantiates its strong antioxidant potential, which may be effective in preventing chronic diseases by reducing oxidative stress.

In vitro Antioxidant and Anti-inflammatory Study

The *in vitro* antioxidant potential of the extract was evaluated with DPPH radical scavenging assay and reducing power assay, while protein denaturation assay, evaluated its ability to inhibit inflammation-related protein damage. Based on the analysis, the methanolic extract of *M. micrantha* leaves exhibited a significant and dose-dependent DPPH radical scavenging activity. Notably, the extract achieved 50% inhibition of DPPH radicals at a concentration of 97.08 ±0.023 µg/mL. The heat-induced protein denaturation is linked to inflammatory conditions, where the inhibitory effect of the extract has been evaluated. These results represent the extract exhibited a dose-dependent inhibition of protein denaturation, indicating effective anti-inflammatory properties, with significant activity observed at 161.62±0.035 µg/mL as IC_{50}. The reducing power assay further revealed a

concentration-dependent increase in the antioxidant activity of the methanolic extract of *M. micrantha* leaves (MME). The extract exhibited strong electron-donating ability, promoting the reduction of Ferric Ions (Fe^{3+}) to Ferrous Ions (Fe^{2+}), with antioxidant activity comparable to that of the standard ascorbic acid.

LC-MS Analysis

The LC-MS data demonstrated around 24 compounds including indole alkaloids, diterpenoids, phytosterols, polyphenols and flavonoid glycosides. In the positive ionization mode 4 peaks and negative ionization mode detected 6 significant peaks.

In the spectrum, Figure 1 shows the highest peak intensity at RT ~38-39 min with m/z 455.3132, occupying 73.46% of the total area. This prominent peak suggests the compound is (1α,3β)-Ergost-5-ene-1,3-diol ($C_{28}H_{48}O_2$), a steroidal metabolite related to ergosterol, found in fungal, plant, and marine sources. The compound was identified with high specificity, showing 89.86% isotope similarity, a score of 38.2, and minimal mass error. Its molecular weight is approximately 416.7 g/mol, and the potassium adduct m/z is 455.33. Phytosterols, like this compound, are fat-soluble and contribute to membrane stability, reported to exert various pharmacological properties that may benefit human health, including cholesterol lowering and cardioprotective effects.

In vivo Cardioprotective Study

The test extract caused no mortality even at 2000 mg/kg in the acute toxicity study, suggesting that the Lethal Dose (LD_{50}) is higher than 2000 mg/kg. Additionally, the cardioprotective effects of the methanolic extract of *M. micrantha* leaves was assessed using isoproterenol-induced cardiomyopathy model in experimental animal.

Cardiac tissue staining with 2,3,5-Triphenyltetrazolium Chloride (TTC)

The TTC dye staining assay evaluated the cardiomyocyte tissue viability, following myocardial infarction induction by ISO in the different experimental groups.

As depicted in Figure 2, the normal group (Figure 2A) illustrated predominantly red-stained myocardial tissue, indicating viable myocardium with intact mitochondria, while pale or white areas represented infarcted tissue. This red coloration results from dehydrogenase activity that converts TTC into a red formazan compound. In the disease control group (Figure 2B), extensive pale regions confirmed significant myocardial injury induced by isoproterenol. Both the standard drug and *M. micrantha* extract-treated groups exhibited largely red-stained tissue, indicating recovery of dehydrogenase activity and viable cells (Figure 2C-2E). The 200 mg/kg dose of MME revealed presence of some pale areas pertaining mild myocardial damage, which was almost entirely reversed in the 400 mg/kg dose. Figure 2F confirmed that MME (400 mg/kg) alone did not induce any pathological changes, demonstrating its safety and non-toxicity to cardiac tissue.

Heart weight index evaluation

Data represented in Table 1 indicated ISO-induced myocardial injury led to a significant elevation in Heart Weight Index (HWI) ($p<0.05$) in the disease control groups compared to the normal group. This reflected cardiac hypertrophy, stress on the cardiac muscle, interstitial edema, inflammation due to oxidative damage, and myocardial necrosis. Reduction in HWI was evident with Enalapril and MME treatment, suggesting moderate protection towards myocardial damage. The group receiving only MME (400 mg/kg) for 21 days had an HWI of 0.32%, indicating no hypertrophic effects and confirming the extract's safety.

Serum analysis

Cardiac marker enzymes CK-MB and LDH were measured in serum to assess cardiac tissue damage. The cardiac marker profiles for all the experimental groups are represented in Table 1. The current study showed a significant increase in the serum levels of cardiac marker enzymes CK-MB and LDH in ISO-treated rats compared to the normal control group ($p<0.05$). However, pretreatment with Enalapril and *M. micrantha* extract significantly reduced these enzyme levels in the ISO-induced rats. Pre-treatment with MME at 400 mg/kg depicted a slight increase level of these markers; however, the levels were significantly lower compared to those administered ISO ($p<0.05$) and developed cardiomyopathy.

Tissue antioxidant studies

LPO and NO levels are commonly used biomarkers for oxidative and nitrosative stress, which are critical indirect indicators of tissue antioxidant status. Elevated levels of LPO and NO indicate impaired antioxidant defense and enhanced oxidative and nitrosative stress. As evident from Table 1, LPO and NO levels were markedly increased ($p<0.05$) in cardiac tissues of the ISO-induced rats, indicating marked oxidative and nitrosative stress from myocardial injury. Treatment with Enalapril (10 mg/kg) effectively reduced both LPO and NO levels ($p<0.05$), demonstrating its known cardioprotective and antioxidant effects. Similarly, the Methanolic extract of plant (MME) exhibited a dose-dependent protective effect, with 400 mg/kg MME significantly reducing LPO and NO levels to near normal, comparable to Enalapril. MME alone at 400 mg/kg did not elevate oxidative markers, suggesting its safety and potential intrinsic antioxidant properties. These findings highlight MME's potential as a therapeutic agent in mitigating ISO-induced myocardial damage.

Histological study

Histopathological examination of cardiac tissue sections revealed normal architecture in healthy control rats (Figure 3A), with dense, elongated myocardial fibers, normal cardiomyocyte nuclei, and erythrocytes in blood vessels. In contrast, control group of rats, induced with ISO (85 mg/kg) and remain untreated, exhibited severe myocardial damage, including necrosis, nuclear pyknosis, and inflammatory cell infiltration, indicating significant injury (Figure 3B).

In contrast, the cardiac tissues from rats treated with Enalapril (Figure 3C) displayed near-normal architecture with preserved myofibrillar striations and continuity. Rats pretreated with *M. micrantha* Extract (MME) at 200 mg/kg illustrated moderate tissue damage, mild neutrophilic infiltration, and intact cardiomyocyte nuclei, without necrosis (Figure 3D). The higher dose of MME (400 mg/kg) (Figure 3E) exhibited significantly improved tissue architecture, with well-preserved cardiac muscle morphology and no focal necrosis, resembling normal histology representing the extract's protective effects against ISO-induced myocardial injury. The group treated with only MME (400 mg/kg) displayed normal myofibrillar structure, without edema or necrosis, indicating the extract's non-toxic effect on cardiac tissue.

DISCUSSION

M. micrantha, a highly invasive plant species in Southeast Asia and the Pacific, significantly threatens agriculture and ecological balance, however found to be beneficial in traditional herbal medicine. The plant contains over 150 phytochemicals,

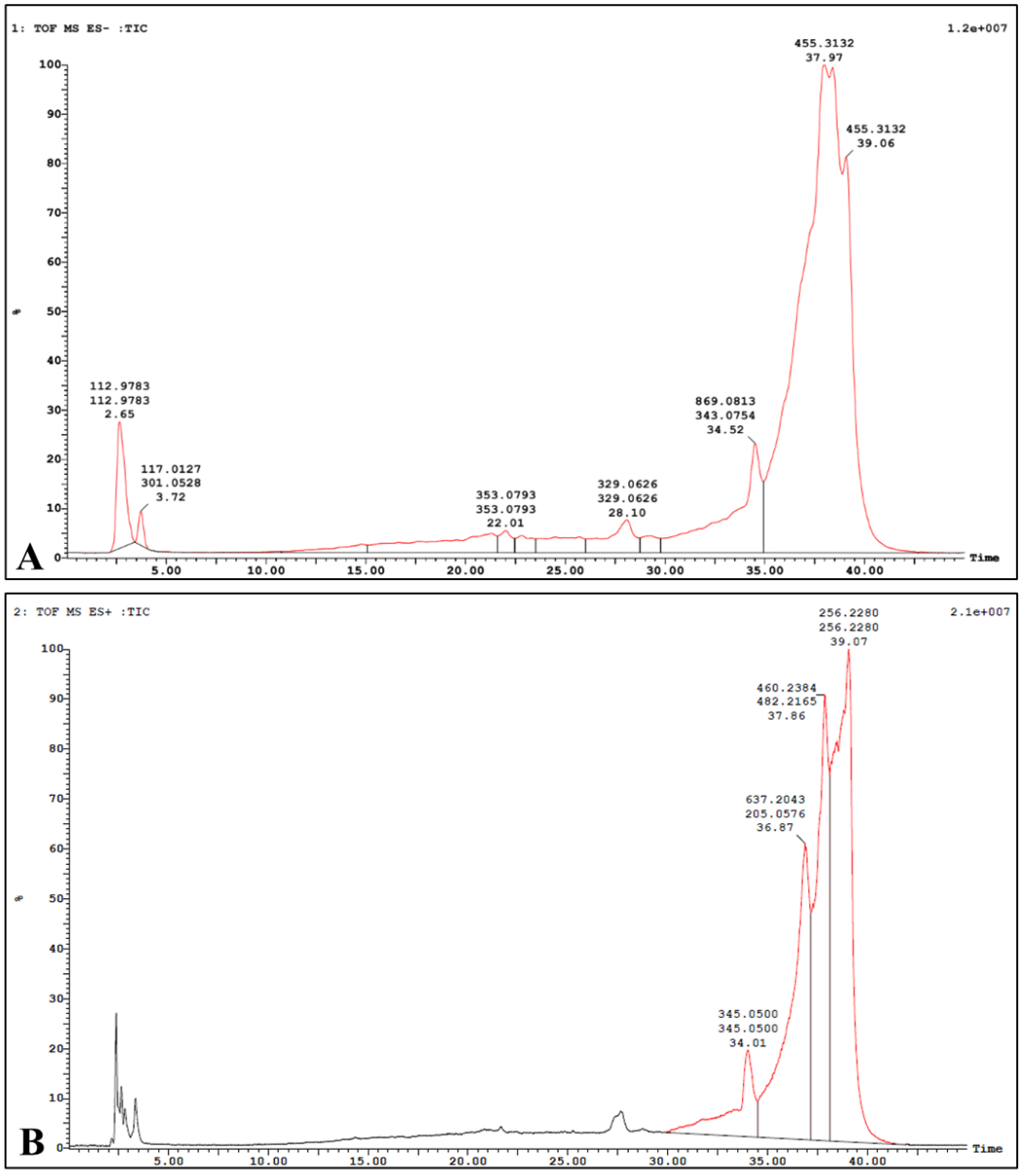

Figure 1: LC-MS Spectra of the methanolic extract of *Mikania micrantha* Kunth leaves. [A] denotes the negative ionization mode and [B] positive ionization mode used.

including essential oils, phenolics, flavonoids, and terpenes, with diverse bioactivities such as antioxidant, anti-inflammatory, antimicrobial, antiparasitic, cytotoxic, antidiabetic, lipid-lowering, wound-healing, and neuroprotective effects.[8,21] A recent study focused on the bioactive properties of its leaf extract, highlighting high flavonoid content (234.22±0.081 mg QE/g) compared to phenolic content (112.86±0.061 mg GAE/g) that substantiated the present study findings. Other research indicates *M. micrantha* contains significant amount of diverse phenolic compounds possesing antioxidant potential, offering health benefit.[22,23] Studies also demonstrated that anthocyanin levels in the plant increase in winter, boosting flavonoid content and enhancing its bioactive properties, supporting its potential for treating oxidative stress-related chronic diseases.[9,24] The methanolic extract of *M. micrantha* exhibited strong antioxidant activity comparable to ascorbic acid, achieving 50% DPPH radical inhibition at 50 µg/mL and showing enhanced ferric ion-reducing power. Dong *et al.* isolated fourteen phenolic compounds from its aerial part, revealing potent free radical scavenging activity, equal to or exceeding those of ascorbic acid.[22] Anthocyanin content, which contributes to antioxidant potential, markedly increases in winter, enhancing the plant's bioactivity.[24] Furthermore, anti-inflammatory effect indicated the extract's capability to inhibit heat-induced protein denaturation, complying with an Indonesian study, which identified thirteen sesquiterpene lactones, including micrantholides, demonstrating potent anti-inflammatory activity.[25]

Limited studies suggest *M. micrantha* has cardioprotective potential, for example, anti-hypercholesterolemic activity of ethyl acetate stem extract, which revealed the improvement in the lipid profiles, inhibition of key enzymes, and lipid peroxidation.[26] In the present study, methanolic leaf extract of *M. micrantha* significantly mitigated ISO-induced myocardial injury in rats. Test extract reduced cardiac hypertrophy and tissue damage in a dose-dependent manner, as evident from decreased HWI and near-normal tissue morphology in TTC staining, particularly at 400 mg/kg, showing effects comparable to Enalapril. Biochemical markers CK-MB and LDH were significantly lowered, and histology confirmed improved myocardial architecture, suggesting efficacy and safety. ISO-induced injury, characterized by oxidative stress and inflammation, was alleviated by *M. micrantha*, as shown by reduced LPO and NO levels. Additionally, Vijayakumar STV *et al.* with their *in silico* analysis reported inhibition of including pro-inflammatory markers including Cyclooxygenase (COX), inducible Nitric Oxide Synthase (iNOS), myeloperoxidase, proteases, and Angiotensin-1 Converting Enzyme (ACE), emphasizing its cardioprotective mechanism.[27]

Literature supports that triterpenes and phytosterols are commonly found in plants of the Mikania family and these phytoconstituents are helpful in cardiovascular disorders.[28-30] In the present study, LC-MS analysis identified (1α,3β)-Ergost-5-ene-1,3-diol, a steroidal compound related to ergosterol, as the most abundant compound in the *M. micrantha* extract, comprising about 73.46% of the total area. The compound is popular for its

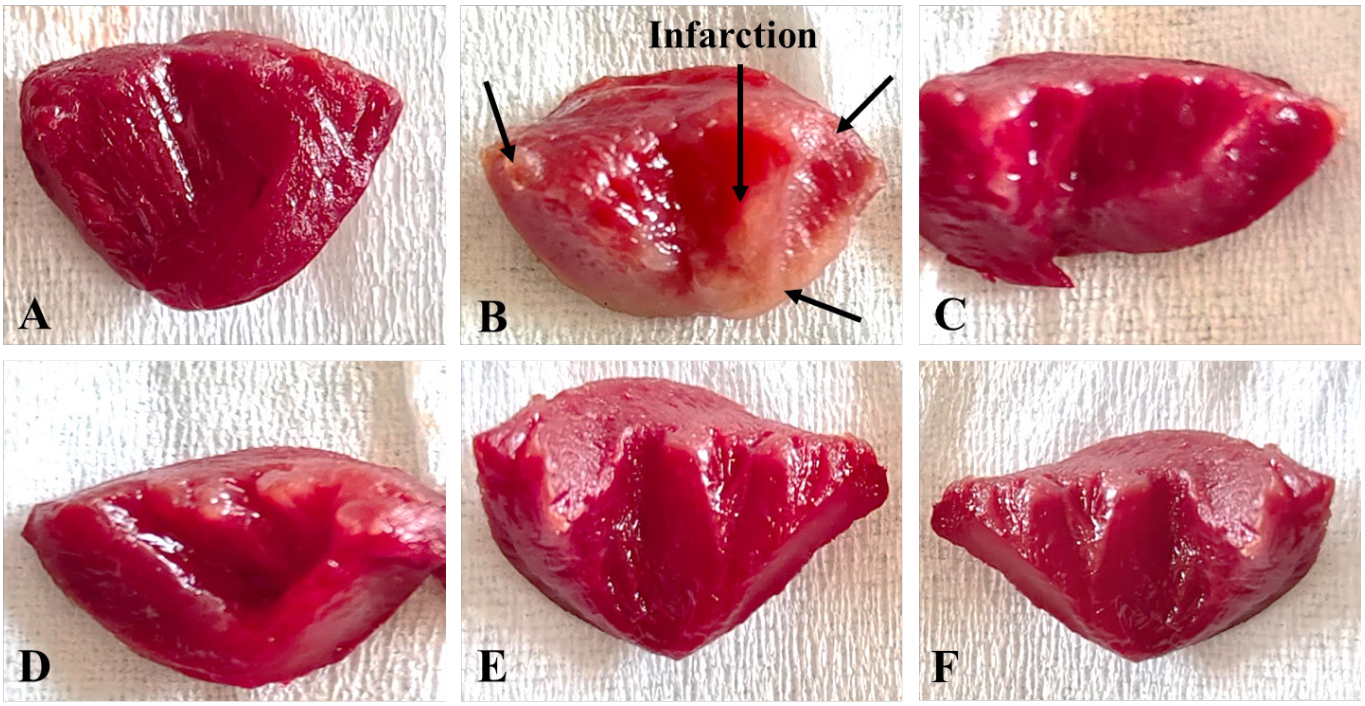

Figure 2: Represents the TTC stained cardiac tissue isolated from the different experimental groups. (A) Normal control, (B) ISO-induced Disease control, (C), (D) & (E) denotes treated with Enalapril 10 mg/kg, MME 200mg/kg & 400mg/kg respectively. (F) is the only extract (400 mg/kg) treated group. Arrow denotes the tissue injury results in infarction.

Table 1: HWI, Serum analysis, and Tissue antioxidant parameters in the experimental groups.

Groups	Heart weight index (HWI)	Serum analysis		Tissue antioxidant parameters	
		LDH (U/L)	CK-MB (U/L)	LPO (µM/mg of protein)	NO (mM/mg of protein)
Normal control	0.27%±0.003	231.64±19.44$^{*\#}$	188.87±4.44*	0.0137±0.0018*	0.63±0.10*
Disease control	0.43%±0.018$^{\$}$	1111.00±27.00$^{\#}$	1284.32±135.54$^{\#}$	0.0379±0.0008$^{\#}$	2.91±0.50
Enalapril 10 mg/kg	0.32%±0.002	426.62±41.11*	329.97±38.89*	0.0161±0.0026*	1.21±0.10*
MME 200mg/kg	0.34%±0.042	997.68±20.33$^{\#}$	838.81±61.11$^{*\#}$	0.0271±0.0011$^{*\#}$	1.13±0.08*
MME 400 mg/kg	0.36%±0.001	636.60±20.00$^{*\#}$	694.38±3.33$^{*\#}$	0.0168±0.0027*	0.99±0.03*
Only MME 400 mg/kg	0.32%±0.029	385.52±14.00*	262.20±8.89*	0.0154±0.0008*	0.63±0.10*

Data are expressed as Mean±SEM (n=5). * and # indicate significance at p<0.05 when compared to disease control group and standard group, respectively. $ denotes significance at p<0.05 compared to the normal control group

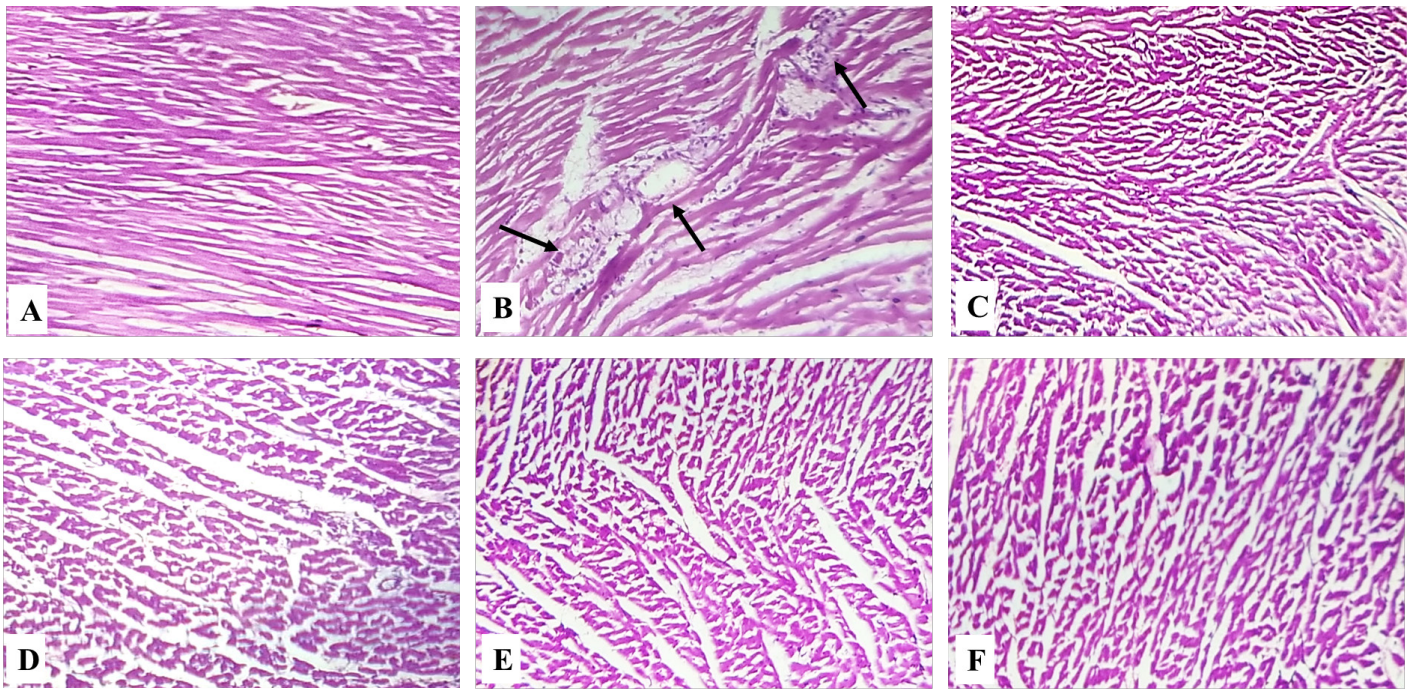

Figure 3: Effect of leaf extract of*Mikania micrantha* (MME) on histopathology of the cardiac tissue (magnification 100x). (A) Healthy Control (B) ISO control, (C) ISO + Enalapril 10 mg/kg group, (D) ISO + MME 200 mg/kg, (E) ISO + MME 400 mg/kg, (F) Only MME 400 mg/kg treated rats. The arrow indicates myocardial damage and infiltrated inflammatory cells.

membrane-stabilizing properties, which might be responsible for the extract's cardioprotective and anti-inflammatory effects. Based on these findings, it can be suggested that the extract's antioxidant properties, likely driven by its substantial presence of bioactive compounds, particularly flavonoids, anthocyanins and phytosterols, play a crucial role in preserving cardiac integrity and function.

CONCLUSION

Mikania micrantha Kunth leaf extract demonstrates significant antioxidant, anti-inflammatory, and cardioprotective effects, likely attributed to its abundant phenolic and flavonoid compounds, along with a high prevalence of phytosterols. Both *in vitro* and *in vivo* results emphasize its potential as a therapeutic agent for combating oxidative stress and inflammation, particularly in cardiovascular diseases. However, additional studies, including

detailed mechanistic evaluation and clinical trials, along with detailed characterization of bioactive compounds are needed to confirm these findings and fully confirm the therapeutic potential of *Mikania micrantha*.

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ABBREVIATIONS

MI: Myocardial infarction; **MME:** *Mikania micrantha* leaf extract; **ISO:** Isoproterenol; **HWI:** heart weight index; **CK-MB:** Creatine kinase-MB; **LDH:** Lactate dehydrogenase; **TTC:** 2,3,5-Triphenyltetrazolium chloride; **GAE:** Gallic acid equivalent; **QE:** Quercetin equivalent; **DPPH:** 2,2-diphenyl1-picryhydrazyl; **LC-MS:** Liquid chromatography mass spectroscopy; **ESI:** Electrospray Ionization; **MRM:** Multiple Reaction Monitoring; **ROS:** Reactive oxygen species; **IC_{50}:** Inhibitory concentration 50%; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **OECD:** Organization for Economic Co-operation and Development; **LPO:** Lipid Peroxidation; **NO:** Nitric Oxide; **MDA:** Malondialdehyde; **TBARS:** Thiobarbituric Acid Reactive Substances; **H&E**: Hematoxylin and Eosin; **SEM:** Standard Error of the Mean; **ANOVA:** Analysis of Variance; **RT**: Retention Time; **m/z**: Mass-to-Charge Ratio; **ABTS:** 2,2′-Azi no-bis(3-ethylbenzothiazoline-6-sulfonic acid); **FRAP:** Ferric Reducing Antioxidant Power; **M. micrantha:** *Mikania micrantha*.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

The methanolic extract of *Mikania micrantha* leaves is rich in diverse phytochemicals, including alkaloids, glycosides, terpenoids, and substantial amounts of phenolic (112.86 mg GAE/g) and flavonoid (234.22 mg QE/g) compounds, which contribute to its strong antioxidant capacity. *In vitro* evaluations demonstrated significant, dose-dependent antioxidant and anti-inflammatory activities, as evidenced by effective DPPH radical scavenging and inhibition of protein denaturation. The extract also exhibited pronounced reducing power, indicating robust electron-donating ability. LC-MS profiling revealed bioactive constituents, including indole alkaloids, diterpenoids, and phytosterols. Among these, (1α,3β)-Ergost-5-ene-1,3-diol—a steroidal compound with evident pharmacological properties was identified as a major component. Isoproterenol-induced myocardial injury model in rats confirmed the extract's cardioprotection effect. TTC staining indicated substantial myocardial protection, particularly at 400 mg/kg, with minimal infarction, along with this, biochemical analyses showed significant decreases in heart weight index, serum CK-MB and LDH levels, and oxidative stress markers (LPO and NO) than control. Histological assessments corroborated these results, revealing preserved myocardial architecture and reduced inflammation and necrosis in treated groups. Overall, these findings demonstrate that *Mikania micrantha* leaf extract possesses potent antioxidant, anti-inflammatory, and cardioprotective properties, supporting its potential as a natural therapeutic candidate for managing oxidative stress and myocardial injury.

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