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Evaluation of cardio protective Phytochemicals from *Phyllanthus maderaspatensis- an in vitro, in vivo and in silico* approach

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ABSTRACT

Background: Plants are used as complementary and alternative medicine for a variety of conditions, regardless of their crude or as their separated pharmacological components. The purpose of this study was to assess in vitro antioxidant and in vivo cardio protective of the *Phyllanthus maderaspatensis*. **Methodology**: Quantitative analysis and Gas Chromatography-Mass Spectrometry (GC-MS) phytochemical study based on solvent method of extraction were carried out. Using the conventional method of antioxidant assay among the leaf extract were ascertained. Isoproterenol (ISO)-treated rats were used for *in vivo* experiment to check the effect of plant extract on cardio protective bio markers. **Results.** There is an increased total antioxidant activity of extract found in a dose-dependent manner. At 50 µg, 19-24% activity and at 400 µg, 79-84% scavenging potential was recorded. The rats receiving the extract showed a decrease in heart weight and an increase in body weight. The mean concentration of cardiac troponin (cTn) T/I in the serum of rats treated with the standard drug reduced effectively (1.13±0.07/0.64±0.05 ng/mL), whereas significantly by the extract (1.25±0.19/0.72±0.08 ng/mL). The creatine kinase MB (CKMB) of the standard was significant at 78.33±6.26IU, and the extract was 83.35±7.51IU. The data showed increased values among the standard and extract compared to the control group but were found to be significantly ($p < 0.001$) decreased among the treated groups. Compared to negative control animals (healthy rats), isoproterenol (ISO)-treated rats had significantly increased levels of aspartate myocardial cell damage. After 5 weeks of treatment, in comparison to those in the positive control (PC), extract-treated rats had significantly ($p <0.05$) elevated levels of serum AST, alanine transaminase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH). Recovery from ISO-induced electrophysiological abnormalities was confirmed by decreased serum cardioprotective markers, mainly troponin and CKMB. The damage to the heart tissues was significantly reduced and found closer to control and standard drug-treated groups at doses of 200 mg/kg body weight (bwt). The data confirm that the cardioprotective benefits are attributed to the presence of antioxidant components in the extract of *Phyllanthus maderaspatensis.*

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INTRODUCTION

Despite tremendous improvements in treatment and prevention over the past few years, myocardial infarction (MI) caused by ischemic cardiovascular disease remains a severe medical illness and a major problem[1]. MI is still a major public health concern that is causing a growing number of deaths in both developed and developing countries[2]. Cardiovascular diseases are a leading reason fatalities worldwide. The formation of endogenous antioxidants and oxidative stress, along with an increase in reactive oxygen species (ROS) in the myocardium, are typically linked to cardiac failure[3]. Conventional medicinal herbs produce a wide range of secondary compounds with pharmacological value to treat oxidative stress-induced conditions[4]. The primary source of medicinal plants' antioxidant action is their medicinally active compounds. Investigation on prophlaxis has consistently identified bioactive substances with antioxidant and cardioprotective prospective, such as flavonoids, phenolic compounds, alkaloids, and terpenoids[5]. Due to new research and studies on their biological characteristics, polyphenols are now highly valued. They are great sources of lipid peroxidation agents and free radicals because they constitute strong antioxidants[6]. According to[7], a variety of sustainable natural plants are explored for their use in health and ecological research, including the prevention of cardiovascular diseases. Therefore, the purpose of this study is to identify the powerful components present in *Phyllanthus* plant extracts that have antioxidant molecule. The current study also entails the cardioprotective activity of *Phyllanthus maderaspatensis. The Phyllanthus (Phyllanthaceae)* genus has more than 1000 species. Ethnic communities in India and other Asian countries have traditionally used herbs from the Phyllanthus genus in traditional folk treatments. Many Phyllanthus plants are vital to the Ayurvedic medical system in India. It seems reasonable that the most of study is concentrated on pharmacognostics and to differentiate biological studies considering the importance and potential of those plants[8].

Materials and methods

Extraction of *Phyllanthus maderaspatensis*

The whole plant was collected from the local area around rasipuram, Namakkal District, Tamilnadu India. The plant was botanically identified and authenticated by *Phyllanthus maderaspatensis* by ABS Botanical Garden (AUT/ MCAS/176). Plants were shed dried and made in coarse powder. The dried powder (100 g) of the plants was extracted using 300 mL of polar, and polar solvents under soxhlet extraction for 6h at 40°C. The solvent phase was collected and dried at vaccum drying. The residues weight was taken and redissolved in respective solvent. The composition of secondary metabolites determined through GCMS.

Quantitative phytochemical analysis (9-11)

The concentration was valuated spectrophotometrically, employing standardized procedures for the quantification of chief phytochemical constituents, including alkaloids, phenols, flavonoids, saponins, and tannins. Total phenol content (TPC) of the extracts was determined by the method of Folin-Ciocalteu, using gallic acid as the standard spectrophotometrically[9]. Flavonoid determination was carried out by the colorimetric method[10] with rutin as the standard. The procedure of[11] was followed for the quantification of tannins. Saponins were quantified following the methodology of Obadoni and Ochuko. Alkaloids were quantified by following the procedure of (Harborne *et al*., 1998).

Antioxidant capacity assays

For antioxidant potential determination different assays were performed to assess the antioxidant prospective against various free radicals and by different mechanisms of action. Extracts, fractions and positive standards (ascorbic acid, Rutin, catechin and gallic acid) 1 mg were liquefied in 1 ml analytical methanol. These solutions were further serially diluted to get 50, 100, 200 and 400µG/mL. DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical scavenging assay, Hydroxyl free radicals scavenging, ABTS*+scavenging and hydrogen peroxide scavenging test were performed with known standard (Ascorbic acid). For ABTS, DPPH and H2O2 scavenging the antioxidant was calculated by using the following formula

Percentage of scavenging = Blank-Test/Blank x 100

The OH scavenging was calculated by using the following formula = 1-Test/Control x 100

Experimental animal

Adult male albino rat (150 gm) was used for this experiment. A total of 20 animals were maintained in the aluminium cages (1 × 0.5 × 0.5 m) in an animal house with well-ventilation at 30 ± 2 °C with 12 h light/dark condition. The humidity 60 ± 5% was maintained under standard laboratory condition. The laboratory animals were maintained according to use

of laboratory animals. This work has been approved by the ethical committee (IAEC/MCAS/3/2022)

Experimental procedure:

The experimental animals were grouped into five having 6 animals each (n = 6). All rats were acclimatized for 10 days before performing the experiments. Isoproterenol was administered intraperitoneally to the rat among group II to V (100 mg/kg bw). To the first group, physiological saline was administered and only isoproterenol was provided to the second group. To the experimental groups III, IV were administered (100 and 200 mg/ kgbw) with plant extract. To group V, Atorvastatin was administered (1.0 mg/kgbw) before intraperitoneal injection of isoproterenol. After the experiment, blood was withdrawn from the experimental and control animals without anticoagulant. Biomarker analysis from the serum sample.

Serum Biomarker analysis

The biomarkers were analyzed from the serum sample. After 48 h of the final treatment, blood was collected from the control and experimental animals. Serum samples were subjected for the determination of LDH, alkaline phosphatase, alanine transaminase, aspartate aminotransferase, CPK. All the biochemical parameters were performed using kinetic method as described by Meril diagnosis kit.

Biomarker analysis from the heart tissues

The experimental animals were sacrificed and heart muscle was separated and weight was recorded. The muscle was repeatedly washed with sodium phosphate buffer (pH 7.0). About 1 g of heart muscle homogenized in cold PBS then it was centrifuged at 10000 rpm for 10 min and clear extract was obtained. It was used for the analyses of CPK, troponin, TBARS and HP Analyses were performed using clinical diagnostic kit (Meril,). The activity of the CK-MB isoenzyme was measured by an immunoinhibition assay. Cardiac TnT was determined by a commercially available enzyme linked immunosorbent assay (ELISA)

Results and Discussion

This study meticulously investigates the effects of *Phyllanthus maderaspatensis* leaf extract (PMLE) on various cardiac parameters, including cardiac troponin levels, cardiac biomarkers, lipid peroxidation products, and the antioxidant system, employing an in vivo experimental model to elucidate its potential cardioprotective properties. The chemical characterization of PMLE was conducted using GC-MS, which revealed a complex spectrum comprising 25 distinct peaks (Figure 1), indicative of the diverse phytochemical constituents present within the extract. Among these, the first eluted compound was identified as 7-Methyl-Z-tetradecen-1-olacetate, which appeared at a retention time of 12.328 minutes and constituted 2.49% of the total extract, while the last eluted compound was characterized as Oxirane, 2-methyl-2-pentyl (CAS), recorded at 20.411 minutes with a relative abundance of 1.14%. Additionally, several notable compounds were identified, including n-Hexadecanoic acid (23.87%), 9-Octadecenoic acid (Z)-(CAS) Oleic acid (11.62%), and Octadecanoic acid (16.91%), which represent significant findings as these compounds have not been previously reported in this plant species (Table 1). Furthermore, a quantitative analysis (Table 2) of the extract revealed the concentrations of various phytochemicals, including alkaloids (10.20 mg/g), flavonoids (9.21 mg/g), tannins (5.15 mg/g), saponins (7.33 mg/g), phenols (6.48 mg/g), and steroids (0.69 mg/g). These findings stand in contrast to those reported by [12], who documented lower levels of flavonoids, steroids, and phenols in their analysis of PMLEE. Conversely, the results of this study align with the observations made by[13], which highlighted the abundance of polyphenols in aqueous ethanolic extracts of Phyllanthus species. Collectively, these findings underscore the potential of Phyllanthus maderaspatensis as a valuable source of bioactive compounds that may confer cardioprotective effects, warranting further investigation into its therapeutic applications in cardiovascular health.

Table 1. List of Compounds identified from GCMS

peak	RT	Area %	Compound
1.	12.328	2.49	7-Methyl-Z-tetradecen-1-olacetate
2.	12.525	6.89	1-Octadecyne
3.	12.794	1.68	2-Hexadecen-1-ol,3,7,11,15-tetramethyl-,[R-
4.	13.000	2.24	Hexadecanal (CAS)Palmiticaldehyde
5.	13.875	23.87	n-Hexadecanoicacid
6.	14.817	0.76	Citronellolepoxid (RoderS)
7.	15.437	4.37	Phytol
8.	15.722	11.62	9-Octadecenoicacid(Z)-(CAS)Oleicacid
9.	15.936	16.91	Octadecanoicacid
10.	16.300	2.92	Octane,1-ethoxy-
11.	16.367	1.15	Ethyl1-hexyl-4-hydroxy-2(1h)-ox
12.	16.458	1.04	cis-Aconiticanhydride
13.	16.550	1.18	2,5,5-trimethyl-3,6-heptadien-2-ol
14.	16.750	1.14	Ethanone,1-(1-hydroxycyclopentyl)-
15.	16.921	1.11	Phenethylamine,N-methyl-.beta.,3,4-tris(trime

16.	17.078	9.08	9-Octadecenamide,(Z)-(CAS)Oleoamide
17.	17.317	0.92	7-Octen-3-ol,2,6-dimethyl-
18.	17.424	1.66	2H-Pyran,2-(3-butynyloxy)tetrahydro-(CAS)
19.	17.525	1.15	1,6:3,4-Dianhydro-2-deoxy-.beta.-D-lyxo-hex
20.	17.708	1.62	Hexadecane,1,16-dichloro-
21.	17.849	1.23	Pyrrolidine-2-one,5-[2-benzoylethyl]-
22.	18.059	2.15	Cyclopentanemethanol,.alpha.,.alpha.-dimethy
23.	18.383	0.90	Propanenitrile,3-ethoxy-(CAS)3-Ethoxyprop
24.	18.725	0.79	4-Methylcyclohexanolacetate
25.	20.411	1.14	Oxirane,2-methyl-2-pentyl-(CAS)1,2-EPOX

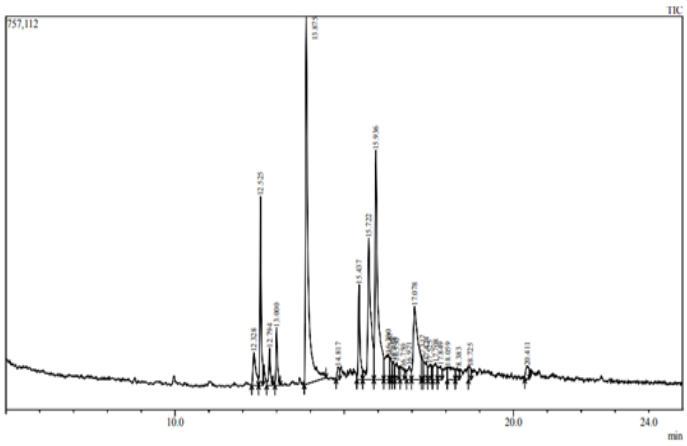

Figure 1. Compounds eluted from GCMS of *Phyllanthus maderaspatensis leaf extract*

Table 2. Phytochemicals estimated from *Phyllanthus maderaspatensis*

S.No.	Phytochemicals	concentration (mg/g)
1	Total alkaloids	10.20±0.03
2	Flavonoids	9.21±0.13
3	Tannins	5.15±0.22
4	Saponins	7.33±0.40
6	Total phenolics	6.48±0.19
13	Steroids	0.69±0.03

The antioxidant ability of PMLE was rigorously evaluated through various assays, including DPPH, ABTS, hydrogen peroxide, and hydroxyl scavenging assays, revealing that the radical scavenging capacity of ascorbic acid consistently surpassed that of the extract in a concentration-dependent manner, as illustrated in (Figure 2). Specifically, the standard ascorbic acid demonstrated more than 70% free radical scavenging activity at a concentration of 200 µg/mL, with calculated IC50 values of 120.48 µg, 116.21 µg, 83.94 µg, and 95.92 µg for the respective assays, as detailed in (Table 3). At a higher concentration of 400 µg/mL, ascorbic acid exhibited over 90% scavenging activity in all assays except for DPPH. In contrast, PMLE displayed less significant scavenging activity at 100 µg/mL, moderate activity at 200 µg/mL, and significant activity at 400 µg/mL, as shown in (Table 4). Notably, the extract demonstrated antioxidant potential ranging from 79% to 84% at maximum concentration, while at 200 µg/mL, the scavenging capacity fell below 70%. The extract's free radical scavenging capacity was less than 45%, whereas ascorbic acid exhibited an activity range of 45% to 55%. The IC50 values for PMLE were determined to be 191.52 µg, 184.33 µg, 148.90 µg, and 191.28 µg for the DPPH, ABTS, hydrogen peroxide, and hydroxyl scavenging assays, respectively, indicating that the extract's IC50 value for DPPH was significantly higher than that of the reference standard ascorbic acid. Interestingly, the ABTS scavenging activity of the extract was found to be 78% higher than that of the standard, while the hydrogen peroxide scavenging capacity of the extract was observed to be two-fold greater than that of ascorbic acid. Furthermore, the hydroxyl scavenging activity of the extract, measured at 96 µg, was greater than that of the standard. Overall, when compared to the standard concentration, the activity of PMLE was less pronounced. These findings align with previous reports by[14] who documented the antioxidant activities of Indian Phyllanthus, and are supported by additional studies indicating that various compounds derived from Phyllanthus species act as efficient scavengers of hydroxyl radicals, as noted by[15] Moreover, the data confirms the findings of[16] who reported that the extract of PMLEE exhibits a marked free radical scavenging effect, further emphasizing the potential of this plant as a source of natural antioxidants.

Table 3. Percentage of free radical scavenging by Standard

Concentration	DPPH	ABTS	H_2O_2	Hydroxyl scavenging
50	27.18±0.67	30.48±0.91	36.12±1.12	34.12±1.02
100	49.52±1.03	52.31±1.05	55.91±1.33	51.72±1.21
200	78.51±1.50	71.71±1.62	77.41±1.50	79.80±1.52
400	88.34±1.65	90.85±1.92	92.12±1.62	90.61±1.79
IC_{50}Value µg/ml	**120.48**µg	**116.21**	83.94	95.92

Table 4. Percentage of free radical scavenging by *Phyllanthus maderaspatensis*

Concentration	DPPH	ABTS	H_2O_2	Hdroxyl scavenging
50	20.47±0.66	22.12±0.84	27.62±0.52	19.64±0.71
100	32.50±1.02	37.23±1.28	42.29±0.78	37.28±0.92

200	62.80±1.39	59.62±1.43	69.82±1.01	60.74±1.41
400	81.45±1.55	83.16±1.81	84.68±1.45	79.85±1.60
IC_{50}Value µg/ml	**191.52**	**184.33**	**148.90**	**191.28**

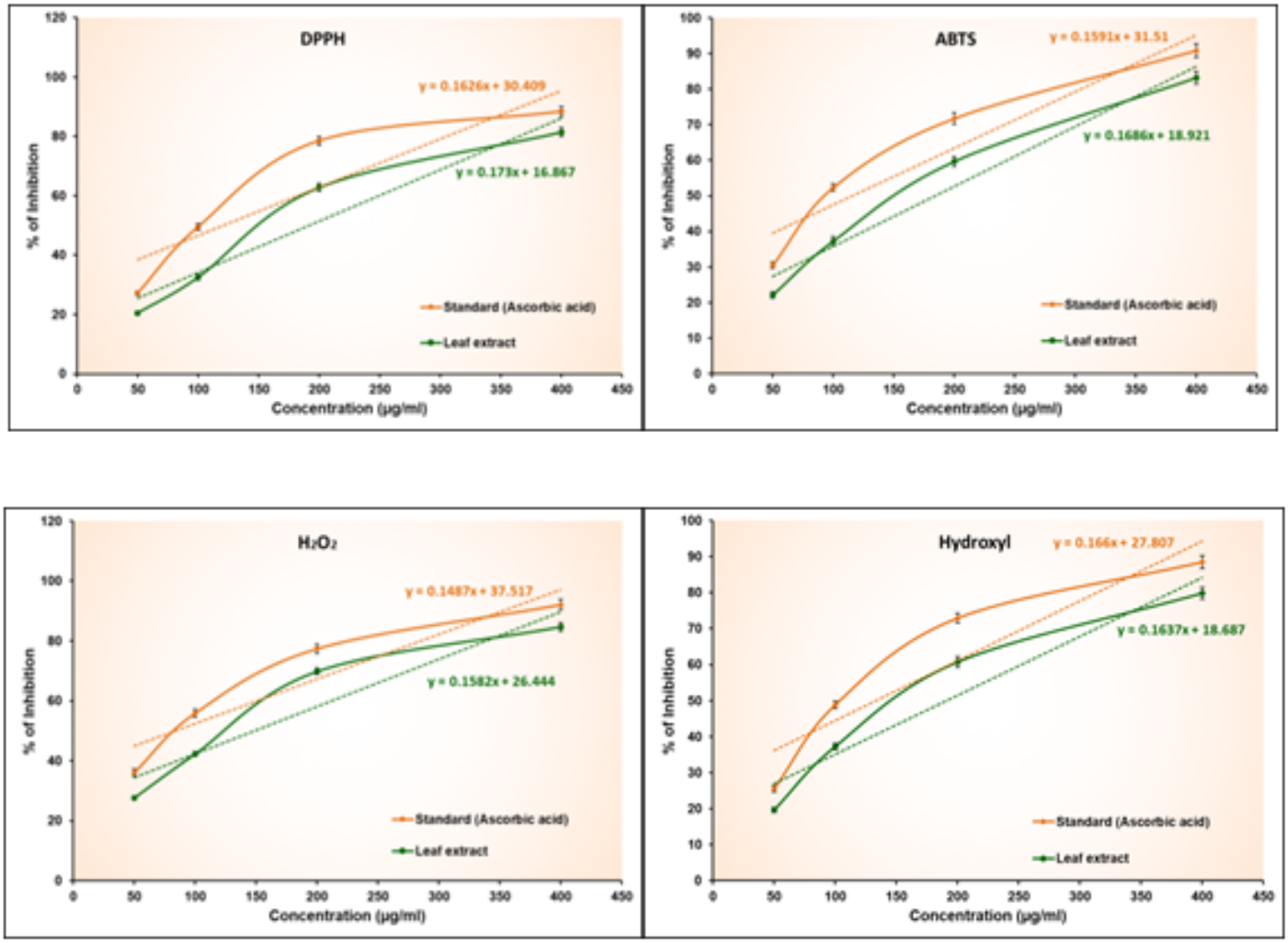

Figure 2. Free radical scavenging activity of Phyllanthus maderaspatensis and standard

The protective properties of PMLE were assessed by monitoring various parameters, including body weight, heart weight, cardiac biomarker enzymes, and cardiac histology at the conclusion of the research duration. Throughout the study period, a general reduction in body weight was observed across all animal groups, with the most significant decrease recorded in the isoproterenol (ISO) induced untreated negative control group, which exhibited a decline from 200 grams to 150 grams. In contrast, both the control and standard-treated animals experienced a 15% reduction in body weight, stabilizing around 178 grams. The average body weight of the animals in group 3, which received PMLEE treatment, showed a more pronounced decrease of 21.5%, resulting in an average weight of 157 grams, while group 4, which received a different treatment regimen, demonstrated a 16% reduction, averaging 168 grams. Regarding heart weight, the control group exhibited an average heart weight of 0.79 ± 0.05 grams, whereas the positive control animals, which were subjected to ISO treatment without any intervention, had an average heart weight of 1.55 grams, indicating significant inflammation of the heart. Notably, treatment with PMLEE at a dosage of 100 mg resulted in a 21% reduction in heart weight compared to the negative control, and at a higher dosage of 200 mg, the reduction was even more substantial at 41.2%. Furthermore, treatment with Atorvastatin, a standard cardioprotective agent, led to a 46% decrease in heart weight relative to the negative control group, as summarized in (Table 5).

Table 5. Effect of ethanol extract of ***Phyllanthus maderaspatensis*** on body weight

Groups	Body weight(g)	Heart weight (g)
Group I–Control	178.5±4.5	0.79±0.05
Group II-ISO induced	150.3±5.7	1.55±0.09

Group III-ISO+EELPM (100mg/kgb.wt)	157.9±6.1	1.12±0.07
Group IV-ISO+EELPM (200mg/kgb.wt)	168.5±5.4	0.91±0.08
Group V–Atorvastatin (Standard)	177.9±6.9	0.83±0.05

When comparing the effects of PMLE to the control group, the extract was found to significantly elevate cardio-hepatic biomarkers, including creatine phosphokinase (CPK), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), as well as cardiac markers such as creatine kinase-MB (CK-MB) and troponin, with the data demonstrating statistical significance at a p-value of less than 0.05. Notably, troponin levels were significantly increased in ISO treated animals compared to the control group, as illustrated in (Figure 3), where Troponin-T levels were found to be 39% higher in the ISO treated groups than in the control. Treatment with PMLEE at a dosage of 100 mg resulted in a 23% reduction in troponin levels, while a higher dosage of 200 mg led to a 27% reduction, and administration of the standard treatment resulted in a reduction of approximately 32%. Statistical analysis revealed that the two-tailed p-value between group II (ISO treated) and group III (PMLE 100 mg) was 0.0503, indicating that this difference is not quite statistically significant. In contrast, the two-tailed p-value between group II and group IV (PMLE 200 mg) was 0.0306, which is considered statistically significant. Furthermore, the two-tailed p-value between group II and group V (standard treatment) was 0.0043, indicating a very statistically significant difference. However, the two-tailed p-value between group IV and group V was 0.3627, suggesting no significant difference, while the p-value between group II and group V was 0.0505, which is also considered not statistically significant.

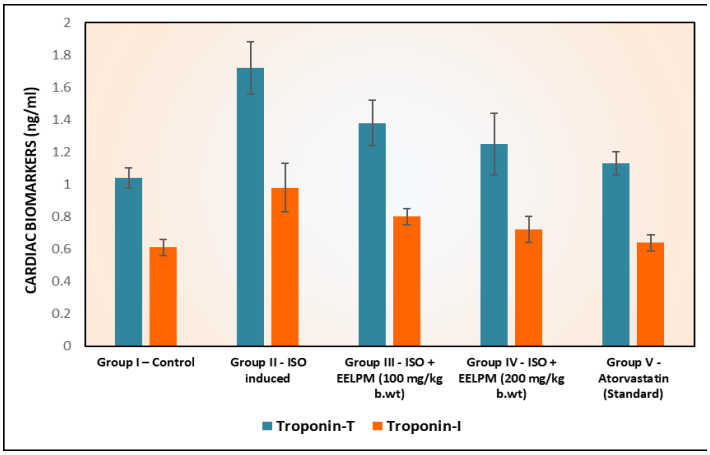

Figure 3. Effect of Troponin biomarkers in serum of ISO induced rats

Troponin-I levels were found to be elevated by 37.7% in the isoproterenol (ISO) administration group compared to the control, with reductions of 18.3%, 26.5%, and 34.6% observed in the groups treated with PMLE at 100 mg, 200 mg, and the standard treatment, respectively, as illustrated in (Figure 4). Statistical analysis revealed that the two-tailed p-value for cTnI between group II (ISO treated) and the standard group was 0.0204, indicating a statistically significant difference. In contrast, the p-value between group II and group III (PMLE 100 mg) was 0.1197, which is not statistically significant, while the p-value between group II and group IV (PMLE 200 mg) was 0.0569, suggesting it is not quite statistically significant. Additionally, the p-value between groups IV and V (standard treatment) was 0.2158, indicating no significant difference, whereas the p-value of 0.0173 between groups III and V was considered statistically significant. Troponin-I is recognized as one of the most sensitive and commonly used biomarkers for cardiac injury, with its release from cardiomyocytes being proportional to the extent of cardiac tissue damage[17]. Throughout the trial period, the creatine kinase-MB (CK-MB) levels in the normal control group remained stable, while a significant increase was noted in the positive control group. Conversely, a decrease in CK-MB levels was observed in groups pretreated with PMLEE and the standard treatment, with the leaf extract demonstrating a significant average reduction of 36.5%, 42.7%, and 46.2% in cardiac biomarkers, as detailed in (Table 6). The ISO group exhibited increased serum levels of biochemical parameters alongside a pronounced decrease in tissue levels, with concentrations of CPK, LDH, AST, ALT, and ALP recorded in untreated control as 128.4, 32.41, 24.32, 19.34, and 24.67 mg in serum, and 95.24, 39.55, 17.88, 16.51, and 133.50 mg in tissue, respectively, as shown in (Figure 5). The administration of PMLE resulted in decreased serum creatine kinase (CPK) levels and elevated tissue concentrations within normal ranges, with the 200 mg dosage proving more effective than the 100 mg dosage, yet still moderate compared to the standard treatment. Furthermore, extract treatment significantly decreased serum levels of LDH, ALP, AST, and ALT, while slightly increasing their concentrations in heart tissue at 100 mg and significantly at 200 mg, although these levels remained comparatively lower than those observed with the standard treatment. When comparing the control group (I) and the standard treatment to group II, significantly higher serum levels of biochemical markers CPK, AST, ALT, ALP, and LDH were noted, alongside a marked decrease in tissue levels (Table 7). In contrast, animals treated with the extract exhibited a substantial decrease in serum biomarkers and an increase in tissue levels, indicating that the heart muscle sustained damage due to the subcutaneous injection of ISO, as evidenced by the significant increase in cardiac marker enzymes[18].

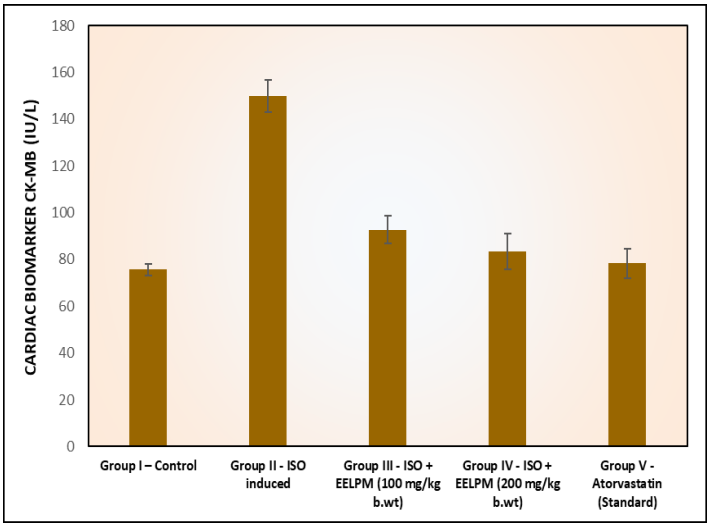

Figure 4. Effect of CK-MB biomarkers in serum of ISO induced rats

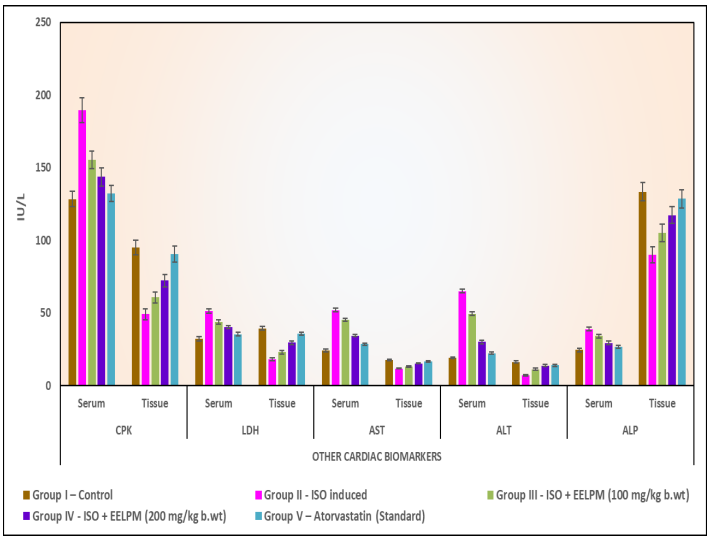

Figure 5 Effect of other cardiac biomarkers in serum of ISO induced rat

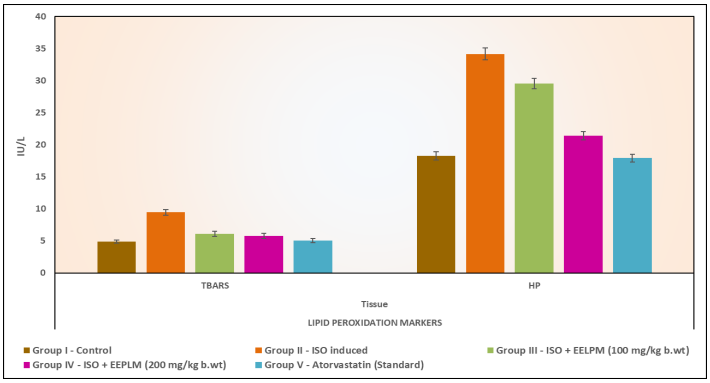

Figure 6 Effect of extract on TBARS and HP in the tissues of ISO induced rats

(Figure 6) illustrates the levels of thiobarbituric acid reactive substances (TBARS) and hydrogen peroxide (HP) in the hearts of both normal and experimental rats, revealing that rats induced with ISO exhibited a significant increase ($p < 0.05$) in TBARS and HP levels compared to normal control rats. This notable elevation in lipid peroxidation products suggests enhanced production of free radicals in the myocardial infarction (MI) model induced by ISO. Oral treatment with *Phyllanthus maderaspatensis* at doses of 100 and 200 mg/kg body weight demonstrated a positive impact on these parameters, with results that were comparable to those of the standard treatment, as summarized in (Table 8). Specifically, the TBARS levels in the negative control group rose to 9.45 ± 0.47, compared to 4.87 ± 0.25 in the normal control group, and were subsequently reduced to 6.13 ± 0.40 and 5.77 ± 0.38 in the groups treated with PMLEE, with the standard treatment achieving a TBARS level of 5.05 ± 0.30. Additionally, hydrogen peroxide levels were nearly doubled in the negative control group (34.18) compared to the control group (18.23) but were effectively reduced by the plant extract at the 200 mg dosage (21.40) and by the standard treatment (17.91). The significant reduction in TBARS values observed in the experimental animals treated with the plant extract indicates that PMLEE could efficiently prevent non-enzymatic lipid peroxidation by effectively trapping free radicals, as supported by findings from[19]. Similar results have been documented in studies involving other plant extracts, as noted[20] and spice compounds[21] further corroborating the potential of *Phyllanthus maderaspatensis* as a protective agent against oxidative stress in cardiac tissues.

Table 6 Effect of cardiac biomarkers in serum of ISO induced rats

GROUPS	Cardiac biomarkers		
	Troponin-T ng/mL	Troponin-I ng/mL	CK-MB IU/L
Group I–Control	1.04±0.06	0.61±0.05	75.61±2.35
Group II-ISO induced	1.72±0.16	0.98±0.15	149.84±6.77
Group III-ISO+EELP-M(100mg/kgb.wt)	1.38±0.14	0.80±0.05	92.63±5.87
Group IV-ISO+EELP-M(200mg/kgb.wt)	1.25±0.19	0.72±0.08	83.35±7.51
Group V-Atorvastatin (Standard)	1.13±0.07	0.64±0.05	78.33±6.26

Table 7 amount of cardiac biomarkers in serum IU/L

GROUPS	OTHER CARDIAC BIOMARKERS									
	CPK		LDH		AST		ALT		ALP	
	Serum	*Tissue*	*Serum*	*Tissue*	*Serum*	*Tissue*	*Serum*	*Tissue*	*Serum*	*Tissue*
Group I–Control	128.45±5.33	95.24±5.13	32.41±1.50	39.55±1.15	24.32±0.87	17.88±0.52	19.34±0.63	16.51±0.56	24.67±1.12	133.50±6.23
Group II-ISO induced	189.64±8.54	49.19±3.63	51.33±1.62	18.23±1.01	52.17±1.14	11.92±0.39	65.22±1.35	7.29±0.41	39.11±1.45	90.27±5.54
Group III-ISO+ EELPM (100 mg/kg b.wt)	155.45±6.11	60.78±3.70	44.07±1.55	23.12±1.17	45.39±0.95	13.22±0.35	49.61±1.22	11.50±0.74	34.20±1.40	105.16±5.81
Group IV-ISO+ EELPM (200 mg/kg b.wt)	143.60±6.37	72.33±4.15	40.51±1.09	29.62±1.22	34.55±0.88	15.47±0.41	30.11±1.08	13.96±0.77	29.36±1.25	117.43±5.94
Group V–Atorvastatin (Standard)	132.18±5.51	90.55±5.60	35.50±1.18	35.81±1.08	28.61±0.61	16.71±0.45	22.52±0.75	14.08±0.81	26.77±0.94	128.69±6.35

CPK: Creatine Phosphokinase LDH: Lactate Dehydrogenase; AST: Aspartate transaminase; ALT: Alanine transaminase; ALP: Alkaline Phosphatase

Table 8 lipid peroxidation markers in the tissues experimental rats

GROUPS	Tissue MARKERS	
	TBARS	**HP**
Group I-Control	4.87±0.25	18.23±0.65
Group II- *Isoproterenol* induced	9.45±0.47	34.18±0.90
Group III- *P maderaspatensis*(100mg/kgb.wt)	6.13±0.40	29.55±0.81
Group IV- *P maderaspatensis* (200mg/kg b.wt)	5.77±0.38	21.40±0.66
Group V- Atorvastatin	5.05±0.30	17.91±0.60

Conclusion

ISO-induced myocardial damage was evidenced by increased activities of cardiac biomarkers, which were subsequently reverted by the administration of PMLEE, suggesting its potential cardioprotective benefits against oxidative damage. The results of this study indicate that PMLEE may effectively mitigate ISO-induced oxidative stress by enhancing endogenous antioxidant levels and preventing the formation of free radicals. Notably, cardiac troponins I (cTnI) and T (cTnT) were identified as extremely sensitive and specific indicators of myocardial cell damage, with the highest values of these biomarkers observed in the extract-treated groups showing a significant correlation with those in the standard treatment group. These findings underscore the ability of PMLEE to reduce the generation of reactive oxygen species (ROS), thereby enhancing antioxidant activity and ultimately lessening the damage sustained by cardiac cells during oxidative stress. The data collectively supports the notion that *Phyllanthus maderaspatensis* possesses valuable therapeutic properties that could be harnessed in the prevention and management of myocardial injury associated with oxidative damage.

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