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RESEARCH ARTICLE

EVALUATION OF CARDIOPROTECTIVE AND ANTIHYPERLIPIDEMIC EFFECTS OF VERNONIA ELAEAGNIFOLIA IN WISTAR RATS

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ABSTRACT

Ischemic heart disease is the most prevalent cause of myocardial infarction, a leading cause of mortality worldwide. Hyperlipidemia is a condition of abnormalincrease inserum LDL, cholesterol and triglycerides levels. The main aim of the current study is to assess the cardioprotective, anti-hyperlipidemic, and anti-oxidant properties of Vernoniaelaeagnifolia's methanolic extract. In vivo evaluation of cardioprotective activity of the methanolic extract of Vernoniaelaeagnifolia was carried out using Aluminium chloride induced cardiotoxicity and Isoprotrenol induced Myocardial Infarction models and anti-hyperlipidemic activity of the methanolic extract of Vernoniaelaeagnifolia was carried out using Triton induced hyperlipidemia model. The preliminary phytochemical screening and GC-MS investigation identified triterpenoids, phytosterols, phenols, fatty acids, and flavonoids. The IC50 values of the test extract were 22.09 µg/mL and 21.78 µg/mL, respectively, in the H2O2 radical scavenging and reducing power assays. AST, ALT, ALP, and lipid profile were elevated due to the induction of isoproterenol and aluminium chloride and significantly decreased by the administration of methanolic extract of Vernoniaelaeagnifoliaat doses of 200 mg/kg and 400 mg/kg and standard (10 mg/kg). Histological observations revealed that rats treated with test extract demonstrated significantly less inflammatory cell infiltration and normal cardiac muscle fibres in isoproterenol-induced myocardial infarction.In Triton-X-100-induced hyperlipidemia, the extract markedly decreased the levels of lipids (Total cholesterol, triglycerides, LDL, and VLDL) and increased the levels of HDL. The presence of terpenoids, phytosterols, phenols, fatty acids, and flavonoids might be responsible for cardioprotective, anti-hyperlipidemic, and antioxidant activities.

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INTRODUCTION

Cardiovascular disease (CVD) is becoming more prominent as the leading cause of mortality. Heart and blood vessel conditions like hyper and hypotension together referred to as CVD. Some other conditions of CVD include Heart attack, stroke, Congestive heart failure, Arrhythmia, Atherosclerosis, Ischemic heart disease, peripheral arterial disease, several types of angina, and coronary heart disease (Prabhakaran D *et al*., 2016). CVD is primarily caused by cardio metabolic, behavioral, environmental, and societal risk factors (Mensah GA *et al*., 2019). Acute myocardial infarction (MI) is the most serious form of ischemic heart disease, results from an imbalance between coronary blood flow and myocardial demand. It is often the end result of a long and complex process, in which, the creation of an occlusive thrombus within a coronary artery leads to cardiac ischemia and infarction. MI causes the heart muscle to suffer severe damage because of a lack of oxygen. Myocardial infarction (MI) is a primary cause of mortality and disability in the industrialized world, as well as a huge socioeconomic burden (Benjamin EJ *et al*., 2019). Some research studies reported that women had a nearly 50% lifetime chance of having heart disease when compared to men. In addition, females had a significant death rate after suffering from acute myocardial infarction (Adhikary D *et al*., 2022).

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Myocardial infarction is frequently responsible for a variety of different health issues as a result of pathophysiological and biochemical changes such as high blood sugar, lipid per oxidation and high blood lipids, and so on. The main factor in the development of cardiovascular disorders, especially myocardial infarction and stroke, is an accumulation of excess lipids in the bloodstream (Upadhyay RK, 2023). Myocardial infarction is caused by a severe and long-term improper balance between myocardial oxygen supply and demand (Hegazy MA *et al*., 2022). The interaction of oxidative stress, inflammation, and hypoxia causes myocardial remodelling in MI (Schirone L *et al*., 2017). Hyperlipidemia is described as abnormal lipids and lipoproteins levels in the blood which is characterized by an increase in total cholesterol, low-density lipoprotein cholesterol, triglycerides, and a decrease in high-density lipoprotein cholesterol. It has been found to be an independent predictor of numerous cardiovascular and cerebrovascular events, leading to increasing advocacy for dyslipidemia prevention and treatment as a critical risk factor, as well as its prognostic relevance in reducing the burden of stroke and myocardial infarction (MI)(Nelson R, 2013). Despite the development of interventional cardiology procedures and medications to minimize the risk of MI, morbidity and mortality continue to rise in the absence of adequate treatments for MI. As a result, novel herbal compounds to prevent MI are urgently required with safe administration and minimal side effects. *Vernoniaelaeaegnifolia* has been traditionally used as a leech repellant. Phytochemical components of *Vernoniaelaeagnifolia* extract include flavonoids, phenolic compounds, tannins, terpenoids, phytosterols, and alkaloids

might be responsible for its pharmacological actions (P. Kattekola, 2020). Few studies reported that Vernoniaelaeaegnifolia has anti-hyperlipidemic, anti-microbial, anti-fungal and anti-oxidant activities(Sultana A*et al*., 2017, Abid S*et al*., 2015). The present study was carried out to evaluate the cardioprotective and anti-hyperlipidemic effects of methanolic extract of aerial parts of *Vernoniaelaeagnifolia*in rats.

MATERIALS AND METHODS

Plant collection and drying: The aerial parts of *Vernoniaelaeagnifolia*were collected from several locations in and around Hyderabad during the month of June 2022. Plant was identified and authenticated by Botanist, Hyderabad. The aerial parts were shade dried for a week and grinded in to a coarse powder using an electrical grinder. The coarse powder was utilized for the extraction procedure and remaining powder was stored.

Preparation of methanolic extract of Vernoniaelaeagnifolia (Soxhlet): The powdered material of *Vernoniaelaeagnifolia*aerial parts were dried and extracted with methanol using thesoxhlation process. The obtained methanolic extract obtained was evaporated to dryness by leaving them at room temperature. Large volumes of medication can be extracted with a considerably lower amount of solvent. This extraction method is cost effective in terms of time, energy, andfinancial investments.

Preliminary phytochemical screening of the extract: The methanolic extract of *Vernoniaelaeagnifolia*(MEVE) was subjected to suitable phytochemical screening tests using established analytical methods to determine the presence of major and secondary metabolic groups such as carbohydrates, flavonoids, saponins, glycosides, alkaloids, tannins, steroids, phenols, proteins, triterpenoids, resins, oils and fats (Evans WC, 2009).

Gas chromatography-mass spectrometry (GC-MS) analysis of MEVE: The GC-MS analysis was performed using a combined 7890A gas chromatograph system and mass spectrophotometer. It was equipped with an HP-5 MS fused silica column (5% phenyl methyl siloxane 30.0 m X 250 µm, film thickness 0.25µm), and it was interfaced with a 5675C Inert MSD with Triple-Axis detector. In order to achieve a column velocity flow of 1.0 mL/min, helium gas was used as the carrier gas. Other GC-MS settings include a 250 °C ion-source temperature, a 300°C interface temperature, a pressure of 16.2 psi, an out time of 1.8 mm, and a 1 µl injector in split mode with a split ratio of 1:50 and an injection temperature of 300 °C. Each component's relative percent amount was computed by comparing its average peak area to total areas.

In vitro Antioxidant Assay: *In vitro* antioxidant activity of methanolic extract of *Vernoniaelaeagnifolia* (MEVE) was carried out by different methods such as the Hydrogen peroxide radical scavenging assay and reducing power methods. Different concentrations of MEVE were prepared by dissolving the extract in methanol at a concentration of 10-50 µg/mL. All the assays were performed in a triplicate manner, average of the three values were considered for compilation.

Hydrogen peroxide radical scavenging activity: Hydrogen peroxide radical scavenging activity was performed according to the method of Sayah K *et al*., 2017. Methanolic extracts of *Vernoniaelaeagnifolia* (10-50 µg/mL) were mixed with 0.6 mL H_2O_2 (40 mM) and phosphate buffer (pH 7.4). The reaction mixtures were kept at room temperature for 10 minutes. After incubation, the reaction mixture was read at 230 nm against a blank solution of phosphate buffer (pH 7.4). Ascorbic acid was used as a standard. The inhibition % was obtained using the formula.

Percentage (%) of inhibition = $[(Abs_{sample} - Abs_{control})/ Abs_{sample}] \times 100$

Where Abs_{sample}= Absorbance of test sample and $Abs_{control}$= Absorbance of control

Reducing power assay: The reducing power assay is carried out as reported by Sayah*et al*., 2017. 1 mL of each extract was combined with 2.5 mL of the phosphate buffer solution (0.2 M, pH 6.6) and 2.5 mL of the 1% potassium ferricyanide aqueous solution. After incubating at 50°C for 20 minutes, 2.5 mL of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. Finally, 2.5 mL of supernatant was combined with 2.5 mL of distilled water and 0.5 mL of aqueous ferric chloride solution FeCl3 (0.1%, w/v). The absorbance was determined at 700 nm. The blank includes of the reactants except the extract. Ascorbic acid was used as a standard.

Acute Toxicity testing: The acute toxicity studies were carried out in accordance with OECD 425 (Organisation for Economic Cooperation and Development) criteria. The current study was conducted in a CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) authorised animal house at Gokaraju Rangaraju College of Pharmacy in Bachupally, Hyderabad, India. (Reg. No. 1175/PO/ERe/S/08/CPCSEA).

Animal Housing: Male Wistar albino rats were housed in poly acrylic cages with a maximum of three rats per cage and a 12 h light/12 h dark cycle. Rats have unlimited access to a normal feed and water. Rats were acclimatized for seven days in the laboratory setting before the study began. Maintenance and care of rats were carried out in accordance with the established guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

In vivomodels for evaluation of cardioprotective activity: *In vivo* evaluation of cardioprotective activity of the methanolic extract of *Vernoniaelaeagnifolia* (MEVE) was carried out using Aluminium chloride induced cardiotoxicity and Isoprotrenol induced Myocardial Infarction models.

Aluminium chloride induced cardiotoxicity model: Thirty healthy wistar albino rats of either sex weighing about 200-250 gm were selected for conduct of the study.Rats were divided into five groups with 6 rats in each group. Rats in all the groups'recieved aluminium chloride (50 mg/kg) *via* drinking water except control group which received only drinking water for 21 days to induce cardiotoxicity. Rats in group III and IV were supplemented with methanolic extract of *Vernoniaelaeagnifolia* at a dose of 200 and 400 mg/Kg body weight orally, once a day for 21 days. Rats in group V were administered with propranolol, a reference standard at a dose of 10 mg/kg bd.wt orally, once a day for 21 days. On day 21, blood samples were collected through retro-orbitatial puncture and the serum was separated by centrifugation at a speed of 2000 rpm for 10 minutes. Serum samples were analysed for various biochemical parameters. Post blood collection, rats were euthanized using carbon dioxide asphyxiation followed by necropsy. Heart was isolated, washed and homogenized in a Tris-HCl buffer of pH 7.4 and then centrifuged at a speed of 5000 rpm for 15 minutes. Supernatant was collected and analyzed for biochemical parameters [Ghorbel I*et al*., 2017, Hawas AM*et al*., 2020].

Isoproterenol induced Myocardial Infarction: Thirty healthy Wistar albino rats of either sex weighing about 200-250 gm were selected for conduct of the study.Rats were divided into five groups with 6 rats in each group. Rats in all the test and reference standard groups'received methanolic extract of *Vernoniaelaeagnifolia* at a dose of 200 and 400 mg/kg bd.wt and Propranol at a dose of 10 mg/kg orally for seven consecutive days. Rats in all the groups received Isoproterenol at a dose of 100 mg/kg bd.wt subcutaneously on 6^{th} and 7^{th} day of the study except normalgroup which received normal saline subcutaneously. At the end of the study, blood samples were collected

via the retro orbital plexus and the serum was separated by centrifugation at a speed of 2000 rpm for 10 minutes. Serum samples were analysed for various biochemical parameters. Post blood collection, rats were euthanized using carbon dioxide asphyxiation followed by necropsy. Heart was isolated, washed and stored in formalin for histopathological observations (Amran AZ*et al*., 2015, Mehdizadeh R*et al*., 2013).

In vivomodels for evaluation of Anti-hyperlipidemic activity: *In vivo* evaluation of anti-hyperlipidemic activity of the methanolic extract of *Vernoniaelaeagnifolia* (MEVE) was carried out using Triton induced hyperlipidemia model.

Triton induced Hyperlipidemia: Thirty healthy male Wistar albino rats weighing about 200-250 gm were selected for conduct of the study. Rats were divided into five groups with 6 rats in each group. Rats in all the groups received a freshly prepared solution of Triton-X-100 (100 mg/Kg bd.wt) in physiological solution by single dose of intraperitonial injection post 18 hours fasted rats(rats were deprived of food but provided access to drinking water) to induce hyperlipidemia. Rats in normal control received normal saline by intraperitoneal injection. Post 72 hours of Triton-X-100 induction, rats in group III, IV and V were supplemented with methanolic extract of *Vernoniaelaeagnifolia* at a dose of 200, 400 mg/kg bd.wt and fenofibrate (65 mg/kg bd.wt) orally for seven days (day4 to day 10). Blood was collected from all the rats on day 1 (prior to induction) and day 10 by retro orbital sinus puncture and centrifuged at 2000 rpm for 10 minutes for serum separation. Serum was analyzed for estimation of biomarkers (lipid profile) (Adigun NS*et al*., 2016).

Biochemical Assays: Serum samples were analyzed for lipid profile such as LDL, HDL and VLDL levels. These assays were carried out using suitable kits and analyzed using semi auto analyzer.

Histopathology: For histological examination, portions of the heart were stored in 10% neutral buffered formalin. The tissues were then processed using an automatic tissue processor, stained with hematoxylin and eosin, and examined under a microscope at 10X and 40 X magnifications.

Statistical Analysis: Statistical analysis was performed by one-way ANOVA followed by Dunnett's multiple comparison tests using Graph Pad Prism version 7.0. $p<0.05$ was considered as statistically significant. Values are presented as mean ± SEM.

RESULTS

Preliminary phytochemical screening of the extract: The preliminary phytochemical screening of the methanolic extract of *Vernoniaelaeagnifolia* is shown in Table 1 and figure 1. Phenols, flavonoids, saponins, tannins, and alkaloids were detected in the plant extract.

Table 1. Preliminary phytochemical analysis of methanolic extract of *Vernonia elaeagnifolia*

S.No	Phytoconstituents	Result
1	Terpenoids	+
2	Sterols	+
3	Phenols	+
4	Fatty acids	+
5	Flavonoids	+
6	Alkaloids	+
7	Tannins	+
8	Saponins	-
9	Carbohydrates	-
10	Proteins	-

Note: + indicates presence; - indicates absence

Fig. 1. Preliminary phytochemical analysis of *Vernoniaelaeagnifolia*

GC-MS studies of methanolic extract of Vernoniaelaeagnifolia: The GC-MS analysis in the methanolic extract of *Vernoniaelaeagnifolia* showed the presence of major phytochemical compounds such as stigmasterol, β-Sitosterol, quinic acid, lupeol, α-amyrin, squalene, n-Hexadecanoic acid, oleic acid, arachidylpalmitate and quercetin. The results were depicted in Figure 2 and Table 2.

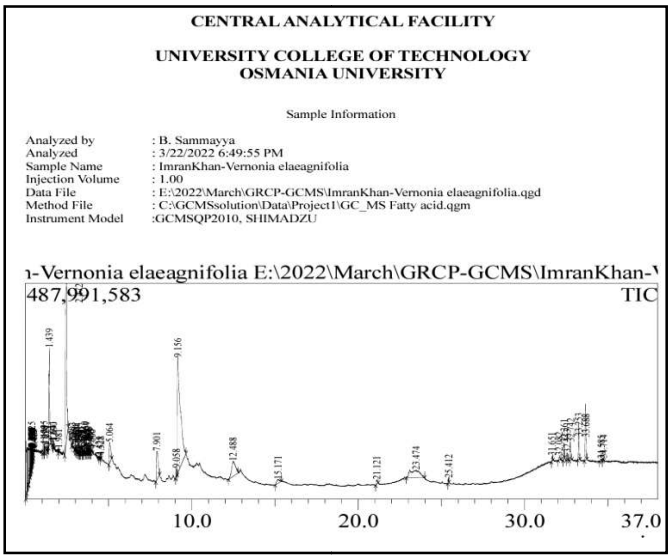

Fig. 2. GC-MS studies of methanolic extract of *Vernoniaelaeagnifolia*

The antioxidant activity of *Vernoniaelaeagnifolia* methanolic extract was screened by hydrogen peroxide radical scavenging assay and reducing power assay.

Hydrogen peroxide radical Scavenging assay: The antioxidant capacity of methanolic extract of *Vernoniaelaeagnifolia*was examined using hydrogen peroxide scavenging activity. The IC_{50} value of MEVE and ascorbic acid was found to be 22.09 µg/mL and 20.80 µg/mL respectively given in table 3.

Reducing power assay: The antioxidant capacity of methanolic extract of *Vernoniaelaeagnifolia* was examined using Reducing power assay. The IC_{50} value of MEVE and ascorbic acid was found to be 21.78 µg/mL and 20.78 µg/mL respectively given in Table 4.

Acute toxicity studies of Vernoniaelaeagnifolia: Mice supplemented with *Vernoniaelaeagnifolia* methanolic extract at a dose of 2000 mg/Kgb.wtdidn't shown any evidence of death. During observation period for 14 days, all the mice were unharmed. Pharmacological testing was carried out at a dose of 200 and 400 mg/kg b.wt.

In vivo Cardioprotective activity

Aluminium chloride induced Myocardial infarction

Effect of MEVE on biomarker Profile: AST, ALT and ALP levels were found to be significantly increased in aluminium chloride induced myocardial infarction rats ($p<0.0001$ vs. normal control). Rats supplemented with MEVE at a dose of 200 and 400

mg/Kgb.wtshown a significant decrease in AST, ALT and ALP levels dose dependently (p<0.0001 vs. disease control). However, rats administered with propranolol 10 mg/Kg b.wtshowed significant decrease in AST, ALT and ALP levels when compared to disease control. The results were depicted in table 5.

Effect of MEVE on Lipid profile: Total cholesterol, triglycerides, low density lipid (LDL), very low density lipid (VLDL) except High density lipid (HDL) were significantly increased in aluminium chloride induced myocardial infarction rats (p<0.0001 vs. normal control).

Table 2. GC-MS study of *Vernoniaelaeagnifolia*

S.No	Retention Time	Name of the Compound	Molecular Weight	Area %	Phytoconstituents
1.	31.651	Stigmasterol	412.69	0.25	Sterols
2.	32.082	β-Sitosterol	414.71	0.19	
3.	15.171	Quinic acid	192.17	0.84	Phenols
4.	32.742	Lupeol	426.72	1.06	Triterpenoids
5.	33.620	α-amyrin	426.70	0.52	
6.	35.520	Squalene	410.73	0.93	
7.	21.121	n-Hexadecanoic acid	256.40	0.30	Fatty Acids
8.	34.850	Oleic acid	282.47	1.20	
9.	29.570	ArachidylPalmitate	536.95	0.93	
10.	23.470	Quercetin	302.23	0.87	Flavonoids

In vitro evaluation of antioxidant activity

Table 3. Antioxidant activity of MEVE by Hydrogen peroxide radical Scavenging assay

Sample	Concentration (µg/mL)	Percentage Inhibition (%)	IC_{50} (µg/mL)
MEVE	10	21.08±0.33	22.09
	20	45.32±0.55	
	30	63.69±0.42	
	40	68.90±0.46	
	50	72.90±0.83	
Ascorbic acid	10	29.96±0.31	20.80
	20	48.06±0.40	
	30	65.30±0.56	
	40	72.12±0.63	
	50	76.32±0.52	

Table 4: Antioxidant activity of MEVE by reducing power assay

Sample	Concentration (µg/mL)	Percentage Inhibition (%)	IC_{50} (µg/mL)
MEVE	10	25.41±0.37	21.78
	20	45.91±0.69	
	30	60.61±0.73	
	40	70.47±0.76	
	50	75.15±0.62	
Ascorbic acid	10	27.23±0.37	20.78
	20	48.11±0.38	
	30	63.31±0.25	
	40	73.27±0.45	
	50	78.33±0.65	

Table 5. Biomarker profile (AST, ALT and ALP)

Treatment	AST		ALT		ALP	
	0^{th} day	22^{nd} day	0^{th} day	22^{nd} day	0^{th} day	22^{nd} day
Normal control	67.40± 0.50	67.5± 0.53	23.57± 0.29	23.38± 0.35	72.87± 0.34	72.73± 0.37
Disease control	77.40± 0.41*	111.8± 0.35*	31.57± 0.27*	78.22± 0.41*	57.90± 0.33*	101.00± 0.67*
MEVE (200 mg/kg)	99.80± 0.92*	88.73± 0.48^{*aA}	34.35± 0.48*	61.52± 0.61^{*aA}	97.92± 0.23*	93.70± 0.47^{*aA}
MEVE (400 mg/kg)	90.87± 0.47*	81.32± 0.44^{*aA}	30.65± 0.43*	40.13± 0.43^{*aA}	86.22± 0.34*	80.38± 0.70^{*aA}
Propranolol (10 mg/kg)	62.40± 0.52*	73.03± 0.83^{*a}	28.00± 0.28*	30.55± 0.45^{*a}	63.88± 0.35*	61.48± 0.27^{*a}

Values were expressed as Mean ± SEM (n=6). One way ANOVA followed by Dunnett's multiple comparison tests. *p<0.0001 vs. Normal control; ^{a}p<0.0001 vs. disease control and ^{A}p<0.0001 vs. Propranolol)

Table 6. Lipid profile (TC, TG, HDL, LDL and VLDL) on Day 0

Treatment	Lipid levels (mg/dL)				
	TC	TG	HDL	LDL	VLDL
Normal control	75.67±0.88	31.33±0.33	52.75±0.55	67.48± 0.97	6.27±0.07
Disease control	96.33±1.05*	51.50±1.12*	20.43±0.39*	99.63±1.75*	10.30±0.22*
MEVE (200mg/kg)	90.5 ±0.76*	46.00±0.82*	27.47±0.79**	98.63±1.30*	9.20±0.16*
MEVE (400mg/kg)	85.17±0.60*	41.50±0.92*	34.39±0.53*	94.47±1.61*	8.30±0.18*
Propranolol (10mg/kg)	80.67±0.56**	36.00±0.89***	42.72±0.70*	89.47±0.99*	7.20±0.18*

Values were expressed as Mean ± SEM (n=6). One-way ANOVA followed by Dunnett's multiple comparison test (*p<0.0001, **p<0.0005, ***p<0.001 vs. Normal control).

Table 7. Lipid profile (TC, TG, HDL, LDL and VLDL) on day 22

Treatment	Lipid levels (mg/dL)				
	TC	TG	HDL	LDL	VLDL
Normal control	76.67± 0.76	33.17±0.48	39.67±0.88	67.97 ± 1.23	6.63 ± 0.10
Disease control	116.67±0.49*	63.67±0.80*	16.17±0.79*	161.97±1.19*	12.73± 0.16*
MEVE (200mg/Kg)	104.50±0.89^{*aA}	59.67±0.76^{*bA}	22.00±0.58^{*aA}	139.97±0.98^{*aA}	11.93±0.15^{*bA}
MEVE (400mg/Kg)	97.50 ±0.56^{*aA}	53.83±0.79^{*aA}	27.33±0.33^{*aA}	121.80±0.85^{*aA}	10.77±0.16^{*aA}
Propranolol (10 mg/Kg)	87.33 ±0.61^{*a}	44.83±0.87^{*a}	33.17 ± 0.60^{*a}	96.80 ± 0.53^{*a}	8.97 ± 0.17^{*a}

Values were expressed as Mean ± SEM (n=6). One-way ANOVA followed by Dunnett's multiple comparison test against *p<0.0001 vs. normal control, ^{a}p<0.0001, ^{b} p<0.005 vs. disease control and ^{A} p<0.0001 vs. propranolol

Supplementation of rats with MEVE at a dose of 200 and 400 mg/Kgb.wt significantly reduced the TC, TG, LDL and VLDL levels ($p<0.0001$, $p<0.005$ vs. disease control). However, rats supplemented with MEVE at 200 and 400 mg/Kgb.wt significantly increased HDL levels in dose dependent manner ($p<0.0001$ vs. disease control). Rats treated with propranolol showed the beneficial effect similar to MEVE supplemented rats. The results were depicted in table 6, 7.

control). Supplementation of rats with MEVE at a dose of 200 and 400 mg/Kg b.wt significantly reduced the TC, TG, LDL and VLDL levels ($p<0.0001$ and $p<0.05$ vs. disease control). However, rats supplemented with MEVE at 200 and 400 mg/Kg b.wt significantly increased HDL levels in dose dependent manner ($p<0.0001$ and $p<0.05$ vs. disease control).

Table 8. Effect of MEVE on biomarker profile (AST, ALT and ALP)

Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)
Normal control	47.98 ±1.08	37.07±1.32	42.32±1.14
Disease control	101.82 ±1.34*	85.68±1.36*	105.75±1.35*
MEVE (200mg/Kg)	91.87±1.05^{*aA}	70.49±1.37^{*aA}	88.62 ±0.49^{*aA}
MEVE (400mg/Kg)	69.28±1.25^{*aA}	58.92±0.48^{*aA}	73.37 ±0.78^{*aA}
Propranolol (10 mg/Kg)	58.02±0.57^{*a}	48.75 ±0.56^{*a}	59.93±0.36^{*a}

Values were expressed as Mean ± SEM (n=6). One way ANOVA followed by Dunnett's multiple comparison tests. $^{*}p<0.0001$ vs. normal control, $^{a}p<0.0001$ vs. disease control and $^{A}p<0.0001$vs. propranolol

Table 9. Effect of MEVE on lipid profile (TC, TG, HDL, LDL, and VLDL)

Treatment	Lipid levels (mg/dL)				
	TC	TG	HDL	LDL	VLDL
Normal control	80.17± 0.6	37.5±0.99	32.95±0.82	88.3± 0.76	7.5± 0.2
Disease control	122.17± 0.48*	71± 0.93*	13.57±0.8*	172.63±0.6*	14.2±0.19*
MEVE (200mg/Kg)	114± 0.97*aA	59.83±0.79*aA	18.08±0.44*bA	155.97±0.79*aA	11.97±0.16*aA
MEVE(400mg/Kg)	99.5±0.99*aA	55.17±0.95*aA	23.17±0.98*aB	134.3± 0.99*aA	11.03±0.19*aA
Propranolol (10mg/Kg)	83.83±0.95**a	47.83±0.79*a	27.17±0.6*a	106.8± 0.86*a	9.57±0.16*a

Values were expressed as Mean ± SEM (n=6). One way ANOVA followed by Dunnett's multiple comparison test $^{*}p<0.0001$ and $^{**}p<0.05$ vs. normal control;$^{a}p<0.0001$ and$^{b}p<0.005$ vs. disease control; $^{A}p<0.0001$ and $^{B}p<0.005$ vs. propranolol

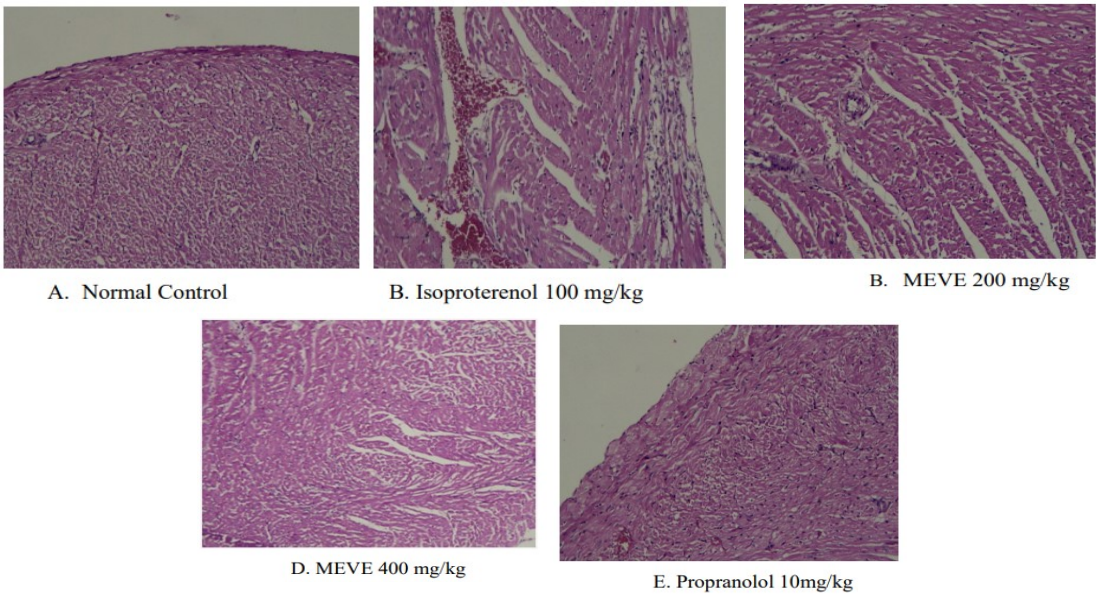

Fig no. 3: Histopathological observations of heart under 100X

Isoproterenol induced myocardial infarction

Effect of MEVE on biomarker Profile: AST, ALT and ALP levels were found to be significantly increased in isoproterenol induced myocardial infarction rats ($p<0.0001$ vs. normal control). Rats supplemented with MEVE at a dose of 200 and 400 mg/Kg b.wt shown a significant decrease in AST, ALT and ALP levels dose dependently ($p<0.0001$ vs. disease control). However, rats administered with propranolol 10 mg/Kg b.wt showed significant decrease in AST, ALT and ALP levels when compared to disease control. The results were depicted in table 8.

Effect of MEVE on Lipid profile: Total cholesterol, triglycerides, low density lipid (LDL), very low density lipid (VLDL) except High density lipid (HDL) were significantly increased in isoproterenol induced myocardial infarction rats ($p<0.0001$ and $p<0.05$ vs. normal

Rats treated with propranolol showed the beneficial effect similar to MEVE supplemented rats. The results were depicted in table 9.

Histopathological observations: Histopathological alterations were examined under light microscope at 100Xpost hematoxylin and eosin staining of isolated rat heart. The myocardial cell membrane and cardiac muscle bundle were found to be normal in normal control rats. However, separation of cardiac muscle fibres, necrosis and inflammation was observed in isoproterenol induced myoxcardial infarction rats. Moderate separation of cardiac muscle fibres and less inflammation is seen upon pre-treatment with MEVE at 200 mg/Kg b.w. whereas, slight separation of cardiac muscle fibres and very less inflammatory cell infiltration was noticed upon pre-treatment with MEVE at 400 mg/Kg b.w. In addition, it is observed that architecture of cardiac muscle fibres was found to be improved and there is a less intensity of inflammation and necrosis upon pre-treatment with propranalol 10 mg/Kg b.w. The pictures were represented in Figure 3.

Table 10. Effect of MEVE on Lipid profile on day 0

Treatment	Lipid levels (mg/dL)				
	TC	TG	HDL	LDL	VLDL
Normal control	171.5 ±0.62	126.3 ± 0.67	51.67 ± 0.84	244.00 ± 0.86	25.27 ± 0.13
Disease control	234.2 ±0.87*	210.2 ± 1.01*	57.83 ± 0.40*	384.3 ± 0.57*	42.03 ± 0.20*
MEVE (200mg/Kg)	272.5±0.92*a	217.0± 0.63*b	43.00±0.89*a	444.3±0.99*a	43.40±0.13*b
MEVE (400mg/Kg)	280.0±0.97*a	232.5 ± 0.76*a	49.33±0.42a	461.0±0.80*a	46.50±0.15*a
Fenofibrate(65 mg/kg)	261.3±0.42*a	225.3±1.20*a	63.33±0.71*a	421.1±0.84*a	45.07±0.24*a

Values were expressed as Mean ± SEM (n=6). One way ANOVA followed by Dunnett's multiple comparison tests. *p< 0.0001 vs. normal control; ap<0.0001 and bp= 0.0001 vs. disease control

Table 11. Effect of MEVE on Lipid profile on day 10

Treatment	Lipid levels (mg/dL)				
	TC	TG	HDL	LDL	VLDL
Normal control	180.40± 0.48	125.7 ± 1.04	52.75 ± 0.55	251.2 ± 0.89	25.15 ± 0.21
Disease control	294.3 ± 0.95*	263.8 ± 0.89*	20.43 ± 0.39*	535.5 ± 0.98*	52.76±0.18*
MEVE(200mg/Kg)	264.4±0.88*aA	215.7±1.09*aA	27.47±0.79*aA	450.4 ± 0.89*aA	43.14±0.22*aA
MEVE (400mg/Kg)	246.8±0.64*aA	194.6±1.10*aA	34.39±0.53*aA	404.8 ± 0.90*aA	38.92±0.22*aA
Fenofibrate (65 mg/kg)	216.2±0.60*a	185.7±0.83*a	42.72 ± 0.70*a	356.9 ± 0.75*a	37.14±0.17*a

Values were expressed as Mean ± SEM (n=6). One way ANOVA followed by Dunnett's multiple comparison tests. *p<0.0001 vs. normal control; ap<0.0001 vs. disease control, and Ap<0.0001vs. fenofibrate

In vivo anti-hyperlipidemic activity using Triton-X-100

Effect of MEVE on Lipid levels : In Triton-X-100 induced hyperlipidemia model, the lipid parameters are observed on 0^{th} day and 10th day. The triton induced group possessed significantly elevated lipid levels when compared with normal control group. On comparison of triton induced disease group with MEVE treated groups at 200 mg/kg, 400 mg/kg and standard Fenofibrate 65 mg/kg showed a significant decrease in lipid profile and with exception of HDL i.e., good cholesterol levels were increased given in table 10 and 11.

DISCUSSION

In the current study, cardioprotective effects of methanolic plant extracts of *Vernoniaelaeagnifolia* extract in myocardial infarction rats were determined. Phytochemical analysis of methanolic extract of *Vernoniaelangifolia* indicated the presence of phyto-compounds with antioxidant and other properties. Flavonoids have been proven to be very efficient scavengers of most oxidising molecules, including singlet oxygen and other free radicals, which have been linked to a variety of illnesses. Flavonoids have antioxidant and mucosal protection properties. Furthermore, some investigations have revealed that flavonoids may have cardioprotective benefits against ischemia (Lecour S *et al*., 2011). Triterpenoids have been shown to relax cardiovascular smooth muscle by inhibiting Ca^{2+} influx or by quenching reactive oxygen species (ROS) and stimulating nitric oxide (NO) generation (Han N*et al*., 2015).Sterols can reduce total cholesterol and low-density lipoprotein-cholesterol levels in the blood and made them an effective dietary supplement for reducing cardiovascular risk (Marangoni F *et al*., 2010). Polyphenols benefit the cardiovascular system by inhibiting platelet aggregation and oxidation of low-density lipoprotein (LDL), easing endothelial dysfunction, lowering blood pressure, strengthening antioxidant defences, and reducing inflammatory reactions (Behl T *et al*., 2020). Hence, the presence of Flavonoids, Triterpenoids, Fatty acids, phenols and sterols in the methanolic extract of *Vernoniaelangifolia*using GC-MS may reflect the plant's multiple therapeutic benefits, including anti-oxidative, cardioprotective and anti-hyperlipidemiccapabilities. Reactive oxygen species levels rise due to overproduction and/or insufficient antioxidant defense, resulting in oxidative stress (Kaur G*et al*., 2006). The capability of MEVE to effectively scavenge hydrogen peroxides and reduce Fe^{3+} to the ferrous form indicates antioxidant and free radical scavenging properties of this extract. This could be due to the presence of flavanoids, phenols, triterpenoids and sterols with established antioxidant properties. These activities could help halt lipid peroxidation by acting as chain breakers. Isoproterenol at a higher dose acts as a synthetic nonselective β-adrenoceptor agonist, and found to cause myocardial infarction in rats. Isoproterenol causes an increase in chronotropism and inotropism due to an imbalance between the supply and demand of oxygen for cardiomyocytes, which in turn causes cardiac hyperfunction. Hypertoxic oxygen-derived free radicals are produced by isoproterenol, causing lipid membrane peroxidation and consequent damage and death of cardiac cells in both the structural and functional domains(Sajid A *et al*., 2022). A well acknowledged mechanism of isoproterenol-induced cardiac necrosis involves the increased generation of cytotoxic free radicals resulting from the metabolic by-products of isoproterenol's auto-oxidation. Aspartate transaminase, alanine transaminase, and alkaline phosphatase are examples of cytosolic enzymes that leak out of injured tissue into the circulation and serve as diagnostic markers when a cell membrane splits or becomes permeable. Plasma membrane permeability or integrity varies depending on the quantity of these cellular enzymes in serum (Farvin KS*et al*., 2004). Pre supplementation of MEVE to MI rats, the marker enzyme (AST, ALT & ALP) activity in the serum reduced. Lipids alter cellular membrane composition, stability, and structure to generate hyperlipidemia and the start of atherosclerosis. Triglyceride and cardiac serum levels were markedly elevated in MI rats by the induction of isoproterenol. In MI rats, HDL cholesterol levels were decreased, but their LDL levels increased as well. These changes may be related to the increased lipid synthesis caused by cardiac cyclic adenosine monophosphate. The risk of developing ischemic heart disease is significantly positively correlated with higher blood levels of low-density lipoprotein (LDL), despite the fact that there is a negative association with good cholesterol. Pre supplementation of MEVE in MI rats resulted in a significant decrease in serum triglycerides, total cholesterol, LDL and VLDL, as well as increase in HDL levels. In addition, histopathological observations of heart shown that pre supplementation of MEVE acts as cardioprotective agent by showing mild to moderate separation of cardiac muscle fibres and less inflammatory cell infiltration when compared to MI rats. Rats exposed to aluminium chloride by drinking water showed an increase in lipid and protein oxidation in the heart tissue, demonstrating the importance of oxidative damage in changing metabolic pathways in the event of aluminium poisoning. Increased intracellular free Fe^{2+} causes membrane damage and lipid peroxidation in rats. Lipid peroxidation and ROS generation are the main causes of Al-induced cell damage. Oxidative stress caused by aluminium interfered with cellular functions and resulted in a lack of

lipoproteins relevant to the heart being produced and secreted. Lipids are thought to be among the most sensitive biological materials in terms of ROS reactivity. The increase in total cholesterol in MI rats may be due to increased hepatic synthesis of cholesterol. Abnormal lipase enzyme activity appears to be one of the key contributing factors to the rise in blood cholesterol, as evidenced by the reduction of high density lipoprotein cholesterol and the increase of LDL (Newairy AS *et al*., 2009). Pre supplementation with MEVE effectively reduced the rise of these markers to values that were near to normal. Gundamaraju R *et al* reported that disturbances in lipid metabolism are the primary cause of cardiovascular illnesses. Triton X-100 is frequently used to cause acute hyperlipidemia in a variety of species by obstructing the clearance of TGs-rich lipoproteins. Post Triton X-100 injection, liver secretes more VLDL while strongly reducing LDL and VLDL catabolism, which accounts for the majority of the substantial rise in plasma cholesterol and TGs (Gundamaraju R *et al*., 2014). In the present study, rats induced with Triton X-100 (100 mg/kg) became hyperlipidemic by increase in serum total cholesterol, trigycerides, LDL and VLDL levels. Pre supplementation of MEVE unequivocally demonstrates that the TGs and cholesterol levels were considerably reduced at doses of 200 and 400 mg/kg. The LDL fraction, which is the target of various hypolipidemic medications, decreased in tandem with the reduction in TC caused byMEVE. This finding implies that the herb extract's ability to decrease cholesterol may stem from the quick breakdown of LDL-C via its hepatic receptors, which then leads to its ultimate excretion as bile acids. It is commonly acknowledged that the atherosclerotic process may be slowed down by the rise in HDL. The findings indicate a decrease in LDL fraction and an increase in HDL cholesterol. These changes may be the consequence of lower absorption of cholesterol through the gastrointestinal tract and an increase in excretion of cholesterol from the body. Increases in HDL-C have been linked to lower coronary risk, according to several studies. The anti-hyperlipidemic activity of MEVE (200 and 400 mg/kg) against Triton X-100 demonstrates a significant drop in TC, TG, LDL-C, VLDL ($P < 0.0001$) and significant rise in HDL-C ($P < 0.0001$) in a dosage dependent manner when compared to the conventional fenofibrate treated group.

CONCLUSION

In the present study, methanolic extract of *Vernoniaelaeagnifolia* was evaluated for its cardioprotective, antihyperlipidemic and antioxidant activities. The GC-MS study revealed the major phytoconstituents present in the extract are triterpenoids like lupeol, α-amyrin, squalene, phytosterols like stigmasterol and βsitosterol, phenols like quinic acid, fatty acids like n-hexadecanoic acid and oleic acid and flavonoids like quercetin might be responsible for its cardioprotective, antihyperlipidemic and antioxidant activities. Pre supplementation of MEVE at 200 and 400 mg/kg b.wsignificantly decreased the serum total cholesterol, triglycerides, LDL and VLDL levels and elevated HDL levels in Triton-X-100 induced hyperlipidemia model. Additionally, pre supplementation of MEVE markedly reduced the elevated biochemical parameters (AST, ALT & ALP) and serum lipid concentrations in isoproterenol induced myocardial infarction and aluminium chloride induced myocardial infarction models. However, in isoproterenol induced myocardial infarction, the histopathological reports of rat heart showed reduced infiltration in inflammatory cells in apex of heart and clearintegrity of myocardial cell membrane. This study's findings suggest *vernoniaelangifolia* acts as cardioprotective, anti-hyperlipidemic and antioxidant agent. Hence, futher research approach is necessary to elucidate the mechanism ofaction of *Vernoniaelaeagnifolia*.

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