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Research Article

Cardioprotective Efficacy of Standardized Extract of Apium Graveolens Against Isoproterenol-Induced Myocardial Toxicity in Rats

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Abstract

Cardiovascular diseases (CVDs) comprise disorders of the heart and blood vessels and still represent a major cause of death globally. CVDs have shown to be responsible for approximately 17.9 million deaths each year, which accounts for 31% of all deaths worldwide. Among the various CVDs, myocardial infarction (MI) is a major cause of mortality and morbidity across the world. The different doses of *Apium Graveolens* (100, 200, and 400 mg/kg) and metoprolol (10 mg/kg) were calculated based on the animal's body weight were administered per oral for 30 days. Parameter for assessment of the effect of *Apium Graveolens* isoproterenol-induced cardiomyopathy in rats: Body weight, Electrocardiographic, Hemodynamic , Heart Weight, Heart/Body Weight, CKMB, LDH, ALP ,Anti-oxidant activity (Total protein, MDA, nitric oxide, GSH and SOD) in cardiac tissue, Histopathology of cardiac tissue. treatment with metoprolol (10 mg/kg) significantly ($P < 0.001$) decreased RR interval as compared to vehicle control rats. Treatment with BS (100 and 200 mg/kg) showed a significant and dose dependent ($P < 0.01$ and $P < 0.001$) decreased in RR interval as compared to vehicle control rats.

Keywords: Cardioprotective Activity; *Apium Graveolens*; Myocardial Toxicity

Introduction

Although clinical care is improved, public awareness is raised and health innovations are widely used, Myocardial infarction (MI) remains the leading cause of death worldwide. It is the acute condition of myocardial necrosis that occurs as a result of imbalance between coronary blood supply and myocardial demand. A significant factor that may prevent or diminish the myocardial damage is the development of collateral circulation through anastomotic channels over a period of time [1]. Myocardial infarction commonly known as heart attack is a disease that occurs when the blood supply to a part of the heart is interrupted, causing death of heart tissue [2]. Oxidative stress resulting from increased production of free radicals plays a major role in CVD such as myocardial infarction, ischemic heart disease, atherosclerosis, congestive heart

failure, cardio myopathy and arrhythmias [3]. Damage to the myocardial cells arises due to the generation of free radicals and reactive oxygen species (ROS). The patient may experience significant disability or die. Experimental and clinical studies have shown that there is increased generation of reactive oxygen species such as superoxide anion ($UO2^-$) and hydroxyl radicals (UOH) in heart failure, which are involved in the formation of lipid peroxides, damage of cell membrane, and destruction of anti-oxidative defense system Therapeutic intervention via suppression of free radical generation and/or enhancement of endogenous antioxidant enzymes may limit the infarct size and attenuate myocardial dysfunction [4].

Materials and Methods

Preparation of and standardization of methanolic extract of *Apium Graveolens*

The standardised extract of *Apium Graveolens* was obtained from Natural Remedies Pvt. Ltd., Bangalore. The methanolic extract of *Apium Graveolens* contain ≥70% boswellic acids.

Standardized extract of *Apium Graveolens*

- **Preparation of test drug solution:** Drug solution of standardized extract of *Apium Graveolens* was prepared by using distilled water a vehicle .
- **Storage of drug solution:** Standardized extract of *Apium Graveolens* powder was stored in a desiccator. A fresh drug solution was prepared for each day's work. The solution was kept in airtight amber-colored bottles and stored at room temperature until ready for use.
- **The volume of drug administration:** The volume of *Apium Graveolens* solution to be administered was calculated based upon the body weight of animals.
- **Route of administration:** The solution of standardized extract of *Apium Graveolens* was administered per oral (p.o.) route.

Metoprolol

- **Preparation of test drug solution:** Drug solution of metoprolol was prepared by using distilled water as a vehicle .
- **Storage of drug solution:** Metoprolol powder was stored in a desiccator. A fresh drug solution was prepared for each day's work. The solution was kept in airtight amber-colored bottles and stored at room temperature until ready for use.
- **The volume of drug administration:** The volume of metoprolol solution to be administered was calculated based upon the body weight of animals.
- **Route of administration:** The metoprolol solution was administered per oral (p.o.) route.

Isoproterenol-induced cardiomyopathy in laboratory animals: The animals were divided randomly into groups with 10 rats per group as follows:

- **Group I:** Normal group :The rats treated with vehicle (distilled water, 15 mg/kg, p.o.) for 30 days and received saline (100 mg/kg, i.p.) on 29th and 30th day
- **Group II:** Vehicle control, The rats treated with vehicle (distilled water, 15 mg/kg, p.o.) for 30 days and received isoproterenol (100 mg/kg, i.p.) on 29th and 30th day
- **Group III:** Metoprolol (10) treated group,The rats treated with metoprolol (10 mg/kg, p.o.) for 30 days and received isoproterenol (100 mg/kg, i.p.) on 29th and 30th day
- **Group IV:** *Apium Graveolens* (100) treated group,The rats treated with *Apium Graveolens* at a dose of 100 mg/kg, p.o for 30 days and received isoproterenol (100 mg/kg, i.p.) on 29th and 30th day.
- **Group V:** *Apium Graveolens* (200) treated group,The rats treated with *Apium Graveolens* at a dose of 200 mg/kg, p.o for 30 days and received isoproterenol (100 mg/kg, i.p.) on 29th and 30th day.
- **Group VI:** *Apium Graveolens* (400) treated group, The rats treated with *Apium Graveolens* at a dose of 400 mg/kg, p.o for 30 days and received isoproterenol (100 mg/kg, i.p.) on 29th and 30th day.

Treatment of *Apium Graveolens* and metoprolol

The different doses of *Apium Graveolens* (100, 200, and 400 mg/kg) and metoprolol (10 mg/kg) were calculated based on the animal's body weight were administered per oral for 30 days.

Parameter for assessment of the effect of *Apium Graveolens* isoproterenol-induced cardiomyopathy in rats

Body weight, Electrocardiographic, Hemodynamic , Heart Weight, Heart/Body Weight, CKMB, LDH, ALP ,Anti-oxidant activity (Total protein, MDA, nitric oxide, GSH and SOD) in cardiac tissue, Histopathology of cardiac tissue.

Parameter for assessment of effect of *Apium Graveolens* on isoproterenol-induced cardiomyopathy in rats

In-vivo parameters

- **Assessment of electrocardiographic abnormalities:** For the measurement of the electrocardiogram, the leads were placed on the right foreleg (negative electrode), left foreleg (positive electrode), and right hind leg (neutral electrode). Electrocardiographic changes (Heart rate, RR, QRS, QT, QTc, PR interval and ST height) were recorded using 8 channels Power Lab System (LabChart 7.3; AD Instrument Pvt. Ltd., Bella Vista, Australia).
- **Hemodynamic measurement:** On last day of study, animals were anaesthetized by urethane (1.25 g/kg) i.p. The right carotid artery of each rat was cannulated for the measurement of systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MABP). The cannula was filled with heparinized saline and connected to pressure transducer to record all the parameters mentioned above. After 30 minutes of stabilization hemodynamic parameters were recorded by eight channel recorder Power lab (AD Instruments, Australia) having LABCHART -7 pro software.
- **Plasma and serum parameters:** The serum was separated by centrifugation using an Eppendorf cryocentrifuge (model no. 5810, Eppendorf, Hamburg, Germany), maintained at 4 ºC and run at a speed of 7000 rpm for 15 min. The levels of serum lactate dehydrogenase (LDH), Creatine Kinase-MB (CK-MB) and alkaline phosphatase (ALP) were measured by a spectrophotometer (UV–visible spectrophotometer, Jasco V-530, Tokyo, Japan) using commercially available reagent kits according to procedure provided by manufacturer (Accurex Biomedical Pvt. Ltd., Mumbai, India and Pathozyme Diagnostics, India).

Ex-vivo parameters

Tissue Parameters

- **Cardiac tissue homogenate preparation:** All animals were sacrificed at the end of study i.e., 30th day and cardiac tissue was immediately isolated. Tissue homogenate was prepared with 0.1M Tris-HCl buffer (pH 7.4) and supernatant of homogenate was employed to estimate total protein, superoxide dismutase (SOD), reduced glutathione (GSH), lipid peroxidation (MDA content), nitric oxide content, membrane bound inorganic phosphate levels
- **Determination of Lipid Peroxidation (MDA content):** It was estimated using the method described by Slater and Sawyer (1971). 2.0 ml of the tissue homogenate (supernatant) was added to 2.0 ml of freshly prepared 10% w/v trichloroacetic acid (TCA) and the mixture was allowed to stand in an ice bath for 15 minutes. After 15 minutes, the precipitate was separated by centrifugation and 2.0 ml of clear supernatant solution was mixed with 2.0 ml of freshly prepared thiobarbituric acid (TBA). The resulting solution was heated in a boiling water bath for 10 minutes. It was then immediately cooled in an ice bath for 5 minutes. The colour developed was measured at 532nm against reagent blank. Different concentrations (0-23 nM) of standard malondialdehyde were taken and processed as above for standard graph.The values were expressed as nM of MDA/mg protein.
- **Determination of Superoxide Dismutase (SOD):** Superoxide dismutase was estimated using the method developed by Misra and Fridovich (1972) [5]. 0.5 ml of tissue homogenate was diluted with 0.5 ml of distilled water, to which 0.25 ml of ice-cold ethanol and 0.15 ml of ice-cold chloroform was added. The mixture was mixed well using cyclo mixer for 5 minutes and centrifuged at 2500 rpm. To 0. 5ml of supernatant, 1.5 ml of carbonate buffer and 0.5 ml of EDTA solution were

added. The reaction was initiated by the addition of 0.4 ml of epinephrine and the change in optical density/minute was measured at 480 nm against reagent blank. SOD activity was expressed as units/mg protein. Change in optical density per minute at 50% inhibition of epinephrine to adrenochrome transition by the enzyme is taken as the enzyme unit. Calibration curve was prepared by using 10-125 units of SOD.

- **Determination of Reduced glutathione (GSH):** Reduced glutathione was determined by the method described by Moron., *et al.* (1979) [6]. Equal volumes of tissue homogenate (supernatant) and 20% TCA were mixed. The precipitated fraction was centrifuged and to 0.25 ml of supernatant, 2 ml of DTNB reagent was added. The final volume was made up to 3ml with phosphate buffer. The colour developed was read at 412 nm against reagent blank. Different concentrations (10-50 gm) of standard glutathione were taken and processed as above for standard graph. The amount of reduced glutathione was expressed as µg of GSH / mg protein
- **Determination of tissue protein:** Protein concentration was estimated according to the method of Lowry et al. (1951) [7] using BSA (bovine serum albumin) as a standard. Briefly, dilute tissue fraction aliquots (0.1 ml) were taken in test tube. To this, 0.8 ml of 0.1 M sodium hydroxide and 5.0 ml Lowry C reagent was added and the solution was allowed to stand for 15 min. Then 0.5 ml of Folin phenol reagent was added and the contents were mixed by vortex mixer. Color developed was measure at 660 nm against reagent blank containing distilled water instead of sample. Different concentrations (40-200 µg) of BSA were taken and process as above for standard graph. The values were expressed as mg of protein/ gm of wet tissue (mg/gm).
- **Determination of Nitrite:** Nitrite was estimated in the cardiac tissue homogenate using the Greiss reagent as per method described by Miranda., *et al.,* 2001 [8]. A measure of 500 µl of Greiss reagent (1:1 solution of 1% sulphanilamide in 5% phosphoric acid and 0.1% napthaylamine diamine dihydrochloric acid in water) was added to 100 µl of post-mitochondrial supernatant and absorbance was measured at 546 nm. Nitrite concentration was calculated using a standard curve for sodium nitrite. Nitrite levels were expressed as µg/ml.

Histopathological analysis

On day 30th the all animals were sacrificed and cardiac tissue were collected. Samples of cardiac tissue were kept in the fixative solution (10% formalin) and it processed for 12 hr using isopropyl alcohol, xylene and paraffin embedded for light microscopic study (Nikon E200).

Paraffin embedded tissue section cut at 5µm thickness were prepared and staining was done by using hematoxylin and eosin as described by Yukari., *et al*., 2004 [9]. Tissue sections were analyzed qualitatively under light microscope (100 ×) for inflammatory influx, myocardial degeneration and necrosis etc.

Statistical analysis

Data analysis was performed using GraphPad Prism 5.0 software (GraphPad, San Diego, USA) [10-22]. Statistical comparisons were made between drug-treated groups and disease control animals (vehicle control). A value of $P < 0.05$ was considered to be statistically significant.

Results and Discussion

Effect of *Apium Graveolens* on isoproterenol-induced alteration in body weight

Body weight (gm) - Mean ± SEM					
Normal	**Vehicle Control**	**Metoprolol (10 mg/kg)**	**BS (100 mg/kg)**	**BS (200 mg/kg)**	**BS (400 mg/kg)**
237.20 ± 4.00	238.00 ± 2.99	240.70 ± 3.90	240.80 ± 4.08	239.30 ± 4.46	239.20 ± 4.76

Table a

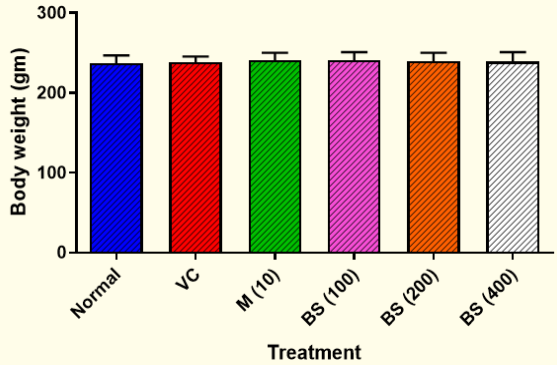

Figure 1: Effect of Apium Graveolens on isoproterenol-induced alteration in body weight.

Data were analyzed by One-Way ANOVA followed by Dunnett's post-hoc test.

The body weight of normal rat, vehicle control and treatment groups rat did not differ significantly. Administration of isoproterenol also did not cause any significant change in the body weight of rats when compared to normal group.

Effect of *Apium Graveolens* on isoproterenol-induced alteration in absolute and relative heart weights

Time (in days)	Absolute heart weight (gm) and Relative heart weight - Mean ± SEM					
	Normal	Vehicle Control	Metoprolol (10 mg/kg)	BS (100 mg/kg)	BS (200 mg/kg)	BS (400 mg/kg)
Heart weight (gm)	0.32 ± 0.02	0.85 ± 0.05$^{###}$	0.41 ± 0.05***	0.78 ± 0.03	0.60 ± 0.03**	0.46 ± 0.04***
Heart weight/Body weight (X10^{-3})	1.35 ± 0.06	3.59 ± 0.21$^{###}$	1.68 ± 0.19***	3.25 ± 0.15	2.53 ± 0.13**	1.93 ± 0.15***

Table b

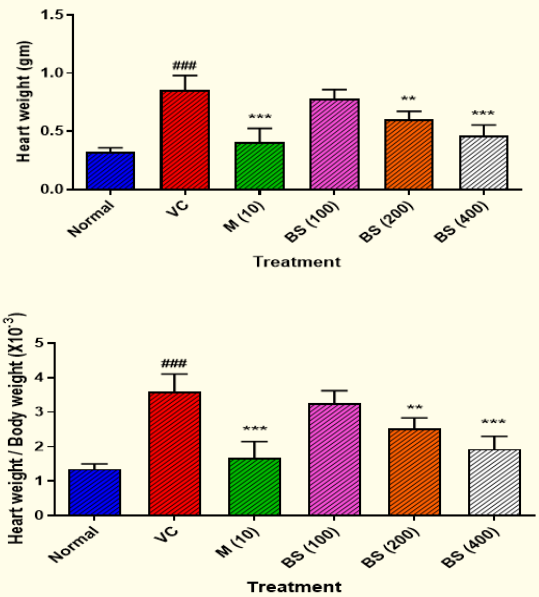

Figure 2: Effect of *Apium Graveolens* on isoproterenol-induced alteration in absolute and relative heart weights. Data were analyzed by One-Way ANOVA followed by Dunnett's. ###P < 0.001 as compared with normal group and **P < 0.01, ***P < 0.001 as compared with Vehicle Control group on respective days.

When compared to normal group, administration of isoproterenol cased a significant increase ($P < 0.001$) in heart weight (absolute) and heart weight to body weight ratio (relative heart weight) in vehicle control group. On the other hand, treatment of metoprolol (10 mg/kg) for for 30 days resulted in the significant attenuation of ratio of heart weight to body weight and heart weight as compared with vehicle control group. When compared with vehicle control rats, BS (100 and 200 mg/kg) treated rats also showed the significant and dose dependant decreased ($P < 0.01$ and $P < 0.001$) in the absolute and relative heart weights. Administration of BS (50 mg/kg) did not show any significant protection against isoproterenol-induced increased cardiac weights.

Effect of *Apium Graveolens* on isoproterenol-induced alteration in electrocardiographic outcomes:

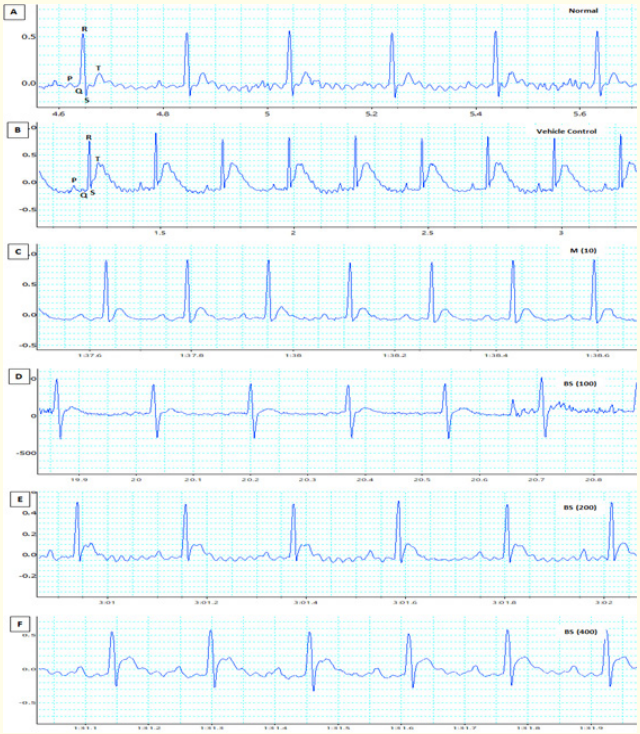

Figure 3: Representative images of ECG recording from normal rats (A), Vehicle Control rats (B), Metoprolol (10 mg/kg) treated rats (C), BS (100 mg/kg) treated rats (D), BS (200 mg/kg) treated rats (E), and BS (400 mg/kg) treated rats (F).

Effect of Apium Graveolens on isoproterenol-induced alteration in heart rate and RR interval

On 30th day, the heart rate in vehicle control group of animals showed significant ($P < 0.001$) decrease when compared to normal group. On the other hand, treatment with metoprolol (10 mg/kg) showed a significant increase ($P < 0.001$) in heart rate when compared to vehicle control group. Treatment with BS (100 and 200 mg/kg) showed significant and dose dependent increase ($P < 0.01$ and $P < 0.001$) in heart rate compared to vehicle control group.

Parameter	Heart rate (BPM) and RR interval (ms) - Mean ± SEM					
	Normal	Vehicle Control	Metoprolol (10 mg/kg)	BS (100 mg/kg)	BS (200 mg/kg)	BS (400 mg/kg)
Heart rate (BPM)	364.70 ± 13.70	268.00 ± 5.82###	322.00 ± 10.64***	274.20 ± 7.80	299.70 ± 11.9**	343.50 ± 13.72***
RR interval (ms)	152.70 ± 4.55	212.50 ± 4.00###	161.80 ± 4.35***	204.70 ± 5.54	176.50 ± 5.57**	170.70 ± 5.43***

Table c

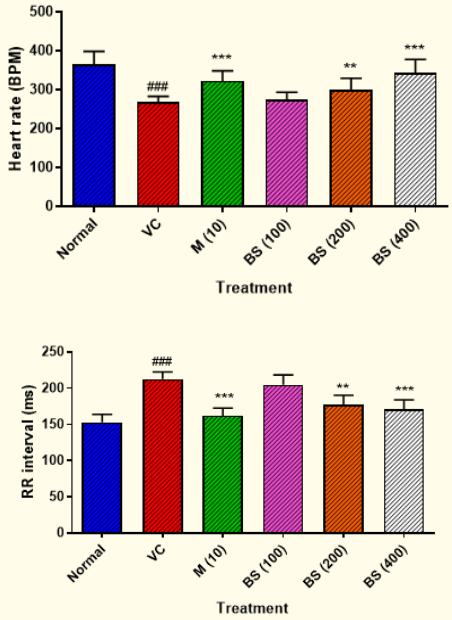

Figure 4: Effect of *Apium Graveolens* on isoproterenol-induced alteration in heart rate and RR interval.
Data were analyzed by One-way ANOVA followed by Dunnett's test. ###P < 0.001 as compared with normal group and **P < 0.01, ***P < 0.001 as compared with Vehicle Control group.

There was a significant ($P < 0.001$) increase in RR interval in vehicle control rats after isoproterenol administration as compared to normal rats. However, treatment with metoprolol (10 mg/kg) significantly ($P < 0.001$) decreased RR interval as compared to vehicle control rats. Treatment with BS (100 and 200 mg/kg) showed a significant and dose dependent ($P < 0.01$ and $P < 0.001$) decreased in RR interval as compared to vehicle control rats.

Effect of *Apium Graveolens* on isoproterenol-induced alteration in QRS interval

There was a significant increase ($P < 0.001$) in the QRS interval after administration of the isoproterenol in vehicle control rats as compared to normal rats. Treatment with metoprolol (10 mg/kg) significantly ($P < 0.001$) decreased the QRS interval compared to vehicle control rats. On the other hand, treatment with BS (100 and

QRS interval (ms) - Mean ± SEM					
Normal	**Vehicle Control**	**Metoprolol (10 mg/kg)**	**BS (100 mg/kg)**	**BS (200 mg/kg)**	**BS (400 mg/kg)**
13.33 ± 0.67	31.17 ± 0.87###	17.17 ± 0.54***	28.00 ± 0.93	22.33 ± 1.17**	20.33 ± 0.88***

Table d

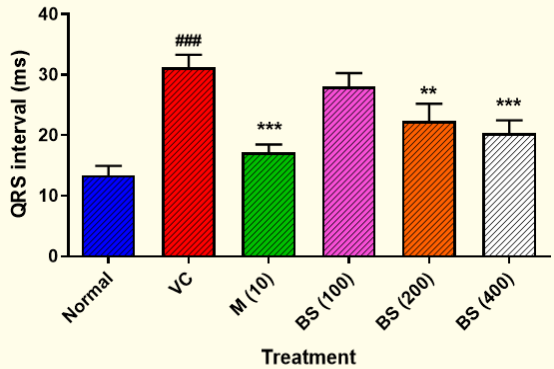

Figure 5: Effect of *Apium Graveolens* on isoproterenol-induced alteration in QRS interval.

Data were analyzed by One-way ANOVA followed by Dunnett's test. ###P < 0.001 as compared with normal group and **P < 0.01, ***P < 0.001 as compared with Vehicle Control group.

200 mg/kg) decreased the QRS interval significantly ($P < 0.01$ and $P < 0.001$) and dose dependently compared to vehicle control rats. When compared to vehicle control rats, BS (100 mg/kg) failed to produce any inhibition in isoproterenol-induced alteration in QRS interval.

Effect of *Apium Graveolens* on isoproterenol-induced alteration in QT interval and QTc interval

On 30th day, the QT and QTc intervals in vehicle control group showed a significant ($P < 0.001$) prolongation post isoprenaline administration when compared to normal group. On the other hand, treatment with metoprolol (10 mg/kg) showed a significant (*P*

Parameter	QT interval (ms) and QTc interval (ms) - Mean ± SEM					
	Normal	Vehicle Control	Metoprolol (10 mg/kg)	BS (100 mg/kg)	BS (200 mg/kg)	BS (400 mg/kg)
QT interval (ms)	48.33 ± 2.77	89.00 ± 2.62###	61.50 ± 3.14***	83.00 ± 3.45	68.67 ± 2.46**	63.00 ± 1.29***
QTc interval (ms)	131.30 ± 4.61	174.80 ± 4.74###	144.50 ± 1.46***	166.70 ± 3.72	147.20 ± 5.26**	143.50 ± 6.16***

Table e

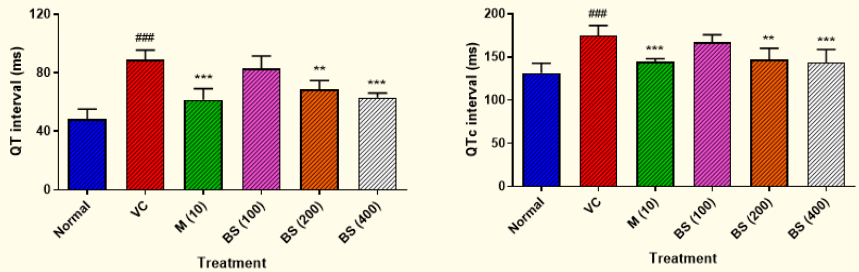

Figure 6: Effect of *Apium Graveolens* on isoproterenol-induced alteration in QT interval and QTc interval

Data were analyzed by One-way ANOVA followed by Dunnett's test. ###P < 0.001 as compared with normal group and **P < 0.01, ***P < 0.001 as compared with Vehicle Control group.

< 0.001) decreased in QT and QTc intervals compared to vehicle control group. Treatment with BS (100 and 200 mg/kg) showed significant and dose dependent decrease ($P < 0.01$ and $P < 0.001$) compared to vehicle control group. BS (50 mg/kg) did not show any significant decrease in QT and QTc intervals compared to vehicle control group.

Effect of *Apium Graveolens* on isoproterenol-induced alteration in PR interval and ST interval

Parameter	PR interval (ms) and ST interval (ms) - Mean ± SEM					
	Normal	Vehicle Control	Metoprolol (10 mg/kg)	BS (100 mg/kg)	BS (200 mg/kg)	BS (400 mg/kg)
PR interval (ms)	14.89 ± 0.55	27.09 ± 0.82$^{###}$	18.54 ± 1.00***	27.07 ± 0.59	23.11 ± 1.09**	21.24 ± 0.66***
ST interval (ms)	20.15 ± 0.51	33.40 ± 0.86$^{###}$	25.21 ± 0.62***	32.39 ± 0.62	30.20 ± 0.61**	25.78 ± 0.67***

Table f

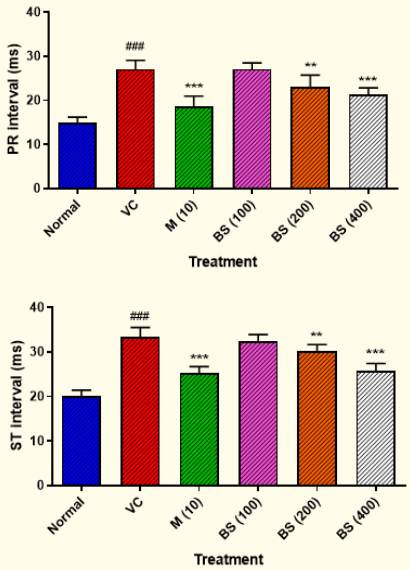

Figure 7: Effect of *Apium Graveolens* on isoproterenol-induced alteration in PR interval and ST interval.
Data were analyzed by One-way ANOVA followed by Dunnett's test. ###P < 0.001 as compared with normal group and **P < 0.01, ***P < 0.001 as compared with Vehicle Control group

Administration of isoprenaline caused significant increased ($P < 0.001$) in PR and ST intervals in vehicle control group when compared to normal group on day 30. When compared with vehicle control group, metoprolol (10 mg/kg) treatment significantly ($P < 0.001$) decreased PR and ST intervals. Treatment with BS (100 and 200 mg/kg) also showed a significant and dose dependant ($P < 0.01$ and $P < 0.001$) decrease in PR and ST intervals as compared to vehicle control group on day 28. BS (100 mg/kg) showed a non-significantly decrease in PR and ST intervals compared to vehicle control group on day 28.

Effect of Apium Graveolens on isoproterenol-induced alteration in SBP and DBP:

Parameter	SBP (mmHg) and DBP (mmHg) - Mean ± SEM					
	Normal	Vehicle Control	Metoprolol (10 mg/kg)	BS (100 mg/kg)	BS (200 mg/kg)	BS (400 mg/kg)
SBP (mmHg)	153.50 ± 3.76	103.30 ± 2.91###	152.30 ± 4.42***	114.50 ± 1.34	130.00 ± 1.84**	136.80 ± 2.86***
DBP (mmHg)	117.00 ± 2.99	85.33 ± 3.54###	112.30 ± 3.75***	93.67 ± 4.57	96.17 ± 4.19**	106.50 ± 3.53***

Table g

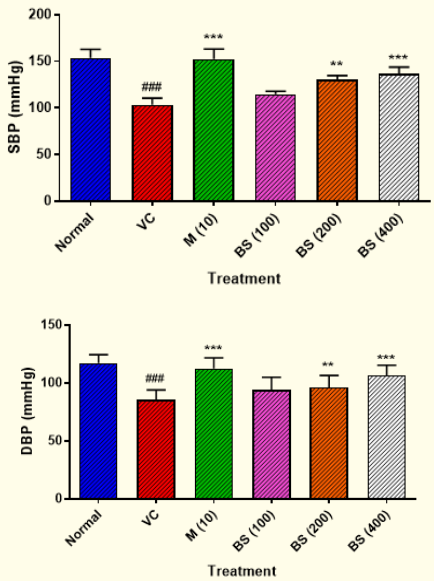

Figure 8: Effect of *Apium Graveolens* on isoproterenol-induced alteration in SBP and DBP.

Data were analyzed by One-way ANOVA followed by Dunnett's test. ###P < 0.001 as compared with normal group and **P < 0.01, ***P < 0.001 as compared with Vehicle Control group.

On 30th day, the systolic BP (SBP) and diastolic BP (DBP) in the vehicle control group was found to be significantly ($P < 0.001$) decrease in normal group. On the other hand, pretreatment of metoprolol (10 mg/kg) for 30 days showed significant ($P < 0.001$) increase in SBP and DBP vehicle control group. Treatment with BS (100 and 200 mg/kg) also significantly and dose dependently ($P < 0.01$ and $P < 0.001$) increased the SBP and DBP when compared with vehicle control group.

Effect of Apium Graveolens on isoproterenol-induced alteration in MABP

The MABP in vehicle control rats significantly induced ($P < 0.001$) as compared to normal rats. This isoproterenol-induced decreased in MABP was significantly attenuated ($P < 0.001$) by the treatment of metoprolol (10 mg/kg) compared to vehicle control group. BS (100 and 200 mg/kg) treatment for 28 days also showed a significant and dose dependant ($P < 0.01$ and $P < 0.001$) increase in MABP as compared to vehicle control rats.

MABP (mmHg) - Mean ± SEM					
Normal	Vehicle Control	Metoprolol (10 mg/kg)	BS (100 mg/kg)	BS (200 mg/kg)	BS (400 mg/kg)
121.50 ± 2.41	90.83 ± 1.72###	117.00 ± 1.29***	99.33 ± 2.86	104.00 ± 2.63**	109.00 ± 2.00***

Table h

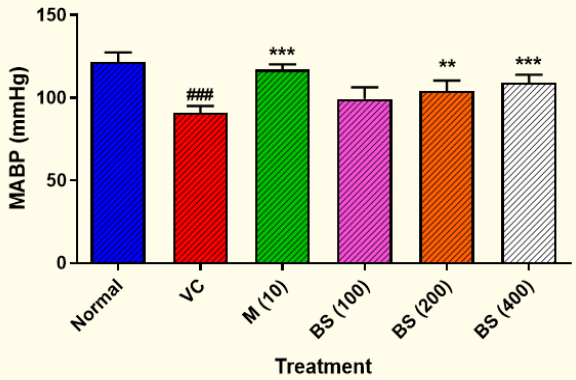

Figure 9: Effect of Apium Graveolens on isoproterenol-induced alteration in MABP.

Data were analyzed by One-way ANOVA followed by Dunnett's test. ###P < 0.001 as compared with normal group and **P < 0.01, ***P < 0.001 as compared with Vehicle Control group.

Effect of *Apium Graveolens* on isoproterenol-induced alteration in CK-MB and LDH levels

The levels of CK-MB and LDH in were significantly ($P < 0.001$) increased in vehicle control group when compared to normal group. On the other hand, treatment with metoprolol (10 mg/kg) significantly ($P < 0.001$) decrease the CK-MB and LDH levels compared to vehicle control group. Treatment with BS (100 and 200 mg/kg) decreased the CK-MB and LDH significantly and dose dependently ($P < 0.001$ and $P < 0.001$) compared to vehicle control group. However, there was non-significant decrease in levels of CK-MB and LDH by BS (100 mg/kg) treated groups compared to vehicle control group.

Parameter	CK-MB (IU/L) and LDH (IU/L) - Mean ± SEM					
	Normal	Vehicle Control	Metoprolol (10 mg/kg)	BS (100 mg/kg)	BS (200 mg/kg)	BS (400 mg/kg)
CK-MB (IU/L)	1058.00 ± 56.33	2099.00 ± 66.24###	1269.00 ± 39.51***	1950.00 ± 37.08	1657.00 ± 50.05**	1317.00 ± 50.54***
LDH (IU/L)	1357.00 ± 73.4	2731.00 ± 62.75###	1667.00 ± 71.75***	2747.00 ± 51.49	2023.00 ± 51.74**	1600.00 ± 110.90***

Table i

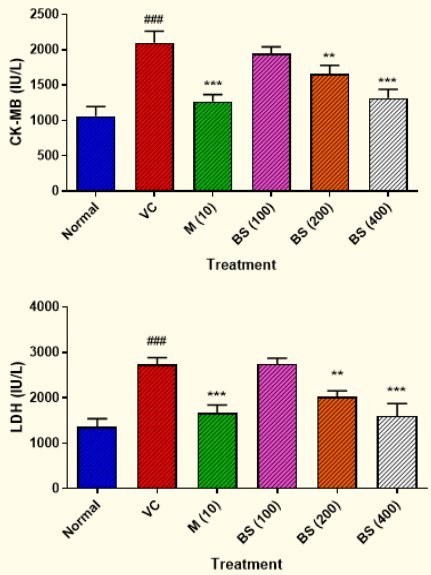

Figure 10: Effect of *Apium Graveolens* on isoproterenol-induced alteration in CK-MB and LDH levels.
Data were analyzed by One-way ANOVA followed by Dunnett's test. ###P < 0.001 as compared with normal group and **P < 0.01, ***P < 0.001 as compared with Vehicle Control group.

Effect of *Apium Graveolens* on isoproterenol-induced alteration in ALP level

The significant increased ($P < 0.001$) in ALP level was found after intraperitoneal administration of isoproterenol in vehicle control rats as compared to normal rats. This decreased level of ALP was significantly attenuated ($P < 0.001$) by metoprolol (10 mg/kg) as compared to vehicle control rats. Treatment with BS (100 and 200 mg/kg) also significantly and dose dependently ($P < 0.01$ and $P < 0.001$) decreased the ALP level compared to vehicle control rats. Rats treated with BS (100 mg/kg) failed to produce significant decrease in the level of ALP as compared to vehicle control rats.

ALP (mg%) - Mean ± SEM					
Normal	**Vehicle Control**	**Metoprolol (10 mg/kg)**	**BS (100 mg/kg)**	**BS (200 mg/kg)**	**BS (400 mg/kg)**
118.80 ± 4.98	339.20 ± 5.20###	138.30 ± 12.82***	316.50 ± 10.27	258.00 ± 7.15**	154.00 ± 11.50***

Table j

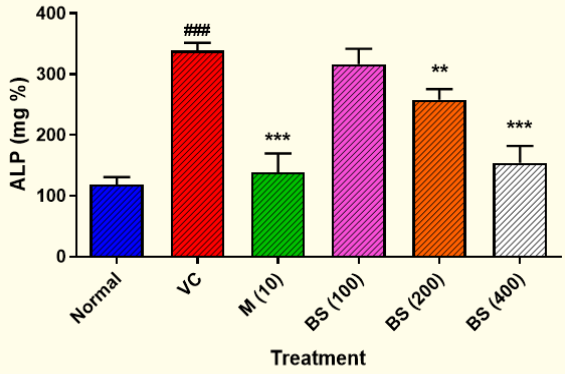

Figure 11: Effect of *Apium Graveolens* on isoproterenol-induced alteration in ALP level.
Data were analyzed by One-way ANOVA followed by Dunnett's test. ###P < 0.001 as compared with normal group and **P < 0.01, ***P < 0.001 as compared with Vehicle Control group.

Effect of *Apium Graveolens* on isoproterenol-induced alteration in cardiac total protein level:

Cardiac total protein (mg/gm) - Mean ± SEM					
Normal	**Vehicle Control**	**Metoprolol (10 mg/kg)**	**BS (100 mg/kg)**	**BS (200 mg/kg)**	**BS (400 mg/kg)**
25.52 ± 3.06	57.85 ± 3.47###	34.86 ± 3.57***	55.19 ± 2.71	48.88 ± 3.15**	37.34 ± 2.60***

Table k

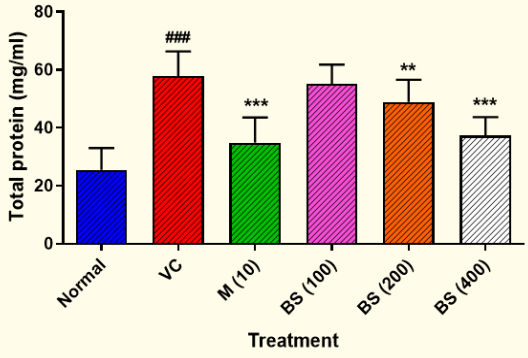

Figure 12: Effect of *Apium Graveolens* on isoproterenol-induced alteration in cardiac total protein level.
Data were analyzed by One-way ANOVA followed by Dunnett's test. ###P < 0.001 as compared with normal group and **P < 0.01, ***P < 0.001 as compared with Vehicle Control group.

There was a significant increase ($P < 0.001$) in cardiac total protein level in vehicle control group when compared to normal group. Administration of metoprolol (10 mg/kg) for 28 days significantly ($P < 0.001$) decrease total protein level in cardiac tissue compared to vehicle control rats. Treatment with BS (100 and 200 mg/kg) also significantly and dose dependently ($P < 0.01$ and $P < 0.001$) decreased the cardiac total protein level compared to vehicle control rats.

Effect of Apium Graveolens on isoproterenol-induced alteration in cardiac SOD and GSH level

Parameter	Cardiac SOD (U /mg of protein) and GSH μg/mg of protein) levels - Mean ± SEM					
	Normal	Vehicle Control	Metoprolol (10 mg/kg)	BS (100 mg/kg)	BS (200 mg/kg)	BS (400 mg/kg)
SOD (U /mg of protein)	9.30 ± 0.44	4.03 ± 0.61###	6.66 ± 0.62***	4.01 ± 0.68	5.59 ± 0.61**	6.21 ± 0.87***
GSH (μg/mg of protein)	0.37 ± 0.02	0.17 ± 0.01###	0.35 ± 0.02***	0.18 ± 0.02	0.25 ± 0.02**	0.32 ± 0.02***

Table l

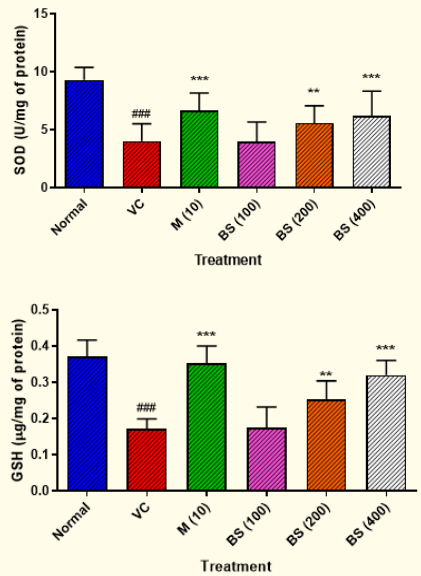

Figure 13: Effect of *Apium Graveolens* on isoproterenol-induced alteration in cardiac SOD and GSH level.
Data were analyzed by One-way ANOVA followed by Dunnett's test. ###P < 0.001 as compared with normal group and **P < 0.01, ***P < 0.001 as compared with Vehicle Control group.

The cardiac SOD and GSH level in the vehicle control rats was significantly decreased ($P < 0.001$) as compared to normal rats. The SOD and GSH level in the cardiac tissue of metoprolol (10 mg/kg) treated rats was significantly increased ($P < 0.001$) as compared to vehicle control rats. The 28 days treatment of BS (100 and 200 mg/kg) significantly and dose dependently attenuated ($P < 0.01$ and $P < 0.001$) this isoproterenol-induced decreased level of SOD and GSH as compared to vehicle control rats.

Effect of Apium Graveolens on isoproterenol-induced alteration in cardiac MDA and NO level

Parameter	Cardiac MDA (nM/mg of protein), nitric oxide (μg/ml) - Mean ± SEM					
	Normal	Vehicle Control	Metoprolol (10 mg/kg)	BS (100 mg/kg)	BS (200 mg/kg)	BS (400 mg/kg)
MDA (nM/mg of protein)	2.48 ± 0.32	7.12 ± 0.29###	3.48 ± 0.29***	6.49 ± 0.35	4.66 ± 0.30**	3.31 ± 0.24***
Nitric oxide (μg/ml)	214.10 ± 15.22	601.70 ± 14.52###	308.70 ± 10.31***	550.90 ± 7.66	492.10 ± 17.84**	349.10 ± 6.36***

Table m

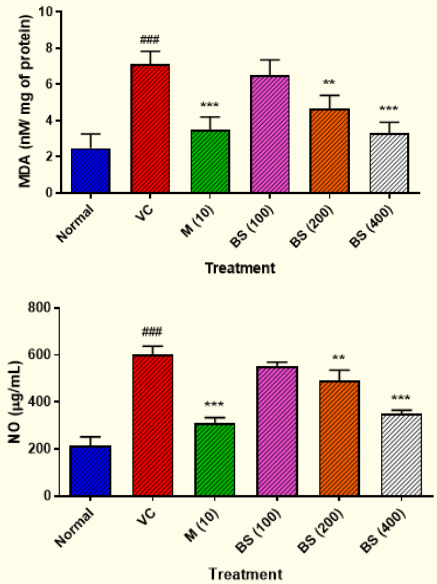

Figure 14: Effect of *Apium Graveolens* on isoproterenol-induced alteration in cardiac MDA and NO level.
Data were analyzed by One-way ANOVA followed by Dunnett's test. ###P < 0.001 as compared with normal group and **P < 0.01, ***P < 0.001 as compared with Vehicle Control group.

There was significant increase in cardiac MDA and NO levels in vehicle control rats as compared to normal rats. When compared to vehicle control rats, the MDA and NO level in cardiac tissue of metoprolol (10 mg/kg) was significantly deceased ($P < 0.001$). BS (100 mg/kg) treatment failed to produce any significant decrease in MDA and NO level compared to vehicle control rats. However, administration of BS (100 and 200 mg/kg) showed significant and dose dependent ($P < 0.01$ and $P < 0.001$) decreased level of MDA and NO as compared to vehicle control rats.

Effect of isoproterenol on histopathological alteration in cardiac tissue

Histopathological observations of the heart from normal group revealed well maintain architecture with normal myocardial fibres and muscle bundles with well-defined boundaries with presence of mild infiltration of neutrophils (Figure A).

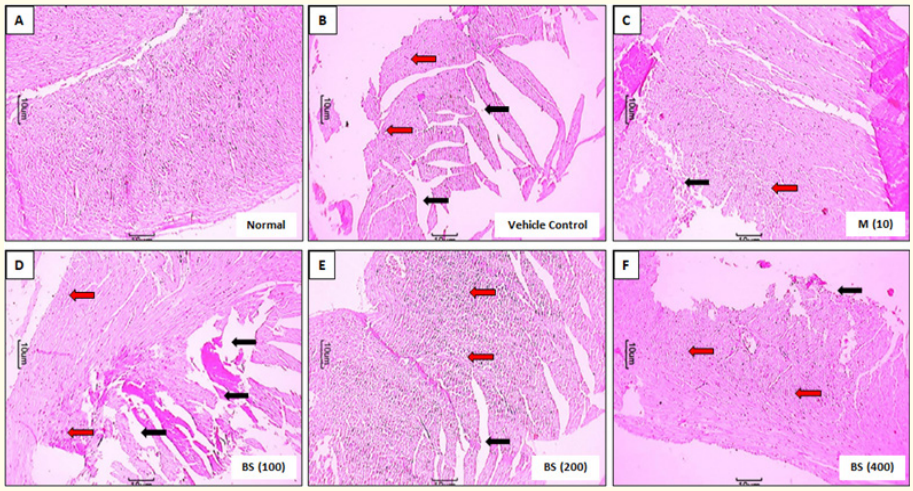

Figure 15: Histopathological representation of cardiac tissue from normal rats (A), Vehicle Control rats (B), Metoprolol (10 mg/kg) treated rats (C), BS (100 mg/kg) treated rats (D), BS (200 mg/kg) treated rats (E), and BS (400 mg/kg) treated rats (F). Stained with H and E (at 100 X). Infiltration of neutrophils (red arrow), necrotic changes in cardiac tissue (black arrow).

The hearts from the vehicle control rats showed severe myocardial degeneration (++++), congestion and oedema (+++) and infiltration of inflammatory cells (+++) with disorganized arrangement of muscle bundles with no well-defined boundaries (Figure B).

Administration of metoprolol (10 mg/kg) showed protection against isoproterenol-induced myocardial damage reflected by mild myocardial necrosis (+), inflammatory infiltration (+), and congestion (+) without any oedema (Figure C).

Heart section from the BS (100 mg/kg) treated rats showed presence of severe myocardial necrosis (+++), inflammatory cell infiltration (+++), congestion and oedema (+++) (Figure D).

However, administration of BS (200 mg/kg) showed reduction in myocardial aberrations indued by isoproterenol up to some extent. It showed moderate myocardial necrosis (++), inflammatory cell infiltration (++), congestion (+++) and oedema (++) (Figure E).

Administration of BS (400 mg/kg) showed reduction in myocardial necrosis (+), inflammatory infiltration (+), congestion (++) and oedema (+) when compared with vehicle control group (Figure F).

Treatment	Infiltration of neutrophils	Congestion	Oedema	Necrotic Changes
Normal	+	-	-	-
Vehicle Control	+++	+++	+++	++++
M (10)	+	+	-	+
BS (100)	+++	+++	+++	+++
BS (200)	++	+++	++	++
BS (400)	+	++	-	+

Table n

Note: 0: no abnormality detected, +: damage/ active changes up to less than 25%, ++: damage/ active changes up to less than 50%, +++: damage/ active changes up to less 75%, ++++: damage/ active changes up to more than 75%

Conclusion

Cardiovascular diseases are the major cause of mortality in the world. CVDs includes coronary artery disease, heart failure, hypertension, orthostatic hypotension, heart valve disease, artherosclerosis, arrhythmia, shock, endocarditis, diseases of aorta, disorders of peripheral vascular system and congenital heart disease. Among CVDs the incidence of myocardial infarction varies greatly. A research study reported that the incidence of MI in India is 64.37/1000 people in men aged between 29-69 years. Plant foods contain wide varieties of nutrient phytochemicals which are synthesized by plants helps in defensing free radicals causing diseases. More than 2000 plants are listed in traditional system of medicines. Several studies have reported that the increase in free radicals formation and oxidative stress have associated with the occurrence of decreased endogenous antioxidants which is one of the mechanism for the development of heart failure. To study possible protective effects of drugs on the myocardial injury from AMI, widely used experimental model is the induction of infarction by means of the administration of isoproterenol in rats. Isoproterenol induces myocardial damage and severe stress in 178 Cardioprotective efficacy of a queous extract of Apium Graveolens against isproterenol induced myocardial infarction in male rats the myocardium and leads to the necrosis of heart muscle similar to the one observed in humans. Several mechanisms are proposed to explain the mechanism of isoproterenol induced myocardial harm. An imbalance between oxygen supply and demand from cardiomyocytes inwardly is related to myocardial hyperfunction due to increase in chronotropism and inotropism as well as hypotension in the coronary bed. Secondly, it is also claimed that there is an elevation of Ca++ over charge inside the cell. In addition, this ion is related to the activation of adenylate cyclase enzyme and the depletion of ATP levels on the course of the events. ISO generates free radicals and stimulate lipid peroxidation which is causative factor for the irreversible damage to the myocardial membrane. Eventually there is an oxidative stress agumentation because of several metabolic products originated from isoproterenol. Considering all available scientific background supporting the pharmacological effects of in Apium Graveolens and keeping in mind the negative impact of cardiovascular deiseases in current population, present study aims was to scientifically evaluate the cardioprotective role of aqueous extract of in Apium Graveolens in Isoproterenol induced myocardial toxicity in rats, an experimental animal model for MI in human beings.

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