STUDY REPORT

STUDY TITLE

Cardioprotective Activity of Madhavprash in Experimental Model of Myocardial Infarction in Rats

TEST ARTICLE

Madhavprash

SPONSOR

Madhavbaug, a Cardiac Rehabilitation Center,
Khopoli, Maharashtra
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TESTING FACILITY

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To Whomsoever It May Concern

I hereby declare that this study report of project entitled, ***"Cardioprotective Activity of Madhavprash in Experimental Model of Myocardial Infarction in Rats"*** constitutes a true and faithful account of the results of this study, to the best of my knowledge. This study was conducted in compliance with the study protocol as amended, and with our facility's standard operating procedures.

Principal Investigator:

Name (signed): _____ Date: 17/03/2022

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Title: Pharmacological Evaluation of Cardioprotective activity of Madhavprash formulation in Experimental Model of Myocardial Infarction in Rats

General: The term Myocardial Infarction is thought to reflect death of cardiac myocytes due to prolonged ischaemia. As such, myocardial infarction is an acute coronary syndrome that can occur during the natural course of coronary atherosclerosis. *[Boersma E., Mercado N. et al., 2003]*

Study details: This study was carried out to evaluate the cardioprotective effect of test formulation in Isoproterenol induced Myocardial Infarction in rat model.

Study schedule: Study was carried out after the availability of animals and formulation.

List of abbreviations: MI: Myocardial Infarction; MDA: Malonaldialdehyde, ISP: Isoproterenol, BPM: Beats per minute, H&E: Haematoxylin and Eosin

GLP compliance statement: This study was conducted in compliance with current principles of Good Laboratory Practices.

Objectives: To study the cardioprotective effect of Madhavprash in rat model of Myocardial Infarction on the basis of hemodynamic parameters, biochemical analysis and histopathological examination.

Materials & Methods:

Animals: Experimental protocol for present study (Protocol no. IAEC/2021/II-R01)reviewed and approved by the Institutional Animal Ethics Committee, DPSRU, New Delhi in accordance to the guidelines of "Committee for the Purpose of Control and Supervision of Experiments on Animals". (CPCSEA, Delhi, India). Albino wistar, male rats weighing (170-200gm) were used in the present investigation. Animals were acclimatized in institutional animal house for one week prior to the use for experimentation with free access to food and water ad libitum.

Study Groups:

Study Groups	Treatment	n	Groups included in study
1	Normal Control	5	√
2	Disease Control	8	√
3	Madhavprash 1.5 (Madhavprash dose 1.5 gm/kg)	8	√
4	Madhavprash 3 (Madhavprash dose 3 gm/kg)	8	√
	Total	29	

- Animals were acclimatized for 7 days prior to the study.
- Wistar albino male rats were allocated to 4 groups:
  Group 1 (control group) with 0.9% NaCl (normal saline) once daily for 1 month;
  Group 2 (ISP control group), in which rats fed normal saline once daily for 1 month and in addition received ISP (85 mg/kg, S.C.) on day 29thand 30that a 24-hour interval;

Treatment groups Group 3, and Group 4 received the test formulation, Madhavprash (MP) in two doses (1.5 g/kg and 3g/kg respectively) once daily for consecutive28 days and then received ISP (85 mg/kg, S.C.) on days 29th and 30th at an interval of 24 hours followed by co- treatment of Madhavprash.

- At the end of the experimental period, on 31st day (24 h after last injection of ISP), all the rats were anaesthetized with Sodium Pentobarbitone (60 mg /kg, I.P.)
- ECG was recorded in all study animals. Mean arterial pressure (MAP) and Heart rate (HR) were analysed.
- Blood sample was collected to separate serum for the determination of biochemical parameters (antioxidant enzymes and MDA).
- Animals were sacrificed with overdose of anaesthesia, heart tissues was excised and rinsed in ice-cold isotonic saline, blotted with filter paper, homogenized in 0.05M ice-cold phosphate buffer (pH 7.4; 1:10 w/v) for biochemical assays.
- Immediately after the sacrifice of the anesthetized animals, their hearts were removed and fixed in 10% formalin solution before being processed for histological examinations with H&E staining.

Observations:

Following parameters were observed:

1) Hemodynamic parameters:

Treatment Groups	**Systolic pressure (mm Hg)**	**Diastolic pressure (mm Hg)**	**MAP (mmHg)**	**HR (BPM)**
Normal Control	215 ± 13	192 ± 20	208 ± 14	328± 24
Disease Control	167 ± 10***	150 ± 10**	159 ± 11***	263 ± 13***
Madhavprash 1.5	192 ± 16##	173 ± 19#	185 ± 19#	280 ± 10#
Madhavprash 3	201± 18##	179± 12##	191± 14##	297 ± 27##

Table 1: MAP and HR. All the data are expressed as Mean ± SD;**p≤ 0.01, ***p≤ 0.001, compared with NC group;#p≤0.05,##p≤ 0.01, compared with DC group. MAP: Mean Arterial Pressure.

Statistical Analysis Report: There is statistically significant difference in value of MAP and HR between Normal Control group and Disease Control group. Group Madhavprash 3 (Madhavprash 3gm/kg) rats possesses more significant values of MAP and HR than MAP and HR values of group Madhavprash 3 (Madhavprash 3 gm/kg) rats as compared with Disease control group rats.There is no significant difference in the MAP and HR values between the treatment groups.

2) Biochemical Analysis

A. In heart tissue:

Groups	GST (ng/mg)	GSH (nm/min/mg protein)	GP (nm/min/mg protein)	GR (nm/min/mg protein)	SOD (ng/mg)	MDA (µM/min/mg of protein)	DPPH (% inhibition of DPPH radical)
NC	840±35	8±0.6	105±6.3	101.7±1.6	15.7±0.50	3.9±0.06	10.1±0.09
DC	627±23***	5±0.5***	46±1.9***	55.5±0.98***	7.0±0.48***	5.2±0.04***	6.9±0.09***
Madhavprash 1.5	700±16$^{\#\#\#}$	6±0.4$^{\#}$	60.6±1.6$^{\#\#\#}$	65.5±1.2$^{\#\#\#}$	9.1±0.51$^{\#\#\#}$	4.3±0.04$^{\#\#\#}$	11.1±0.10$^{\#\#\#}$
Madhavprash 3	783±19$^{\#\#\#}$	7±0.4$^{\#\#\#}$	84.6±1.5$^{\#\#\#}$	89.5±1.4$^{\#\#\#}$	13.2±0.21$^{\#\#\#}$	5.0±0.04$^{\#\#\#}$	13.0±0.14$^{\#\#\#}$

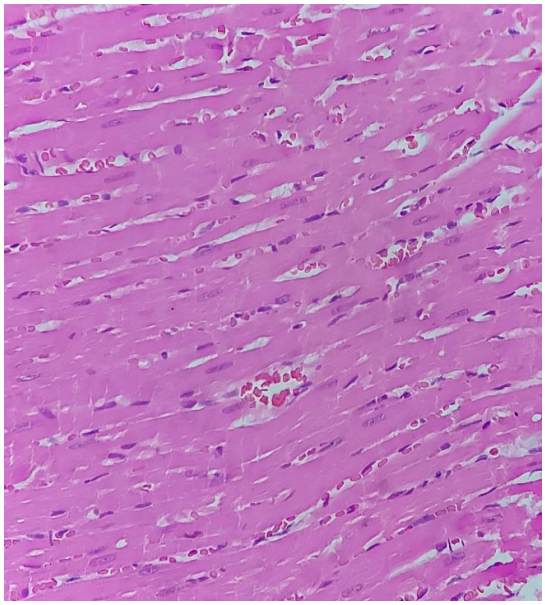
Table 2: Biochemical estimation in heart tissue. All the data are expressed as Mean ± SD;***p≤ 0.001, compared with NC group; $^{\#}$p≤0.05, $^{\#\#\#}$p≤0.001 compared with DC group

B. In Serum:

Groups	GST (ng/mg)	GSH (nm/min/mg protein)	GP (nm/min/mg protein)	GR (nm/min/mg protein)	SOD (ng/mg)	MDA (µM/min/mg of protein)	DPPH (% inhibition of DPPH radical)
NC	502±17	5±0.6	80.9±1.52	77.6±0.72	10.2±0.50	1.23±0.04	6.1±0.22
DC	395±16***	4±0.6*	40.0±1.44***	45.4±0.52***	6.1±0.23***	1.94±0.04***	3.9±0.08***
Madhavprash 1.5	444±8$^{\#\#\#}$	4±0.4	50.2±1.45$^{\#\#\#}$	57.4±0.52$^{\#\#\#}$	7.2±0.13$^{\#\#\#}$	1.43±0.02$^{\#\#\#}$	7.9±0.12$^{\#\#\#}$
Madhavprash 3	483±11$^{\#\#\#}$	5±0.4$^{\#}$	61.8±1.65$^{\#\#\#}$	67.1±0.77$^{\#\#\#}$	8.9±0.15$^{\#\#\#}$	1.72±0.01$^{\#\#\#}$	10.4±0.14$^{\#\#\#}$

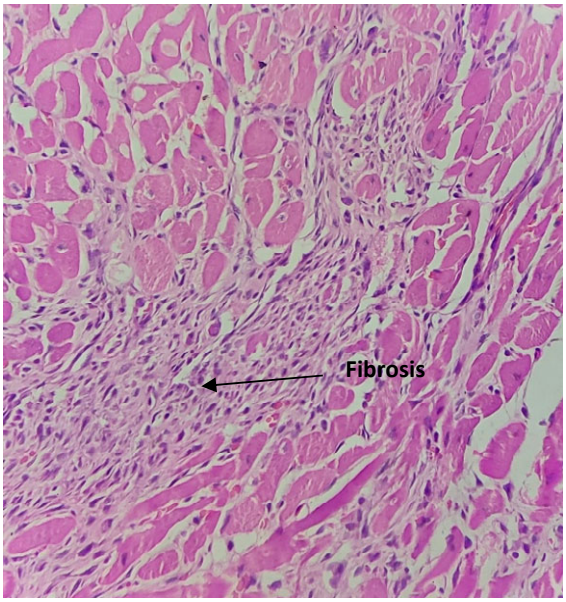
Table 3: Biochemical estimation in blood serum. All the data are expressed as Mean ± SD;*p≤ 0.05, ***p≤ 0.001, compared with NC group; $^{\#}$p≤0.05, $^{\#\#\#}$p≤0.001 compared with DC group

3). Histological evaluation:



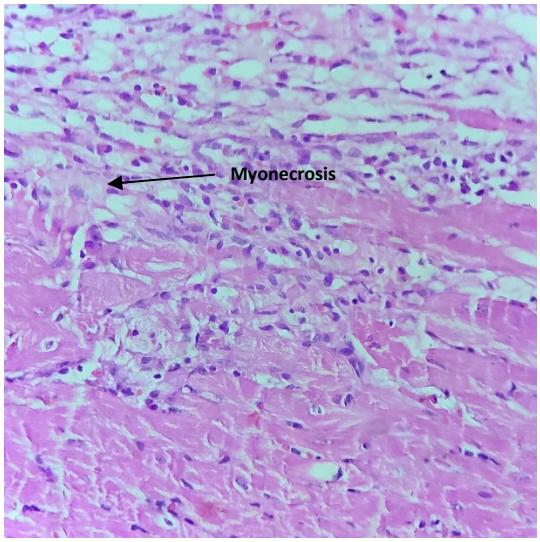
NORMAL CONTROL

Section examined shows normally arranged and maintained polarity of endocardum, myocardium, epicardium. Myocytes arranged in muscle bundles separated by fibrous tissue. Individual myocytes form a syncytium with end to end junctions (intercalated disc). Each myocytes contains a central ovoid nucleus with bland chromatin and central clear zone (H&E, 400X).



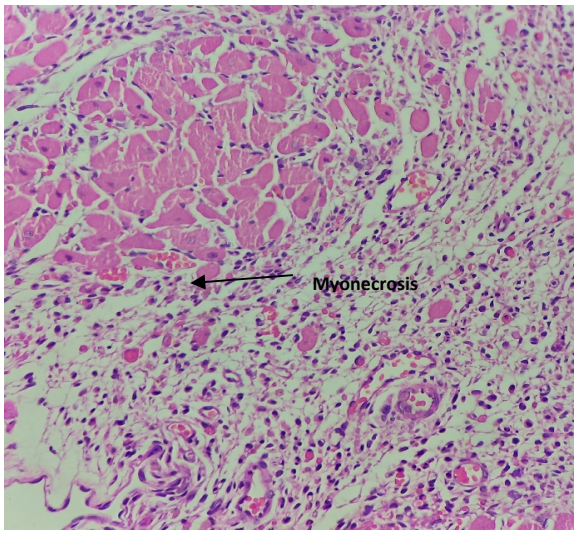
DISEASE CONTROL

Section examined shows normally arranged and maintained polarity of endocardium, myocardium, epicardium. Myocytes arranged in muscle bundles separated by fibrous tissue. Individual myocytes form a syncytium with end to end junctions (intercalated disc). Each myocytes contains a central ovoid nucleus with bland chromatin and central clear zone. Focal areas in myocardial shows fibroblastic proliferation with interspersed capillaries (H&E, 400X).



MADHAVPRASH 1.5

Section examined shows normally arranged and maintained polarity of endocardium, myocardium, epicardium. Myocytes arranged in muscle bundles separated by fibrous tissue. Individual myocytes form a syncytium with end to end junctions (intercalated disc). Each myocytes contains a central ovoid nucleus with bland chromatin and central clear zone. At places in myocardial shows fibroblastic proliferation with interspersed blood vessels. Epicardium shows moderate inflammatory cell infiltrate. Focal areas of myonecrosis (1-2%) are also seen. (H&E, 400X).



MADHAVPRASH 3

Section examined shows normally arranged and maintained polarity of endocardum, myocardium, epicardium. Myocytes arranged in muscle bundles separated by fibrous tissue. Individual myocytes form a syncytium with end to end junctions (intercalated disc). Each myocytes contains a central ovoid nucleus with bland chromatin and central clear zone. Epicardium shows moderate inflammatory cell infiltrate with edema, interspersed blood vessels and areas of myonecrosis (1-2%). Myocardium shows mild fibrosis (H&E, 400X).

Figure: Histopathological examination showing effect of different treatments on myonecrosis in MI induced rat heart tissue as compared with normal control

Grade of H&E staining

Group	**Myonecrosis (Focal area)**	**Fibroblast Proliferation**	**Inflammatory cell infilteration**	**Edema**
Normal Control	(-)	(-)	(-)	(-)
Disease Control	(++)	(++)	(++)	(++)
Treatment 1	(+)	(+)	(+)	(+)
Treatment 2	(+)	(+)	(+)	(+)

(-), (+), (++) denotes minimal, mild and moderate level respectively

Conclusion: Biochemical analysis data predicts that Madhavprash formulation possess antioxidant activity more at dose of 3 gm/kg. Madhavprash 3 have shown significant reduction in area of myonecrosis in T2 group animal myocardium and with moderate inflammatory cell infiltration in epicardium as compared to Disease control animal myocardium and Treatment 1 group animal myocardium.

Madhavprash treatments have improved the MAP and HR in respective groups rats as compared to Disease control group rats more significant with Madhavprash 3.

References:

1. Boersma, E., Mercado, N., Poldermans, D., Gardien, M., Vos, J., & Simoons, M. L. (2003). Acute myocardial infarction. The Lancet, 361(9360), 847–858. doi:10.1016/s0140-6736(03)12712-2.

2. Singal PK, Dhalla AK, Hill M, Thomas TP. Endogenous antioxidant changes in the myocardium in response to acute and chronic stress conditions. Mol Cell Biochem1993;129:179–86

3. Nandave, M. et al. Moringa oleifera leaf extract prevents isoproterenol-induced myocardial damage in rats: evidence for an antioxidant, antiperoxidative, and cardioprotective intervention. J Med Food. 2009;12(1):47-55.

4. K. Karthikeyan, B.R. Sarala Bai, S. Niranjali Devaraj, Cardioprotective effect of grape seed proanthocyanidins on isoproterenol-induced myocardial injury in rats, International Journal of Cardiology,2007; 115 (3),326-333.

5. Nandave, M., Mohanty, I., Nag, T. C., Ojha, S. K., Mittal, R., Kumari, S., & Arya, D. S. Cardioprotective response to chronic administration of vitamin E in isoproterenol induced myocardial necrosis: Hemodynamic, biochemical and ultrastructural studies. *Indian journal of clinical biochemistry: IJCB*,2007; *22*(1), 22–28.