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Research article

Experimental investigation of marigold extract: Modulates isoproterenol induced myocardial ischemia in rats

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Abstract

Ischemic heart disease is a major leading cause of morbidity and mortality. Myocardial infarction is a condition where loss of myocytes takes place due to prolonged ischemic condition and adrenergic over activation. High oxidative stress and myocardial inflammation play key role in the pathogenesis of myocardial infarction. In myocardial ischemia, generation of reactive oxygen species (ROS) and decreased level of antioxidant defense mechanism is associated with its main pathophysiology. Experimentally, Isoproterenol-induced myocardial infarction with β -adrenergic sympathetic overactivation. The β -adrenergic $G_{\alpha s}$ signalling activation results in the elevation of cAMP through adenylyl cyclase activation, leading to phosphorylation of L-type calcium channels and elevation of intracellular calcium concentration, results in ultrastructural changes, alteration of gene transcription and activation of calcium dependent endonuclease (DNase I), causing loss of myocytes through apoptosis. However, in present research, Marigold extract ameliorate the cardio-toxic effect of isoproterenol, significantly prevent the damages induced by isoproterenol on histopathological and biochemical changes in rat model of myocardial infarction. Furthermore, Marigold extract *Calendula Officinalis* have a high potent antioxidant potential. Thereby, this study discuss information gleaned for various herbal drugs suggesting that adding herbs to daily life serve as scrumptious and sensible way to keep heart healthy.

Keywords: Myocardial infarction, reactive oxygen species, antioxidant enzymes, Marigold extract

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1. Introduction

Ischemic heart disease is a leading cause of mortality worldwide, and its prevalence is incessantly increasing worldwide [1, 2]. Myocardial infarction is a condition in which loss of myocytes takes place due to prolonged ischemic condition and adrenergic overactivation [3, 4]. Myocardial infarction occurs as a result of coronary artery obstruction, thrombotic occlusion and coronary spasm-associated myocardial ischemia [5, 6]. High oxidative stress and myocardial inflammation play key roles in the pathogenesis of myocardial infarction [7, 8]. In addition, increased migration of neutrophils to myocardial ischemic tissue contributes to the pathogenesis of myocardial injury [9]. Acute myocardial infarction is associated with various symptoms such as sudden chest pain radiating to the left arm and shoulder, shortness of breath, anxiety, palpitation, and nausea, vomiting and sweating [10]. The chronic and abnormal β -

adrenergic receptor overactivation induces cardiac toxicity and myocardial infarction by switching on serial of events. These include activation of $G_{\alpha s}$, elevation of cyclic adenosine monophosphate (cAMP) levels, calcium overload, coronary spasm and reduction in coronary flow [11, 12, 13]. Isoproterenol, a synthetic catecholamine and a non-selective β -adrenergic agonist, is often employed in high dose to induce experimental myocardial infarction in order to study cardioprotective anti-infarct effects of pharmacological interventions [14, 15, 16]. Isoproterenol-induced myocardial infarction is associated with β -adrenergic sympathetic overactivation. The β -adrenergic $G_{\alpha s}$ signalling activation results in the elevation of cAMP through adenylyl cyclase activation, leading to phosphorylation of L-type calcium channels and elevation of intracellular calcium concentration. This results in ultrastructural changes, alteration of gene transcription and activation of calcium dependent endonuclease (DNase I), causing loss of myocytes

through apoptosis [17]. It should be noted that elevation in the level of calcium is also a mediator of myocardial necrosis induced by isoproterenol [18]. Induction of high oxidative stress in the heart is one of key events that could contribute to isoproterenol-induced experimental myocardial infarction [19, 20].

In recent years, natural products received much attention as therapeutic agents to treat cardiovascular and metabolic disorders as they are associated with less adverse effects. *Calendula officinalis* (*C. officinalis*), also known as marigold, a yellow or orange-colored flowers are used as food dye, spice, and tea as well as tincture, ointment or cosmetic cream. Although the genus *Calendula* is usually indigenous to the southern European region including Italy, Malta, Greece, Turkey, Portugal, and Spain [21], it is nowadays cultivated in many temperate regions of the world depending on its commercial value. Since *C. officinalis* is grown in northern parts of Africa, it is also named as "African marigold" [22]. It has been widely used on the skin to treat minor wounds, infections, burns, bee stings, sunburn, warts, and cancer. Most scientific evidence regarding its effectiveness as a wound-healing agent is based on animal and laboratory studies, while human research is virtually lacking. *C. officinalis* has many pharmacological properties. It is used for the treatment of skin disorders, pain and also as a bactericide, antiseptic and anti-inflammatory. Butanolic fraction of *C. officinalis* possesses a significant free radical scavenging and antioxidant activity [23]. *C. officinalis* flowers are believed to be useful in reducing inflammation, wound healing, and as an antiseptic. *C. officinalis* is used to treat various skin diseases, ranging from skin ulcerations to eczema. Internally *C. officinalis* has been used for stomach ulcers and inflammation. The flavonoids, found in high amounts in *C. officinalis*, are responsible for its anti-inflammatory activity; triterpene saponins may also be important. *C. officinalis* also contains carotenoids. As study in women receiving radiation therapy to the breast for breast cancer reports that *C. officinalis* ointment applied to the skin at least twice daily during treatment reduces the number of people experiencing severe dermatitis (skin irritation, redness, pain) [24]. *C. officinalis* is highly effective for the prevention of acute dermatitis of grade 2 or higher and should be proposed for patients undergoing post operative irradiation for breast cancer [25]. The methanolic extract and its 1-butanol-soluble fraction from *C. officinalis* flowers are found to show a hypoglycemic effect, inhibitory activity of gastric emptying, and gastroprotective effect [26]. Acute toxicity studies in rats and mice indicate that the extract is relatively non-toxic. Animal tests showed at most minimal skin irritation, and no sensitization or phototoxicity. Minimal ocular irritation is seen with one formulation and no irritation with others. Six saponins isolated from *C. officinalis* flowers are not mutagenic in an Ames test, and a tea derived from *C. officinalis* is not genotoxic in *Drosophila melanogaster* [26]. The plant has been reported to contain mainly carotenoids, flavonoids, phenolic acids, and triterpenes [27-30]. The flower per

se, flower extracts, flower essential oil, and seed oil of *C. officinalis* are cosmetic ingredients and the plant has been presented as a new source for cosmetic industry [31]. *C. officinalis* have been found to be usually safe [32], although very rare occurrence of contact dermatitis due to Compositae (Asteraceae) plants should be taken into account [33]. On the other hand, marigold is shown to be a promising dye plant for obtaining natural colors [34, 35]. According to ancient records [36], the *Calendula* flowers are used as a symbol of remembrance and believed to give great forces of warmth and benign compassion to the human soul, especially helping to balance the active and receptive modes of communication. Besides, the *Calendula* essential oil has been reported to be used in care of the elderly [37]. Taking this information on *Calendula* into account, in this study, we aimed to examine inhibitory activity of the n-hexane, dichloromethane, acetone, ethyl acetate, methanol, and water extracts of the leaf and flowers of *Calendula arvensis* and *C. officinalis* L. against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), which are the key enzymes for the treatment of Alzheimer's disease and, lately, Down syndrome [38]. Since neurodegeneration is strongly associated with oxidative damage [39], antioxidant activity of the extracts is tested by 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric ion chelating capacity, and ferric-reducing antioxidant power (FRAP) assays at 250, 500, and 1000 µg mL⁻¹. Total phenol and flavonoid contents of the extracts are calculated spectrophotometrically. The extracts and quercetin dilutions (500 µg) are mixed with 95% ethanol (1500 µL), aluminum chloride reagent (100 µL), and sodium acetate (100 µL) (Emir Kimya, Turkey) as well as distilled water (2800 µL). Following incubation for 30 min at room temperature, absorbance of the reaction mixtures is measured at wavelength of 415 nm with a Unicam 4802 UV-visible double beam spectrophotometer (USA). The total phenol and flavonoid contents of the extracts are expressed as gallic acid and quercetin equivalents (mg/g extract), respectively. The pharmacological activity of marigold is related to the content of several classes of secondary metabolites such as essential oils, flavonoids, sterols, carotenoids, tannins, saponins, triterpene alcohols, polysaccharides, a bitter principle, mucilage, and resin [27]. Bilia et al., 2001 [40] found that marigold flowers contain rutin, isoquercitrin, quercetin-3-O-rutinosylrhamnoside, isorhamnetin-3-O-rutinosylrhamnoside, isorhamnetin-3-O-glucosylglucoside, and isorhamnetin-3-O-glucoside. According to the fact that marigold contains polyphenols, the assessment of its antioxidant properties is of great interest in the understanding of positive effect of these compounds especially in phytotherapy. In addition to its anti-inflammatory action, Marigold Extract: *Calendula Officinalis* have a potent anti-oxidant potential. However, the protective effect of Marigold Extract on experimentally isoproterenol induced myocardial infarction has not been investigated. Therefore, the present study has been designed to investigate the effect of Marigold Extract: *Calendula officinalis* against

isoproterenol-induced myocardial infarction in rats.

2. Materials and methods

Wistar albino rats of either sex weighing about 200-300 g were used in the present study. The animals were acclimatized in the 'Institutional animal house' and maintained on rat chow and tap water. Rats were allowed ad libitum access to food and water. They were exposed to normal day and night cycles. The experimental protocol employed in this study received approval from the 'Institutional Animal Ethics Committee' under the guidelines given by the 'Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)'.

2.1 Experimental protocol

There are six groups employed in present study, and each group comprised six rats. Isoproterenol is dissolved in normal saline (NaCl, 0.9% w/v).

Group I (Normal Control), rats are maintained on standard food and water, and no treatment shall be given.

Group II (Isoproterenol Control), rats are administered isoproterenol (85 mg/kg/day) subcutaneously for last two consecutive days of 30 days experimental protocol.

Group III (Marigold extract *per se*), normal rats are administered Marigold extract (200 mg/kg/day, *p.o.*) for 30 days.

Group IV (Marigold extract Pre-treated), rats are pre-treated with Marigold extract (100 mg/kg/day, *p.o.*) for 30 days. These rats, on last two consecutive days (day 29 and day 30), are administered isoproterenol (85 mg/kg/day, *s.c.*) one hour after Marigold extract administration.

Group V (Marigold extract Pre-treated), rats are pre-treated with Marigold extract (150 mg/kg/day, *p.o.*) for 30 days. These rats, on last two consecutive days (day 29 and day 30), are administered isoproterenol (85 mg/kg/day, *s.c.*) one hour after Marigold extract administration.

Group VI (Marigold extract Pre-treated), rats are pre-treated with Marigold extract (200 mg/kg/day, *p.o.*) for 30 days. These rats, on last two consecutive days (day 29 and day 30), are administered isoproterenol (85 mg/kg/day, *s.c.*) one hour after Marigold extract administration.

2.2 Induction of experimental myocardial infarction

Experimental myocardial infarction is induced in rats by administration of isoproterenol hydrochloride (85mg/kg/day, *s.c.*) for last two consecutive days of 30

days experimental protocol dissolved in freshly prepared normal saline (NaCl, 0.9% w/v).

2.2.1 Assessment of myocardial infarction

The isoproterenol induced myocardial injury is assessed by estimating the release of lactate dehydrogenase (LDH) and creatine kinase (CK-MB) in the blood serum and measuring the infarct size in the heart. The release of LDH and CK-MB is noted by commercially available kits.

2.2.2 Assessment of myocardial infarct size

The heart is removed from the animal. Both auricles, root of aorta and right ventricle are excised, and the left ventricle is kept overnight at -4° C. Frozen ventricle is sliced into sections of 2-3 mm in thickness. The slices are incubated in 1% 2,3,5- triphenyltetrazolium chloride (TTC) solution in 0.1 M Tris buffer of pH 7.8 for 20-min at 37° C. The TTC stain reacts with dehydrogenase enzyme in the presence of cofactor NADH to form formazan pigment in viable cells, which are brick red in colour. The infarcted cells that have lost dehydrogenase enzyme remain unstained. Thus, the infarcted portion of the myocardium remains unstained while the normal viable myocardium is stained brick red with TTC staining. The infarct size is measured macroscopically using the volume method [41-43].

2.2.3 Estimation of CK-MB

The amount of released CK-MB in the blood serum is estimated by immunoinhibition method using the commercially available enzymatic kit (Crest Biosystems, Goa, India). It is based on the principle that CK-M fraction of CK-MM and CK-MB in the sample is completely inhibited by CK-M antibody present in the reagent. Then, the activity of CK-B fraction is measured, and the CK-MB activity is expressed in IU/L. The procedure for CK-MB estimation follows. Briefly, 0.8 mL of enzyme reagent and 0.05 mL of blood serum are taken out in a glass tube and incubated at 37° C for 5-min. Then, 0.2 mL of starter reagent is added to the reaction mixture with thorough mixing. The absorbance is noted against blank, and the initial absorbance is noted after 5-minute (A) and thereafter 1 minute (B) 2 minute (C) 3 minute (D) at 340 nM. The mean change in absorbance per minute is noted and the CK-MB activity is calculated using the following formula:

CK-MB activity (IU/L) 37° C = $\Delta A/\text{min} \times 6666$

Where, ΔA is $[(B-A) + (C-B) + (D-C)]/3$

2.2.4 Estimation of LDH

The amount of released LDH in the blood serum is estimated by UV-kinetic method using the commercially available enzymatic kit (Crest biosystem, Goa, India). It is based on the principle that LDH catalyzes the reduction of pyruvate to lactate accompanied by the simultaneous

reduction of NADH into NAD. The rate of oxidation of NADH to NAD is proportional to a decrease in absorbance which is proportional to the LDH activity in the sample.



The procedure for LDH estimation follows. Briefly, 1 mL of working reagent (Mixed contents of bottle of L2 (starter reagent) and bottle L1 (buffer reagent) is added to 0.05 mL of blood serum in a glass tube with thorough mixing. The absorbance of test is noted against blank exactly after 1min. (A) and thereafter at 2 minute (B), 3 minute (C) and 4 minute (D) at 340 nM. The mean change in absorbance per minute is noted, and LDH activity (expressed in International Units per Litre (IU/L) is calculated using the following formula:

$$\text{LDH activity (IU/L)} \quad 25^\circ \text{C}/30^\circ \text{C} = \Delta A/\text{min} \times F$$

$$\text{Where } \Delta A = [(C-A) + (D-B)]/2 \times F \text{ and } F=3333$$

2.2.5 Assessment of oxidative stress

The left ventricle is minced and homogenized using potassium chloride (KCl) 1.15 %, in a ratio of 1 g of wet myocardial tissue to 10 mL of 1.15 % KCl. The tissue homogenate is used to estimate thiobarbituric acid reactive substances (TBARS) and reduced form of glutathione (GSH).

2.2.6 Estimation of myocardial TBARS

The quantitative measurement of TBARS in the rat heart is performed⁴⁴⁻⁴⁵. The reaction mixture is prepared by mixing 0.2 mL of tissue homogenate, 0.2 mL of 8.1 % sodium dodecyl sulfate, 1.5 mL of 20 % acetic acid solution (adjusted to pH 3.5 with NaOH) and 1.5 mL of 0.8 % aqueous solution of thiobarbituric acid (TBA). The reaction mixture is made up to 4.0 mL with distilled water and then incubated at 95° C for 60 minute. After cooling in tap water, 1.0 mL of distilled water and 5.0 mL of the mixture of n-butanol and pyridine (15:1 v/v) are added to reaction mixture and shaken vigorously using vortex shaker. The test tubes are centrifuged at 4000 rpm for 10 minute (REMI Cooling Centrifuge, India). The absorbance of developed pink color is measured spectrophotometrically at 532 nM. The standard curve using 1, 1, 3, 3-tetramethoxypropane (1-10 nM) is plotted to calculate the concentration of TBARS, and the results are expressed as nM/g wet weight of myocardial tissue.

2.2.7 Estimation of reduced glutathione

The myocardial GSH level is estimated using the methods described by [46-47]. The supernatant of heart homogenate of the rat is mixed with 10 % w/v trichloroacetic acid in 1:1 ratio and centrifuged at 4° C for 10 minute at 5,000 rpm. The supernatant (0.5 mL) is mixed with 2 mL of 0.3 M disodium hydrogen phosphate buffer (pH 8.4) and 0.4 mL of distilled water. Then, 0.25

mL of 0.001 M freshly prepared DTNB [5, 5'-dithiobis (2-nitrobenzoic acid) dissolved in 1 % w/v sodium citrate is added to the reaction mixture and then incubated for 10-min. The absorbance of the yellow-colored complex is noted spectrophotometrically at 412 nM. A standard curve is plotted using the reduced form of glutathione (0.1–1 μM), and the results are expressed as μM/g wet weight of myocardial tissue.

2.3 Measurement of C- reactive protein (CRP)

The serum CRP, a marker of inflammation, is measured using an immunoassay kit (Immunospec Corporation, CA, and USA). This estimation is done from Nalwa laboratories Pvt. Ltd., Hisar, India. Quantitative measurement of CRP is done in rat serum by turbidimetric immunoassay method. In this method, CRP in the sample binds to specific anti-CRP antibodies, which have been absorbed to latex particles and agglutinates. -The agglutinate is proportional to the quantity of CRP in the sample. The actual concentration is then determined by interpolation from a calibration curve prepared from calibrators of known concentrations. The CRP concentration is calculated by using formula:

$$\text{CRP concentration in mg/L} = \frac{(A_2 - A_1) \text{ sample}}{(A_2 - A_1) \text{ calibrator}} \times \text{calibrator concentration}$$

$$\text{Calibrator concentration} = 150 \text{ mg/ L}$$

$$A1 = \text{Initial concentration}$$

$$A2 = \text{Final concentration}$$

2.4 Histopathological assessment

The histological assessment of the myocardium is performed where the heart is rapidly dissected and fixed immediately with saline and then fixed in 10% buffered neutral formalin solution, Five-micrometers thick serial histological sections are obtained from the paraffin blocks and stained with hematoxylin and eosin, after being dehydrated in alcohol (starting from 80% to absolute alcohol) and subsequently cleared with xylene. The histological findings are reported in order to describe the sites of the lesions; no morphometric evaluations are made in this preliminary study. The photomicrographs are shot using Motic Microscope BA310 (Motic, USA) at 40 X to assess the integrity of myocardium.

2.5 Statistical analysis

The results are expressed as mean ± standard deviation (SD). The data obtained from various groups are statistically analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. A 'p' value of less than 0.001 is considered statistically significant, and the 'p' values are of two-tailed.

3. Result

Administration of Marigold extract *per se* (200 mg/kg/day *p.o.*, 30 days) did not produce any significant effect on various parameters observed in normal rats. Isoproterenol (85 mg/kg/day *s.c.*) in rats twice at an interval of 24 hrs on last two consecutive days (day 29 and 30) induce myocardial infarction. Myocardial injury was evaluated by estimating serum parameters like CK-MB and LDH level, and myocardial infarct size was measured in rat tissue at the end of 4 week of experimental protocol. In addition, the serum CRP level was also noted.

3.1 Effect of Isoproterenol on heart weight/body weight ratio

The effect of Marigold extract treatment on heart weight to body weight ratio is depicted in figure.... There was no significant difference in the body weight between the

treated and normal control groups. While, isoproterenol treated animals showed a significant reduction in body weight. The heart weight/body weight ratio were increased significantly ($p < 0.001$) in isoproterenol administered rats, when compared with normal control rats. Marigold extract pre-treated rats, showed significant ($P < 0.001$) reduction in heart weight/body weight ratio as compared to isoproterenol treated rats.

3.2. Effect of marigold extract on myocardial infarct size

Increase in myocardial infarct size was seen in isoproterenol treated group as compared to normal control group. The results obtained were statistically significant in reducing myocardial infarct size. Pre-treatment with Marigold extract (100, 150 & 200 mg/kg/day *p.o.*, 4 weeks) significantly attenuated isoproterenol induced myocardial infarct size changes, when compared to normal control group [Figure 1].

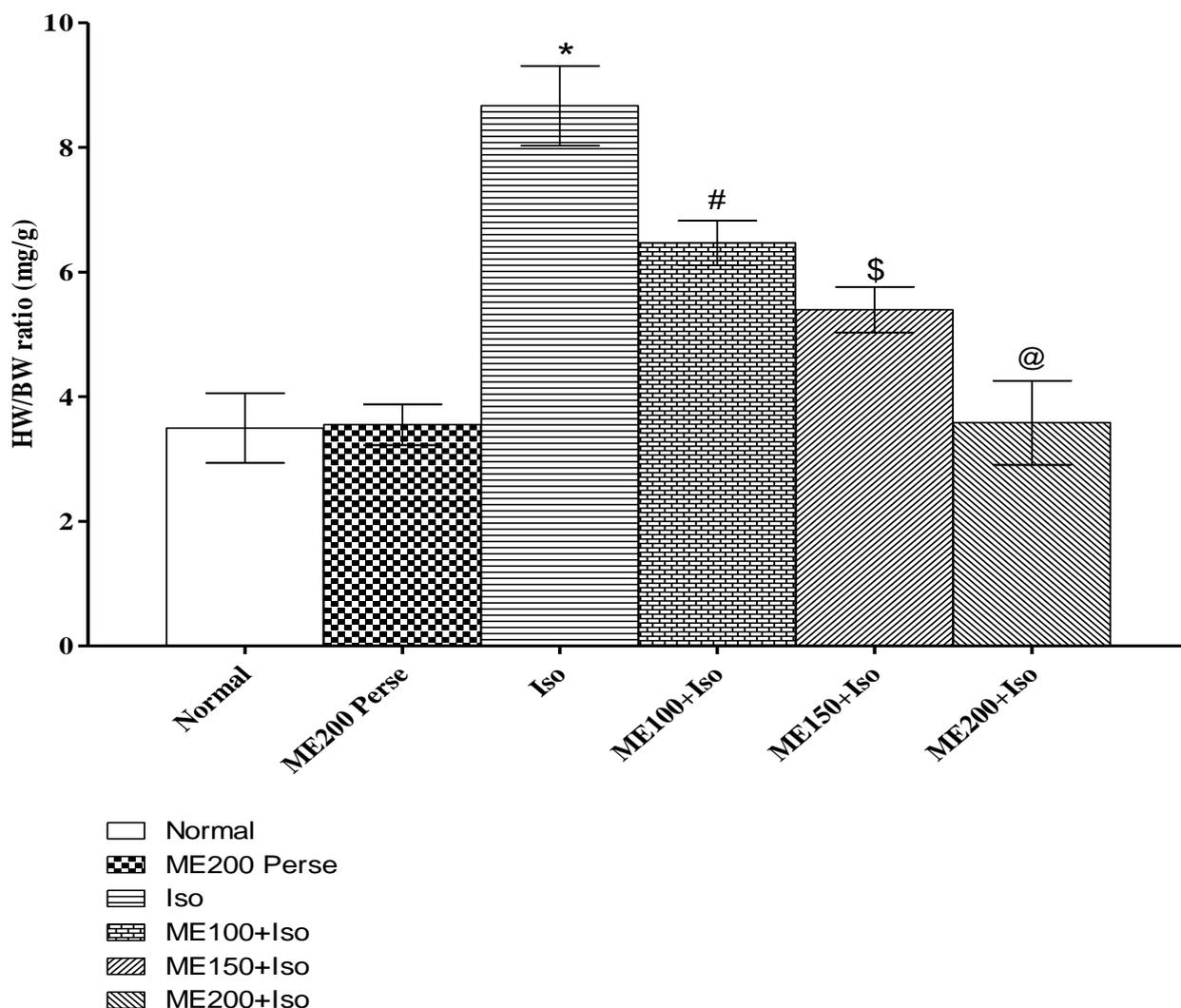


Figure 1: Effect of Marigold Extract on HW / BW Ratio (mg/g)

Values were expressed as mean ± SD.

*, p<0.001 versus Normal Control & Marigold extract 200 Perse; #, p<0.001 versus Isoproterenol Control; \$, p<0.001 versus Marigold extract 100; @, p<0.001 versus Marigold extract 150.

Table 1: Effect of Marigold Extract on HW / BW Ratio (mg/g)

Groups	HW / BW Ratio (mg/g)
Normal	3.498±0.5597
ME200 Perse	3.553±0.3267
Iso	8.668±0.6414*
ME100+Iso	6.467±0.3559#
ME150+Iso	5.395±0.3676\$
ME200+Iso	3.582±0.6745@

3.3 Effect of Marigold extract on serum CK-MB and LDH levels

Discernible increase in serum LDH and CK-MB was noted in isoproterenol induced rats as compared to normal control rats. Pre-treatment with Marigold extract (100, 150 & 200 mg/kg/day *p.o.*, 30 days) restore isoproterenol induced alterations of serum diagnostic marker enzymes to normal. The results obtained were statically significant in reducing CK-MB [Figure 2] and LDH [Figure 3] levels in isoproterenol treated rats as compared to normal control rats.

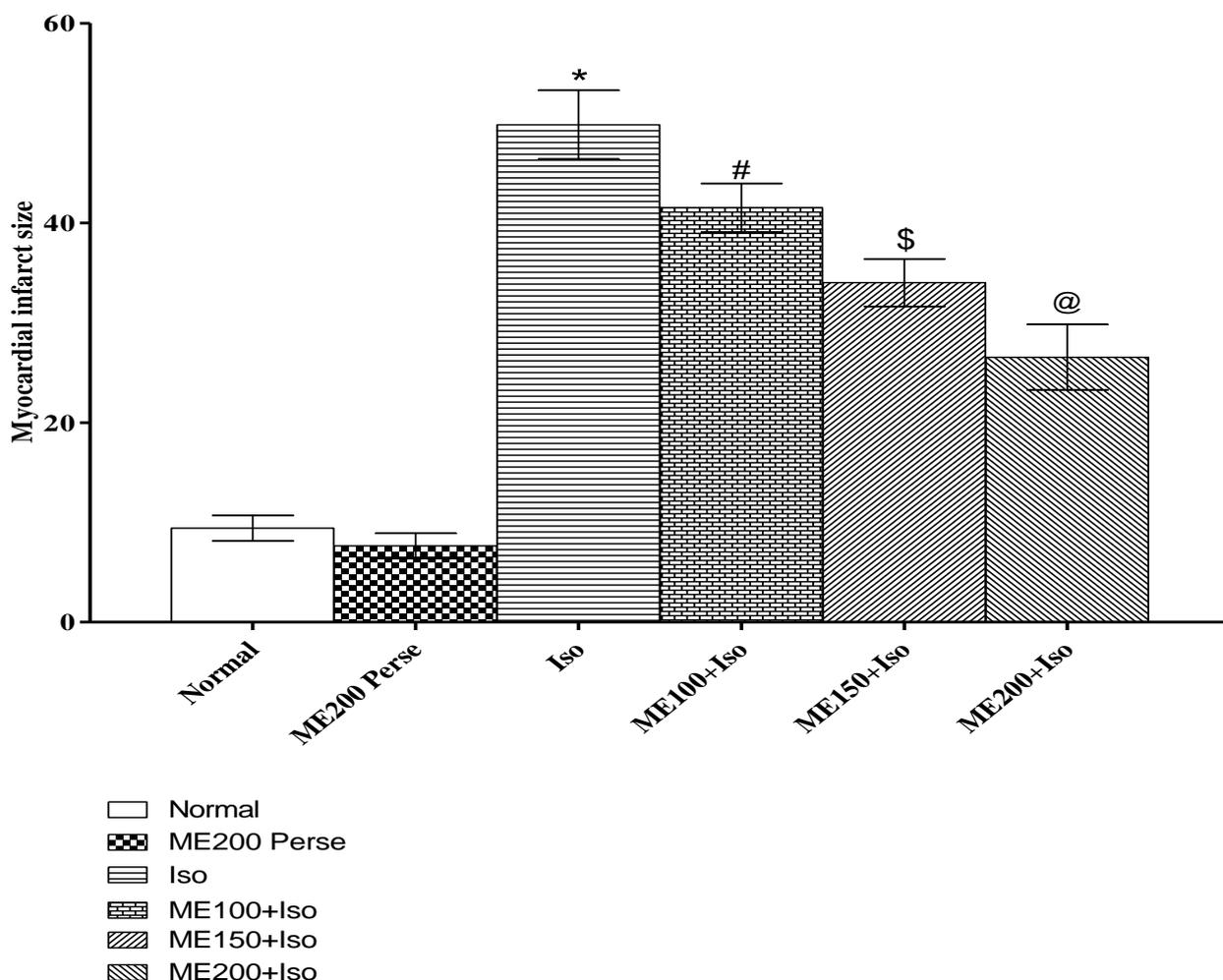


Figure 2: Effect of Marigold extract on myocardial infarct size

Values were expressed as mean ± SD.

*, p<0.001 versus Normal Control & Marigold extract 200 Perse; #, p<0.001 versus Isoproterenol Control; \$, p<0.001 versus Marigold extract 100; @, p<0.001 versus Marigold extract 150.

Table 2: Effect of Marigold extract on myocardial infarct size

Groups	Myocardial infarct size
Normal	9.417±1.270
ME200 Perse	7.650±1.252
Iso	49.83±3.430*
ME100+Iso	41.50±2.429#
ME150+Iso	34.00±2.366\$
ME200+Iso	26.54±3.276@

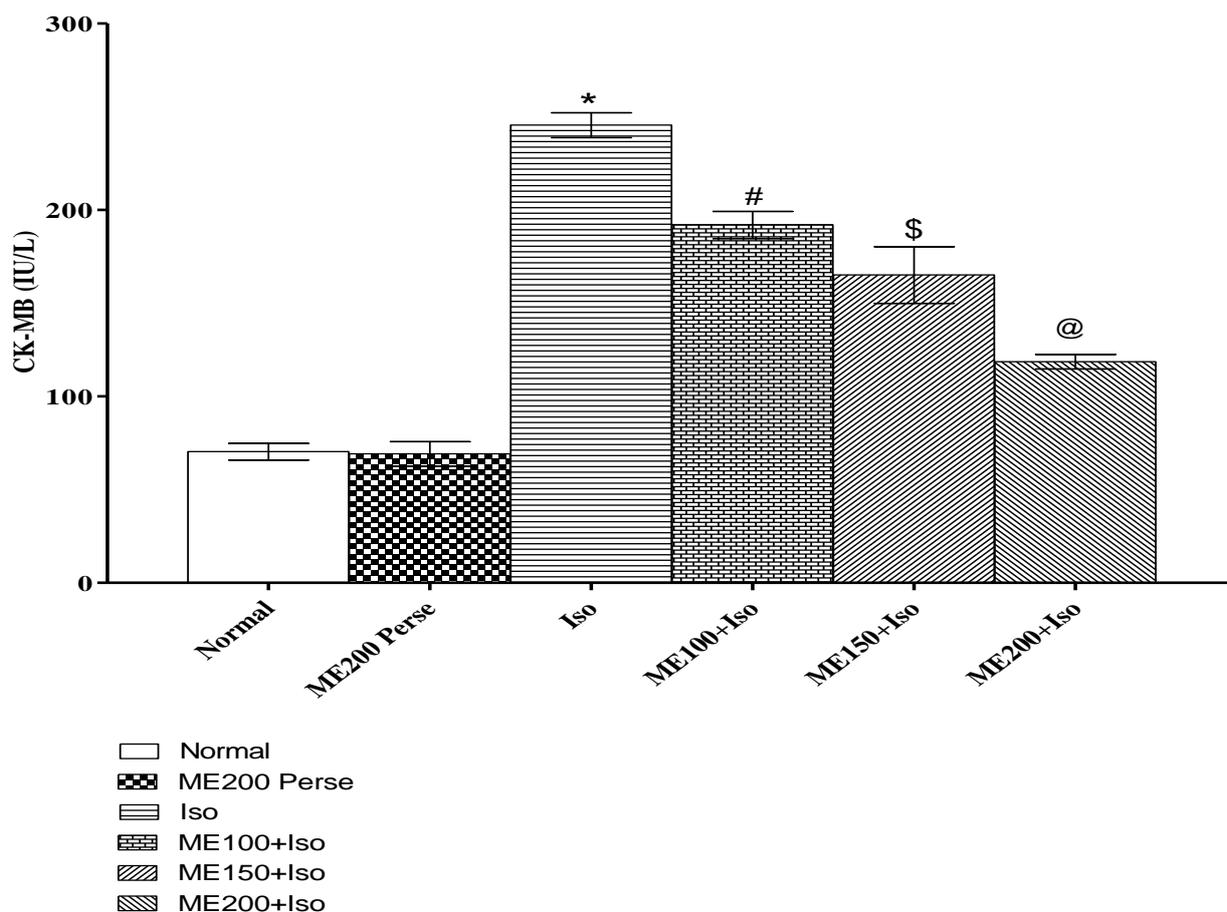


Figure 3: Effect of Marigold extract on serum CK-MB (IU /L)

Values were expressed as mean ± SD.

*, $p < 0.001$ versus Normal Control & Marigold extract 200 Perse; #, $p < 0.001$ versus Isoproterenol Control; \$, $p < 0.001$ versus Marigold extract 100; @, $p < 0.001$ versus Marigold extract 150.

Table: 3 Effect of Marigold extract on serum CK-MB (IU /L)

Groups	Serum CK-MB (IU /L)
Normal	70.37±4.414
ME200 Perse	69.17±6.528
Iso	245.5±6.653*
ME100+Iso	192.0±7.266#
ME150+Iso	165.2±15.17\$
ME200+Iso	118.6±3.940@

3.4 Effect of Marigold extract on myocardial oxidative stress

Administration of isoproterenol on last two consecutive days of 30 days experimental protocol (day 29 and day 30) showed marked increase in myocardial TBARS, when compared to normal rats. In addition, the myocardial concentration of reduced GSH was decreased in isoproterenol treated rats as compared to normal rats. Beneficial effect of Marigold extract was obtained and the results were statically significant showing decrease in myocardial TBARS and restoration of reduced GSH. Treatment with Marigold extract (100, 150 & 200 mg/kg/day *p.o.*, 4 weeks) significantly attenuated isoproterenol induced increase in myocardial TBARS

[Figure 4] and decrease in myocardial reduced GSH level [Figure 5].

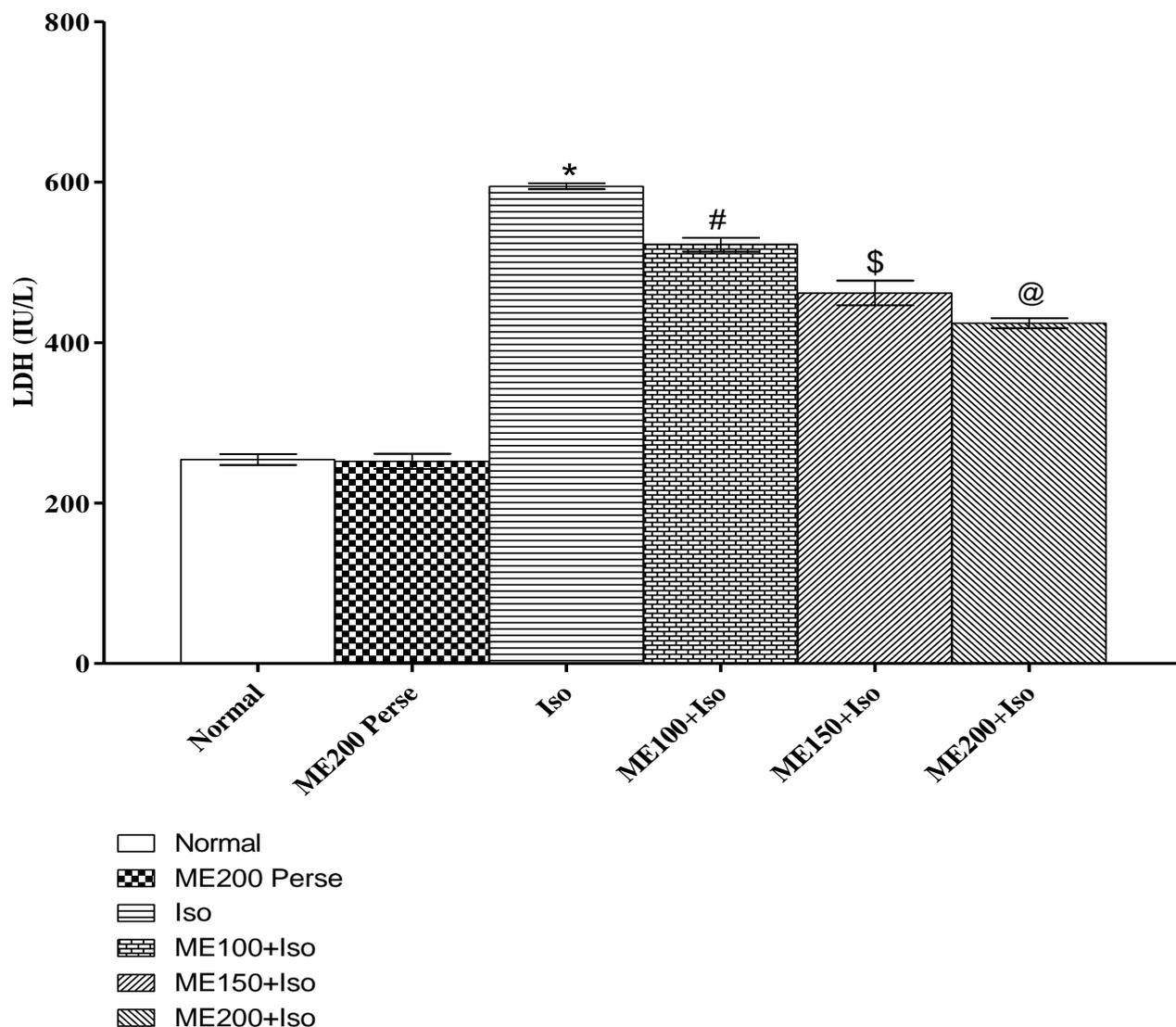


Figure 4: Effect of marigold extract on serum LDH (IU/L)

Values were expressed as mean ± SD.

*, $p < 0.001$ versus Normal Control & Marigold extract 200 Perse; #, $p < 0.001$ versus Isoproterenol Control; \$, $p < 0.001$ versus Marigold extract 100; @, $p < 0.001$ versus Marigold extract 150.

Table 4: Effect of marigold extract on serum LDH (IU/L)

Groups	Serum LDH (IU/L)
Normal	254.3±6.778
ME200 Perse	252.0±9.466
Iso	594.7±3.659*
ME100+Iso	522.2±8.519#
ME150+Iso	461.7±15.37\$
ME200+Iso	424.2±5.960@

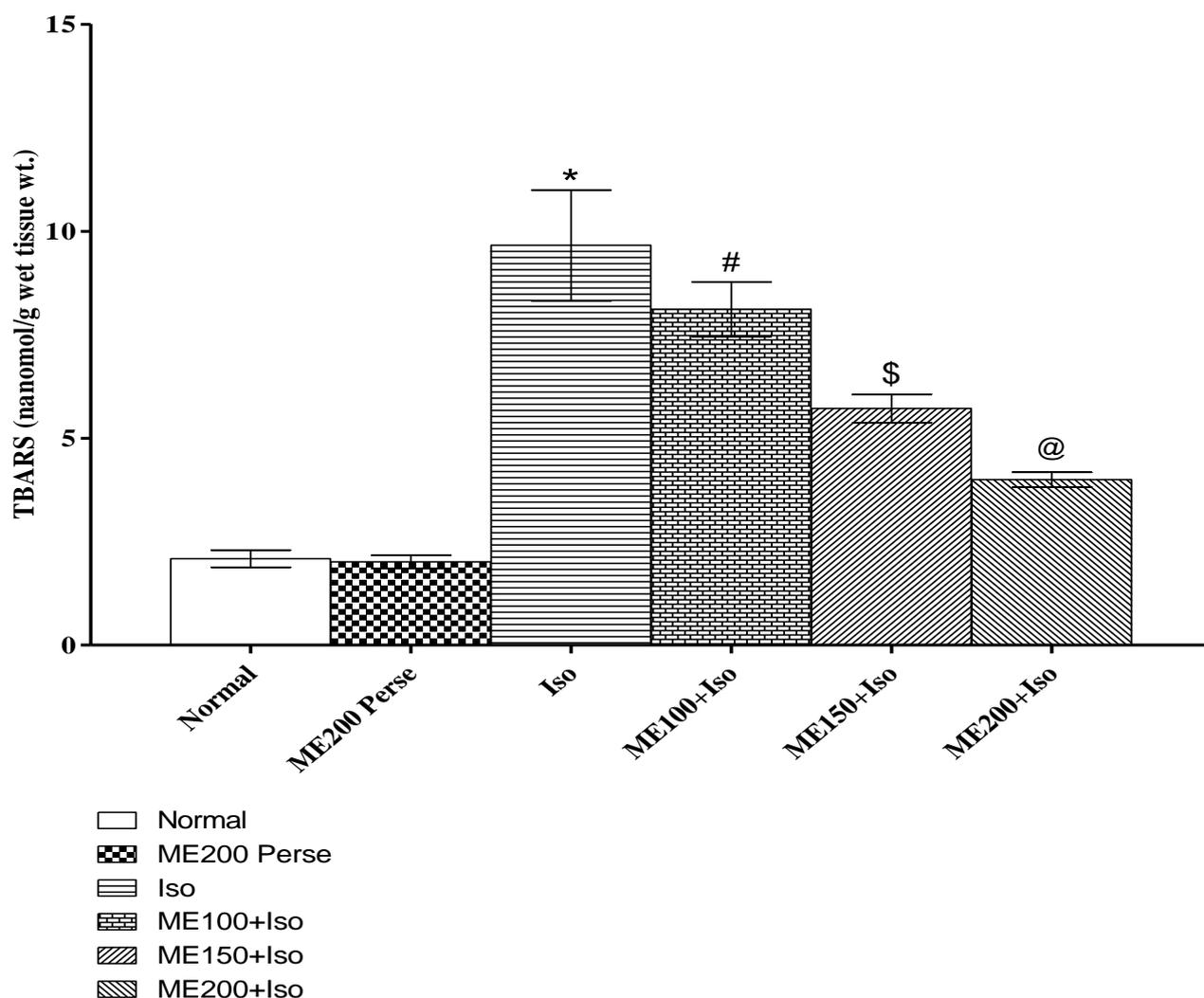


Figure 5: Effect of Marigold extract on myocardial TBARS (nanomolar/ g wet tissue wt)

Values were expressed as mean ± SD.

*, $p < 0.001$ versus Normal Control & Marigold extract 200 Perse; #, $p < 0.001$ versus Isoproterenol Control; \$, $p < 0.001$ versus Marigold extract 100; @, $p < 0.001$ versus Marigold extract 150.

Table: 5 Effect of Marigold extract on myocardial TBARS (nanomolar/ g wet tissue wt)

Groups	Myocardial TBARS (nanomolar/ g wet tissue wt)
Normal	2.087±0.2066
ME200 Perse	2.010±0.1592
Iso	9.660±1.339*
ME100+Iso	8.117±0.6585#
ME150+Iso	5.717±0.3430\$
ME200+Iso	3.997±0.1777@

3.5 Effect of Marigold extract on C-reactive protein (CRP)

Isoproterenol control group significantly showed marked increase in CRP level (3.0 mg/dL), when compared to

normal control (1.0 mg/dL). Increased CRP level in isoproterenol-induced rat was significantly attenuated in pre-treated Marigold extract group (1.5 mg/dL).

3.6 Effect of Marigold extract on histological changes

Histological changes were evaluated by light microscopy, the structural architecture of normal rats fraying without infarction, where isoproterenol treated rats showed severe patches of necrotic tissue, inflammatory cells and edema. In Marigold extract pre-treated group, there were only mild inflammatory cells, edema, and necrotic patches. The treatment effect of Marigold extract restores the structural features of myocardial tissue [Figure 6].

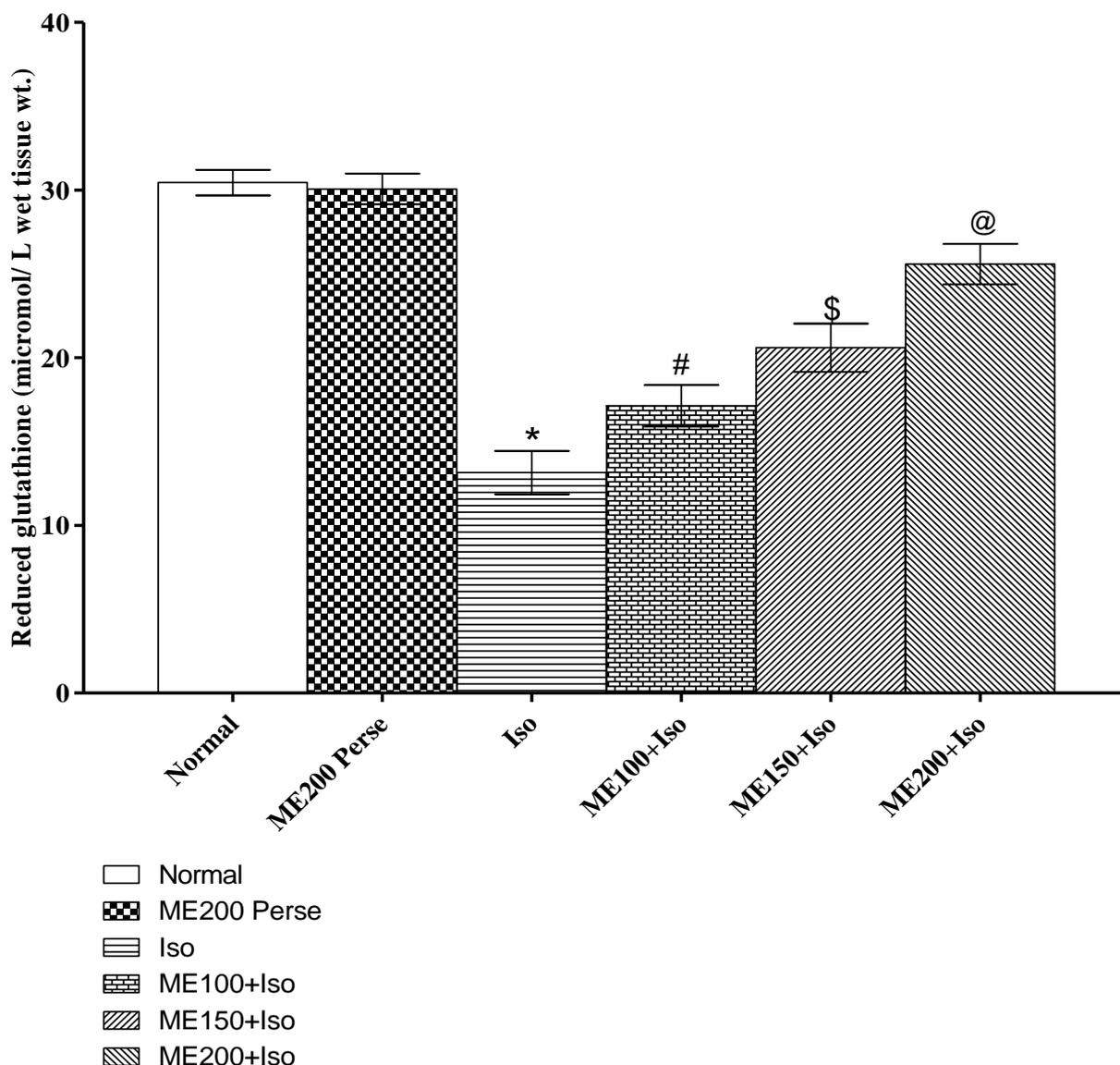


Figure 6: Effect of Marigold extract on myocardial GSH (micromolar/g wet tissue wt)

Values were expressed as mean ± SD.

*, $p < 0.001$ versus Normal Control & Marigold extract 200 Perse; #, $p < 0.001$ versus Isoproterenol Control; \$, $p < 0.001$ versus Marigold extract 100; @, $p < 0.001$ versus Marigold extract 150.

Table: 6 Effect of Marigold extract on myocardial GSH (micromolar/g wet tissue wt)

Groups	Myocardial GSH (micromolar/g wet tissue wt)
Normal	30.45±0.7635
ME200 Perse	30.07±0.9245
Iso	13.15±1.291*
ME100+Iso	17.13±1.236#
ME150+Iso	20.60±1.437\$
ME200+Iso	25.58±1.201@

3.7 Histopathological analysis

3.7.1 Normal Control: The myocardium showed adequate cellularity and normal morphology. Myocytes were healthy and there was no evidence of myocyte necrosis, nuclear pyknosis, vascular proliferation, macrophage activity, scar formation, or muscle hypertrophy.

3.7.2 Isoproterenol Control: The morphological changes occur in myocardium that was strongly suggestive of isoproterenol-induced myocardial injury were seen. Large areas of coagulative necrosis were seen

with marked congestion of subendocardial blood vessels with a large infarct with margin showing inflammation and hyperemia. Nuclear pyknosis and clumping of cytoplasm was evident throughout areas of necrosis.

3.7.3 Marigold extract *per se*: The myocardium showed similar pathology as observed in normal control group.

3.7.4 Marigold extract pre-treated: There was few occurrence of congestion of subendocardial blood vessels with edema of myocardium and mild inflammation. Increased areas of scar formation, vascular proliferation and macrophage activity were indicative of better healing following myocardial infarction throughout treatment with Marigold extract.

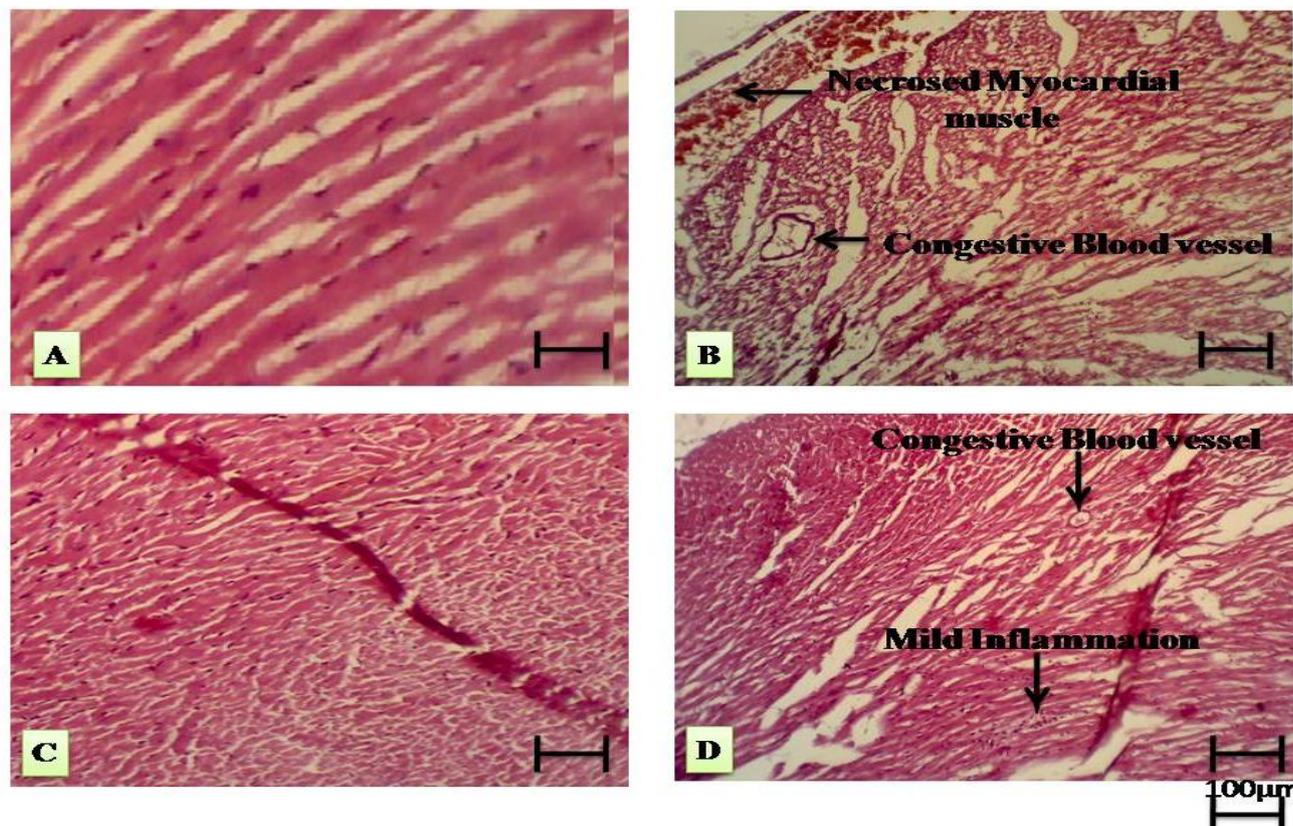


Figure 7: Effect of Marigold Extract on Histopathological estimation

Figure represents:-

A; Normal Control,

B; Isoproterenol Control,

C; Marigold Extract *per se*,

D; Marigold Extract Pre-Treated.

4. Discussion

The present study demonstrates that the Marigold extract efficiently protect the myocardium against isoproterenol-induced myocardial ischemia. Isoproterenol, a synthetic catecholamine and β -adrenergic agonist, has been found to induce myocardial injury in rats [48-49]. The pathological events caused by isoproterenol in experimental animals mimic the injuries that occur in human myocardium. Zhou *et al.*, 2008 have reported that maximum dose of isoproterenol induce subendocardial myocardial ischemia, hypoxia, necrosis, and finally results in fibroblastic hyperplasia with decreased myocardial compliance and inhibition of diastolic and systolic function. These changes, closely resembles local myocardial infarction like pathological as well as structural changes observed in human myocardial

infarction [50-51]. Further, studies suggest that administration of isoproterenol (85 mg/kg) leads to biochemical and morphological alterations in the heart tissue of experimental animals similar to those observed in human myocardial infarction⁴⁸. Thus, isoproterenol induced myocardial infarction is widely used model for evaluating cardioprotective drugs and studying myocardial consequences of ischemic disorders [52]. In accordance with above studies, isoproterenol (85 mg/kg, *s.c.*) was used in present study, for induction of myocardial infarction in experimental animals. Treatment with Marigold extract (100, 150 & 200 mg/kg, *p.o*) produced significant prevention from destruction of normal myocardial architecture induced by isoproterenol (85 mg/kg, *s.c.*) as revealed by evidences of histopathological study⁵². In the normal group adequate cellularity and normal morphology of myocardium is

seen, whereas in isoproterenol-induced group large areas of coagulative necrosis, inflammation and hyperemia were observed indicating myocardial injury. Marigold extract treated group showed few occurrence of congestion of subendocardial blood vessels and mild inflammation, which indicative of better were healing following myocardial infarction. These findings suggest that Marigold extract have protective action against isoproterenol-induced myocardial infarction. Marigold extract have a potent antioxidant potential. In the present study the degree of oxidative stress in the experimental rats was assessed by estimating thiobarbituric acid reactive substances and reduced glutathione. An increase in the levels of thiobarbituric acid reactive substances and decrease in reduced glutathione level in the heart tissue of isoproterenol-induced rats were observed. Marigold extract treatment significantly reverse the levels of markers which cause oxidative stress proving its antioxidant potential. It has been also reported that CK-MB is present in the higher proportion and concentration in the injured myocardium and also referenced as early marker of myocardial infarction. Moreover, LDH levels get elevated, when there is tissue inflammation and necrosis. Numerous researchers have also reported the elevated levels of CK-MB and LDH in isoproterenol-induced myocardial infarction [53-55]. In accordance with the above studies the present results also showed increase in CK-MB and LDH levels in isoproterenol group. Treatment with Marigold extract significantly decreases the levels of CK-MB and LDH, when compared to isoproterenol control animals. Further, isoproterenol injected rats showed a significant rise in serum CRP level indicative marker of inflammation. CRP has been used as a sensitive predictor of acute cardiovascular events, when compared with other widely used biomarkers [56]. The observational studies have reported that serum CRP concentrations are inversely associated with dietary intake of fruits, vegetables and tea, which are rich in poly-phenolic antioxidants [53, 57]. Treatment with Marigold extract significantly reduced the elevated CRP levels suggesting its potent antioxidant and anti-inflammatory activity. Isoproterenol treatment significantly increased the heart weight to body weight ratio showing isoproterenol mediated cardiac hypertrophy in rats [53, 58]. There was a significant difference in the heart weight to body weight ratio between normal control and isoproterenol control group. Marigold extract treatment significantly reduced the heart weight to body weight and attributes its cardioprotective effect in the present results.

It is concluded from the present study that Marigold extract improves necrotic damage, restore antioxidant defense, reduce oxidative stress and inflammation induced by isoproterenol and prove its cardioprotective action against myocardial infarction.

5. Summary

The present study investigated the effect of Marigold extract in isoproterenol-induced myocardial infarction in

rats. The administration of isoproterenol (85mg/kg/day *s.c*) induced myocardial infarction by making biochemical and histological changes. Further, hematoxylin-eosin staining of heart revealed altered myocardial infarct size suggesting that isoproterenol administration impaired myocardium. In addition, isoproterenol-induced myocardial infarction is the result of elevated HW/BW ratio, and serum CK-MB and LDH levels. Isoproterenol administration also produced oxidative stress by increasing TBARS and decreasing the level of reduced glutathione. In addition, histopathological studies revealed the development of coagulative necrosis, congestion of subendocardial blood vessels, large infarct with margin showing inflammation and hyperemia in isoproterenol group. Intriguingly, administration of Marigold extracts (100, 150 & 200 mg/kg/day, *p.o.*) through antioxidant and anti-inflammatory mechanism attenuated isoproterenol-induced myocardial infarction. Marigold extract reduce myocardial infarct size, HW/BW ratio, and serum CK-MB and LDH levels. Boswellic acids ablate oxidative stress by reducing TBARS and increasing GSH levels. Further, Marigold extract treatment indicated better healing of myocardium by making pathological changes such as low congestion of blood vessels, less edema and inflammation. Hence, it is summarised that Marigold extract has myocardial protective potential.

6. Conclusion

Marigold extract significantly prevent the damages induced by isoproterenol on histopathological and biochemical changes in rat model of myocardial infarction. Marigold extract ameliorate the cardio-toxic effect of isoproterenol in rat heart. The present study provides experimental evidences that Marigold extract augmented the myocardial antioxidant enzymes level, preserved histoarchitecture and improved cardiac performance by changing marker level following isoproterenol administration. These findings suggest the beneficial cardioprotective effects of Marigold extract on rat heart against experimental myocardial infarction and it should further be explored against other cardiac complications.

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