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Research Article

HYDROALCOHOLIC EXTRACT OF *MATRICARIA CHAMOMILLA* LINN. AMELIORATES LIPIDS, LIPOPROTEINS, AND PARAOXONASE IN ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTION IN WISTAR RATS

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ABSTRACT

Objective: The objective of this study was to evaluate the effect of the hydroalcoholic extract of *Matricaria Chamomilla* Linn. (CHAE) on lipids, lipoproteins, and antioxidants activity in isoproterenol (ISO)-induced myocardial infarcted rats.

Methods: ISO (85 mg/kg, s.c.)-induced myocardial infarction for 2 consecutive days at an interval of 24 h. Rats were pretreated with CHAE (100 and 200 mg/kg, oral) for a period of 20 days and ISO was injected on 21 and 22 days at 24 h intervals and after 24 h, blood was collected through retro-orbital plexus for the estimation of lipids, lipoproteins, and antioxidants assay.

Results: In the present study, ISO caused a significant increase in the concentration of total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), very LDL-C, and lipid peroxidation whereas a significant decrease in the concentration of high-density lipoprotein-C. ISO administration also significantly decreased the activities of paraoxonase (PON) enzyme. Oral pre-treatment of CHAE at doses of 100 and 200 mg/kg body weight (bw) for 20 days challenged with a concurrent injection of ISO (85 mg/kg bw) on 21 and 22 days significantly attenuated these alterations and restored the levels of lipids and lipoproteins. In addition, CHAE significantly elevated the serum antioxidants enzyme PON and catalase (CAT).

Conclusion: The report revealed that pre-treatment with CHAE ameliorated lipid and lipoprotein and increased the antioxidant PON and CAT activity and decreased LPO level in ISO-treated male albino Wistar rats.

Keywords: *Matricaria chamomilla* Linn., Myocardial infraction, Isoproterenol, Paraoxonase, Lipid peroxidation, Catalase.

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INTRODUCTION

Myocardial infarction (MI) or heart attack is the leading cause of death for both men and women all over the world. MI occurs when blood supply is insufficient to the myocardium, death of myocardial muscle occurs, a condition known as ischemia. Prolonged ischemia of the myocardium leads to necrosis of myocardium cell, which is referred to as MI [1]. MI is the acute condition that occurs due to imbalance between coronary blood supply and demand [2]. The risk factors of cardiovascular diseases (CVDs) are high levels of total cholesterol (TC), triglycerides (TGs), low-density lipoprotein cholesterol (LDL-C), and apolipoproteins A-I and low levels of high-density lipoprotein cholesterol (HDL-C). Cardiovascular diseases are directly or indirectly related to oxidative stress that causes molecular and cellular damage. Numerous medicinal plants have been evaluated for cardiovascular diseases in India and various parts of the world using cardiotoxic models [3].

Isoproterenol (ISO) is a β-adrenergic agonist that causes severe stress on the myocardium in heart muscle, leading to the necrosis of myocardium [4]. ISO causes an increase in the levels of serum and myocardial lipids and also increases the level of LDL-C in the blood that causes the blockage of arteries favoring CVD [5]. ISO stimulates lipid peroxidation (LPO) by inducing free radical production, which may be a causative factor of irreversible damage to the myocardial membranes [6]. Oxidative stress and oxygen-free radicals together lead to the generation of atherosclerotic lesions by the formation of oxidized LDL from LDL, which is the underlying cause of MI [7].

Matricaria chamomilla Linn. (CHAE) (synonym: Matricaria recutita) commonly known as chamomile or German chamomile belongs to the family Asteraceae [8,9]. Chamomile as a whole plant has been used traditionally in different forms for the treatment of multiple medical complaints such as common cold, bronchitis, gastrointestinal spasms, epilepsy, hypertension, neuralgia, toothache, dysmenorrhea, eczema, impetigo, indigestion, colic, and diarrhea [10-12]. Its flowers are also used as carminative and antipyretic, while its oil has been used in rheumatism, flatulence, and colic [13,14].

Phytochemical studies revealed the presence of alpha-bisabolol, cis-spiroethers sesquiterpenes (anthecotulid), cadinene, farnesene, furfural, spathulenol, and proazulene (Matricaria and matricin) as plant constituents. The presence of tannin in chamomile has also been detected <1% [15,16].

Pharmacological investigations showed that *Matricaria chamomilla* possesses anti-inflammatory [17], antispasmodic [18], antibacterial [19], digestive [20], antioxidant, and antidiabetic [21,22] activities.

Paraoxonase (PON) is synthesized in the liver and is bound to plasma HDL-C [23]. PON has treated as a component of the plasma antioxidant system. This enzyme prevents the oxidation of LDL-C and acts as a protective enzyme against atherogenesis. PON protects against atherosclerosis by reducing HDL-C peroxidation and protect plasma membranes from free radical injury [24]. Human serum PON has been shown to hydrolyze oxidized lipids and thus to decrease oxidative stress on serum lipoproteins [25]. Low activity of serum PON has been reported in diseases associated with hypercholesterolemia, atherosclerosis and increased prevalence of CVD [26].

In the present study, we assessed the ameliorative effect of CHAE on lipids, lipoproteins, catalase (CAT) activity, LPO, and PON in ISO-administered myocardial infarcted rats.

MATERIALS AND METHODS

Collection and authentication of plant material

Matricaria chamomilla collected from local areas of Coimbatore district, Tamil Nadu, India. The collected flower parts of *Matricaria chamomilla* were authenticated by Dr. S Rajan, Field Botanist, Survey of Medicinal Plants and Collection Unit, Central Council for Research in Homoeopathy, Department of AYUSH, the Nilgiris, Tamil Nadu.

Extraction procedure

The dried plant material weighing 500 g used for extraction and soaked in 70% ethanol for 3 days, with occasional shaking. The soaked material was filtered through a muslin cloth and then through a Whatman qualitative Grade 1 filter paper. This procedure repeated 3 times and the combined filtrate was evaporated using a rotary evaporator to get the final aqueous-ethanolic extract of *Matricaria chamomilla*, yielding 25% w/w [27].

Experimental animals

Healthy, adult Male Wistar albino rats (180–250 g) obtained from the central animal house facility, JSS College of Pharmacy, Udhagamandalam, Tamil Nadu. The animals were exposed in a well-ventilated room and were exposed to 12 h day and night cycle with a temperature between 22±3ºC. The animals were housed in large spacious, hygienic polypropylene cages during the course of the experimental period and fed with water *ad libitum*. All the experiments were performed after prior approval from the Institutional Animal Ethics Committee (JSSCP/IAEC/M.PHARM/PH.CPLOGY/04/2013-14).

Induction of experimental MI

ISO was dissolved in normal saline and was subcutaneously injected into rats (85 mg/kg) at 24 h intervals for 2 days to induce experimental MI [28].

Experimental design

After a 1-week acclimation period, animals randomly divided into four groups (with six rats in each group) and treated as follows:

Group I – Untreated control rats
Group II – Pre-treatment of rats with ISO (85 mg/kg bw)
Group III – Pre-treatment of rats with CHAE 100 mg/kg (oral) + ISO (85 mg/kg)
Group IV – Pre-treatment of rats with CHAE 200 mg/kg (oral) + ISO (85 mg/kg)

CHAE was solubilized in distilled water. CHAE orally pre-treated to the rats for 20 days using an intragastric tube. ISO was solubilized in distilled water and administered to the rats by subcutaneous injection for the past 2 consecutive days. Animals were sacrificed by cervical decapitation. Blood was collected from heart puncture to separate serum and plasma. Tissue samples were separated and refrigerated at 80 LC.

Biochemical measurements

The levels of serum TC and TGs were estimated using diagnostics kits from Erba Diagnostics (Mumbai) as described by Allian *et al*. [29]. HDL-C was estimated by utilizing the kit of Siemens Diagnostics Ltd., India, as described by Richmond [30]. Very LDL-C (VLDL-C) was calculated as VLDL-C = TG/5, whereas LDL-C was calculated as LDL-C = TC − (HDL-C + VL DL-C). Malondialdehyde (MDA) level was measured to estimate LPO by the method of Okhawa *et al*. [31]. PON activity was assayed by the method of Gan *et al*. [32].

Statistical analysis

Results analyzed statistically by performing one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests to assess differences between the groups. Data considered statistically significant at $p<0.05$. All the statistics were performed using GraphPad Prism, version 6.

RESULTS AND DISCUSSION

Effect of CHAE on lipids and lipoproteins

Fig. 2, and Tables 1 and 2 depict the effect of CHAE on serum lipids and lipoproteins (TC, TG, HDL-C, VLDL-C, and LDL-C) in normal and ISO-administered groups. Rats injected with ISO exhibited a significant ($p<0.05$) increase in the levels of serum TC, TG, LDL-C, and VLDL-C, except HDL-C which showed a significant ($p<0.05$) decrease

Table 1: Effect of CHAE on cholesterol and triglycerides levels

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Control	55.80±1.881	56.92±2.150
ISO 85 mg/kg	86.17±1.748	185.2±2.151
CHAE 100 mg/kg	65.62±1.957	139.2±3.195
CHAE 200 mg/kg	63.73±2.035	79.43±2.997

Values represent mean±SEM; n=6; #p<0.05 versus control. *p<0.05 versus ISO one-way ANOVA followed by Tukey's multiple comparisons test

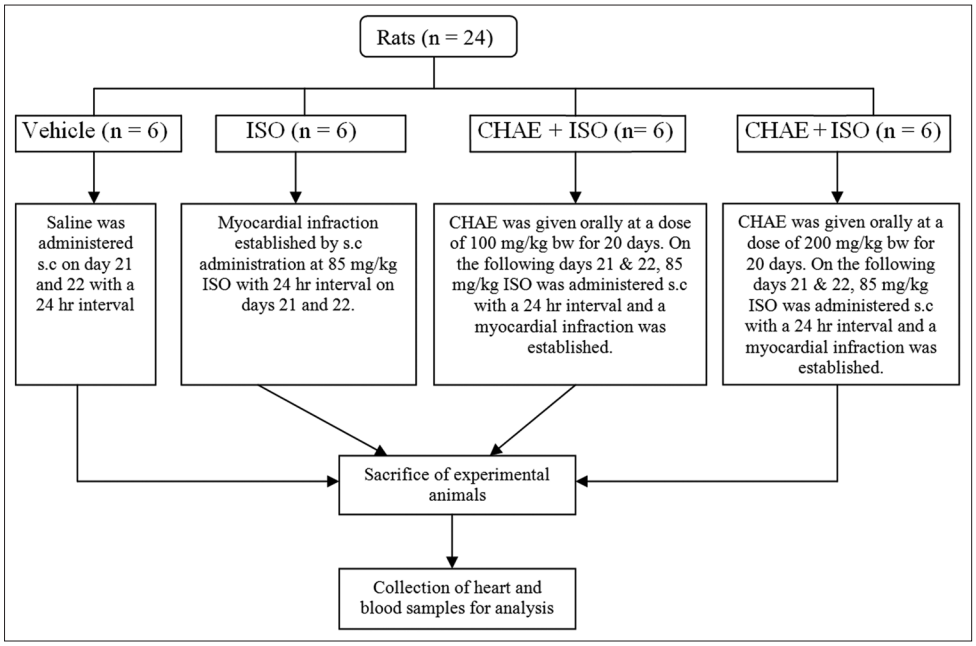

Fig. 1: Schematic representation of the experimental design of the study

Table 2: Effect of CHAE on LDL-C, VLDL-C, and HDL-C levels

Groups	LDL-C (mg/dl)	VLDL-C (mg/dl)	HDL-C (mg/dl)
Control	10.17±0.587	11.386±0.43	34.24±2.038
ISO 85 mg/kg	36.16±0.03	370.05±0.43	12.96±1.361
CHAE 100 mg/kg	20.66±0.21	27.84±0.64	17.12±1.525
CHAE 200 mg/kg	20.78±0.656	15.886±0.6	27.06±2.091

Values represent mean±SEM; n=6; #p<0.05 versus control. *p<0.05 versus ISO one-way ANOVA followed by Tukey's multiple comparison tests. LDL-C: Low-density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol

when compared to control rats. Pre-treatment with CHAE (100 and 200 mg/kg bw) dose dependently decreased serum TC, TG, LDL-C, and VLDL-C levels significantly (p<0.05) and increased serum HDL-C levels significantly (p<0.05) when compared to ISO administered rats. Treatment of CHAE at 200 mg/kg bw in ISO-administered rats decreased the serum TC and LDL-C almost to near normal but not significantly (p<0.05) when compared to the control rats.

Lipids play a significant role in CVD. Hyperlipidemia and hypercholesterolemia are vital risk factors in the progress of MI. ISO-administered MI is allied with elevated levels of circulatory lipids. In this study, ISO-administered rats showed significantly increased levels of TC, TGs in serum. The increased level of cholesterol in ISO-treated rats is due to an increased level of LDL-C taken from the blood circulation. Increased level of TGs is the prime risk factor of MI that is associated with cardiovascular disturbances. CHAE treatment ameliorated lipids and lipoproteins with a significant increase in HDL-C levels and a decrease in TC, TGs, LDL-C, and VLDL-C levels, which may be due to the hypocholesterolemia and hypolipidemic activities of CHAE [33].

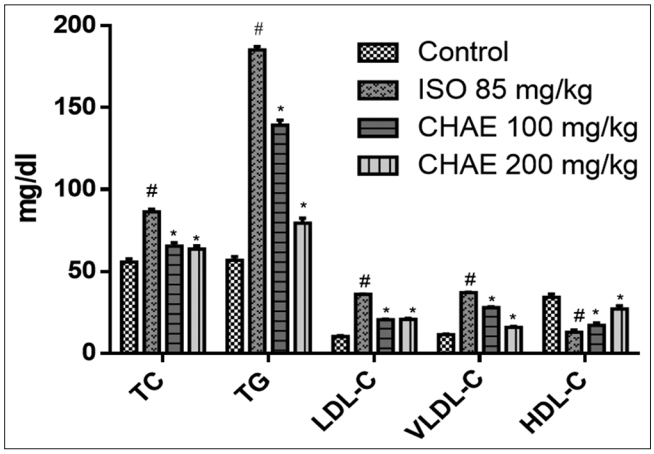

Fig. 2: Effect of CHAE on lipids and lipoproteins in the serum of untreated and isoproterenol-administered rats. The bars represent mean±SEM; n=6; #p<0.05 versus control. *p<0.05 versus ISO one-way ANOVA followed by Tukey's multiple comparison tests

Effect of CHAE on LPO

Fig. 3 represented the effect of CHAE on serum LPO marker MDA in control and ISO-administered rat groups. Rats administered with ISO showed significant (p<0.05) increase in the level of MDA in serum when compared to control rats. CHAE (100 and 200 mg/kg bw) dose decreased the level of MDA significantly (p<0.05) in serum as compared to ISO alone administered rats.

LPO plays a crucial role in the toxicity of heart and liver. LPO is an important pathogenic event in myocardial necrosis and accumulation of lipid hydroperoxides which reflects damage to the cardiac constituents [34]. The free radicals mediate membrane damage that may increase the level of lipid peroxides in ISO-administered MI. The present study revealed a significant increase in the level of MDA in the serum of ISO-administered rats. CHAE pre-treatment to ISO-treated rats minimized MDA content, clearly exhibiting that CHAE inhibited the LPO. The inhibition of LPO may be due to the antioxidative activities of CHAE [35].

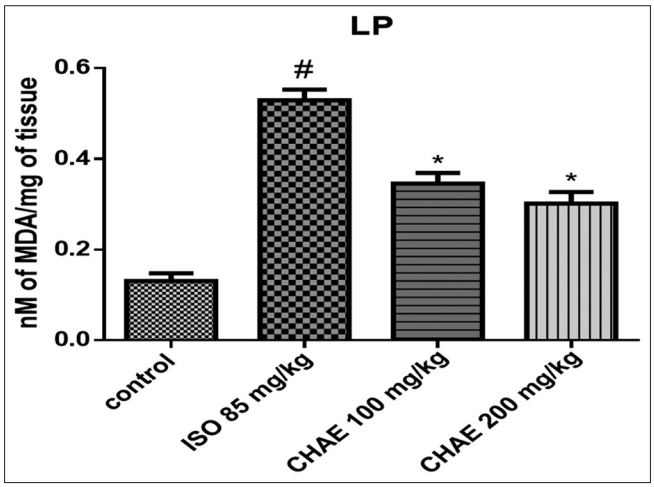

Fig. 3: Effect of CHAE on lipid peroxidation in the serum of untreated and isoproterenol-administered rats. The bars represent mean±SEM; n=6; #p<0.05 versus control. *p<0.05 versus ISO one-way ANOVA followed by Tukey's multiple comparison tests

Effect of CHAE on CAT

Fig. 4 shows the effect of CHAE on the activity of CAT in the serum of control and ISO-administered rats. Rats administered with ISO significantly (p<0.05) decreased the activities of CAT in the heart as compared to normal control group. However, treatment with CHAE (100 and 200 mg/kg) significantly prevented the reduction in the activities of antioxidant CAT as compared to ISO alone administered rats.

CAT is a common enzyme, found in nearly all living organisms. It catalyzes hydrogen peroxide into water and oxygen and protects organisms from free radicals. CAT has one of the highest turnover numbers of all enzymes; one CAT molecule can convert millions of hydrogen peroxide molecules to water and oxygen each second. CHAE pre-treatment to ISO-treated rats increases the CAT level in serum, clearly exhibiting that CHAE enhanced the CAT activity.

Effect of CHAE on PON enzyme

Fig. 5 depicts the effect of CHAE on the activity of serum PON in control and experimental rats. Significant (p<0.05) decrease in the levels of serum PON observed in rats administered with ISO as compared to control rats. CHAE (100 and 200 mg/kg bw) pre-treatment for a period of 20 days increased the activity of serum PON significantly (p<0.05) when compared to ISO alone administered rats.

CHAE treatment has been enhanced PON activity in ISO-treated groups. CHAE may directly elevate serum PON activity because both *in vitro* and *in vivo* introduction of antioxidant molecules were shown to preserve PON activity. CHAE may also favorable to PON by its anti-hypercholesterolemia and antioxidant activities.

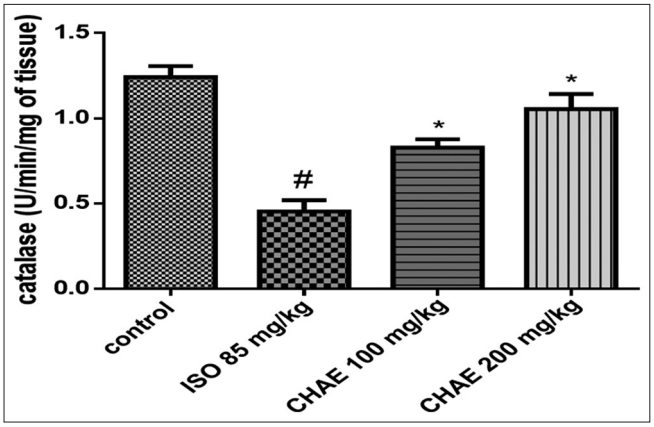

Figure 4: Effect of CHAE on CAT level in the serum of untreated and isoproterenol-administered rats. The bars represent mean±SEM; n=6; #p<0.05 versus control. *p<0.05 versus ISO one-way ANOVA followed by Tukey's multiple comparison tests

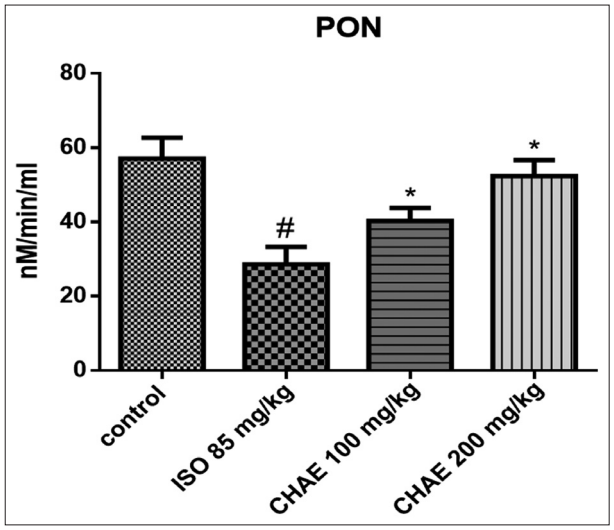

Fig. 5: Effect of CHAE on paraoxonase in the serum of untreated and isoproterenol-administered rats. The bars represent mean±SEM; n=6; #p<0.05 versus control. *p<0.05 versus ISO one-way ANOVA followed by Tukey's multiple comparison tests

CONCLUSION

The conclusion of our study is pre-treatment with CHAE exhibits ameliorative effects in ISO injected myocardial infraction rats by modulating lipids, lipoproteins, and antioxidants enzymes such as LPO, CAT, and PON at the dose of 100 mg/kg and 200 mg/kg bw. The possible mechanism of CHAE cardioprotection is due to its anti-hypercholesterolemic, antihyperlipidemic, and antioxidant actions.

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AUTHORS' CONTRIBUTION

VR designed an entire study project, contributed to experiment finalization and implementation, manuscript editing, and finalization. GA designed and performed experiments.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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