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# EFFECT OF OG-1 CARDIOACTIVE PRINCIPLE FROM CHLOROFORM EXTRACT OF *Ocimum gratissimum (Linn.)* LEAVES ON ISCHAEMIA AND REPERFUSION-INDUCED MYOCARDIAL INJURY

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## ABSTRACT

***Objective**:The present study has been designed to investigate efficacy Of OG-1 cardioactive principle from chloroform extract of Ocimum gratissimum (Linn.) Leaves on ischaemia and reperfusion-Induced myocardial injury.*

***Methods:** Various extracts of O. gratissimum (Linn.) leaves were prepared viz. Pet.ether, chloroform, acetone and methanol. Among all the extracts chloroform extract reduce myocardial injury. The chloroform extract further purified using column chromatographic technique that results four major fractions viz F1, F2, F3 and F4. Among these fractions F4 was found to be hot fraction. OG1 (active principle) was isolated from the F4 using column chromatography technique and evaluated for cardioprotective effect.*

***Results**: The present study demonstrated that Chloroform extract of O.gratissimum (Linn.) leaves and further fraction F4 of chloroform extract significantly prevented myocardial infarct size as compared with that of standard ramipril. OG1 (active principle) was isolated from fraction F4 of chloroform extract showing cardioprotective effect at dose level of 50mg/kg body weight as compared with that of standard ramipril (1mg/kg body weight).*

***Conclusion:**These results suggest that OG1 is active cardioprotective principle of O. gratissimum (Linn.) chloroform leaves extract.*

## *KEY WORDS*

*Ischaemia, Reperfusion, Ocimum gratissimum, Ramipril, Chromatography.*

## 1. INTRODUCTION

As per *Ayurveda*, Indian medicinal plants are rich sources of substances that have several therapeutic properties including cardio protection. About 75-85% of the world's population, plant derived products is still play an essential role in primary health care, mainly in the developing countries. The use of herbal preparation is increasing in the treatment of cardiovascular disease because of various possible mechanism involved in the cardio protection. Therefore, herbal extracts that are traditionally used, evaluated against in limiting the deleterious effects of ischaemia and reperfusion- induced myocardial injury. Furthermore, the results are statistically analysed and validated for prophylactic approaches and as an adjunct to standard treatment of ischaemia and reperfusion-induced myocardial injury [4] [5]. The word ischaemia is

derived from "ischo" meaning to hold back and "hamia" meaning blood. Thus, myocardial ischaemia means decrease in coronary blood flow which is unable to meet the oxygen demand of myocardium. The persistent myocardial ischaemia leads to death of cardiomyocytes leading to myocardial infarction [1]. Reperfusion is prerequisite to salvage ischaemic myocardium. The restoration of blood flow after transient ischaemia has been noted to produce myocardial hibernation, myocardial stunning, reperfusion induced arrhythmia, endothelial dysfunction leading to no-reflow state and extension of infarct size. Reperfusion after exacerbate myocyte cell death and increase in infarct size, a phenomenon termed as 'reperfusion injury' [2] [3].

Genus *ocimum* is widespread over Asia, Africa and Central and southern America. Tulsi was probably first put to cultivation in India. Among the plants known for medicinal value, the plants of genus *ocimum* belonging to family *lamiaceae* are very important for their therapeutic potentials. *Ocimum sanctum* L. (tulsi), *ocimum gratissimum* (ram tulsi), *ocimum canum* (dulal tulsi), *ocimum basilicum* (ban tulsi), *ocimum kilimandscharicum, ocimum ammericanum, ocimum camphora* and *ocimum micranthum* are grown in different parts of the world and are known to have medicinal properties[6] [7].

*Ocimum gratissimum* found throughout India as a common weed in road sides and in waste places.The most important aroma components are euginol, 1, 8-cineole, α-pinene, β-pinene, (Z)-ocimene, α-terpineole, (E)-β-caryophyllne, α-humulene, γ-muurolene, β-selinene, α-selinene. The composition of the essential oil is known to depend on climate, soil, genetic strain and season [8]. In order to establish the therapeutic uses of genus ocimum in modern medicine, in last few decades several Indian scientists and researchers have studied the pharmacological effects of essential oils and extract of tulsi on immune system, central nervous system, gastric system, reproductary system, blood biochemistry and described the therapeutic significance in management of various ailments[9] [10].

## 2. MATERIALS AND METHODS

### 2.1 Drugs and chemicals:

Ramipril is taken as a gift sample from USV Baddi, Himanchal pradesh, Himanchal, India. All the reagents used in this study were of analytical grade and were always freshly prepared before use.

### 2.2 Plant material:

Leaves of *Ocimum gratissimum* (Linn.) was collected from Indian Institute of Integrative Medicine, Jammu (Formerly Regional Research Laoratiory), CSIR, India. The reference number is [RRL (J) OG-14].

### 2.3 Animals:

Adult Wister rats of either sex, weighing 250 to 300g were used in the study. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (Reg.no.273/CPCSEA). Animals were obtained from IVRI Bareilly, India and were maintained under standard laboratory conditions in the departmental animal house of SBSPGI, Dehradun, Uttarakhand, India.

### 2.4 Preparation of extracts:

The fresh leaves of *O. gratissimum* (Linn.) were dried in shade and room temperature for 2days followed by drying [40-50$^{o}$C] for 3-4hrs and powdered to obtained coarse powder. 1.5kg of powder of *O. gratissimum* (Linn.) leaves were extracted with pet ether, chloroform, acetone and methanol to get four extracts. The solvent was removed by evaporation under reduced pressure to obtain a semisolid mass. The resultant extracts were kept in a desicator

followed by weighing to give percentage yield of each extract.

**2.5 Isolation and purification of principle constituent from active fraction:**

The chloroform extract showing good cardioprotective effect was subjected to column chromatography using silica gel mesh size 200-400µ and Pet ether: ethyl acetate as mobile phase in different ratio leadind to the isolation of four fractions, F1, F2, F3 and F4. The cardioprotective activity was evaluated for all four fractions in which fraction F4 of chloroform extract was found significantly effective. Further purification of fraction F4 as above said process resulted in isolation of OG1, OG2 and OG3. Among all these the compound OG1 further evaluated for cardioprotective activity and it was more significant than other compounds.

**2.6 Acute toxicity study:**

Albino mice of 10 animals per group and weighing 20-25g were administered graded dose (100-2000 mg/kg body weight, orally) of the chloroform extract of O.gratissimum (Linn.). After administration of extract mice were observed for toxic effects after 48hr of treatment. The toxicological effects were observed in terms of mortality expressed as $LD_{50}$. The number of animals dying during the period was noted. The $LD_{50}$ of the extract was determined by Litchfield and Wilcoxon, 1949 method [11]. No mortality was observed therefore the extract is safe to use even at the doses of 2000mg/kg of body weight orally.

**2.7 Isolated rat heart preparation:**

Rats were heparinised (500 IU/L, i.p.) and sacrificed after 20min by cervical dislocation. The heart was rapidly excised and immediately mounted on Langendorff's apparatus [12]. The temperature was maintained at 37°C by circulating hot water. The preparation was perfused with krebs Henseleit (K-H) buffer (NaCl 118 Mm; KCl 4.7 Mm; $CaCl_2$ 2.5 Mm; $MgSO_4.7H_2O$ 1.2 mM; $KH_2PO_4$ 1.2mM; $C_6H_{12}O_6$ 11 mM), pH 7.4 and bubbled with 95% $O_2$ and 5% $CO_2$. The coronary flow rate was maintained 6-9 ml/min and perfusion pressure was kept constant at 70 mmHg. Global ischemia was produced for 30min by completely closing the inflow of physiological solution and followed by 120min of reperfusion. The coronary effluent was collected before ischaemia, immediately, 5min, 30min and 120min after reperfusion for estimation of LDH and CK-MB.

**2.8 Assessment of myocardial injury:**

The myocardial infarct size was measured using the triphenyltetrazolium chloride (TTC) staining method. The level of LDH and CK-MB (Siemens Medical Solution Diagnostic Ltd.,Baroda, India)in coronary effluents was estimated using commercially available kits. Values of LDH and CK-MB were expressed in international units per litre (IU/L).

**2.9 Assessment of myocardial infarct size:**

Heart was removed from the Langendorff,s apparatus. Both the auricals and the root of aorta were excised, and ventricles were kept overnight at temperature of -4°C. Frozen ventricles were sliced into uniform sections of 1-2mm thickness. The slices were incubated in 1% w/v TTC solution in 0.2M Tris-chloride buffer,pH 7.8 for 20min at 37°C. The normal myocardium was stained brick red while the infracted portion remained unstained. Infarct size was measured by macroscopic volume method [13].

**3. Experimental protocol:**

In all groups, isolated rat heart was perfused with K-H solution and allowed to stabilize for 10min.

**Group 1: (Sham control; n=5)** After stabilization isolated rat heart was perfused continuously with K-H buffer for 160min. without subjecting it to global ischaemia.

**Group 2: (Vehicle control; n=5)** Rats were administered 1% Tween 80 orally for 7days;

thereafter, on the 7th day ,isolated rat heart after stabilization, was subjected to 30min of global ischaemia followed by reperfusion for 120min.

**Group 3: (Standard; n=5)** Ramipril (1mg/kg) was dissolved in 1% Tween 80 and administered orally once daily to rats for 7days; thereafter, on the 7th day ,isolated rat heart after stabilization, was subjected to 30min. of global ischaemia followed by reperfusion for 120min.

**Group 4: (Chloroform extract; n=5)** Chloroform extract (100mg/kg) was dissolved in 1% Tween 80 and administered orally once daily to rats for 7days; thereafter, on the 7th day ,isolated rat heart after stabilization, was subjected to 30min of global ischaemia followed by reperfusion for 120min.

**Group 5: (Fraction 4 of Chloroform extract; n=5)** Fraction 4 of chloroform extract (100mg/kg) was dissolved in 1% Tween 80 and administered orally once daily to rats for 7days; thereafter, on the 7th day, isolated rat heart after stabilization, was subjected to 30min of global ischaemia followed by reperfusion for 120min.

**Group 6: (Active principle OG1of chloroform extract; n=5)** Active principle OG1 of chloroform extract (50mg/kg) was dissolved in 1% Tween 80 and administered orally once daily to rats for 7days; thereafter,on the 7th day ,isolated rat heart after stabilization, was subjected to 30min of global ischaemia followed by reperfusion for 120min.

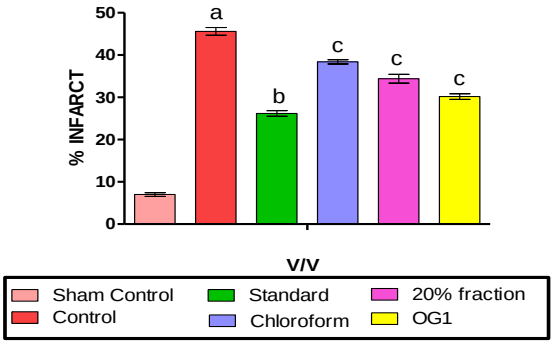


**Figure 1: Assessment of Myocardial Infarct Size**. Infarct size was measured by volume method. Values are expressed as mean ±SEM .a= P<0.05 vs. Sham control; b= P<0.05 vs. Control; c= P<0.05 vs. Standard. ANOVA followed by Tukey's multiple comparison test

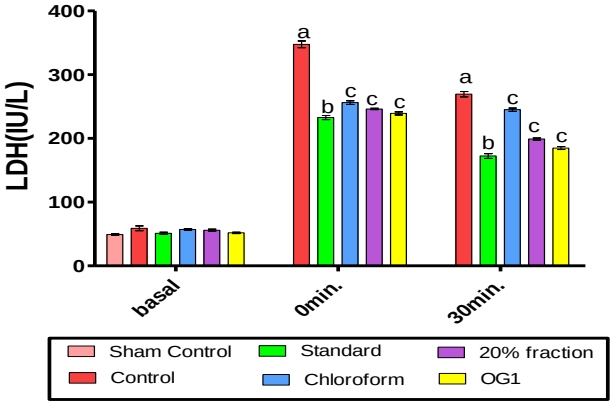


**Figure 2: Effect of Ischaemia and Reperfusion on LDH release.** LDH was estimated in coronary effluent after stabilization (Basal), Immediately (Imm'Rep.) and 30min. after reperfusion (30' Rep.). Values are expressed as mean ±SEM .a= P<0.05 vs. Sham control; b= P<0.05 vs. Control; c= P<0.05 vs. Standard. ANOVA followed by Tukey's multiple comparison test.

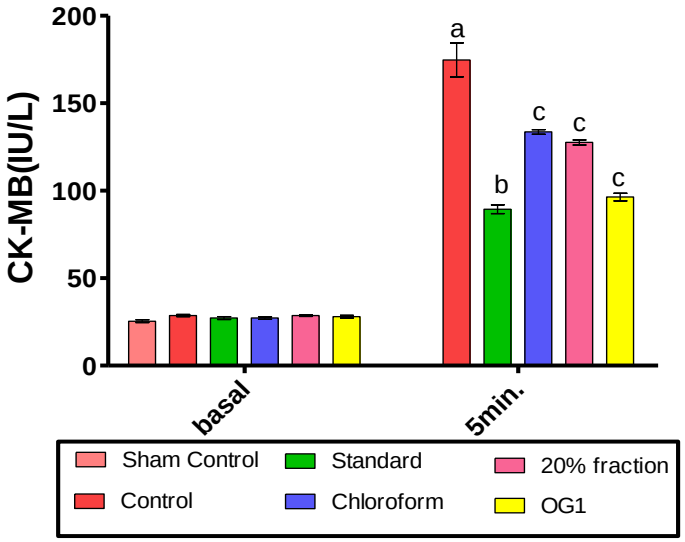


**Figure 3: Effect of Ischaemia and Reperfusion *on* CK-MB release**. CK-MB was estimated in coronary effluent after stabilization (Basal) and 5min. after reperfusion (5' Rep.). Values are expressed as mean ±SEM .a= P<0.05 vs. Sham control; b= P<0.05 vs. Control; c= P<0.05 vs. Standard. ANOVA followed by Tukey's multiple comparison tests.

**4. Statistical Analysis:**

All values for enzymatic data (LDH and CK-MB) and infarct size were expressed as mean ±SEM. Statistical analysis was performed using Graph Pad Prism Software.The values were statistically analysed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Value of P<0.05 was considered to be statistically significant.

## 5. RESULTS

### 5.1 Effect on Myocardial Infarct Size:

Various extracts of *O.gratissimum* (Linn.) leaves viz. petroleum ether, chloroform, acetone and methanol were evaluated on ischaemia and reperfusion induced increase in myocardial infarct size,respectively. Among all the extracts chloroform extract of *O.gratissimum* (Linn.) leaves found to be active. Further purification of active extract was carried out using column chromatography which resulted isolation of four fraction viz. F1, F2, F3 and F4. Which were again evaluated for above said effect and among all the fractions fraction F4 significantly attenuated ischaemia and reperfusion induced increase in myocardial infarct size. Further purification of fraction F4 resulted in isolation of OG1, OG2 and OG3. OG1 further evaluated for cardioprotective activity and it was more significant than other compounds. However, treatment with standard (ramipril,1mg/kg) was significantly more effective to reduce myocardial infarct size as compared to compound OG1, measured by macroscopic volume method (Figure 1).

### 5.2 Effect on Ischaemia and Reperfusion Induced release of LDH

Various extracts of *O.gratissimum* (Linn.) leaves viz. Petroleum ether, chloroform, acetone and methanol were evaluated on ischaemia and reperfusion induced increase in release of LDH in coronary effluent measured immediately and 30 min. after reperfusion, respectively. Similarily, among all the extracts chloroform extract of *O.gratissimum* (Linn.) leaves and the isolated

fraction F4 from chloroform extract significantly reduced release of LDH in coronary effluent. Further the active principle OG1 isolated from fraction F4 of chloroform extract significantly attenuated release of LDH in coronary effluent measured immediately and 30 min. after reperfusion. Moreover, treatment with standard (ramipril,1mg/kg) markedly reduced release of LDH in coronary effluent as compared to active compound OG1, measured immediately and 30 min. after reperfusion (Figure 2).

**5.3 Effect on Ischaemia and Reperfusion Induced release of CK-MB**

Various extracts of *O.gratissimum* (Linn.) leaves viz. Petroleum ether, chloroform,acetone and methanol were evaluated on ischaemia and reperfusion induced increase in release of CK-MB measured in coronary effluent collected after 5min. of reperfusion, respectively. Similarily, among all the extracts chloroform extract of *O.gratissimum* (Linn.) leaves and the isolated fraction F4 from chloroform extract significantly reduced release of CK-MB in coronary effluent. Further the active principle OG1 isolated from fraction F4 of chloroform extract significantly attenuated ischaemia and reperfusion induced increase in release of CK-MB in coronary effluent collected after 5 min. of reperfusion. Moreover, treatment with standard (ramipril,1mg/kg) markedly reduced release of CK-MB in coronary effluent as compared to the active compound OG1, collected 5 min. of reperfusion (Figure 3).

## 6. DISCUSSION

Inspite of the disadvantage of high mortality, high heart rate and high rate of drug metabolism, albino rats are used in the present study because they are small in size having low cost and readily available. Moreover histological sectioning and quantification is easy in rat hearts due to small size. Isolated perfused rat heart preparation has been employed in the present study because it permits the use of pharmacological interventions without any interference due to change in systemic circulation. Various extracts of *O.gratissimum* (Linn.) leaves viz. petroleum eather, acetone, chloroform and methanol at a dose level of 100mg/kg were evaluated for ischaemia and reperfusion induced myocardial injury. Further purification of active extract was carried out using column chromatography which resulted isolation of four fraction viz. F1, F2, F3 and F4. These fractions were again evaluated for above said effect and among all the fractions F4 significantly attenuated ischaemia and reperfusion induced increase in myocardial infarct size. Further purification of fraction F4 resulted in isolation of OG1, OG2 and OG3. All the compounds were screened for above said activity.Compound OG1 significantly decreased the infarct size, release of LDH and CK-MB in coronary effluent during perfusion period compared to control group. The present findings suggests that compound OG1 from fraction F4 of chloroform extract of *O.gratissimum* (Linn.) leaves significantly effective to ameliorate myocardial ischaemic injury as compared to ischaemia and reperfusion induced control group. Moreover some extensive work in this direction could also lead to explore the possible mechanism of *O.gratissimum* (Linn.) against ischaemia and reperfusion induced myocardial injury. Further, chemical structure elucidation of active principle OG1 is in progress.

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