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Analysis of maslinic acid and gallic acid compounds as xanthine oxidase inhibitors in isoprenaline administered myocardial necrotic rats



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ABSTRACT

Objective: This research designed to analyze the *in vivo* and *in silico* ameliorative action of maslinic acid (MA) and gallic acid (GA) on reactive oxygen species generating enzyme xanthine oxidase (XO) in isoprenaline or isoproterenol (ISO) induced myocardial infarcted rats.

Methods: Albino Wistar rats were categorized into four groups with eight rats in each group. A dose of 15 mg/kg of MA and GA were pretreated to each MA and GA groups for seven days. A dose of 85 mg/kg of ISO administered to the ISO group along with MA and GA groups except normal group on two consecutive days of pretreatment. All animals sacrificed and the heart tissues were collected for the analysis of XO. The *in silico* molecular docking analysis of the compounds MA and GA with XO was analyzed by using Gold 3.0.1 software.

Results: XO enzyme levels were significantly increased in the heart homogenate of ISO administered rats when compared to normal rats. Pretreatment of MA and GA to ISO treated rats significantly brought XO enzyme to the near normal levels which indicate the protective action of MA and GA against myocardial necrosis. The *in vivo* results were further supported by the *in silico* molecular docking study which revealed the inhibition of XO enzyme by the formation of enzyme and ligand complex with the compounds MA and GA.

Conclusion: MA and GA compounds manifested the ameliorative effect against ISO administrated myocardial necrosis by inhibiting the free radical generating enzyme XO which is evidenced by both *in vivo* and *in silico* studies.

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1. Introduction

The changes in modern life style of human beings along with living environment leads to increase in the occurrence of cardiovascular diseases (CVD) and the mortality rate (Yazouli et al., 2018). Myocardial infarction (MI) or heart attack, myocardial

ischemia and atherosclerosis are the major CVD which contributes higher deaths worldwide. The interruption of blood supply to the heart induces the necrosis of myocardium known as MI (Rona, 1985).

Isoproterenol (ISO) is a synthetic chemical used to induce MI which exhibits the changes in animal model that represents the similar changes to human MI (Shaik et al., 2020b). ISO generates reactive oxygen species by undergoing autoxidation and produce free radicals which leads to MI (Barbaras et al., 1988). Among many proposed mechanisms for myocardial damage, the free radicals accumulation have also been implicated in the pathophysiology of ISO induced MI in animals (Davel et al., 2014).

Xanthine oxidase (XO) is a homodimer oxidoreductase enzyme with molecular weight of 300 kDa (Malik et al., 2019). It is a crucial

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enzyme of purine metabolism which directly or indirectly associated in many diseases like cancer, diabetes and CVD (Krakoff and Meyer, 1965). XO catalytically oxidizes xanthine and oxygen. This oxidation leads to the generation of superoxide anion or hydrogen peroxide free radicals which proceeds towards the formation of oxygen reactive species as free radicals. The excess amounts of reactive oxygen species causes to many pathological disorders like inflammatory diseases, hypertensive disorders and CVD (Berry and Hare, 2004). XO has been regarded as a potential drug target in the treatment of CVD. XO inhibitors are categorized into purine derived inhibitors such as allopurinol (Inkster et al., 2007) and non purine derived inhibitors such as febuxostat (Takano et al., 2005), unfortunately which exhibited adverse side effects (Sagor et al., 2015; Nepali et al., 2011). This focuses the importance and need of research to find the XO inhibitors without side effects in the treatment of CVD.

In market many drugs are available for the treatment of CVD, but unfortunately these remedies causing severe adverse side effects. So there is a lot of demand for the natural compounds without any side effects or with few side effects (Krushna et al., 2017). Maslinic acid (MA) is a triterpenoid and gallic acid (GA) is a phenolic acid which are the natural compounds widely distributed in medicinal plants (Shaik et al., 2012; Lozano-Mena et al., 2014). These compounds have been proved for their pharmacological and biochemical activities (Dzubak et al., 2006). In our previous research, MA and GA have been tested on paraoxonase enzyme (Hussain Shaik et al., 2012), on ATPase enzymes and on the lipid metabolizing enzymes (Shaik et al., 2020b) for the cardioprotection (Shaik et al., 2020a). The present research has been designed to execute the *in vivo* and *in silico* cardioprotection of MA and GA on reactive oxygen species generating enzyme XO in ISO induced MI rats

2. Materials and methods

2.1. Experimental rats

The acclimatization of Wistar strain male rats weighing about 130–170 g to animal house condition was carried out for 7 days with 12 h light and dark cycles in hygienic conditions. The standard pellet diet was provided and water *ad libitum*. The animal study was conducted by following the guidelines approved by Institutional Animal Ethics Committee (Reg. No. 470/01/a/CPCSEA), S.K. University, Anantapuramu, A.P., India.

2.2. Chemicals

MA procured from Cayman chemicals, U.S.A. GA purchased from SRL chemicals, India. ISO bought from Sigma, U.S.A. All other chemicals used were of analytical grade.

2.3. Experimental procedure

The dose of MA was determined by dose dependent study using two different doses of 7.5 mg/kg and 15 mg/kg from which the higher dose was found to be effective and selected in the current experimental study.

Five groups of rats maintained with eight rats in each group. The groups categorized as control rats, MA treated rats with 15 mg/kg dose, ISO treated rats with 85 mg/kg dose, ISO treated rats pretreated with MA 15 mg/kg dose and ISO treated rats pretreated with GA 15 mg/kg dose. MA, GA and ISO were dissolved in sodium carboxymethyl cellulose, saline and distilled water respectively. The treatment was conducted for seven days followed the administration of ISO for two consecutive days to induce MI.

The rats were anaesthetized with thiopentone sodium (35 mg/kg) and sacrificed by cervical decapitation. The heart tissues were collected and washed with isotonic saline. The heart homogenate was centrifuged to obtain supernatant and used for biochemical analysis.

2.4. Xanthine oxidase (XO) analysis

The activity of the enzyme XO was measured according to the method described by (Bergmeyer and Gawehn, 1974). The test sample mixture with final volume of 3.0 ml, consisted 1.9 ml of potassium phosphate buffer (50 mM) pH 7.5, 1.0 ml of xanthine (0.15 mM) and 0.1 ml of enzyme source heart homogenate. Blank was used with 0.1 ml deionized water instead of enzyme source. The absorbance change was recorded at 290 nm for 5 min. 1 unit of activity has been defined as the conversion of 1.0 μ M of xanthine to uric acid per minute at pH 7.5 at 25 °C.

2.5. Molecular docking analysis

2.5.1. Collection of structures

The protein structures of XO were collected from the database of protein data bank (PDB). After collecting the structure it proceeded towards the removal of chains and hetero atoms which were not necessary and active site in the protein was predicted.

2.5.2. Active site identification

Computed atlas of surface topography of proteins (CASTP) server (Binkowski et al., 2003) was applied for the detection of the active site of XO. A new program, CAST was used for measuring protein cavities and pockets which is followed precise computational geometric procedures that include alpha shape and discrete flow theory. The CAST specifically detects and analyses pockets and pocket mouth openings along with cavities. This program specifies the following parameters such as pockets openings, buried cavities, atoms lining pockets, pockets and cavities total volume and area, mouth openings area and circumference.

2.5.3. Virtual screening of maslinic acid (MA) and gallic acid (GA) through molecular docking

Genetic optimization of ligand docking (GOLD) version 3.0.1 (Gareth Jones et al., 1997) is a docking program used for virtual screening of MA and GA through docking. The MA and GA used for docking were minimized and are docked to the active sites of XO. The derivatives interactions with the residues of active sites were analyzed by calculating molecular mechanics. At the time of docking process, default speed of algorithm was set and the ligand binding with active residues of protein were defined within a 10 Å radius. 100 poses were set for each inhibitor and was allowed for early termination when the ligand exhibited 3 bound conformations within 1.5 Å root mean square deviation (RMSD). When docking completed, the interactions of ligand with the protein along with the individual binding poses were analyzed. Among them the most energetic and best favorable conformation of ligands were selected.

2.5.4. Gold score fitness function

Gold Score fitness function was calculated by the following formula. The fitness function optimization was carried out to predict the ligand binding positions.

$$\text{Gold score fitness} = S(\text{hb_ext}) + 1.3750 * S(\text{vdw_ext}) + S(\text{hb_int}) + 1.0000 * S(\text{int})$$

$$S(\text{int}) = S(\text{vdw_int}) + S(\text{tors})$$

Abbreviations in formula denoted as below:

S (hb_ext): Protein-ligand hydrogen bond score,

S (vdw_ext): Protein-ligand van der Waals score,

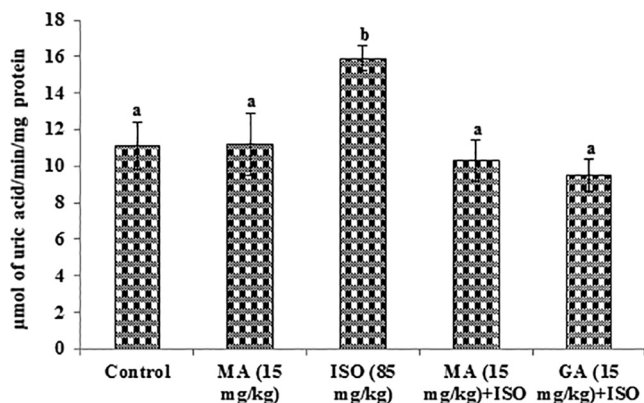


Fig. 1. Effect of maslinic acid (MA) and gallic acid (GA) on xanthine oxidase (XO) in the heart homogenate of isoproterenol (ISO) administered rats. Values are mean \pm S.D. (n = eight rats). Values not shared a common superscript (a and b) differ significantly from each other ($p < 0.05$, Duncan's multiple range test).

S (hb_int): Score from intramolecular hydrogen bond in the ligand and

S (vdw_int): Score from intramolecular strain in the ligand.

2.6. Statistical analysis

One way analysis of variance was executed continued by Duncan's multiple range test with SPSS software. Values expressed with means \pm S.D and $p < 0.05$ considered as significant.

3. Results and discussion

3.1. In vivo xanthine oxidase (XO) inhibitory activity

Fig. 1 revealed the effect of MA and GA on XO in control and ISO treated rats. ISO induced cardiotoxicity demonstrated a significant ($p < 0.05$) increase in XO activity when compared to control rats. Pretreatment with MA in 15 mg/kg and GA in 15 mg/kg doses significantly ($p < 0.05$) decreased XO activity and maintained to near normal in ISO treated rats when compared to ISO alone treated rats. There is no significant change with treatment of MA (15 mg/kg) alone.

The present study has been focused to develop the low toxic or nontoxic cardio protective compounds by regulating the XO

enzyme. XO is the oxygen free radicals generating enzyme that dominantly affects to the damage of myocardium (Chambers et al., 1985). During ischemia, XO acts on xanthine and hypoxanthine and triggers the generation of oxygen free radicals (Raghuvanshi et al., 2005). It has been reported that XO can damage the myocardium by both structural alteration and functional depression (Prasad et al., 1993). The present study revealed that MA and GA pretreatment in ISO administered rats significantly decreased the activity of XO by inhibiting the generation of superoxide free radicals. It has been reported that XO inhibitor allopurinol blocked the oxidative stress, fibrosis and cardiac necrosis in ISO treated rats by reducing the marker enzymes, lipid peroxidation due to antioxidant activity (Sagor et al., 2015). Also, it has been revealed that generation of reactive species and expression of inflammatory enzymes during inflammation were regulated by MA (Huang et al., 2011; Márquez Martín et al., 2006; Qian et al., 2011). The present results are in accordance with the earlier results where *Terminalia pallida* fruit ethanolic extract showed cardioprotection by inhibiting the enzyme XO in ISO administered MI rats by exhibiting antioxidant activity (Althaf Hussain et al., 2018). In addition, it has been showed that *in silico* designed and synthesized rutin derivatives reported as XO inhibitors (Malik et al., 2019). Our study clearly revealed that MA and GA compounds inhibited the XO enzyme with both *in vivo* and *in silico* evidences.

3.2. In silico molecular docking of maslinic acid (MA) and gallic acid (GA) with xanthine oxidase (XO)

The collected XO structure (PDB ID: 2E1Q) (**Fig. 2**) has 1307 amino acids and containing six domain regions including iron binding domain (67–162), molybdopterin dehydrogenase, FAD binding domain (206–370), CO dehydrogenase flavoprotein (392–502), aldehyde oxidase and xanthine dehydrogenase domains (562–668; 676–1211) and sodium pump decarboxylase domain (1223–1261).

3.2.1. Active site identification

The active site residues of XO were predicted (**Fig. 3**) by using CASTP server and the position of active site along with amino acids were selected for docking.

3.2.2. Docking of inhibitors

With the preliminary inhibitory results of MA and GA, new structural features and functionalization requirements were pro-

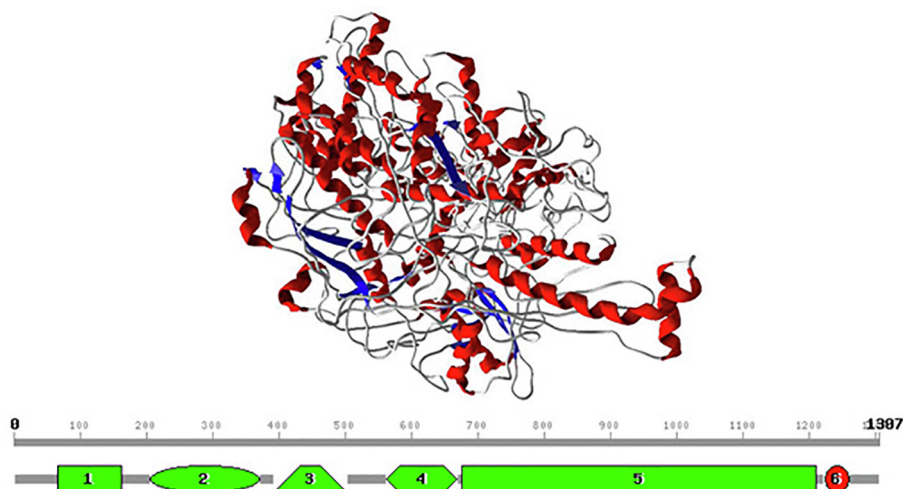


Fig. 2. Three dimensional structure of xanthine oxidase (XO) with twenty four helices and sixteen sheets; containing six domain regions.

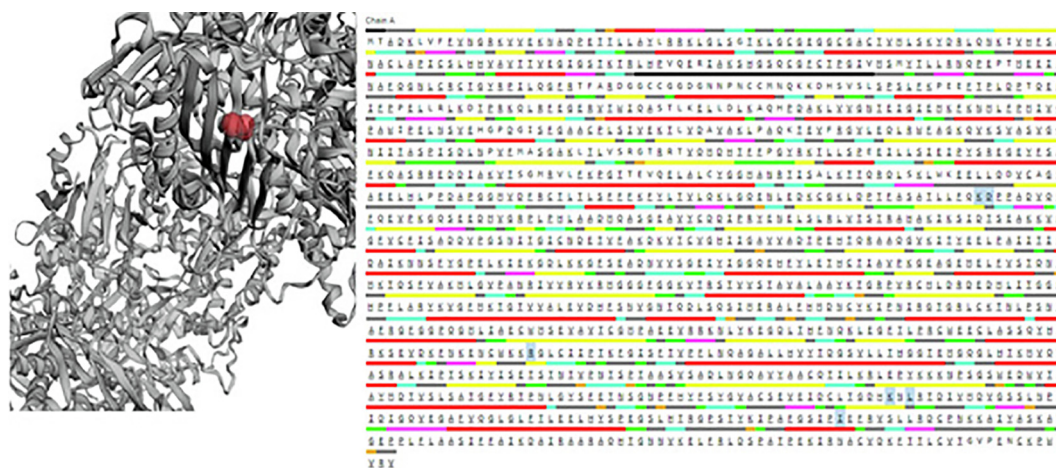


Fig. 3. Prediction of binding sites (red color) in xanthine oxidase (XO); amino acids involved in active site were predicted (grey color).

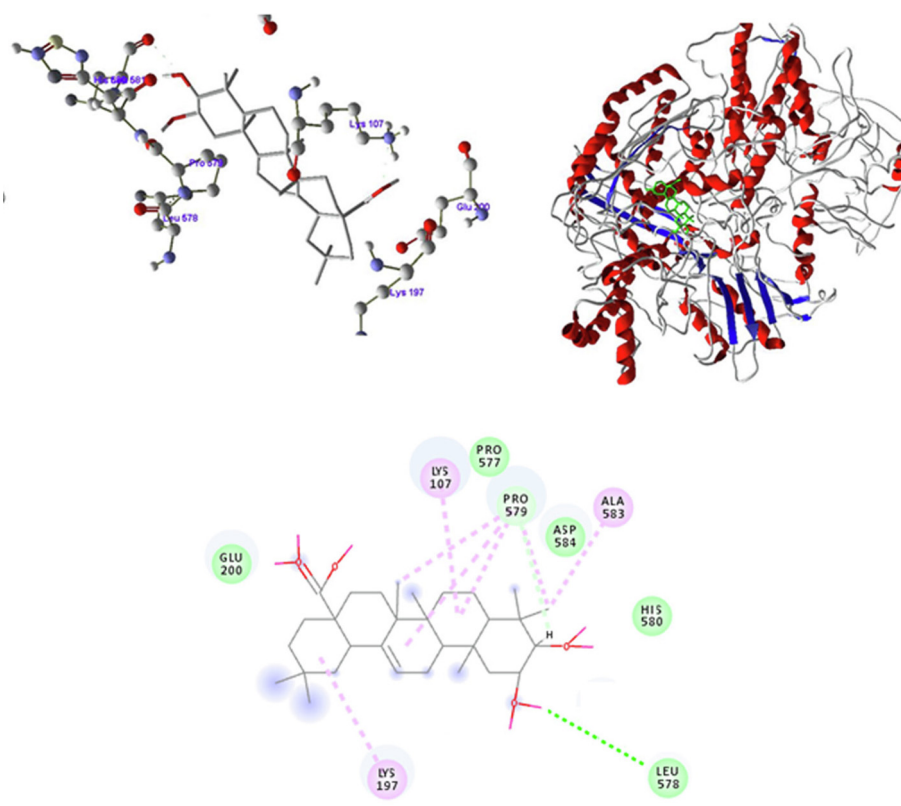


Fig. 4. Docking studies of maslinic acid (MA) with xanthine oxidase (XO).

posed for these that could increase affinity with XO. MA and GA were demonstrated for docking studies by using GOLD 3.0.1 to evaluate better phytochemical which inhibit XO, which is based on genetic algorithm of Cambridge crystallographic data center, Cambridge, U.K. In the docking of MA and GA showed inhibition with XO.

Among the MA and GA, MA showed higher affinity towards XO.

MA docked to XO with one hydrogen bond and catalytic residue involved in docking is PRO579 (Fig. 4). The bonds present in the XO-MA complex and the distances and angles of the bonds are rep-

resented in Table 1. GA docked to XO with one hydrogen bond and catalytic residue involved in docking is GLY1040 and also three other pi bonds are formed (Fig. 5). Docking of MA and GA inhibitors with active site of XO performed by employing GOLD software and the docking evaluations were executed based on Gold fitness function. The docking results revealed that the conservation of amino-acid residues with XO exhibits a crucial role to maintain the functional conformation which is directly contributed in donor substrate binding. The current result revealing the interaction of domain and inhibitors is helpful to understand the mechanism of inhibitor binding with domain.

Table 1
Hydrogen bonding interactions of maslinic acid (MA) and gallic acid (GA) compounds with target proteins of xanthine oxidase enzyme.

Protein	Compounds	No. of bonds	Atoms Protein	molecule	Bond length (Å)	Docking score (K.cal/mol)
Xanthine oxidase	GA	3	GLY1040, ILE1191, ALA1190	O ₃	1.43	−30.88
	MA	7	LYS107, ALA583, LYS197	H ₂ O	1.23	−113.06

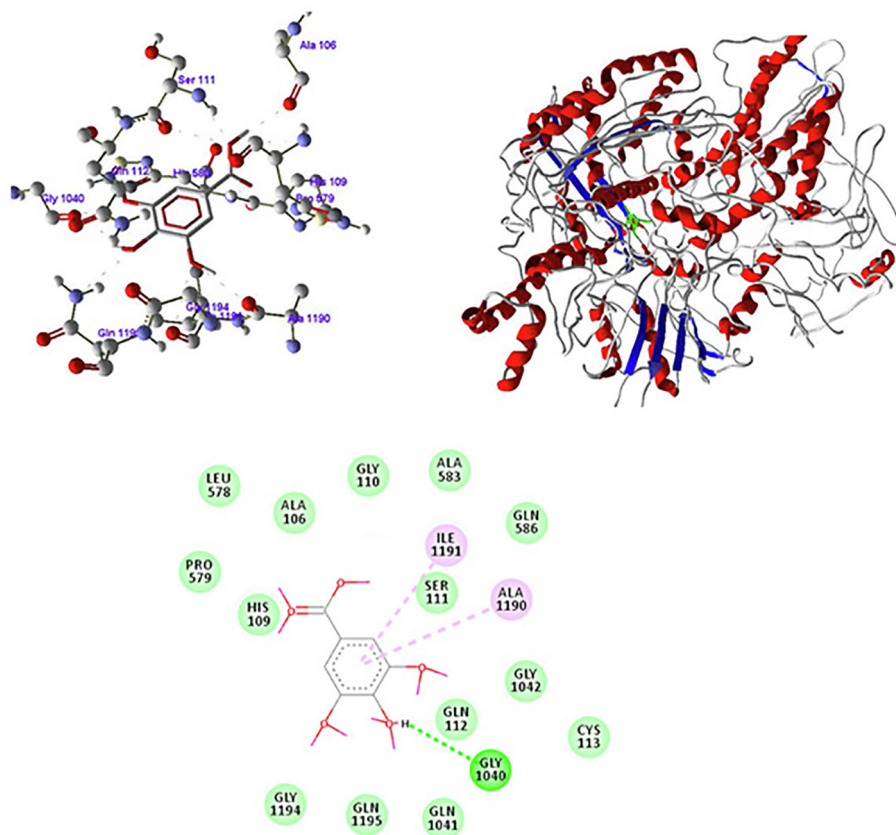


Fig. 5. Docking studies of gallic acid (GA) with xanthine oxidase (XO).

3.2.3. Gold score fitness function

MA exhibited the total gold score fitness function with XO is −113.06 Kcal/mol, whereas GA showed the total gold score fitness function with XO is −30.88 Kcal/mol. This revealed that MA has excellent inhibitory activity on XO. In this study gold fitness function has chosen other than chem score fitness function as the earlier one is marginally better than the later one. Among the MA and GA, the fitness function is highest for MA and can be used for further investigations.

4. Conclusion

In conclusion, the present study clearly revealed that the compounds MA and GA inhibited the free radical generating enzyme XO in ISO administered MI rats. Furthermore, the *in silico* molecular docking studies confirmed that the MA and GA compounds effectively act against the XO enzyme and offered the cardioprotection. MA and GA could be applied in the treatment of XO associated diseases especially CVD.

Declaration of Competing Interest

None.

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