CHAPTER 6

DISCUSSION

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3-Methyl-4-substituted benzylidene-pyrazol-5-ones (**PYZ1- PYZ10**) were synthesized by the condensation of 3-methyl-pyrazol-5-one with substituted aliphatic and aromatic aldehydes. Their structures have been elucidated by sophisticated instrumental analysis such as UV, IR, ^{1}H NMR, Mass spectral data and elemental analysis. **(Fig: 5.1 - 5.40)**

Toxicological studies of the test compounds (as suspension in 0.5%w/v carboxy methyl cellulose) were carried out by Up and Down method in oral dose of 2000 to 5000 mg/kg body weight in albino rat. The rats were continuously observed for 8hr for any signs of acute toxicity such as increased/decreased motor activity, ataxia, tremors, convulsions, sedation, lacrimation etc. After 24hrs the rats were sacrificed, then stomach, intestine and liver were examined under the magnifying lens for any ulcer-hemorrhagic spots. Since no such spots were detected, it is concluded that the drugs are nontoxic up to that dose limit. Acute toxicity and gross behavior studies revealed that the entire series of compounds in the present investigation were found to be nontoxic up to 5000 mg/kg body weight.**(Table: 5.1.)**

Lipid metabolism plays an important role in myocardial injury produced by ischemia by deposition of cholesterol ion coronary arteries (Mathew et al., 1981). Abnormalities in lipid profile (LDL, VLDL, HDL TG, TC, creatinine and TP) are associated with increased risk of myocardial infarction. High level of circulating cholesterol and its accumulation in the heart tissue is usually accompanied by cardiovascular damage (Mediene-Benchekor et al., 2001). According to Patsch, 1993 and Ng et al., 1997, patients with cardiovascular diseases experience markedly elevated triglycerides and reduced HDL levels. It can be attributed that metabolism of triglyceride-rich lipoproteins present in HDL, particularly the sub-fraction HDL2 which has a negative association with cardiovascular risk. Hence the estimation of lipid profile can be directly correlated with the intensity of myocardial injury.

The Isoproterenol elevated the levels of total cholesterol, total protein, LDL, VLDL, triglycerides; and decreased HDL in serum. It causes hyperlipidemia and it increases the LDL cholesterol in the blood, which in turn leads to harmful deposits in the arteries

thus favoring coronary heart diseases (CHD) (Rajdurai and Prince, 2006). In the present study also, isoproterenol administration caused a significant rise in the serum lipids thereby increases lipid biosynthesis and lipid peroxidation. Rats treated with pyrazolone derivatives showed decreased concentration of serum total cholesterol, triglycerides, LDL cholesterol indicates the beneficial effects of pyrazolone derivatives in reducing hyperlipidemia caused by Isoproterenol. All the pyrazolone derivatives (10mg/kg b.w) have controlled the elevated lipid profile to near normal level. Amongst the entire compounds, **PYZ8, PYZ9, PYZ10** had shown profound effect to lower the various lipids. **(Table: 5.2 and Fig 5.41-Fig: 5.52.)** The results are statistically significant at $p < 0.01$ level.

The administration of Isoproterenol in rats resulted significant increase in the serum levels of cardiac marker enzymes such as LDH, SGOT, SGPT and ALP. Isoproterenol induced myocardial injury has been reported to alter membrane permeability (Mathew *et al.*, 1981) and to cause leakage of marker enzymes of cardiac damage (LDH, CPK, AST, ALT and ALP) into the blood stream(Khalid and Ashraf, 1993; Mohanty *et al.*, 2004). All the synthesized compounds (10 mg/kg b.w) brought back the elevated myocardial injury markers to near normal level. **(Table.5.3).**Comparatively, the compounds **PYZ8, PYZ9** and **PYZ10** had shown profound effect to lower the myocardial injury markers such as LDH, SGOT, SGPT and ALP. The results are statistically significant at $p < 0.01$ level.

Pyrazolone derivatives pre-treatment improves cardiac antioxidant status in isoproterenol induced myocardial injury by effective scavenging of free radicals generated during oxidation of lipids thus collectively contributing to its overall antioxidant and anti ischemic activity. The significantly decreased activities of endogenous enzymatic antioxidants (CAT,GSH and SOD) observed in the heart of ISO-treated rats were improved following pretreatment with pyrazolone derivatives for 5 days. All the test compounds had shown significant activity to elevate reduced level of enzymatic antioxidant. **(Table: 5.4 and Fig: 5.53 - Fig 5.55.)** Amongst them, **PYZ8, PYZ9, PYZ10** had shown to improve the level of endogenous enzymatic antioxidants effectively. The results are statistically significant at $p < 0.01$ level.

The present study has clearly demonstrated that the pyrazolone derivatives have antioxidant activity which could prevent the occurrence of heart related diseases. Significantly elevated activities levels of SOD and CAT recorded in pyrazolone treated group could be due to its potent free radical scavenging ability. GSH scavenges singlet oxygen, superoxide and peroxy radicals to form oxidised glutathione and other disulfides (Meister, 1984). Also, antioxidant compounds have been shown to increase glutathione reductase activity that maintains GSH in a reduced state (Mohanty *et al.*, 2004). The elevated GSH content observed in pyrazolone treated groups may be due to its enhanced synthesis. ISO treatment is also known to create an imbalance between enzymatic as well as non enzymatic antioxidant defense system leading to production of free radicals that induce myocardial injury and lipid peroxidation.(Ojha *et al.*, 2008). The significant decrement in lipid peroxidation in pyrazolone derivatives treated group further justifies the role of pyrazolone derivatives as a potent antioxidant and free radical scavenger. These results are in conformity with reports that have been demonstrated the modulation of cellular antioxidant activities by treatment with pyrazolone derivatives (David et al., 2006).

Pyrazolone pre-treatment to isoproterenol treated rats provide cardio protection by inhibiting the formation of free radicals generated during oxidation of lipids thus inhibiting peroxidation of membrane lipids and preventing subsequent leakage of soluble enzymes. Pyrazolone derivatives pre-treatment appears to improve the status of enzymatic antioxidants that further contributes to its overall cardioprotective property. Hence, it can be concluded that pyrazolone derivatives pretreatment provides cardio protection myocardial injury via multiple mechanisms. It was assessed by estimation of Malonaldehyde (MDA) and 4-Hydroxyl-2-noneal (4-HNE) as lipid peroxidation markers in myocardial ischemic reperfusion injury by Langendorff isolated rat heart model. The quantification of MDA and 4-HNE can be directly correlated with the lipid peroxidation inhibition capacity of the pyrazolone derivatives. The toxic radical's quantification is also an indicator to monitor the overall progress of lipid peroxidation which is associated with myocardial ischemic reperfusion injury.

The antioxidant activity of pyrazolone derivatives was compared with standard antioxidant (ascorbic acid). Comparatively, all the compounds have shown significant antioxidant effect where as **PYZ7, PYZ8, PYZ9** and **PYZ10** having effective role to control both MDA and 4-HNE generation. **(Table: 5.5, Fig: 5.56 and Fig 5.57.)** All the experimental data were statistically significant at $p<0.05$ level.

Histopathological effects of **(Fig: 5. 60, 5.61 and 5.62.)** show the light micrograph of control heart showing normal architecture and integrity of myocardial cell membrane. Severe degenerations of the myofibrils, with focal necrosis, vacuolated cytoplasm , lymphocytic infiltration in sub endocardial region indicative of infarct like lesions as reported in various studies (Teerlink et al., 1994; Grimm et al., 1998). Isoproterenol intoxication also induced eosinophilic cytoplasm, focal hemorrhage and with inflammatory cell infiltrations. Scrutiny of cardiac tissue of isoproterenol + pyrazolone group revealed that there was minimum damage to the myocardium with much reduced myonecrosis and lymphocyte infiltration than ISO treated group. The animals pretreated with PYZ1-PYZ10 showed better-preserved appearance of cardiac muscle fibers with slight degeneration and some leukocyte infiltration.

Myocardial injury is associated with inflammatory response (Entman et al., 1991). Endothelial injury plays a critical role in the pathogenesis of myocardial ischemia-reperfusion (I/R) injury by setting the stage for adherence of neutrophils to the vascular endothelium and subsequent development of inflammatory component of the I/R. The suppression of polymorph nuclear leukocytes (PMNs) infiltration and inhibiting NF-kappa B (NF-kB) activation diminishes I/R damage and potentially offers myocardial protection (Kim et al., 2009). The high mobility group box 1 protein (HMGB1) maintains the nucleosome structure and regulates gene transcription, can be released by necrotic cell or activated innate immune cells (Lotze and Tracey, 2005). Preconditioning with HMGB1 protects against myocardial I/R injury. It is binding to its receptors activates intracellular signaling pathways, such as the NF-kB pathway, which induces downstream cytokine release (Fiuza et al., 2003).

Based on the above facts it can be assessed that PYZ1-PYZ10 derivatives could protect the heart from the injury induced by ischemia and inflammation on rat myocardial I/R injury . All the synthesized compounds were screened for anti-inflammatory activity against Carrageenan- induced paw edema in rats. When compared with the control, all the compounds showed reduction in edema volume with prominent percentage inhibition to the inflammatory response ranging from 44% to 65% at 4^{th} hour of observation. Compounds **PYZ 5, PYZ 6, PYZ 7** and **PYZ 10** were found to have a potent anti-inflammatory response at $p<0.01$ levels **(Table: 5.6 Fig 5.58)**

Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1-2 h) carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissues surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorph nuclear cells and prostaglandins produced by tissues macrophages (Brito and Antonio, 1998; Gupta et al., 2006). The pyrazolone derivatives reduced the carrageenan induced paw edema in rats and all the compounds are active. It may be due to inhibition of cyclooxygenase enzyme followed by prevention of inflammatory mediator's release. There is increasing evidence that lysosomal enzymes play an important role in the development of acute and chronic inflammation. (Anderson *et al.* 1971; Jannoff and Zweifach, 1964).

Most of anti-inflammatory drugs exert their beneficial effect by inhibiting either release of lysosomal enzymes or by stabilizing lysosomal membrane which is one of the major events responsible for the inflammatory process. The experimental findings of pharmacological parameters suggest that pyrazolone derivatives are the promising non steroidal anti-inflammatory agents.

The underlying mechanism by which pyrazolone derivatives improved myocardial function in I/R rats might be partially associated with decrease of PMNs infiltration and the infiltrating PMNs is activated by an ischemic insult, directly contact and injure neighboring myocyte via the release of inflammatory substances (Vinten , 2004). PMNs activation in reperfusion areas has detrimental consequences on cardiac

function (Shandeyla et al., 1993). It induces post ischemic damage by liberating reactive oxygen metabolites, hydrolytic enzymes, and eicosanoids, which would lead to microvascular injury (Zimmerman and Granger, 1990), leukocyte accumulation was increased (Abe et al., 2008). Pyrazolone reduced the PMNs infiltration in ischemic myocardial tissue. Pyrazolone derivatives prevented I/R-induced myocardial injury and inflammation partially due to inhibition of neutrophils infiltration since the compounds are having significant anti-inflammatory response.

Acetic acid which is used as an inducer for writhing syndrome (Koster et al., 1959) causes analgesia by releasing of endogenous substances, which then excite the pain nerve ending. The abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandins. It is possible that pyrazolone exert an analgesic effect probably by inhibiting prostaglandin synthesis. Perusal of the results on their analgesic activity by tail-flick method revealed that almost all of them exert significant activity. Amongst them, compounds **PYZ1, PYZ 4, PYZ 5, PYZ 8** and **PYZ 10** were found to have an effective analgesic response at $P < 0.01$ level **(Table: 5.7.and Fig 5.59.).**

The perusal of results of antipyretic study revealed that **PYZ9** significantly reversed hyperthermia similar to standard drug paracetamol (100 mg/kg, p.o) as shown in **Table: 5.8.** Fever results due to generation of mediators such as IL-1β, IL-6, interferons and TNF-α cytokines increase the synthesis of prostaglandin which elevates the body temperature. From the results of antipyretics study, it can be suggested that pyrazolone derivatives produce the antipyretic action by inhibiting the prostaglandin synthesis by blocking cycloxygenase isoenzymes, platelet thromboxane synthesis and prostanoids synthesis (Graham and Scott, 2003; Bentur and Cohen, 2004).

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