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Pharmacological and Toxicological Evaluation of Some Novel 2-substituted 4, 5-diphenyl Imidazole Derivatives

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Abstract: Background: Imidazole derivatives are reported to possess wide spectrum of pharmacological activities. This study describes the pharmacological and toxicological studies of five imidazole derivatives namely IMZ1: 2-phenyl-4,5-diphenyl imidazole, IMZ2: 2-[4-methoxyphenyl]-4,5-diphenyl imidazole and IMZ3: 2-[2-nitrophenyl]-4,5-diphenyl imidazole, IMZ4: 2-[4-N,N-dimethylaminophenyl]-4,5-diphenyl imidazole, IMZ5: 2-[3-methoxyphenyl]-4,5-diphenyl imidazole derivatives. **Result:** Analgesics, anti-inflammatory, antipyretic, anticonvulsant studies of imidazole derivatives at 400 mg kg^{-1}, p.o. have shown significant activity as compared to control. Tail-flick method and acetic acid induced writhing test was used to screen analgesic activity. Carrageenan induced rat paw edema model and Freund's adjuvant-induced polyarthritis model was used to assess the anti inflammatory activity. Amongst five imidazole derivatives, IMZ5 was found to be more active. Based on the result of pharmacological studies, IMZ5 was selected for toxicological studies by Up and Down Procedure. Acute toxicity studies revealed that 2-[3-methoxyphenyl]-4, 5-diphenyl imidazole derivatives are non toxic in rats up to 5000 mg kg^{-1}, p.o. The sub acute toxicity study of IMZ5 showed that slight alteration in Hb content, RBC and WBC count. In biochemical analysis, level of blood glucose and bilirubin reduced to some extent where as AST, ALT and ALP level elevated. Histopathological studies revealed that there was mild toxicity on liver and kidney at 1000 mg kg^{-1}, p.o but no toxic effects were observed on heart. **Conclusion:** All the tested derivatives retained the above mentioned pharmacological activities due to the basic imidazole heterocycle. Since, there was mild toxic profile on this nucleus, further structural exploitation may yield safe therapeutic drug candidates.

Key words: Imidazole, anti-inflammatory, anticonvulsant, anticancer, cardioprotective

INTRODUCTION

Imidazole is an aromatic five-membered heterocycle with two nitrogen atoms. This aromatic heterocyclic moiety present in alkaloid from plant secondary metabolites. Imidazole nucleus forms the main structure of some well-known components of human organism, i.e., the amino acid histidine, vit-B12, a component of DNA base structure (purines), histamine and biotin (Athereden and Bentley, 1996).

Imidazoles are present in many antifungal, anti protozoal and anthelmintic medications. It is part of the theophylline molecule, found in tea leaves and coffee beans which stimulates the central nervous system. It is present in the anticancer drug mercaptopurine which combats leukemia by interfering with DNA synthesis. It is also present in the structure of many natural or synthetic drug molecules, i.e., cimetidine, azomycine, metronidazole, clonidine, losartan and phenytoin (Lednicer and Mitscher, 2005; Laurence *et al*., 2000).

Nitro imidazoles are associated with antimicrobial activity, whereas imidazolines are often present in drugs acting as adrenergic agents. Nitroimidazole forms the basis for an extensive class of agents used in the treatment of infections by the protozoans (Lednicer and Mitscher, 2005; Habbu *et al*., 2009). Moreover, imidazoles are reported to show a broad spectrum of pharmacological activities and many of these have gained wide acceptance in clinical practice. Therefore imidazole derivatives are potent source of drugs in the category of anti-tumor, antifungal, cardioprotective, alpha- blocker, CNS-depressant, antimicrobial and anticonvulsant drugs (Gopalakrishnan *et al*., 2005). Also, there are evidences for imidazole derivatives to be used for the inhibitions of the enzymes like Acyl CoA Cholesterol-Acyl Transferase (ACAT) and HMG CoA Reductase (HMGR). These

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compounds are also potentially useful for the prevention or treatment of neurological dysfunctions such as dystonias, dyskinesias, akathisia, tremor and spasticity, treatment of spinal cord injury, neuropathy, migraine and vigilance disorders, sleep disorders, pain disorders, cranial trauma. These diphenyl imidazole compounds are potentially useful for the treatment of cardiovascular disorders, metabolic disorders, reproductive, endocrine and gastrointestinal disorders (Nafziger *et al*., 1991).

Hence, these derivatives are the versatile source of drug candidates for alleviating most of the devastating disease conditions. In this perspective, it has been decided to screen the pharmacological and toxicological activities of some newer imidazole derivatives.

MATERIALS AND METHODS

Test compounds: Imidazole derivatives IMZ1-IMZ5 (Fig. 1) investigated in the present study were synthesized, characterized as per the reported procedure (Umit *et al*., 2001).

Chemicals and drugs: Carboxy methyl cellulose (CMC), acetic acid, eosin, hematoxylin, dextrene polystyrene xylene, paraffin and xylene were purchased from S. d fine, Mumbai, India. Carrageenan, Freund's adjuvant, Pentylenetetrazole and Brewer's yeast were procured from Sigma, USA. Indomethacin, phenytoin, paracetamol and aspirin were obtained from Cipla pharmaceuticals, Sikkim, India and pentazocine from Ind-Swift L., Baddi, India as gift sample.

Biochemical diagnostic kits: Cholesterol, Direct HDL-Cholesterol, Triglycerides, Glucose, AST (GOT), ALT (GPT) and Alkaline Phosphatase kits were purchased from Span Diagnostics Ltd., Surat, India.

Animals: Male and female albino mice (Swiss, 20-25 g), rats (Sprague-Dawley, 100-150 g) and rabbits (New Zealand strain, 1.5-2 kg) were used as experimental animals. The animals were housed under standard conditions of temperature (24±1°C), relative humidity (65±10%) and 12 h light/dark cycle environment. During the study period, guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Institutional Animals Ethics Committee (IAEC) were followed for the maintenance of animals and the experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) No: IAEC HPI/09/60/ IAEC/077.

Experimental procedure: The animals were divided into five groups of six in each. The first group was treated as control and received 1% CMC, second group received the standard drug (Indomethacin 10 mg kg^{-1}, p.o., Pentazocine 3.9 mg kg^{-1}, i.p., Paracetamol 100 mg kg^{-1}, p.o. and Aspirin100 mg kg^{-1}, p.o.) and rest of the groups were administered test compounds at 400 mg kg^{-1} p.o.

Pharmacology: All the compounds prepared herein were screened for their analgesic, anti-inflammatory, antipyretic and anticonvulsant activities. The analgesic activity was carried out by Tail-flick method reported by

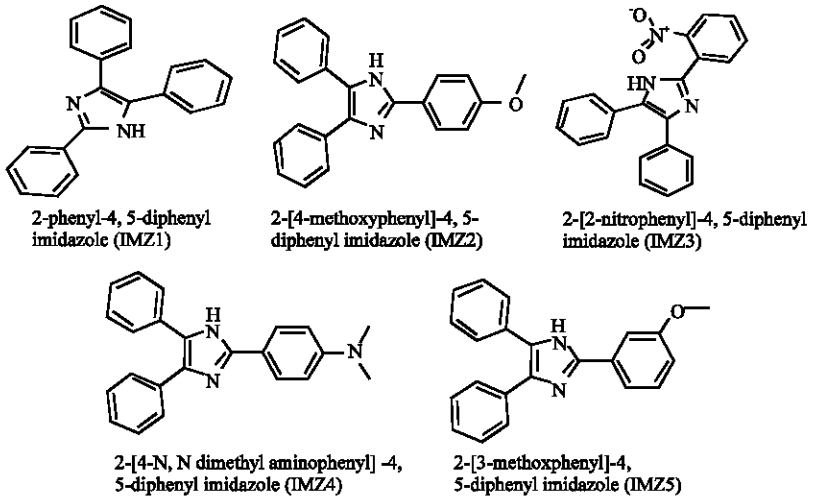

Fig. 1: Structure and nomenclature of imidazole

D'Amour and Smith (1941) and acetic acid induced writhing test by Koster *et al.* (1959). The anti-inflammatory activity of the test compounds was evaluated by carrageenan induced rat paw edema model described by Winter *et al.* (1962) and Freund's adjuvant-induced polyarthritis model by Newbould (1963). The antipyretic activity was performed on rabbits of either sex according to the reported method described by Szreder (1990). Maximal electroshock method by Swinyard (1972) and Pentylenetetrazole-induced seizures method by Loscher *et al.* (1991) were used to study the anticonvulsant activity.

Toxicology: The Up and Down Procedure (Dixon, 1999) was adopted to evaluate the acute and sub acute toxicity of imidazole derivatives. At the end of the study period on 29th day the animals were anaesthetized with chloroform and blood samples immediately collected by cardiac puncture for hematological and biochemical analysis. Necropsy of all the animals was carried out and selected organs like the heart, liver and kidney were removed and preserved. The organs were physically examined, weighed and samples were collected for histopathological examinations.

Biochemical assay: The non-heparinized blood was allowed to coagulate before being centrifuged (4000 rpm for 20 min) and the serum separated. The levels of Cholesterol (Chol.), Glucose (Glu) Triglycerides (TG), High Density Lipoprotein Cholesterol (HDLC), Total Bilirubin (TB), Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP) were estimated using commercially available standard kits (Erba diagnostic kit and Span diagnostics Ltd. India) on an automatic analyzer (Merck P. Ltd).

Hematological assay: At the end of the experimental period, the next day after an overnight fasting, blood was collected directly from heart and used for the analysis of hematological parameters viz., Hemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, Hematocrit and blood clotting time.

RBC, WBC count and hemoglobin estimation: The RBC count, WBC count and hemoglobin estimation were carried out by the method of Wintrobe *et al.* (1976) and Armour *et al.* (1965).

Differential leukocyte count, platelet count and hematocrit: The heparinized blood was analyzed by digital automatic hematology analyzer/blood cell counter (Care Well Biotech Pvt. Ltd, India) in Ashok Laboratories, Jodhpur Park, Kolkata-32, India and the results were obtained.

Blood clotting time: It was assessed by the method of Ghai (1990).

Histopathology: Liver, heart and kidney of animals from control and treated groups were dissected into small sections and preserved in cedar wood oil. Infiltration was done by dipping the tissues in xylene: paraffin wax in 1:1 ratio for one hour at 60°C and then tissues were dipped in molten paraffin for one hour at 60°C. The processed tissues were embedded in the molten wax for section cutting. Thin section of the paraffin blocks containing tissue was done using rotary microtome. Then the slides were stained with eosin and hematoxyline and mounted with dextrene polystyrene xylene and examined microscopically for pathological examination.

Statistical analysis: All the results were expressed as mean±SEM. The results were analyzed by one way analysis of variance (ANOVA) followed by Dunnett's test (Dunnett, 1955). The p value <0.01 and <0.05 were considered statistically significant.

RESULTS

Table 1 shows the analgesic activity by tail flick and acetic acid induced writhing methods using pentazocine and indomethacin as standard drugs, respectively. All the compounds showed significant analgesic activities in both methods. IMZ5 has shown more prominent analgesic response at 400 mg kg^{-1} b.wt. since it was found to be more effective at the end of 3 h (5.11±0.33) compared to the standard drug indomethacin (10 mg kg^{-1}, p.o., 7.87±0.30).

Anti inflammatory activity was screened by carrageenan-induced acute inflammation model and adjuvant- induced arthritis model and results are presented in Table 2. The results of carrageenan induced model revealed that IMZ5 was more active at 4 h (35.08% inhibition) of paw odema which is higher than all other derivatives. The results of adjuvant-induced arthritis model showed that IMZ5 (29.26% inhibition), has shown profound response than all other imidazole derivatives after 30 days administration.

From Table 3, the perusal of results of antipyretic study revealed that significantly all the compounds reversed hyperthermia similar to standard drug paracetamol (100 mg kg^{-1}, p.o.) as shown in Table 3. Amongst them, IMZ5 was found to be more active (39.59°C) which is similar to standard paracetamol (39.57°C).

Table 1: Analgesic activity of Imidazole derivatives

	Tail flick method				
	Average tail withdrawal time (sec)				Writhing test
Compd (mg kg^{-1})	0 h	1 h	2 h	3 h	No. of writhings
Control	2.11±0.16	2.13±0.21	2.46±0.21	2.78±0.72	60.24±1.27
Standard (a and b)	2.24±0.16	5.30±0.31**	7.19±0.30**	7.87±0.30**	20.06±0.17**
IMZ1(400)	2.10±0.15	3.50±0.23*	3.67±0.21*	3.86±0.33*	45.73±0.21*
IMZ2(400)	2.00±0.25	3.44±0.21*	5.13±0.21**	5.46±0.43**	51.18±1.80
IMZ3(400)	2.24±0.21	3.55±0.31*	4.49±0.67*	4.93±0.16*	40.71±0.63**
IMZ4(400)	2.00±0.12	3.09±0.32	3.34±0.27*	3.66±0.31*	58.35±0.61
IMZ5(400)	2.26±0.30	3.23±0.30*	5.23±0.41**	5.11±0.33**	37.33±0.42**

Values are expressed as Mean±SEM, n = 6. *p<0.05, **p<0.01 compared with vehicle control (ANOVA followed by Dunnett's test). a: Pentazocine (3.9 mg kg^{-1}, i.p.) used as standard drug in tail flick method, b: Indomethacin (10 mg kg^{-1}, p.o.) used as standard drug in writhing test

Table 2: Anti inflammatory activity of imidazole derivatives

	Paw edema volume in mL (% inhibition of Paw edema)					
	Carrageenan-induced paw edema method				Freund's adjuvant method	
Compd (mg kg^{-1})	1 h	2 h	3 h	4 h	0 h	18 h
Control	1.23±0.01	1.19±0.03	1.17±0.01	1.14±0.02	0.69±0.01	1.19±0.01
Standard	0.87±0.02**	0.81±0.01**	0.71±0.02**	0.52±0.01**	0.68±0.02	0.83±0.02**
(a and b)	(29.26)	(31.93)	(39.31)	(54.38)		(50.79)
IMZ1 (400)	1.03±0.01	0.97±0.01	0.89±0.02**	0.79±0.01**	0.66±0.01	1.08±0.02*
	(16.26)	(18.48)	(23.93)	2(9.41)		(15.07)
IMZ2 (400)	1.09±0.01	1.06±0.02	0.98±0.01*	0.92±0.01*	0.67±0.02	1.16±0.01
	(11.38)	(13.82)	(16.23)	(19.29)		(19.84)
IMZ3 (400)	0.98±0.02*	0.92±0.02**	0.83±0.01**	0.74±0.01**	0.68±0.01	1.01±0.03*
	(22.32)	(23.68)	(26.97)	(33.33)		(27.77)
IMZ4 (400)	1.08±0.01	1.01±0.01	0.96±0.02*	0.87±0.01*	0.67±0.02	1.10±0.01
	(12.19)	(15.12)	(17.94)	(23.68)		(16.66)
IMZ5 (400)	0.97±0.01*	0.90±0.01**	0.82±0.01**	0.73±0.02**	0.66±0.01	1.01±0.01*
	(21.13)	(24.36)	(29.91)	35.08)		(29.36)

Values are expressed as Mean±SEM, n = 6. *p<0.05, **p<0.01 compared with vehicle control (ANOVA followed by Dunnett's test). a: Indomethacin (10 mg kg^{-1}, p.o.) used as standard drug in Carrageenan-induced paw edema method, b: Diclofenac (100 mg kg^{-1}, p.o.) used as standard drug in Freund's adjuvant method

Table 3: Effect of imidazole derivatives on yeast induced pyrexia in rabbits

	Rectal temperature in °C			
Treatment (mg kg^{-1}, p.o.)	0 h	1 h	2 h	3 h
Control	39.91±0.02	40.87±0.07	40.99±0.01	41.03±0.03
Paracetamol (100)	39.33±0.11	39.81±0.01**	39.71±0.02**	39.55±0.02**
IMZ1 (400)	39.32±0.15	40.83±0.09	40.32±0.03*	39.72±0.01*
IMZ2(400)	39.36±0.05	40.78±0.02	40.61±0.02	40.43±0.08
IMZ3 (400)	39.24±0.02	40.27±0.02*	39.82±0.02**	39.64±0.02**
IMZ4 (400)	39.32±0.02	40.82±0.01	40.76±0.02	40.61±0.02
IMZ5 (400)	39.31±0.01	39.93±0.03*	39.85±0.01**	39.59±0.03**

Values are expressed as Mean±SEM, n = 6. *p<0.05, **p<0.01 compared with vehicle control (ANOVA followed by Dunnett's test)

Antiepileptic studies revealed that all the compounds have shown significant protection against both maximum electroshock-induced convulsions and pentylenetetrazole induced convulsions. Amongst them IMZ5 was found to be more active (103.33±1.79 sec Table 4). Acute toxicity and gross behavior studies revealed that no signs and symptoms of toxicity and mortality were observed. Hence, the test compounds in the present investigation were found to be nontoxic up to 5000 mg kg^{-1} b.wt. Hematological changes have been observed such as slight depletion of hemoglobin, hematocrit, RBC and WBC count in dose dependent manner of IMZ5 treated rats on either sex (Table 5).

The level of lymphocyte and monocyte is reduced to some extent as compared to control in dose dependent manner in IMZ5 treated groups of either sex. The platelets count is increased in treated rats in dose dependent manner. The clotting time is altered in IMZ5 treated groups of either sex and change is within the normal range (2.35±0.35-2.61±0.27×10^{3}mm^{-3}). Biochemical

Table 4: Antiepileptic activity of Imidazole derivatives in rats

	Electro-shock induced convulsions method				Pentylenetetrazole induced seizures method			
Treatment dose (mg kg^{-1} b.wt.)	Flexor (sec)	Extensor (sec)	Clonus (sec)	Stupor (sec)	Straub's tail phenomenon time of onset (sec)	Jerky movement time of onset (sec)	Onset of convulsions (sec)	Death (%)
Control	2.79±0.23	8.133±0.21	2.93±0.30	124.63±1.08	57.16±2.04	65.21±3.09	68.83±1.58	100
Standard (a and b)	2.26±0.26	0.26±0.20**	2.11±0.36	88.0±1.30	274.83±1.57	283.14±2.01	295.19±2.83	0
IMZ1 (400)	2.64±0.21	0.41±0.24*	2.67±0.28	121.23±1.02	62.56±2.47	69.8±5.37	72.4±3.12	0
IMZ2(400)	2.71±0.29	0.89±0.21	2.91±0.39	122.45±1.43	65.0±1.96	68.15±1.34	72.9±1.53	0
IMZ3 (400)	2.54±0.25	0.33±0.21**	2.83±0.30	109.6±1.07	67.83±1.93*	73.53±1.98*	79.19±0.91*	0
IMZ4 (400)	2.74±0.28	1.02±0.29	2.78±0.42	119.3±1.69	60.33±1.40	69.83±1.59	70.19±1.03	0
IMZ5 (400)	2.53±0.30	0.31±0.41**	2.38±0.25	103.33±1.79	71.33±1.38	81.35±0.98**	87.34±1.5**	0

Values are expressed as Mean±SEM n = 6. *p<0.05, **p<0.01 compared with vehicle control (ANOVA followed by Dunnett's test). a: Phenytoin (25 mg kg^{-1} i.p.) used as standard drug in electro-shock induced convulsions method, b: Diazepam (25 mg kg^{-1} i.p.) used as standard drug in pentylenetetrazole induced seizures method

Table 5: Hematological parameters of IMZ5 treated rats

	Male			Female		
	Control	500 mg kg^{-1}	1000 mg kg^{-1}	Control	500 mg kg^{-1}	1000 mg kg^{-1}
RBC[a]	8.7±0.23	8.4±0.57*	7.2±0.56*	8.45±0.34	8.27±0.64*	8.31±0.78*
Hb[b]	15.62±0.98	15.23±0.76*	14.34±0.72*	13.9±0.57	13.83±0.78*	13.43±0.38*
Hr[c]	45.56±2.08	43.67±1.34*	41.23±2.08*	44.56±2.56	43.23±1.9*	42.43±1.3*
WBC[d]	5.35±0.19	5.08±0.16*	4.69±0.14*	5.45±0.17	5.78±0.21*	5.97±0.21*
Lymp.[e]	71.21±2.21	69.9±2.16*	68.4±1.78*	83.43±1.65	82.67±1.69*	82.54±1.29*
Mono.[f]	3.93±0.39	4.0±0.71*	4.1±0.86*	2.5±0.81	3.01±0.65*	3.19±0.73*
Eosi.[g]	3.1±0.03	3.2±0.04*	4.3±0.08*	0.6±0.01	2.8±0.05*	3.54±0.05*
PLT.[h]	902.1±98	945.2±102*	988.9±79*	901.6±78.9	957.6±47.9*	948.3±50.7*
CIT.[i]	2.01±0.25	2.55±0.16*	2.35±0.35*	2.67±0.19	2.49±0.25*	2.61±0.27*

Data are expressed as Mean±SEM n = 5. All the *p values are <0.05 as compared to respective controls by Dunnett's test. [a]Red blood cell (x10^{6} mm^{-3}), [b]Hemoglobin concentration (g dL^{-1}), [c]Hematocrit (%), [d]White blood cell (x10^{3} mm^{-3}), [e]Lymphocyte (%), [f]Monocyte (%), [g]Eosinophilic leukocyte (%), [h]Platlets (x 10^{3} mm^{-3}), [i]Clotting time (min)

Table 6: Biochemical parameters of IMZ5 treated rats

	Male			Female		
	Control	500 mg kg^{-1}	1000 mg kg^{-1}	Control	500 mg kg^{-1}	1000 mg kg^{-1}
AST[b]	173.6±29.5	174.2±21.4	184.6±19.6	167.7±12.5	175.6±19.5	181.47±15.3
ALT[c]	48.2±3.9	48.7±3.5	51.3±0.69	48.09±0.92	50.2±0.45	59.2±0.89
ALP[h]	39.2±0.55	37.4±0.76	38.6±0.89	37.4±0.46	38.2±0.65	38.7±0.83
HDL[d]	35.1±4.28	38.2±5.1	41.5±4.9	36.89±3.4	36.1±2.4	37.3±2.1
Chol[e].	56.3±3.2	55.4±3.7	66.3±4.6	55.8±3.9	56.4±5.9	69.7±4.8
TG[f]	42.8±4.9	41.9±3.57	44.7±2.89	46.1±3.86	46.3±3.71	46.89±3.18
Glu[g].	100.3±1.87	97.3±3.7	86.81±4.1	119.2±2.67	101.09±4.3	89.32±2.59
TB[a]	0.23±0.05	0.25±0.06	0.26±0.03	0.22±0.03	0.23±0.07	0.25±0.02

Data are expressed as Mean±SEM n = 5. *p<0.05, **p<0.01 compared with vehicle control (ANOVA followed by Dunnett's test) [a]Total bilirubin (mg dL^{-1}), [b]Aspartate transaminase (U L^{-1}) S.G.O.T, [c]Alanine transaminase (U L^{-1}) S.G.P.T, [d]High density lipoprotein (mg dL^{-1}), [e]Cholesterol (mg dL^{-1}), [f]Triglycerides (mg dL^{-1}), [g]Glucose (mg dL^{-1}), [h]Alkaline Phosphatase (U L^{-1})

parameters showed that decrease of total bilirubin and blood glucose in both 500 and 1000 mg kg^{-1} b.wt. dose of IMZ5 treated groups as compared to control. The elevation of lysosomal enzymes such as ALP, AST and ALT level were observed in animals with treated IMZ5 in dose dependent manner. The changes were observed in HDL, cholesterol and triglycerides level but within the normal range (Table 6).

The histopathological studies revealed that some toxic changes have been observed in IMZ5 treated animals at cellular level. The structural changes like cytoplasm vacuolation and degranulation of nucleus in hepatic cells were also observed. Dilated globules and atrophied glomeruli were seen in kidney. Interestingly no toxic effects were observed on myocardium in Fig. 2.

DISCUSSION

The mechanism for testing analgesic effect was selected such that both centrally and peripherally mediated effects were investigated. The acetic acid induced abdominal constriction and tail immersion methods elucidated peripheral and central activity, respectively. This test is very useful not only for

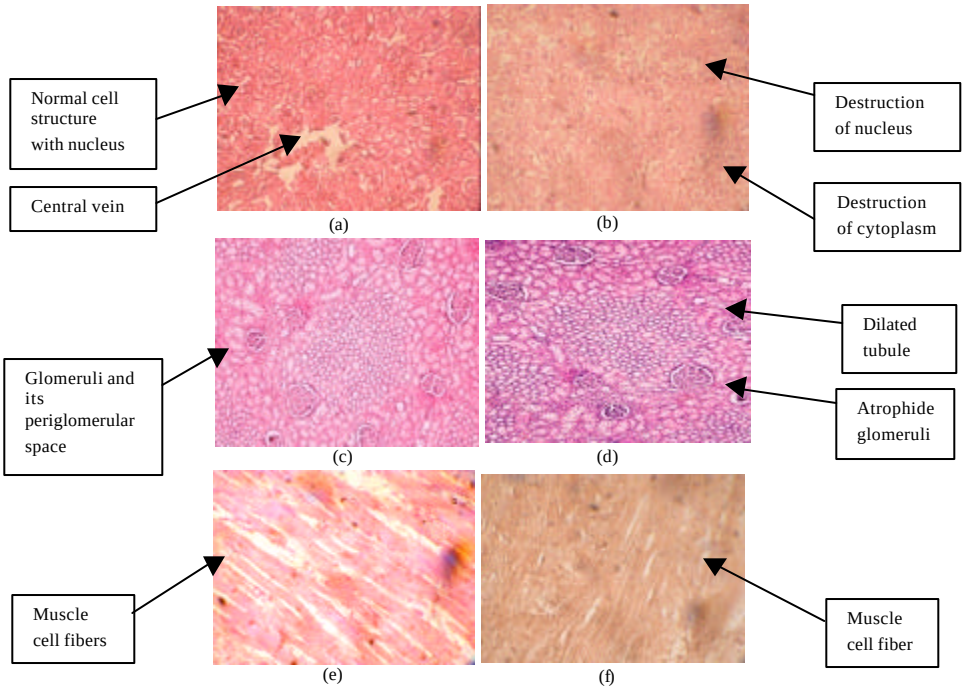

Fig. 2: Histological changes of liver, kidney and heart from IMZ5 treated rats. (a) liver from control, (b) IMZ5 liver from treated, (c) kidney from control, (d) IMZ5 kidney from treated, (e) heart from control and (f) IMZ5 heart from treated rats

assessing analgesic drugs but also helping in the elucidation of mode of action. 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. Prostaglandins cause pain (Roberts and Morrow, 2001) and sensitize the skin to painful stimuli (Dray, 1995) probably because they sensitize pain receptors to mechanical and chemical stimulation (Roberts and Morrow, 2001) such as the pain eliciting effect of mediators (e.g., histamine, kinins etc) which are released in tissue injury and inflammation. The inhibition of the synthesis of pro-inflammatory prostaglandins is one of such therapeutic targets to which some of the potent analgesic and anti-inflammatory agents of clinical relevance (e.g., NSAIDs) owe their activity (Flower and Vane, 1974). The imidazole derivatives administered orally (400 mg kg^{-1}); significantly inhibit acetic acid induced writhing in rats. The result firmly suggests that the mechanism of action of imidazoles may be linked to lipoxygenase and/or cycloxygenase inhibition. It is possible that imidazoles exert an analgesic effect probably by inhibiting prostaglandin synthesis by blocking cycloxygenase enzyme.

The non steroidal anti-inflammatory drugs exert anti-inflammatory effect principally by inhibiting the synthesis of prostaglandin an eicosanoid mediator of the inflammatory response (Foegh and Ram, 2001). The most widely used primary test to screen new anti-inflammatory agent's ability to reduce local edema induced in the rat paw by injection of an irritant agent. Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The carrageenan test was selected because of its sensitivity in defecting orally active anti-inflammatory agents particularly in the acute phase of inflammation (Di Rosa *et al*., 1971). The sub plantar injection of carrageenan in rats leads to paw edema. The early phase (1-2 h) carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorph nuclear cells and prostaglandins produced by tissue macrophages (Antonio and Souza Brito, 1998). The imidazole

derivatives reduced the carrageenan induced paw edema in rats. It may be due to inhibition of cyclooxygenase which activates prostaglandin synthesis followed by prevention of inflammatory mediator's release. The result of analgesic and anti inflammatory activity of these derivatives was in consistent with the result of Lombardino and Wiseman (1974).

In Freund's adjuvant-induced polyarthritis model, treatment with imidazole derivatives showed significant inhibitory effect on injected hind paw edema and maximum inhibition was observed on the 30th day. In the present study, the increased lymphocyte count and migration of leucocytes into the inflamed area of arthritic rats were significantly prevented with the treatment of the imidazole derivatives and the standard drug as reflected from the significant decrease in total WBC count (Saraf *et al*., 1989).

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 imidazole derivatives produce the antipyretic action by inhibiting the prostaglandin synthesis by blocking cycloxygenase isoenzymes, platelet thromboxane synthesis and prostanoids synthesis (Graham and Scott, 2003).

Inflammatory process is characterized by the involvement of multiple inflammatory cells of the WBC (Kytridis and Manetas, 2006). WBC and indices relating to it such as lymphocytes usually show increase in activity in response to toxic environment (Robins, 1974). In this study, WBC was significantly altered. The lymphocytes, the main effector cells of the immune system (McKnight *et al*., 1999) showed marginal decrease thus suggesting that the imidazoles only exerted minimal challenge on the immune system of the animals.

There is increasing evidence that lysosomal enzymes (ALT, AST and ALP) play an important role in the development of acute and chronic inflammation (Janoff and Zweifach, 1964; Anderson *et al*., 1971).

Most of anti-inflammatory drugs exert their beneficial effect by inhibiting either release of lysosomal enzymes or by stabilizing lysosomal memberane which is one of the major events responsible for the inflammatory process. The stabilization of lysosomal membranes is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases which cause further tissue inflammation and damage upon extracellular release (Chou, 1997). Some NSAIDs like indomethacin and acetylsalicylic acid are known to possess membrane stabilizing properties (Murugesh *et al*., 1981; Furst and Munster, 2001), which may contribute to the potency of their anti-inflammatory effect. In a toxic environment, blood level of AST and ALT are known to significantly increase (Adam, 1998; Crook, 2006). These two classical enzymes are reliable indices of liver toxicity. From the sub acute toxicity it can be assumed that the increased level of alkaline phosphatase, AST and ALT level may be responsible for the tissues damages in the liver and kidney. This was confirmed by the histological study in which tissue morphology showed mild changes on liver and kidney.

The histopathological studies revealed that IMZ5 has mild toxicity on liver and kidney with the exception on heart. This supports that imidazoles have no toxic effect on heart on 500 and 1000 mg kg^{-1} b.wt. The present experimental findings of both pharmacological and toxicological parameters enable to claim that imidazole derivatives are the promising non steroidal anti-inflammatory and antiepileptic agents. Hence, it can be concluded that it is worthwhile to modify the structure to obtain more potent and less toxic compound.

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