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Zinc-indomethacin complex: synthesis, physicochemical and biological evaluation in the rat

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Abstract

In continuation of our work on zinc complexes of acidic NSAIDs in order to improve their therapeutic index, zinc complex of indomethacin was synthesised and characterised by IR, NMR, UV, DSC, atomic absorption spectroscopy and elemental analysis. The pH-solubility profile at 25°C and in vitro release pattern at 37°C by dissolution method were determined for the zinc complex and compared with that of indomethacin. Zinc-indomethacin complex showed almost double the solubility and rate of dissolution at pH 6.0 as compared to the parent drug. Anti-inflammatory studies (using carrageenan-induced hind paw edema method) showed that the zinc complex is 2.99-times more potent than indomethacin and 2.55-times more potent than the corresponding physical mixture of indomethacin and zinc sulphate. ANOVA followed by Duncan's new multiple range test indicated a statistically significant difference ($p < 0.01$) among them. Ulcerogenic effects of the zinc complex were observed at 1.5-times the ED_{50} of indomethacin as well as at 1.5-times its own ED_{50}, in rats. The lesion indices obtained were compared with that of indomethacin (at 1.5-times its ED_{50}) and control and were statistically evaluated using the Kruskal-Wallis rank test. They were found to be significantly different ($p < 0.001$). The zinc complex at 1.5-times its own ED_{50} was found to be the safest with practically no ulcers at all. These studies indicate that the dose of indomethacin and hence its ulcerogenic effects may be reduced appreciably by complexing it with zinc, with no change in its therapeutic action.

Keywords: Indomethacin; Zinc-indomethacin complex; pH-solubility profile; Dissolution study; Anti-inflammatory activity; Anti-ulcerogenic activity

1. Introduction

Indomethacin, [1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]acetic acid, is a frequently prescribed non-steroidal anti-inflammatory agent with anti-inflammatory, analgesic and antipyretic properties but its efficacy is offset by significant incidence of gastrointestinal ulceration and haemorrhage. The absorption of indomethacin is associated, within minutes, with an increase in membrane permeability, leading to an abnormal efflux of Na^+ and K^+ ions into the luminal fluid and back-diffusion of H^+ ions from the lumen into the gastric mucosa. This results in an immediate reduction in mucosal potential difference (Rainsford and Willis, 1982; Price and Fletcher, 1990). Indomethacin also inhibits gastric mucosal secretion, active bicarbonate secretion from gas-

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tric mucosa and reduces mucosal blood flow (Schoen and Vender, 1989). At the same time, there is a statistically significant reduction in fundic prostaglandin levels paralleled by severe gastric mucosal damage (Rainsford and Willis, 1982).

Many attempts have been made to reduce the untoward side effects associated with NSAIDs. It has been demonstrated that Zn^{2+} possesses an anti-ulcerogenic profile (Fraser et al., 1972; Frommer, 1975) besides having anti-arthritic effects of its own (Simkin, 1976). Recently, we reported that the zinc complex of aspirin has a better therapeutic index than aspirin with improved physicochemical characteristics (Singla and Wadhwa, 1994). The data of anti-inflammatory studies using carrageenan induced hind paw edema model in rats indicated that the zinc-aspirin complex is 2.64- and 1.73-times more potent than aspirin alone and than the corresponding physical mixture of aspirin and zinc sulphate, respectively. In addition, this complex has been found to be the least ulcerogenic.

In this study, we report the synthesis, characterisation, physicochemical and biological evaluation of the zinc-indomethacin complex.

2. Materials and methods

2.1. Materials

Indomethacin (form I) of USP grade was procured from Ranbaxy Laboratories Ltd, New Delhi (India) while λ-carrageenan type IV was purchased from Sigma Chemical Co., USA. Zinc sulphate heptahydrate, sodium bicarbonate and all other chemicals of analytical reagent grade, and triple-distilled water, were used in all studies. Male albino rats (Porton strain) obtained from the Central Animal House, Panjab University, Chandigarh (India) were used for biological studies.

2.2. Preparation of zinc complex of indomethacin

Indomethacin (17.9 g, 0.05 mol) was dissolved in acetone (200 ml) while sodium bicarbonate (4.2 g, 0.05 mol) was dissolved in water (200 ml). The two solutions were mixed and after the effervescence stopped, a solution of zinc sulphate heptahydrate (7.2 g, 0.025 mol) in water (200 ml) was added dropwise with continuous agitation. The resultant white precipitate was filtered, washed first with water and then with acetone. Finally, it was dried under vacuum to give the zinc complex of indomethacin (yield: 18 g, 88.32%); m.p. 232°C (decomp.).

2.3. Instrumental methods of analysis

Infrared (IR) spectra up to 400 cm^{-1} were recorded on a Perkin Elmer 882 infrared spectrophotometer using potassium bromide pellets. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on Varian EM 390-NMR (90 MHz) and Bruker AC 300 F-NMR (300 MHz) spectrometers using tetramethylsilane as internal standard and $CDCl_3$, DMSO-d_6 as solvents.

Elemental analysis was performed on a Perkin Elmer 2400 CHN analyser. Zinc content was determined using a Pye Unicam SP 2900 atomic absorption spectrophotometer. A Karl-Fischer moisture automatic titration apparatus was used for the determination of water content. Ultraviolet (UV) spectra were recorded on a Milton Roy Spectronic 1201 UV-Vis spectrophotometer while differential scanning calorimetry (DSC) was conducted on a DuPont 2000 apparatus. The sample size used for DSC was about 5 mg and the scanning speed was 10°C/min.

2.4. pH-solubility studies of indomethacin and its zinc complex

The solubility was determined at 25°C in the pH range of 1.75–6.65 in Britton and Robinson modified universal buffer solutions consisting of acetate, phosphate and borate buffers, 0.05 M each, adjusted to a constant ionic strength (μ) of 0.175 with potassium chloride. An excess of the drug was agitated with 10 ml of each buffer in a shaking water bath (Haake, SWB 20, Germany) maintained at 25 ± 0.2°C for a time period sufficient to achieve a steady-state concentration. It was then filtered at 25°C, the equilibrium pH of

the filtrate was recorded and after making appropriate dilutions with a mixture of methanol and phosphate buffer of pH 6.0 (1:1), it was analysed spectrophotometrically at 318 nm using a standard plot of indomethacin in the same media.

2.5. Dissolution studies

Dissolution studies were performed for indomethacin and its zinc complex in a USP XXI Type 2 Dissorate Dissolution Tester (Model SC-2, Scientific Instruments and Technology Corp., NJ) using 1000 ml of phosphate buffer of pH 6.0, stirred at 100 rpm and maintained at 37 ± 0.2°C. Each sample equivalent to approx. 25 mg of indomethacin, passed through 40 mesh and retained on 80 mesh, was added to the medium in a powdered form. At appropriate time intervals, 10-ml aliquots were withdrawn and filtered, and replaced with an equal volume of fresh medium. The concentration of indomethacin dissolved was determined spectrophotometrically at 318 nm.

2.6. Determination of anti-inflammatory activity

The carrageenan-induced hind paw edema test in rats developed by Winter et al. (1962) was carried out. Young male rats, fasted overnight and weighing 100–150 g, were randomly divided into different groups, each consisting of a minimum of six rats. Test animals were administered orally aqueous suspensions (0.5 ml 100 g^{-1}) of indomethacin (3.0–24.0 mg kg^{-1}), zinc-indomethacin complex (1.71–13.68 mg kg^{-1} equivalent to 1.5–12.0 mg kg^{-1} of indomethacin), and its physical mixture (1.5–12.0 mg kg^{-1} of indomethacin plus 0.6–4.8 mg kg^{-1} of zinc sulphate). The vehicle alone in an equivalent quantity was used as a placebo for the control group. Both food and water were withdrawn during the test. Paw edema was induced 1 h after the drug or placebo administration in the right hind paw of each rat by a subplantar injection of 0.1 ml of a 1% w/v suspension of carrageenan in distilled water. The volume of injected paw was measured immediately (0 min) and at 30, 60, 90, 120 and 180 min after the injection using a plethysmometer (UGO Basile 7150, Comerio, Italy). The paw swelling was expressed as per cent edema relative to the initial (0 min) hind paw volume. The mean value of per cent edema ± SE was determined for each time interval and per cent inhibition of edema produced by each drug-treated group was calculated with respect to the control group. The plot of per cent inhibition at 3 h after injection vs log dose was used to determine the value of ED_{50} of the drug administered.

2.7. Determination of ulcerogenic effects

The approach of Dearden and Nicholson (1984) for determining the lesion index (LI) of NSAIDs at their anti-inflammatory ED_{50} values, was used. Since the ED_{50} of indomethacin (6.555 mg kg^{-1}) was not sufficient to give values of LI high enough so as to differentiate between the drug and its zinc complex, LI was determined for indomethacin and zinc-indomethacin at 1.5-times the above-mentioned ED_{50} (9.83 mg kg^{-1}) on an equimolar basis. Zinc-indomethacin complex was tested at an additional dose of 1.5-times its own ED_{50} (3.75 mg kg^{-1} equivalent to 3.29 mg kg^{-1} of indomethacin). The vehicle alone in an equivalent quantity was used as a placebo for the control group.

Male rats, fasted for 36–48 h and weighing 150–230 g, were randomly divided into four homogeneous groups, each consisting of a minimum of five rats. They were given aqueous suspension of the drug or placebo orally four times over a 2 day period. During this period no food and water were allowed. On the day after the final dose, the animals were killed, their stomachs removed, opened along the length of greater curvature and cleared of debris. Gastric mucosal damage was examined by focussing under a dissecting microscope (10 ×) and scored according to the severity of damage. A lesion index was computed for each rat by counting the number of lesions (x) in each of different size classes (y) and adding the products of x and y.

2.8. Statistical analyses

For parametric data obtained from the carrageenan-induced paw edema test, statistical sig-

nificance of the mean per cent edema of each drug-treated group at each time interval was determined with respect to that of the control group, using Student's t-test (unpaired, two-tailed). Linear regression analysis was performed on log dose vs per cent inhibition of edema at 3 h data to calculate the correlation coefficient (r), slope and intercept (y-axis) and ED_{50} with 95% confidence limits. Statistical significance of the correlation coefficient was also determined using two-tailed Student's t-test. Analysis of variance (ANOVA) one-way was performed at each dose level to evaluate the statistical significance of the differences in edematic responses caused by different treatments. In cases where the F ratio was found to be statistically significant, it was followed by Duncan's new multiple range test to determine which of the multiple treatment(s) was actually responsible for making the F ratio significant.

For non-parametric data obtained from tests to determine ulcerogenic effects, the Kruskal-Wallis rank test was used to evaluate the statistical significance of the differences in lesion indices obtained with different treatments.

To determine whether the differences obtained between the dissolution profiles of indomethacin and its zinc complex were significant, Student's t-test was applied to the pair of average values of per cent drug released for each sampling time.

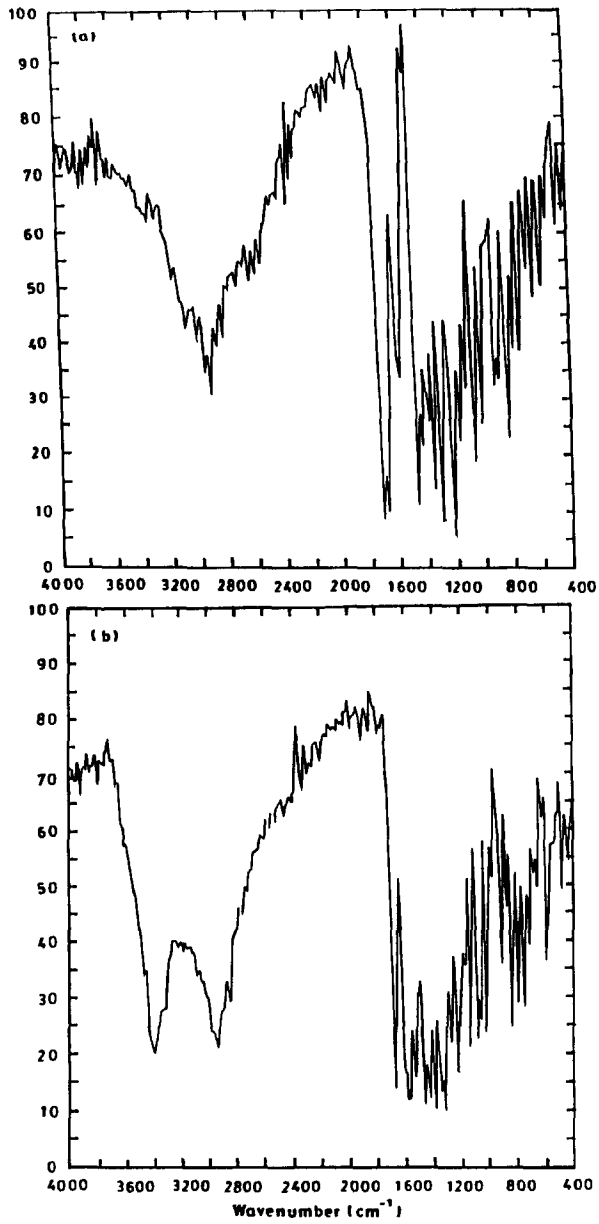


Fig. 1. IR spectra of (a) indomethacin and (b) zinc-indomethacin.

3. Results and discussion

3.1. Characterisation of zinc complex of indomethacin

In this study, a method involving the use of aqueous solutions was employed to prepare zinc-indomethacin complex. This method was more economical and less time-consuming than the method reported earlier (Singla et al., 1990). The hydrated zinc complex thus synthesised was characterised using a combined technique of spectral and elemental analyses.

The major IR peaks (Fig. 1) obtained for indomethacin and its hydrated zinc complex are as follows:

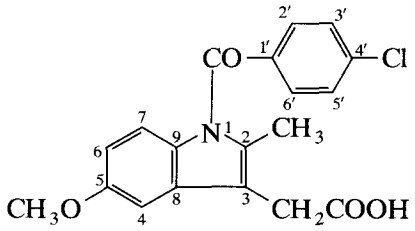
IR (indomethacin in KBr): 3400–2400 (carboxyl OH stretching), 1719 (carboxyl C=O stretching), 1692 (amide C=O stretching), 1309 (carboxyl C-O stretching), 924 (carboxyl OH bending out of plane) cm^{-1}.

IR (zinc indomethacin in KBr): 1678 (amide C=O stretching), 1586 (asymmetrical stretching of carboxylate anion), 1326 (symmetrical stretching of carboxylate anion) cm^{-1}.

Examination of the IR spectra of both the compounds revealed a definite shift in absorption for the carboxyl group and the disappearance of carboxyl OH stretching and bending. The shift occurred in the direction of longer wavelength, indicating that the carboxyl group of indomethacin is strongly involved in complexation with zinc. Donation of electrons to metal produces a lower excitation state and therefore shifts to longer wavelength (Williams et al., 1976).

Comparison of the ^{1}H-NMR spectra of the two compounds did not show much difference as the peak for proton could not be obtained in the case of indomethacin due to exchange broadening involving the water in the solvent.

The formation of the zinc complex was further confirmed by ^{13}C-NMR spectra which revealed a strong shift in the absorption of -COOH and slight shifts in the case of -CH_2, C-3, C-7 and C-8 as is clear from the data given below (Scheme 1):



Scheme 1. Structure of indomethacin.

^{13}C-NMR (indomethacin in $CDCl_3$-DMSO-d_6): δ 172.15 (-COOH), 167.74 (N-C=O), 155.64 (C-5), 138.30 (C-4'), 135.16 (C-8), 133.90 (C-1'), 130.95 (C-2', C-6'), 130.67 (C-2), 130.39 (C-9), 128.89 (C-3', C-5'), 114.57 (C-7), 113.23 (C-3), 111.16 (C-6), 101.45 (C-4), 55.295 (-OCH_3), 29.82 (-CH_2) and 13.17 (-CH_3) ppm.

^{13}C-NMR (zinc indomethacin in $CDCl_3$-DMSO-d_6): δ 176.57 (-COOH), 167.73 (N-C=0), 155.49 (C-5), 138.07 (C-4'), 134.34 (C-8), 134.06 (C-1'), 131.15 (C-2', C-6'), 130.94 (C-2), 130.34 (C-9), 128.87 (C-3', C-5'), 115.47 (C-7), 114.34 (C-3), 110.93 (C-6), 101.95 (C-4), 55.235 (-OCH_3), 31.34 (-CH_2) and 13.27 (-CH_3) ppm.

Elemental analysis of the hydrated zinc-indomethacin complex showed it to possess a ligand-metal ratio of 2:1 with two molecules of water as water of crystallisation. Anal: (Calc. for $C_{38}H_{34}N_2O_{10}Cl_2Zn$) C, 55.99; H, 4.20; N, 3.44; and Zn, 8.02. Found: C, 54.84; H, 4.39; N, 3.25; and Zn, 8.81.

The presence of two molecules of water in the zinc complex was further confirmed by Karl-Fischer testing for water determination which gave the water content equal to 5.05% (Calc. for $2H_2O$: 4.42%).

The ultraviolet spectra of indomethacin and its zinc complex in a mixture of methanol and phosphate buffer of pH 6.0 (1:1), after dissolving each of the two drugs in a minimum quantity of dimethylformamide, showed maximum absorption at 318 nm. The linearly regressed equation calculated from the standard plot of indomethacin in the medium and wavelength mentioned above, gave the content of indomethacin equal to 87.435% in the zinc complex (Calc. for $(C_{19}H_{15}ClNO_4)_2Zn \cdot 2H_2O$: 87.56%)

DSC curves for indomethacin, the physical

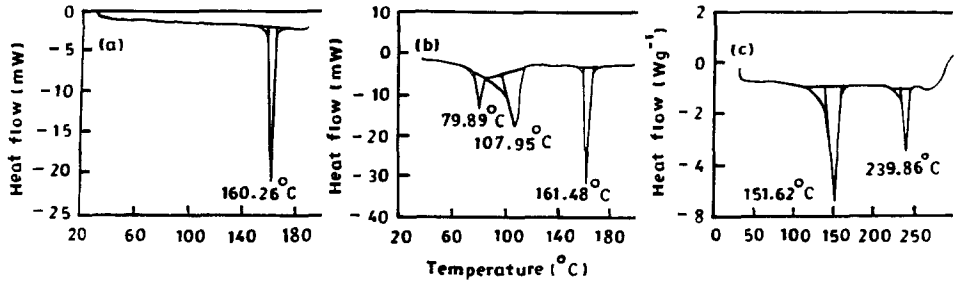


Fig. 2. DSC curves of (a) indomethacin; (b) physical mixture of indomethacin and zinc sulphate; and (c) zinc-indomethacin.

mixture of indomethacin and zinc sulphate, and zinc-indomethacin complex, are shown in Fig. 2. The endothermic peak of indomethacin at 160.26°C (Fig. 2a) disappeared completely in the zinc complex (Fig. 2c), with the appearance of two new endothermic peaks. The first one at 151.62°C is broader and is probably due to loss of water of crystallisation of the zinc complex, as broader endotherms indicate a slow change in heat capacity and cover behaviours like dehydration (Willard et al., 1986). The second peak at 239.86°C is sharper and is probably due to melting with decomposition, of the zinc complex as sharp endotherms are indicative of crystalline rearrangements, fusions or solid state transitions of relatively pure materials (Willard et al., 1986).

3.2. pH-solubility profile

The buffer pH-solubility profiles of indomethacin and its zinc complex at 25°C shown in Fig. 3 indicate that in the buffer pH range 4.65–6.65, the solubility of the zinc complex, in terms of indomethacin, is 1.5–2.5-times that of indomethacin.

It is well established that the absorption of an orally administered drug is mainly governed by its solubility and membrane-water partition coefficient. It has been found that drugs having an octanol-water partition coefficient of 100 or more (log $P > 2$) are well absorbed, provided they are given in doses that do not exceed their solubility in the gastric medium (Yalkowsky and Morozowich, 1980). This means that if log P is greater than 2.0, it is the solubility of the drug which plays a more important role in absorption. Since the values of log P for indomethacin as well as its zinc complex are greater than 3.0 (exact values could not be determined with accuracy because of very high log P values), an increase in the solubility of zinc-indomethacin complex may probably enhance its rate and extent of absorption as compared to the parent drug.

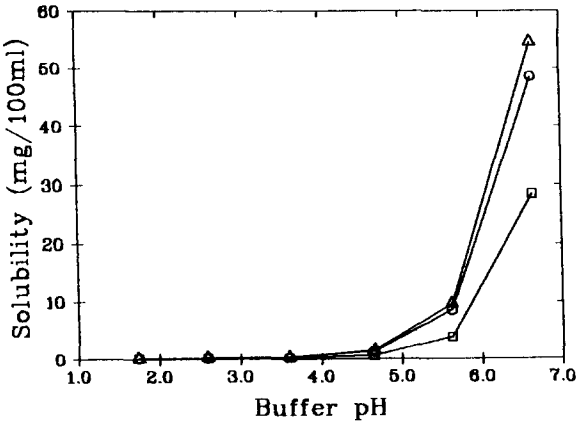


Fig. 3. pH-solubility profiles at 25°C: (□) indometachin, (△) zinc-indomethacin complex and (○) zinc-indomethacin complex in terms of indomethacin.

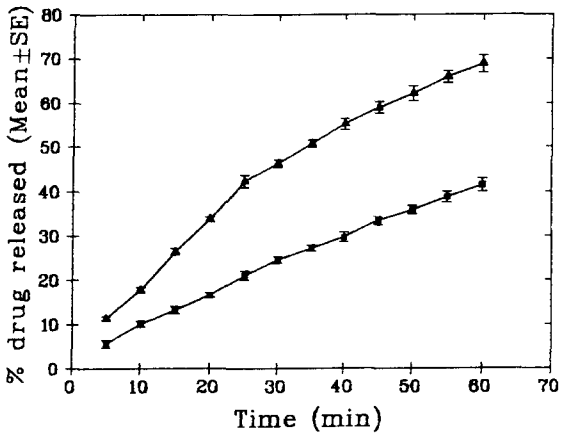


Fig. 4. Dissolution profiles at 37°C in phosphate buffer of pH 6.0: (■) indomethacin and (▲) zinc-indomethacin complex.

3.3. In vitro release pattern (dissolution studies)

The dissolution profiles of indomethacin and its zinc complex at 37°C in pH 6.0 phosphate buffer are shown in Fig. 4. It is evident from the profiles that the release of indomethacin in vitro is significantly enhanced by complexation with zinc at all time intervals studied ($p < 0.001$). At 60 min, nearly 70% of indomethacin is dissolved from the zinc complex as compared to just 40% from drug alone. The enhanced dissolution rate of the zinc complex is in direct correlation with its increased solubility (C_s) at the same pH (Fig. 3), thus conforming to Noyes-Whitney's equation for rate of dissolution.

Table 1
Effects of oral administration of indomethacin, zinc complex of indomethacin and physical mixture of indomethacin and zinc sulphate on carrageenan-induced paw edema in rats

Treatment	Dose (mg kg^{-1})	No. of rats	Edema (%) ± SE										Inhibition (%) ± SE	Statistical parameters after linear regression of log dose vs % inhibition data	ED_{50} (mg kg^{-1}) with 95% confidence limits
			30 min		60 min		90 min		120 min		180 min				
Control	–	29	22.82	±2.41	32.28	±3.25	45.82	±3.59	64.68	±3.53	80.29	±1.51	–		
Indomethacin	3.0	6	40.07	±1.05	36.96	±2.08	44.40	±3.21	44.64 [b]	±6.36	49.22 [d]	±7.22	38.68 ± 8.99	% inhibition = 31.2204 log dose + 24.5067 $r = 0.9979$ ($p < 0.01$)	6.555 ± 1.17
	6.0	6	27.30	±7.04	34.00	±7.21	37.05	±4.12	32.02 [d]	±7.38	40.44 [d]	±8.01	49.63 ± 9.98		
	12.0	6	13.33	±2.53	22.62	±2.77	19.48 [c]	±2.39	25.59 [d]	±2.20	33.16 [d]	±3.60	58.69 ± 4.48		
	24.0	6	14.72	±2.52	15.14 [b]	±1.68	16.63 [c]	±1.48	17.60 [d]	±2.32	26.50 [d]	±2.31	66.99 ± 2.87		
Zinc complex of indomethacin	1.71 (1.50) [a]	6	11.30	±2.35	23.07	±6.55	20.83 [c]	±4.60	31.33 [d]	±5.77	46.06 [d]	±2.32	42.62 ± 2.89	% inhibition = 41.9490 log dose + 35.7269 $r = 0.9989$ ($p < 0.01$)	2.50 ± 1.32 (2.19 ± 1.16)
	3.42 (3.0)	6	23.04	±2.31	23.46	±2.76	27.36 [b]	±4.22	39.12 [c]	±5.17	34.68 [d]	±3.94	56.80 ± 4.91		
	6.84 (6.0)	6	17.90	±5.87	23.54	±4.21	28.02 [b]	±3.98	30.51 [d]	±5.72	25.90 [d]	±5.97	67.73 ± 7.44		
	13.68 (12.0)	6	16.96	±3.74	21.58	±2.59	23.05 [c]	±2.59	19.98 [d]	±2.64	15.19 [d]	±1.20	81.07 ± 1.49		
Indomethacin plus zinc sulphate	1.5 + 0.6	6	12.35	±2.02	20.29	±2.02	30.47	±2.22	43.05	±2.94	61.75 [d]	±2.58	23.07 ± 3.22	% inhibition = 48.8559 log dose + 13.5294 $r = 0.9782$ ($p < 0.05$)	5.58 ± 1.70
	3.0 + 1.2	7	23.14	±2.47	31.98	±3.22	34.94	±4.14	48.92 [b]	±5.08	49.49 [d]	±6.13	38.35 ± 7.64		
	6.0 + 2.4	6	19.73	±1.85	26.90	±3.87	25.94	±2.14	33.46 [d]	±2.88	43.58 [d]	±2.71	45.71 ± 3.37		
	12.0 + 4.8	8	8.76 [c]	±2.25	15.11	±3.76	18.56 [d]	±4.02	28.08 [d]	±7.02	24.38 [d]	±6.28	69.64 ± 7.83		

[a] Values in parentheses are the equivalent quantities of indomethacin.
[b] $p < 0.05$, [c] $p < 0.01$, [d] $p < 0.001$: significantly different from control (when determined by Student's t-test).

Table 2
Analysis of variance (ANOVA) and Duncan's new multiple range test for the values of mean per cent edema produced by different groups

Treatment	Dose (mg/kg)	Analysis of variance (ANOVA) one way					Duncan's new multiple range test: $A_k(5\%\text{ allowance}) = \frac{t_k}{\sqrt{2}}\sqrt{\left[S^2(1/n_i + 1/n_j)\right]}$
		Source	Sum of squares	Degrees of freedom	Mean square	F ratio	
Control	–	treatment	15402.244	3	5134.0813	41.3994	$X_{con.} - X_{comp.} = 45.61 > 11.0198$ [b]
Indomethacin	3.0	error	5456.584	44	124.0133	($p < 0.01$)	$X_{ind.} - X_{comp.} = 14.545 > 13.1403$ [b]
Zinc-indomethacin	3.42 (3.0) [a]	total	20858.828	47			$X_{phy.} - X_{comp.} = 14.81 > 13.3189$ [b]
Indomethacin plus zinc sulphate	3.0 + 1.2						$X_{con.} - X_{ind.} = 31.065 > 10.7372$ [b] $X_{con.} - X_{phy.} = 30.80 > 9.5834$ [b] $X_{phy.} - X_{ind.} = 0.265 < 12.6617$
Control	–	treatment	22229.639	3	7409.88	62.9214	$X_{con.} - X_{comp.} = 54.39 > 10.7384$ [b]
Indomethacin	6.0	error	5063.862	43	117.764	($p < 0.01$)	$X_{ind.} - X_{comp.} = 14.54 > 12.8049$ [b]
Zinc-indomethacin	6.84 (6.0)	total	27293.501	46			$X_{phy.} - X_{comp.} = 17.68 > 13.4696$ [b]
Indomethacin plus zinc sulphate	6.0 + 2.4						$X_{con.} - X_{ind.} = 39.85 > 10.4631$ [b] $X_{con.} - X_{phy.} = 36.71 > 9.9468$ [b] $X_{phy.} - X_{ind.} = 3.14 < 12.8049$
Control	–	treatment	38134.946	3	12711.6487	127.3754	$X_{con.} - X_{comp.} = 65.095 > 9.8854$ [b]
Indomethacin	12.0	error	4490.852	45	99.7967	($p < 0.01$)	$X_{ind.} - X_{comp.} = 17.965 > 12.3995$ [b]
Zinc-indomethacin	13.68 (12.0)	total	42625.798	48			$X_{phy.} - X_{comp.} = 9.185 < 11.0258$
Indomethacin plus zinc sulphate	12.0 + 4.8						$X_{con.} - X_{ind.} = 47.13 > 9.1567$ [b] $X_{con.} - X_{phy.} = 55.91 > 8.5757$ [b] $X_{ind.} - X_{phy.} = 8.78 < 11.0258$

[a] Values in parentheses are the equivalent quantities of indomethacin.
[b] $p < 0.05$.

3.4. Biological activity of zinc complex of indomethacin

3.4.1. Anti-inflammatory activity

As can be seen from Table 1, the inhibition of edema at 3 h after carrageenan injection is dose-dependent in all the three cases. The values of ED_{50} for each treatment and other statistical parameters calculated after linear regression of log dose vs per cent inhibition of edema data are also listed. One-way ANOVA, when carried out at each dose level, indicated a statistically significant difference ($p < 0.01$) in the edematic responses for different treatments, as shown by the F ratios in Table 2. This was followed by Duncan's new multiple range test which showed that per cent edema obtained with zinc-indomethacin complex was significantly different ($p < 0.05$) from the control as well as from the parent drug at all the doses, and from the physical mixture at 3.0 and 6.0 mg kg^{-1} equivalent of indomethacin. Per cent edema obtained in the case of indomethacin and physical mixture was significantly different from the control at all the doses but no statistically significant difference was found between the two, when compared with each other.

The values of ED_{50} in Table 1 show that the zinc-indomethacin complex is 2.99-times more potent as an anti-inflammatory agent than pure indomethacin. This may be attributed mainly to a greater rate and extent of absorption of indomethacin from the complexed form, which may be due to an appreciable increase in solubility and enhanced rate of dissolution of the zinc complex, as mentioned earlier (Fig. 3 and 4). Although zinc is known to exhibit anti-inflammatory activity of its own through various mechanisms (Nugteren et al., 1966; Chvapil et al., 1972; Chvapil, 1973; Karl et al., 1973; Yamamoto and Takahashi, 1975), its contribution towards increase in potency seems to be minimal in the case of zinc-indomethacin as compared to zinc-aspirin where it was quite significant (Singla and Wadhwa, 1994). The reason behind this difference may be that the content of zinc is very small in zinc-indomethacin, i.e., 0.60 mg kg^{-1} (at the ED_{50} of indomethacin) as compared to 34.60 mg kg^{-1} of zinc (at the ED_{50} of aspirin) in the zinc-aspirin complex. The same reason may be given to explain the increase in potency of only 1.17-times in the case of the physical mixture of indomethacin and zinc sulphate with respect to indomethacin as

Table 3
Values of lesion index (LI) and their corresponding ranks, obtained for each rat in different groups

Rat	Indomethacin (9.83 mg kg^{-1}) ($n = 10$)		Zinc-indomethacin (9.83 mg kg^{-1} equiv. of indomethacin) ($n = 11$)		Zinc-indomethacin (3.29 mg kg^{-1} equiv. of indomethacin) ($n = 5$)		Control ($n = 5$)	
	LI	Rank	LI	Rank	LI	Rank	LI	Rank
1	120.0	26	24.00	12	0.50	3	0.00	1
2	25.0	13	113.00	25	0.50	3	3.25	9
3	20.5	11	43.75	20	0.50	3	4.00	10
4	30.5	16	57.25	23	1.50	7	3.00	8
5	53.0	22	29.00	15	0.75	5.5	0.75	5.5
6	32.5	17	50.50	21	–	–	–	–
7	128.5	27	36.00	19	–	–	–	–
8	33.1	18	134.25	30	–	–	–	–
9	73.5	24	132.25	29	–	–	–	–
10	176.0	31	28.88	14	–	–	–	–
11	–	–	129.88	28	–	–	–	–
Av. LI	69.26		70.80		0.75		2.20	
ΣRank		205		236		21.5		33.5
Av. Rank		20.5		21.45		4.3		6.7

compared to 1.52 in the case of aspirin (Singla and Wadhwa, 1994).

3.5. Ulcerogenic effects

The physical mixture of indomethacin and zinc sulphate was not tested for ulcerogenicity since it did not possess any significant advantage over indomethacin, as can be seen from their respective ED_{50} values in Table 1. The values of the lesion indices in Table 3 show that the degree of ulceration was almost identical in the drug and its zinc complex, when administered at the same dose level of 9.83 mg kg^{-1} of indomethacin, and this was much higher than that of the zinc complex corresponding to 3.29 mg kg^{-1} of indomethacin, where it was almost zero. These lesion indices (LI) were analysed statistically for significance by the Kruskal-Wallis rank test (Schefler, 1980) and the Kruskal-Wallis statistic (H) was calculated as:

$$H = \frac{12}{n_T(n_T+1)}\left[\frac{\left(\sum R_a\right)^2}{n_a} + \frac{\left(\sum R_b\right)^2}{n_b} + \frac{\left(\sum R_c\right)^2}{n_c} + \frac{\left(\sum R_d\right)^2}{n_d}\right] - 3(n_T+1)$$

where ΣR_a, ΣR_b, ΣR_c and ΣR_d are the sums of ranks for indomethacin, zinc-indomethacin (corresponding to 9.83 mg kg^{-1} of indomethacin), zinc-indomethacin (corresponding to 3.29 mg kg^{-1} of indomethacin) and control, respectively. n_a, n_b, n_c and n_d denote the respective number of rats used and n_T is the total number of rats. A value of H equal to 19.9503 was obtained ($H_{crit.}$ = 16.30), which indicated the existence of statistically significant differences between the groups at the 0.001 probability level.

The lesion index of the zinc complex at 1.5-times the ED_{50} of indomethacin was equal to the LI of an equivalent amount of indomethacin (Table 3). This is in contrast with the direct gastroprotective effect exhibited by zinc in the case of zinc-aspirin complex (Singla and Wadhwa, 1994). This may be attributed to two factors. Firstly, indomethacin undergoes extensive enterohepatic circulation in the bile (Brune et al., 1987) with possible intestinal reflux into the stomach (Price and Fletcher, 1990), leading to extensive gastric mucosal damage, this action being very rare in the case of aspirin (CSM Update, 1986). Zinc-indomethacin complex, after being absorbed, has the same fate as indomethacin, leading to the same degree of ulceration when administered at an equivalent dose. The second factor may be the negligible direct gastroprotective effect shown by a very small content of zinc (0.9 mg kg^{-1}) present in zinc-indomethacin complex, even at 1.5-times the ED_{50} of indomethacin, as compared to the high zinc content (34.60 mg kg^{-1}) in zinc-aspirin complex at the ED_{50} of aspirin.

Zinc-indomethacin complex, when administered at 1.5-times its own ED_{50} dose level, was the safest with practically no ulcers at all. This is in accordance with the findings of Dearden and Nicholson (1984) that as anti-inflammatory potency of a NSAID increases, its gastric irritancy as measured by LI_{50}, i.e., the LI at its anti-inflammatory ED_{50} (which is a clinically realistic measure) decreases.

4. Conclusion

The zinc complex of indomethacin has been synthesised by a modified method, characterised and has been found to possess a ligand-metal ratio of 2:1. In spite of a very small content of zinc present in it as compared to zinc-aspirin, zinc-indomethacin is 3-times more potent than the parent drug which may be mainly due to an appreciable increase in its solubility and dissolution rate. Its ulcerogenic effects are negligible when administered at 1.5-times its own ED_{50}. This indicates that the dose of indomethacin and hence its ulcerogenic effects may be reduced significantly by complexing it with zinc, without affecting its therapeutic action.

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