Analgesic Activities of Synthesized Divalent Metal Complexes of Tolfenamic Acid

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ABSTRACT: The objective of the study was to synthesize and to uncover the potentially interesting biological peripheral and central analgesic activities of some new divalent metal complexes of tolfenamic acid. The inception of the study was carried out with the formation of complexes $[Cu(tolf)_2(H_2O)]_2$, $[Co(tolf)_2(H_2O)_2]$ and $[Zn(tolf)_2(H_2O)]$ through the reaction of tolfenamic acid, a potent anti-inflammatory drug, with Cu, Co and Zn salts. Characterization of the metal complexes of tolfenamic acid was furnished with spectral (FTIR, UV-Visible) and calorimetric (DSC) analysis. The peripheral analgesic activities of the complexes were investigated by acetic acid-induced writhing method. In peripheral analgesia model, Cu, Co and Zn complexes of tolfenamic acid showed significantly promising analgesic potency at a dose of 25 mg/kg body weight with percentage of inhibition of acetic acid induced writhing by 49.67% ($p < 0.01$), 67.32% ($p < 0.001$) and 72.55% ($p < 0.001$), respectively compared to the standard tolfenamic acid 46.41%. Radiant heat tail-flick test was used to observe the central analgesic activity which showed analgesic effect evidenced by % elongation time (34.59%, 25.34% and 53.08%), respectively compared to that of morphine 2 mg/kg body weight at an equimolar dose of tolfenamic acid at 10 mg/kg body weight after 30 minutes. In the final analysis, it can be stipulated that newly synthesized stable metal complexes of tolfenamic acid have promising pharmacological effects like peripheral and central analgesic potency. Therefore, these complexes may be the better therapeutic options in the near future however it needs further extensive analysis in the pharmacokinetics, pharmacodynamics and toxicology perspective.

Key words: Tolfenamic acid, metal complex, characterization and analgesic activity.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are also known as non-steroidal anti-inflammatory agents/analgesics (NSAIAs) or non-steroidal anti-inflammatory medicines (NSAIMs). These drugs have proved to be effective in the treatment of acute and chronic painful and inflammatory musculoskeletal conditions.[1]

Tolfenamic acid *i.e.*, 2-[bis (3-chloro-2-methyl-phenyl)-amino] benzoic acid or Htolf is a potent, well-tolerated NSAID with anti-inflammatory, analgesic and antipyretic effects.

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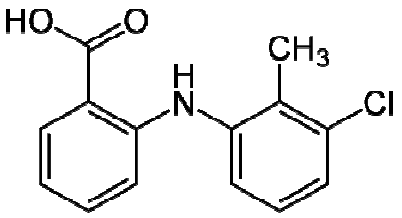

Tolfenamic acid

Tolfenamic acid acts by inhibiting prostaglandin and leukotriene synthesis. It is used to treat the symptoms of migraine. Chemically, it resembles mefenamic and flufenamic acids, other fenamates in clinical use. Tolfenamic acid inhibits COX-2 pathway preferentially.[2] Transition metals have an important place within medicinal biochemistry. Many

researchers have shown significant progress in utilization of transition metal complexes as drugs to treat several human diseases like carcinomas, lymphomas, infection, inflammation, diabetes and neurological disorders. Transition metals exhibit different oxidation states and can interact with a number of negatively charged molecules. This activity of transition metals has started the development of metal-based drugs with promising pharmacological application and may offer unique therapeutic opportunities.[3] Metal-based drug is a research area of increasing interest for inorganic pharmaceutical and medicinal chemistry and has concentrated much attention as an approach to new drug development.

Recent studies revealed that, in addition to arthritis and pain, cancer and neurodegenerative diseases like Alzheimer's disease could potentially be treated with COX-2 inhibitors. Tolfenamic acid selectively inhibits COX-2 pathway to exert its anti-inflammatory, analgesic and antipyretic effects.

The undertaken study was focused on the synthesis of some novel metal complexes (Cu, Co and Zn complexes) of tolfenamic acid to screen better pharmacological profile compared to parent drug and also to investigate peripheral and central analgesic activities of metal complexes of tolfenamic acid.

MATERIALS AND METHODS

Drugs and materials. Tolfenamic acid was obtained from Eskayef Bangladesh Limited. Chemical salts of highest purity were collected from Advanced Chemical Industries Limited. Morphine was purchased from Popular Pharmaceuticals Ltd. Vincristine sulfate and ciprofloxacin were collected from Square Pharmaceuticals Ltd., Bangladesh.

Solvents and reagents. Dimethyl sulfoxide and sodium bicarbonate were purchased from Merck, Darmstadt, Germany. Acetone, Tween-80, methanol and 1, 1-dipheny-l-2-picryl hydrazyl were collected from Sigma Chemicals, USA. Normal saline was obtained from Opsonin Pharma Ltd. All chemicals and reagents were of analytical grade.

Synthesis of metal complexes. To a solution of tolfenamic acid (0.262 g, 1 mmol) in methanol (5 ml) was added a solution of $CuSO_4.5H_2O$ (0.249 g, 1 mmol) in methanol. Drops of sodium methanoate (MeONa) were added till the apparent pH value was ~7. The reaction mixture was stirred at room temperature for 2 h & cooled to 5°C in a refrigerator for 4 h. The precipitate was collected by filtration, washed with a mixture of H_2O: MeOH (1:5) & dried in a vacuum to afford $[Cu(tolf)_2(H_2O)]_2$. A solution of $CoCl_2.6\ H_2O$ (0.095 g, 0.4 mmol) in MeOH (5 ml) and a solution of $ZnSO_4.7\ H_2O$ (0.115 g, 0.4 mmol) in MeOH (5 ml) were added to a solution of tolfenamic acid (0.209 g, 0.8 mmol) in MeOH (5 ml) separately. Drops of a methanolic solution of 1N NaOH were added to two different solutions until the apparent pH value was ~7. The two mixtures were stirred at room temperature for 1 hour and cooled to 5°Cin a refrigerator for 4hours. After the addition of a few drops of distilledH_2O, a pale pink powder and a light yellow powder were precipitated respectively. The two precipitates were collected by filtration and recrystallized from hot MeOH. The powders were washed with cold MeOH: H_2O (5:1) and dried in vacuum to afford $[Co(tolf)_2(H_2O)_2]$ and $[Zn(tolf)_2(H_2O)]$ respectively. The yield values were 53%, 32% and 15% of tolfenamic acid complexes with copper, cobalt and zinc, respectively.

Experimental animals. Adult Swiss-albino mice 25-30 g ages of 4-5 week were used as experimental animal. They were housed properly and kept under well controlled temperature with humidity of 60-70% in the animal house and kept before the test for 5-7 days because of their environmental sensitivity.

Instrumental measurements. FT-IR Spectra were analyzed with FT-IR 8400S Shimadzu, Japan, spectrophotometer within the range 4000-400 cm^{-1} to determine the functional groups. A Shimadzu Model-UV-1800 spectrophotometer was used to observe UV-Vis spectra of tolfenamic acid and its metal complexes. DSC is a thermo-analytical measurement technique and was performed by DSC-60 WS,

Shimadzu, Japan furnished with computer and convenient software program.[4]

Peripheral analgesic activity. Peripheral analgesic activity can be evaluated by acetic acid-induced Writhing method.[5] Acetic acid was administered intra-peritoneally to the experimental animals to create pain sensation. As a result, the animals squirms their body at regular interval out of pain. This squirm or contraction of the body is termed as "writhing". As long as the animals feel pain, they continue to give writhing. Each writhing is counted and taken as an indication of pain sensation. Any substance that has got analgesic activity is supposed to lessen the number of writhing of animals within in a given time frame and with respect to the control group. The writhing inhibition of positive control was taken as standard and compared with test samples and control. As positive control, any standard NSAID drug can be used. In the present study, tolfenamic acid was used as a standard drug. The test was performed by taking samples at doses of 25 mg/kg body weight. The degree of analgesia or the percentage of inhibition of writhing was calculated by using the following formula:

(Mean of control group – Mean of treated group)/ Mean of control group × 100.

The result of statistical analysis for animal experiment were expressed as mean ± SEM; n=5 considering 95% confidence level at $p<0.05$ being considered as significant.

Central analgesic activity. Tail flick assay of animal models was used to determine central analgesic response.[6] A hot wire was applied to mice tail, which acted as pain stimulus. When the stimulus exceeded the threshold, rat showed instant withdrawal of its tail.[7] In this experiment, test samples and saline were administrated orally. Tail flicking time was taken by analgesiometer (Medicraft-India). For making the wire hot, current was passed through the wire. The animals flick the tail aside. The time of withdrawal of the tail was recorded. Percentage of time elongation was calculated using the following formula:

% elongation of reaction time = (Average reaction time of test group - Average reaction time of control group)/ Average reaction time of control group.

The central analgesic activity of the test samples were compared in respect to morphine. Statistical analysis was done using Statistical Package for Social Science (SPSS) software by one-way ANOVA considering 95% confidence level at $p<0.05$ being considered as significant.

Statistical analysis. Statistical analyses were done by using the Statistical Package for Social Science version (SPSS)16.0 software, and statistical differences between groups were analyzed by one-way analysis of variance ANOVA followed by Dunnet t-tests. Data's were represented as means ± SEM and differences were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

An entrenched method which was slightly modified to prepare the metal complexes of tolfenamic acid was followed which was more economical.[8] The metal complexes of tolfenamic acid were characterized by both spectral and physicochemical analysis.

FTIR. From the IR spectra of the synthesized metal complexes showed a characteristic bands of absorption by which they were identified.[9] As the carboxyl H-atom is more acidic than the amino H-atom the deprotonation occurs in the COOH group. This is confirmed by the IR spectra of the complexes, showing the characteristic bands for the secondary amino groups and for the coordinated carboxylate group. A broad absorption at 3600-3400 cm^{-1} in the spectra of the complexes was attributed to the presence of coordinated H_2O shown if table 1.The absence of large systematic shifts of the NH bands (in the region of 3360-3310 cm^{-1} due to N-H stretch & 1550-1450 cm^{-1} due to N-H bend) in the spectra of the complexes compared with those of the ligand tolfenamic acid indicates that there is no interaction between the NH group and the metal ions. The corresponding bands of C=O conjugation of the carboxyl group of the prepared complexes are at

1615 – 1580 cm^{-1}. When compared with that of the ligand tolfenamic acid, it is found that there are significant differences in the frequency of the complexes & ligands which indicate the formation of metal complex with ligand tolfenamic acid which are presented in figure 1.

Table 1. Assignment of the bands of tolfenamic acid and its metal complexes.

Compounds	Frequency (cm^{-1})	Tentative assignment
T	3339	N-H stretch of secondary amine
	1499	N-H bend of secondary amine
	1566	C=O conjugation of -COOH
T-Cu	3400	Coordinated H_2O
	3340	N-H stretch of secondary amine
	1500	N-H bend of secondary amine
	1581	C=O conjugation of -COOH
T-Co	3624	Coordinated H_2O
	3324	N-H stretch of secondary amine
	1494	N-H bend of secondary amine
	1578, 1607	C=O conjugation of -COOH
T-Zn	3409, 3508	Coordinated H_2O
	3337	N-H stretch of secondary amine
	1461	N-H bend of secondary amine
	1582, 1614	C=O conjugation of -COOH

(T: Tolfenamic acid, T-Cu: Copper complex of Tolfenamic acid, T-Co: Cobalt complex of Tolfenamic acid, T-Zn: Zinc complex of Tolfenamic acid)

Table 2. UV-visible spectrometric data of tolfenamic acid and its metal complexes.

Compound	λ_{max}	Absorbance
T	287	1.208
T-Cu	287	0.331
T-Co	287	0.181
T-Zn	287	0.043

(T: Tolfenamic acid, T-Cu: Copper complex of Tolfenamic acid, T-Co: Cobalt complex of Tolfenamic acid, T-Zn: Zinc complex of Tolfenamic acid).

UV. Tolfenamic acid and its metal complexes were mixed in chloroform solution and UV-visible light was introduced set at 200 – 400 nm range to observe characteristic absorbance. By observing the ultraviolet spectra of tolfenamic acid and its metal complexes revealed that there was clear hypochromic shift given in figure 2. The respective absorbance of tolfenamic acid and its complexes are shown in table 2. All three metal complexes (Cu, Co and Zn) showed hypsochromic shift from parent tolfenamic acid. Tolfenamic acid and its complexes showed peaks of different intensity in the same wavelength, which supported the formation of complexes.

DSC. DSC is an established method for the analysis of synthetic drugs. There was a detectable change in the melting point of the complexes of tolfenamic acid compared with parent tolfenamic acid. The characteristic endothermic peak of tolfenamic acid was at 214.59°C and completely disappears for all the complexes shown in figure 3. The sharper peaks at 77.17°C, 80.40°C and 59.57°C for tolfenamic-Cu, tolfenamic-Co and tolfenamic-Zn correspondingly were probably due to evaporation of water of crystallization. It indicates the gradual change in heat capacity along with evidence of dehydration. The peaks at 200.97°C, 110.55°C and 99.44°C for tolfenamic-Cu, tolfenamic-Co and tolfenamic-Zn complexes, consequently showed the melting point of the metal complexes. Therefore, presence of

Table 3. Peripheral analgesic activity of tolfenamic acid and its metal complexes.

Groups	Dose (mg/kg b.w.)	Number of writhing	% of Inhibition of writhing
Control	-	76.5 ± 15.87	-
Standard(Tolfenamic acid)	25	41 ± 9.12**	46.41
Tolfenamic acid-Copper	25	38.5 ± 10.98**	49.67
Tolfenamic acid-Cobalt	25	25 ± 9.68***	67.32
Tolfenamic acid-Zinc	25	21 ± 7.42***	72.55

Number of writhing expressed as the mean ± SEM. Significant at ***p<0.001, **p<0.01, *p<0.05 compared to control group.

characteristic new endothermic peaks and changes of melting point may be the evidence of complexation.

Peripheral analgesic activity. The peripheral analgesic activities of metal complexes of tolfenamic acid in acetic acid-induced writhing method are given in table 3. The test was performed by taking samples at doses of 25 mg/kg body weight. Cu, Co and Zn complexes of tolfenamic acid showed significant analgesic activity with percentage of inhibition of acetic acid induced writhing 49.67% ($p < 0.01$), 67.32% ($p < 0.001$) and 72.55% ($p < 0.001$), respectively compared to the standard tolfenamic acid (46.41%, $p < 0.01$).

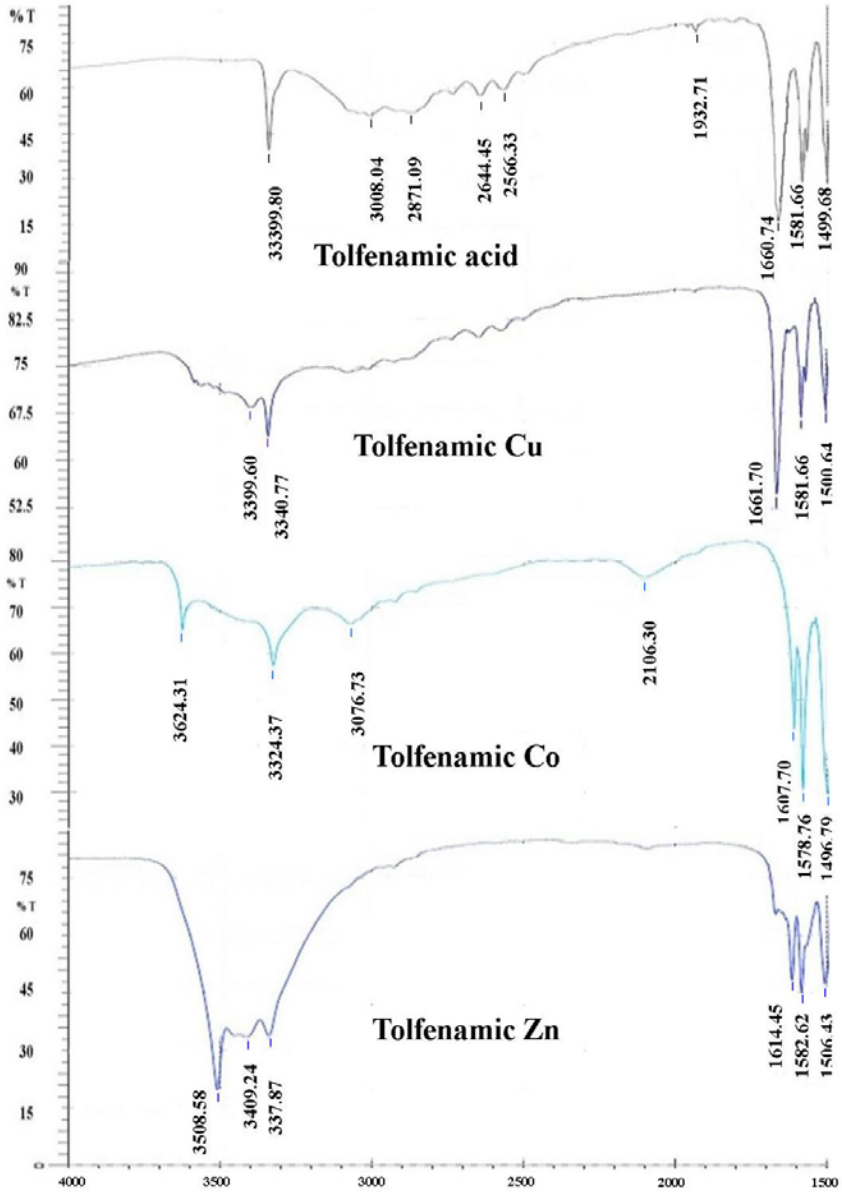

Figure 1. Combined FTIR spectrum of tolfenamic acid and its complexes.

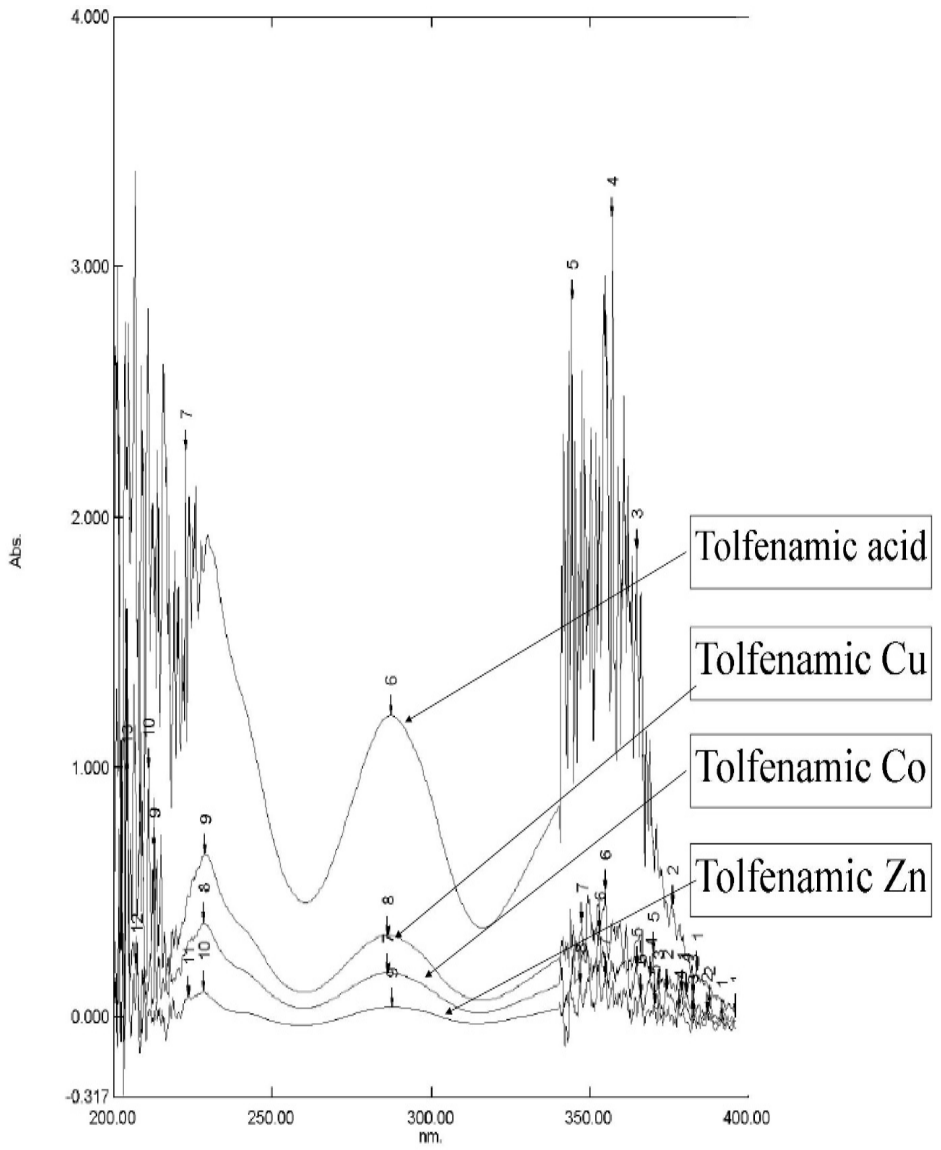

Figure 2. Combined UV- visible spectrum of tolfenamic acid and its complexes.

Table 4. Central analgesic activity of tolfenamic acid and its metal complexes.

Groups	Dose (mg/kg b.w.)	Reaction time (sec)		
		% Elongation (30 min)	% Elongation (60 min)	% Elongation (90 min)
Control	-	5.84 ± 0.44	6.62 ± 0.20	6.98 ± 0.20
Standard	2	15.3 ± 0.89, 161.99***	14.08 ± 0.76, 112.69***	11.54 ± 0.40, 65.33***
Tolfenamic acid	10	7.46 ± 0.49, 27.74*	7.08 ± 0.36,6.95	7.06 ± 0.21, 1.15
Tolfenamic acid-Copper	22.9	7.86 ± 0.37, 34.59**	7.24 ± 0.29, 9.37	7.1 ± 0.31, 1.72
Tolfenamic acid-Cobalt	23.4	7.32 ± 0.30, 25.34*	7.12 ± 0.22, 7.55	7.06 ± 0.22, 1.15
Tolfenamic acid-Zinc	23.3	8.94 ± 1.04, 53.08*	7.5 ± 0.63, 13.29	7.12 ± 0.49, 2.01

Each value expressed as the mean ± SEM. Significant at ***p<0.001, **p<0.01, *p<0.05 compared to control group.

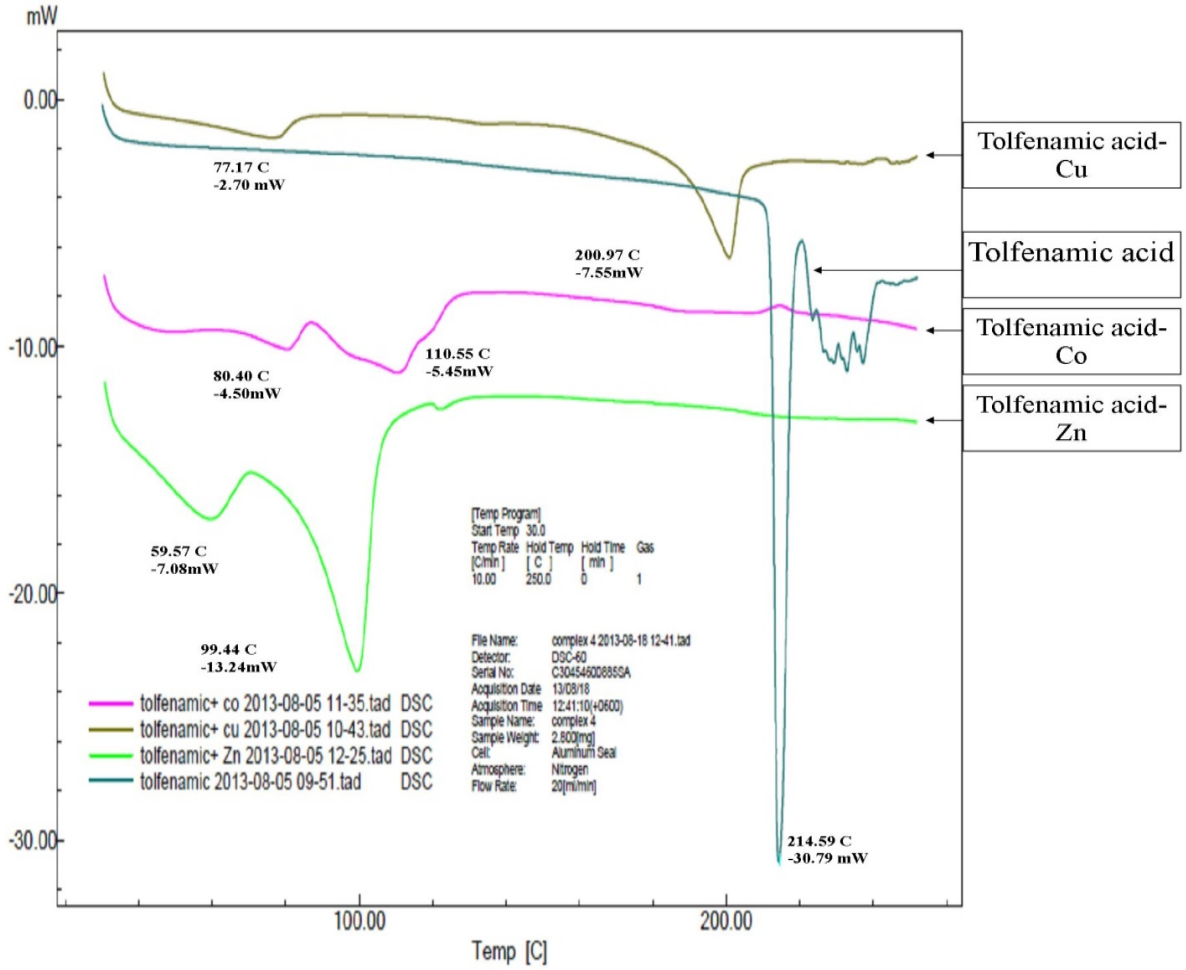

Figure 3. Combined DSC thermogram of tolfenamic acid and its complexes.

Central analgesic activity. The central analgesic activities of metal complexes of tolfenamic acid in tail flick method are given in table 4. In this experiment, metal complexes were given at an equimolar dose of tolfenamic acid at 10 mg/kg body weight individually. Cu, Co and Zn complexes of tolfenamic acid (with % elongation time of 34.59%, 25.34% and 53.08%, respectively) showed significant analgesic effect compared to that of morphine (2 mg/kg body weight) at an equimolar dose of tolfenamic acid at 10 mg/kg body weight after 30 minutes.

CONCLUSION

Scanty level of pharmacological investigation on divalent metal complexes of tolfenamic acid was a key driver to design our study and to search for new pharmacological properties such as peripheral and central analgesic potency; to prepare new compounds, i.e., complexes of tolfenamic acid with essential metal ions, which probably would exhibit improved or different biological properties compared to the parent NSAID. The Co and Zn complexes of tolfenamic acid possess considerably better analgesic potency than the parent tolfenamic acid and further bioactivity guided investigation can lead to the development of new classes of analgesic drugs.

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