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A comprehensive in vitro biological investigation of metal complexes of tolfenamic acid

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ABSTRACT

Objective: The inquisitive objective of the study was to observe the antimicrobial, cytotoxicity, and antioxidant activities of some newly synthesized metal complexes of tolfenamic acid.
Methods: While antimicrobial activity was studied by disk diffusion method, cytotoxicity was studied by performing brine shrimp lethality bioassay. Moreover, DPPH radical scavenging potential was observed to determine the antioxidant property of the complexes.
Results: From the disk diffusion antimicrobial screening of tolfenamic acid and its metal complexes, it was found out that considerable antimicrobial activity in terms of zone of inhibition against the tested organisms had been demonstrated by Cu and Zn complex of tolfenamic acid. In addition, the brine shrimp lethality bioassay corroborated that tolfenamic acid and Cu, Co, Zn complexes of the parent NSAID exhibited cytotoxicity with LC_{50} values 1.23 ± 0.91 μg/ml, 1.12 ± 0.12 μg/ml, 1.17 ± 0.56 μg/ml, 1.35 ± 0.24 μg/ml respectively, compared to the vincristine sulfate had LC_{50} value of 0.82 ± 0.09 μg/ml. Furthermore, 1,1-diphenyl-2-picrylhydrazyl assay revealed that in comparison with standard BHT had IC_{50} of 11.84 ± 0.65, Cu and Co complex of tolfenamic acid exhibited significant antioxidant or radical-scavenging properties with IC_{50} values 13.61 ± 0.58 μg/ml and 15.38 ± 0.09 μg/ml, respectively.
Conclusion: It can be postulated that metal complexes of tolfenamic acid have auspicious pharmacological effects: antimicrobial, cytotoxicity, and antioxidant potency. Hence, these complexes might have better therapeutic responses in future; notwithstanding, it needs further detailed analysis in other pharmacological perspectives.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are the agents which play a vital role in the field of biological chemistry. These drugs have shown promising effects on the treatment of painful and inflammatory musculoskeletal disorders.[1] Furthermore, in the field of therapeutics, the metal complexes are the subjects of extensive study.[2–6] Because the metal complexes exhibit various mechanisms of biological activities, they are the best contender of alternate Drug delivery system. Recently, studies have shown that metal complexes enhances the pharmacological potency when bind with a drug molecule; eventually, some biological responses which are far beyond the parent drug's activities.[7,8]

A transition metal can exist in several oxidation states and interact with many different negatively charged ligands to form complexes.[9] Many such complexes may have therapeutic potentials. In fact, complexes of transition metals are showing promising results in the treatment of carcinomas, lymphomas, infections, inflammation, diabetes, and neurological disorders.[10]

Today, Multi-Drug Resistant (MDR) microorganisms – the so called superbugs have become a great risk to the public health in many parts of the world. Many studies have shown that the metal complexes can possess strong anti-microbial activity against these resistant microbes. Sometimes, these complexes can have stronger activity compared to conventional drugs. Such activity may be due to the metal ion itself, the ligand or a combination of both.[11,12] In this research, a vast range of microorganisms were used in the disk diffusion method to investigate the anti-microbial properties of these complexes under study. Since many metal complexes show

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efficacy against cancer, investigation of potential antitumor or antineoplastic activity in the metal complexes under study was undertaken. Preliminary screening by the brine shrimp lethality bioassay was performed under controlled in vitro conditions. This test can also give an insight into the potential toxicity profile of the compounds. Finally, antioxidant activity of these metal complexes is also investigated. Antioxidant property is related to protecting biological systems against the potential detrimental effects of oxidative stress or more specifically free radicals.[13] Recently, it is receiving a lot of attention from researchers due to its relation with DNA damage, protein modification, and enzyme activity among others.[14]

There are no known research studies on these biological activities of metal complexes of tolfenamic acid; thus, the present study was focused to investigate antimicrobial, cytotoxic, and antioxidant activities of metal complexes of tolfenamic acid.

2. Material and methods

2.1. Drugs and materials

Tolfenamic acid, at highest purity, was collected from – renowned – Eskayef Bangladesh Limited. Salts of Cu, Co, and Zn were obtained from ACI Pharmaceuticals Ltd. Moreover, Vincristine sulfate and ciprofloxacin were purchased from Square Pharmaceuticals Ltd.

2.2. Solvents and reagents

Acetone, Tween-80, methanol, and 1,1-diphenyl-2-picrylhydrazyl were obtained from Sigma Chemicals, USA, and also Dimethyl sulfoxide and sodium bicarbonate were collected from Merck, Darmstadt, Germany. Normal saline was purchased from Opsonin Pharma Ltd; all chemicals and reagents were of analytical grade.

2.3. Synthesis of complexes

Equimolar solutions of $CuSO_4{\cdot}5H_2O$, $CoCl_2{\cdot}6H_2O$, and $ZnSO_4{\cdot}7H_2O$ in methanol (5 ml) were added to a solution of tolfenamic acid in methanol (5 ml), separately. After that, drops of a methanolic solution of 1 N sodium hydroxide were added to three different solutions until the apparent pH value was ~7; and, the mixtures were stirred for several hours at room temperature and cooled to refrigerator until the temperature dropped to 5 °C. With the addition of distilled water, significant amount of precipitation was formed, separately. The precipitates were obtained by filtration and recrystallization; and, the powders were washed with cold MeOH : H_2O (5 : 1) and finally dried in vacuum to afford the complexes.[15]

2.4. Experimental animals

Artemia salina Leach (brine shrimp eggs) collected from pet shops was kept properly under controlled temperature and was used as the test organism.[16] Ten (n = 10) living shrimps were added to each of the test tubes containing 5 ml of simulated sea water.

2.5. Antimicrobial activity

Tolfenamic acid and its complexes were tested for antimicrobial activities by the standardized disk diffusion method.[17,18] In vitro antimicrobial screening was done against numerous strains of bacteria and fungi. The obtained results were compared with standard antibiotic, ciprofloxacin.

2.6. Cytotoxic activity

Cytotoxicity was evaluated by using brine shrimp lethality test according to the reported method.[19,20] In this test, dimethyl sulfoxide and vincristine sulfate were used as negative control and positive control, respectively. Ten matured shrimps were applied to each of all test tubes of tolfenamic acid and its complexes. After 24 hours, the morbidity of brine shrimps was observed. An approximate linear correlation was observed by plotting logarithm of concentration versus percentage of mortality.

2.7. Antioxidant activity

The antioxidant activity of tolfenamic acid and its complexes was assessed by 1,1-dipheny-l-2-picryldrazyl and estimated by reported methods.[21–23] Here, butylated hydroxyl toluene was used as standards and DPPH solution was used as control.[24] The absorbance was measured by UV spectrophotometer at a wave length of 570 nm.

Inhibition of free radical was estimated by following equation:

$$\text{Inhibition of free radical } \% = (A_c - A_s)/A_c \times 100 \text{ where,}$$

Ac = Absorbance of the control and As = Absorbance of the tolfenamic acid and its complexes. The 50% inhibitory concentration IC_{50} was calculated by plotting the inhibition concentration versus standard tolfenamic acid complex concentration.

2.8. Statistical analysis

Statistical analyses were done by using the Statistical Package for Social Science (SPSS) version 16.0 software, and statistical differences between groups were analyzed by one-way analysis of variance ANOVA followed by Dunnett's t-tests.[16] Data's were presented as means ± SEM of three parallel measurements and differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Characterization of metal complexes

Characterization of the complexes of tolfenamic acid was done by analyzing FTIR spectra, UV–vis Spectra, and DSC thermograms.[15]

3.2. Antimicrobial activity

To determine antimicrobial activity of metal complexes of tolfenamic acid, they were tested against some gram positive and some gram negative bacteria and some fungi.[25] Here, five gram positive bacteria namely, *B. cereus*, *B. megaterium*, *B. subtilis*, *S. lutea, and Staph. aureus* as well as eight gram negative bacteria namely, *E. coli, P. aeruginosa, S. paratyphi, S. typhi, V. parahemolyticus, S. boydii, S. dysenteriae*, and *V. mimicus* and also three fungi namely, *C. albicans*, *A. niger,* and *S. cerevacae* were used. In this study, tolfenamic acid complexes showed mild to moderate antimicrobial activity except cobalt complex of tolfenamic acid. The zone of inhibition for tolfenamic acid-copper and tolfenamic acid-zinc were 18–20 mm and 10–12 mm respectively, which was compared to standard ciprofloxacin with an inhibition zone of 44–46 mm as mentioned in Table 1.

3.3. Cytotoxic activity

The results obtained from brine shrimp lethality assay are presented in Table 2. The LC_{50} denoted the concentration by which

Table 1
Antimicrobial activity of tolfenamic acid and its metal complexes.

Test organism	Determination of zone of inhibition (mm)				
	TOL	TOCU	TOCO	TOZ	CIP
Gram positive bacteria					
Bacillus cereus	–	18	–	10	45
Bacillus megaterium	–	18	–	11	45
Bacillus subtilis	–	19	–	11	45
Sarcinalutea	–	18	–	10	45
Staphylococcus aureus	–	19	–	10	45
Gram Negative bacteria					
Escherichia coli	–	20	–	12	44
Pseudomonas aeruginosa	–	18	–	10	45
Salmonella paratyphi	–	19	–	11	46
Salmonella typhi	–	19	–	12	46
Vibrio parahemolyticus	–	19	–	10	44
Shigellaboydii	–	18	–	11	45
Shigelladysenteriae	–	19	–	10	45
Vibrio mimicus	–	18	–	10	45
Fungi					
Candida albicans	–	18	–	11	45
Aspergillus niger	–	19	–	11	45
Sacharomycescerevacae	–	18	–	11	45

TOL = Tolfenamic acid, TOCU = Cu complex of Tolfenamic acid, TOCO = Co complex of Tolfenamic acid, TOZ = Zn complex of Tolfenamic acid, CIP = Ciprofloxacin (Standard).

Table 2
Cytotoxic activity of tolfenamic acid and its complexes.

Test samples	LC_{50} (μg/ml)
Vincristine sulfate (VS)	0.82 ± 0.09
Tolfenamic acid	1.23 ± 0.91***
Cu complex of tolfenamic acid	1.12 ± 0.12***
Co complex of tolfenamic acid	1.17 ± 0.56***
Zn complex of tolfenamic acid	1.35 ± 0.24**

Data of LC_{50} represents mean ± SEM of triplicate analysis and significant at ***$p < 0.001$, **$p < 0.01$ compared to vincristine sulfate.

50% of shrimps were killed.[26] Here, tolfenamic acid-Cu complex and tolfenamic acid-Co complex exhibited significantly highest cytotoxicity with the LC_{50} values of 1.12 ± 0.12 μg/ml, 1.17 ± 0.56 μg/ml respectively, compared to the vincristine sulfate having LC_{50} value of 0.82 ± 0.09 μg/ml. Also, tolfenamic acid and tolfenamic acid-Zn complex exhibited cytotoxicity with the LC_{50} values of 1.23 ± 0.91 μg/ml, 1.35 ± 0.24 μg/ml, respectively.

3.4. Antioxidant activity

The antioxidant activity of tolfenamic acid and its complexes are shown in Table 3. Here, tert-butyl-1-hydroxytolune (BHT) was used as reference standard.[27] In this case, parent tolfenamic acid, tolfenamic acid-Cu complex, tolfenamic acid-Co complex and tolfenamic acid-Zn complex showed significant free radical scavenging activity with IC_{50} value 20.56 ± 0.71 μg/ml, 13.61 ± 0.58 μg/ml, 15.38 ± 0.09 μg/ml and 47.28 ± 0.89 μg/ml, respectively.

Table 3
Antioxidant activity of tolfenamic acid and its complexes.

Sample	% of inhibition	Free radical scavenging activity IC_{50} μg/ml
tert-Butyl-1-hydroxytolune (BHT)	65.74 ± 0.15	11.84 ± 0.65
Tolfenamic acid	31.63 ± 0.47	20.56 ± 0.71
Cu complex of tolfenamic acid	61.18 ± 0.41***	13.61 ± 0.58***
Co complex of tolfenamic acid	56.59 ± 0.14**	15.38 ± 0.09**
Zn complex of tolfenamic acid	47.28 ± 0.89**	16.21 ± 0.16**

Values are expressed as the mean ± SEM of triplicate analysis and significant at ***$p < 0.001$, **$p < 0.01$ compared to standard BHT.

4. Conclusion

Pharmacological investigation on metal complexes of tolfenamic acid was the main purpose of designing the study. In addition, search for new pharmacological responses such as antioxidant, anticancer, and antimicrobial potency was, also, a key drive to prepare the new compounds; therefore, complexes of tolfenamic acid, which might exhibit enhanced biological properties than its parent compound. The Co and Cu complex can be developed as antitumor drugs having very promising antioxidant properties. They can also be used for their cytoprotective role in neurodegenerative diseases. Moreover, the Cu and Co complexes possess significant cytotoxic potency compared to vincristine, which may lead to the development of new anticancer drugs; however, further researches are needed to test the compound against other anticancer agents. Emergence of Cu and Zn complexes of tolfenamic acid as future antimicrobial agents can be predicted and bioactivity guided studies can be further performed with a view to developing new classes of antimicrobial agents.

Conflict of interest

We declare that we have no conflict of interest.

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