INTRODUCTION

Inflammation is a protective response of body’s immune system to harmful stimuli such as pathogen, irritants, allergens and trauma. The classic symptoms of inflammation are heat, redness, swelling, pain and dysfunction of the organs involved [1,2]. While inflammation may be a normal response, chronic inflammation results in destruction of normal connective tissue due to the activities of catalytic enzymes and cytokines[3,4]. Anti-inflammatory refers to a drug that reduces inflammation. Anti-inflammatory drug can help improve sense of well being by relieving pain, reducing swelling, slowing down or stopping joint damage, and increasing ability to function. Anti-inflammatory includes non-steroidal anti-inflammatory drugs (NSAIDs), enzymes, and steroids. Non-steroidal anti-inflammatory drugs are the one kind of pharmaceuticals used for the relief of mild to severe pain. Inhibition of the COX-1 and COX-2 enzyme systems, and subsequent down-regulation of PG synthesis, is the well-accepted mode of action of NSAIDs[5,6]. Aspirin, ibuprofen, IndOm, flurbiprofen and meloxicam etc., are some of the well known COX inhibitors. NSAIDs act at additional sites in the inflammatory cascade, e.g. they may block tumor necrosis factor-alpha (TNF-α), which also controls the PGs[7].

Many of today’s anti-inflammatory drugs are associated with problematic renal, gastrointestinal and cardiovascular side effects. Salicylamide[8] and its simple derivatives encompass significant biological activities like antifungal, anti-inflammatory, analgesic, antipyretic activities. The derivatives with active structural moieties like N, N-dimethyl-3-phenyl[9], N-benzyl, N-chlorobenzyl[10] and N-heterocyclics[11] have improved activity. Synthesis and study of metal complexes with anti-inflammatory drugs as ligands is a research area of considerable interest, particularly complexes which exhibit synergistic activity between metal and drug[12-14]. Anti-inflammatory drugs with metal centers show improved activity and lesser side effect[2]. Motivated by above mentioned findings the anti-inflammatory, analgesic and antipyretic properties of N, N’ - propylenebis(salicylamide) metal complexes were screened.

MATERIALS AND METHODS

The N, N’ - propylenebis(salicylamide) (ligand, BA) and its Co(II), Ni(II), Cu(II) and Zn(II) complexes were prepared as per the literature procedure[15], and were washed thoroughly with ether and chloroform. The purified compounds were used for pharmacological studies.
Animal: The pharmacological studies were carried out at Department of Pharmacology, Sankaralingam Bhuvaneswari College of Pharmacy, Sivakasi. Adult albino rats of either sex weighing 100-140 grams were used for the study. All animals were kept in polypropylene cages in the animal house facility of Department of Pharmacology and maintained under standard laboratory conditions. They were fed with a standard diet and water ad libitum. All experiments were conducted after overnight fasting but there was free access to water. All the experimental protocols were approved by the Institutional Animal Ethical Committee.

Evaluation of Anti-inflammatory activity — Carrageenan induced paw edema method: The anti-inflammatory activity was assessed by the method suggested by Winter et al, using carrageenan as phlogistic agent[16]. Albino rats of either sex weighing between 100-140 g were used for all studies. The animals were divided into seven groups with each group having four animals. The animals were fasted over night prior to the experimental procedure but had free access to water. The solid samples were suspended in 0.5% w/v Sodium carboxy-methyl cellulose and administered orally 30 min before injection of carrageenan (0.1 mL of 1% w/v solution) in normal saline into subplantar region of right hind paw of each rat. The contalateral paw was injected with an equal volume of saline. The control group, standard group and test groups received normal saline, diclofenac sodium (100 mg/kg) and the test samples (200 mg/kg) respectively. The effective dose of the test compounds was assigned as per the similar works carried out with salicylamide and related compounds[17,14]. The left and right paw volumes were measured plethysmographically at each hour, for 4 h. The percentage inhibition of edema has been calculated by the following formula.

\[
\text{% inhibition} = 100 \times \frac{(V_c - V_t)}{V_c}
\]

where, Vc is volume of the edema in control and Vt is volume of the edema in animals treated with standard drugs/samples.

Evaluation of Analgesic activity — Tail immersion test: Wister albino rats were screened for its sensitivity by placing the tip of the tail (last 1–2 cm) gently in warm water maintained at 55°C (±2°C). Any albino rats flicking the tail within 5 sec were selected for the study. The selected rats were divided into seven groups of four animals each. The control, standard and test groups received normal saline, pentazocine (4 mg/kg, ip) and test samples (200 mg/kg, po) suspended in 0.5% w/v CMC respectively. After drug treatment, the basal reaction time of all groups of animals was noted at different time intervals viz., 1, 2, 3 and 4 h.

Evaluation of Anti-pyretic activity — Brewer’s yeast induced pyrexia: The antipyretic activity was evaluated using Brewer’s yeast-induced pyrexia in rats. The initial rectal temperatures were recorded using digital tele thermometer. Fever was induced by subcutaneous injection of 15% aqueous suspension of sterile Brewer’s yeast (10 mL/kg). The animals that developed satisfactory pyrexia (0.5 or more raise in rectal temperature) after 18 h were used. Control group received normal saline and paracetamol (100 mg/kg, po) served as standard drug. The test samples were administered to different groups (200 mg/kg) orally as a suspension in 0.5% w/v CMC. After drug administration the rectal temperature was recorded at 0, 1, 2, 3 and 4 h.

Statistical analysis: All the data were expressed as mean ± S.E. Statistical significance of the difference between control and treated group were assessed by the method of student’s t-test. P<0.05 was considered as statistically significant.

RESULTS AND DISCUSSION
The anti-inflammatory effects of all the five compounds are presented in Table 1 as the difference in paw volume and % inhibition. The ligand showed only 33 % activity. The Co(II) and Cu(II) complexes showed 94 % and 92 % inhibition with p < 0.001 respectively at the end hour of experiment as standard drug, diclofenac (94 %, p < 0.001) for the dose level taken. The drug action i.e. reduction of paw volume is observed from 2nd h for standard drug (100 mg/kg, po). But for Co(II) and Cu(II) complexes (200 mg/kg, po), it is observed even from 1st h. The Zn(II) complex showed 73 % inhibition with p < 0.01.

The tail withdrawal reflux times for untreated and treated groups are given in Table 2. The standard drug showed peak activity at 2nd h, and at the 4th h its activity was reduced to half the extent. The ligand and Ni(II) complex showed less activity. The Co(II) and Cu(II) complexes showed peak activity at 3rd h, and Zn(II) complex at 4th h with the significance of p < 0.01. The results of effect of ligand and complexes on yeast induced pyrexia in albino rats were depicted in Table 3. The Co(II), Cu(II) and Zn(II) complexes showed 1.05, 0.85, 0.78°C
The painful reactions in animals were mediated by histamine and 5-hydroxytryptamine, secondary phase is mediated by bradykinin notably the endogenous nonapeptide bradykinin produced by kallikrein. The final phase is attributed to local production of prostaglandins (PG) of E series. The N, N’ - propylenebis(salicylamide) is a salicylic acid derivative and it will also belong to non-steroidal anti-inflammatory drug. It showed only mild activity (33 %). Activities of all the complexes are higher than ligand particularly Co(II), Cu(II) and Zn(II) complexes. It is well known that Co(II), Cu(II)[13,20,21] and Zn (II)[12,22] complexes of many non-steroidal anti-inflammatory drugs exhibited enhanced anti-inflammatory action. Even, Co(II)[23,24] and Cu(II)[25] complexes of many non-anti-inflammatory agents exhibited anti-inflammatory action, where the ligand may simply acts as a carrier that brings metal to the therapeutic target. During inflammation higher concentration of zinc ions were found in inflamed tissues. This opens the view of the anti-inflammatory properties of zinc[26]. The same activity was also reported for copper[21,27,28]. There is an increased demand for Cu(II) during inflammatory conditions[29,30]. So, with the added effect of metal ions, all the complexes showed enhanced activity, especially for Co(II), Cu(II) and Zn(II) complexes. The anti-inflammatory activity of test samples concludes that few compounds are efficient cyclooxygenase inhibitor. Usually cyclooxygenase inhibitors have analgesic activity. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non-steroidal anti-inflammatory drugs inhibit only peripheral pain[31,32]. But some of the NSAIDs are reported to have both[33,34]. Tail immersion method was used to evaluate analgesic activity involving centrally mediated mechanism. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems[35-37]. The painful reactions in animals were produced by thermal stimulus that is by dipping the tip of the tail in hot water. Analgesic effect against thermal noxious stimuli may be elicited through opioid receptors or through modulation of several neurotransmitters involved in relevant phenomena[38]. The tail withdrawal reflex time for test group was found to be higher than control group. This suggests that the test samples have central mechanism of analgesic action. But, the extent of activity showed by the test samples is less than that of the standard drug pentazocine. The ligand and Ni(II) complex showed poor activity compared to standard drug and other complexes. The Co(II), Cu(II) and Zn(II) complexes showed appreciable activity but the results are only the half extend compared to standard drug.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose p.o (mg/kg)</th>
<th>Paw volume (mm)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>control</td>
<td>0.37±0.08</td>
<td>0.60±0.09</td>
<td>0.74±0.08</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>0.39±0.04</td>
<td>0.81±0.04</td>
<td>0.53±0.05</td>
</tr>
<tr>
<td>BA</td>
<td>0.69±0.18</td>
<td>1.10±0.19</td>
<td>1.12±0.19</td>
</tr>
<tr>
<td>Co</td>
<td>0.62±0.04</td>
<td>0.51±0.04</td>
<td>0.33±0.03#</td>
</tr>
<tr>
<td>Ni</td>
<td>0.62±0.09</td>
<td>0.64±0.07</td>
<td>0.59±0.09</td>
</tr>
<tr>
<td>Cu</td>
<td>0.62±0.03</td>
<td>0.55±0.02</td>
<td>0.40±0.02#</td>
</tr>
<tr>
<td>Zn</td>
<td>0.78±0.11</td>
<td>1.32±0.09</td>
<td>1.07±0.09</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM (standard error of the mean) *p < 0.001, **p = 0.001, #p < 0.01 compared to control
Subcutaneous injection of Brewer’s yeast induces pyrexia by increasing the synthesis of prostaglandin. It is considered as a useful test for the screening of plants materials as well as synthetic drugs for their antipyretic activity[39, 40]. The experimental rats showed a mean increase of about 1.2-1.5°C in rectal temperature 24 h after the yeast injection. Yeast induced fever is a pathogenic fever. Its etiology comprises of production of prostaglandins[41]; particularly PGE2 appears to be a final pathway responsible for fever induced by several pyrogens[42]. The inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action[43] as that of paracetamol and the inhibition of prostaglandin can be achieved by blocking the cyclo-oxygenase enzyme activity. The Co(II) and Cu(II) showed considerable inhibition. The fall in elevated body temperature of experimental animals showed that for Co(II) complex there was a gradual and maximum reduction in temperature up to 4 h. But for Cu(II) and Zn(II) complexes, efficient reduction in temperature of 0.83°C and 0.7°C were observed within 3 h and 2 h respectively; compared to Co(II) complex at same time period (3 h, 0.75°C and 2 h, 0.6°C). Less useful inhibition was observed for ligand (0.4°C) and Ni(II) complex (0.45°C).

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