CHAPTER V

ANTINFLAMMATORY
AND
ANTIMICROBIAL
5.1. ANTI-INFLAMMATORY EVALUATION OF SYNTHESIZED COMPLEXES: GENERAL INTRODUCTION:

Inflammation is a tissue reaction to infection, irritation or foreign substance. It is a part of the host defence mechanism, but when it becomes great it is a hopeless condition. Aging is also considered to be an inflammatory response. There are several tissue factors or mechanisms that are known to be involved in the inflammatory reaction such as release of histamine, bradykinin and prostaglandins. The development of non-steroidal anti-inflammatory agents in recent years have contributed a lot in not only overcoming the human sufferings (such as Arthritis Osteoarthritis, Ankylosing Spondylitis, Gout, Rheumatic fever, Systemic lupus, Eythematos, Psoriasis and Poly arthritis nodosa), but also has helped in understanding the tissue mechanism of inflammation.

The inflammatory response, consists of three successive phases:

(i) Increased vascular permeability with resulting oedema and swelling.
(ii) Cellular infiltration and phagocytosis.
(iii) Proliferation of the fibroblasts, synthesizing new connective tissue to repair the injury.

The most widely used primary test to screen new nonsteroidal anti-inflammatory agents measures the ability of a compound to reduce local oedema induced in the rat paw by infection with irritant carrageenan, which is a mucopolysaccharide derived from Irish sea mass, chondrus crispus.

A variety of recent observations indicated that copper complexes when administered in conjunction with anti-inflammatory drugs exhibit synergistic activity. It has also been found that the copper complexes of some anti-arthritic drugs are themselves more active as anti-inflammatory agents than respective parent compounds. The molecular basis of action of the copper and other first-row transition metal complexes with well-established drug such as diclofenac sodium is not as yet clear and same is the case with metal (II) Tungstate and our metal (II) tellurite.

We therefore, have undertaken the investigation of the interaction of metal
(II) tellurite [where metal (II) = Co (II), Ni(II) and Cu(II)] ions with a widely used anti-inflammatory ligand 2,2'-Bipyridyl.

Anti-inflammatory agents are classified as follows:

1. Steroidal anti-inflammatory agents: They exert their action by inhibiting the release of phospholipids in lipoxygenase are pathway which inhibit the release of arachidonic acid from membrane.

2. Non-steroidal anti-inflammatory agents: They are said to inhibit biosynthesis of prostaglandin at cyclo-oxygenase pathway.

Mechanisms of reducing inflammation:

- By inhibition of oxidative phosphorylation.
- By inhibition of mucopolysaccharide biosynthesis.
- By stabilization of mast cell and erythrocytes.
- By inhibition of leukocyte migration.
- By Fibrinolytic activity.
- By the effect on collagen metaboslim.
- By prevention of leukocyte accumulation.
- By inhibition of enzyme (5- hydroxy trytophen decarboxylase).

5.1.1. SCREENING METHODS:

Various screening methods for evaluating antiinflammatory activity have been classified as follow:

(1) Non-immunological method
(2) Immunological method
(3) Miscellaneous method

(1) Non Immunological Methods:

(A) Method for evaluating antiinflammatory activity of acute inflammation: It is of the following types:-

(i) Carrageenan induced hind paw oedema methods\textsuperscript{2,7}.
(ii) 5- hydroxy tryptamine induced paw oedema method\textsuperscript{8}.
(iii) Histamine induced hind paw oedema method\textsuperscript{9}. 
(iv) Formalin induced hind paw method^{10}.
(v) Hyaluronidase induced hind paw oedema method^{11}.
(vi) Turpentine oil induced arthritis in knee joint^{12}.

(B) Method for evaluating anti-inflammatory activity of subacute inflammation: It is of the following types:-

(i) Carrageenan granuloma pouch technique^{12}.
(ii) Cotton pellet granuloma^{13}.

(C) Method for evaluating anti-inflammatory activity of chronic inflammation:

(i) Formaldehyde induced arthritis method^{14}.

2. Immunological Methods:

These are of two types:

(i) Adjuvant arthritis method^{15}.
(ii) Tuberculin sensitivity test method^{16}.

3. Miscellaneous methods:

These are of two types:

(i) Urate crystal induced synostitis method^{17}.
(ii) U. V. erythema method^{16,18}.

5.1.2 EXPERIMENTAL:

In the present study the anti-inflammatory activity of the synthesized complexes were evaluated by the carrageenan induced rat paw oedema by taking phenylbutazone as control^{19}.

5.1.3 TOXICOLOGY:

DETERMINATION OF LEthal DOSE (LD_{50})^{20}

LD_{50} is defined as the dose which is lethal to 50% of the animal group. In the present study the procedure adopted by Horn^{21} was followed. The animals were divided into different groups of four each. These were administered with 500,800, 1000,1200 mg/kg of the synthesized complexes and observation was made for mortality upto 24 hours.

LD_{50} for the synthesized complexes E and F was found [Table - 5.1]. But for the
complexes A, B, C and D no mortality was observed upto the dose of 1200 mg/kg body weight. Therefore, the LD_{50} of these complexes was not determined.

Table - 5.1. LETHAL DOSE (LD_{50}) OF SYNTHESIZED COMPLEXES

<table>
<thead>
<tr>
<th>Complex No.</th>
<th>% Mortality at dose mg/kg</th>
<th>LD_{50} mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>500</td>
<td>800</td>
</tr>
<tr>
<td>A. CoTeO_{3}.H_{2}O</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B. NiTeO_{3}.2H_{2}O</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C. CuTeO_{3}.H_{2}O</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D. [Co(Bipy.)<em>{2}(H</em>{2}O)<em>{2}]TeO</em>{3}.H_{2}O</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E. [Ni(Bipy.)<em>{3}]TeO</em>{3}.6H_{2}O</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F. [Cu(Bipy.)<em>{2}]TeO</em>{3}.3H_{2}O</td>
<td>0</td>
<td>50</td>
</tr>
</tbody>
</table>

5.1.4 DETERMINATION OF ANTI-INFLAMMATORY ACTIVITY:

Carrageenan induced paw oedema method of winter et al.\textsuperscript{22}, is the simplest and most widely used model for the evaluation of anti-inflammatory activity of the synthesized complexes.

Anti-inflammatory studies were performed plethysmographically to measure carrageenan induced paw volume following the method of Winter et al.\textsuperscript{22}.

The paw volume of the rat right hind paw was measured by “Plethysmograph”.

Eight groups (each group consists of four rats) of albino rats with average weights of 100 gm were taken. Carrageenan (1% W/V solution, in sterilized saline) was taken as phlobistigic agent.

Six groups of albino rats were injected with synthesized complexes, (100 mg/kg body weight). One group were administered with standard Phenylbutazone 30 mg/kg body weight orally. The last group was taken as control group (0.3 ml of 0.9 % saline administered introperitonially). After 30 minutes of administration of the doses 0.1ml of carrageenan (1% W/V) was injected into subplantar tissue of the right hind paw of each animal of all the groups. The paw volume was determined in all the groups (administered with complexes) “Plethysmographically” before and after four hours of
carrageenan treatment and these were compared with the control group. The mean increase of the volume of the right hind paws of these animals was compared with the standard (Phenylbutazone);

The percent inhibition of inflammation was calculated by the method of Newbould\textsuperscript{23} using the following formula:

\[
\text{Percent Inhibition} = 100 \left(1 - \frac{a - x}{b - y}\right)
\]

Where 
- \(x\) = Mean foot volume of rats before the administration of carrageenan injection in the test and the standard group.
- \(a\) = Mean foot volume of the rats after the administration of carrageenan injection in the test and the standard group.
- \(y\) = Mean foot volume of rats before the administration of carrageenan in the control group.
- \(b\) = Mean foot volume of rats after the administration of carrageenan injection in the control group.

**TABLE 5.2 ANTIINFLAMMATORY ACTIVITY OF THE SYNTHESIZED COMPLEXES**

<table>
<thead>
<tr>
<th>Complex No.</th>
<th>Complex Dose mg/kg body wt.</th>
<th>No. of Rats used in each group</th>
<th>Mean Initial paw volume (ml) 0.0 hrs.</th>
<th>Mean Final paw volume (ml) 3 hrs.</th>
<th>Mean change in paw volume (Final-Initial)</th>
<th>Percent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>4</td>
<td>0.54</td>
<td>1.56</td>
<td>1.02</td>
<td>-</td>
</tr>
<tr>
<td>A. CoTeO\textsubscript{2}H\textsubscript{2}O</td>
<td>100</td>
<td>4</td>
<td>0.55</td>
<td>1.37</td>
<td>0.82</td>
<td>19.60</td>
</tr>
<tr>
<td>B. NiTeO\textsubscript{2}H\textsubscript{2}O</td>
<td>100</td>
<td>4</td>
<td>0.55</td>
<td>1.43</td>
<td>0.88</td>
<td>13.72</td>
</tr>
<tr>
<td>C. CuTeO\textsubscript{2}H\textsubscript{2}O</td>
<td>100</td>
<td>4</td>
<td>0.62</td>
<td>1.57</td>
<td>0.95</td>
<td>6.86</td>
</tr>
<tr>
<td>D. [Co(Bipy.\textsubscript{2})(H\textsubscript{2}O\textsubscript{2})\textsubscript{2}TeO\textsubscript{2}H\textsubscript{2}O</td>
<td>100</td>
<td>4</td>
<td>0.57</td>
<td>1.36</td>
<td>0.79</td>
<td>22.54</td>
</tr>
<tr>
<td>E. [Ni(Bipy.\textsubscript{2})\textsubscript{2}TeO\textsubscript{2}6H\textsubscript{2}O</td>
<td>100</td>
<td>4</td>
<td>0.62</td>
<td>1.28</td>
<td>0.66</td>
<td>35.29*</td>
</tr>
<tr>
<td>F. [Cu(Bipy.\textsubscript{2})\textsubscript{2}TeO\textsubscript{2}3H\textsubscript{2}O</td>
<td>100</td>
<td>4</td>
<td>0.68</td>
<td>1.24</td>
<td>0.56</td>
<td>45.0**</td>
</tr>
<tr>
<td>STANDARD</td>
<td>30</td>
<td>4</td>
<td>0.66</td>
<td>1.10</td>
<td>0.44</td>
<td>56.86</td>
</tr>
</tbody>
</table>

**STANDARD** - Phenylnbutazone (30 mg/kg)

** = Highly significant (P \leq 0.01)

* = Moderate significant (P < 0.05)

Bipy = 2,2'-Bipridyl (C\textsubscript{10}H\textsubscript{8}N\textsubscript{2})
BAR GRAPH REPRESENTING ANTI-INFLAMMATORY ACTIVITY (% INHIBITION) BY SYNTHESIZED COMPLEXES VS STANDARD.

<table>
<thead>
<tr>
<th>COMPLEX NO.</th>
<th>% INHIBITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>19.60</td>
</tr>
<tr>
<td>B</td>
<td>13.72</td>
</tr>
<tr>
<td>C</td>
<td>6.86</td>
</tr>
<tr>
<td>D</td>
<td>22.54</td>
</tr>
<tr>
<td>E</td>
<td>35.29</td>
</tr>
<tr>
<td>F</td>
<td>45.00</td>
</tr>
<tr>
<td>STANDARD</td>
<td>56.86</td>
</tr>
</tbody>
</table>

A. CoTeO₃.H₂O,          B. NiTeO₃.2H₂O
C. CuTeO₃.H₂O,          D. [Co(Bipy.)₂(H₂O)₂]TeO₃.H₂O
E. [Ni(Bipy.)₃]TeO₃.6H₂O, F. [Cu(Bipy.)₂]TeO₃.3H₂O
5.2 ANTIMICROBIAL ACTIVITY OF THE SYNTHESISED COMPLEXES:

General Introduction To Antimicrobial Activity:

Micro-organism, which were first observed by Anton van Leeuwenhoek (1673), are minute living things individually too small to be seen with the naked eye. The term includes bacteria, fungi (Yeasts and Molds), Protozoanes, and microscopic algae.

Agostine Bass\textsuperscript{24} offered experimental proof that a living organism could cause disease in others.

Microbe may be the most significant life which sharing this planet with humans. They may play beneficial role in maintaining life or undesirable role in causing human disease.

A large number of microorganism give their crucial contributions to the welfare of the world’s inhabitants. Many act as miniature benefactors, helping to maintain a balance of living organisms and chemicals in our global environment. Marine and fresh water microorganisms from the basis of the food chain in oceans, lakes and rivers. Soil microbes help to breakdown wastes and incorporate nitrogen gas from the air into organic compounds, chemical element in the land and air are thus recycled. Certain bacteria and microscopic algae have important roles in photosynthesis, a food and oxygen generating process is critical to our life on earth.

Bacteria are single celled microorganisms that lack a nuclear membrane but are metabolically active and divide by binary fission. These organisms exists every where in both parasitic and free living forms because they have a remarkable capacity to adopt different environments.

Microorganisms, also provide chemical benefits besides natural aids to health. They can be cultured and may be used in the production of acetone, glycerin, organic acids, enzymes, alcohols and many drugs in a more easier and cheaper way than chemists can synthesize them. Microorganisms also help to produce vinegar, pickles, alcoholic beverages, buttermilk cheese and bread\textsuperscript{25}.

In the present age a large number of common diseases of man and animals which are caused by bacterial infections are treated by antibacterial drugs and may require a
long treatment time for their cure. However the present research work has shown that some complexes could be of better use i.e. are more potent than the respective drug against these diseases because these complexes can easily be metabolised into man and animal systems alongwith their quick curative effect.

Bacteria are one celled organism usually spherical, rodlike or branched. They can be discribed as procaryotic, because they have no nucleus. Bacteria are classified into two groups on the basis of staining by the gram (+) ve and gram (-) ve.

(i) **gram (+)ve**: Bateria are those which retain the crystal violet and appear deep violet in colour.

(ii) **gram (-)ve**: Bacteria are those which lose the crystal violet and counter stained by the safranin and hence appear red in colour.

The reason behind this difference is due to their cell-wall composition.

1. High lipid content (11-22%) of gram negative bacteria than gram positive bacteria (1-4%).
2. Higher percentage of peptidoglycon in gram positive bacteria than gram negative bacteria²⁶.

The term *infection* which is derived from the latin *inficere* meaning "to pur or dip into, to stain" implies the presence of microorganisms in or on the body of the host. The role of antibacterial agents becomes more important in our daily life due to the different and easy methods of the spread of bacterial infections including easy carriers like air, water, dust, various food articles and even physical contact²⁷ to inhibit the growth and multiplication of bacteria intensities, the agents which are either bacteriostatic or bactericidal²⁸,²⁹ are required.

(i) **Bacteriostatic agent**: One which reversibly stops growth as a result the number of viable organisms remains constant for hours;

(ii) **Bactericidal agent**: Cause a progressive decrease in the number of viable organisms or kills the bacteria.

The following are the ideal characteristics occurs in an antibacterial agent²⁶.

1. They should have the ability to destroy or inhibit many different species of pathogenic
microorganisms (broad spectrum).

2. They should prevent the ready development of resistant forms of the parasites.
3. They should not produce the undesirable side effects in the host.
4. They should not eliminate the normal microbial flora of the host.
5. Control of microbial enzymes.

The Mechanism of Action of Antibacterial Agents:

1. Inhibition of bacterial cell wall synthesis.
2. Inhibition of nuceleic acid synthesis.
3. Inhibition of bacterial protein synthesis.
4. Inhibition of cytoplasmic membrane function.

The antibacterial activity is studied in vitro in order to determine.

(a) The potency of an antibacterial agent in solution.
(b) The sensitivity of the selected microbe to the known concentration of the drug.

Fungi are heterotrophic microorganisms. They are eukaryotic. They may be unicellular (yeast) or multicellular (mushrooms). The most typical fungi are molds. The word fungi is a general term that includes both yeasts and molds. The former are spherical oral or elongated cells that usually reproduce by budding and form mucoid colonies on agar media. The latter consist of elongated branching cells or hyphae, tangled mass of hyphae constitute mycelia and these appear as dry colonies on agar surfaces or in natural habitats.

The benefits from the growth of fungi may outweigh their harmful effects. Basically fungi are important in the conversion of organic materials into humus. Which permits the survival of higher plants and animals. They may serve as food for animals and are useful in conferring desirable flavars upon other foods. The antibiotics produced by fungi and actinomycetes have been of value in the battle against harmful bacteria.

Fungi are extremely common and are wide spread in distribution but fortunately only a few of them are pathogenic.

The word ‘fungicide’ is derived from the Latin word fungus as microscopic plant
and ‘caedo’ as kill the term is applied to the higher plants to kill the parasites fungi or to prevent the development of fungal disease without seriously injuring the host plant. Nowadays, it is used in the sense of measure to control fungi present in any environment.

The last few years have also seen the isolation of some wide spectrum complexes which have became very important due to their antimicrobial activity. Pathogens can be removed from the human body by complexation with life essential metals and the ligand administered. With these ends in view and to make an humble contribution to a distant goal, the present investigation deals with the study of the chemical nature and the antimicrobial activity of the synthesised complexes in vitro against the following bacteria and fungi described as under.

**BACTERIA**

1. *Bacillus subtilis*:

It is a gram positive bacteria and found in soil, water milk and hay. It was described by Ehrenberg in 1938. It causes anthrax, an acute specific disease of cattle, sheep and swine. In man it may give rise to conjunctivities, may invade blood stream in cachetic disease and furuncle which ulcerates and discharges a seropurulent exudate which may heal and disappear or gangrene may set in followed by septicaemia. This usually terminates fatally in about five days.

2. *Staphylococcus aureus*:

It is gram positive bacteria. The misease is characterised by inflammation, necrosis and abscess formation. Every tissue and organ is susceptible for invasion by these cocci.

3. *Salmonella typhi*:

It is a gram negative bacteria. It’s infection in human beings is known as ‘typhoid’.

4. *Escherichia coli*:

It is a gram negative bacteria. It was first isolated by Escherich (1885) from the faeces of an infant. Its shape is plump rod like (1-2μ in length and 0.6μ in width). The urinary tract infection in humans is the most common infection caused by this bacteria. In addition to urinary infection it causes appendicitic, peritonite epidermic diarrhoea,
peritonities septicaemia and meningitits.

Fungi

1. *Candida albicans*:

   It belongs to the class deutoeromycetes and often isolated from warm-blooded animals, including humans, where it exists as part of the normal flora of mucous membrane. It was the first fungus reported to cause disease in man (*Langebeke*, 1839). In laboratory cultures, it forms white or creamy, opaque paste like colonies. This fungus may become pathogenic, causing candidiasis, a disease that may take a number of forms in humans include cutaneous candidiasis, oral candidiasis (thrush), broncho candidiasis, pulmonary candidiasis, and vulvovaginal candidiasis.

2. *Chrysosporium pannicale*:

   It is a storage fungi, capable of growing in seeds. Results in decrease in germinability, discolouration and total spoilage.

3. *Trichoderma viridae*:

   It is ubiquitons soil fungus and produces white, yellowish or green colonies when cultured. It is used in the commercial production of the enzyme cellulose. *Trichoderma viridae* grow on dead or living aerial parts of plant quite successfully and inhibit or prevent the development of pathogenic micro-organisms there.

4. *Rhizopus stolonifer*:

   It is a commonly occurring fungi and also known as 'bread mold'. It causes the decay of sweet potato.

Identification of the organisms:

The microorgan bacteria were obtained from MTCC Institute of Microbial technology Chandigarh. The fungi were procured from Deptt. of Botany Hari Singh Gour Vishwavidyalaya Sagar.

Bacteria and fungi were identified by using the following stains:

1. Schiff technique - periodic acid
2. Gram's stains
4. Latophenol, cotton blue stains for fungi.

5.2.1. EVALUATION TECHNIQUES $^{41,42}$

The following two methods are most commonly used for testing the microbial susceptibility to chemotherapeutic agents$^{25,43}$.

(a) Disk Diffusion Method

(b) Tube Dilution and Agar Dilution Tests

Various techniques which are used for the evaluation of antimicrobial activity can be summarized as follows$^{44}$:

(i) Agar-Streak Dilution Method

(ii) Serial-Dilution Method

(iii) Agar-Dilution Method can be carried out by

(a) Cup plate Method

(b) Cylinder Method

(c) Paper Disc Method

(iv) Turbidimetric Method

In the determination of paper antimicrobial activity the following conditions must be fulfilled:

1. Proper sterilization of the required material e.g. Nutrient broth, Groth Media, Petry dishes etc.

2. Aseptic environment should be maintained throughout the study.

3. Condition should be maintained unchanged.

4. Proper condition for groth should be provided.

5. Intimate contact between the test organism and the substance to be evaluated.

6. Measurment of Zone of inhibition should be done correctly.

5.2.2. EXPERIMENTAL

Preparation of samples solution:

Test Solution: The solution of the synthesized complexes having a different concentration (0.01M, 0.05M, 0.1M) were made in distilled water.

Standard Solution: Streptomycin was dissolved in distilled water having a
concentration of 40 mg/ml (w/v).

For the present study the filter paper disc diffusion plate\textsuperscript{45-55} method has been adopted and its details are given below.

**Preparation of Culture Media for Bacteria:**

The nutrient agar media having following composition was used for studying antibacterial activity\textsuperscript{56-58}.

- Beef extract 1.0g
- Yeast extract 2.0g
- Peptone 5.0g
- Sodium chloride 5.0g
- Agar 20.0g
- Distilled water 1000 ml (qs)

Accurately weighed quantities were dissolved in the prescribed amount of freshly prepared distilled water. The pH was adjusted to 7.2-7.4 by adding 0.1 N NaOH solution. The media was then autoclaved at 15 lbs for 30 min.

**Preparation of culture media for fungi:**

For the present study potato dextrose agar (PDA) medium having the following composition was used for preparing slants and plates.

- Potato slices 200 gm
- Dextrose 25 gm
- Agar-Agar 20 gm
- Distilled water 1000 ml (qs)

All the components were dissolved in freshly prepared distilled water and were sterilized in an autoclave at 15 lbs pressure for 30 minutes.

**APPARATUS:**

Glass petridishes of 10cm diameter with covers and filterpaper disc of 6mm diameter prepared from whatman filterpaper No.1 were used. These were sterilized prior to use.
STERILIZATION:

The sterilization of media, petridishes, culture tubes, conical flasks, forceps, scissors, loops, paper disc and other materials was done by autoclaving them at 15 lb/sq. inch pressure for 30 minutes in an autoclave. All the tubes and flasks were plugged with cotton. These were wrapped in brown papes tied with thread and then placed in autoclave.

INCUBATION:

The bacteria were inoculated by seeding the nutrient agar media cooled down to 45°C while fungi were inoculated with their spore suspensions (2 x 10⁶ spores/ml).

For studying antibacterial activity of complexes, the culture tubes and the seeded petri-dishes were incubated in an electrically heated incubator at 37°C for 24 hours.

The incubation for antifungal activity was performed at room temperature (30±2°C) for 48 hours.

Preparation of Inoculum: (Stock Culture and Inoculum):

The bacteria were sub-cultured on the nutrient agar plates. The inoculum of bacteria was prepared by transferring a loopful of corresponding organisms from the stock culture into the sterilized broth and incubated.

In the case of fungi the same procedure was adopted using PDA media and incubated as mentioned.

Preparation of Plates:

Nutrient agar and PDA media were sterilized and 15-20 ml of the required media was added to each sterilized petridish. After sufficient colling 2 ml of 24 hours broth cultue of bacteria at 37°C and 2 ml of 48 hours broth cultures of fungi at room temperature were then added to the respective plates and mixed thoroughly by rotary motion of plates, which gave the uniform distribution of the culture.

5.2.3. MEASUREMENT OF ANTIBACTERIAL/ANTIFUNGAL ACTIVITY:

Antibacterial/antifungal activity of complexes was determined by standard paper disc method of Raper et. al.,⁵⁹ sterilized Whatman filter paper no. 1 disc (diameter 6 mm) were dipped into the complex solution of different concentration 0.01 M, 0.05M,
| S.No. | Complexes           | \begin{tabular}{c|ccc|ccc|ccc|ccc|ccc}  
 & \textbf{Bacillus subtilis} & \textbf{Staphylococcus aureus} & \textbf{Salmonella typhi} & \textbf{Escherichia coil} \\
 \hline
 & 0.01M & 0.05M & 0.1 M & 0.01M & 0.05M & 0.1 M & 0.01M & 0.05M & 0.1 M & 0.01M & 0.05M & 0.1 M \\
 1.  & CoTeO₃·H₂O & 9 & 11 & 12 & 9 & 12 & 20 & 12 & 12 & 12 & 9 & 11 & 12 \\
 2.  & NiTeO₃·2H₂O & 8 & 9 & 10 & 8 & 10 & 17 & 8 & 8 & 8 & 8 & 9 & 10 \\
 3.  & CuTeO₃·H₂O & 10 & 10 & 11 & 10 & 15 & 19 & 10 & 11 & 11 & 8 & 10 & 11 \\
 4.  & [Co(en)₃]TeO₃·3H₂O & 8 & 10 & 14 & 10 & 12 & 21 & 8 & 10 & 16 & 10 & 13 & 25 \\
 5.  & [Ni(en)₃]TeO₃·5H₂O & 9 & 18 & 24 & 7 & 15 & 22 & 9 & 17 & 23 & 19 & 19 & 29 \\
 7.  & [Co(Bipy.)₂(H₂O)₂] TeO₃·H₂O & 8 & 11 & 13 & 7 & 19 & 18 & 35 & 38 & 40 & 8 & 11 & 14 \\
 8.  & [Ni(Bipy.)₃] TeO₃·6H₂O & 8 & 10 & 14 & 10 & 15 & 22 & 39 & 43 & 50 & 8 & 10 & 12 \\
 10. & [Co(Phen)₃] TeO₃·4H₂O & 10 & 12 & 18 & 10 & 19 & 28 & 31 & 35 & 40 & 7 & 11 & 14 \\
 11. & [Ni(Phen)₃] TeO₃·6H₂O & 9 & 14 & 19 & 10 & 12 & 17 & 33 & 37 & 48 & 9 & 12 & 16 \\
  \end{tabular} | \} \\

* Inclu ding diameter of filter paper disc (6mm)  
* en = C₂H₄N₂ (1,2-diaminoethane)  
* Bipy. = C₁₀H₁₂N₂ (2,2-Bipyridyl)  
* Phen. = C₁₂H₁₄N₂ (1,10-phenanthroline)
0.1M and were thoroughly moistened with the synthetic complexes solution and control. They were placed on seeded nutrient agar /PDA plates aseptically. These petridishes were incubated at 37°C for 24 hours, whereas the plates with fungi were kept at room temperature for 72 hours. After this period the effects were observed by measuring the diameter of zone of inhibition with the help of divider and scale and compared with that of the standard.

The results are listed in Table 5.3 to 5.6 and shown in Fig. 5.1 to 5.9.

Table 5.4. : IN VITRO ANTIBACTERIAL ACTIVITY OF ALOCOHOLIC EXTRACT OF THE CONTROLS.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the organisms tested</th>
<th>Zone of inhibition standard streptomycin 40 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Bacillus subtilis</em></td>
<td>45.5</td>
</tr>
<tr>
<td>2.</td>
<td><em>Staphylococcus aureus</em></td>
<td>34.0</td>
</tr>
<tr>
<td>3.</td>
<td><em>Salmonella typhi</em></td>
<td>55.0</td>
</tr>
<tr>
<td>4.</td>
<td><em>Escherichia Coil</em></td>
<td>40.0</td>
</tr>
</tbody>
</table>

Table 5.5. : IN VITRO ANTIFUNGAL ACTIVITY OF ALOCOHOLIC EXTRACT OF THE CONTROLS.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the organisms tested</th>
<th>Zone of inhibition standard streptomycin 40 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Candida albicans</em></td>
<td>42.0</td>
</tr>
<tr>
<td>2.</td>
<td><em>Chryosporium pannicale</em></td>
<td>38.0</td>
</tr>
<tr>
<td>3.</td>
<td><em>Trichoderma viridae</em></td>
<td>40.0</td>
</tr>
<tr>
<td>4.</td>
<td><em>Rhizopus stolonifer</em></td>
<td>46.0</td>
</tr>
</tbody>
</table>
### TABLE 5.6. ANTIFUNGAL ACTIVITY OF SYNTHESIZED COMPLEXES AT DIFFERENT CONCENTRATION.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Complexes</th>
<th>Strains of Fungus/Zone of inhibition (mm*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Candida albicans</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01M 0.05M 0.1M</td>
</tr>
<tr>
<td>1.</td>
<td>CoTeO$_3$.H$_2$O</td>
<td>9     11 14</td>
</tr>
<tr>
<td>2.</td>
<td>NiTeO$_3$.2H$_2$O</td>
<td>8     12 14</td>
</tr>
<tr>
<td>3.</td>
<td>CuTeO$_3$.H$_2$O</td>
<td>8     9   13</td>
</tr>
<tr>
<td>4.</td>
<td>[Co(en)$_3$]TeO$_3$.3H$_2$O</td>
<td>nil nil nil</td>
</tr>
<tr>
<td>5.</td>
<td>[Ni(en)$_3$]TeO$_3$.5H$_2$O</td>
<td>nil nil nil</td>
</tr>
<tr>
<td>6.</td>
<td>[Cu(en)$_2$]TeO$_3$.4H$_2$O</td>
<td>nil nil nil</td>
</tr>
<tr>
<td>7.</td>
<td>[Co(Bipy)$_2$(H$_2$O)$_2$] TeO$_3$.H$_2$O</td>
<td>11   14 21</td>
</tr>
<tr>
<td>8.</td>
<td>[Ni(Bipy)$_3$] TeO$_3$.6H$_2$O</td>
<td>9     17 23</td>
</tr>
<tr>
<td>9.</td>
<td>[Cu(Bipy)$_3$] TeO$_3$.3H$_2$O</td>
<td>8     13 19</td>
</tr>
<tr>
<td>10.</td>
<td>[Co(Phen)$_3$] TeO$_3$.4H$_2$O</td>
<td>15    17 20</td>
</tr>
<tr>
<td>11.</td>
<td>[Ni(Phen)$_3$] TeO$_3$.6H$_2$O</td>
<td>15    18 22</td>
</tr>
<tr>
<td>12.</td>
<td>[Cu(Phen)$_3$] TeO$_3$.H$_2$O</td>
<td>17    19 22</td>
</tr>
</tbody>
</table>

* Including diameter of filter paper disc (6mm)

* en = C$_2$H$_8$N$_2$ (1,2-diaminoethane),

* Bipy. = C$_{10}$H$_8$N$_2$ (2,2-Bipyridyl)

* Phen. = C$_{12}$H$_{14}$N$_2$ (1,10-phenanthroline)
FIG. 5.1 - PHOTOGRAPH SHOWING ZONE OF INHIBITION OF METAL ION (A) Co (II), (B) Ni (II) and (C) Cu (II) BASE ON STAPHYLOCOCCUS AUREUS BACTERIA.

FIG. 5.2 - PHOTOGRAPH SHOWING ZONE OF INHIBITION OF 1-2 DIAMINO ETHANE LIGAND AND ITS COMPLEXES WITH METAL ION (A) Co (II), (B) Ni (II) AND (C) Cu (II) ON BACILLUS SUBTILIS BACTERIA.
FIG. 5.3 - PHOTOGRAPH SHOWING ZONE OF INHIBITION OF 2,2'-BIPYRIDYL LIGAND AND ITS COMPLEXES WITH METAL ION (A) Co (II), (B) Ni (II) & (C) Cu (II) ON ESCHERICHIA COLI, BACTERIA.

FIG. 5.4 - PHOTOGRAPH SHOWING ZONE OF INHIBITION OF 1,10-PHENANTHROLINE LIGAND AND ITS COMPLEXES WITH METAL ION (A) Co (II), (B) Ni (II) and (C) Cu (II) ON ESCHERICHIA COLI, BACTERIA.

FIG. 5.5 - PHOTOGRAPH SHOWING ZONE OF INHIBITION OF 2-2' BIPYRIDYL LIGAND AND ITS COMPLEXES WITH METAL ION (A) Ni (II) & (B) Cu (II) ON SALMONELLA TYPHI, BACTERIA.
FIG. 5.6 - PHOTOGRAPH SHOWING ZONE OF INHIBITION OF METAL ION (A) Co (II), (B) Ni (II) and (C) Cu (II) BASE ON RHIZOPUS STOLONIFER FUNGI AND (D) Ni (II) BASE WITH TRICHOSTERMA VIRIDAE, FUNGI

FIG. 5.7 - PHOTOGRAPH SHOWING ZONE OF INHIBITION OF 1-2 DIAMINO ETHANE LIGAND AND ITS COMPLEXES WITH METAL ION (A) Co (II), (B) Ni (II) AND (C) Cu (II) ON TRICHOSTERMA VIRIDAE, FUNGI
FIG. 5.8: PHOTOGRAPH SHOWING ZONE OF INHIBITION OF 2,2'-BIPYRIDYL LIGAND AND ITS COMPLEXES WITH METAL ION (A) Co (II), (B) Ni (II) AND (C) Cu (II) ON CANDIDA ALBICANS, FUNGI

FIG. 5.9: PHOTOGRAPH SHOWING ZONE OF INHIBITION OF 1,10-PHENANTHROLINE LIGAND AND ITS COMPLEXES WITH METAL ION (A) Co (II), (B) Ni (II) AND (C) Cu (II) ON RHIZOPUS STOLONIFER, FUNGI
5.3 RESULTS AND DISCUSSION

The present study concern with the synthesis of complex metal (II) tellurite with organic ligand 2,2'-Bipyridyl. These complexes as well as metal bases were evaluated for anti-inflammatory activity.

(i) TOXICOLOGY: The lethal doses as determined are given in table 5.1. The LD$_{50}$ of complexes E and F were detected. But for the other complexes no mortality occurred up to a dose of 1200 mg/kg body weight. LD$_{50}$ of complex E and F was found to be 850 mg/kg and 750 mg/kg body weight respectively.

(ii) ANTIINFLAMMATORY ACTIVITY: The results are given in table 5.2. It was found that the complexes E \{[Ni(C$_{12}$H$_{8}$N$_{2}$)$_{3}$]TeO$_{3}$,6H$_{2}$O\} and F\{[Cu(C$_{12}$H$_{8}$N$_{2}$)$_{2}$]TeO$_{3}$,3H$_{2}$O\} are more active than the parent metal (II) tellurite. These complexes show better antiinflammatory activity than the 2,2'-Bipyridyl organic ligand. In the present study, it was observed that activity of synthesised metal (II) tellurite.

In the present study was observed that activity of synthesised metal (II) tellurite complexes [Ni (II) and Cu (II) tellurite complexes of 2,2'-Bipyridyl ligand] showed better activity than the copper (II) tellurite complex of the same ligand. The metal (II) tellurite B and C have shown only a negligible response but, A (cobalt (II) tellurite) exhibited good anti-inflammatory activity.

(iii) ANTIMICROBIAL ACTIVITY: Most of the complexes have been found to be very toxic against these pathogens. The table 5.3 to 5.6. and Fig. 5.6 to 5.9 records the inhibition zone of the complexes at three concentration (0.01M, 0.05M, 0.1M) against bacteria/fungi. It has been found that 9 (nine) complexes and metal (II) tellurite (where M(II) = Co(II), Ni(II) and Cu(II)) have a remarkable antibacterial/antifungal activity. It has been found that the all Bipy./Phen. with ligand complexes showed maximum and remarkable antibacterial activity against solmonella typhi bacteria as compared to the parent metal (II) tellurite. All complexes effected by all bacteria 0.01M concentration showed in smallest inhibition but 0.1M concentration higher inhibition.

Inhibition zone of the complexes against fungi have been recorded in table- 5.6. [Co(en)$_{3}$]TeO$_{3}$,3H$_{2}$O, [Ni(en)$_{3}$]TeO$_{3}$,5H$_{2}$O and [Cu(en)$_{2}$]TeO$_{3}$,4H$_{2}$O have no zone of
inhibition against *candida albicans* and *chrysosporium pannicale*. The data reveal that all complexes showed maximum and remarkable inhibition zone against 0.1M concentration by all fungi. It is also clear from data that the microbial activity is a function of the concentration of the complex. The activity increases with the increase in concentration which evident from the increased zone of inhibition.

Higher bactericidal activity of certain metal complexes than the pure complex may be due to the fact that complexation with metal imparts some important characteristics to the complex, which are helpful in its biological activity e.g. low dissociation constant (strong-metal bond), special redox-potential, electron distribution and solubilities. It also helps in the natural process of bond formation and bond cleavage and the group transfer reaction. As a result, the metal complex has increased duration of action and posses enhanced blood concentration, which may probably be due to a comparatively faster diffusion of the metal complex as a whole through the organism, due to its more liposoluble nature (more covalent metal to ligand bond) on being coordinated with the metal ion forming stable complexes.

The higher biocidal activity of the metal complex may also be due to the combined bioactive effect of the metal and the ligand and the higher concentration of the ligand in the complex (1:2 M:L ratio). Further, the antigrowth (inhibition) of the bacteria species may be due to the exchange of trace metals of the metalloenzymes with the metal ions of the complex under test and/or due to steric control of the encumbered and bulky complex molecule. The results of present study clearly indicates the formation of (M:L) 1:2 complexes with involvement of N-atom in metal to ligand-bond resulting in a sufficient high covalent-nature of complex molecules and hence lipid-solubility.

The activity of complex depends on its bioavailability, which in turn depends, apart from other factors, upon its particle size. It has been shown that reduction in particle size increases activity, it increases the solubility of the complex and hence its bioavailability. In most of the cases the complexes having high activity have microparticle size, which helps their higher solubility.
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