

Synthesis, Characterization, Spectral Analysis & Pharmacological Evaluation of Different 1, 4-Naphthoquinone Derivatives and their Metal Chelates

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ABSTRACT

The aim of this work is to synthesize, characterize, spectral analysis and pharmacological evaluation of 1,4-naphthoquinone derivatives and their metal chelates. Continuing our work with another derivatives of 1,4-naphthoquinone ligands, this work had been constructed for synthesis of new ligands derived from 1,4-naphthoquinone. which characterized on the basis of elemental analysis, electronic, IR, H- NMR spectral data. The synthesized ligands have been carried out to achieve the coordination behavior towards bi-valent metal ions like cobalt, zinc and copper. The solid chelates of the different ligands were prepared and subjected to analytical techniques such as elemental analyses, spectroscopic techniques including H-NMR, and IR spectroscopy, and thermal analyses techniques. The chelates were found to have octahedral geometry. The pharmacological activity of the prepared ligands and their binary metals complexes were also screened.

Keywords: 1,4-Naphthoquinone, Metal Complexes, Analgesic Activity, NMR Spectroscopy

INTRODUCTION

In the Synthetic chemistry research the organic chemist attempts to design and synthesize a drug or a pharmaceutical agent which will benefit human being to relieve from various illness. Immediately in the past centenary the standard drugs were primarily discovered by modification of innate matter or by chemical fusion.

Now in this century traditional lines in between physical, chemical and biological, sciences were effaced and in the present millennium, new borderline investigations such as molecular level biology, pharmacology, biological medicine, cellular biology, genetics and other began to capture the interest of chemical scientist with the race to discover novel drug molecules..

Naphthoquinone and their derivatives:

Most of Naphthoquinone are mainly obtained from natural source. The naphthoquinone are having different biological activities. Chemically naphthoquinone are the class of organic compounds derived from Naphthalene. Normally these exist in three isomeric forms. i.e.

- 1] 1,2-Naphthoquinone
- 2] 1,4-Naphthoquinone
- 3] 2,6-Naphthoquinone.

1,2-Naphthoquinone is a polycyclic aromatic organic compound found to be one of the important metabolite of naphthalene. The gathering of this poisonous metabolite in rats has been shown to cause damage to eye and cataract formation.

1,4-Naphthoquinone is yellow to green powder with an odour of benzoquinone. It is insoluble in water. It reacts along several bases and acids eliminating heat and flammable gases. It also react with some aldehyde and perchloric acid. When 1,4-Naphthoquinone is heated to decomposition emit toxic fumes and smoke.

Juglones have been specially assigned to an interesting class of compounds in which the -OH group is placed at ortho or para positions to single one of the carbonyl groups.

SYNTHESIS OF DIFFERENT 1,4-NAPHTHOQUINONE DERIVATIVES & THEIR METAL CHELATES

General Method for synthesis of Metal chelates of different 1,4-Naphthoquinone derivatives:-

Step.1)Preparation of Substituted 1,4-Naphthoquinone Ligand Solution:- The different Substituted 1,4-Naphthoquinone Ligands were weighed appropriately to obtain their accurate 2×10^{-3} M i.e. 0.002M solutions. The weighed ligands were dissolved in sufficient

amount of methanol to dissolve the ligand completely and diluted with distilled water. In case of each experiment, clear solution of the ligand was obtained.

Step.2) Preparation of the different metal acetate salt Solution: Aqueous solution of the different metal ions corresponding to $1 \times 10^{-3} \text{M}$ i.e. 0.001M were got up by mixing the correct amount of different metal acetate in distilled water.

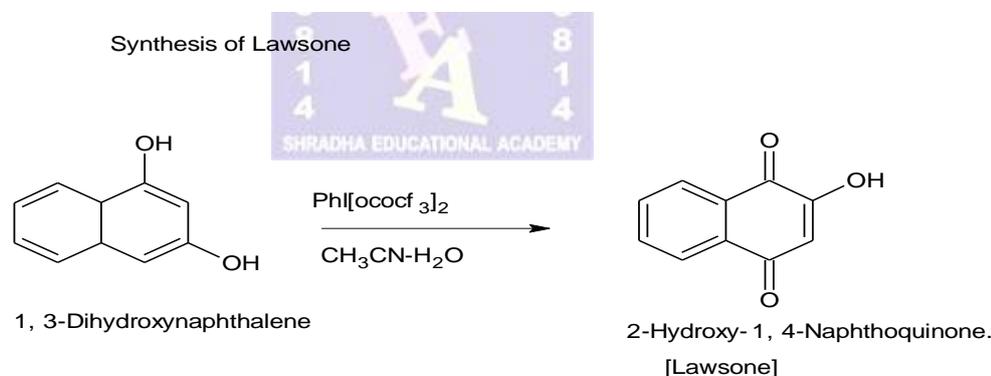
Step.3) Preparation Aqueous Ammonia solution [10%]: It was got up in the distilled water and can be used for adjusting the pH of reaction mixture.

Step.4) Synthesis of different Metal Chelates of different 1,4-Naphthoquinone derivatives:- The different substituted 1,4-Naphthoquinone ligand solutions of $2 \times 10^{-3} \text{M}$ as prepared above was taken in a 3 neck-flask. The different metal ion solution of $1 \times 10^{-3} \text{M}$ was a mixed drop by drop from a dropping funnel into the ligand solution, and was stirred continuously. After the addition of the solution the aqueous Ammonia solution [10%] was added drop wise to adjust pH 5-6. The flask containing the reaction mixture was then kept overnight in refrigerator, after stirring the resultant chemical reaction mixture approximately about sixty minutes i.e. one hour then reaction mixture subjected to filter under vacuum. Room temperature was maintained. Then the mixture was thoroughly washed by fresh distilled water and then washed with methyl alcohol. Lastly the different metal chelates of substituted 1,4-Naphthoquinones were dried in vacuum desiccator.

Synthesis of 2-Hydroxy-1,4-Naphthoquinone [Lawsone] and Their Metal Chelates.:

Synthesis of 2-Hydroxy-1,4-Naphthoquinone [Lawsone]: Analogous oxidation of 1,3-dihydroxynaphthalene in presence [bis(trifluoroacetoxy)iodo]benzene in $\text{CH}_3\text{CN}-\text{H}_2\text{O}$ gives lawsone.

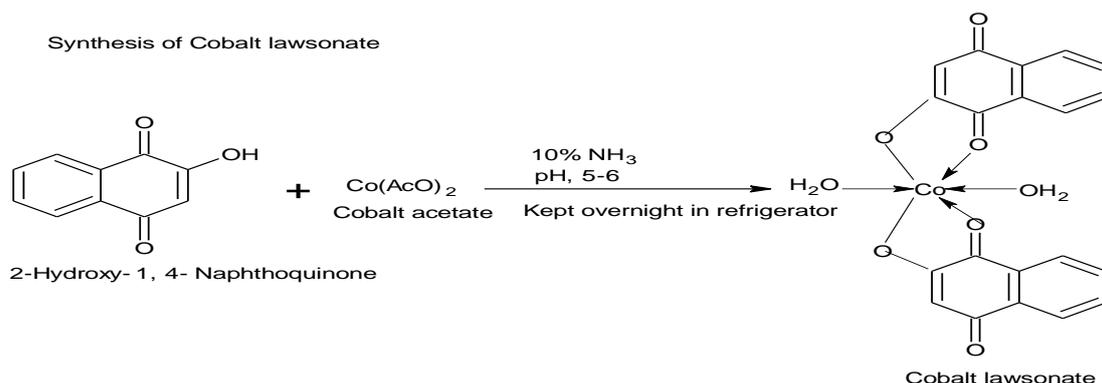
Scheme of Synthesis:-



Synthesis of Metal [Co,Cu,Zn&Cd] chelates of Lawsone:

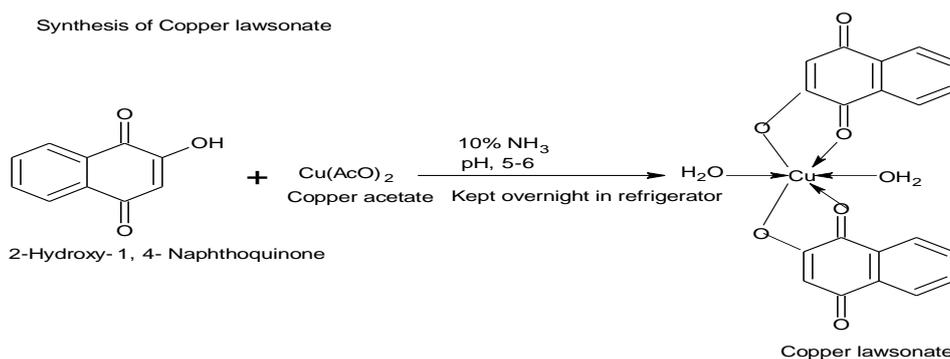
A. Synthesis of Cobalt [Co]Lawsonate:

Scheme of Synthesis



B. Synthesis of Copper [Cu]Lawsonate:

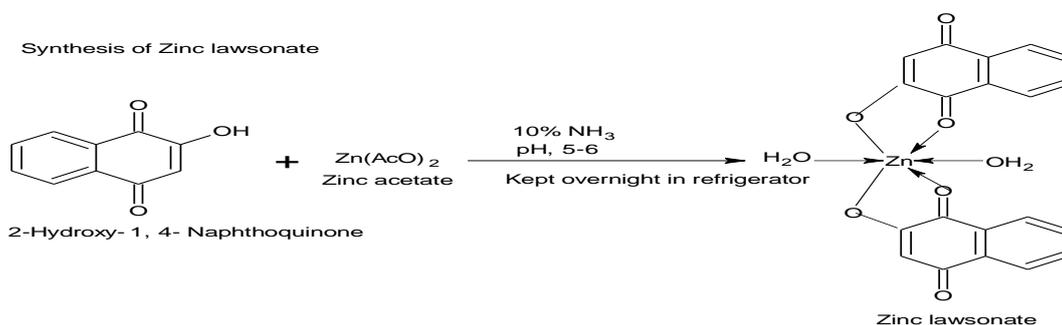
Synthesis of Copper lawsonate



C.Synthesis of Zinc [Zn]Lawsonate:

Scheme of Synthesis

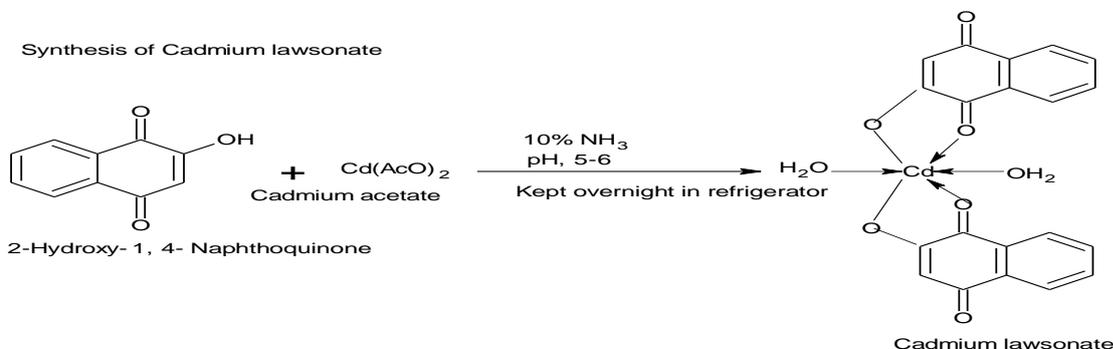
Synthesis of Zinc lawsonate



D.Synthesis of Cadmium [Cd]Lawson ate:

Scheme of Synthesis

Synthesis of Cadmium lawsonate

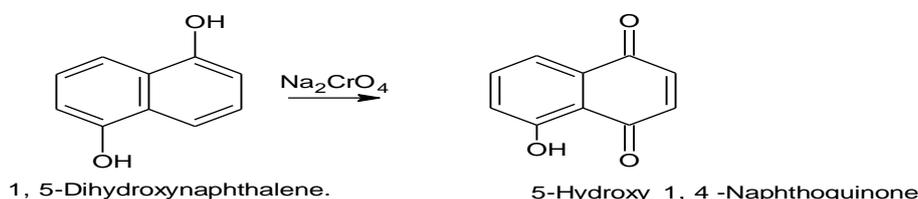


Synthesis of 5-Hydroxy-1,4-Naphthoquinone [Juglone] and Their Metal Chelates.:
Synthesis of 5-Hydroxy-1,4-Naphthoquinone [Juglone]:

Sodium dichromate in water will be carefully added to conc. H_2SO_4 . To this slurry add, 1,5-dihydroxy naphthalene. The mixture will then heated to no more than 50°C . for about 30 minutes. Crude product can be recrystallized from hexane. Yellow orange needles will produced with M.P. $148-153^\circ\text{C}$.

Scheme of Synthesis

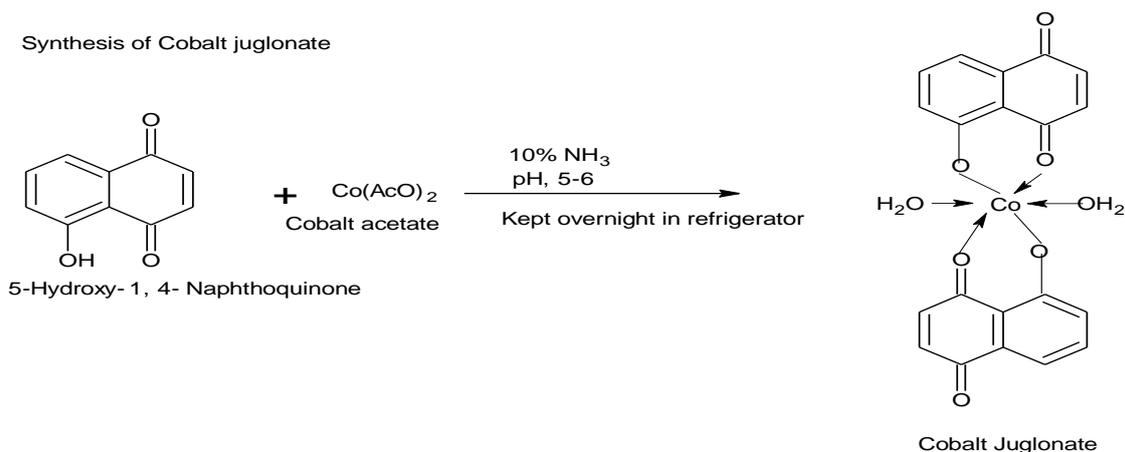
Synthesis of Juglone



Synthesis of Metal [Co,Cu,Zn&Cd] chelates of Juglone:

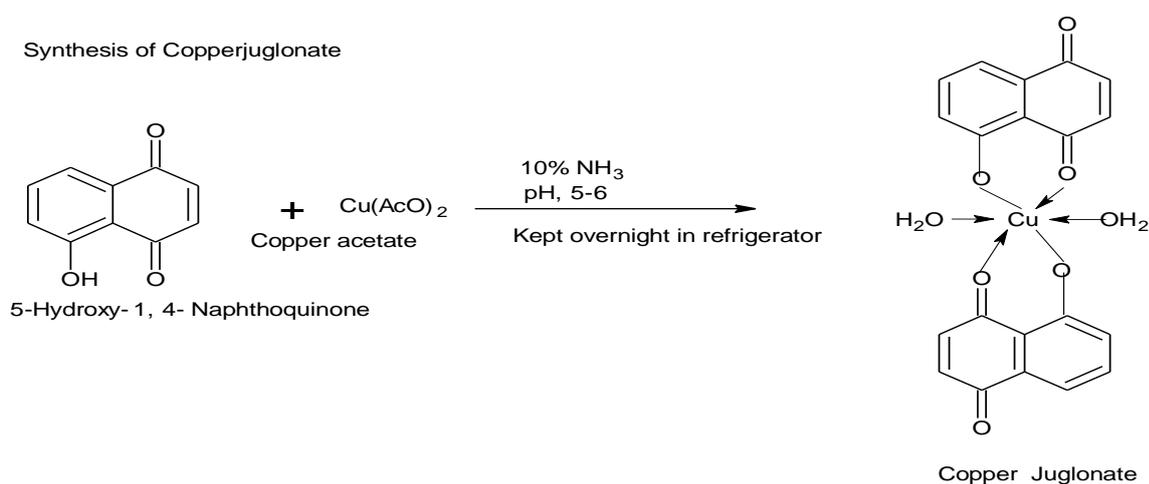
A.Synthesis of Cobalt [Co]Juglonate:

Scheme of Synthesis



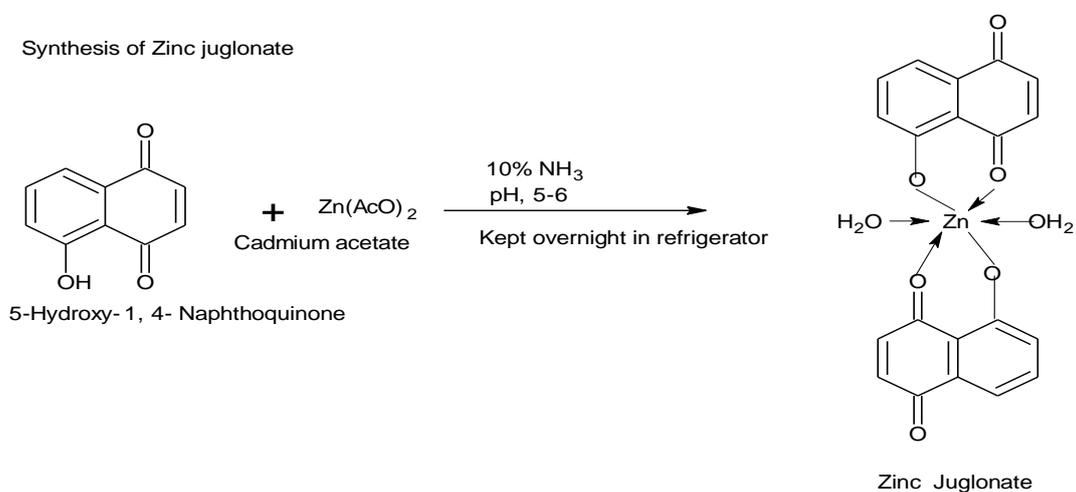
B.Synthesis of Copper [Cu] Metal Juglonate:

Scheme of Synthesis

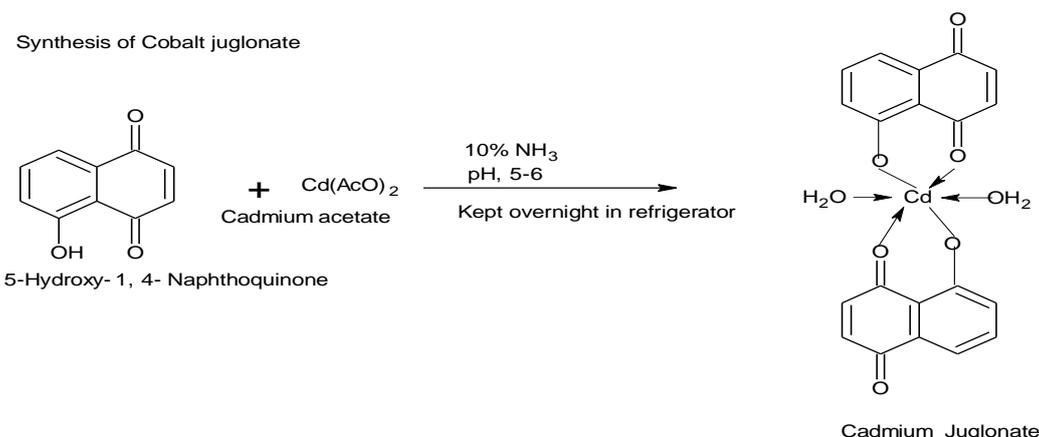


C.Synthesis of Zinc [Zn] MetalJuglonate:

Scheme of Synthesis



Scheme of Synthesis

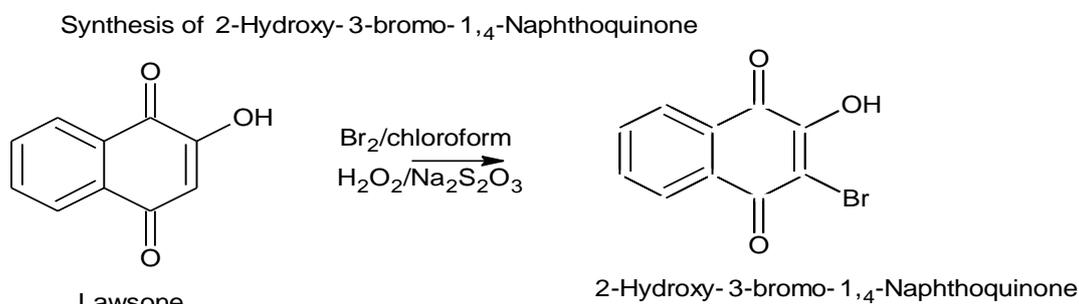


Synthesis of 2-hydroxy-3-bromo-1,4-naphthoquinone and Their Metal Chelates:

Synthesis of 2-hydroxy-3-bromo-1,4-naphthoquinone:

A solution of lawsone was stirred in chloroform and then cooled to 20^{0C}. Later 2N H₂SO₄ was added to it. The resulting mixture was slowly added to bromine and 0.7ml of solution H₂O₂ 30%. The reaction was observed constantly by TLC until all the lawsone was consumed. After the reaction came to room temperature, a solution of Na₂S₂O₃ 10% was added to eliminate any residues of bromine. The reaction mixture was extracted with chloroform. The combined organic phase was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to get the final product.

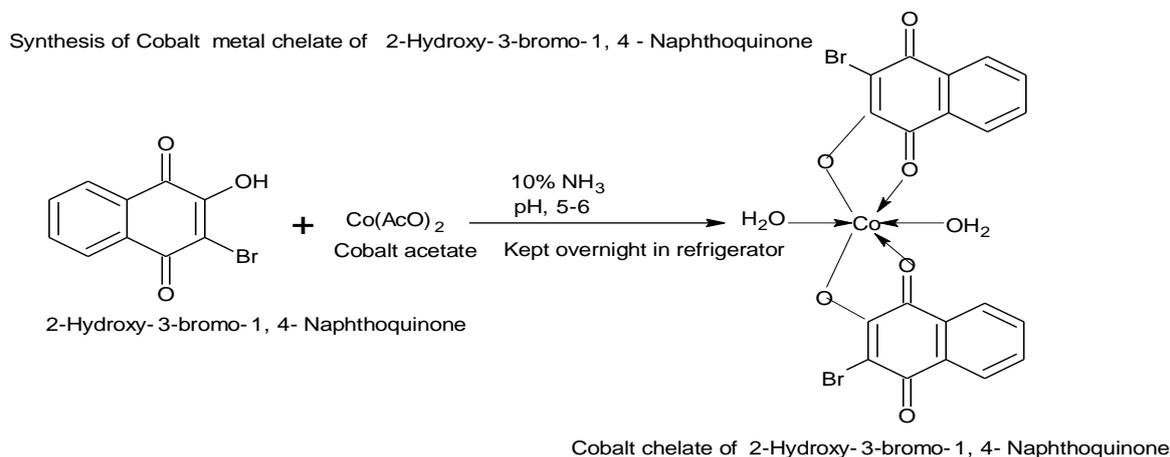
Scheme of Synthesis



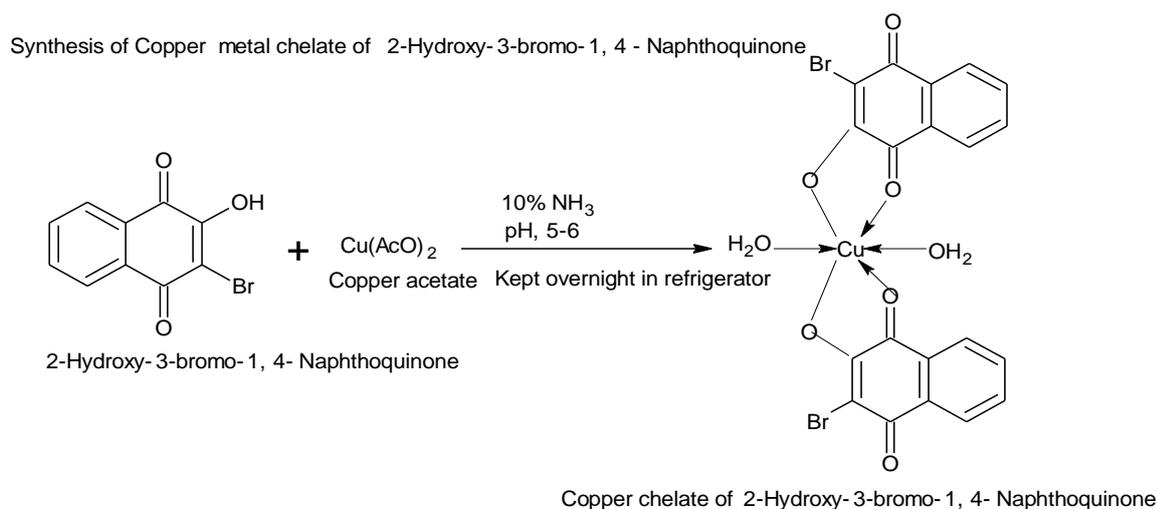
Synthesis of Metal [Co,Cu,Zn&Cd] chelates of 2-Hydroxy-3-bromo-1,4-naphthoquinone:

A.Synthesis of Cobalt [Co] Metal Chelate of 2-Hydroxy-3-Bromo-1,4-naphthoquinone:

Scheme of Synthesis

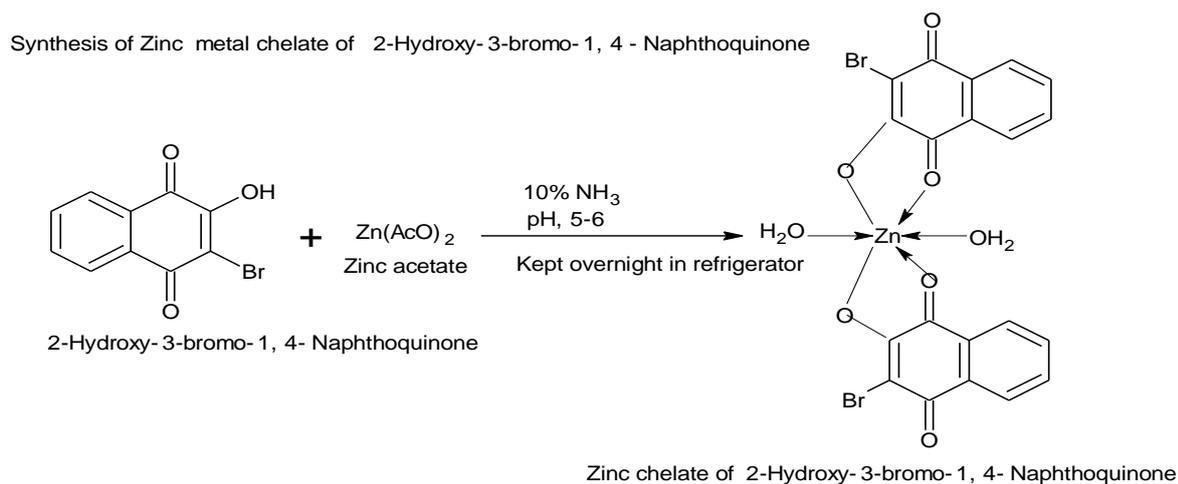


Scheme of Synthesis



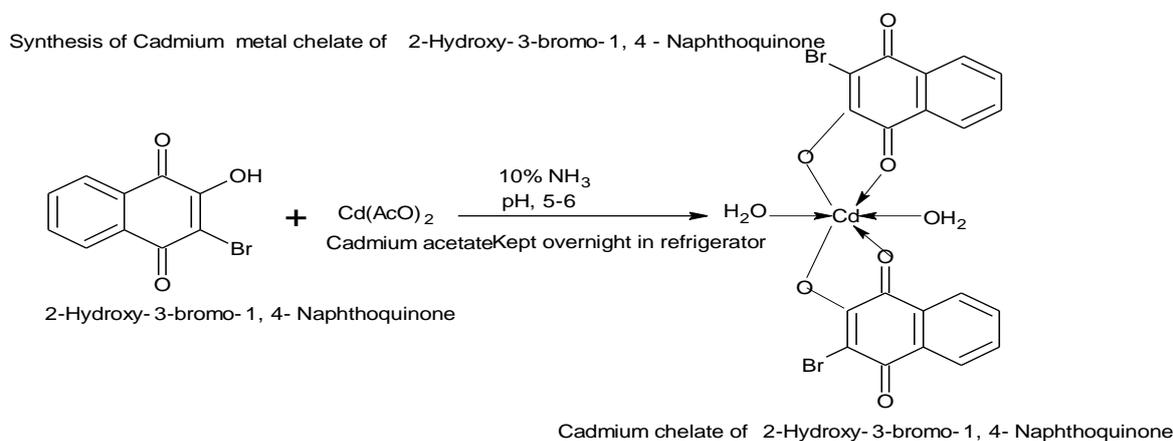
C.Synthesis of Zinc [Zn] Metal Chelate of 2-Hydroxy-3-Bromo-1,4-naphthoquinone:

Scheme of Synthesis



D.Synthesis of Cadmium [Cd] Metal Chelate of 2-Hydroxy-3-Bromo-1,4-naphthoquinone:

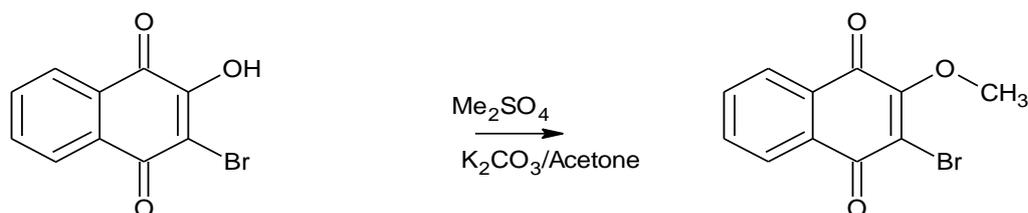
Scheme of Synthesis



Synthesis of 2-methoxy-3-bromo-1,4-naphthoquinone and their Metal chelates:

Scheme of Synthesis

Synthesis of 2-Methoxy- 3-bromo- 1, 4-Naphthoquinone



2-Hydroxy- 3-bromo- 1, 4-Naphthoquinone

2-Methoxy- 3-bromo- 1, 4-Naphthoquinone

CHARACTERIZATION OF 1,4-NAPHTHOQUINONE DERIVATIVES AND THEIR METAL CHELATES

In this chapter the different physical characters of metal chelates of 1,4-Naphthoquinone derivatives and their metal chelates described in chapter-II. By

1. Elemental Analysis
2. Physical Constant(M.P.) determination
3. IR Spectral Studies
4. NMR Spectral Studies.

Elemental Analysis of 1,4-Naphthoquinone Derivatives And Their Metal Chelates.:

All the compounds synthesized were subjected for elemental analysis of C,H,O and N contents by Perkin Elmer Elemental analyser. The C,H, O & N contents of all compounds are shown in table.

Table1: Elemental Analysis of Synthesized compounds.

Sr. No	Compound	Molecular Formula	Mol.Wt gm/mole	Elemental Analysis					
				% C		% H		% O	
				Cal.	Found	Cal.	Found	Cal.	Found
1	A	C ₁₀ H ₆ O ₃	174	68.96	68.88	3.44	3.36	27.58	27.49
2	A-1	Co[C ₁₀ H ₅ O ₃] ₂ .2H ₂ O	441	54.42	54.38	3.17	3.09	29.02	28.95
3	A-2	Cu[C ₁₀ H ₅ O ₃] ₂ .2H ₂ O	445.5	53.87	53.81	3.14	3.07	28.73	28.66
4	A-3	Zn[C ₁₀ H ₅ O ₃] ₂ .2H ₂ O	447.4	53.64	53.57	3.13	3.08	28.61	28.54
5	A-4	Cd[C ₁₀ H ₅ O ₃] ₂ .2H ₂ O	494.4	48.54	48.49	2.83	2.74	25.89	25.82
6	B	C ₁₀ H ₆ O ₃	174	68.96	68.88	3.44	3.36	27.58	27.49
7	B-1	Co[C ₁₀ H ₅ O ₃] ₂ .2H ₂ O	441	54.42	54.38	3.17	3.09	29.02	28.95
8	B-2	Cu[C ₁₀ H ₅ O ₃] ₂ .2H ₂ O	445.5	53.87	53.81	3.14	3.07	28.73	28.66
9	B-3	Zn[C ₁₀ H ₅ O ₃] ₂ .2H ₂ O	447.4	53.64	53.57	3.13	3.08	28.61	28.54
10	B-4	Cd[C ₁₀ H ₅ O ₃] ₂ .2H ₂ O	494.4	48.54	48.49	2.83	2.74	25.89	25.82
11	C	C ₁₀ H ₆ O ₃ Br	254	47.24	47.16	2.36	2.27	18.89	18.80
12	C-1	Co[C ₁₀ H ₅ O ₃ Br] ₂ .2H ₂ O	601	39.93	39.84	2.32	2.24	21.29	21.21
13	C-2	Cu[C ₁₀ H ₅ O ₃ Br] ₂ .2H ₂ O	605.5	39.63	39.54	2.31	2.24	21.13	21.07
14	C-3	Zn[C ₁₀ H ₅ O ₃ Br] ₂ .2H ₂ O	607.4	39.51	39.43	2.30	2.22	21.07	21.01
15	C-4	Cd[C ₁₀ H ₅ O ₃ Br] ₂ .2H ₂ O	654.4	36.67	36.59	2.14	2.05	19.56	19.48
16	D	C ₁₁ H ₇ O ₃ Br	267	49.43	49.35	2.62	2.53	17.98	17.89
17	D-1	Co[C ₁₁ H ₇ O ₃ Br] ₂ .2H ₂ O	629	41.97	41.88	2.86	2.77	20.35	20.27
18	D-2	Cu[C ₁₁ H ₇ O ₃ Br] ₂ .2H ₂ O	633.5	41.67	41.59	2.84	2.76	20.20	20.14
19	D-3	Zn[C ₁₁ H ₇ O ₃ Br] ₂ .2H ₂ O	635.4	41.55	41.47	2.83	2.75	20.14	20.06
20	D-4	Cd[C ₁₁ H ₇ O ₃ Br] ₂ .2H ₂ O	682.4	38.69	38.62	2.64	2.55	18.76	18.38

Physical Constant(M.P.) determination of different 1,4-Naphthoquinone Derivatives and Their Metal Chelates.:

The melting points of the compounds that were synthesized were determined with the help of digital melting point apparatus. The Melting point of various synthesized compounds are shown as in the following table.

Table 2: Physical Constant(M.P.) determination of different 1,4-Naphthoquinone Derivatives And Their Metal Chelates.:

Sr. No.	Compound No.	Melting Point [0°C]	% Yield
1	A	191-193	72
2	A-1	210-212	65
3	A-2	218-220	63
4	A-3	220-222	65
5	A-4	210-212	68
6	B	164-165	68
7	B-1	208-210	56
8	B-2	209-211	56
9	B-3	210-212	55
10	B-4	221-223	54
11	C	192-194	92
12	C-1	210-212	78
13	C-2	198-200	72
14	C-3	210-212	85
15	C-4	206-208	81
16	D	186-188	80
17	D-1	201-203	78
18	D-2	198-200	76
19	D-3	199-201	75
20	D-4	202-204	72

Infrared Spectral Studies:

IR Spectra of different 1,4-Naphthoquinone derivatives and their metal chelates:

Infrared Spectrum of synthesized compounds were scanned in the range 400-4000cm⁻¹ by using ATR(Attenuated Total reflection) sampling technique used coupled with FTIR. This specific arrangement enables samples to be examined directly in any state, solid or liquid without requiring any further preparation.

The Infra Red Spectra of different 1,4-Naphthoquinone Derivatives And Their Metal Chelates were recorded and are shown in the fig 3.1-3.50. The anticipated IR spectral frequencies of all different 1,4-Naphthoquinone Derivatives And Their Metal Chelates are shown below the respective spectral diagram.

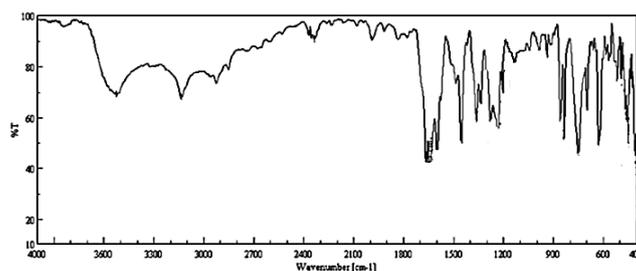


Fig. 1: IR Spectrum of Compound – A

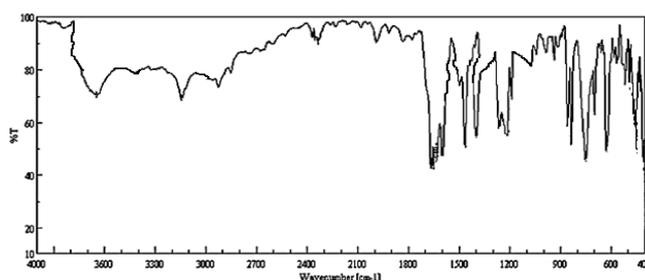


Fig. 2: IR Spectrum of Compound – A-1

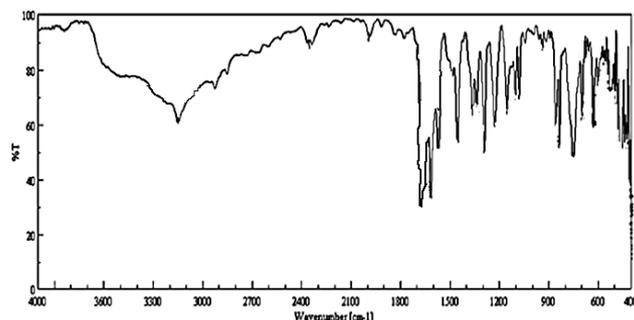


Fig.3: IR Spectrum of Compound – A-2

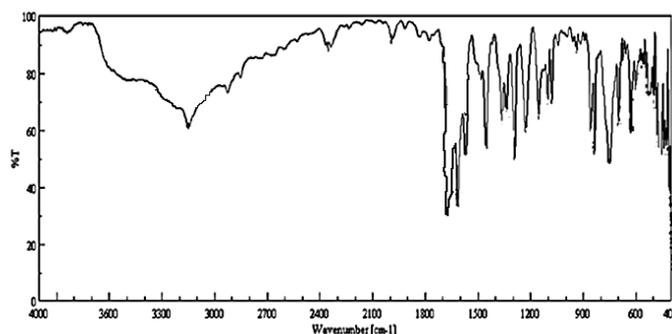


Fig. 4: IR Spectrum of Compound – A-3

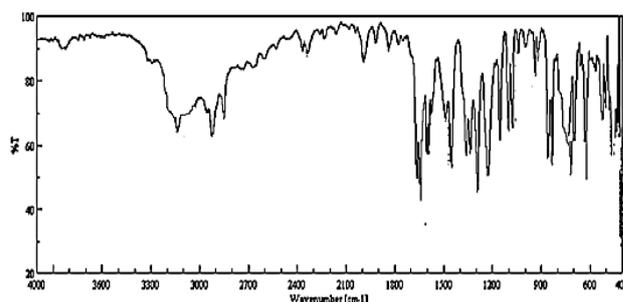


Fig. 5: IR Spectrum of Compound – A-4

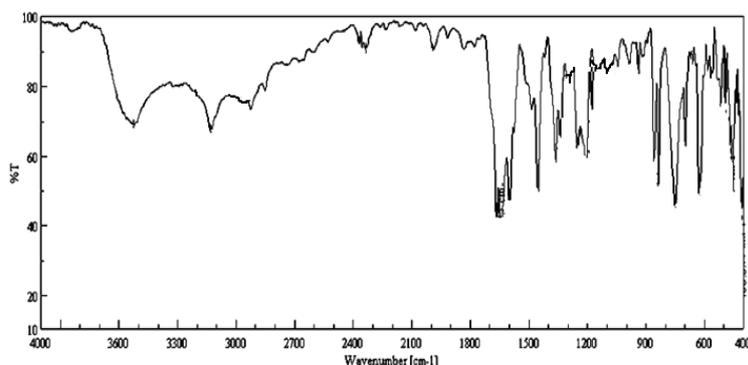


Fig 6: IR spectra of Compound- B.

NMR Spectral Studies:

¹H NMR Spectra of different 1,4-Naphthoquinone derivatives and their metal chelates:

There is complete dissociation of required amount of sample in the appropriate volume of suitable NMR solvent in step-in running of NMR spectrum. Carbon tetrachloride [CCl₄], Deuteron chloroform {CDCl₃}, Deuteron methyl sulphoxide [DMSO] etc. are commonly

used solvents for this purpose. TMS is generally utilized as internal standard for measuring the position of ^1H , ^{13}C in the NMR spectrum for various reasons. Firstly, it gives a single sharp peak. Secondly, it is chemically inert and miscible with a large range of solvents. It can be easily removed in case the sample has to be recovered because it is extremely volatile. Finally, it does not involve in the process of intermolecular association with the sample.

The different synthesized 1,4-Naphthoquinone derivatives and their metal chelates were dissolved in appropriate NMR solvents, $[\text{CCl}_4]$, Deuteron chloroform $[\text{CDCl}_3]$ and Deuteron methyl sulphate $[\text{DMSO}]$ and run in the NMR spectrum using TMS as internal standard to get the ^1H , NMR Spectra of different 1,4-Naphthoquinone Derivatives and their metal and the respective spectra were recorded. They are shown in the fig 3.51-3.70. The anticipated NMR spectral chemical shift δ values of all different 1,4-Naphthoquinone Derivatives and their metal chelates are given in the table.

HNMR Spectra of different 1,4-Naphthoquinone derivatives and their metal chelates.

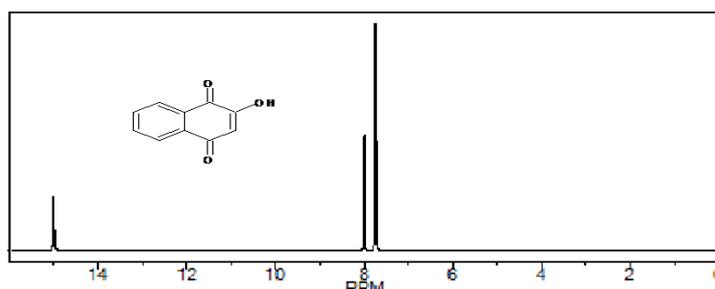


Fig 21: NMR spectra of Compound A

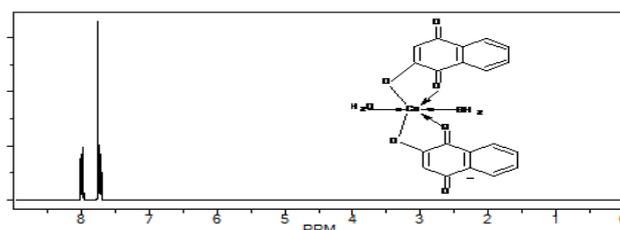


Fig 22: NMR spectra of Compound A-1

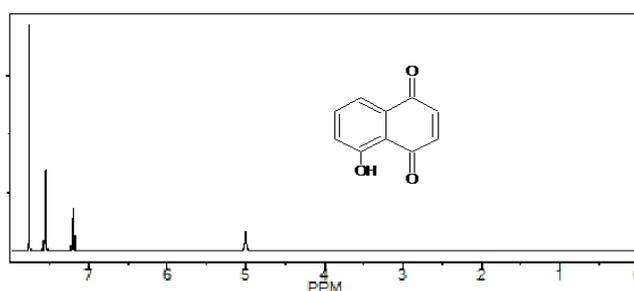


Fig 23: NMR spectra of Compound B

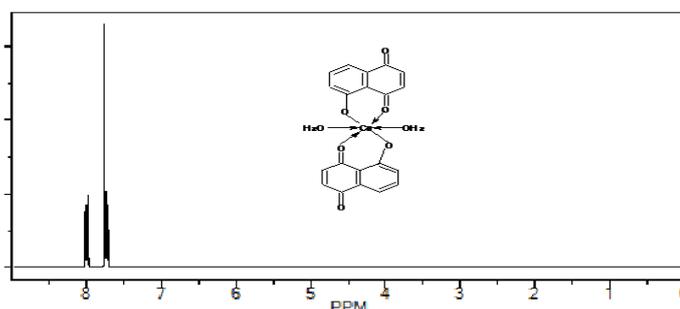


Fig 24: NMR spectra of Compound B-1

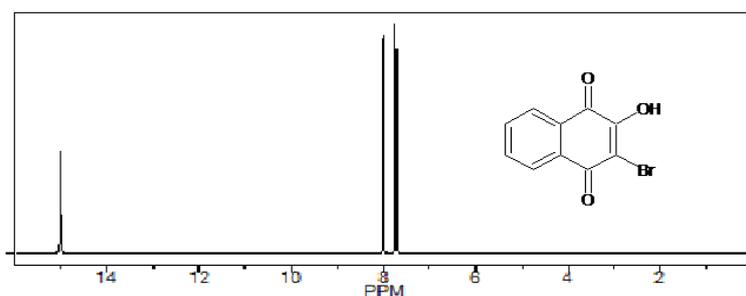


Fig 25: NMR spectra of Compound C

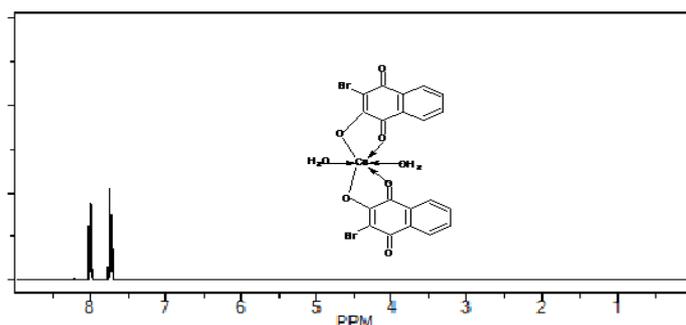


Fig 26: NMR spectra of Compound C-1

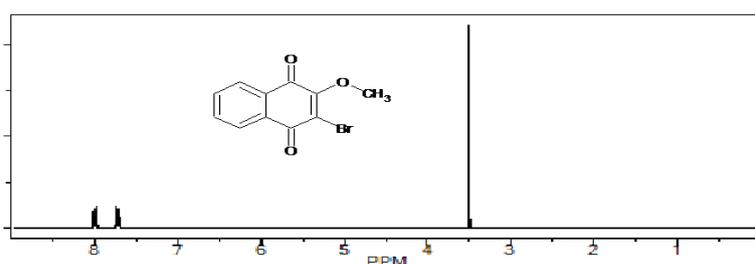


Fig 27: NMR spectra of Compound D

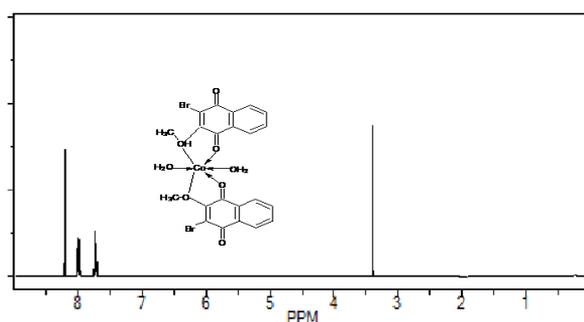


Fig 28: NMR spectra of Compound D-1

ANALGESIC ACTIVITY OF DIFFERENT 1,4-NAPHTHOQUINONE DERIVATIVES AND THEIR METAL CHELATES.

Experimental methods For Analgesic effects

Various procedures are there for screening of drug for analgesic activity. In most of them one or other type of stimulus can be applied for producing pain reaction. The various methods are classified according to the type of stimulus used.

1. Thermal stimulus

- Hot plate test.
- Radiant heat method using analgesiometer or electric bulb as the source.

2. Mechanical stimulus

- a) Tail clip
- b) Randal Sellito test

3. Chemical stimulus

- a) Writhing by acetic acid or phenylquinone

4. Electric stimulus

- a) Pododolorimeter
- b) Rectodolorimeter

The Randall Sellito test and the chemically induced writhing tests are used to evaluate the analgesic activity of peripherally acting drugs. The other methods are chiefly meant to evaluate drugs that act by central mechanisms. Other methods like the tail clip, tail flick and hot plate methods can also be used for screening of non-narcotic drugs.

EVALUATION OF ANALGESIC ACTIVITY OF SYNTHESISED COMPOUNDS.:-

Material and methods:

Materials:-

Different 1,4-Naphthoquinone derivatives, Albino mice, Diclofenac Sodium. Carboxy Methyl cellulose and Analgometer were used as received.

Acute Toxicity Studies: The acute toxicity study, before analgesic activity was carried out by Karber's method^[246], and the LD₅₀ value of synthesized compounds were found to be 200mg/kg body weight of albino mice. Hence the ED₅₀ was selected as 20mg/kg body weight of albino mice for the present study.

Study of Analgesic activity of compounds,

1. Tail Flicking Method/ Heat Conduction Method^[247]: Swiss albino mice were used for the experiment. They weighed 20-30gm and consisted of both the sexes. They were maintained under controlled condition of light (12 hr) and temperature 25±1⁰c. in the animal house. These rats were grouped into twelve [12] groups of six animals each. Group-I was administered only carboxymethyl cellulose, and it served as control group. Group-II was the standard group and it was administered Sodium Diclofenac (10 mg/kg. i.p) Group-III-XII received oral 20mg/kg body weight of synthesized different 1,4-Naphthoquinone derivatives. A period of 60 minutes was allowed to elapse after oral administration of synthesized compounds and half an hour after the intraperitoneal injection of sodium salt of Diclofenac. After this the tip of the tail of every rat was immersed up to the five centimeter into the hot water which was maintained at about 57⁰c and the stimulus response time was taken for the immediate withdrawal of the tip of the tail of the rat from the hot water. During this the cut off time period of 12 seconds was maintained.

The results are shown in table and represented in graph.

2. Eddy's Hot Plate Method^[248]:

In this method the albino species mice group of 12 mice in each group six mice were selected. The first Group-I considered as the group of control and it received only carboxymethyl cellulose. Group-II, that was considered to be the standard group, received Sodium Diclofenac (10 mg/kg. i.p). Group-III-XII received oral 20mg/kg body weight of synthesized different 1,4-Naphthoquinone derivatives. After sixty minutes post oral administration of synthesized compounds and 30 minutes after i.p. injection of Sodium Diclofenac, the mice were individually placed on the Hot plate where a temperature of 55⁰C was deliberately maintained. The responses noted in this case were licking of paw or jumping of animal whichever is first observed was noted down. Here the time period of 15 seconds was maintained as cut off period.

The results are shown in table and represented in graph.

Statistical analysis:

All the experimental data obtained were analysed statistically. The data were subjected for One way ANOVA test and further followed by Dunnett's test which will enable us to individual comparison of groups with the controlled group. The p values less than 0.001 were considered to be significant.

All values of statistical analysis are expressed as mean ± SEM.

**Table 3: Analgesic activity of Compounds (A-B)
 [A,A₁,A₂,A₃,A₄,B,B₁,B₂,B₃&B₄] By Tail Flick Method.**

Sr. No.	Groups	Dose [mg/kg]	Reaction Time (seconds)
1	Group-I (Control)	0.1ml of 1% w/v of CMC.	2.6±0.254
2	Group-II(Standard)	10	11.2±0.123
3	Group-III (Compd.A)	20	5.8±0.316
4	Group-IV(Compd.A ₁)	20	7.5±0.354
5	Group-V(Compd.A ₂)	20	7.1±0.324
6	Group-VI(Compd.A ₃)	20	8.1±0.215
7	Group-VII(Compd.A ₄)	20	6.5±0.381
8	Group-VIII(Compd.B)	20	5.9±0.421
9	Group-IX(Compd.B ₁)	20	7.3±0.354
10	Group-X (Compd. B ₂)	20	7.2±0.214
11	Group-X (Compd. B ₃)	20	8.0±0.316
12	Group-X (Compd. B ₄)	20	6.4±0.381

Values are expressed as mean ± SEM; n=6; p<0.001

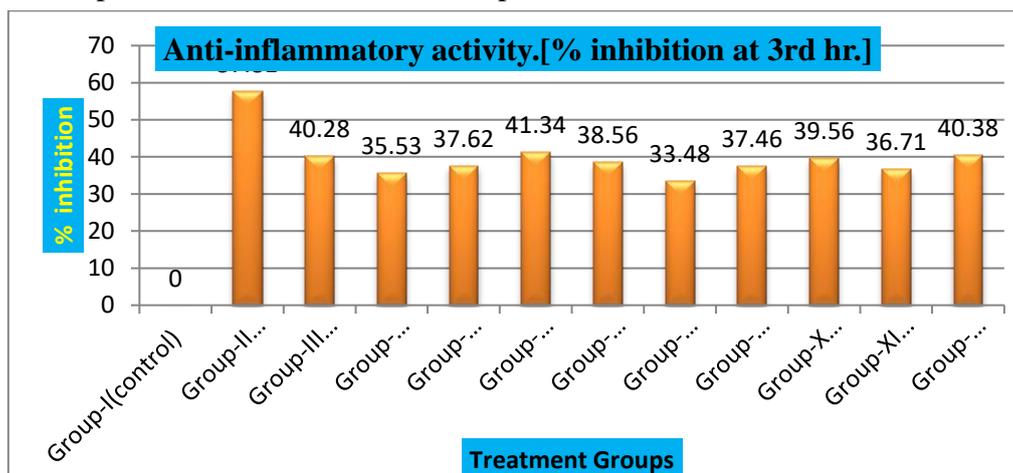


Fig. 36: Histogram showing anti-inflammatory(% inhibition) activity of Compounds.[C,C₁,C₂,C₃,C₄,D,D₁,D₂,D₃&D₄] .

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