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Jointly organized by:

Automation, Computer Science and Technology Department, Kryvyi Rih National University, Ukraine
'Research Culture Society'

and

'Scientific Research Association'

Conference Special Issue - 31

November - 2021

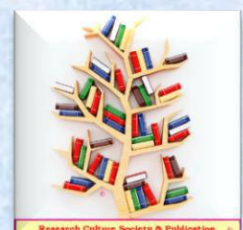


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EURASIAN CONFERENCE
ON
SCIENCE, ENGINEERING &
TECHNOLOGICAL
INNOVATIONS

(ECSETI – 2021)

20 & 21 Nov, 2021

Conference Special Issue - 31

The Managing Editor:

Dr. Chirag M. Patel

(Research Culture Society & Publication)

Jointly Organized By :

**Automation, Computer Science and Technology
Department, Kryvyi Rih National University, Ukraine**

Research Culture Society

and

Scientific Research Association

Eurasian Conference on Science, Engineering & Technological Innovation

20 & 21 Nov, 2021

(Conference – Special Issue)

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About the organizing Institutions:

Kryvyi Rih National University is one of the largest education institutions of the central region of Ukraine for qualified personnel training in metallurgical, mining, engineering and technological specializations. Scientific subjects performed by the university aimed to increasing the efficiency of production and control processes, power saving and environmental protection.

Automation, Computer Science and Technology Department

ACST department trains specialists in computer science, automation and computer-integrated technologies. The main educational and scientific areas are: information support for decision making, , development and implementation of process automation and mechatronics systems.

‘Research Culture Society’ is a Government Registered Scientific Research organization. Society is working for research community at National and International level to impart quality and non-profitable services. Society has successfully organized 100+ conferences, seminars, symposiums and other educational programmes at national and international level in association with different educational institutions.

Objectives of the International Conference :

Our main objective is to promote scientific and educational activities towards the advancement of common citizen’s life by improving the theory and practice of various disciplines of science and engineering. The aim of the conference is to provide an interaction stage for researchers and practitioners from academia and industries to deal with state-of-the-art advancement in their respective fields.



KRIVYI RIH NATIONAL UNIVERSITY

Prof. Natalia Morkun

Head of Department of Automation,
Computer Sciences and Technologies

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MESSAGE

Dear Colleagues!!!

I am proud to be the part of Organizational Committee of two-day International online “Eurasian Conference on Science, Engineering & Technological Innovations - 2021”, jointly organized by ‘Research Culture Society’, ‘Scientific Research Association’ and Department of Automation, Computer Sciences and Technologies, Kriviy Rih National University (20 & 21 Nov, 2021).

We have an exciting program at this conference that will allow participants to reflect upon and celebrate their accomplishments, renew friendships and extend networks, and jointly explore current and future research directions. I hope that all participants will have a productive and fun-filled time at this online conference.

I sincerely hope that this conference will deliberate and discuss all the different facets of this exciting topic and come up with recommendations that will lead to a better world.

I wish the conference great success.

Prof. Natalia Morkun



Message

Dear Professional Colleagues.

I am happy that Automation, Computer Science and Technology Department, Kryvyi Rih National University, Ukraine in collaboration with 'Research Culture Society' (Government Registered Scientific Research organization, India) are organizing 'Eurasian Conference on Business, Management, Social and Economical Advancements' during 20 & 21 Nov, 2021.

The aim of the conference is to provide an interaction stage for researchers, practitioners from academia and industries to deal with state-of-the-art advancement in their respective fields. The main objective is to promote scientific and educational activities towards the advancement of common citizen's life by improving the theory and practice of various disciplines of science and engineering. Provide the delegates to share their new ideas and the application experiences face to face.

I believe, this International Conference will help in redefining the strong connection between science, engineering and technology students and academicians from different institutions. An additional goal of this international conference is to combine interests and scientific research related to basic, applied and allied sciences, engineering and technology to interact with members within and outside their own disciplines and to bring people closer for the benefit of the scientific community worldwide.

My best wishes to the committee members, speakers and Participants of this scientific conference.

Dr.C. M. Patel

Director, Research Culture Society.

Conference Committee

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Dr. Pokkuluri Kiran Sree, Professor, Dept. of CSE, Sri Vishnu Engineering College for Women, Bhimavaram, India.

Dr. Jessica Chocha, Assistant Professor, Bhauddin Science College, Junagadh, Gujarat, India

Keynote Speakers:

GAGIK SHMAVONYAN (Ph.D), Professor & Research Scientist, National Polytechnic University of Armenia, Department of Microelectronics and Biomedical Devices, Yerevan, ARMENIA Research Scientist, Institute for physical research (IPR), Solid state physics Laboratory, NAS Armenia, Ashtarak-2, ARMENIA.

Dr. Amit Parikh, Professor & Principal, Mehsana Urban Institute of Sciences, Ganpat University, Gujarat, India.

Dr. Shailesh Shah, Associate Professor, Faculty of Tech & Engineering, The Maharaja Sayajirao University of Baroda., India

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Rania Lampou, STEM instructor and an ICT teacher trainer, the Greek Ministry of Education - the Directorate of Educational Technology and Innovation, Greece.

Prof.Om Kumar Harsh, Honorary Pro-Chancellor (Additional) Glocal University, India and Former V.C. Tania University, India

Prof. Natalia Morkun, (Ph.D), Head, Automation, Computer Science and Technology Department, Kryvyi Rih National University, Ukraine

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Vitalii Tron (Ph.D), Associate professor, Automation, Computer Science and Technology Department, Kryvyi Rih National University.

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Dr. Sanjay Gaur, Associate Professor, Jaipur Engineering College & Research Center, Jaipur, India.

INDEX

PAPER ID	Table of Contents	Page No.
a)	About the organizing Institutions: Objectives of the International Conference :	3
b)	Message of HOD, Automation, Computer Sciences and Technologies, Kriviy Rih National University, Ukraine	4
c)	Message of Director, Research Culture Society.	5
d)	Conference Committee	6
e)	Keynote Speakers	7
f)	Table of Contents	8
	Paper Title & Author Name	
ECSETI01	Biological Importance of Selenosemicarbazone -- Rinku Malhi	9-16
ECSETI02	Undergraduate Chemistry Students' Perspective about Flipped Learning on solving Organic chemistry problems: A Case Study - - Dr. Sajith S.	17-19
ECSETI03	AMELIORATIVE EFFECT OF ANNONA MURICATA LEAF EXTRACT ON FIPRONIL INDUCED BIOCHEMICAL AND HISTOLOGICAL CHANGES IN OREOCHROMIS MOSSAMBICUS -- Reena Michael, Dr. M. L. Joseph	20-45
ECSETI04	Study of coordination behaviour of some pyrazine derivative -- Shailesh Kumar, Alok kumar and Bijay kumar	46-50
ECSETI05	ANNEALING EFFECT ON CHARACTERISTICS OF NICKEL-TUNGSTEN ALLOY THIN FILMS -- Dr. T. Baskar ; Dr.A.Shaji George	51-55
ECSETI06	Education Data Mining: Future Prospects and Potential Benefits -- Mr. Rajinder Kumar, Dr.Garima Bansal	56-63
ECSETI07	Heart Rate Measurement from Fingertip Using Microcontroller -- Borchala Namomsa Dareje	64-70
ECSETI08	Analysis of Frequently Failed Transmission Lines in India and Innovative Solutions for the Better Operation and Maintenance of the Towers -- Malar Kodi, Naqui Anwer and M. Tamil Selvan	71-77

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Biological Importance of Selenosemicarbazone

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Abstract: *Selenium is a p-block element with atomic number 34, valence electronic configuration $[Ar] 3d^{10} 4s^2 4p^4$ and also a member of chalcogen family. Selenium is an essential trace element in having its own codon in mRNA that specifies its insertion into selenoproteins as selenocysteine and this insertion for selenoprotein production has some implications for the requirement of selenium for cancer prevention and this element also involved in different physiological functions of the human body, having its chemoprevention properties. Its deficiency explained by low 60-70% of normal selenoenzyme activity levels in brain and occurs only when a low selenium level is linked with an additional stress. One analogues of selenium is selenosemicarbazone. Reaction of $KSeCN$ with hydrazine hydrate and cyclohexanone in acidic medium resulted into formation of cyclohexanone selenosemicarbazone. Anti-tubercular activity of the compound is also investigated.*

Keywords : *Selenium, cyclohexanone selenosemicarbazone, anti-tubercular activity.*

1. INTRODUCTION OF SELENIUM :

SELENIUM (Se) is a p-block element with atomic number 34. It is a member of chalcogen family. It occurs in organic and inorganic form. In inorganic, including selenide(-2), selenite(+4) and selenate(+6) and in organic, it is found in amino acids selenomethionine, selenocystine and methylselenocysteine. It was discovered in 1817 by Jones Berzelius and Johan Gottlieb, greek selen meaning 'Moon'

Selenium compounds exist in -2,+2,+4 and +6 oxidation state. The first organoselenium compound isolated was diethyl selenide in 1836. Now it is classified in various forms such as Selenol($RSeH$), Diselenides($R-Se-Se-R$), Selenylhalide($R-Se-Cl$), Selenides($R-Se-R$), Selenoxihalides($R-Se(O)-R$), Selenenic acids ($R-Se-OH$), Selenuranes, Seleniranes are three-membered rings (parent: C_2H_4Se), Seleninic acids ($RSe(O)OH$), Selones($RC=Se$). Organoselenium compounds are specified but useful collection of reagents useful in organic synthesis, they are excluded from processes useful to pharmaceuticals. Other forms of organoselenium compounds are Chalcogen compounds. Selenium, in the form of organoselenium compounds, is an essential micronutrient whose absence from the diet causes cardiac muscle and skeletal dysfunction. Organoselenium compounds are required for cellular defense against oxidative damage and for the correct functioning of the immune system. They may also play a role in prevention of premature aging and cancer. Selenocysteine, called the twenty-first amino acid, is essential for ribosome-directed protein synthesis in some organisms. More than 25 selenium-containing proteins (selenoproteins) are now known. Most selenium-dependent enzymes contain Selenocysteine, which is related to cysteine analogue but with selenium replacing sulfur. This amino acid is encoded in a special manner by DNA.

Selenium is naturally occurring element which is widely distributed in the earth's crust. Selenium appears in number of allotropes (black, red and gray). The most important physical characteristics are its electrical properties in spite of vapour pressure, density, volume, atomic weight etc. It is used in the manufacture of transistors for computers, cellular phones and hand-held electronic games, in the form of photoconductor, in solar cells, in lithium-selenium batteries, for glass production, in photoconductors, in electrolysis cells, and its photovoltaic and photoconductive properties are useful in photocopying, photocells, light meters, in paints, enamels, rubber and in the toning of photographic prints, multiple wave length anomalous dispersion and single wave-length anomalous dispersion phasing, increases the permanence of images.⁷⁵Se is used as a gamma source in industrial radiography, as a catalyst in the preparation of antidandruff radiography. As selenium is gaining increasing importance and synthesis of organoselenium compounds continues to be a very active research area due to their distinct physical, chemical and biological properties. As selenium is a semiconductor and has both a photovoltaic action means conversion of lights to electricity and photoconductive action means electrical resistance decreases with increased illumination and it is therefore useful in photocells, solar cells and in the manufacture of transistors for computers, cellular phones, in lithium selenium batteries, hand-held electronic games, photocopying and in the toning of photography prints. It can also convert AC electricity to DC electricity and therefore it is used in rectifiers. The biggest commercial uses for selenium are glass making as some selenium compounds decolourise glass and other give a deep red colour and also used in paints, enamels, rubber and pigments.

Besides this, living forms of selenium plays a significant role in biological chemical processes and selenium containing compounds exhibit various activities –1. Induce cell death in various cells

- Anti Malaria
- Anti-Microbial
- Anti-Inflammatory
- Anti-Oxidant
- Anti-Tumor agent
- Cytotoxic activity
- Anti-Proliferative
- Pro-apoptotic activity
- Anti-Cancer
- Free-radical scavenging activity
- Anti-viral activity
- Cell cycle perturbation
- Preventing heart diseases
- Regulating blood pressure
- Activate and de-activate various thyroid hormones and their metabolites
- Reduction of antioxidant enzyme

Selenium is assigned as an element that is homologous to oxygen and sulfur in the periodic table. It has similar characteristics to oxygen and sulfur but also different specific features and reactivity compared with them. Selenium is a micronutrient essential for mammals. It plays a crucial role in the mammalian defense system against oxidative stress, since it's essential for the activity of glutathione peroxidase (GPx). In addition, it's essential for the activity of various enzymes such as thioredoxin reductase, iodothyronine deiodinase, selenophosphate synthetase, and selenoprotein.

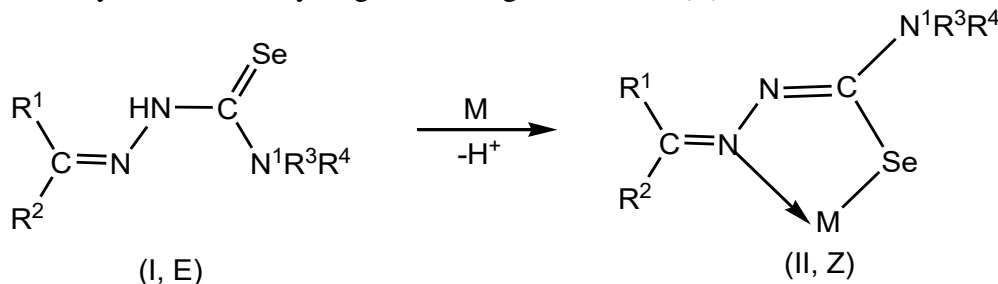
Selenium is an essential trace element that has been widely studied because of its chemo preventive properties. It's deficiency creates a number of serious diseases such as cancer, diabetes and HIV/AIDS and tuberculosis. Living forms of selenium plays a significant role in bio-chemical processes

and it is also known that selenium containing compounds exhibit specific characteristics and various activities in many drug candidates including a human breast cancer cell growth, uridine phosphorylase inhibitor, antitumor agent, potent inhibitor of 5- lipoxygenases, RAR(Retinoic acid Receptor) agonist, mimics of the glutathione peroxidase enzyme, antioxidant, anti-malaria antimicrobial, anti-inflammatory antiviral activity, cytotoxic activity, anti-proliferative, pro-apoptotic activity, reactive oxygen species scavenging activities, for the treatment of tumors and cancers inhibition of migration and in vasion in vitro cell cycle perturbation , preventing function of some forms of cancer, induce cell death in various cells, induces apoptosis through mitochondria pathway in caner celle as selenium is a component of several selenoproteins , preventing heart diseases and regulating blood pressure , reduction of antioxidant enzyme, activate and de-activate various thyroid hormones and their metabolites and cofactor for thyroid hormone deiodinases, bearing enzyme in some plants and animals generates reducing enzyme ribonucleotide reductase. Increased dietary selenium reduces the effects of mercury toxicity, including prostate, colon and liver cancer, leukemia and lymphoma.

Mainly selenium was considered as toxic during 1957 when the scientist Schwarz and Foltz et al. postulated that it is very healthy in low concentration. After that, it was found that deficiency of selenium can cause many diseases and it is recognized as an essential element. Now a-days, biological activity of selenium compounds are in hundred million dollars range. SELECT (Selenium and Vitamin E Cancer Prevention Trial) clinical trial involves examining the role of selenium in protecting against prostate cancer.

2. Selenium Element Related Analogues :

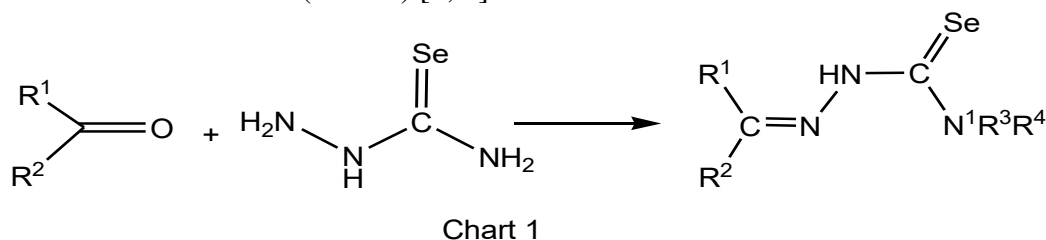
Selenium analogous of selenosemicarbazone have been investigated to a much smaller extent due to controversial about selenium impact on health. First, this element was marked as toxic, but in 1980s, it was found to be essential in humans (Combs Jr. 2015). Selenosemicarbazones are schiff bases characterized by the presence of selenium and azomethine nitrogen offering a ready co-ordination site due to the presence of a lone pair of electrons on these donor atoms. In natural form, a selenosemicarbazone exists in E-mode (I) and binds to metal centre via selenium atom, however, on deprotonation of hydrazine N²H hydrogen, it change to Z mode (II) and form a chelate ring.



3. Methods of Preparation of Selenosemicarbazone :

Selenosemicarbazones can be prepared by various methods. Brief description of these methods is given below:

Method I. Selenosemicarbazones can be prepared by direct condensation of aldehyde or ketone with selenosemicarbazide in methanol (Chart I) [1, 2].



Method II. Reaction of hydrazine hydrate with KSeCN and aldehyde / ketone in acidic medium resulted into respective selenosemicarbazone (Chart 2) [3, 4].

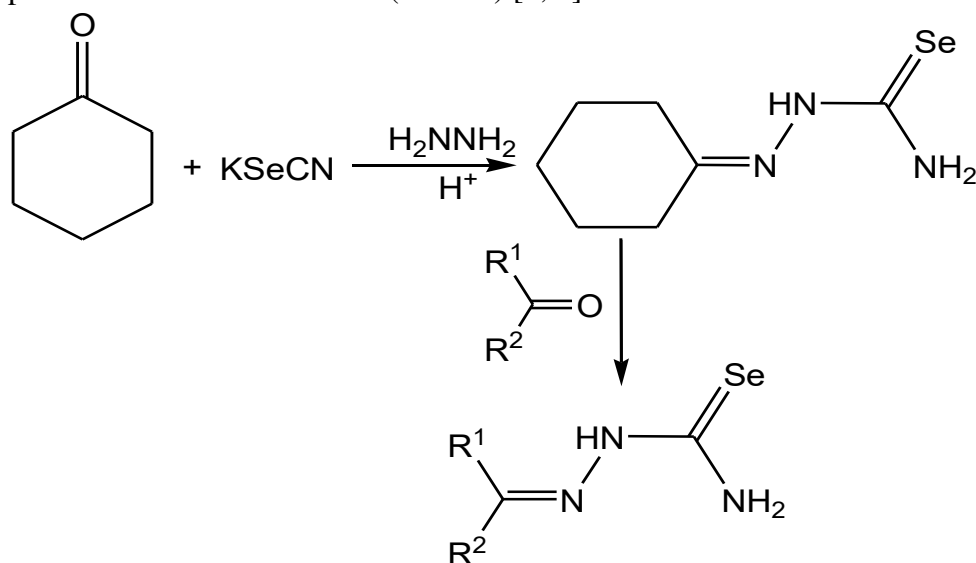
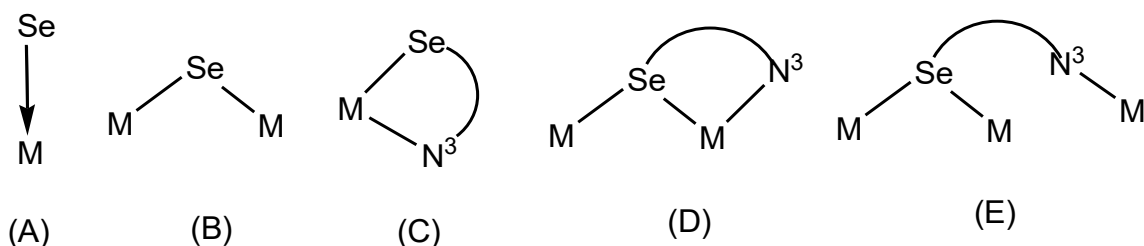


Chart 2

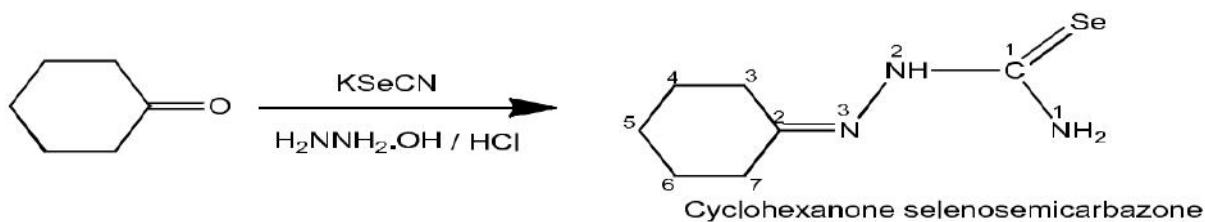
Selenosemicarbazone can adopt a variety of different coordination modes. Selenosemicarbazone coordinate as bidentate ligands via azomethine nitrogen and thione/thiol to selenium. When additional coordination functionality is present in the proximity of donating centres, the ligands will coordinate in a tridentate manner. Various bonding modes of selenosemicarbazones are: i) selenium bonded terminally, η^1 -Se (mode A) [5,6]; ii) selenium atom bridges two metal centers, μ -Se (mode B) [7,8]; iii) chelation by N, Se- (mode C) [9]; iv) chelation by N, Se- cum-Se-bridging (mode D) [10]; Se-bridging-cum-Se, N- bridging (mode E).



The metal complex can be more active than the free ligand. Some of the synthesized selenosemicarbazone and their complexes showed good biological activity such as antifungal, antiparasitic, antibacterial, antimalarial, anticancer, antioxidant and antidiabetic activity. Even some of the comparative studies explained that selenosemicarbazones showed similar or although better biological activities than the corresponding thiosemicarbazones and their complexes while oxygen analogues showed the lower activity.

4. Synthesis of Cyclohexanone Selenosemicarbazone :

A solution of hydrazine hydrate, 3.75 g in a mixture of ethanol (100ml) and water (2 ml) was prepared. To it was added mixture of KSeCN (3.6 g) dissolved in 1ml of water and 8 ml cyclohexanone. The resulting mixture was refluxed for 2-3 hours. During refluxing pH was maintained upto 2.6 by adding HCl. The mixture is filtered to avoid any grey selenium (if deposited). The filtrate was evaporated in rotary evaporator to get white compound, which was then dissolved in CHCl_3 and filtered through molecular sieves. After evaporation white solid was formed and collected.



Characterization of Cyclohexanone selenosemicarbazone

Yield	70 %
Melting Point	180-182°C
IR (cm ⁻¹) (Figure 1)	(NH ₂) 3362s, 3225s; (–NH–) 3157s; (C–H _{cyclo}), 2986s, 2854s; (C=N) + v (C=C) + (NH ₂) 1591, 1489s, 1454s; (C=Se) 856s
¹ HNMR (δ, ppm) (Figure 2)	9.23 s (1H, N ² H), 7.65 s (1H, N ¹ H ₂), 7.15 s (1H, N ¹ H ₂), 2.32-1.54 m (10H, Cy ring proton)
¹³ C NMR (δ, ppm) (Figure 3)	185.4 (C ¹), 136.1, (C ²) 103.4 (C ³), 135.6 (C ⁴), 115.2, (C ⁵), 112.4 (C ⁶), 115.4 (C ⁷), 131.8 (C ⁸), 125.4 (C ⁹), 119.7 (C ¹⁰).

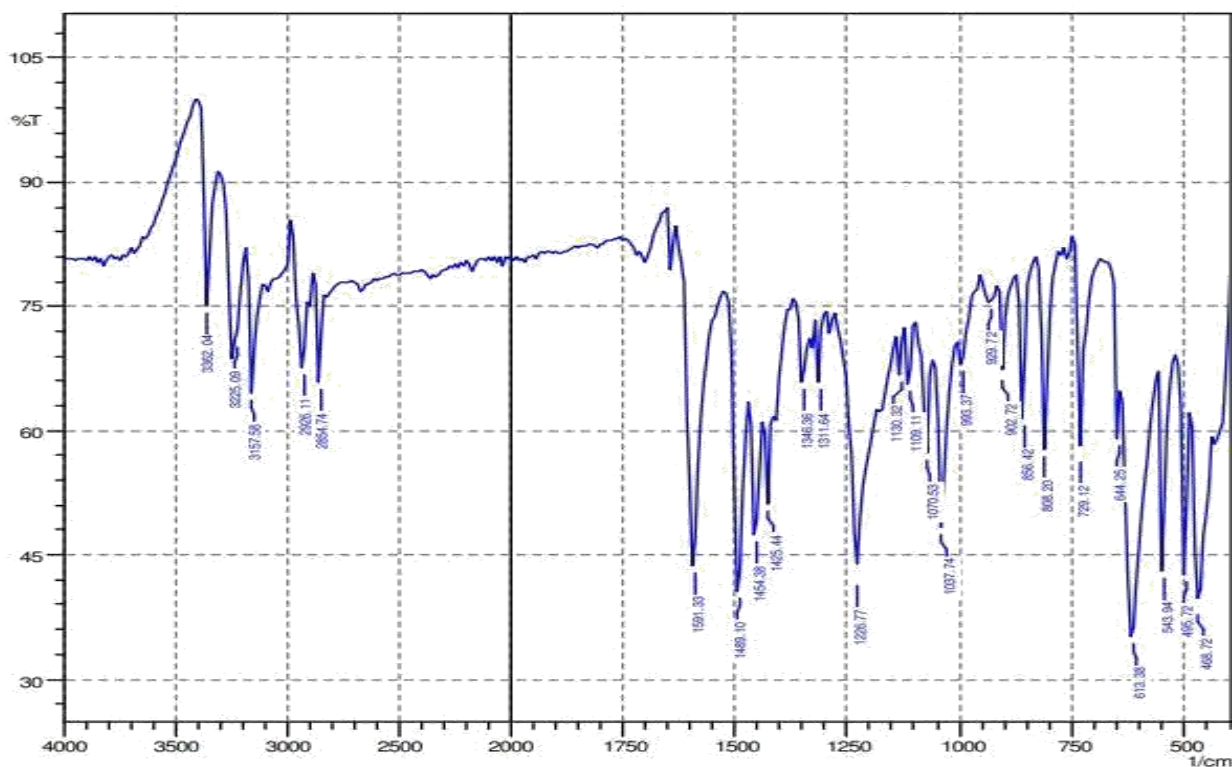


Figure 1. IR spectrum of Cyclohexanone selenosemicarbazone

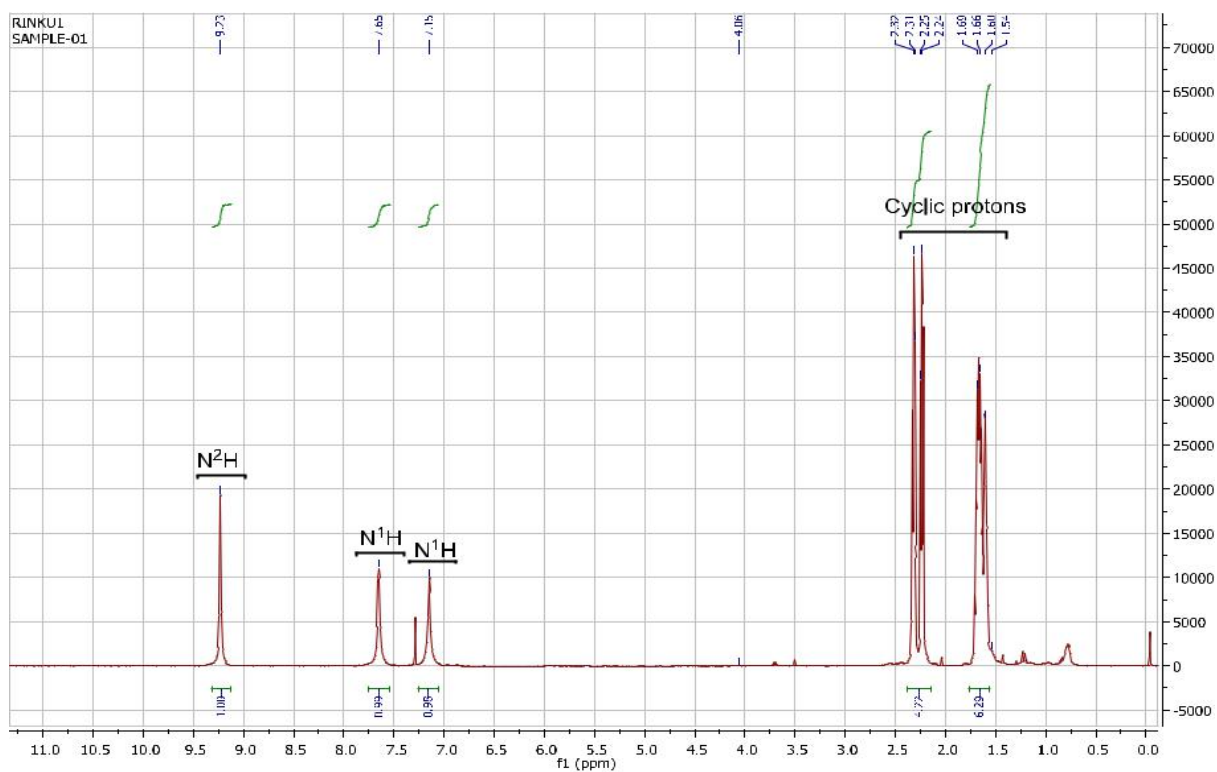


Figure 2. ^1H NMR spectrum of Cyclohexanone selenosemicarbazone

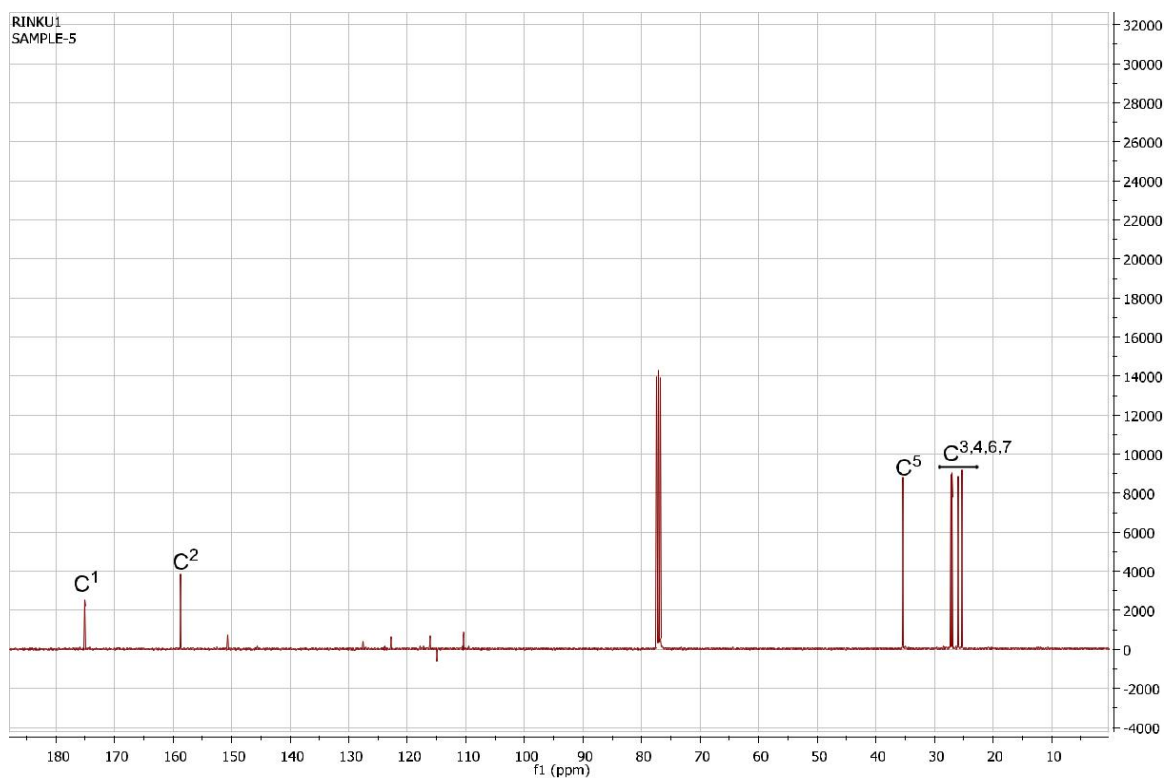


Figure 3. ^{13}C NMR spectrum of Cyclohexanone selenosemicarbazone

5. Discussion on biological activity (anti-tuberculosis activity):

The anti-tuberculosis activity of cyclohexanone selenosemicarbazone was evaluated against *M. tuberculosis* at various concentration 100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml, 6.25µg/ml, 3.12µg/ml, 1.6µg/ml and 0.8µg/ml respectively. The inhibition zone was given in table-1 and shown in figure 4. The MIC value for HCysesc is 50µg/ml, 12.5µg/ml 50µg/ml and 1.6µg/ml respectively. This anti-TB activity of HCysesc is better than the second line standard drugs Isoniazid, Ethambutol and Pyrazinamide(MIC =1.6 µg/ml, 3.2 µg/ml, 3.125µg/ml and 0.8 µg/ml).

Table 1. Minimum Inhibitory Concentration of compounds against *M. Tuberculosis* H37RV)

S= Sensitive, R = Resistant

S. No Compound	Mycobacterium tuberculosis H37RV strain	
	MIC (µg /mL)	

		100	50	25	12.5	6.25	3.12	1.6	0.8
1.	Cyclohexanone selenosemicarbazone	S	S	S	S	S	S	S	R
2.	Isoniazid	S	S	S	S	S	S	S	R
3.	Ethambutol	S	S	S	S	S	S	S	R
4.	Pyrazinamide	S	S	S	S	S	S	R	R



(a)



(b)

Fig- 4. Diagram showing anti-tubular activity of (a) compounds (b) standard drugs

6. CONCLUSION :

Selenium related analogues selenosemicarbazone shows better anti-tubercular activity as compared with standard drugs and more sensitive towards bacterial. So selenosemicarbazone presents good biological activity.

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**Undergraduate Chemistry Students' Perspective about
Flipped Learning on solving Organic chemistry problems:
A Case Study**

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***Abstract :** Blended learning is an educational strategy which combines the traditional classroom with on-line activities[1]. The flipped classroom as a key component of blended learning, through which authentic assessment development exercises on the learning model can be done in the classroom by direct lecturers. The teacher becomes facilitator and helps students in the transition process from knowledge to the acquisition of skills [2]. The implementation of flipped learning in chemistry is rapidly growing over the past decades. Traditional lessons are replaced by interactive forms of teaching chemistry. This study was conducted among the undergraduate chemistry students. A flipped classroom was introduced in Organic Chemistry topic with a large number of application exercises. The students had to be creative to find solutions to the problems and to understand the best learning practices individually. Feedback regarding their flipped learning experience was analysed.*

***Keywords:** Online Learning, Blended learning, flipped classroom, Organic chemistry, students' perspectives.*

1. INTRODUCTION :

Blended learning is an educational strategy which combines the traditional classroom with on-line activities. Blended learning is becoming the essential part of the education technology[4]. The flipped classroom as a key component of blended learning, through which authentic assessment development exercises on the learning model can be done in the classroom by direct lecturers. Flexible timetable, involvement of students in the learning process and increase of students' academic performance are the major outcome of Flipped Learning mode [5]. The flipped classroom technology is a new approach to learning where the teacher becomes a supporter and facilitator[1][4].

2. Methodology :

This study was conducted among the postgraduate chemistry students. A flipped classroom was introduced in Organic Chemistry topic with a large number of application exercises. The students had to be creative to find solutions to the problems and to understand the best learning practices individually. Feedback regarding the flipped learning experience was collected to evaluate academic performance and perception towards flipped class-rooms in comparison to traditional teaching. An online questionnaire consisting of 16 questions (Table 1) was formulated for the students (n=21). The questions were sent via google form and all the students were asked to take part in the survey.

Participants were UG chemistry students (n=21). Out of the 21 responses obtained, 16 were female and 5 were male. Blended learning (BL) was not introduced in the year of 2020 and all lectures for UG Chemistry students were delivered via online. Flipped classroom sessions were introduced for Organic Chemistry topic in the year of 2021. Flipped Classroom was introduced by incorporating exercises and problems on Organic Chemistry relating to the online and traditional lectures. The results of the 2020-2021 batch of students were compared with the previous batch. Student feedback for the blended learning experience was collected via questionnaire. Suggestions to improve the blended learning experience was also collected from the students. Data collection techniques were conducted by with questionnaires. Statistical analysis was also done to assess any statistical significance.

3. Results and discussion :

Sl No	Question	Response %				
		1	2	3	4	5
1	Do you believe that it is very important for you to be able to learn through video lectures?	81.08	8.1	5.4	-	-
2	Would you like to see the videos of lab techniques uploaded to Moodle?	86.5	5.4	8.1	-	-
3	Do you think that lab experiments can be effectively done via flipped classroom?	67.56	10.8	5.4	8.1	8.1
4	Do you think that e-learning resources would help your learning in chemistry?	67.56	8.1	21.62	2.7	-
5	Do you like receiving instruction through the flipped method?	75.67	5.4	5.4	5.4	8.1
6	Were the pre-reading materials (e-Lecture/reference/others) were available on e-learning portal before the Flipped Classroom (FC) ?	81.08	13.51	5.4	-	-
7	Do you feel comfortable with learning through the flipped method?	67.56	13.51	2.7	8.1	8.1
8	Do you feel more motivated in Flipped Classroom (FC) mode.	75.67	10.8	5.4	5.4	2.7
9	Was adequate time provided to spend on learning through the flipped method?	59.45	10.8	21.62	8.1	-
10	Do you think that it takes a longer amount of time to learn when my teacher uses the flipped method?	86.5	10.8	2.7	-	-
11	Do you think that the activities during Flipped Classroom (FC) improved your understanding of the key concepts?	75.67	13.51	10.8	-	-
12	Do you think that more lectures should be incorporated in the Flipped Classroom (FC) mode?	86.5	10.8	2.7	-	-
13	Do you believe that the Flipped Classroom (FC) session was inspiring to pursue for further learning?	16.21	2.7	-	21.65	59.45
14	Was the facilitator able to provide clarification on difficult concepts during the Flipped Classroom (FC) activity?	59.45	10.8	21.62	8.1	59.45
15	Was Learning through the flipped method is difficult?	94.59	5.4	2.7	-	-
16	Do you think you could focus on your study more effectively with the assistance of Moodle ?	59.45	10.8	21.62	8.1	-

The results showed that blended learning media for flipped classroom model to use in the evaluating the quantum chemistry learning. 67.56% students felt comfortable with learning through the flipped method. 86.5% students felt more motivated in Flipped Classroom (FC) mode. 86.5% students believe that the Flipped Classroom was inspiring to pursue for further learning. 75.67% students think that more lectures should be incorporated in the Flipped Classroom (FC) mode. The majority of students reported blended learning could focus study more effectively. Students expressed satisfaction with blended learning as a new and effective learning approach.

4. CONCLUSION:

Organic chemistry is a discipline that is focused around problem solving and therefore is ideally suited to flipped learning. The study evaluated the effectiveness of flipped learning for the delivery of quantum chemistry module. Flipped classroom sessions were introduced to one module only, a limitation for the study. A large sample size would improve the statistical significance of the study. It is worth mentioning, that limited time is a major problem. Further research is needed to establish the academic outcomes of flipped learning at various levels of undergraduate disciplines.

Acknowledgments

I would like to thank all the students and faculty members of our college for their support and inspiration.

Conflict of Interest

The author declares that no conflict of interest with regard to this study.

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**AMELIORATIVE EFFECT OF *ANNONA MURICATA* LEAF
EXTRACT ON FIPRONIL INDUCED BIOCHEMICAL AND
HISTOLOGICAL CHANGES IN
*OREOCHROMIS MOSSAMBICUS***

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Abstract: The current experiment was an approach to measure the ameliorative effect of *Annona muricata* leaf extract on fipronil induced biochemical and histological changes in the kidney and gills of *Oreochromis mossambicus*. The experiment was carried out in 2 distinct experimental units with each unit having 4 groups – a control and three sublethal concentrations of fipronil which were fed with normal feed and *Annona muricata* plant extract supplement. The antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) showed decreased activity on exposure to fipronil for 15 days and 30 days. This indicates the stressed condition of *O. mossambicus* on exposure to fipronil that was reduced in *A. muricata* extract supplemented groups indicating the ameliorative effect of leaf extract. Kidney and gills of fish exposed to fipronil showed different histopathological alterations. Several histopathological and biochemical changes were observed in fish organs would serve useful purpose in evaluating the toxic effects of fipronil.

Keywords: Fipronil, *Annona muricata*, *Oreochromis mossambicus*, antioxidant enzymes, histopathology.

1. INTRODUCTION:

Fipronil is a broadspectrum insecticide belongs to the class phenylpyrazole used in agriculture which exhibit selective toxicity to pests like insects, flees, ticks, lice, ants, termites, cockroaches and crustaceans. Studies shows that fipronil have greater affinity for GABA regulated chloride channels within insects than the GABA receptors in mammals (Hainzl and Casida, 1996). The use of this pesticide has raised concerns for its harmful effects on human health and the environment (Tingle *et al.*, 2003).

Fipronil exhibits toxic effects on non-target organisms such as aquatic invertebrates like blue crab, giant prawn, water flea, estuarine mysid shrimp (Key *et al.*, 2003) vertebrates like fish (Beggel *et al.*, 2010) some reptiles, (Peveling and Demba, 2007) birds (Kitulagodage *et al.*, 2011) and mammals (de Oliveira *et al.*, 2012). Fipronil exposure in rats have induced oxidative stress in brain, liver and kidney (Badgujar *et al.*, 2015). The effects of fipronil toxicity on rainbow trout (*Oncorhynchus mykiss*) were determined by studying the marker enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), malondialdehyde (MDA), and caspase 3 activity in liver and gill tissue and caspase-3 activity was increased in liver and gill tissue whereas

fipronil toxicity inhibited antioxidant enzyme activities in rainbow trout liver and gill tissues (Ucar *et al.*, 2020).

Annona muricata is a vast source of enzymatic antioxidants and non-enzymatic antioxidants (Vijayameena *et al.*, 2013). The stem bark extract of *A. muricata* showed protective effects on the oxidative stress induced by CCl₄ in rat (Olakunle *et al.*, 2014). It is a rich source of various phytochemicals like flavonoids, terpenoids, steroids, saponins and Cardiac glycosides. These phytochemicals are attributed with free radical scavenging which is the key mechanism in various diseases and this property when used as an ameliorating activity renders protective adaptations in various organisms exposed to toxicity linked to oxidative stress.

Any change within the environmental condition leads to stress within the organisms and this is reflected as a change in histological characteristics of the organ. In toxicity studies the histopathological features of kidney and gills are used as biomarker. Fipronil exposure caused histopathological alterations in the fish *Caspian kutum* liver tissue that included pyknosis, sinusoid dilation and vacuolization (Ardeshtir *et al.*, 2017). Gills showed focal necrosis and sloughing, edema, extensive hyperplasia and congestion (El Murr *et al.*, 2015). Fipronil exposed silver catfish showed hyperplasia, lamellar fusion and congestion (Ghisi *et al.*, 2015).

2. MATERIALS AND METHODS:

Technical grade fipronil (99%) was procured from RFCL limited, New Delhi, Art No: P-738N. It was stored under refrigerated conditions. Due to low solubility in water, a stock solution of 100mg was prepared in 5% acetone solution.

Oreochromis mossambicus was collected from culture farms of the state fisheries station, Kerala State Agricultural University, Kochi, India and transported to laboratory in aerated tanks. In laboratory conditions the fish was stocked in larger tanks of 500 litre capacity and was left undisturbed overnight. The fish was given an antibiotic treatment of 0.001% for three days. Water was exchanged every day for first three days followed by exchange of 50% water for every two days. Fish was kept in aerated tanks for two week acclimatization and provided with commercial dry feed pellets.

The experiment was carried out in tanks of 50 litre capacity (60x30x30cm). The tanks were filled with 15 ppm potassium permanganate and kept overnight. The tank was properly cleaned with water thrice and chlorine free bore well water was added up to the 40 litre mark. The water in the aquarium was renewed every day and proper photoperiod of 13h light/11h dark was maintained. Fish weighing 30±2.9 g of length 13.62±1.8 cm was used for study. Fish was introduced at a stocking density of 10 fish/ aquarium and feeding was stopped 24 hours prior to start of experiment. Aeration was maintained with an air stone and a plastic regulator. The tanks were covered by meshed lids.

The experiment setup in 2 distinct experimental Units with each unit having 4 groups – a control and three concentrations of fipronil – 1/5th, 1/10th and 1/15th of LC₅₀ value. Each group had three replicates. Unit 1 the fishes were fed with normal feed and exposed three different concentrations of fipronil along with the control. Unit 2 to in addition to the control the three groups were present that were fed with *A. muricata* plant extract as supplement.

Unit -1

- Group A1 - control + normal feed
- Group A2 - 1/5 fipronil +normal feed
- Group A3 - 1/10 fipronil +normal feed
- Group A4 - 1/15 fipronil + normal feed

Unit – 2

- Group B1 - control + *A. muricata* plant extract supplement

- Group B2 - 1/5 fipronil + *A. muricata* plant extract supplement
Group B3 - 1/10 fipronil + *A. muricata* plant extract supplement
Group B4 - 1/15 fipronil + *A. muricata* plant extract supplement

The fishes were collected on day 15 and day 30 of experiment for biochemical analysis.

FORMULATION AND PREPARATION OF EXPERIMENTAL DIET

Ingredients included crude protein(fat free, Hi Media Laboratories Ltd), vitamin mix (Hi Media Laboratories Ltd), Sunflower oil(procured locally), carboxymethyl cellulose(CMC)(Hi Media Laboratories Ltd), starch(procured locally), crude fibre (procured locally), Butylated Hydroxy Toluene (BHT)(Hi Media Laboratories Ltd), and Betaine hydrochloride (Hi Media Laboratories Ltd)

Crude protein, crude fibre, starch and Sunflower oil were mixed together in an earthen vessel (Table 1.) The dough after mixing was kept for an hour for proper conditioning and the later steamed for 10 minutes in a pressure cooker. Vitamin mix, Vitamin C, BHT, CMC, and Betaine chloride were mixed after the dough was completely cooled. Later pellets were prepared with the hand pelletizer of 1 mm diameter. The pellets were sun dried for 5 to 6 hours and kept in oven overnight at 50 OC for complete drying. The pellets were stored in airtight containers.

Ingredients	Unit 1	Unit 2
Crude protein	50.00	50.00
Sunflower oil	8.00	4.00
Crude Fibre	4.00	8.00
Starch	30.00	30.00
Vitamin C	0.01	0.01
Vitamin Mix	1.96	1.96
Carboxymethyl Cellulose	2.00	2.00
Betaine Hydrochloride	0.02	0.02
Butylated Hydroxyl Toluene	0.01	0.01
<i>Annona muricata</i> leaf extract	0	4.00
Total Percent	100.00	100.00

Table 1: Composition of experimental diet as expressed in percent.

BIOCHEMICAL ANALYSIS

On day 15 and day 30 the experimental fishes were killed by decapitation and liver, gills and kidney were dissected out and kept at -20°C until analysis. For biochemical studies these tissues were homogenized for 5 min in ice-cold 0.1M Tris-HCl buffer solution with pH 7.2 (115 w/v) using a Polytron 220mogenizer (Polytron model PT 3000, Switzerland) and centrifuged at 5000 RPM for 20 minutes(Remi, India). The supernatant was collected for the antioxidant enzyme studies.

SUPEROXIDE DISMUTASE (SOD) (EC 1.15.1.1)

Superoxide dismutase was assayed according to a modified procedure of Das *et al.*, (2000). In this method, 1.4 ml aliquot of the reaction mixture (comprising 1.11 ml of 50 mM phosphate buffer, pH 7.4, 0.075 ml of 20 mM L-Methionine, 0.04 ml of 1% (v/v) Triton X-100, 0.075 ml of 10 mM hydroxylamine hydrochloride and 0.1 ml of 50 mM EDTA) was added to 100 µl of the homogenate and incubated at 30°C for 5 minutes. 80 µl of riboflavin was then added and the tubes were exposed to 20W-Philips fluorescent lamps for 10 minutes. After the exposure time, 1 ml of Greiss reagent (mixture of equal volume of 1% sulphanilamide in 5% phosphoric acid) was added and absorbance

of the colour formed measured at 543 nm. One unit of enzyme activity was measured as the amount of SOD capable of inhibiting 50% of nitrite formation under assay condition.

CATALASE (CAT) (EC 1.11.1.6)

Catalase (CAT) was estimated by the method of Sinha et al., (1972). The reaction mixture 1.5 ml volume contained 1.0 ml of 0.01 M phosphate buffer (PH 7.0) 0.1 ml of tissue homogenate and 0.4 ml of 2 M H₂O₂. The reaction was stopped by the addition of 2.0 ml dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). Then the absorbance was measured at 530 nm; CAT activity was expressed as n moles of H₂O₂ decomposed/min/mg protein

GLUTATHIONE PEROXIDASE (GPx) (EC 1.11.1.9)

GPx activity was determined following the method of Rotruck *et al.*, (1973). To 1.0 mL of phosphate buffer (0.1 M, pH7.4) taken in a tube, 0.5 mL Sodium azide solution (29.25 mg in 15.0 mL of buffer), 0.5 mL of EDTA solution (50.4 mg in 15.0 mL of buffer), and 100.0 μ L of the enzyme were added and mixed well. To this mixture, 0.5 mL glutathione (36.75 mg in 15.0 mL of buffer) was added and incubated at 37°C for 10 minutes, followed by the addition of 1.0 mL of hydrogen peroxide (freshly prepared by mixing 240 mL of hydrogen peroxide in 40.0 mL of buffer). The control contained all the reagents except the enzyme. After the incubation period, aliquots (1.0 mL) of the samples (both test and control) were taken in a tube to which 2.0 mL of Meta phosphoric acid and 1.0 mL of DTNB (5, 5'- dithio-bis-2- nitrobenzoic acid) reagent were added. The absorbance was then read at 412 nm in a Spectrophotometer. The enzyme activity is expressed as n moles of GSH oxidized min⁻¹ mg⁻¹ protein.

HISTOLOGICAL ANALYSIS

For histological and histopathological studies, the organs kidney and gills were taken from fishes (Unit 1 and Unit 2) at the end of exposure regimen (15 & 30 Days). The dissected organs were cut to thickness of about 5 mm and placed in Bouin's fixative for 24 hours. After fixation the fixed tissues were processed for paraffin embedment. Sections were cut at 4 microns using the rotary microtome and the ribbon formed was collected using a brush and transferred to a water bath. The sections when mounted on a clear glass slide smeared with Meyer's egg albumin. Allowed the slide to dry on a hot plate for 10 minutes so that the section adhere to the glass slide. The slides were dried in an oven for 30 minutes at 55°C. The slide deparaffinised by xylene and absolute alcohol ratio of 1:1 (Xylol) for two changes for 5 minutes each. They were brought down to water by immersing in descending series (95%, 90%, 80%, 50% and 30%) of alcohol for 4 minutes each. This was followed by washing the slides under running tap water for 20 minutes. The slides were passed through two sets of hematoxylin stain for 5 minutes each. To remove excess stain that was not adhered to the tissue the slides were washed under running tap water for 15 minutes. The slides were then dehydrated by passing through graded alcohol (30%, 50%, 70%, 90%) to 90%. The slides were then counter stained by 0.49% Eosin in 90% alcohol for two changes of 5 minutes each. The slides were dipped in 95% alcohol and absolute alcohol for 2 to 3 minutes for two changes. Lastly the slides were cleared with xylene for two changes and the slides were mounted in DPX medium. The slides were kept in a dessicator to prevent changes and were examined under a stereomicroscope (Leica) with camera attachment and were photographed at different power resolutions. The nucleus stained blue and cytoplasm pink.

STATISTICAL ANALYSIS

Results were statistically analysed using one way Anova test using the statistical software SPSS version 20. Data were presented as Mean \pm SD. Significantly different means were compared with

Tukey's post hoc multiple comparison test. Results obtained showed p-value ≤ 0.05 which implies that means are significantly different.

3. RESULT :

MEDIAN LETHAL CONCENTRATION, LC_{50}

Sl.No.	Concentration in $\mu\text{g/L}$	Log Concentration	No. of Fishes Exposed	No. of Fishes died at 96hr	Percentage Mortality	Probit Mortality
1.	2	0.3010	10	2	20.00	4.158
2.	3	0.4771	10	3	30.00	4.476
3	4	0.6021	10	5	50.00	5.000
4	5	0.6990	10	6	60.00	5.253
5	6	0.7782	10	8	80.00	5.842
6	7	0.8451	10	9	90.00	6.282
7	8	0.9031	10	10	100.00	-

Table 2: Table showing percent mortality of *Oreochromis mossambicus* exposed to different concentration of fipronil for 96 hours using Probit analysis.

Data obtained for 96 hour acute toxicity using static renewal test for studying median lethal concentration, LC_{50} of fipronil to *O.mossambicus* is given in table 2. The median lethal concentration, LC_{50} was $3.74\mu\text{g/L}$ calculated using Probit analysis (Fig. 1). The LC_{50} values showed an increase with increase in concentration of fipronil.

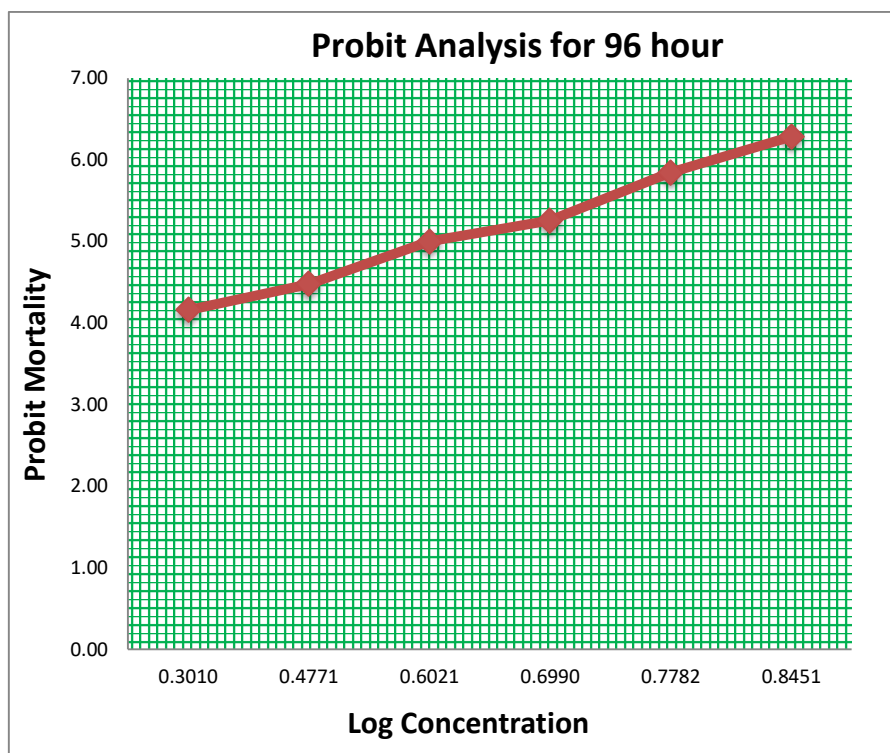


Fig 1: Graphical representation of LC_{50} of *Oreochromis mossambicus* exposed to different concentration of fipronil for 96 hours using Probit analysis.

BIOCHEMICAL ANALYSIS

In this study we investigated the catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzyme level in organs like kidney and gills of *Oreochromis mossambicus* as a

result of exposure to different concentrations of fipronil and *Annona muricata* leaf extract to reduce the harmful effects of the phenylpyrazole fipronil. Treatment of *Oreochromis mossambicus* with fipronil fed with normal feed alone had significant effect ($p < 0.05$) on the activity of CAT, SOD and GPX. These three biochemical assays showed a decreased activity than the control group. The fipronil and *Annona muricata* leaf extract together exposed in Unit 2 all 4 groups showed a reduction in CAT, SOD and GPx activity which was significant than control group ($p < 0.05$). For 15 days and 30 days the reduction was significant ($p < 0.05$) but the *Annona muricata* extract exposed groups of Unit 2 showed increased activity of CAT, SOD and GPx than Unit 1 groups for 15 days and 30 days exposure. A dose dependent reduction in the activity of endogenous antioxidant enzymes CAT, SOD and GPx was observed for all the three concentrations 1/5, 1/10 and 1/15 when compared to the control group enzyme activity of gills and kidney exposed to fipronil alone for 15 and 30 days.

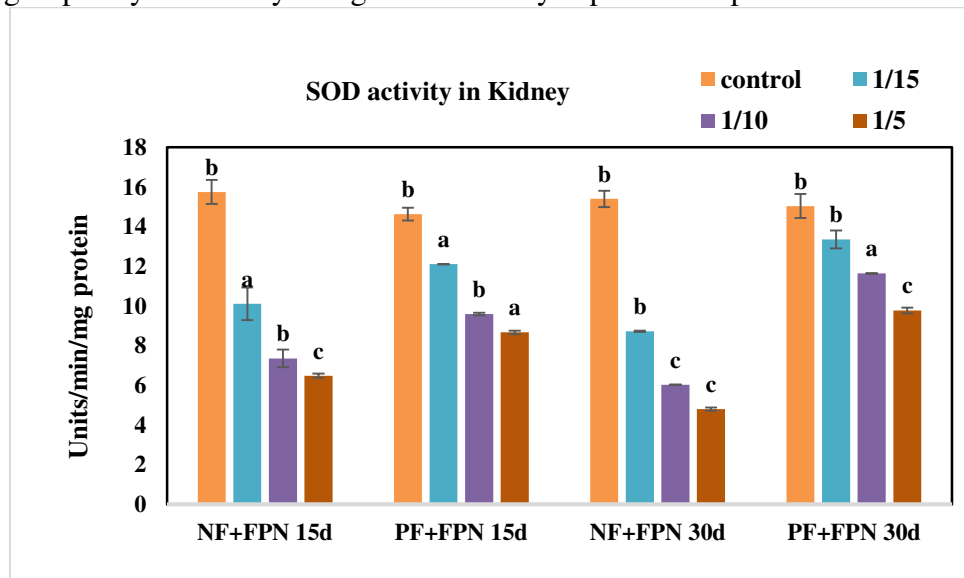


Fig 2: Superoxide dismutase (SOD) activity in kidney tissue of *Oreochromis mossambicus* exposed to 1/15, 1/10 and 1/5 of LC50 value of fipronil (unit 1 with normal feed and unit 2 with *A.muricata* leaf extract) 15 days and 30 days of exposure and control. Values are represented as Mean \pm SD. Means having the same letters are not significantly different from each other, $p < 0.05$.

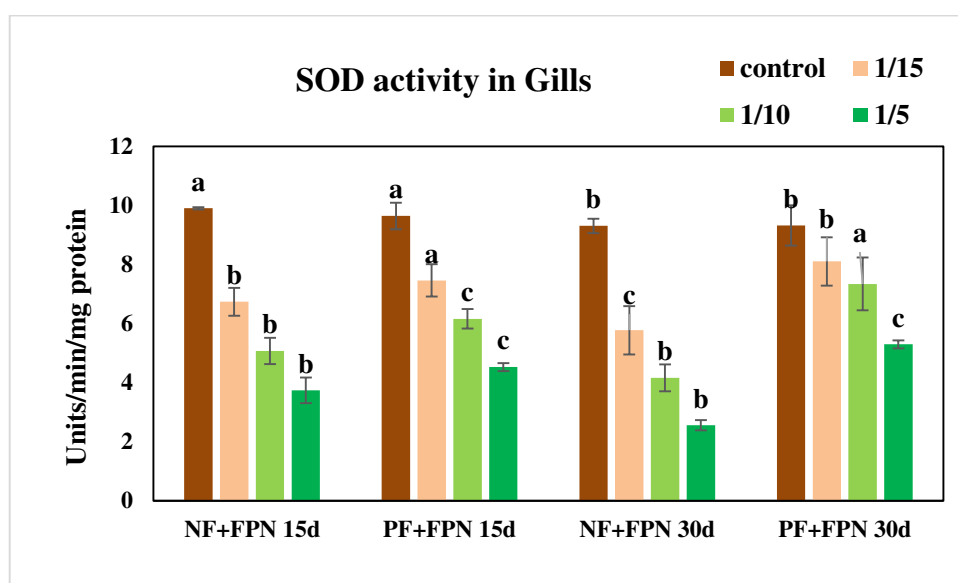


Fig.3: Superoxide dismutase (SOD) activity in gill tissues of *Oreochromis mossambicus* exposed to 1/15, 1/10 and 1/5 of lc50 value of fipronil (unit 1 with normal feed and unit 2 with *A.muricata*

leaf extract) 15 days and 30 days of exposure and control. Values are represented as Mean \pm SD. Means having the same letters are not significantly different from each other, $p < 0.05$.

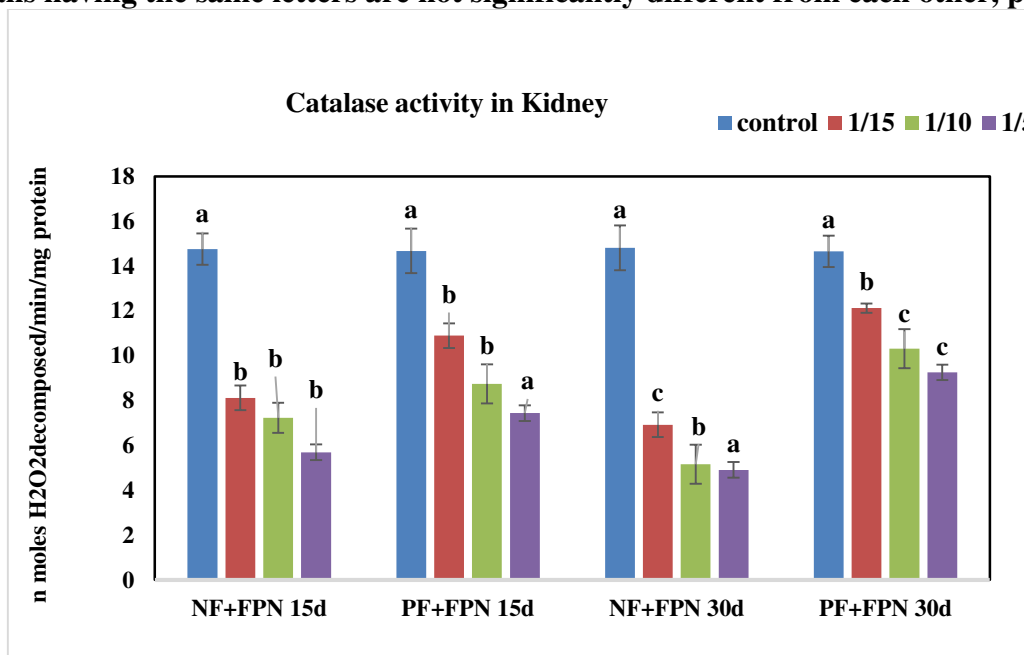


Fig.4: Catalase activity in kidney tissue of *Oreochromis mossambicus* exposed to 1/15, 1/10 and 1/5 of LC_{50} value of fipronil (unit 1 with normal feed and unit 2 with *A. muricata* leaf extract) 15 days and 30 days of exposure and control. Values are represented as Mean \pm SD. The letters a, b, c, d were used to indicate significantly different groups ($p < 0.05$) within the same column within each experimental treatment group ($n = 20$).

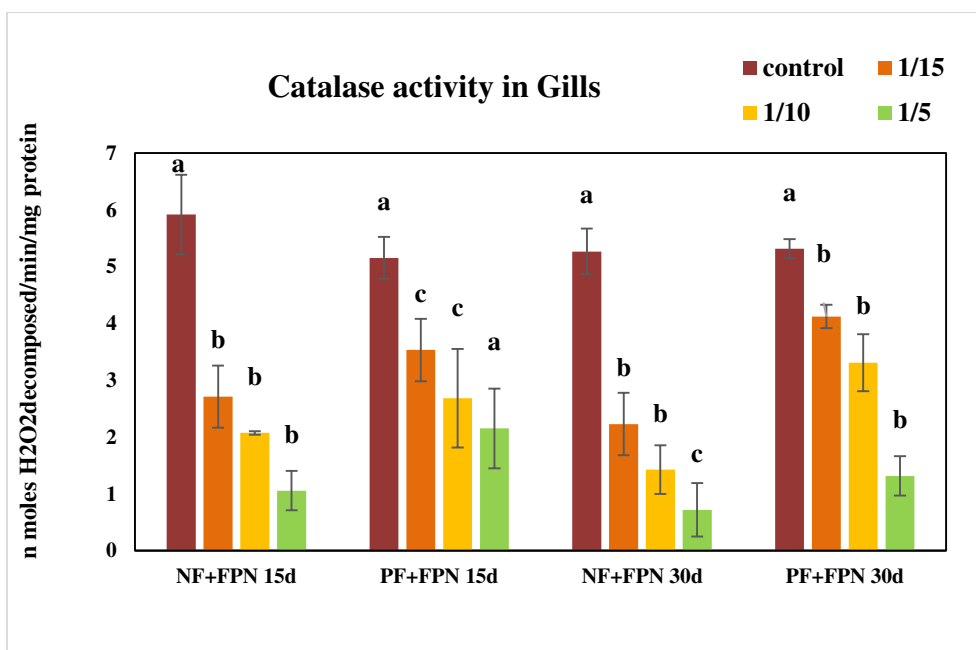


Fig. 5: Catalase activity in gill tissue of *Oreochromis mossambicus* exposed to 1/15, 1/10 and 1/5 of LC_{50} value of fipronil (unit 1 with normal feed and unit 2 with *A. muricata* leaf extract) 15 days and 30 days of exposure and control. Values are represented as Mean \pm SD. Lowercase letters (a, b, c, d) indicate significant differences among the same column within each experimental treatment group, $p < 0.05$. $n = 20$

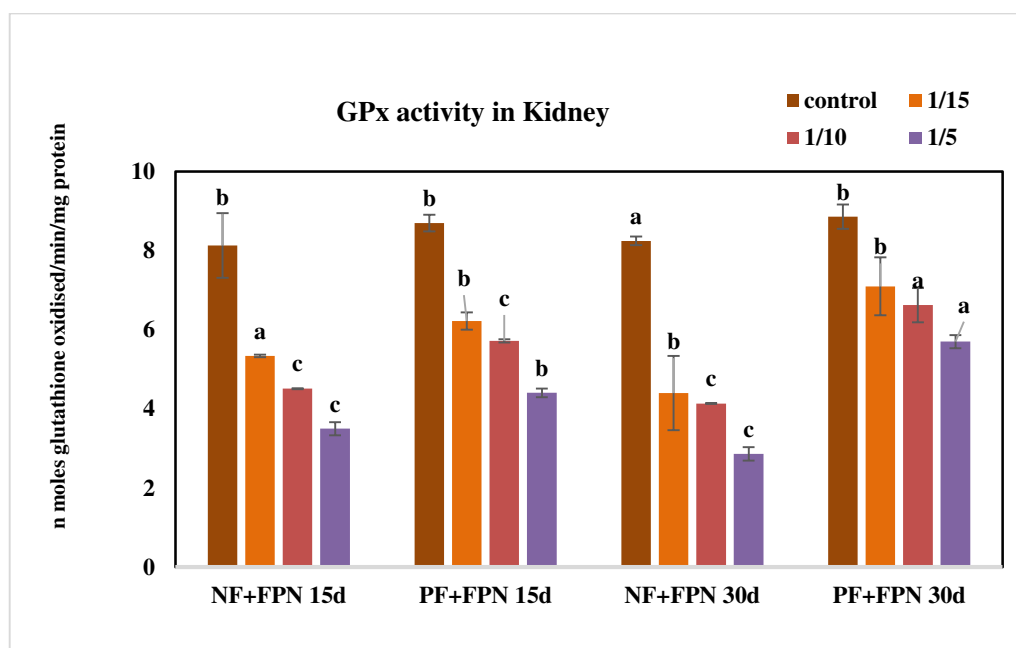


Fig 6: Glutathione peroxidase activity in kidney tissue of *Oreochromis mossambicus* exposed to 1/15, 1/10 and 1/5 of lc_{50} value of fipronil (unit 1 with normal feed and unit 2 with *A. muricata* leaf extract) 15 days and 30 days of exposure and control. Values are represented as Mean \pm SD. The letters a, b, c, d were used to indicate significantly different groups ($p < 0.05$) within the same column within each experimental treatment group ($n = 20$).

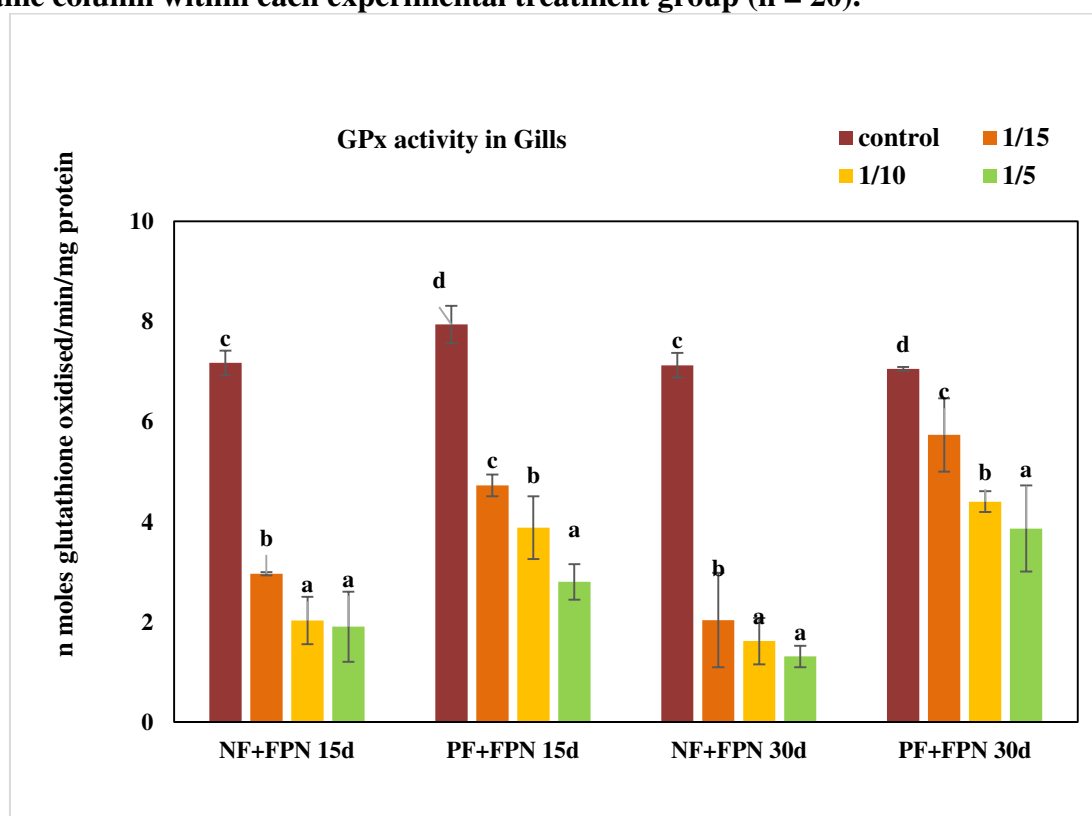


Fig 7: Glutathione peroxidase activity in gill tissue of *Oreochromis mossambicus* exposed to 1/15, 1/10 and 1/5 of lc_{50} value of fipronil (unit 1 with normal feed and unit 2 with *A. muricata* leaf extract) 15 days and 30 days of exposure and control. Values are represented as Mean \pm SD. The letters a, b, c, d were used to indicate significantly different groups ($p < 0.05$) within the same column within each experimental treatment group ($n = 20$).

Kidney in control group

Nephrons are the functional unit of kidney. Consists of two parts glomerular capsule and the tubules-renal tubule, convoluted tubule and proximal tubule. The glomerular capsule is made of single flattened epithelial layer and the renal tubules are made of single layer of cuboidal epithelial cells with densely arranged microvilli in lumen. The distal convoluted tubules are also composed of cuboidal epithelial cells with no microvilli. Proximal segment is formed of columnar epithelial cells with nucleus. Hemopoietic tissues interspaced between the tubules (Fig.8A,B,C,D).

Changes in kidney observed on exposure to 1/15 of LC₅₀ fed with Normal Feed (NF) for 15 and 30 days

At a concentration of 1/15 of LC₅₀ for 15th day of exposure kidney shows a renal parenchyma with moderate acute or chronic interstitial inflammation, along with a few dilated Bowman's capsules there is cloudy swelling and cellular hypertrophy and nuclear hypertrophy occurred (Fig.9).

At a concentration of 1/15 of LC₅₀ for 30th day of exposure kidney shows a renal parenchyma with moderate acute or chronic interstitial inflammation, along with several dilated Bowman's capsules, progressive glomerulosclerosis (Fig.10)

Other changes noticed were decreased Bowman's space and at times blood droplets were observed in Bowman's space. The tubules showed degeneration and disorganization and also reduced tubular lumen were also seen.

Changes in kidney observed on exposure to 1/10 of LC₅₀ fed with Normal Feed (NF) for 15 and 30 days

On the 15th day at 1/10 of LC₅₀ exposure of fipronil treated kidney cells shows renal parenchyma with mild chronic inflammation, dilated Bowman's capsule, mild patchy glomerulosclerosis. Eosinophilic granules were observed in cytoplasm. Melanomacrophages aggregates were observed (Fig. 11). Bowman's space showed decrease and at times blood was seen in Bowman's space. Tubules showed degeneration.

On the 30th day at 1/10 of LC₅₀ treated kidney cells shows renal parenchyma with mild chronic inflammation, dilated Bowman's capsule, increased glomerulosclerosis (Fig.12).

Changes in kidney observed on exposure to 1/5 of LC₅₀ fed with Normal Feed (NF) for 15 and 30 days

On the 15th day renal parenchyma with diffuse mild chronic interstitial inflammation and glomerulosclerosis (Fig.13). On the 30th day of exposure renal parenchyma with diffuse mild chronic interstitial inflammation and glomerulosclerosis (Fig.14). Nucleus showed irregular shape and hypertrophy along with displacement towards lateral side. Cellular hypertrophy and atrophy were observed. Cytoplasmic vacuolation and dilated Bowman's capsule were also seen.

Changes in kidney observed on exposure to 1/15 of LC₅₀ fed with Plant extract supplemented Feed (PE) for 15 and 30 days

On the 15th day renal parenchyma with mild acute on chronic interstitial inflammation, along with a few dilated Bowman's capsule. (Fig.15). On the 30th day renal parenchyma with mild chronic interstitial inflammation, along with a few dilated Bowman's capsule. (Fig.16)

Changes in kidney observed on exposure to 1/10 of LC₅₀ fed with Plant extract supplemented Feed (PE) for 15 and 30 days

On the 15th day renal parenchyma with mild chronic interstitial inflammation, dilated Bowman's capsule, mild patchy glomerulosclerosis. (Fig.17). On the 30th day renal parenchyma with mild chronic inflammation, dilated Bowman's capsule, mild patchy glomerulosclerosis (Fig.18).

Changes in kidney observed on exposure to 1/15 of LC₅₀ fed with Plant extract supplemented Feed (PE) for 15 and 30 days

On the 15th day renal parenchyma with diffuse mild chronic interstitial inflammation, and glomerulosclerosis (Fig.19). On the 30th day of fipronil exposure supplemented with plant extract sections of kidney showed renal parenchyma with diffuse mild chronic interstitial inflammation, and glomerulosclerosis (Fig.20). The Bowman's capsule showed a reduction in space. Regenerating tubular structures were also seen.

Gill histology in control fish

The histology of gills of the control fish exhibited normal gill structure. The gill arches are four pairs of bony structures that lie in the pharynx. In control fish the holobranches arranged in two rows of four arising from these gill arches forming the sides of the pharynx. Projecting from the posterior edge of the gill arch arises each holobranch consisting of two hemibranches. These hemibranches are arranged so that their ends touch adjacent holobranches. From the gill arches radiate double rows of primary filament or the primary lamellae that are seen in pairs. The primary lamellae are arranged from the gill arch like teeth of a comb. Perpendicular to the primary lamellae numerous secondary lamellae arise that increase surface area of primary lamellae. The surface area of the primary lamellae is covered by a thick mucous secreting epidermal layer and within this mucous layer is embedded the salt secreting chloride cells. Secondary lamellae are flattened and covered by single layer of inter digitating squamous epithelium and supported by intermitting pillar cells. The pillar cells contain contractile protein and are primarily concerned with providing support. From the lamellar epithelium arise villi. Gaseous exchange is maintained by counter current mechanism of blood flow in secondary epithelium (Fig.21 A,B,C,D).

Changes in gills observed on exposure to 1/15 of LC₅₀ fed with Normal Feed (NF) for 15 and 30 days

At 1/15 of LC₅₀ of fipronil for 15 days of exposure the changes observed in the gill parenchyma, with mild sloughing and lymphocytic inflammation (Fig.22). Other changes observed was atrophy of gill lamellae, fusion of gill filaments at the base region or complete fusion between two or more epithelial cells of lamellae, degeneration and necrosis of the epithelial cells, visible disorganization and rupture of the epithelial cells of secondary lamellae. The changes caused by fipronil was dose dependent. Gills were larger than normal size accompanied with increase in the number of mucous cells. Swollen and edematous epithelial cells of primary and secondary lamellae filled with red blood cells were observed. Histological sections of gill parenchyma, with mild sloughing and lymphocytic inflammation was observed. The fish gill at the concentration of 1/15 of LC₅₀ fipronil on 30th day showed edematous gills with increased number and size of mucous cells. The epithelial cells of primary and secondary lamellae were normal. Histological sections of gill parenchyma, exhibited mild sloughing but lymphocytic inflammation was absent (Fig.23).

Changes in gill observed on exposure to 1/10 of LC₅₀ fed with Normal Feed (NF) for 15 and 30 days

At 1/10 of LC₅₀ of fipronil for 15 days of exposure showed gill parenchyma with patchy epithelial hyperplasia and basal fusion of secondary lamellae (Fig.24) Lamellar telangiectasis or aneurysm was frequently observe with increase in concentration and duration of exposure. Red blood cells were highly concentrated especially near the gill filament and necrotic regions. Gill parenchyma was with patchy epithelial hyperplasia and basal fusion. The subepithelial vessels showed congestion and perivascular lymphocytic inflammation.

At 1/10 for 30 days of exposure showed gill parenchyma with patchy epithelial hyperplasia and basal fusion. The subepithelial vessels showed minor congestion and perivascular lymphocytic inflammation was completely absent. (Fig.25).

Changes in gill observed on exposure to 1/5 of LC₅₀ fed with Normal Feed (NF) for 15 and 30 days

On the 15th day of exposure of 1/5 of LC₅₀ showed gill with lamellar capillary congestion and diffuse mild chronic inflammation. (Fig.26). The changes observed are rupture and swelling of branchial epithelial tissue, necrosis within epithelial cells, fusion of secondary lamellae, hypertrophy of the mucoid cells and dissociation between the epithelial cells and pillar cells.

On the 30th day of exposure of 1/5 of LC₅₀ showed gill with lamellar capillary congestion and recovery from swelling of the capillaries (Fig.27).

Changes in gills observed on exposure to 1/15 of LC₅₀ fed with Plant extract supplemented Feed (PE) for 15 and 30 days

At lower concentration of fipronil exposure of 1/15 of LC₅₀ supplemented with plant extract feed on the 15th day of exposure the gills retained its normal shape and structure with few lamellar disorganization. Lymphocytic infiltration was clearly visible at different regions of the gill parenchyma. Lymphocytic infiltration was observed with mild sloughing and blood congestion. Hypertrophy of epithelial cells were also observed (Fig.28).

On the 30th day of exposure, swelling of the secondary lamellae showed recovery. Fusion of the secondary lamellae was common. Gill parenchyma with mild sloughing and lymphocytic inflammation also showed recovery (Fig.29).

Changes in gills observed on exposure to 1/10 of LC₅₀ fed with Plant extract supplemented Feed (PE) for 15 and 30 days

At concentration of 1/10 of LC₅₀ of fipronil supplemented with plant extract for 15 days of exposure, the changes observed in the gill deviated from normal structure included gill filament fusion and epithelial hyperplasia and necrosis of the epithelial cells and hypertrophy were also observed. The sub epithelial vessels showed congestion and perivascular lymphocytic inflammation. (Fig.30).

At 1/10 of LC₅₀ for 30 days of exposure, the changes observed in the gill parenchyma with patchy epithelial hyperplasia, and basal fusion. The sub epithelial vessels showed congestion and perivascular lymphocytic inflammation. (Fig.31).

Changes in gills observed on exposure to 1/5 of LC₅₀ fed with Plant extract supplemented Feed (PE) for 15 and 30 days

At a concentration of 1/5 of LC₅₀ of fipronil supplemented with plant extract for 15 days of exposure, the commonest anomalies found were lamellar capillary congestion, with diffuse moderate inflammation (Fig.32). The changes included swelling and hyperplasia of the epithelial cells and lifting of the lamellar epithelium and disorganization of the secondary lamellar epithelial lining.

On 30th day of exposure, the commonest anomalies found were lamellar capillary congestion, with diffuse moderate chronic inflammation (Fig.33). With increased duration of exposure along with supplemented plant extract the hyperplasia was less, with few secondary lamellae fused when compared to 15 days of exposure at same concentration. Blood congestion was also observed along with lymphocyte infiltration.

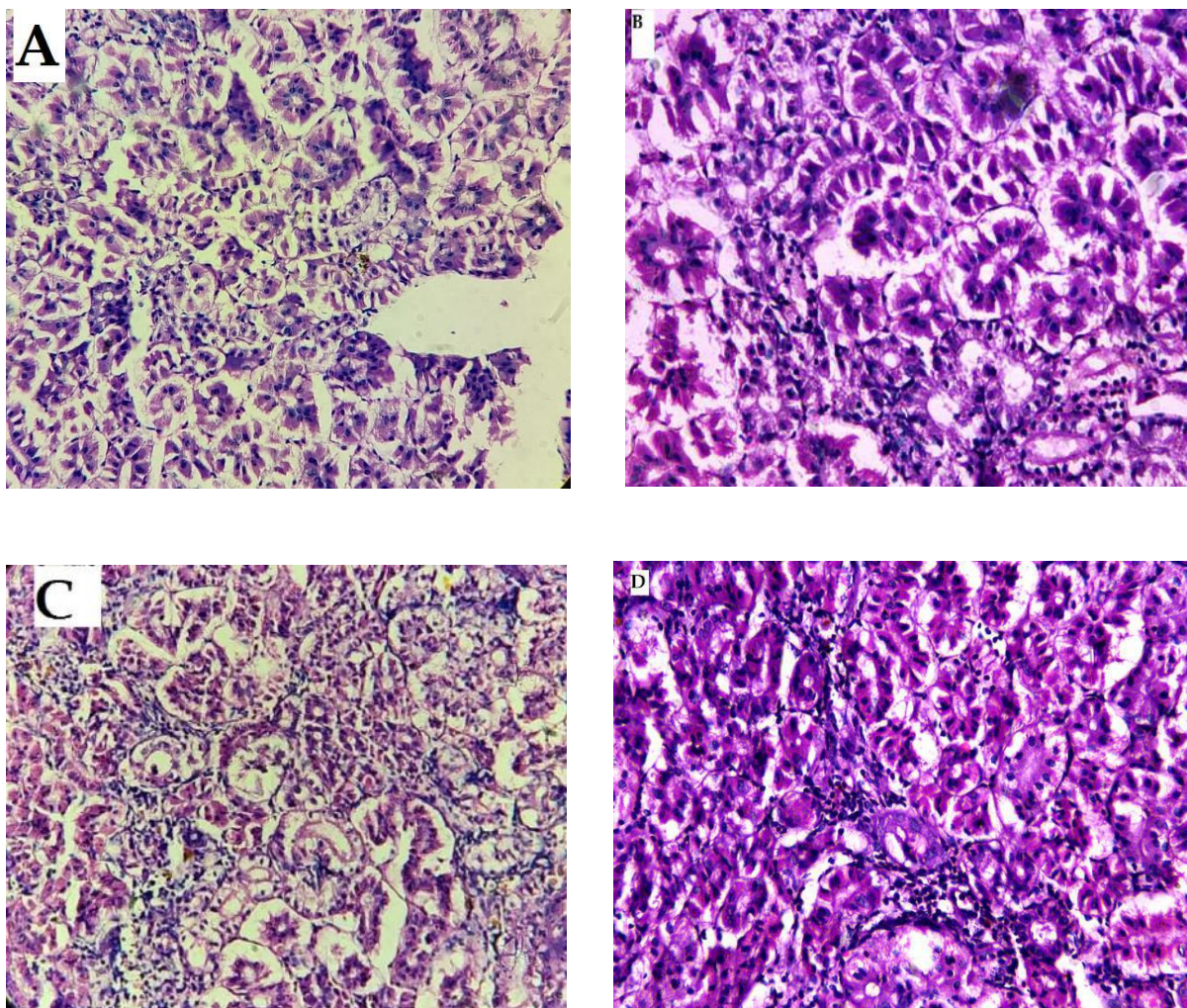


Fig 8: Light microscopic photograph of Haematoxylin and Eosin stained sections of kidney of *Oreochromis mossambicus*. A) Control with normal feed 15 days B) Control with normal feed 30 days C) Control with *A. muricata* plant extract supplemented feed 15 days D) Control with *A. muricata* plant extract supplemented feed 30 days.

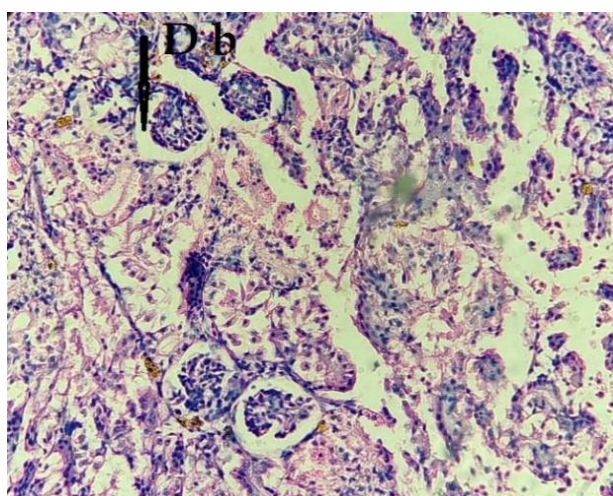


Fig 9: Light microscopic photograph of Haematoxylin and Eosin stained sections of kidney of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/15 of LC₅₀ value and fed with normal feed for 15 days. Shows sections of renal parenchyma with moderate acute on chronic interstitial inflammation, along with a few dilated Bowman capsules(Db).

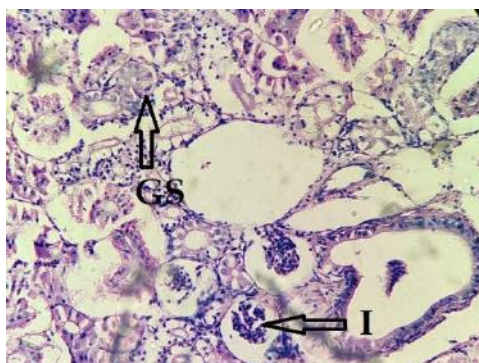


Fig 10: Light microscopic photograph of Haematoxylin and Eosin stained sections of kidney of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/15 of LC₅₀ value and fed with normal feed for 30 days. Shows sections of renal parenchyma with moderate acute or chronic interstitial inflammation(I), along with several dilated Bowman capsules, progressive glomerulosclerosis (GS).

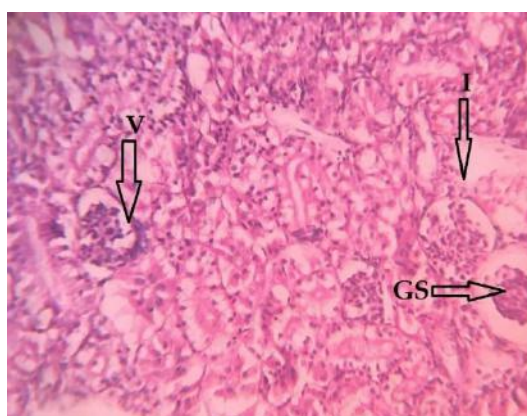


Fig 11: Light microscopic photograph of Haematoxylin and Eosin stained sections of kidney of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/10 of LC₅₀ value and fed with normal feed for 15 days. Sections of renal parenchyma with mild chronic inflammation (I), dilated Bowman capsule(V), mild patchy glomerulosclerosis(GS).

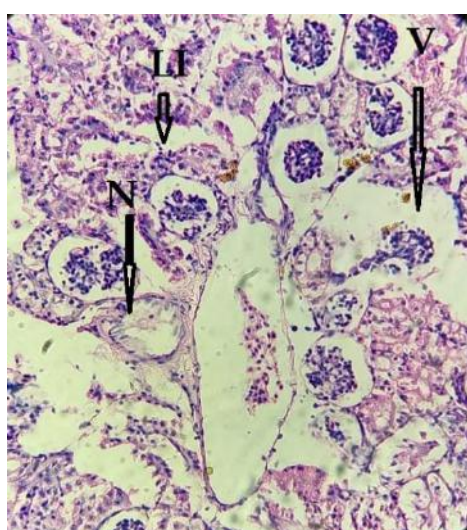


Fig 12: Light microscopic photograph of Haematoxylin and Eosin stained sections of kidney of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/10 of LC₅₀ value and fed with normal feed for 30 days. Sections of renal parenchyma with mild chronic inflammation, lymphocyte infiltration(LI), dilated Bowman capsule(V), necrosis (N) increased glomerulosclerosis.

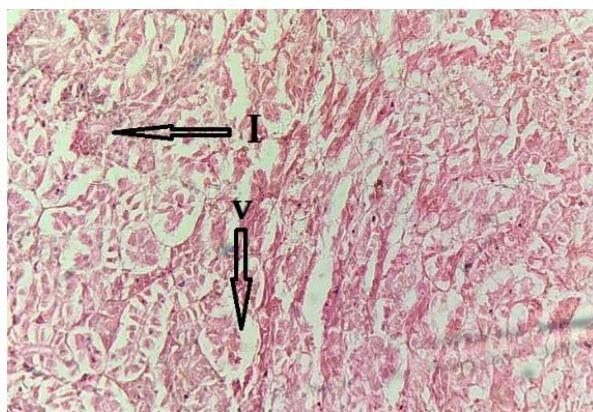


Fig 13: Light microscopic photograph of Haematoxylin and Eosin stained sections of kidney of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/5 of LC₅₀ value and fed with normal feed for 15 days. Sections of renal parenchyma with diffuse, mild chronic interstitial inflammation (I) dilated Bowman's capsule and glomerulosclerosis.

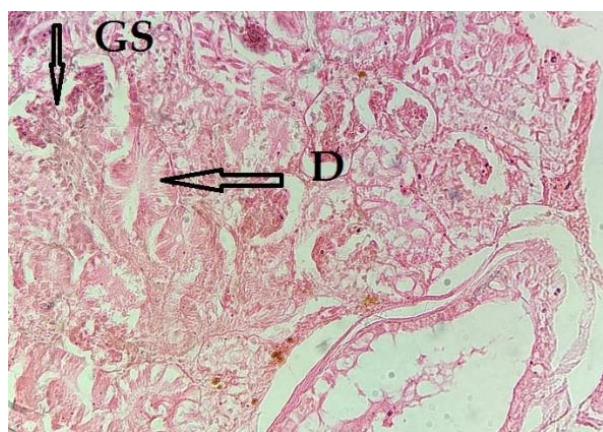


Fig 14: Light microscopic photograph of Haematoxylin and Eosin stained sections of kidney of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/5 of LC₅₀ value and fed with normal feed for 30 days. Sections of renal parenchyma with diffuse, mild chronic interstitial inflammation and glomerulosclerosis (GS) and degeneration (D).

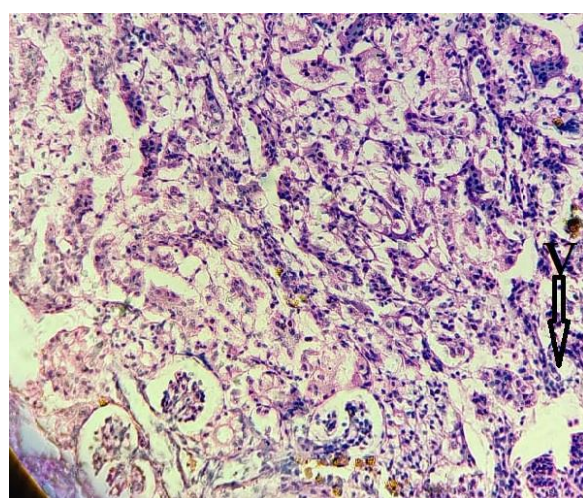


Fig 15: Light microscopic photograph of Haematoxylin and Eosin stained sections of kidney of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/15 of LC₅₀ value and fed with *A. muricata* leaf extract feed for 15 days. Shows sections of renal parenchyma with mild acute on chronic interstitial inflammation, along with a few dilated Bowman's capsule (V).

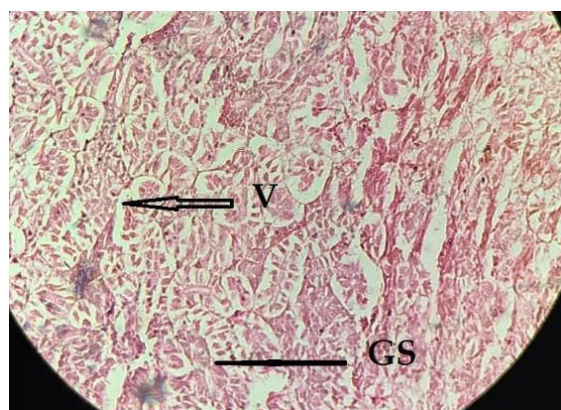


Fig 16: Light microscopic photograph of Haematoxylin and Eosin stained sections of kidney of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/15 of LC₅₀ value and fed with *A. muricata* leaf extract feed for 30 days. Shows sections of renal parenchyma with mild chronic interstitial inflammation, along with a few dilated Bowman's capsule (V) and glomerulosclerosis(GS).

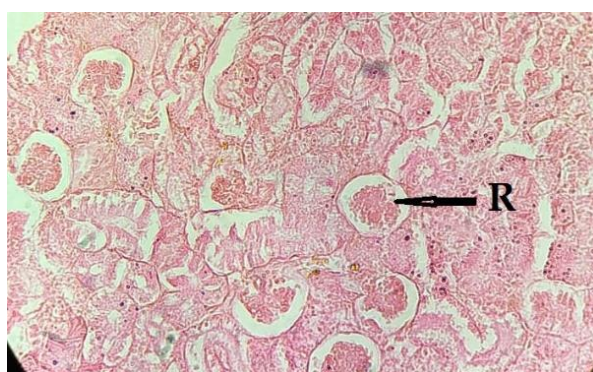


Fig 17: Light microscopic photograph of Haematoxylin and Eosin stained sections of kidney of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/10 of LC₅₀ value and fed with *A. muricata* leaf extract feed for 15 days. Sections of renal parenchyma with mild inflammation, dilated Bowman's capsule, mild patchy glomerulosclerosis and regenerative cells.

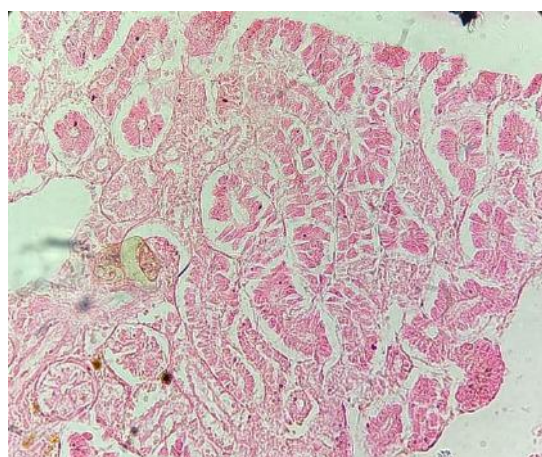


Fig 18: Light microscopic photograph of Haematoxylin and Eosin stained sections of kidney of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/10 of LC₅₀ value and fed with *A. muricata* leaf extract feed for 30 days. Sections of renal parenchyma with mild chronic inflammation, dilated Bowman's capsule, mild patchy glomerulosclerosis.

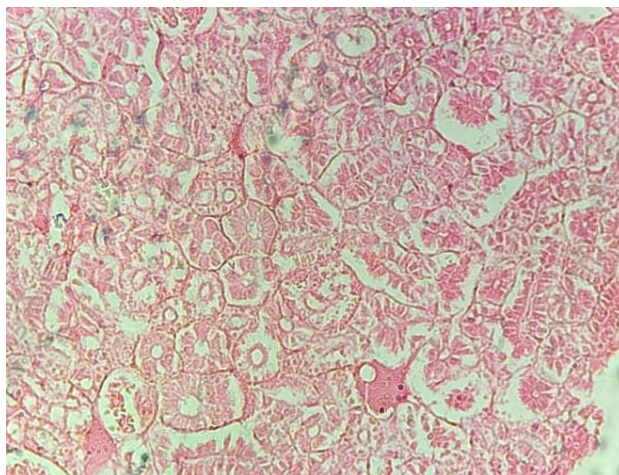


Fig 19: Light microscopic photograph of Haematoxylin and Eosin stained sections of kidney of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/5 of LC₅₀ value and fed with *A. muricata* leaf extract feed for 15 days. Sections of renal parenchyma with diffuse mild chronic interstitial inflammation and glomerulosclerosis.

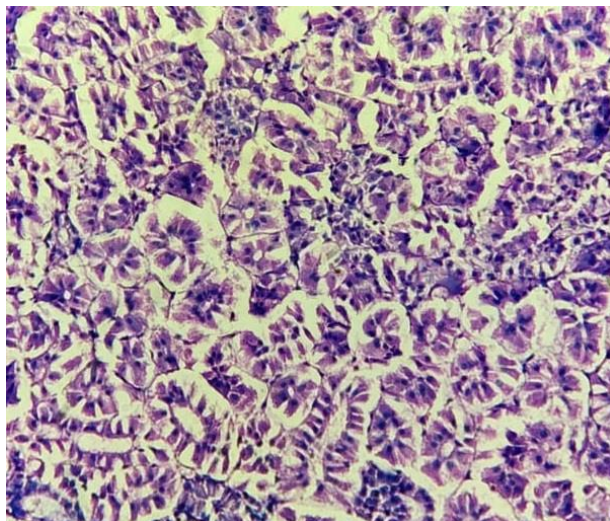
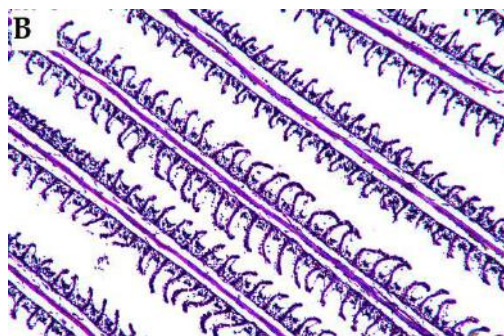
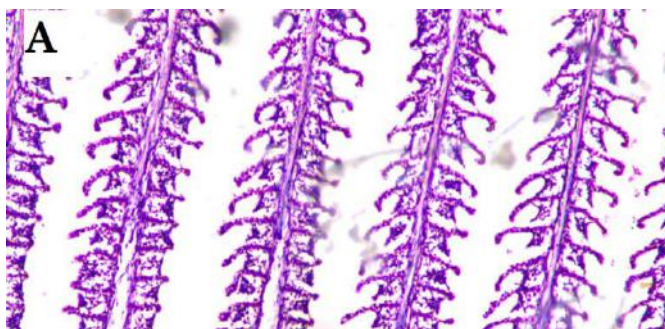


Fig 20: Light microscopic photograph of Haematoxylin and Eosin stained sections of kidney of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/5 of LC₅₀ value and fed with *A. muricata* leaf extract feed for 30 days. Sections of renal parenchyma with diffuse mild chronic interstitial inflammation and glomerulosclerosis.



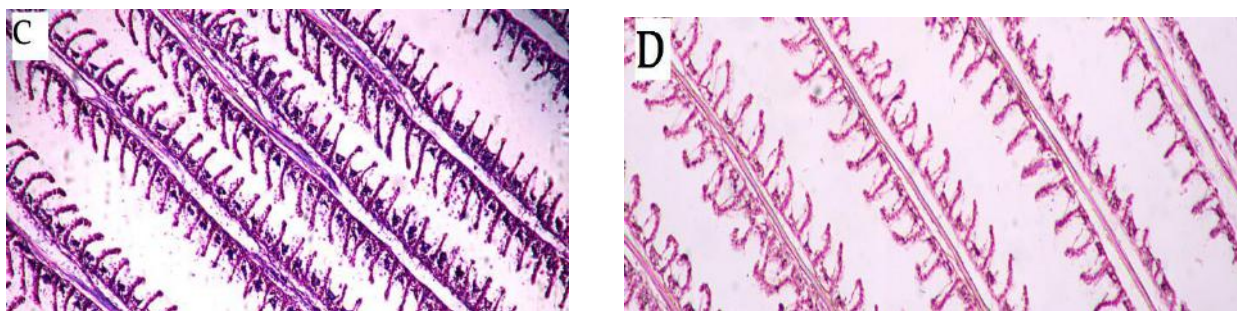


Fig 21: Light microscopic photograph of Haematoxylin and Eosin stained sections of gills of *Oreochromis mossambicus*. A) Control with normal feed 15 days B) Control with normal feed 30 days C) Control with *A. muricata* plant extract supplemented feed 15 days D) Control with *A. muricata* plant extract supplemented feed 30 days.

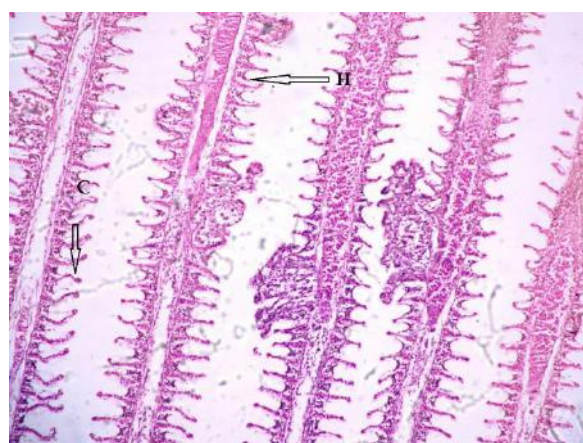


Fig 22: Light microscopic photograph of Haematoxylin and Eosin stained sections of gills of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/15 of LC_{50} value and fed with normal feed for 15 days. Shows sections of gill parenchyma, hyperplasia of gill epithelium (H), curling of secondary gill lamellae (C), with mild sloughing and lymphocytic inflammation.

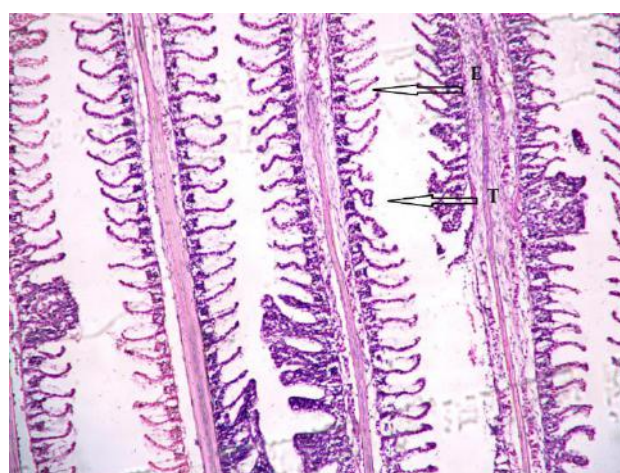


Fig 23: Light microscopic photograph of Haematoxylin and Eosin stained sections of gills of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/15 of LC_{50} value and fed with normal feed for 30 days. Shows sections of gill parenchyma, with mild sloughing, telangiectasia (T), epithelial uplifting of secondary gill lamellae (E) and lymphocytic inflammation.

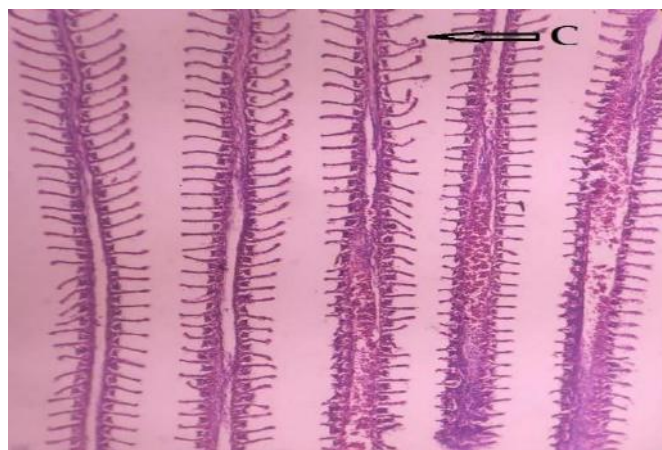


Fig 24: Light microscopic photograph of Haematoxylin and Eosin stained sections of gills of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/10 of LC₅₀ value and fed with normal feed for 15 days. Sections of gill parenchyma with patchy epithelial hyperplasia, curling of secondary gill lamellae (C) and basal fusion. The subepithelial vessels show congestion and perivascular lymphocytic inflammation.

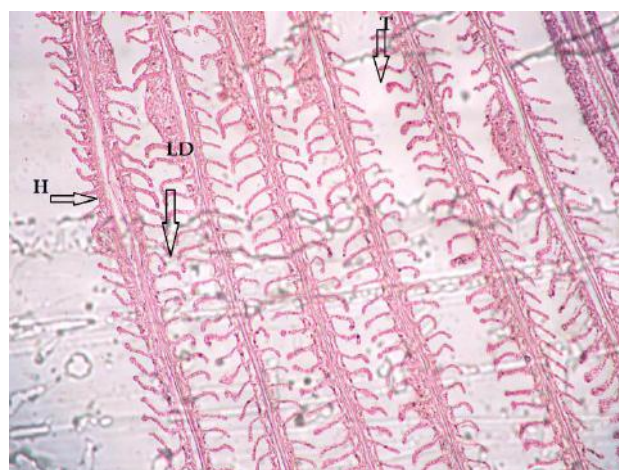


Fig 25: Light microscopic photograph of Haematoxylin and Eosin stained sections of gills of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/10 of LC₅₀ value and fed with normal feed for 30 days. Sections of gill parenchyma with patchy epithelial hyperplasia (H), Telangiectasia (T), lamellar disorganization (LD) and basal fusion. The subepithelial vessels show congestion and perivascular lymphocytic inflammation.

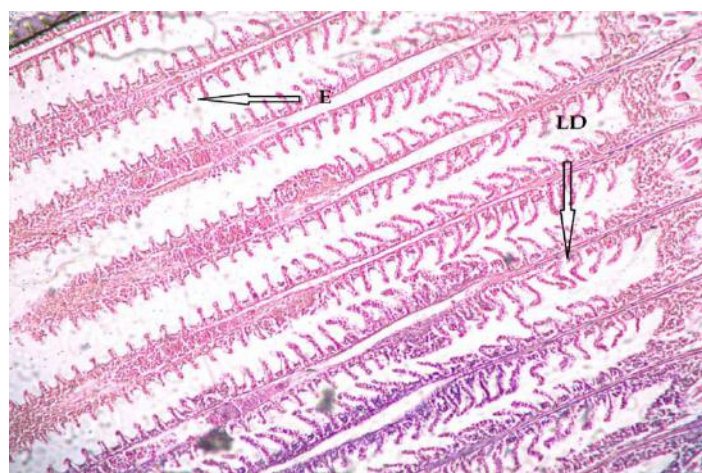


Fig 26: Light microscopic photograph of Haematoxylin and Eosin stained sections of gills of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/5 of LC₅₀ value and fed

with normal feed for 15 days. Shows sections of gill parenchyma, with mild sloughing lamellar disorganization (LD) epithelial uplifting of secondary gill lamellae (E) and lymphocytic inflammation.

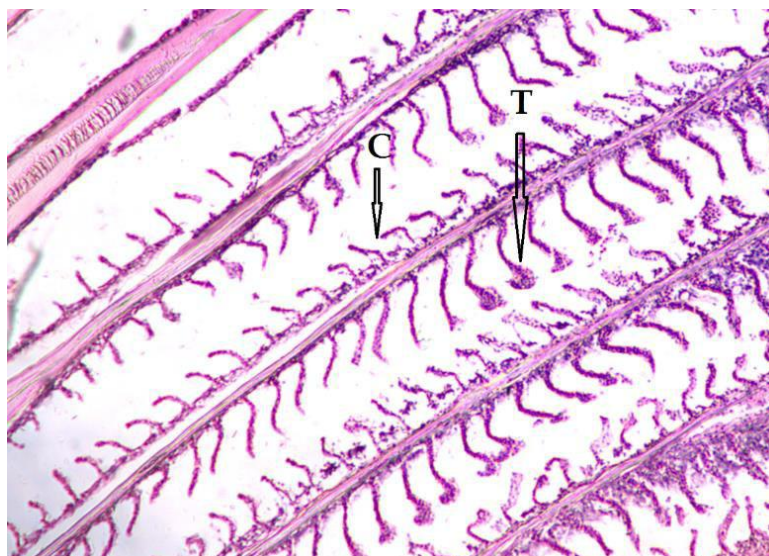


Fig 27: Light microscopic photograph of Haematoxylin and Eosin stained sections of gills of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/5 of LC₅₀ value and fed with normal feed for 30 days. Sections of gill with lamellar capillary congestion, Telangiectasia (T), curling of secondary gill lamellae (C) and diffuse chronic inflammation.



Fig 28: Light microscopic photograph of Haematoxylin and Eosin stained sections of gills of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/15 of LC₅₀ value and fed with *A. muricata* leaf extract feed for 15 days. Shows sections of gill parenchyma, with mild sloughing, hyperplasia of gill epithelium (H), lamellar disorganization (LD) and lymphocytic inflammation.

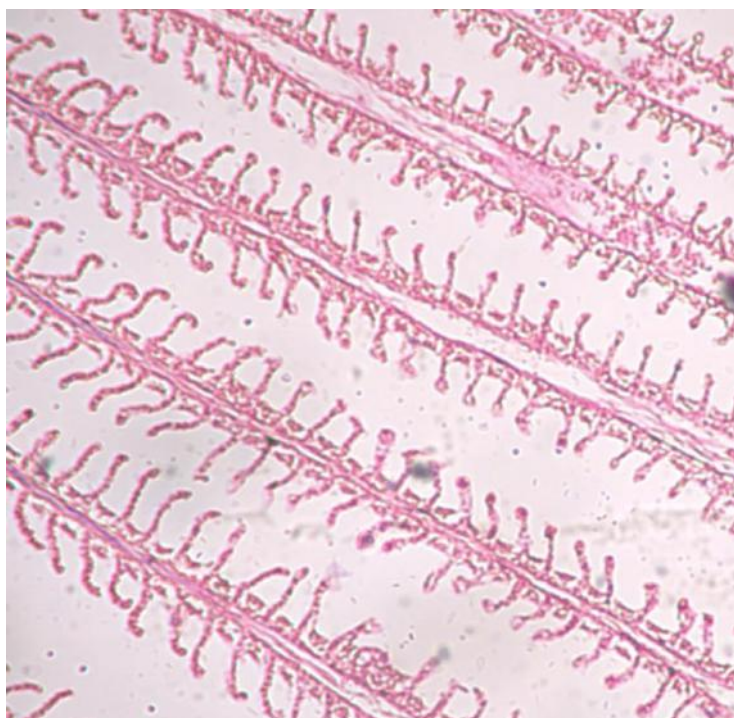


Fig 29: Light microscopic photograph of Haematoxylin and Eosin stained sections of gills of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/15 of LC₅₀ value and fed with *A. muricata* leaf extract feed for 30 days. Shows sections of gill parenchyma, with mild sloughing and lymphocytic inflammation.

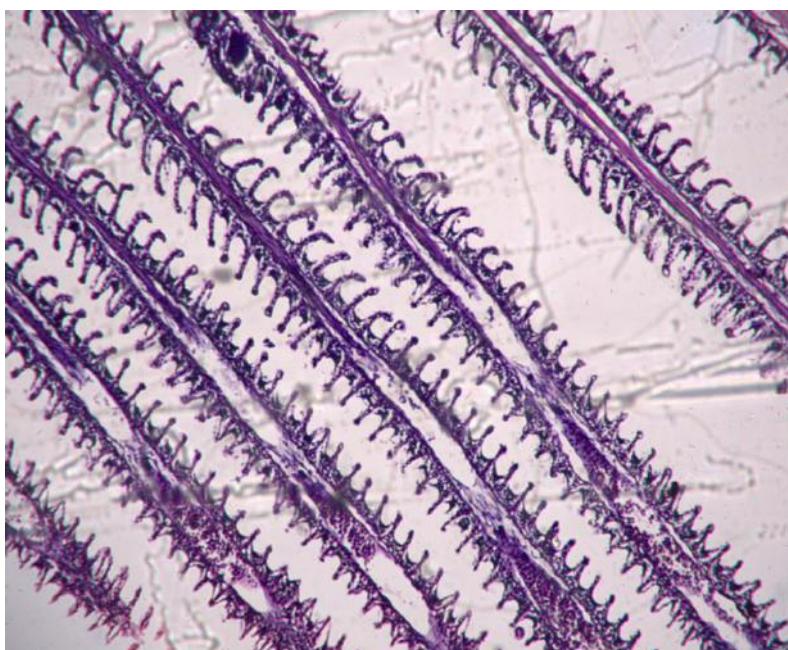


Fig 30: Light microscopic photograph of Haematoxylin and Eosin stained sections of gills of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/10 of LC₅₀ value and fed with *A. muricata* leaf extract feed for 15 days. Sections of gill parenchyma with patchy epithelial Hyperplasia and basal fusion. The subepithelial vessels show congestion and perivascular lymphocytic inflammation.

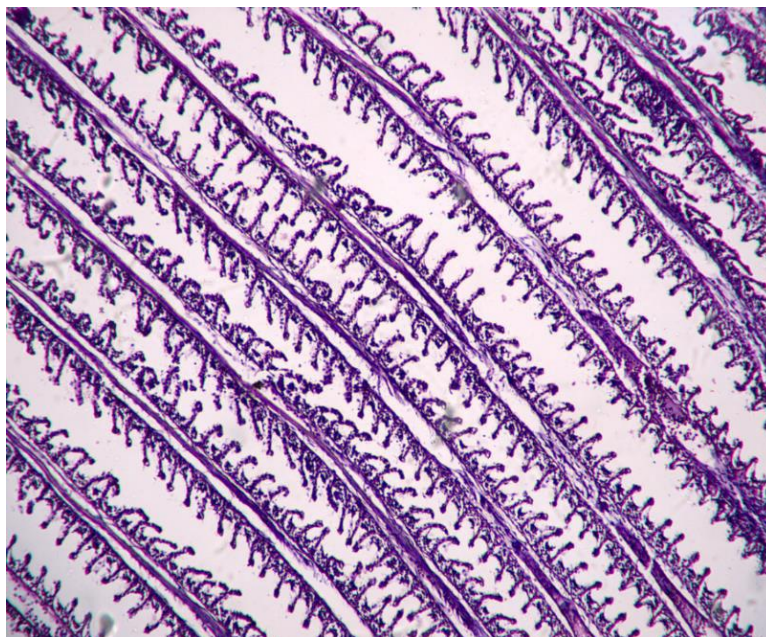


Fig 31: Light microscopic photograph of Haematoxylin and Eosin stained sections of gills of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/10 of LC₅₀ value and fed with *A. muricata* leaf extract feed for 30 days. Sections of gill parenchyma with patchy epithelial Hyperplasia and basal fusion. The subepithelial vessels show congestion and perivascular lymphocytic inflammation.



Fig 32: Light microscopic photograph of Haematoxylin and Eosin stained sections of gills of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/5 of LC₅₀ value and fed with *A. muricata* leaf extract feed for 15 days. Sections of gill with lamellar capillary congestion, with diffuse moderate chronic inflammation.

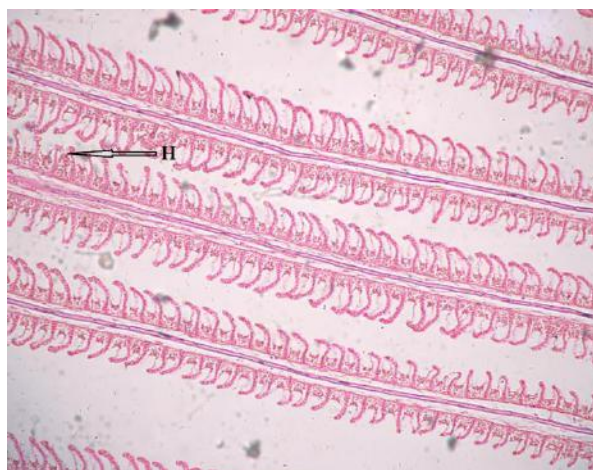


Fig 33: Light microscopic photograph of Haematoxylin and Eosin stained sections of gills of *Oreochromis mossambicus* exposed to fipronil at a concentration of 1/5 of LC₅₀ value and fed with *A. muricata* leaf extract feed for 30 days. Sections of gill with lamellar capillary congestion, with diffuse moderate chronic inflammation.

4. DISCUSSION:

The impact of pesticide exposure depend on the concentration of the pesticide, ability to induce adverse effect by penetrating into the organism, by intoxication leading to stressed condition of nontarget organism or indirectly altering its physicochemical environment. The median lethal concentration, LC₅₀ of fipronil was 3.74 µg/L on *Oreochromis mossambicus*. Similar results are seen in nile tilapia, *Oreochromis niloticus* that showed an LC₅₀ of 42 µg/L (Tingle *et al.*, 2003). The 96 hours LC₅₀ value of fipronil to cyprinus fry was 0.428 µg/L. The values showed gradual decrease with increase in exposure time (Gupta *et al.*, 2014). The LC₅₀ of *Daphnia pulex* was about 16 µg/L (Stark and Vargas, 2005).

The present study recorded a reduction in SOD enzyme activity on exposure to fipronil that might be ascribed to the role of an antioxidant by this enzyme for the conversion of the free radical formed (O₂⁻) to H₂O₂ (Mossa *et al.*, 2015; Badgujar *et al.*, 2016; Kartheek and David, 2018). Fipronil exhibited dose-dependent inhibition of antioxidant enzymes SOD, CAT and GPx in liver and gill tissues at different doses in rainbow trout, *Oncorhynchus mykiss* (Ucar *et al.*, 2021).

The teleostean kidney is one of the main organs affected by xenobiotics in the water. Kidney is concerned with excretion of nitrogen waste and these toxic substances reach kidney the gross histopathologic changes observed include tubular degeneration, glomerular capillary dilation, congestion, distorted bowman's capsule vacuolization and alteration in renal corpuscles. (Takashima and Hibiya, 1995). Thophon *et al.*, (2003) observed alterations in the tubules and glomerulus in the perch (*Lates calcarifer*) exposed to cadmium. Handy and Penrice (1993) also observed melanomacrophages and dilated Bowman's capsule in the kidney of trout (*Salmo trutta*) and tilapia (*Oreochromis mossambicus*) exposed to toxicants. Veiga *et al.*, (2002) Also observed similar operation on exposure to organic contaminants in fish. Exposure to unknown pollutants also resulted in kidney damage in fishes (Schwaiger *et al.*, 1997).

The immune response observed in kidney exhibited both specific and nonspecific reaction towards stressors. In the present study dilation of the tubules and cloudy swelling within the tubular cells were observed. The alterations in kidney showed regeneration on exposure to fipronil when supplemented with plant extract of *Annona muricata*. The gross pathological changes in kidney were most prominent in groups exposed to fipronil indicating the renal toxicity induced by the phenylpyrazole. The lymphocyte infiltration was observed as presence of small granules in the cytoplasm which was characterized with the presence of eosinophilic granules in the cells (Hinton

and Lauren, 1990) leading to tissue necrosis (Takashima and Hibiya, 1995). Saxena and Saxena (2008) reported that congestion and hemorrhages in kidney, engorged blood vessels and extravagated erythrocytes could be seen in tissue sections of sampled kidney of fish after exposure to water polluted with heavy metals. Moderate lymphocytic infiltration was observed in kidney that was visible as large blue stain aggregates of round cells.

Kidney showed degenerative changes in the parenchyma. Cellular structure of tissues was changed and homogenous structure of tissues was lost in the liver and kidney of treated fishes. The development of nephron occur throughout fish life and this growth is more prominent during young stages (Reimschuessel, 2001). Present study showed regeneration of tubules and new nephrons in groups exposed to fipronil and supplemented with *Annona muricata* leaf extract which was also observed by Hinton and Lauren (1990) during the process of the recovery of the damaged kidney in fish but complete it regeneration was not observed as it is about two months for tubules to re generate completely (Gemhofer *et al.*, 2001). Similar observation have also been made in the cod (*Microgadus tomcod*) collected from contaminated streams (Cormier *et al.*, 1995) , and in gold fish, *Oncorhynchus mykiss*, *Brachydanio rerio* and *Oreochromis mossambicus* exposed to contaminants that cause necrosis and vacuolization. There is evidence that xenobiotics has endocrine disrupting potential (Gray and Foster, 2003). Histopathologic changes observed in the present study are either defence mechanism of the fish against fipronil or may be the result of direct toxic effect of fipronil.

The gills are the primary internal organ that comes in direct contact with surrounding aquatic medium with larger proportion of surface area directly bathing the blood vessels in the surrounding aquatic medium (Wood and Soivio, 1991), facilitating gaseous exchange. The histological alterations attributed to prolonged exposure to fipronil might lead to respiratory, osmoregulatory and circulatory impairment and similar observations were recorded. (Fernandes *et al.*, 2008). Exposure to lead for 30 days caused intralamellar hyperplasia and excess mucus secretion in gill filament (Weber, 1991) that lowered the diffusion of gases inhibiting the transportation abilities and on exchange capacity of the gill surface that resulted in decreased activity as expressed by slow swimming capacity (Tabche *et al.*, 1990). Hypertrophy was reported by Alvarado *et al.*, (2006) where epithelial thickness was produced by elevated levels of chloride cells that migrated to the edge of the secondary lamellae. In *Oreochromis mossambicus* exposed to fipronil poisoning exhibited hyperplasia of the epithelial cells in the secondary lamellae. Similar observations were made in metal poisoning (Roncero *et al.*, 1990). The hyperplasia was more severe in normal feed supplemented fish when compared to plant extract supplemented fish showing that the reduced hyperplasia may be due to the immuno stimulants present within *Annona muricata*.

5. CONCLUSION:

The protective effect of *A. muricata* leaf extract against fipronil toxicity is well evidenced by the increased activity of SOD, CAT and GPx. Results indicated that the supplement with *A. muricata* leaf extract mitigated fipronil induced oxidative damage. The antioxidant potential of *A. muricata* may be attributed to its phytochemical constituents. In the present study the histological examination of kidney and gill provide information on the toxicity of fipronil and the effect of the plant extract of *Annona muricata* on the toxicity of fipronil. Study clearly show the immunostimulant activity of *Annona muricata* leaf extract that inhibited the toxic effects on the cellular level of organism.

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Study of coordination behaviour of some pyrazine derivative

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Abstract: A novel series of Co(II) and Ni(II) complexes with Schiff base derived from pyrazine-2-carboxamide and 3-chloro-furan-2-carbaldehyde have been synthesized. The metal complexes were characterized by micro elemental analysis, molar conductivity, magnetic susceptibility, FTIR, and electronic absorption spectra. The metal (II) complexes are coloured and stable in air. In the metal complexes the metal -ligand ratio found to be 1:2. The sharp band appeared at 1610 cm⁻¹ is characteristics of the azomethine group present in the Schiff base ligand. This band was shifted to lower frequency (1585 – 1590 cm⁻¹) in all the metal (II) complexes, which indicates the coordination of the azomethine nitrogen to metal ion in complexes. The lower molar conductivity data of the complexes in DMF solution indicates they are non- electrolytic in nature. The spectral data indicate that the Schiff base ligand behaves as neutral bidentate and coordinated with metal ion by azomethine nitrogen and furan oxygen atom to the metal ion. On the basis of elemental analysis, magnetic susceptibility and electronic spectral data, geometry of complexes was proposed to be octahedral in nature.

Keywords: Pyrazine-2-carboxamide, 3-chloro-furan-2-carbaldehyde, metal salts, Schiff base.

1. INTRODUCTION: -

A large number of Schiff base compounds have been prepared by the condensation of a carbonyl compound and a primary amine under suitable conditions having nitrogen, oxygen and sulphur atoms as their donor sites. The chemistry of -C=N moiety bearing chelators and their metallic compounds have been an interesting aspect of bioinorganic chemistry in the last two decades owing to their vast applications. Schiff bases may be represented as R-CH=N-R' where R and R' may be alkyl, aryl or heterocyclic¹⁻⁴. Schiff bases of aromatic aldehydes are more stable than aliphatic aldehydes due to effective conjugation. The presence of azomethine group, which is responsible for stability, reactivity and biological activity of Schiff bases and their metal complexes. Schiff bases and their metal complexes are used in catalyst, antimicrobials, antioxidants, dyes optical materials and analytical chemistry. Due to chelation transition metal Schiff base complexes are more stable and have wide applications in antibacterial, antifungal, antiviral, anticancer, and anti-inflammatory⁵⁻⁸. Pyrazines are most important classes of heterocyclic compounds that can be obtained naturally or can be synthesized chemically. Pyrazine is a weaker base than pyridine and pyrimidine. Pyrazine and alkyl pyrazine are flavour and aroma compound found in baked and roasted goods. Pyrazines gained attention from the food industry as important ingredients in raw and roasted foods⁹⁻¹². Pyrazine is recognized to universally occur in wildlife as vital component of fused oil, cocoa bean, green peas, molds resembling *Aspergillus flavus*, *A. oryzae*, etc and have been engaged as synthetic forerunner of bioactive compounds. An extensive range of therapeutic active compounds of pyrazine and its applications in antibiotics is becoming progressively vast mostly with the

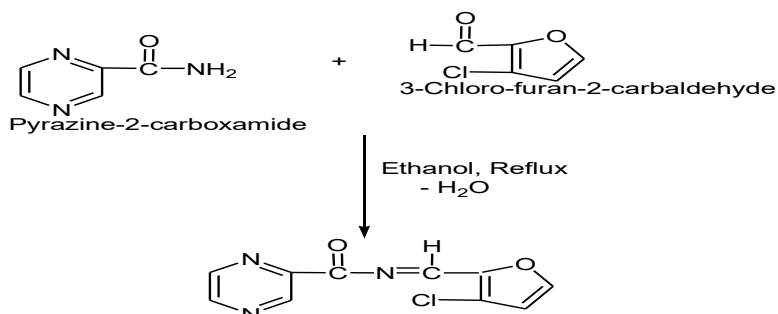
functionalized derivatives. Pyrazine derivatives are documented to display a wide range of pharmacological activities consisting of anticancer, antitubercular, diuretic, antidiabetic, insecticide and nematocide¹³⁻¹⁵. The drugs bearing pyrazine structure have remained famous chemotherapeutic agents and are applied as inhibitor against various tumor enzyme targets. we report here the Study of coordination behaviour of some pyrazine derivative.

2. MATERIALS AND METHODS:-

The chemicals used in the present work were of Anal-R grade and were used without further purification. The elemental analysis (CHNS) data was obtained using Perkin-Elmer elemental analyzer. The molar conductivity of the complexes in DMF solution (10^{-3} M) were measured by using DI-909 digital conductivity meter. The IR spectra of the ligand and metal complexes were recorded on Shimadzu FTIR spectrophotometer using KBr disc. The magnetic susceptibility data were measured by Gouy method using Hg [Co (SCN)₄] as a calibrant. The electronic spectra of the complexes were recorded by using Shimadzu model UV-1601 spectrophotometer in DMSO solution.

Synthesis of Schiff base Ligand (L): -

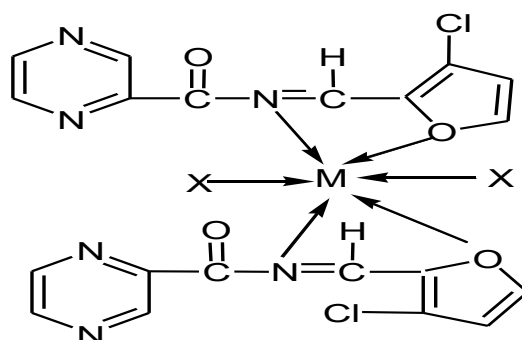
The Schiff base ligand N-(3-Chloro- furan-2-ylmethylidene) -pyrazine-2-carboxamide was prepared by adding 2 mmol of pyrazine-2-carboxamide in 20 ml ethanol to 2 mmol of 3-chloro-furan-2-carbaldehyde in 20 ml ethanol. The resulting solution was refluxed for 2-3 h. On cooling the reaction mixture, the yellow crystalline solid was formed. The precipitate is washed with ethanol and diethyl ether several times and is then recrystallized with ethanol to obtain the required Schiff base ligand.



N-(3-Chloro- furan-2-ylmethylidene) -pyrazine-2-carboxamide

Synthesis of metal (II) complexes:-

The metal (II) complexes were prepared by mixing (50 ml) ethanolic solution of metal (II) chloride or acetate with the (50 ml) ethanolic solution of Schiff base in a metal-ligand ratio 1:2. The resulting mixture was refluxed on water bath for 3-4 h. The complex obtained in each time was cooled, filtered and washed with acetone and recrystallized with ethanol and dried over anhydrous KOH in a desiccator.



Proposed structure of metal (II) complexes.

M = Co⁺² and Ni⁺², X = Chloride and acetate.

3. RESULTS AND DISCUSSION: -

The analytical and physical properties of Schiff bases and their metal (II) complexes are given in Table-1. The spectral, analytical, theoretical and magnetic data validated the molecular formula and structures of the synthesized Schiff base ligand and metal (II) complexes. All metal (II) complexes are coloured and stable in air. All experimental data obtained strongly confirmed with theoretical data and were in agreement with suggested elemental analysis for all the synthesized complexes. The metal ligand ratio in all the metal complexes have 1:2. Molar conductance and magnetic moment data of metal (II) complexes are given in Table 2. The lower molar conductance values of metal (II) complexes in DMF solution indicate their non-electrolytic in nature. All metal (II) complexes are paramagnetic in nature due to unpaired electrons present in d-orbital of metal (II) ion.

Table 1. Physical Properties and Analytical data of Schiff base and its metal(II) Complexes

Compounds	Color	% Analysis Found (Calc)			
		C	H	N	M
Schiff base	Yellow	50.95 (50.89)	2.52 (2.49)	17.83 (17.79)	—
[Co(L) ₂ Cl ₂]	Brown	39.94 (39.91)	1.99 (1.96)	13.97 (13.86)	9.80 (9.78)
[Ni(L) ₂ (OAc) ₂]	Green	39.95 (39.90)	1.99 (1.94)	13.98 (13.91)	9.77 (9.73)

Table 2. Molar Conductance and Magnetic Moment data of metal(II) Complexes

Compounds	Molar conductance ($\Omega^{-1}\text{cm}^2\text{mol}^{-1}$)	μ_{eff} (B.M)	Magnetic nature
[Co(L) ₂ Cl ₂]	12	4.84	Paramagnetic
[Ni(L) ₂ (OAc) ₂]	10	2.88	Paramagnetic

IR Spectra: -

The I.R spectral data of Schiff base ligand and metal (II) complexes are given in Table 3. The band appeared at 1610 cm^{-1} is characteristics of the azomethine group present in the Schiff base ligand. This band was shifted to lower frequency ($1585 - 1590\text{ cm}^{-1}$) in all the metal (II) complexes, which indicates the coordination of the azomethine nitrogen to metal ion in complexes. A broad band at 1200 cm^{-1} is present in pyrazine ring of Schiff base ligand and it remains unshifted in the metal complexes, which indicates nitrogen atoms of pyrazine ring is not involved in coordination to the metal ion. A sharp band due to $\nu(\text{C-O})$ furan appears at 820 cm^{-1} in the Schiff base, which shifted to lower frequency ($780 - 790\text{ cm}^{-1}$) in all the metal (II) complexes, which indicates the coordination of the furan oxygen to metal ion in complexes.

Table 3. IR spectral data of Schiff base ligand and its metal (II) complexes

Compounds	$\nu(\text{C=O})$	$\nu(\text{C=N})$	$\nu(\text{C-O})$ Furan ring
Schiff base	1680	1610	820
$[\text{Co}(\text{L})_2\text{Cl}_2]$	1710	1585	790
$[\text{Ni}(\text{L})_2(\text{OAc})_2]$	1730	1590	780

Electronic absorption spectra: -

The electronic absorption spectra of metal (II) complexes recorded in DMF solution. The electronic spectra of the Co(II) Complex shows three bands at 12560, 16410 and 19310 cm^{-1} assigned to $4\text{T}_{1g}(\text{F}) \rightarrow 4\text{T}_{2g}(\text{F})$, $4\text{T}_{1g}(\text{F}) \rightarrow 4\text{A}_{2g}(\text{F})$ and $4\text{T}_{1g}(\text{F}) \rightarrow 4\text{T}_{1g}(\text{P})$. These data and the magnetic moment value of 4.84 B.M, which suggested the octahedral geometry of Co(II) complex. The Ni (II) complex shows three bands at 10460, 12672 and 19610 cm^{-1} assigned $3\text{A}_{2g}(\text{F}) \rightarrow 3\text{T}_{2g}$, $3\text{A}_{2g}(\text{F}) \rightarrow 3\text{T}_{1g}(\text{F})$ and $3\text{A}_{2g}(\text{F}) \rightarrow 3\text{T}_{1g}(\text{P})$. These data and the magnetic moment value of 2.88 B.M, which suggested octahedral geometry of Ni(II) complex.

4. CONCLUSION: -

The Schiff base derived from pyrazine-2-carboxamide and 3-chloro-furan-2-carbaldehyde have been synthesized. The metal complexes were characterized by micro elemental analysis, molar conductivity, magnetic susceptibility, FTIR, and electronic absorption spectra. The metal (II) complexes are coloured and stable in air. In the metal complexes the metal -ligand ratio found to be 1:2. The sharp band appeared at 1610 cm^{-1} is characteristics of the azomethine group present in the Schiff base ligand. This band was shifted to lower frequency (1585 – 1590 cm^{-1}) in all the metal (II) complexes, which indicates the coordination of the azomethine nitrogen to metal ion in complexes. A sharp band due to $\nu(\text{C-O})$ furan appears at 820 cm^{-1} in the Schiff base, which shifted to lower frequency (780 – 790 cm^{-1}) in all the metal (II) complexes, which indicates the coordination of the furan oxygen to metal ion in complexes. The lower molar conductivity data of the complexes in DMF solution indicates they are non- electrolytic in nature. The spectral data indicate that the Schiff base ligand behaves as neutral bidentate and coordinated with metal ion by azomethine nitrogen and furan oxygen atom to the metal ion. On the basis of elemental analysis, magnetic susceptibility and electronic spectral data, geometry of complexes was proposed to be octahedral in nature.

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ANNEALING EFFECT ON CHARACTERISTICS OF NICKEL-TUNGSTEN ALLOY THIN FILMS

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Abstract: *Electroplating at room temperature was used to create NiW alloy thin films. After that, the electroplated NiW thin films were annealed at 200 degrees Celsius. The texture of NiW deposited films is oriented in the FCC phase. Morphological, structural, and mechanical characterizations were performed on them. On the surface, NiW films were brilliant and uniformly covered. NiW film deposition were also nanoscale, with an average crystalline size of roughly 92 nm. After annealing, the micro hardness of NiW was 138 VHN.*

Keywords: *Electroplating, electrolytic bath, crystalline size, VSM, Ni-P, X-ray diffraction, VHN, SEM.*

1. INTRODUCTION:

MEMS devices such as tiny actuators, sensors, micro motors, and frictionless micro gears require soft magnetic materials. Ferromagnetic films and alloys have unique features compared to non-magnetic films and alloys due to their use in magnetic storage media, magnetic sensors, and other applications [1-4]. Ni-Fe, Ni-W, and Co-Fe alloys have been widely employed due to their low coercivity and high saturation magnetization. Because the crystal structure of Ni-W alloys has the greatest influence on their magnetic properties, a detailed examination of this engineering substance is required for its application areas [5-8]. Several tests on the effects of various deposition parameters on the magnetic characteristics of Ni-W films have been undertaken in recent years by scientists. Nickel and its alloys have a number of advantages, including excellent wear and corrosion resistance [9-12]. As a result, in the industry, nickel deposition is required to improve wear and corrosion resistance as well as magnetic characteristics. Electrochemical techniques, such as electrodeposition and electroless deposition, are ideally adapted to meet high yield and low cost criteria [13-14]. The effects of annealing on NiW films were investigated in this work.

2. EXPERIMENTAL PART:

Electrodeposition of NiW alloy films were prepared with electrolyte baths consisting Sodium tungstate (15 g/l), Nickel sulphate (30 g/l), Ammonium sulphate (40 g/l), Boric acid (10 g/l), and Saccharin (10 g/l) and operating at temperature (30 °C). The deposition process took 15 minutes

to complete. Copper and stainless steel substrates with dimensions of 1.5 cm x 7.5 cm were used as cathode and anode in this study [6-8]. By adding ammonia solution, the pH of the electrolytic solution was set to 6.0, and the electroplating procedure was carried out with a current density of 3 mA/cm². After 15 minutes, the copper or cathode was gently removed from the bath and dried for a few minutes [10-14]. Then electroplated NiW thin films was annealed at 200 °C. Scanning Electron Microscope was used to describe the surface nature of NiW films. Energy-dispersive X-ray spectroscopy was used to look at the atomic composition of film deposits, and X-ray diffraction was used to look at the crystal structure of the deposits. Vickers Hardness Test was used to determine the micro hardness of the films.

3. RESULTS AND DISCUSSION:

3.1 ELEMENTAL COMPOSITION OF NiW THIN FILMS

The elemental composition of NiW films was determined by EDAX analyser. The obtained data by this analyser are shown in Table 1. From result, after annealing , tungsten increased and nickel decreased .

Table 1: EDAX analysis of thin films

S. No	Condition	Ni Wt%	W Wt%
1.	NiW (30°C)	72.21	27.79
2	NiW (Annealed 200°C)	68.45	31.55

3.2 MORPHOLOGICAL OBSERVATION

Surface appearance of NiW thin films at 30°C and annealed thin film were analysed by Scanning Electron Microscope (SEM) images and they are shown in Fig 1. The thin films are bright and uniformly coated on the surface. They are crack free by appearance.

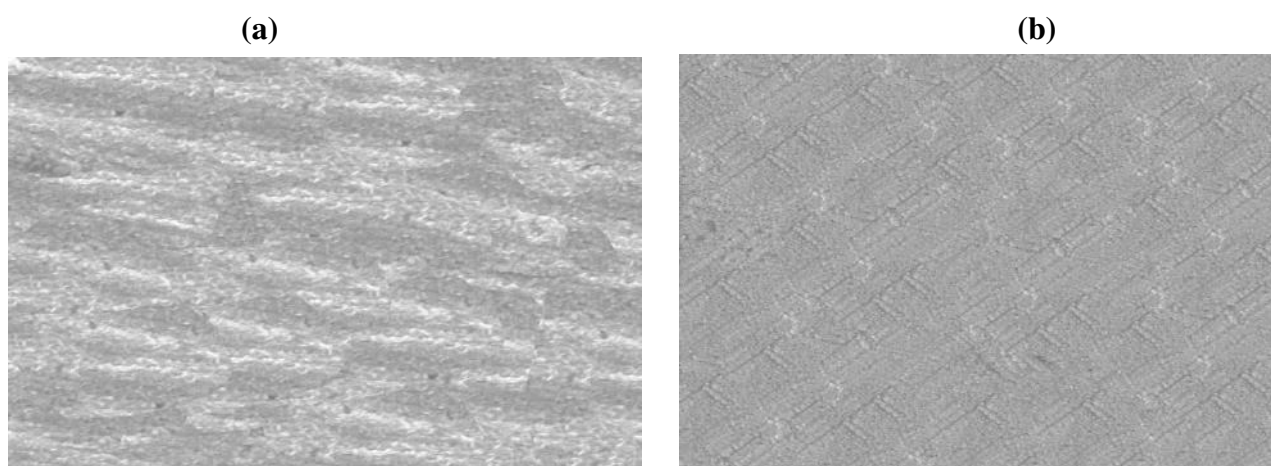


Figure 1. SEM images of thin films (a) NiW (30°C) (b) NiW (Annealed 200°C)

3.3 STRUCTURAL CHARACTERS

Structural characteristic (from XRD Data) results of deposited materials prepared with temperature 30°C and annealed thin film are shown in figure 2. From XRD pattern of NiW, crystal formation of deposits can be concluded. The size of crystals of can be determined by formula

$$\text{Crystal Size (D)} = (0.955 \lambda) / \beta \cos \theta$$

Where, β is FWHM at 2θ , λ is wavelength of incident light. The XRD results of NiW films have shown face centred cubic phase with three diffraction peaks. The nano crystallite deposits was obtained

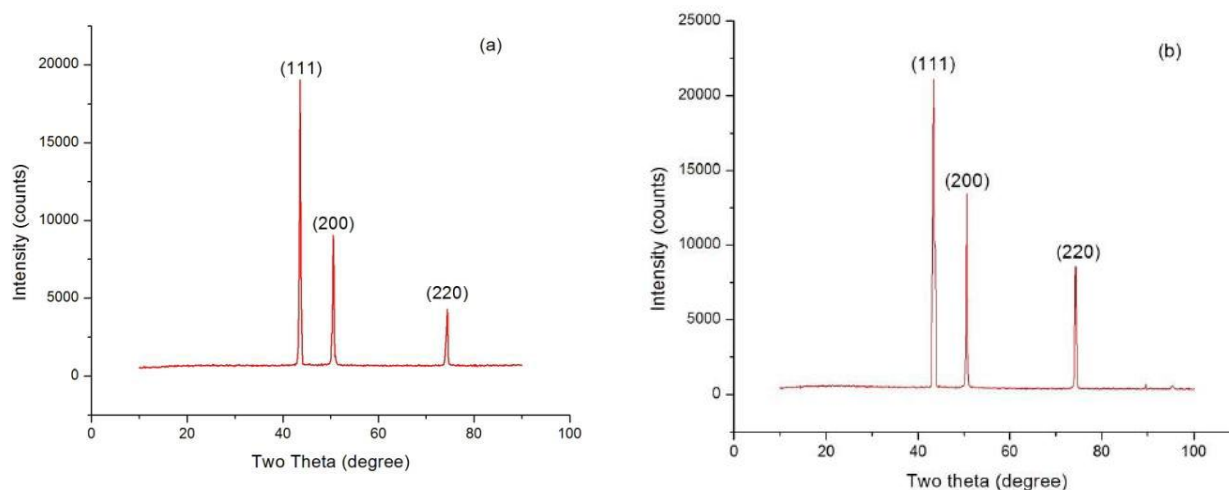


Figure.2. XRD patterns (a) NiW (30°C) (b) NiW (Annealed 200°C)

The crystallite sizes of NiW deposits are tabulated in table 2. Annealing process decreases the crystal size.

Table.2: NiW alloy films -Structural properties

S.No	Condition	2 θ (deg)	d (Å ⁰)	Particle Size(D) (nm)
1	NiW (30°C& without Adenine)	43.47	1.6012	95.67
2	NiW (Annealed 200°C)	42.74	1.6512	89.04

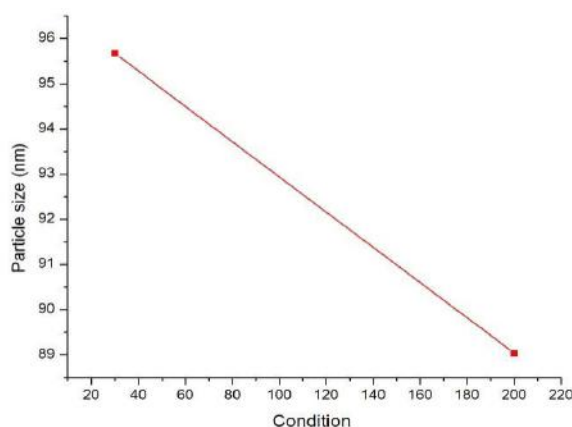


Figure.3. Particle size changes with condition

3.4 MECHANICAL PROPERTIES

Micro hardness measurement of deposits was done by Vickers hardness tester. The hardness values of thin films at room temperature 30°C and annealed thin film are shown in table 3. Annealing process increases the hardness, because of onset formation of crystal deposits during electro deposition process.

Table.3: NiW alloy films -Hardness

S.No	Condition	Hardness VHN)
1	NiW (30°C)	124
2	NiW (Annealed 200°C)	138

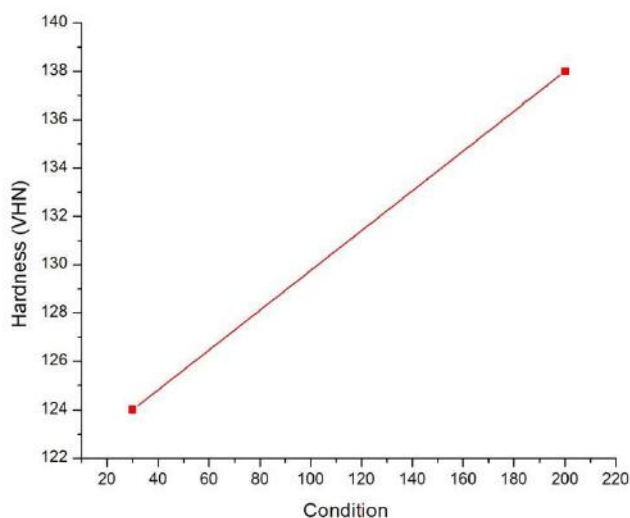


Figure.4. Hardness changes with condition

4. CONCLUSION :

An alloy thin films NiW has been prepared by electro deposition method. The characteristics of NiW films were observed. From EDAX result, tungsten increased and nickel decreased after annealing. The XRD results of NiW films have shown face centred cubic phase with three diffraction peaks. The thin films prepared with annealing process are bright and uniformly coated on the surface. They are crack free by appearance. The hardness values of thin films after annealing process increases .

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Education Data Mining: Future Prospects and Potential Benefits

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Abstract: *In the twenty-first century humans are engaged in many technologies to fit in the society. Nearly every day, humans use enormous amounts of data from a variety of sources. As the information is readily accessible in a variety of forms, it is feasible to take appropriate action with any of it. Not only must this data be analysed, but smart decisions should also be taken, and the data must be preserved. Data should be obtained from the database and should be used to make a better choice as and when desired by the client which is referred to as data mining or the Knowledge Discovery in Database. Students may now access their courses from any location via the internet and participate in learning activities. As a result of their learning management system activities, students generate a vast quantity of data that can be used to improve the learning environment and help them learn better. Students' learning and institutional effectiveness benefit greatly from data mining. More data can be obtained from educational institutions and queries may be run on it to make this strategy effective and broaden its reach. As a result, pupils' progress may be more effectively monitored.*

Key words: *Big Data, Educational Data Mining (EDM), Algorithm, Knowledge Discovery in Database (KDD), e-learning.*

1. INTRODUCTION:

In the twenty-first century humans are engaged in many technologies to fit in the society. Nearly every day, humans use enormous amounts of data from a variety of sources. There are many different ways to store data: papers, graphic formats, video, and audio recordings are just a few examples. Because the information is readily accessible in a variety of forms, it is feasible to take appropriate action with any of it. Not only must this data be analysed, but smart decisions should also be taken, and the data must be preserved. Data should be obtained from the database and used to make a better choice as and when desired by the client. In fact, this method is referred to as data mining or the Knowledge Discovery in Database (KDD). To a considerable extent, information technology's focus on finding meaningful information in massive data sets has shifted towards "Data Mining" since many believe that they are "data rich but information poor."

Analyzing internal assessments and end-of-semester evaluations may help enhance student performance in an educational institution. Internal evaluation includes teacher-led activities such as quizzes, seminars, attendance, and lab practicals. Students' communication skills and paper presentations from their academic days are also essential to study how to enhance student performance along with the internal evaluation.

Several new pedagogical advancements have been made as a consequence of education research. The number of learning settings based in the community has risen. Users engage in modern learning

environments through participating in online communities such as discussion forums, online chats, instant messaging clients, and other learning management system. Flipped Classroom is a recent learning style that heavily relies on online activities. To enhance the learning experience, a number of frameworks and models have been developed for online learning management systems. Low-cost cell phones, made possible by open source initiatives entering mobile computing, are becoming widely used. Students are now accessing educational materials on their smartphones. Students may now access their courses from any location via the internet and participate in learning activities. As a result of their learning management system activities, students generate a vast quantity of data that can be used to improve the learning environment and help them learn better. Aside from information gathered through student activities, educational institutions also develop their own data as they employ programmes to keep track of courses, classes, and students. Conventional data processing approaches can't handle the volume of data accessible in the aforementioned conditions. Traditional data processing programmes have their limits, thus educational institutions have begun researching "Big Data" solutions to handle educational data.

In order to comprehend how and why data mining works, you must first grasp a few basic ideas. To begin, classification, categorization, estimation, and visualization are all integral parts of data mining. Classification identifies and differentiates research topics by forming relationships and clusters within each group of subjects. To handle categorical outcomes like "persist" or "dropout" and "transfer" or "remain," categorization employs rule induction algorithms. Predictive functions or probability are part of estimation, and they deal with continuous outcome variables like a student's GPA or pay. Visualization is significantly more complicated than pie or bar charts, using dynamic graphs to show mathematically generated rules and scores. Higher education institutions may classify students, or estimate the probability of various outcomes, such as transferability, persistence, retention, and course achievement, for a full examination of student attributes.

2. LITERATURE REVIEW:

- Pandey and Pal studied the performance of 60 students from a degree college affiliated with Dr. R. M. L. Awadh University in Faizabad, India, as part of their research. To figure out whether a student is interested in language learning, they use the association rule.
- A study conducted by Khan on 400 students from Aligarh Muslim University's senior secondary school in Aligarh, India, examined the predictive value of various measures of cognition, personality, and demographic variables for success in the science stream at the higher secondary level. Of the 400 students, 200 were male and 200 were female. After dividing the population of interest into small groups called clusters, a random sample from each cluster was chosen and analysed further. This selection was made using cluster sampling approach. Girls from affluent families had greater academic accomplishment in science, whereas males from less affluent families had higher academic achievement overall.
- Bhardwaj and Pal studied the performance of 300 students from five different degree colleges in India who were enrolled for BCA programme at Dr. R. M. L. Awadh University. The study used Bayesian classification method on 17 attributes discovered a strong correlation between student academic performance and factors such as a student's grade in the senior secondary exam, residence, teaching medium, and mother's qualification, as well as other habits and family annual income.
- At Yarmouk University in Jordan in 2005, a decision tree model was used by Al-Radaid et al. to predict the final grade of C++ course students. Classification was performed using three distinct methods: ID3, C4.5, and the Naïve Bayes, with the results showing that the Decision Tree model had superior prediction than the other two.

3. APPLICATIONS IN EDUCATION:

There are a number of ways in which big data concepts may be used in learning analytics, as described below:

- **Prediction of Performance**

The performance of a student may be anticipated by looking at how the student interacts with the other students and instructors in a learning environment.

- **Detection of Attrition Risk**

The danger of students dropping out of courses may be foreseen and actions can be made at the beginning of the course to retain students by evaluating their behaviour.

- **Data Visualization**

Educational data reports get more complicated as the amount of data increases. To detect patterns and relationships in the data, visualization methods may be used to represent it.

- **Feedback that is both intelligent and actionable**

Learners will be able to participate and perform better if learning systems deliver intelligent and rapid feedback in response to their input.

- **Recommendation of Courses/Programs**

Students' actions may be analysed to identify their interests, and thus new courses can be proposed to them. This approach won't stray students into majoring in something they ultimately don't prefer.

- **Estimation of student skills**

Estimation of the student's acquired level of skill

- **Observation of Conduct**

Monitoring student conduct in community-based activities or games that contribute to the development of a student model

- **Organizing pupils into groups and encouraging teamwork**

- **Analysis of social networks**

- **Creating concept maps**

- **Putting together instructional resources**

- **Preparation for the future and setting goals**

4. PERFORMANCE IMPROVEMENT IN EDUCATION SECTOR:

- ❖ **Data Mining In Education**

Students and educational institutions both get benefited from using data mining methods for the purpose of discovering new information. Data based on expert knowledge aids the educational decision-making process. Through the use of educational data mining, we can improve our knowledge of how students learn by identifying educational trends such as better course selection and in-house training. Students' academic performance has been linked to things including their mother's qualifications and their family's wealth according to a study that used linear regression analysis. In order to enhance retention, educational improvement, and learning outcomes for students, data mining approaches can be used. To find patterns and relationships in massive amounts of data, data mining methods are utilised. These approaches assist to make better decisions by uncovering previously unknown information. They argue that data mining software should be built such that it enables users to evaluate data from many perspectives, to classify the information, and to summarise the outcomes of the analysis. Data mining may be used in both on-campus and online learning. An extensive set of algorithms, filters and visualisation approaches are available as part of the basic data-mining toolset. DB Miner, Clementine, Intelligent Miner, Rapid Miner, and Weka are some examples of data mining tools. Student attitudes about educational patterns or trends, interest in technology and teaching methods are studied via the use of questionnaires and feedback forms. The obtained data is processed using techniques such as decision trees, neural networks, and so on. Mining models include Decision Trees, Naive Bayes, Support Vector Machines, Linear Regressions, Minimum

Description Lengths, K-means, and O-Clusters, to name just a few. These models may provide information on student behaviour patterns, course behaviour patterns, retention predictions, course suitability predictions, and a personalised intervention strategy.

- **Visualization of Statistics**

Tsantis & Castellani claim that student log history and data may be useful in evaluating an e-learning system since they provide insight into how students are using the system. Web-based educational systems capture student data like his maximal interest in particular technologies or interest in answering surveys, etc. by using information visualisation methods. Techniques for visualising interactions in online groups, social networking sites, and so on are used. In addition, teachers who can alter the produced graphical representations and capture the attention of their learners might be benefitted from these strategies.

- ❖ **Web-Based Data Mining**

"Web mining is used to extract relevant information from web data," as suggested by Srivastava et al. Web mining is a method for extracting usable information from the contents of web pages, while web use mining is another approach for detecting relevant patterns in data created by client-server activities on one or more web locations.

- **Clustering, classification, and the identification of outliers**

Classification techniques include clustering and classification. Clustering is unsupervised, whereas classification is supervised. Classification and prediction are two strategies that are closely connected. Outliers are observations that are exceptionally big or tiny in comparison to the other values in a dataset. Classification predicts class labels, whereas prediction predicts continuous-valued functions. The Liu decision tree, also known as the C5.0 algorithm, and data cube technologies are utilised to manage classroom procedures, according to Chen. Induction analysis aids in the identification of possible student groups with shared traits. Talavera and Gaudioso suggest utilising clustering to mine student data in order to find patterns that represent user behaviour.

- **Adaptive and intelligent web-based educational systems**

According to Tang et al. web-based educational systems are adaptive and intelligent which. Introduce the notion of data clustering for web-based learning and to assist in the resolution of learner-based challenges. Based on the chronology and content of the sites they viewed, they identify groups of students who have similar learning characteristics.

- ❖ **Association Rule Mining**

Association rule mining is a common mining approach for finding relationships between groups of objects in huge datasets. IF-THEN statements are used to link one or more dataset characteristics with one another.

- **Web-based courses in particular:**

Ha et al. examine the navigational structure of webpages accessed by learners from web-based virtual classrooms, e-learning portals, and web pages.

- **Web-based educational systems that are adaptive and intelligent:**

In a tailored e-learning content recommender system, Lu employs association fuzzy rules. To uncover links between a student's needs and a list of learning resources, fuzzy matching criteria are utilised. Romero et al. propose to provide feedback to writers who built courses and deduced associations from student use data using grammar-based genetic programming combined with optimization approaches.

- ❖ **Text mining**

Text mining is similar to online content mining in that it utilises text data as a source for the mining process. Machine learning and data mining are only a few of the topics covered in this multidisciplinary field, which also includes statistics, information retrieval, and natural language processing. Datasets such as full-text documents, HTML files, emails, and so on may be mined using text mining.

❖ Web-based educational systems

For shared learning, data mining and text mining technologies are utilised in Web-based educational systems. For broader communication analysis, text mining is utilised on a discussion board. Learners choose the appropriate category for his or her opinion, and the system assigns peer ratings to each learner's contribution.

• Well-known learning content management systems

Dringus and Ellis suggest that text mining be used to evaluate interactions in ad hoc discussion boards. Text mining algorithms may also be used to assess how far a thread or user group debate has progressed. Data may be extracted from pdf interactive multimedia compositions to aid in the statistical assessment of multimedia presentations and the extraction of pertinent data. Online-based educational systems capture a vast quantity of data from students' web log histories, which may then be analysed to find useful trends.

• Adaptive and intelligent web-based educational systems

Tang et al. suggest creating a tailored web-based application that allows for mining of the courseware's foundation and structure. Articles for remote learning students are chosen using keyword-driven text mining algorithms.

5. EDUCATIONAL DATA MINING: CHALLENGES :

In this section, some of the significant research problems that was highlighted in three workshops will be illustrated. The research problems are divided into five categories:

- Improving data mining methods' scalability
- Non-vector data mining
- Data mining on a large scale.
- Making data mining methods and environments more user-friendly.
- Data mining privacy and security concerns.

Improving data mining methods' scalability

The majority of data mining methods now days presume that the data is small enough to fit in memory. Although enormous data sets are often stated to be successful, this is mainly the consequence of sampling large data sets until they fit in memory. Scaling data mining algorithms is a significant difficulty:

- a) There is an increase in the number of recordings or observations.
- b) As the number of characteristics per observation grows, so does the number of attributes per observation.
- c) As the number of predictive models or rule sets utilised to evaluate a set of data grows, so does the complexity of the analysis.
- d) As interactivity and real-time response become more prominent.

Not only must distributed, parallel, and out-of-memory versions of existing data mining methods be invented, but also whole new algorithms. For example, today's association algorithms can examine out-of-memory data in one or two passes, needing just the storage of some auxiliary data.

Extending data mining algorithms to new data types.

The vast majority of data mining algorithms now use vector-valued inputs to do their calculations. Expanding data mining algorithms to operate with different kinds of data is a significant task.

- a) Process and time-series data.
- b) Data that is not organised, such as unstructured text.
- c) Documents in HTML or XML format that are semi-structured.
- d) Data derived via multimedia and group collaboration.
- e) Data that is organised hierarchically and spans many scales.
- f) Collection-valued data.

Creating algorithms for distributed data mining (DDM).

The majority of today's data mining techniques call for consolidating all data to be mined into a single data warehouse. A major problem is to build distributed versions of data mining algorithms that allow data mining to be performed while still preserving part of the original data. To manage the meta-data and mappings necessary for mining dispersed data, proper protocols, languages, and network services are also essential. In order to mine the data generated by wireless and ubiquitous computing environments, new methods and systems must be created.

- **Ease of Use**

Today's data mining is a semi-automated process at best, and that may be the case in the future as well. While on the other hand, creating data mining tools that even novice users can utilise is a significant problem. Improving the user interface is one relevant method. Other relevant strategies include facilitating casual browsing and visualising large, dispersed data sets, establishing systems to manage meta-data, and creating user-friendly data mining languages and protocols. Another important fundamental challenge is the development of data mining and knowledge discovery environments that address the collection, processing, mining, and visualisation processes, as well as the collaborative and reporting aspects required when working with data and information derived from them.

- **Integrity and Security.**

Useful information may be gleaned from large amounts of data by using data mining techniques. The misuse of data mining is becoming more and more likely as digital data becomes more widely accessible. A major problem is to build next-generation data mining systems from the ground using privacy and security models and protocols suited for data mining.

7. TRENDS THAT EFFECT DATA MINING:

This section discusses five external factors that will have a major influence on data mining in the next years.

Trends in the Data: The growth of digital data over the last two decades is perhaps the most important external development. Over this time span, the volume of data has expanded by six to 10 times. Networks make a lot of this data readily available. Contrarily, the number of researchers and analysts who have had access to this data has grown steadily during the same time period. Statistics, for example, has seen a very stable number of new Ph.D.s graduate each year. You have two choices: either much of your information will be read-only in the future or tools like data mining will need to be created to help you analyse your information more efficiently, remove unnecessary data, and extract useful insights.

Trends in hardware and software: Quantitative and statistical analysis of big data sets is required for data mining. Increasing workstation memory and processing speed make it possible to mine data sets that were too huge to mine using existing methods and methodologies. SMP workstations and high performance workstation clusters have commoditized high performance computing, making it possible to address data mining challenges that were previously only accessible by employing the world's greatest supercomputers.

Network Trends: Sites will be linked at OC-3 (155 MBits/sec) speeds or faster with the next generation internet (NGI). This is a 100-fold increase in speed over present networks. Using current methods and methodologies, it is now feasible to correlate remote data sets with this form of connectedness. New protocols, algorithms, and languages are also being created to make it easier to mine dispersed data on today's and tomorrow's networks, respectively.

Trends in Scientific Computing: Simulation is now considered a third form of research by scientists and engineers, as previously noted. For experiments or simulations that produce enormous data sets, data mining and knowledge discovery play a critical role in connecting the three modalities of science: theory, experiment, and simulation.

Business Trends: It is more important than ever before for organisations to be more lucrative while also reacting quickly and providing higher-quality services, all while employing fewer people and

spending less money. Because of these demands and limitations, data mining becomes a critical corporate tool for identifying opportunities and minimising risks associated with consumers and their transactions.

8. CONCLUSION:

There are various well-established fields of study such as e-learning, web mining, text mining, etc. that are relevant to Educational Data Mining. Educational data is analysed using Data Mining techniques to extract relevant information from the massive amounts of data that are collected. This study reviews KDD and fundamental data-mining methods in order to combine research in this field. Methods and approaches for making data relevant are developed in the KDD area. Data Mining Strategies may be used in the educational sector to produce software and visualisation techniques that not only forecast student success in exams but also assist us to group those students who need more attention in their studies. Students' learning and institutional effectiveness benefit greatly from data mining. More data can be obtained from educational institutions and queries may be run on it to make this strategy effective and broaden its reach. As a result, pupils' progress may be more effectively monitored. This overview of Data Mining's Knowledge Discovery viewpoint can assist researchers discover patterns in educational data sets.

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Heart Rate Measurement from Fingertip Using Microcontroller

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Abstract: Heart rate is a very vital health parameter that is directly related to the soundness of the human cardiovascular system. It is a physiological parameter that is measured in a wide variety of situations. Thus by determining the health status and fitness of a person, it will help us to give a measure of energy expenditure of an individual. Conventional systems comprise of a belt worn around a user's chest, carrying a heartbeat sensor and a radio transmitting measured data to a wrist-worn display unit. Thus, the existing system makes use of hardware that can be complex, costly and inaccurate. Our project aims at making a Heart rate for the person to measure the heart rate without specially visiting the doctor at home at one time investment. This will save time and money at a great extent and is very useful in emergency. The heart rate of a healthy adult at rest is around 72 beats per minute (bpm). Heart rate measurement is an important diagnostic tool because it reflects our state of mind and physical condition. So, we develop a project which will be an indication of how healthy our body is, by measuring and monitoring the heartbeat. Our project presented here uses a fingertip sensor that monitors the heartbeat and displays its value on the LCD connected to the microcontroller.

Key Words: Heart Rate Measurement (HRM), Wollega University, Micro C PRO, "Heart Rate Measurement using Microcontroller"

1. INTRODUCTION:

Background of the Project

Heart rate measurement indicates the soundness of the human cardiovascular system. Our project demonstrates a technique to measure the heart rate by sensing the change in blood volume in a finger artery while the heart is pumping the blood. It consists of an infrared LED that transmits an IR signal through the fingertip of the subject, a part of which is reflected by the blood cells. The changing blood volume with heartbeat results in a train of pulses at the output of the photo diode, the reflected signal is detected by a photo diode sensor, the magnitude of which is too small to be detected directly by a microcontroller. Therefore, a two-stage high gain, active low pass filter is designed using two Operational Amplifiers (OpAmps) to filter and amplify the signal to appropriate voltage level so that the pulses can be counted by a microcontroller. Current medical techniques for monitoring the heart rate and other vital signs use electrodes attached to the body, which are impractical for patients who want to move around. These techniques pose a problem especially in the rural areas where we do not have the required infrastructure and commuting to the nearest hospital or primary health center is a daunting task. Keeping in mind the problems faced by people, we have designed a system which is portable, can be effectively and efficiently used without the need of a doctor in close proximity. It also saves the valuable time of both the patient and the doctor. our project has been divided into various chapters giving a detailed description of the project components and also for a better understanding of the project's working. Towards the end we have drawn a conclusion to this

project giving its future scope. Additional information related to the project has been given in the appendices. I have also mentioned the references from where we got help for completion of our project.

2. Statement of the Problem

The human health is one of the most important concerns in the world today. People nowadays have no health awareness. Besides that, the person cannot update their health condition continuously to the doctor. The problem of patients dying is associated with cardiac arrests. Current medical technique for monitoring the heart rate uses electrodes attached to the body, which is impossible for patients who want to move around even to move around and even to afford the treatment. The heart is a very delicate organ in the human body (once it stops beating, nothing else matters). Thus, If early actions are taken (and on time) the heart condition can be managed effectively and many patients can be cured and saved. The problem of patients slumping and dying is associated with cardiovascular arrests and can be checkmated this sensitive and highly effective device. This device has an outstanding advantage that it is easy to handle. Heartbeat monitors are part of the most vital tools needed in first aid kit for saving lives. Unlike the x-ray, the heartbeat monitor does not impose any hazard to the human health. There are devices in the market which can provide raw measurement data of the patients to the doctors, but the patients may not be able to interpret the medical measurement into a meaningful diagnosis due to their limited medical background. On the other hand, if raw medical data is delivered to the doctor, time is wasted and may pose a problem, but in emergencies waste of time can never be tolerated. Most of the products available in the market have this drawback of limitation in flexibility and portability.

3. Objectives :

General Objective

- The project general objective was Heart Rate Measurement from Finger Tip Using Microcontroller.

Specific Objectives

- To help the family members to keep track of the health condition of their loved ones by knowing there result of: Heart beat in their day to day life
- In the case of an abnormality in the health condition for those with heartbeat defects, means beyond the rated normal heart rate and below the system gives on time result and alarms

Wireless Heart Rate Tele monitor for Home Care

The limitations are: It use only for home and it is not mobile

Sustainable Wireless Sensor Device for Heartbeat Monitoring With Energy Harvester

More energy is realized, it needs energy harvesting and energy is generated by vibration and human motion and Viruses can attack the system.

Our project work is used for every user to check their heart rate and they can check whether the heart rate is normal or not, if person heart rate is not normal they must go to clinic and check by doctor. The previous system use electrodes attached to the body, which are impractical for patients who want to move around but our system removes this type of method. The person can move anywhere and measure its heart rate.

4. Design and Methodology:

The methodology employed and consideration takes into account for this project. It begins with the discussion of the project methodology, followed by system design procedure, techniques and tools utilized in this work.

Start, Problem Statement, Literature Review, Overall the Study of the System, Data Collection and Analysis, Simulation and Testing, Conclusion and Recommendation.

Flow Chart of the Heart Rate Measurement from Finger Using Microcontroller

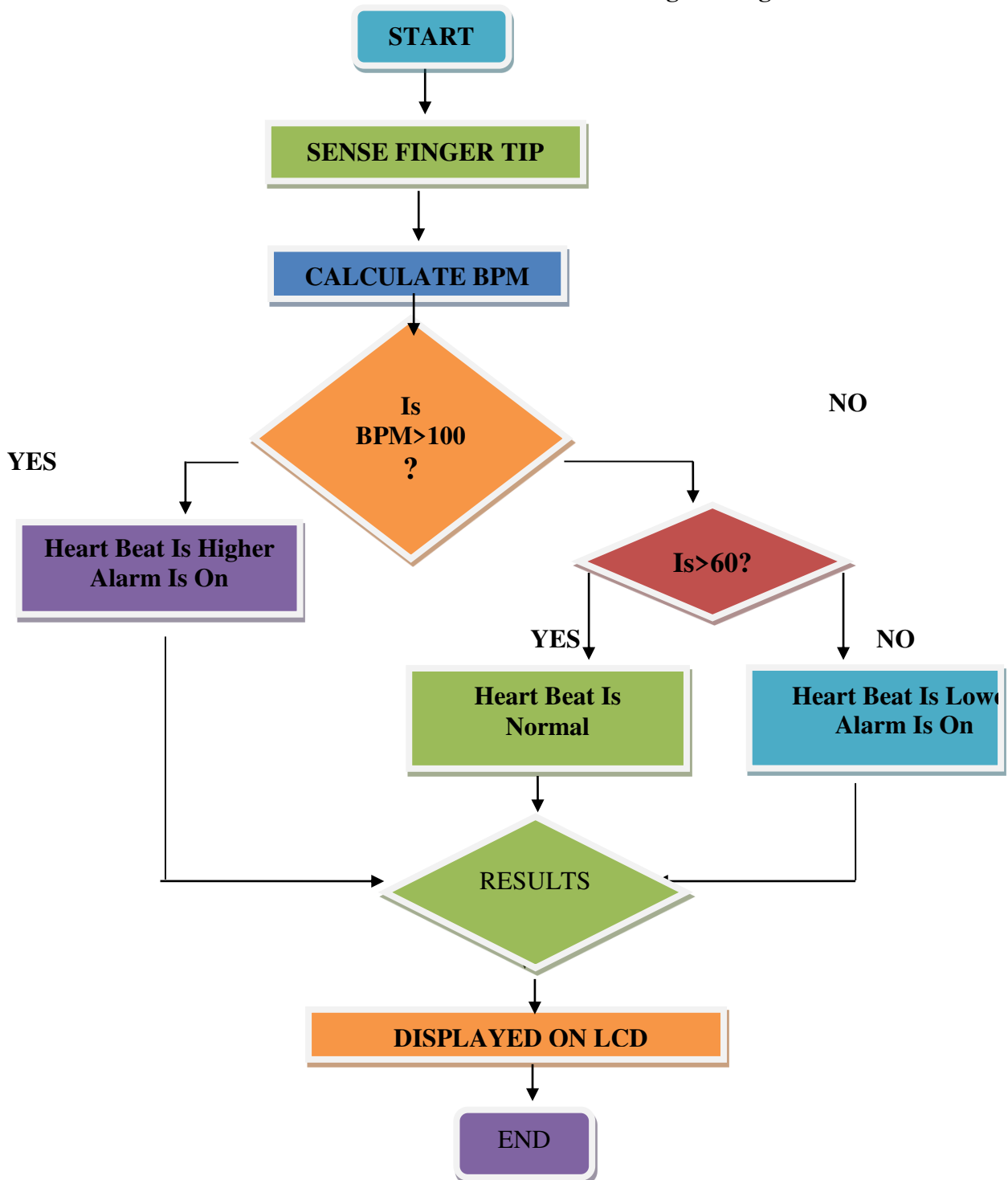


Fig 1 flow chart of heart rate measurement from finger using microcontroller

Algorithm for Flow Chart Implementation

Step 1: Start, Step 2: sense fingertip for 15secs, Step 3: the pulse out from Op Amp is counted using timer in pic microcontroller, Step 4: Final result displayed on LCD and Step 5: End.

System Description

Heart rate measurement indicates the soundness of the human cardiovascular system. This project demonstrates a technique to measure the heart rate by sensing the change in blood volume in a finger artery while the heart is pumping the blood. It consists of an infrared LED that transmits an IR signal through the fingertip of the subject, a part of which is reflected by the blood cells. The reflected signal is detected by a photo diode sensor. The changing blood volume with heartbeat results in a train of pulses at the output of the photo diode, the magnitude of which is too small to be detected directly by a microcontroller. Therefore, a two-stage high gain, active low pass filter is designed using two Operational Amplifiers (Op Amps) to filter and amplify the signal to appropriate voltage level so that the pulses can be counted by a microcontroller. The heart rate is displayed on a liquid crystal display (LCD). The microcontroller used in this project is PIC16F877A.

System Design

Block diagram of heart rate measurement from fingertip using microcontroller.

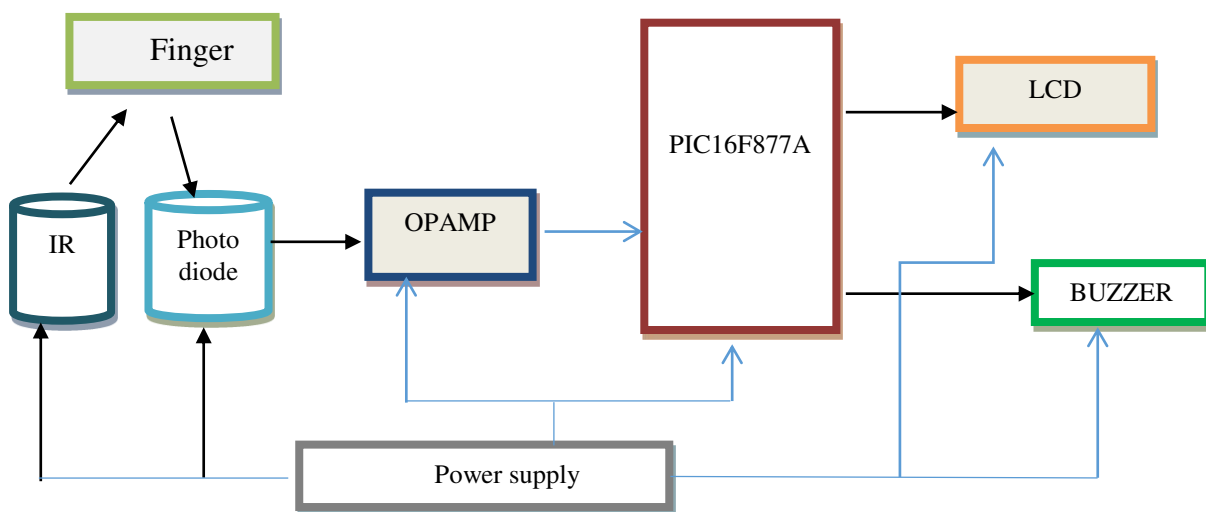


Fig: 2 block diagram of the heart rate measurement from fingertip using microcontroller

Simulation result and discussion

The program I used in this project is written in MicroC PRO for PIC. All the codes are tested and simulated using proteus ISIS-Professional. The external timer we have used is a crystal Oscillator and generate 10MHz.

Software Testing Implementation

The code is written in C language with Mikro C compiler and loaded with Mikroprog suit for PIC kit on the microcontroller, for the simulation purpose we use proteus software to test the system ideally, and to see the simulation we use pulse generator as fingertip. first amplifier circuit amplify the input pulse and give to the next op-amp to filtering purpose and then give to the microcontroller the microcontroller have timer/counter in the internal module to count all received pulse from external calculate bpm (beat per minute) and display the result on the LCD display.

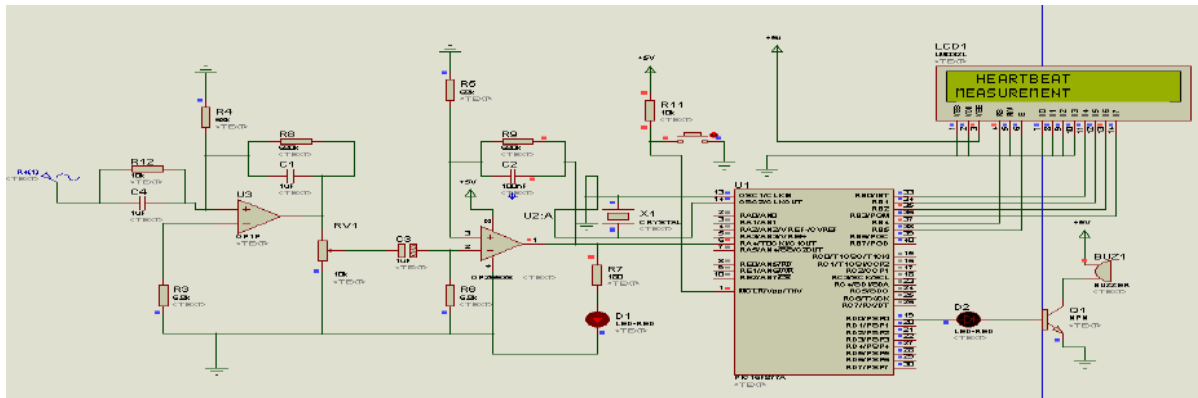


Fig 3 Circuit diagram of heart rate measurement using microcontroller

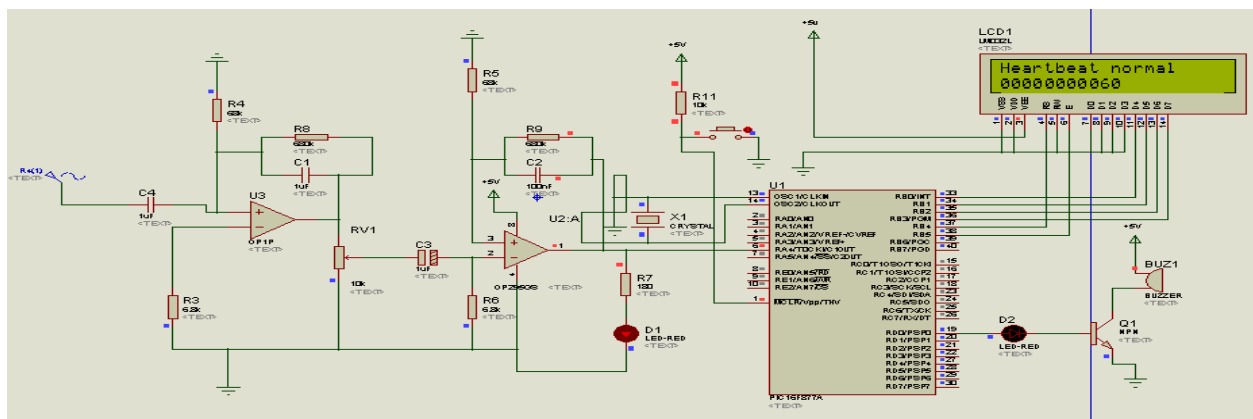


Fig 4 when heartbeat is normal
 Heartbeat is normal [Pulserate \geq 60and $<$ 100]

As shown on the LCD display the person heart beat is normal this implies it is at rest does not perform any activity. Also the led beside transistor does not glow due to heart beat is at rest and the alarm is off.

The Figure 4.1 below shows the overall software design configuration on Proteus System design

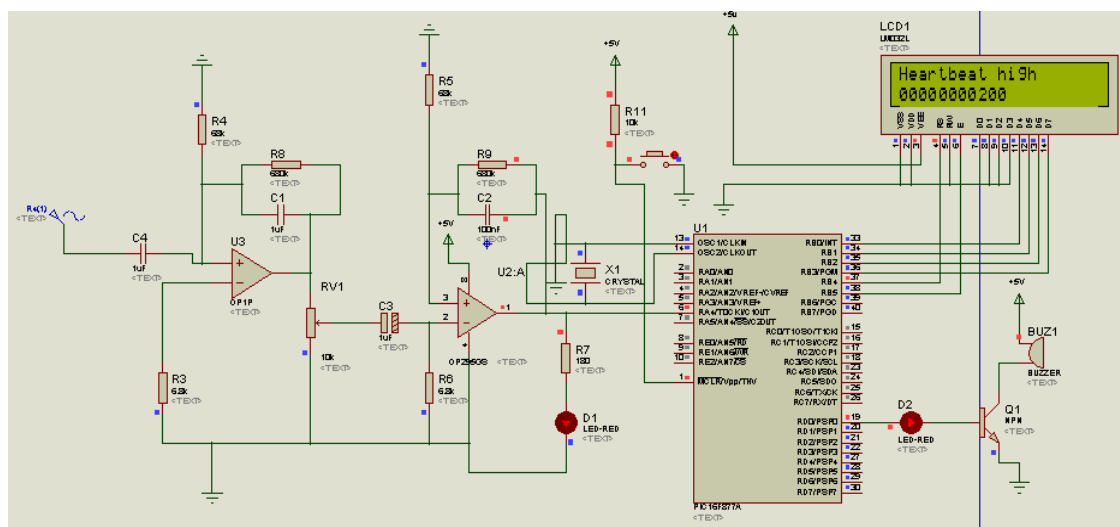


Fig 4.1 when heart beat high

Heart beat is high [Pulse rate>100]

As we see from the simulation result of above fig the heartbeat of the person is high due pulse high. Thus both led glow and alarm is on.

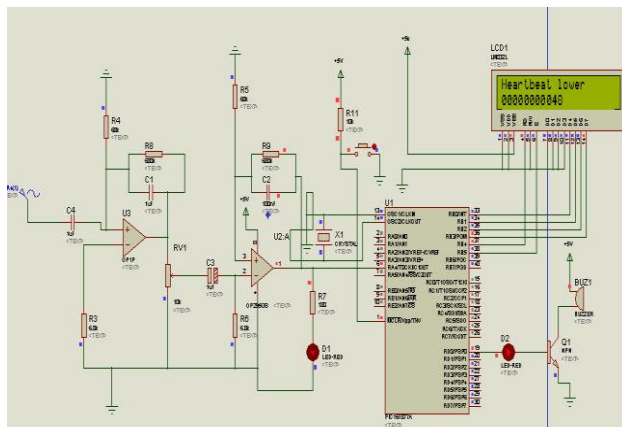


Fig 4.2 when heart beat is lower
Heart beat low (pulse is <60)

As we see from the above fig the heart beat is low so led glow and alarm is on.

Conclusions and Recommendation

5. Conclusions :

In this project, a microcontroller based Medicare device for heart rate measurement has been presented. The system allows health personal to monitor heart rate without requiring the physicians. The different group of people such as aged, illiterate and children benefited from the device. The device is efficient and easy to use. Could be used in clinical and nonclinical environments. It can also be easily used by individual users, e.g. athletes during sporting activities. Lists of accomplishments include:

Adequately acquiring signal due to the change in the volume of blood flow through the finger.

Functional heart rate counter

Functional notification and alarm system.

LCD heart rate display

Use of low power components for battery operation

6. Recommendation :

This thesis focus on the analysis of heart rate measurement from fingertip using microcontroller is better than conventional health monitoring that has a many advantage. However, there is improvement that can be made to the firmware portion of the project that would result in more reliable system. Looking ahead, as microcontrollers get more and more advanced, there will be shift from analog amplification to digital amplification. This will significantly reduce the surface area consumed by a circuit and would lead to a smaller, more compact and portable system.

More can be done in process leading to accusation of these small signals. There are many challenges that still pose big problems in design of the systems like this. For future work the device further improved by addition of monitoring device that used to detect heart beat abnormalities of physically challenged individuals without hands. The device can be reconfigured and interfaced with the GSM modem and Bluetooth technology.

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Analysis of Frequently Failed Transmission Lines in India and Innovative Solutions for the Better Operation and Maintenance of the Towers

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Abstract: Supply of continuous power will be the most important aspect of a transmission line network during emergency time. Many Transmission Lines in India are constructed long time back. So, they are having old aging transmission system life and aging equipment which leads to higher probabilities of failure, higher maintenance cost and higher replacement cost. So, aging equipment needs to be replaced and this replacement should be planned in coordination with capacity additions in any. As the demand for power is increased and various technological development has been tested and approved for better equipments to be selected with reference to the geographical location and weather condition of the line routes. Nowadays, many high voltage transmission lines have been developed worldwide and are successfully being operated in developed nations.

In Indian transmission scenario are progressing towards establishing 765 KV lines to strengthen in recent trends its transmission infrastructure. Massive expansion of the inter-state transmission system is under way to provide to the transmission requirement of new generation projects. Transmission line failures occur due to harsh weather and non-climatic reasons such as human errors and mechanical failures. While it is impossible to prevent all such situations, electrical utility officials and contractors must be equipped to meet such emergencies. Emergency Restoration System Towers were a temporary solution designed to bypass the existing transmission towers of any voltage in any terrain. They will be used until the main line is re-conducted or restored. Planning includes determining which transmission lines are important and the possible ways in which they can fail and how best to restore them. This includes general information about existing structures and foundations, data on past weather related failures, weather criteria and structural loading and the extent of damages. To compound this difficult situation, large amounts of inventory were required in order to have spare lattice towers available for emergency restoration work. In India, Power Grid Corporation of India inherited more than 100 different types of designs of transmission suspension towers and transmission tension or dead-end towers. In this paper, it has been analysed for various reasons for frequent failure of transmission lines in various parts of India. Also, analysed various necessity for the transmission line utilities to take precautionary actions to avoid and reduce these disruptions. Then discussed about various recent innovation technologies and transmission components which can be used for the better operation and maintenance of the transmission lines in Indian conditions and standards adopted.

Key Words: Transmission Line, TL Failures, Operation and Maintenance, Innovative Technology.

1. INTRODUCTION:

All the high voltage transmission lines are exposed to a certain level of damage risk, threatening to disrupt or impair critical power supply infrastructure and affect public safety. Utilities cannot avoid these emergencies, therefore, it is important to know their cause and quickly, efficiently respond to the problem on timely manner. Emergency situations may include failure of structural systems and foundations, failure of conductor splices and damage to conductor strands leading to conductor failure, etc. Transmission line tower damage may also occur due to degradation of conductors due to wild fires, flood, insulator breakage, failure of cross-arms and buckling of tower angle members or connection failures.

Transmission line maintenance is a frequent process and involves cleaning insulators, the elements located at the top of the tower which hold the cables that transport power (conductors). In order to get rid them of dust and other particles suspended in the air that build up, operators spray them with pressurized demineralized water. To do so, they have got to climb the 35- to 50-meter-high tower structure beforehand or go up in a crane to place themselves in front of them. This manoeuvre sometimes also entails replacing broken components with new parts.

2. Objectives:

High voltage transmission lines carry electrical energy from power generating stations to long distances and reaches substations. These transmission lines are passing through a wide range of terrains, climates and physical environments and are always at risk to experience catastrophic events, both weather-related and man-made. Weather-related events include high winds, ice buildup, ice storms, flash floods, rock or mud slides, erosion of foundations, etc. Sudden power failures caused by nature are very common, however, non-weather related events are just as likely. These events include human error, vehicle accidents, vandalism, terrorism, design faults and poor maintenance practice.

3. Methodology:

Due to the vast expanse of India and the variation of the weather conditions, Power Grid Corporation of India is subjected to transmission lines that occasionally fail due to natural disasters. These natural disasters are a result of mudslides, heavy ice, cyclones and floods. Traditionally, restoration of these transmission lines was completed using existing spare of normal towers. The typical self supporting lattice type towers used in India were erected using derricks and in some cases a complete new foundations has to be constructed. These processes are very time consuming and often resulted in prolonged outages. In many of the sites of these damaged transmission structures were difficult to access physically. The loss of these towers would sometimes result in backing down the generation and load shedding at industrial area in India. When major natural disasters occurred, it was calculated that lost Gross Domestic Product for India could be eighteen times the value of the power that was lost.

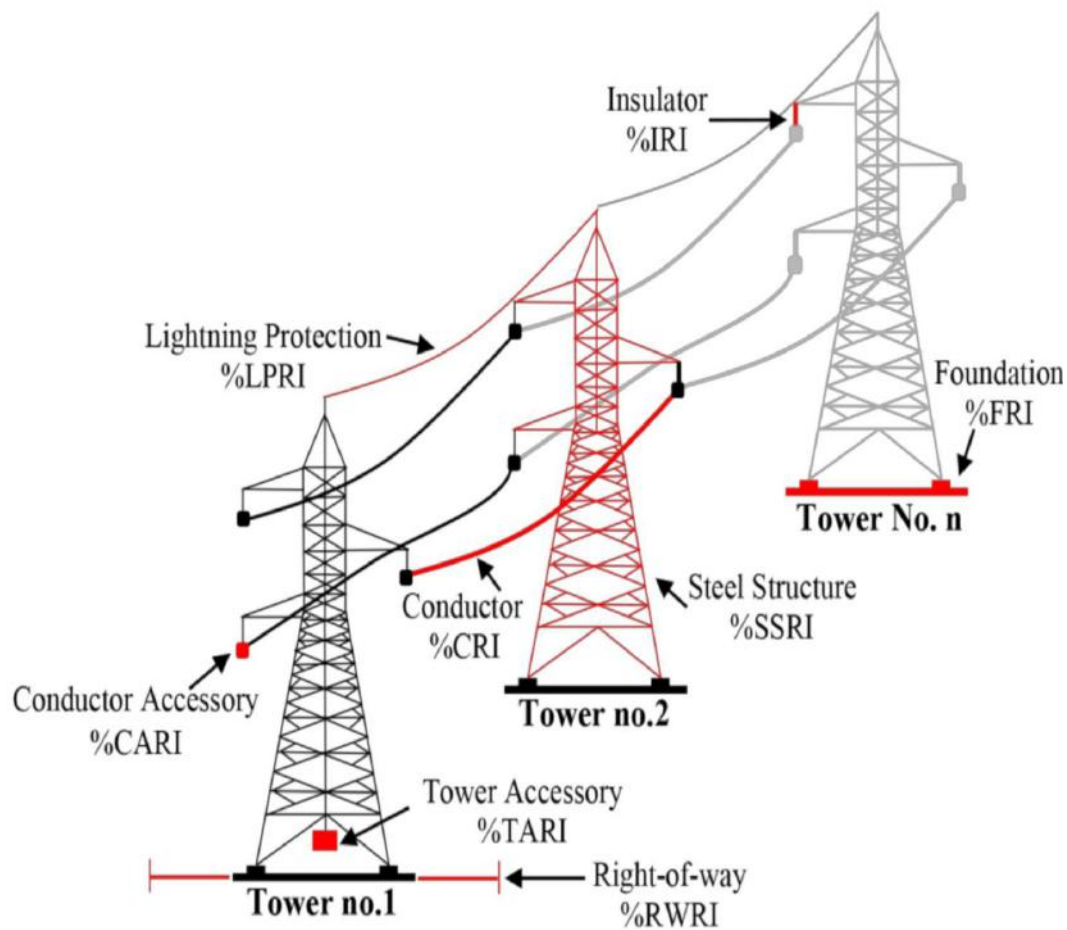


Figure 1: Various Transmission Line Components for maintenance

Transmission line inspections are carried out either by walking in the terrain or from the airborne every one to three years. The following issues, among others, are observed in the inspections as

1. Vegetation in the transmission line area, new structures, excavations
2. Possible inclination of tower structures, condition of steel parts, birds' nests, woodpecker holes
3. Integrity and condition of insulators and conductors.

Special inspections are also carried out whenever necessary, such as decay damage in towers, earthing, insulators and couplings.

4. Analysis and Discussion:

When a high voltage transmission line goes down with one or several damaged towers, the responsible utility agency incurs huge monetary losses and hundreds of non-transmission hours. Given that the total losses and damages are directly proportional to the outage duration, time is a crucial factor in reinstating or remediating the failed towers. In some cases, the process of formally rebuilding a new line with original towers can take as long as 5 to 6 weeks.



Figure 2: Typical Transmission Line failure in many places

However, by using an effective Emergency Restoration Plan, the failed transmission structures can be replaced in a few hours depending on the nature and depth of the damage. Proper planning benefits not only for maximizes restoration efficiency but can also minimize inventory levels.

An effective Emergency Restoration Plan contains three essential elements like Planning, Emergency Materials and Training. To perform any quick emergency restoration work, critical materials must be readily available for installation. The inventory must include standard wire sizes in sufficient quantities as may required more. If the line consisted of poles, then the inventory stock must have a reasonable number of poles of similar size and strength of the same line. Structures used for restoration work can be modular, temporary or permanent type.

An important role of any restoration is the training of field workers in the erection of the replacement structures, stringing and guying operations. A prompt mobilization of the trained work force is vital to the process of emergency restoration.

In the real application of the tilt monitoring system, by establishing and verifying various theoretical calculation models among environmental information such as the inclination angle of the transmission line conductor, the inclination angle of the insulator tower, the ambient temperature, humidity and the wind direction, the inclination of the tower is given to improve the system and the accuracy of the calculation.



Figure 3: Maintenance of Tower components in using tilt monitoring system

The restoration process consisting of simple design with standard weather criteria for the re-route, which required towers and other materials shifted to the affected site.

Normally, a double circuit line will be transferred to three sets of ERS towers with two towers in each set thereby forming a double circuit. Surveying and transportation of huge materials to site is a major challenge. The real challenge will be installing the anchors as we can see at the time of the standard towers. If the soil is too soft that it was not suitable for installing any type of anchors. Modular supports were specially designed by considering the guy angles and transportation to the site easily. In ERS towers, the long modular steel supports offer the large width, which is necessary for both connecting the guy wires and also increasing the stability of the ERS structure installed.

One of the noteworthy features of ERS structures installation are easy and quick. The ERS structures does not require any foundation and can be set up on any soil type. The base of the tower contains a four meter box upon which strong metallic angles can be built. These towers are light in weight and can be transported easily using helicopters or manually.

5. Challenges and Innovative Solutions :

Traditional power transmission tower maintenance mainly depends on regular inspections and human observations, which are very necessary safety protection methods. However, these methods have certain subjectivity and some parameters are difficult to measure by manual method. It is not easy to find problems in real time and cannot meet the constraints of real-time monitoring of the tower.

The restoration process consisted of the determining the cause of failure as due to the galloping, replacing the existing hardware fittings and adding more vibration dampers to the conductors to increase the damping.

The three restoration activities discussed above highlight the importance of utilizing obtainable inventory, creating quick construction methods, deployment of temporary structures and why conformance to design protocols is critical to avoid hardware failures.



Figure 4: Maintenance using Innovative Technology

Even after carrying out condition monitoring and preventive maintenance, the break-down of transmission lines cannot be ignored, mainly because of natural calamities. Restoration of damaged and collapsed transmission line tower can take a very long time which can vary from three to four weeks depending on site conditions, availability of spare towers, requirement of pile foundation etc. Because of the Power Grid has been deployed the IEEE Standard 1070 Emergency Restoration System for restoration of failed towers. Manual erection of the ERS tower takes minimum of three to four days when restoring a collapsed tower. When hydraulic cranes or helicopters can be deployed, the restoration work can be done in 2-3 days time.

Since acquiring the emergency restoration systems, Power Grid has used these systems many times for either emergency restoration on transmission line or maintenance activities. The results to date have met all of Power Grid's expectations. As a result of Power Grid's preplanning for potential emergencies on their regional grids, major savings have been achieved by both the Power Grid and by the economy of India. From the experience to date, Power Grid has modified their emergency restoration program by keeping emergency restoration materials which have been made more decentralized throughout India in their regional offices in order to reduce the deployment time.

6. CONCLUSION:

In all these cases, the utility demand is to restore the power system to normalcy rapidly. The primary concern in Emergency Restoration is to revive the transmission network, get the line back into normal operation as quickly as possible and to restore electricity supply to the affected consumers at the earliest. Another important concern is to minimize the economic impact of the costs associated with the devastation and rebuilding. While total the cost to rebuild or restore a failed transmission line is inversely proportional to the restoration time, the total losses are directly proportional to the outage time. Therefore, one can view any Emergency Restoration Plan as a combination of technical or engineering processes with the financial planning.

Use of composite insulated cross arm will reduce the cross-arm load on the structure and also the clearance required for insulator swing. These towers are best suitable to maintain uninterrupted power supply, when tower failure occurs. These ERS towers bypass the original transmission system to restore power at the earliest, while the original tower is being rectified. These towers are very

much useful for restoring power supply following various natural disasters for conducting scheduled maintenance work on the existing towers without much interruptions.

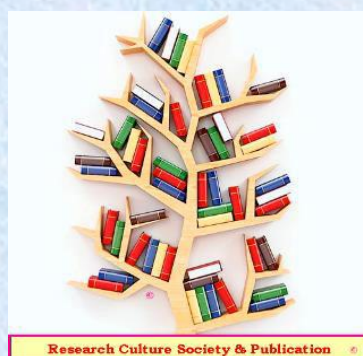
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