RESEARCH PAPER

NEPHROPROTECTIVE POTENTIAL OF *ACONITUM HETEROPHYLLUM* LEAVES IN A LEAD ACETATE INDUCE KIDNEY INJURY MODEL

Mansi Rawat[1], Dr. Waseem Khan[2], Dr. Sanjay Singh[3]

[1]Research Scholar, Departmen of Pharmacology, Siddhartha Institute of pharmacy, Sahashtradhara Road Dehradun, Uttarakhand, India, 248001

[2] Professor & Head of Pharmacology, Siddhartha Institute of pharmacy, Sahashtradhara Road Dehradun, Uttarakhand, India, 248001

[3]Principal, Siddhartha Institute of pharmacy, Sahashtradhara Road Dehradun, Uttarakhand, India, 248001

Abstract

This experimental study was done to determine the nephroprotective outcome of leaves of Aconitum heterophyllum on lead acetate induced toxicity in wistar albino rats. In this study the animals were grouped in 5 different groups having 6 rats in every group. The Groups I, II, III, IV and V were the Normal Control, Disease control, Standard Control, Test Group a and Test Group b respectively. In Group I no treatment was given it was only given normal saline. In group II as the rats were given lead acetate prepared with normal saline solution according to their body weight with 60 mg/kg dose to induce nephrotoxicity orally for 28 days. In group III the rats were given lead acetate same as disease group but they were also given the treatment ie, $CaNa_2$ EDTA intraperitoneally for last 5 days. In IV group the rats were given lead acetate with *Aconitum heterophyllum* extract lower dose ie, 250 mg/kg and in V group the rats were given lead acetate with *Aconitum heterophyllum* extract higher dose ie, 500 mg/kg both for 28 days. The nephroprotective activity of Aconitum heterophyllum was evaluated by the estimation of serum creatinine, serum urea, blood urea nitrogen (BUN) and inorganic phosphorus. The oxidative stress biomarkers were also studied and they were Malondialdehyde (MDA) and Glutathione (GSH). Histopathological examinations were also done of the kidneys of each group to see the possible changes in structure of the cells. It was noted that the methanolic extract Aconitum heterophyllum leaves shows nephroprotective activity. It is able in protecting the kidney cells from the damage caused by heavy metal and also able in reversing the changes which was caused by these agents.

Key words: - Aconitum heterophyllum, Nephroprotective, Lead acetate, $CaNa_2$ EDTA

How to cite this article: Rawat M, Khan W, Singh S. Nephroprotective Potential of Aconitum heterophyllum Leaves in a Lead Acetate Induce Kidney Injury Model. Int J Drug Deliv Technol. 2026;16(63s):1412-1420. DOI: 10.25258/ijddt.16.63s.140

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The human body is composed of many organs and organ system and every organ has its own importance. One such organ system is excretory system. When excretory system gets affected then body's basic metabolic activities gets affected. Kidney acts as the main excretory organ of the renal system. The kidneys are made up of millions of structural and functional unit called as nephrons. The main work of the kidney is to filter the fluids from renal blood flow by leaving all the essential components in blood [1]. The kidney internally has a very complex and unique structure. The kidney has a blood volume of 25-30% out of total cardiac output. This volume of blood gets filtrated and removes all the toxins present in blood. The GFR of a healthy kidney is 120- 125 ml/min. When GFR is too low, the filtration of metabolic waste products is insufficient. Conversely, an excessively high GFR overwhelms the renal tubules' ability to reabsorb salt and water effectively. To regulate these fluctuations, the kidneys rely on autoregulation, which operates through two key mechanisms. First The myogenic response, where increased stretch in the afferent arterioles triggers smooth muscle contraction to reduce blood flow. Second the Tubuloglomerular feedback, in which the macula densa detects changes in sodium and fluid flow in the distal nephron [2].

Renal disease can be elaborated as progressive retardation in functioning of kidney which is indicated with two main ways one is the abnormal functions of the kidney ie, proteinuria (if more than 150 mg of protein is present in individual's urine per day) or morphological changes in kidney and the other measure is low levels of GFR <60 mL/min/1.73m^2 if it persists for three months or more [3]. Nephrotoxicity can be caused by some drugs. It can be of two types, dose dependents toxicity and dose independent.

The dose dependent nephrotoxicity has predictable symptoms and most of the time it can be treated by reducing the amount of drug or completely stop it. The dose independent nephrotoxicity does not have these type of symptoms and it can not be treated many times [4]. Nephrotoxicity can be cause by the heavy metals and they are present in environment. Heavy metals can affect both humans and animals. There very less amount is also harmful. They accumulated with time and reach to their maximum toxic done. Heavy metals can alter the normal physiology of the cell. They affect by mechanism by increasing oxidative stress, gene expression and mitochondrial damage [5].

One such heavy metal is lead (Pb). Lead salts are created by its abnormal cycle in fuel and ignition in automobile engines. Lead enters the body through food, drink, and the air and constitutes as one of the four metals that have the most detrimental impact on human health.

Because lead is poisonous and non-biodegradable, it seriously damages the coastlines and aquatic life. Plants consume the major part of lead from the soil. Environmental variables such as pH and plant species affect how soluble lead is [6]. Lead is not metabolised like other compounds inside the human body instead it gets oxidized by the P 450 system. It get stored inside the body and after sometime it gets back into the blood stream causing toxicity. That's why lead is said to be one of the toxic metals in the environment because having a minimum half life of around 10 years, it remains forever in the body [7].

When lead enters inside the body it creates reactive species which destroy the cells. Whole process of oxidative stress occurs inside mitochondria. Redox imbalance process in renal disease can be understood by three main mechanisms which are nicotinamide adenine dinucleotide phosphate oxidase (Nox) stimulation, xanthine oxidoreductase (XOR) activation & irregular functioning of mitochondria. In kidney reactive oxygen species are majorly formed via mitochondrial respiratory chain & NADPH oxidase (NOX). When oxidative destruction occurs inside kidney it damages DNA, RNA, proteis and lipids so, it is very important to take a measure of oxidative stress that occurs during kidney disease [8].

The most studied biomarkers are MDA, thiobutyric acid reactive substances ($TBARS_S$), 4-hydroxynoneal. MDA causes proteins and nucleic acids to malfunction by binding through covalent bonds to them. HNE serves as a key mediator in oxidative stress because of its capability of forming harmful abducts with protein leading to inflammation & ultimately in the progression of CKD [9]. Protein carbonyl (PCO), plasma protein disulphide formation, AOPPs, and dityrosines are a result of protein oxidation. ROS easily attack DNA guanine bases, forming 8-OHdG, which binds to thymidine, a biomarker of mutagenesis due to oxidative stress [10].

There are many conventional medicines which can help during lead poisoning. One of the main and most effective method is chelation therapy. Chelation can be defined as a process where a particular chelating agent enters the body and bind with the heavy metal. CT is approved as a medical treatment for heavy metal poisoning [11]. We are using Calcium disodium ethylenediaminetetraacetic acid ($CaNa_2$ EDTA) in this study. It's a synthetic polyamino carboxylic acid that can bind to a metal ion at six coordination sites because it functions as a hexadentate chelating agent. It is a substituted diamine, a strong metal linking agent, and a very stable molecule that is frequently sold by its sodium salts. Due to its structure it is the primary cure for lead poisoning. It is given intravenously for better action [12]. There are many adverse effect of these conventional medicines that's why it is important to use a alternate treatment for heavy metal poisoning.

One alternate method for these conventional medicines is the use of herbal compounds. One such plant is *Aconitum heterophyllum.* It is a critically endangered herb that is found in the temperate and alpine regions of India, Nepal and Bhutan at a height of 2400 – 4000m above the sea level. It is called by different names in different parts of India like atis, ativika, atibaje, ativisha, patish, patrees, batis, atividdyam, ataich. suklakanda, ghunavallabha, kshmira and shishubhaishajya. It has some other synonyms like greenish Himalayan monkshood. It has a wide variety of bioactive substances, such as the poisonous alkaloids atisine, heteratisine, and aconitine, are responsible for its therapeutic actions. It has analgesic, anti-inflammatory, antipyretic, antibacterial, immunomodulatory & antioxidant qualities are all influenced by these chemicals. It has diterpenoid Alkaloids (Main Active Group) and compounds like Atisine, Heteratisine, Heterophylline, Heterophyllidine, Napelline, Aconitine, Quercetin, Kaempferol and Gallic acid derivatives are found [13].

AIM

To evaluate the nephroprotective acitivity of leaves of aconitum heterophyllum in lead acetate induced wistar albino rats.

MATERIALS AND METHODS

Animals

The study was done on the male albino wistar rats. The approval of animal study protocol was done through institute of pharmacy ie, the "Siddhartha Institute of Pharmacy, Dehradun"

and their committee is the IAEC (Institutional Animal Ethical Committee) that reviewed and approved the protocol and procedures employed in the experiment. Form B [as per rule 8 (a) for submission of research protocols] was submitted for permission of animal experiments to the Institutional Animal Ethics Committee (IAEC). The form was passed by IAEC and all the protocol was done inside the animal house which is approved through CPSCEA.

The rats were acclimatized for 10 days prior to protocol. The relative humidity and temperature were according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPSCEA) ie, 55% ±5% and 25˚C ±1˚C respectively. The standard feed and water was given properly to all the animals. The age of the rats was six to eight weeks. The weight of all the rats was between 125 to 220 grams. The rats were taken from Shri Guru Ram Rai University, Patel Nagar, Dehradun, Uttarakhand – 248001. The whole experimental process was done according to CPSCEA and under the supervision of IAEC.

Chemicals

Lead acetate [Pb (CH3COO)2], Calcium disodium Ethylenediaminetetraacetic acid (CaNa2 EDTA) were taken from the laboratory of institute.

Collection and authentication of Plant Material

The plant EMPLOYED in this study was *Aconitum heterophyllum*. The plant was collected from Uttarkashi district of Uttarakhand in the month of September. The leaves were kept aside for further use. After that the herbarium was prepared for the authentication procedure of plant because it is very important to authenticate the plant before the study. The plant was authenticated from Dr. K. Madhava Chetty, a botanist, Sri Venkateswara university in Tirupati, Andhra Pradesh. The authentication certificate was provided and is preserved for the further use. Then leaves were dehydrated firstly in shade & then in the sunlight. Once the leaves were completely dry then they were triturated into a powder with the help of mortar pestle. Then it was run through sieve to get a smooth powder.

Extract preparation

Methanol was used as a solvent for extraction process. Firstly the cotton bed was made inside the extractor and the downward orifice was also covered by it. Then 300 gm of plant material was filled inside the extractor and it was again covered by a cotton bed. Then the extractor was attached with condenser. One pipe was attached to the inlet of condenser by one side and other side by a water source. The another pipe was attached to the outlet of the condenser. 270 ml of solvent was then poured inside from the condenser and 30 ml of the solvent is kept in round bottom flask. A few porcelain pieces were kept inside the RBF to avoid splitting of solvent during heating. The heating mantle was turned on and let the cycles run. The process ran for around 40 cycles. After all the cycles were complete. The solution was filtered and taken in a china dish and was kept in water bath and let the solvent evaporate until a semisolid extract of dark green colour was left behind [14].

Study design

According to the protocol we have received 30 animals. All rats distributed in 5 groups, with 6 rats in each. The Groups I, II, III, IV and V were the Normal Control, Disease control, Standard Control, Test Group a and Test Group b respectively. In Group I no treatment was given it was only given normal saline. In Group II as the name suggested disease group so, the rats were given lead acetate prepared with normal saline solution according to their body weight with 60 mg/kg dose to create nephrotoxicity orally for 28 days. In Group III the rats were given lead acetate same as disease group but they were also given the treatment ie, CaNa2 EDTA intraperitoneally for last 5 days. In Group IV the rats were given lead acetate with *Aconitum heterophyllum* extract lower dose ie, 250 mg/kg for orally for 28 days. In Group V the rats were given lead acetate with *Aconitum heterophyllum* extract lower dose ie, 500 mg/kg orally for 28 days.

Evaluation of kidney function

After 24 hour of the completion of protocol the rats were sacrificed and samples were collected from each group & analysis of various parameters was done ie, serum creatinine by Jaffe's method [15], serum urea and blood urea nitrogen (BUN) by diacetylmonoxime method [16] and inorganic phosphorus by isobutyl alcohol method [17].

Evaluation of Oxidative stress markers

As the oxidative stress is the major effect of lead ingestion so it is very important to determine the levels of oxidants and antioxidants. The estimation of biomarkers was done to check the possible effect of *Aonitum heterophyllum* extract on reversing the kidney functions. The levels of Malondialdehyde (MDA): Lipid peroxidation assay [18] and Glutathione (GSH): Assay for antioxidant activity [19] were estimated.

Histopathology

On 29th day the animals were sacrificed by overdose of anesthesia. The abdomens of rats were cut down and the kidneys were isolated. Kidney were set in 10% neutral buffered

formalin, submerged in paraffin, sectioned (4–5 µm thickness), and stained with hematoxylin and eosin (H&E). Histological changes, including tubular degeneration, glomerular damage, and interstitial inflammation, will be evaluated under a light microscope.

Statistical analysis

The data was indicated as mean ± standard deviation (SD). Statistical comparisons between groups will be performed using one-way ANOVA followed by Tukey's post hoc test. The threshold for statistical significance is characterized as a p-value of less than 0.05.

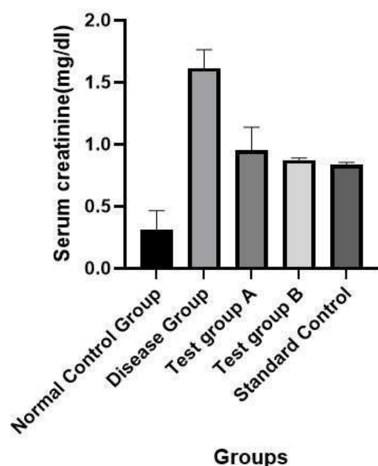
RESULTS

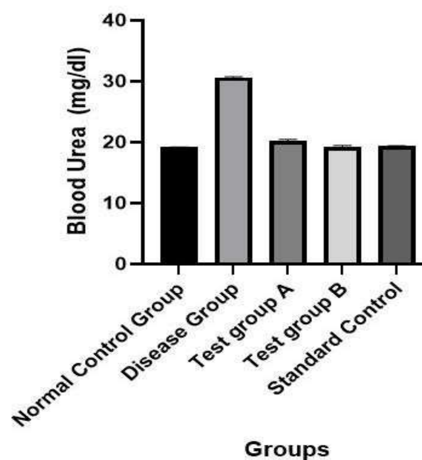
The results of lead acetate induced nephrotoxicity are shown in table. It shows the affect of lead acetate on different kidney function test parameters. To know the toxic effect the levels of serum urea, creatinine, blood urea nitrogen and inorganic phosphorus were determined. The levels of these were highest in group II because it was the disease group. The levels of these serums were less in group III. IV and V in comparison to group II. The oxidative stress markers were also estimated and they are also shown in table. The level of MDA which is the oxidative marker was high in the groups with most toxicity ie, group II and less in groups which received treatment like group III, IV and V. The level of GSH was high in treatment groups because the rats of these groups were able to form the antioxidants.

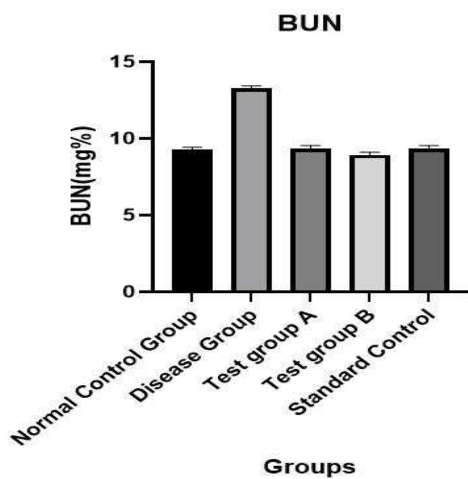
Histopathological examination was done and microscopic images are attached in figure 1, 2, 3, 4 & 5 for the Group I- Normal Control, Group II- Disease Control, Group III- Standard Control, Group IV- Test group a (*Aconitum heterophyllum*, 250mg/kg) and Group V- Test group b (*Aconitum heterophyllum*, 500mg/kg) respectively. Group I shows the normal cell structure. Group II shows changes in parenchymal of renal. It is also shows that necrosis in tubular, thyroidization, modest congestion in renal, moderately lymphocytic infiltration in the interstitium. Morphology of glomeruli appeared normal but increase mesangial cell and congestion in some glomeruli are noted in histology. This shows that the cells were highly toxic. Group III shows that mild necrosis, thyroidization, minimal infiltration of lymphocytic in interstitium and glomeruli appeared almost normal are noted in histology. Group IV shows that mildly recovery changes in parenchymal of renal. It is also showed moderate necrosis in tubular, thyroidization, modest congestion, infiltration in lymphocytic mild to the moderate in the interstitium. Increase mesangial cell with congestion in some of glomeruli are noted in histology. Group V shows some modest recovery changes in parenchymal of renal. It is also shows that thyroidization, tubular necrosis, congestion, infiltration in lymphocytic in interstitium, increase mesangial cell with congestion in glomeruli were better than test group a which shows a sign of recovery.

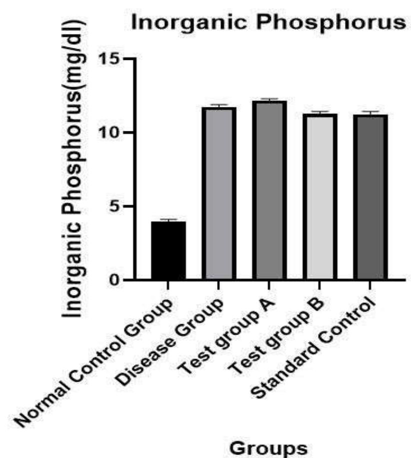
S. No.	**Group Name**	**Serum Ceratini**	**Serum Urea**	**BUN** (mg%	**Inorganic Phosphorus**	**MDA** (ug/ml)	**GSH** (mg/

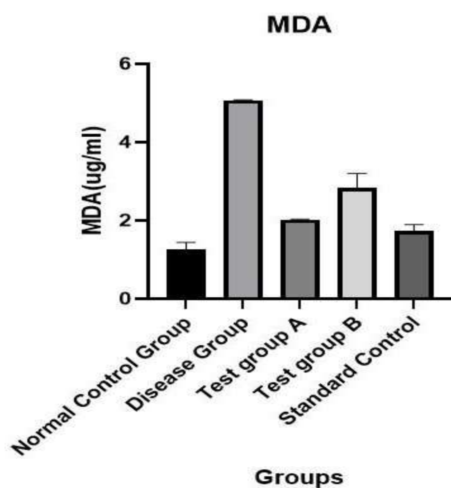
		ne (mg/dl)	(mg/dl)	)	(mg/dl)		dl).
1.	Group I-Normal Control	0.0317 ± 0.147	19.19 ± 0.016	9.250 ± 0.187	3.983 ± 0.116	1.250 ± 0.187	20.00 ± 1.414
2.	Group II-Disease Control	1.617 ± 0.147	3.060 ± 0.141	13.25 ± 0.187	11.73 ± 0.163	5.070 ± 0.014	14.18 ± 0.147
3.	Group III-Standard Control (CaNa2 EDTA)	0.835 ± 0.018	19.39 ± 0.018	9.350 ± 0.187	11.23 ± 0.196	1.733 ± 0.163	19.83 ± 1.169
4.	Group IV-Test group a (Aconitum heterophyllum 250 mg/kg)	0.950 ± .0187	20.25 ± 0.187	9.350 ± 0.187	12.17 ± 0.121	2.020 ± 0.014	16.83 ± 1.169
5.	Group V-Test group b (Aconitum heterophyllum 500 mg/kg)	0.873 ± .016	19.25 ± 0.187	8.933 ± 0.163	11.25 ± 0.187	2.835 ± 0.367	21.27 ± 0.163






BUN
BUN(mg%)
15
10
5
0
Normal Control Group
Disease Group
Test group A
Test group B
Standard Control
Groups


Inorganic Phosphorus
Inorganic Phosphorus(mg/dl)
15
10
5
0
Normal Control Group
Disease Group
Test group A
Test group B
Standard Control
Groups


MDA
MDA(ug/ml)
6
4
2
0
Normal Control Group
Disease Group
Test group A
Test group B
Standard Control
Groups

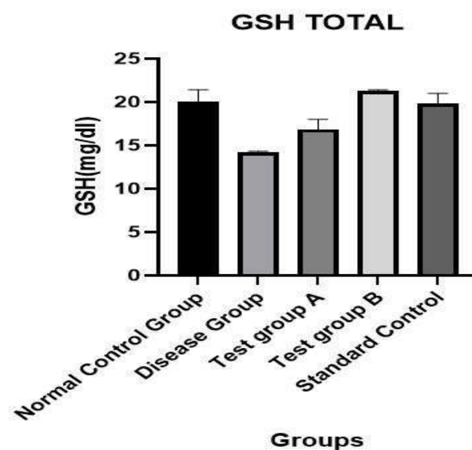

GSH TOTAL
GSH(mg/dl)
25
20
15
10
5
0
Normal Control Group
Disease Group
Test group A
Test group B
Standard Control
Groups

Fig 1: Normal Control Group

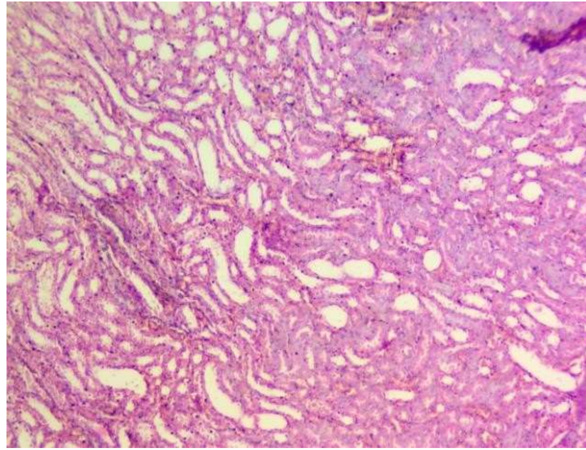
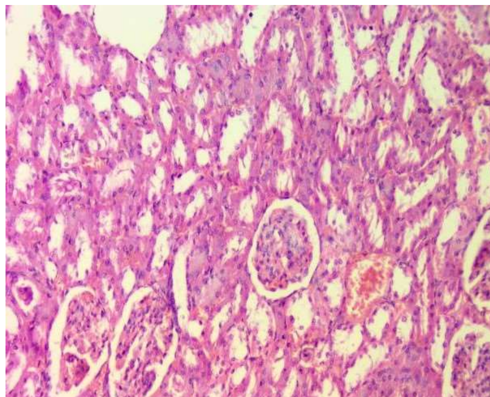


Fig 2: Disease Group



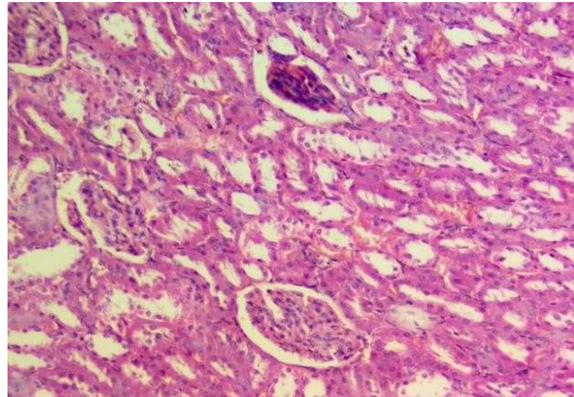
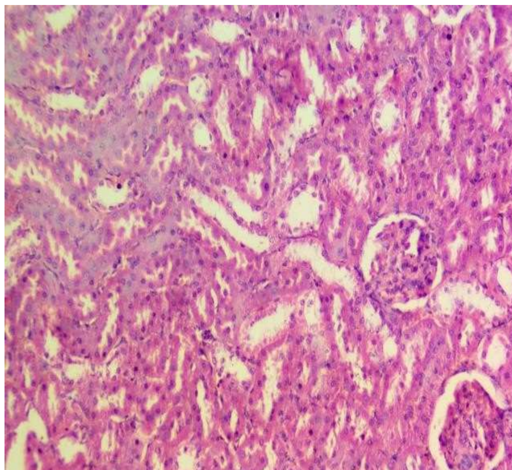


Fig 3: Standard Group



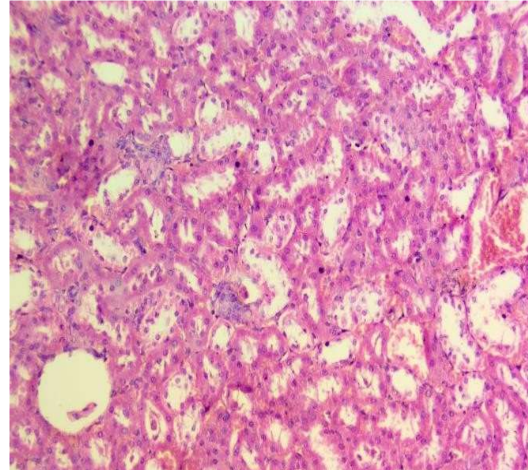
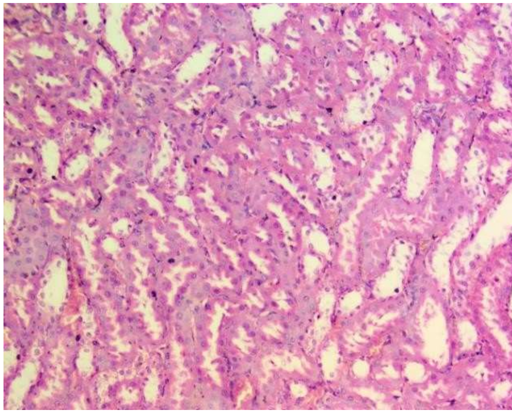
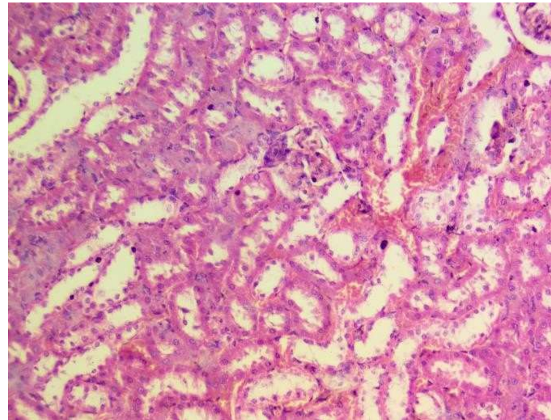


Fig 4: Test Group a





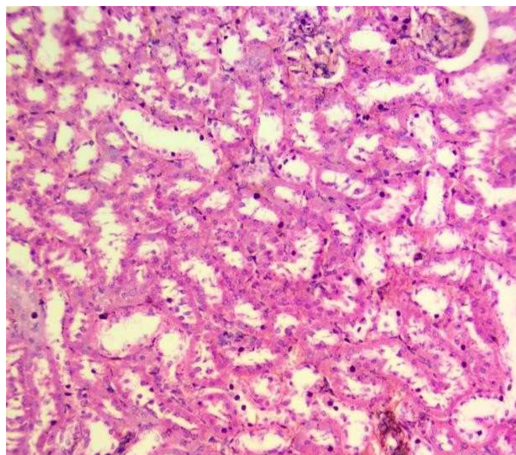
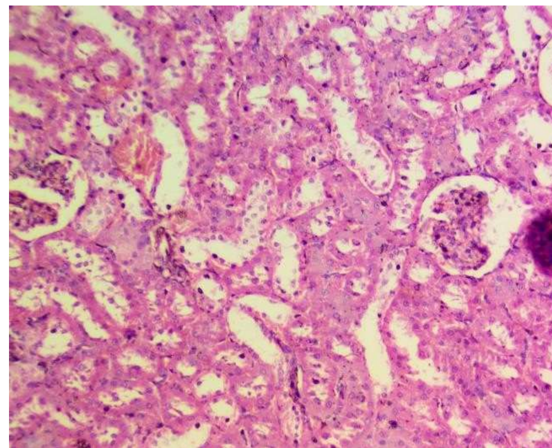


Fig 5: Test Group b



DISCUSSION

This experimental study was done to check whether the *Aconitum heterophyllum* leaves has nephroprotective activity or not. The protocol was prepared & it was passed by IAEC. After approval we had received the animals. The protocol was of 28 days. According to the protocol we have received 30 animals. After receiving the animals they were under acclimatization period for 10 days to make them habitual with the environment. Then the protocol was started after acclimatization period. All rats distributed in 5 groups, with 6 rats in each. The Groups I, II, III, IV and V were the Normal Control, Disease control, Standard Control, Test Group a and Test Group b respectively.

In group I no treatment was given it was only given normal saline.

In group II as the name suggested disease group so, the rats were given lead acetate prepared with normal saline solution according to their body weight with 60 mg/kg dose to create nephrotoxicity orally for 28 days. This was the most toxic group among all. This toxicity was confirmed by running the confirmatory test after two weeks of inducing toxicity in rats.

In group III the rats were given lead acetate same as disease group but they were also given the treatment ie, $CaNa_2$ EDTA intraperitoneally for last 5 days. These rats were showing less toxicity symptoms s compared to the group II.

In IV group the rats were given lead acetate with *Aconitum heterophyllum* extract lower dose ie, 250 mg/kg and in V group the rats were given lead acetate with *Aconitum heterophyllum* extract higher dose ie, 500 mg/kg both for 28 days. The animals in group III, IV and V were given $CaNa_2$ EDTA, 250 mg/kg plant extract and 500 mg/kg *Aconitum heterophyllum* extract respectively that's why they showed a decrease in toxicity parameters like KFT and oxidative biomarkers.

The KFT was done for all the groups. Additionally oxidative stress biomarkers were also evaluated. One was MDA to check the oxidative stress level and other was GSH to check the antioxidant level. The MDA was increased in group II the most but it was slightly lower in groups III, IV and V because the treatment was also going on. While the GSH was increased in groups III, IV and V showing that the *Aconitum heterophyllum* extract has also the strength to form antioxidant markers which can help in reducing the amount of free radicals in

case of heavy metal poisoning.

At last the histopathology was also done of the kidneys from each group and the slides are attached above which also shows that the cells of group II were suffering from necrosis and the cells of group III were having mild necrosis. While the cells of group IV had recovered a lot and of group V had left with little bit of damaged part of cells. The group V treated more the cells as compared to group IV because group V had a higher dose. So, it can be said that the leaves of plant *Aconitum heterophyllum* has nephroprotective activity and can be used in case of heavy metal poisoning.

CONCLUSION

This study was done on the topic "Nephroprotective potential of Aconitum heterophyllum leaves in a Lead acetate induce kidney injury model" in which methanolic extract of plant was used and the toxicity induction done by lead acetate in albino wistar rats. The main goal was to know the kidney protection of Aconitum heterophyllum leaves in heavy metal toxicity like lead. The induction of lead showed both physical and chemical changes and morphological also. There were different conclusion from this study like: -

Lead, a heavy metal can cause toxicity at cellular level to organisms.

When lead was given to rats there were many toxicity symptoms like weight loss, diarrhea, irritation, itching and being lethargic. Group II was showing all these symptoms. In group III, IV and V all these symptoms were not present but only a few of them like weight loss and diarrhea. It can be concluded that lead has major negative effects if consumed by any age group.

The kidney function tests were also done for all the groups which included the serum creatinine, serum urea, BUN and the inorganic phosphorus. The statistical analysis of these tests was also done and the significance values were note down and according to that the study w found to be significant.

The estimation of oxidative stress markers was also done which included MDA and GSH. These levels was found to be satisfactory as mentioned above.

The histopathological testing was also done and results were also satisfying as mentioned above.

The lower value of significant represent higher accuracy in result and all graphs level of significant are less than 0.05. It can be concluded that the *Aconitum heterophyllum* leaves extract has the antioxidant, anti- inflammtory and nephroprotective activity by the fact that it was able in reversing the toxic effects that was caused by lead acetate.

FUTURE PERSPECTIVE

The study of the experimental topic "Nephroprotective potential of *Aconitum heterophyllum* leaves in a Lead acetate induce kidney injury model" was performed successfully and show positive result. In this experimental study disease is Nephrotoxicity which is induced through heavy metal or chemical lead (lead acetate) that represent the toxicity were preventable by the natural material of plant. So, Plant extract contained some of the constituent which is help to preventable and improve this condition. In future the activity of plant will be remarkable and clinical research in the plant of *Aconitum heterophyllum* for nephrotoxicity and any other toxicity can be done. The Plant *Aconitum heterophyllum* contains many chemical constituents which are used for study in different diseases and conditions. It is also used in the formulation of dosage form and prepared novel formulation with aim of novel drugs. The *Aconitum heterophyllum* consisted antioxidant and anti-inflammtory property which is used in future for cancers and heart related diseases.