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Evaluation of Nephroprotective and Antioxidant Activity of Leaves of Aerva Lanata

[1]Ram Prasad Upreti, [2]Arbind Kumar Mandal, [3]Kusu Susan Cyriac
Karnataka College of pharmacy #33/2, Thirumenahalli, Bangalore – 560064, India.Department of pharmacology.
Corresponding Author: Ram Prasad Upreti

Abstract: The herbal drug Aerva lanata, basically the decoction of leaves of Aerva lanata. Linn -the plant which isbeing commonly used in Siddha System of Medicine, is evaluated for Nephroprotective activity in animal model. Toevaluate the efficacy of Aerva lanata in the management of Renal function impairment. The Nephroprotectiveactivity of the drug in Gentamicin models was evaluated in Wistar albino rats. The rats in prophylactic group weretreated with the decoction of leaves of Aerva lanata at the low dose of 150 mg and 600 mg/ kg. The Gentamicin models of rats treated with the drug at the dose of 600.0 mg/kg orally for 10 days showed significant reduction inthe level of Blood urea ($P < 0.02$) and Serum Creatinine with the significance of ($P < 0.05$). Histopathology alsoreveals the reduction in the degree of renal damage. These findings suggest that the drug possess Nephroprotective activity with minimal toxicity and could offer promising role in the management of renal damage caused byNephrotoxins like Gentamicin.

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I. INTRODUCTION

In the medical field Impairment of renal function is one of the challenging problem causing mortality and morbidity, for which the replacement therapy (dialysis or transplantation) becomes necessary, and is associated with complications in virtually of all organ systems. Hence there is a continuous search for agents which provide protection against the renal impairment caused by drugs. This fact drove the author to go for a native solution through Aerva lanata.

In general, the Plant Aerva lanata has significant Pharmacological activities like Diuretic, Anti-inflammatory and Lithotriptic action [1] .The Antioxidant, Cytotoxic and Hepatoprotective activity of Aerva lanata has also been demonstrated by Scientific studies and published in Journals. The plant contains flavonoids such as Kaempferol 3-rhamnoside and Kaempferol 3 –galactoside which are well known potent antioxidant and free radical scavengers. [2]. As per the Siddha Literatures the herb Aerva lanatais indicated for Neeradaippu[3] - the pathological conditions of which can easily be correlated with the Impairment of Renal function. Moreover Aerva lanatawas mentioned as 'Uppuchathai nasamakki' in the Siddha text Bohar Nigandu 1200 [4] which means that this drug can remove the metabolic waste products from blood. These Siddha Literary evidences makes the author to evaluate the
Nephro protective action of Aerva lanata decoction in rats.

EXPERIMENTAL SECTION

1.1. PREPARATION OF DRUG

1.1.1. Collection of Plant material and Authentication:

Plants were collected from Tirupati, Andhra Pradesh local area in the month of September 2018 and was authenticated by the botanist. The leaves were washed in pure water, cut into
small pieces and dried in sun shade. The dried plant is powdered and preserved.

1.1.2. Preparation of drug:

The powdered plant was boiled with water of four times that of drug and condensed into ¼th of it. The decoction wasmade in such a way that 1 ml of decoction contains 50 mg of drug i.e, 5% extract. The drug was daily prepared justbefore administration because of the short shelf life of the drug (3 hours).

1.2. BIOCHEMICAL ANALYSIS

The Biochemical analysis of the drug was done in Rohana Veterinery Diagnostics Pvt. Ltd. to determine themetals and minerals in Aerva lanata, by means of Atomic Absorption Spectrometer (AAS) with air

– acetylene. The XRF analysis of the drug Aerva lanatawas done in Central Electro Chemical Research Institute, Karaikudi.

1.3. PHARMACOLOGICAL STUDIES

The pharmacological action of the drug Aerva lanatawas carried out in Rohana Vetinery lab, Bengaluru, Study was conducted after obtaining Institutional Animal Ethical Committee clearance

1.3.1. Experimental Animals:

Healthy adult male albino rats (120 – 250gms) of Wister strain were used for the study. The rats were housed inpolypropylene cages and maintained under standard conditions (Temperature range: 65-75oF and Humidity range:40-70%). The animals had free access to standard pellet diet and water utilizing aqua guard.

1.3.2. Drugs and chemicals:

The chemicals used for the study are Gentamicin Injection, Estimation kit forBlood urea, Serum creatinine, Serum total protein, albumin and globulin (Rohana Veterinery Diagnostics Pvt. Ltd. Bengaluru)

5.2 Result of Acute Oral Toxicity Study:

The LD50 of the extract of Aerva lanata was found to be 2000mg/kg after performing the acute oral toxicity studies. (Already reported).

1.3.3. Induction of Nephrotoxicity and drug feeding schedule:

All the animals (24 Males) were weighed and randomly divided into four groups comprising of six rats in each. Theexperimental protocol for Gentamicin induced nephrotoxicity is cited in **Table 1**. The kidney damage was inducedby subcutaneous injection of Gentamicin @ 80 mg/kg on 6th day onwards in Groups II, III and IV. Group I and IVwere kept as normal (Saline) and toxic control group, respectively. On the other hand, Group II and III were treatedwith Aerva lanata @ 150.0 mg/kg and Aerva lanata@ 600 mg/kg orally, respectively. The dose of thesamples were collected through retro orbital sinus of all the animals on 11th day of experiment. The bloodsamples were estimated for biochemical parameters such as Blood urea nitrogen, Serum creatinine, Total protein,Albumin and Globulin. After blood collection all the animals were weighed and euthanized under ether anesthesia and the kidneys were collected, weighed and preserved in neutral buffered 10% (V/V) formalin for histopathology. These were processed for paraffin embedding using ethyl alcohol as de-hydrant and xylene as clearing agent.

Paraffin sections of kidney, about 4-5 μm thickness, were stained with haematoxylin and eosin. These sections wereexamined for histopathological changes and the cellular alterations were scored as Nil, +, ++ and +++ for No, Mild,Moderate and Severe damage, respectively.

Experimental model for Gentamicin induced Nephrotoxicity

Group no=6	Drug treatment	Route(mg/kg)/bw	Duration	Days of withdrawal of blood and kidney	Purpose
1	saline	P.O	1^{st}-10^{th}	11^{th} day	Control
2	Saline + Gentamicin	P.O 100 mg/kg IP	1^{st}-10^{th} 5^{th}-10^{th}	11^{th} day	Produce kidney injury
3	Gentamicin + Aerva lanata	100mg/kg IP 150mg/kg PO	5^{th}-10^{th} 1^{st}-10^{th}	11^{th} day	Protective effect
4	Gentamicin+ Aerva lanata	100mg/kg IP 300mg/kg PO	5^{th}-10^{th} 1^{st}-10^{th}	11^{th} day	Protective effect
5	Gentamicin+ Aerva lanata	100mg/kg IP 600mg/kg PO	5^{th}-10^{th} 1^{st}-10^{th}	11^{th} day	Protective effect
6	Gentamicin+ Vitamin E	100mg/kg IP 200mg/kg IP	5^{th}-10^{th} 1^{st}-11^{th}	11^{th} day	Protective effect

1.3.4. Statistical Analysis:

The data collected were subjected statistical analysis using unpaired t-test. Thestatistical significance of difference was taken as $P < 0.05$.

II. RESULTS AND DISCUSSIONS

1.4. Biochemical Reports:

The Biochemical analysis of the drug Aerva lanatareveals the presence of Minerals with the percentage of Selenium in0.043%, Manganese in 0.053%, Lead in 0.003%, Copper in 0.002%, Potassium in 2.075% and Calcium.

X-Ray fluorescence studies on the drug Aerva lanatareveals the presence of minerals namely Zinc, Iron, Calcium andPotassium.

Sl.No.	Constituents	Tests	Result
1.	Flavonoid	Zinc Hydrochloric acid test	+
		Lead acetate test	+
		Shinodas test	+
		Ferric chloride test	+
2.	Sterols	Liebermann burchard test	-
		Salkowski test	-
3.	Terpenoids	Terpenoid test	-
4.	Alkaloids	Mayers test	+
		Wagers test	+
		Drangendoffs test	+
		Hagers test	+
		Tannic acid test	+
5.	Saponin	Foam test	+
6.	Tannin	Ferric chloride test	+
		Gelatin test	+
		Lead acetate test	+
		Alkaline reagent test	+
7.	Phenols	Ellagic acid test	+
		Phenols test	+
8.	Glycosides	Keller killiani test	+
		Concentrated sulphuric acid	+
		Molischs test	+

Where :(+) represents - present and (-) represents – absent

1.5. Pharmacological Reports:

In the present study the mean Blood urea, Serum creatinine, Total protein, Albumin and Globulin value of eachgroup of rats at the 11th day of the experiment is compared with the values of Nephrotoxic control group. Meanlevels of Blood urea, Serum creatinine, Total protein, Albumin and Globulin are presented in **Table 2** and **Table 3**;the representing Charts in **Figure1, 2 and 3.**

In this study the rats included in Group IV (Nephrotoxic control) showed significant increase in Blood urea level on comparison with the values of Group I ($P < 0.001$). In the group III (Aerva lanata@ 150.0 mg/kg orally for 10days) there was significant reduction in Blood urea levels as compared to that of Group IV ($P < 0.05$). In the group V (@ 600.0 mg/kg orally for 10 days) there was significant reduction in Blood urea levels as compared to that of Group IV ($P < 0.02$).

Change in body weight

S.N	Groups	Average body weight	
		Day 0	Day 11
1	I	165.41±0.029	173±0.034
2	II	203±0.021***	164±0.018***
3	III	180.12±0.056##	165±0.068##

4	IV	186.24±0.018##	177.53±0.028##
5	V	212.14±0.031###	207.56±0.042###
6	VI	174.21±0.014##	180.25±0.019#

The data are expressed as Mean ±S.E.M (n=6) rats in each group
***P<0.001 when compared with Normal Control
###P<0.001 when compared with Gentamicin (100mg/kg) control

Group 1: Control (normal saline 10ml/kg)
Group 2: Diseased
Gentamicin (80mg/kg) group
Group 3: Low dose
Drug extract(150mg/kg) + Gentamicin(100mg/kg) group

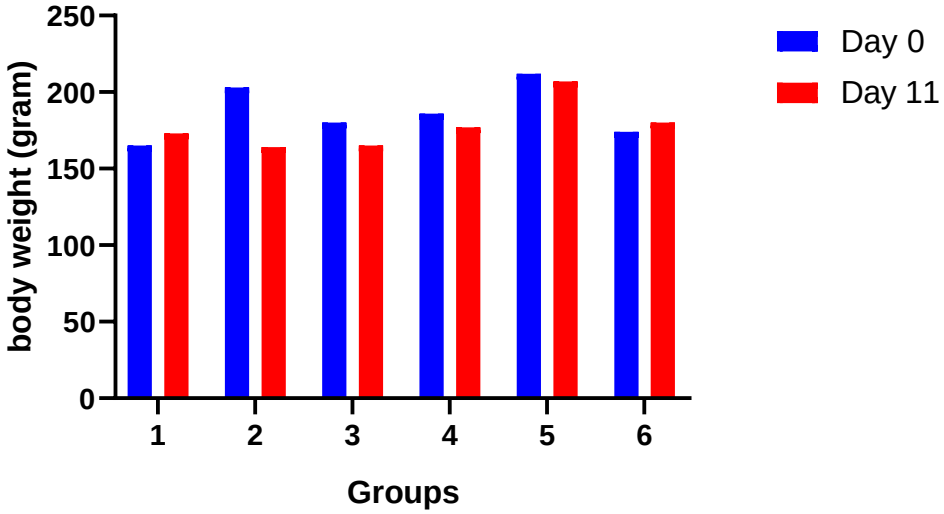


Fig 1- Body weight of rats on oth day vs. 11th day

Group 4: Medium **dose**
Drug extract (300mg/kg) + Gentamicin (100mg/kg) group
Group 5: High dose
Drug extract (600mg/kg) +Gentamicin (100mg/kg)
Group 6: Standard
Vitamin E 200mg/kg+Gentamicin (100mg/kg)

5.4 Effect of the gentamicin, standard and test drug on the wet kidney weight.

A significant increase (P<0.001) of kidney weight in gentamicin treated group when compared with the control group and dose dependent decrease (P<0.001) in kidney weight was observed on animals pretreated with MEAL along with gentamicin. These values are tabulated below.

Table No.5- Effect of graded oral doses of MEAL on the average kidney weight in gentamicin induced rat.

	Groups	Weight of wet kidneys (mg)
1	I	1.347±0.007
2	II	1.964±0.006***
3	III	1.752±0.004##
4	IV	1.649±0.006##
5	V	1.416±0.003##
6	VI	1.365±0.004##

The data are expressed as Mean ±S.E.M (n=6) rats in each group
***P<0.001 when compared with Normal Control
###P<0.001 when compared with Gentamicin (100mg/kg) control

Group 1: Control (normal saline 10ml/kg)
Group 2: Diseased
Gentamicin (100mg/kg) group
Group 3: Low dose
Drug extract(150mg/kg) + Gentamicin(100mg/kg) group
Group 4: Medium dose
Drug extract (300mg/kg) + Gentamicin (100mg/kg) group
Group 5: High dose
Drug extract (600mg/kg) +Gentamicin (100mg/kg
Group 6: Standard
Vitamin E 200mg/kg+ Gentamicin (100mg/kg)

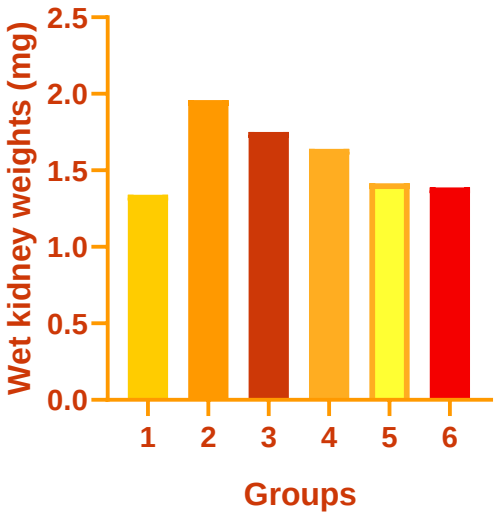


Fig 2- Weight of wet kidneys of various groups

Group 5: High dose
Drug extract (600mg/kg) +Gentamicin (100mg/kg
Group 6: Standard
Vitamin E 200mg/kg+Gentamicin (100mg/kg)

5.5 Effect of the gentamicin, standard and test drug on urine volume:

A significant decrease (P<0.05) in urine volume was seen in gentamicin treated group when compared with the control group and dose dependent increase (P<0.05) in urine volume was observed on animals pretreated with MEAL along with gentamicin. These values are tabulated below.

Table No.6- Effect of graded oral doses of MEAL on the average urine volume in gentamicin induced rat

S.N	Groups	Urine volume (ml)
1	I	12.016±0.033
2	II	5.042±0.002**
3	III	13.264±0.022##
4	IV	15.086±0.009##
5	V	18.015±0.013##
6	VI	19.057±0.008##

The data are expressed as Mean ±S.E.M (n=6)rats in each group
**P<0.05 when compared with Normal Control
##P<0.05 when compared with Gentamicin (100mg/kg) control

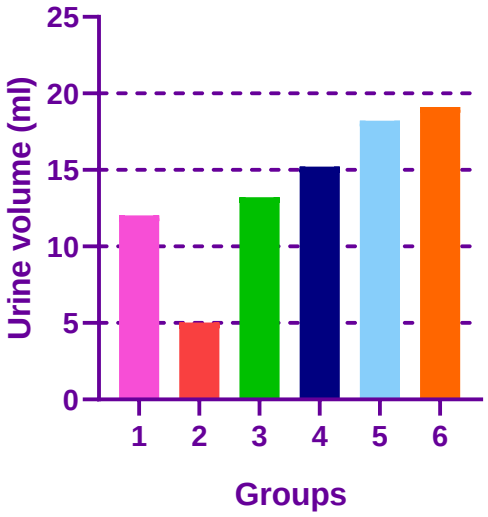


Fig 3- Urine volume of various groups

5.6.1 Effect of the gentamicin, standard and test drug on blood urea nitrogen:

A significant increase (P<0.05) in blood urea nitrogen was seen in gentamicin treated group when compared with the control group and dose dependent decrease (P<0.05) in blood urea nitrogen was observed on animals pretreated with MEAL along with gentamicin. These values are tabulated below.

Table No.7- Effect of graded oral doses of MEAL on the Blood urea nitrogen in gentamicin Induced rat.

S.N	Groups	Blood urea nitrogen (mg/dl)
1	I	48.034±0.013
2	II	128.043±0.012**
3	III	71.059±0.058##
4	IV	58.003±0.006##
5	V	47.023±0.007##
6	VI	49.059±0.023##

The data are expressed as Mean ±S.E.M (n=6)rats in each group
**P<0.05 when compared with Normal Control
##P<0.05when compared with Gentamicin (100mg/kg) control

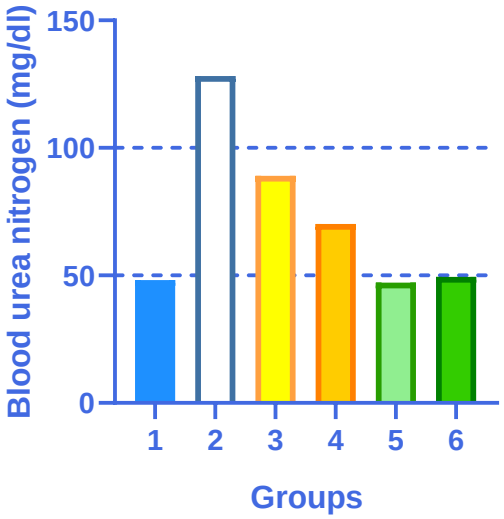


Fig 4- Serum blood urea nitrogen of various group of animals

5.6.2 Effect of the gentamicin, standard and test drug on serum uric acid:

A significant increase (P<0.05) in serum uric acid was seen in gentamicin treated group when compared with the control group and dose dependent decrease (P<0.05) in serum urea was observed on animals pretreated with MEAL along with gentamicin. These values are tabulated below.

Table No.8- Effect of graded oral doses of MEAL on the serum uric acid in gentamicin induced rat.

S.N	Groups	Serum uric acid (mg/dl)
1	I	1.769±0.001
2	II	3.217±0.001**
3	III	2.520±0.006##
4	IV	2.203±0.004##
5	V	1.87±0.001##
6	VI	1.825±0.003##

The data are expressed as Mean ±S.E.M (n=6) rats in each group
**P<0.05 when compared with Normal Control
##P<0.05 when compared with Gentamicin (100mg/kg) control.

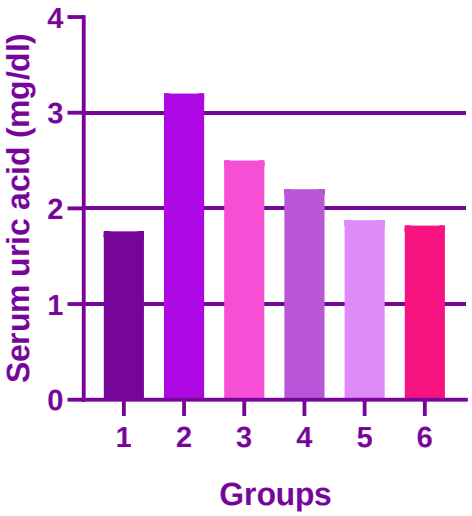


Fig 5- Serum uric acid of various groups

5.6.3 Effect of the gentamicin, standard and test drug on serum creatinine:

A significant increase (P<0.05) in serum creatinine was seen in gentamicin treated group when compared with the control group and dose dependent decrease (P<0.05) in serum urea was observed on animals pretreated with MEAL along with gentamicin. These values are tabulated below.

Table No.9- Effect of graded oral doses of MEAL on the serum creatinine in gentamicin induced rat.

S.N	Groups	Serum creatinine (mg/dl)
1	I	0.869±0.007
2	II	2.497±0.0006**
3	III	1.721±0.001##
4	IV	1.359±0.004##
5	V	0.979±0.007##
6	VI	0.915±0.003##

The data are expressed as Mean ±S.E.M (n=6) rats in each group
**P<0.05 when compared with Normal Control
##P<0.05 when compared with Gentamicin (100mg/kg) control

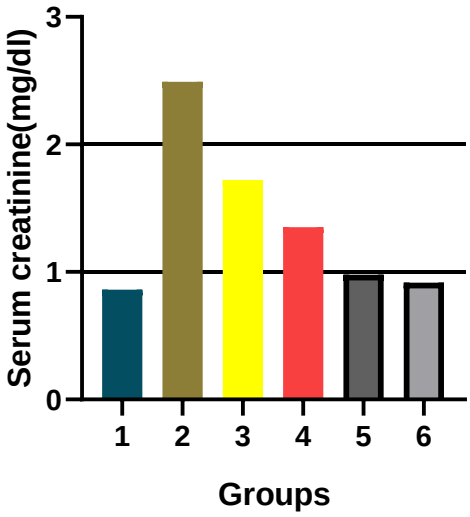


Fig 6- Serum creatinine of various groups of animals

5.6.4 Effect of the gentamicin, standard and test drug on serum total protein:

A significant increase (P<0.05) in serum total protein was see in gentamicin treated group when compared with the control group and dose dependent decrease (P<0.05) in serum total protein was observed on animals pretreated with MEAL along with gentamicin. These values are tabulated below

Table No.10- Effect of graded oral doses of MEAL on the serum total protein in gentamicin induced rat.

S.N	Groups	Serum total protein (mg/dl)
1	I	6.212±0.007
2	II	8.314±0.002**
3	III	7.210±0.001##
4	IV	7.411±0.003##
5	V	6.721±0.006##
6	VI	6.49±0.004##

The data are expressed as Mean ±S.E.M (n=6) rats in each group
**P<0.05 when compared with Normal Control
##P<0.05 when compared with Gentamicin (100mg/kg) control

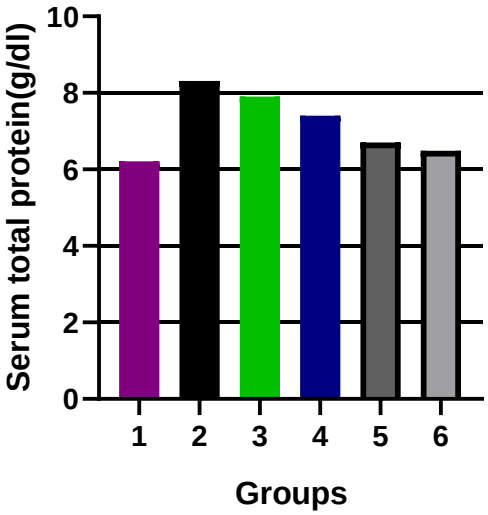


Fig 7- Serum total proteins of various groups of animals

5.6.5 Effect of the gentamicin, standard and test drug on serum albumin:

A significant increase in serum albumin (P<0.05) was seen in gentamicin treated group when compared with the control group and dose dependent decrease (P<0.05) in serum albumin was observed on animals pretreated with MEAL along with gentamicin. These values are tabulated below.

Table No.11- Effect of graded oral doses of MEAL on the serum albumin in gentamicin induced rat.

S.N	Groups	Serum albumin(g/dL)
1	I	2.334±0.001
2	II	3.705±0.0001**
3	III	2.571±0.006##
4	IV	2.725±0.001##
5	V	2.904±0.003##
6	VI	2.405±0.001##

The data are expressed as Mean ±S.E.M (n=6) rats in each group
**P<0.05 when compared with Normal Control
##P<0.05 when compared with Gentamicin (80mg/kg) control

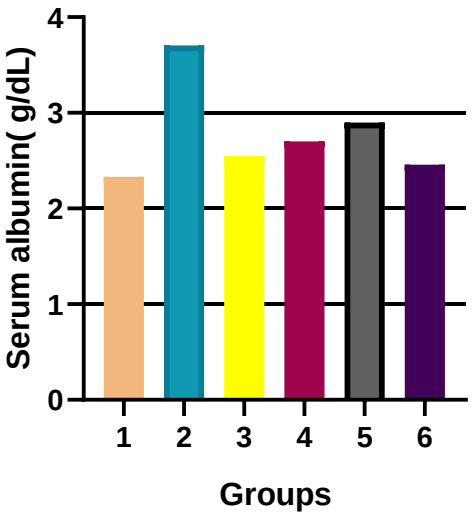


Fig 8- Serum albumin level of various groups of rats

5.6.6 Effect of the gentamicin, standard and test drug on serum chloride:

A slight increase (P<0.05) in serum chloride was seen in gentamicin treated group when compared with the control group and dose dependent decrease(P<0.05) in serum chloride was observed on animals pretreated with MEAL along with gentamicin. These values are tabulated below

Table No.12- Effect of graded oral doses of MEAL on the serum chloride in gentamicin induced rat.

S.N	Groups	Serum chloride (mmol/l)
1	I	193.213±0.003
2	II	262.208±0.0001**
3	III	243.414±0.004##
4	IV	225.029±0.007##
5	V	214.284±0.001##
6	VI	210.307±0.006##

The data are expressed as Mean ±S.E.M (n=6) rats in each group
**P<0.05 when compared with Normal Control
##P<0.05 when compared with Gentamicin (100mg/kg) control

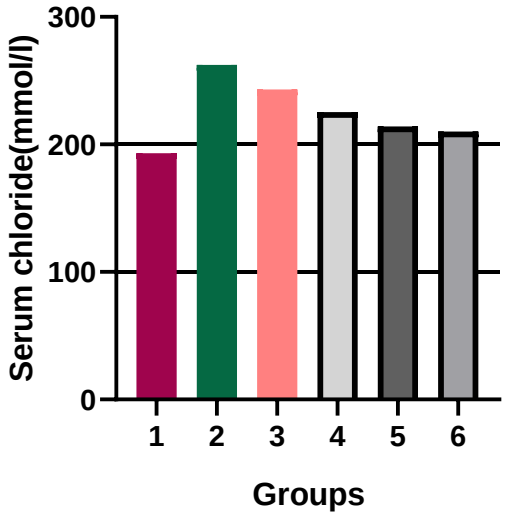


Fig 9- serum chloride of various groups of rats

5.6.7 **Effect of the gentamicin, standard and test drug on serum sodium**.

No significant changes in serum sodium levels are observed in gentamicin treated group and treatment groups. These values are tabulated below

Table No.13- Effect of graded oral doses of MEAL on the serum sodium in gentamicin induced rat.

S.N	Groups	Serum sodium (mmol/l)
1	I	142.021±0.321
2	II	147.384±0.227*
3	III	153.820±0.492#
4	IV	159.204±0.325#
5	V	162.923±0.273#
6	VI	158.235±0.459#

The data are expressed as Mean ±S.E.M (n=6) rats in each group
**P<0.05 when compared with Normal Control
##P<0.05 when compared with Gentamicin (100mg/kg) control

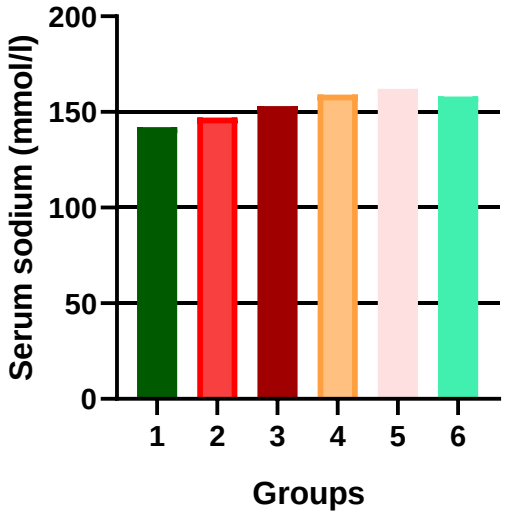


Fig 10- Serum sodium of the various groups.

5.6.8 Effect of the gentamicin, standard and test drug on serum potassium:

A slight increase($P<0.05$) in serum potassium was seen in gentamicin treated group when compared with the control group and dose dependent decrease ($P<0.05$) in serum potassium was observed on animals pretreated with MEAL along with gentamicin. These values are tabulated below.

Table No.14- Effect of graded oral doses of MEAL on the serum potassium in gentamicin induced rat.

S.N	Groups	Serum potassium(mmol/l)
1	I	4.876±0.003
2	II	8.152±0.005**
3	III	7.213±0.0001##
4	IV	6.319±0.004##
5	V	5.217±0.006##
6	VI	4.982±0.003##

The data are expressed as Mean ±S.E.M (n=6) rats in each group
**P<0.05 when compared with Normal Control
##P<0.05 when compared with Gentamicin (100mg/kg) control

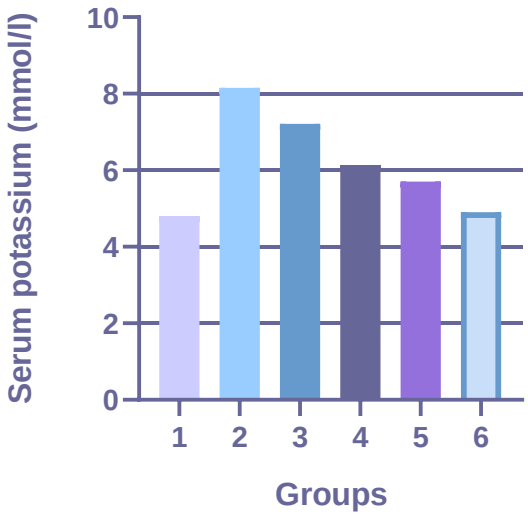


Fig 11- Serum potassium of various groups of rats.

5.6.9 Effect of the gentamicin, standard and test drug on urea:

A significant increase ($P<0.05$) in urea was seen in gentamicin treated group when compared with the control group and dose dependent decrease ($P<0.05$) in urea was observed on animals pretreated with MEAL along with gentamicin. These values are tabulated

Table No.15- Effect of graded oral doses of MEAL on the urinary urea in gentamicin induced rat.

S.N	Groups	Urea (mg/dl)
1	I	35.692±0.003
2	II	93.212±0.0001**
3	III	72.237±0.006##
4	IV	59.335±0.004##
5	V	41.882±0.007##
6	VI	37.204±0.0006##

The data are expressed as Mean ±S.E.M (n=6) rats in each group
**P<0.05 when compared with Normal Control
##P<0.05 when compared with Gentamicin (100mg/kg) control

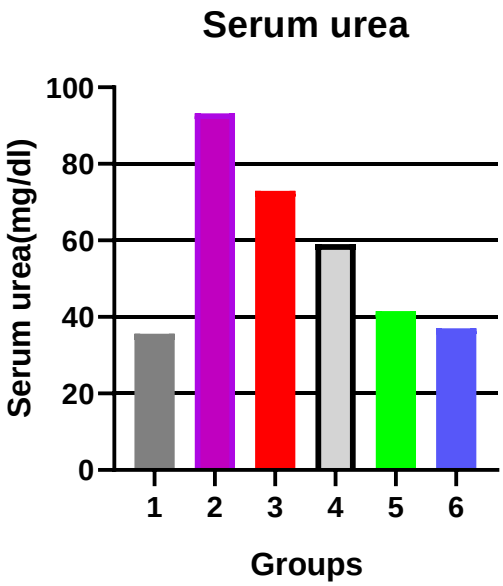


Fig 12- Serum urea of various groups of rats.

5.6.10 Effect of the gentamicin, standard and test drug on urinary creatinine:

A significant increase (P<0.05) in urinary creatinine was seen in gentamicin treated group when compared with the control group and dose dependent decrease (P<0.05) in urinary creatinine was observed on animals pretreated with MEAL along with gentamicin. These values are tabulated.

Table No.16- Effect of graded oral doses of MEAL on the urinary creatinine in gentamicin induced rat.

S.N	Groups	Urinary creatinine(mg/dL)
1	I	4.184±0.491
2	II	13.205±0.566**
3	III	10.453±0.225##
4	IV	7.348±0.001##
5	V	5.127±0.003##
6	VI	4.923±0.001##

The data are expressed as Mean ±S.E.M (n=6) rats in each group
**P<0.05 when compared with Normal Control
##P<0.05 when compared with Gentamicin (100mg/kg) control

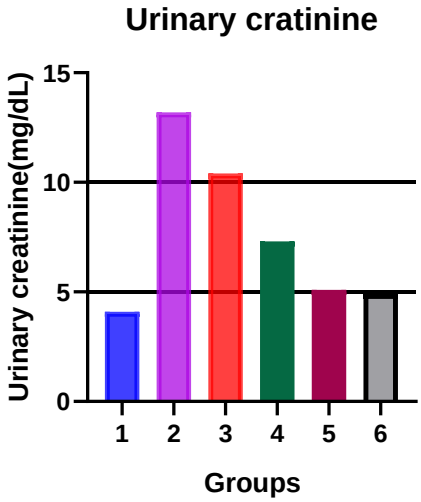


Fig 13- Urinary creatinine of the different groups of rats.

5.6.11Effect of the gentamicin, standard and test drug on Urinary total protein:

A significant increase(P<0.05) in urinary total protein was seen in gentamicin treated group when compared with the control group and dose dependent decrease(P<0.05) in urinary total protein was observed on animals pretreated with MEAL along with gentamicin. These values are tabulated

Table No.17- Effect of graded oral doses of MEAL on the urinary total protein in gentamicin induced rat.

S.N	Groups	Urinary total protein(g/L)
1	I	1.49±0.006
2	II	2.903±0.112**
3	III	2.433±0.023##
4	IV	1.957±0.003##
5	V	1.734±0.016##
6	VI	1.512±0.034##

The data are expressed as Mean ±S.E.M (n=6) rats in each group
**P<0.05 when compared with Normal Control
##P<0.05 when compared with Gentamicin (100mg/kg) control

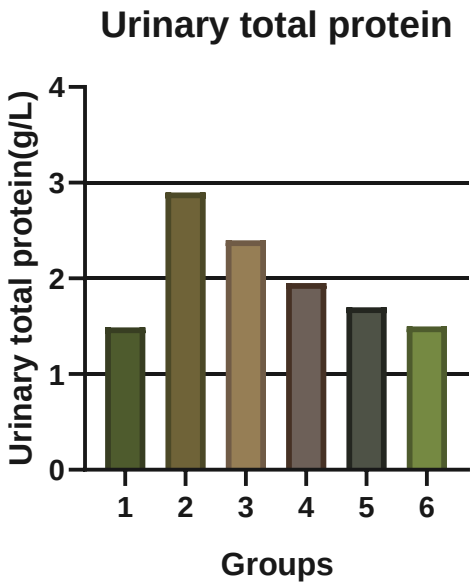


Fig 14- Urinary total protein of different groups of rats.

5.6.12 Effect of the gentamicin, standard and test drug on urinary uric acid:

A slight increase(P<0.05) in urinary uric acid was seen in gentamicin treated group when compared with the control group and dose dependent decrease(P<0.05) in urinary uric acid was observed on animals pretreated with MEAL along with gentamicin. These values are tabulated

Table No.18- Effect of graded oral doses of MEAL on the urinary uric acid in gentamicin induced rat.

S.N	Groups	Urinary uric acid(µmol/L)
1	I	10.357±0.932
2	II	14.763±0.557**
3	III	12.252±0.004##
4	IV	11.209±0.001##
5	V	10.953±0.003##
6	VI	10.562±0.011##

The data are expressed as Mean ±S.E.M (n=6) rats in each group
**P<0.05 when compared with Normal Control
##P<0.05 when compared with Gentamicin (100mg/kg) control

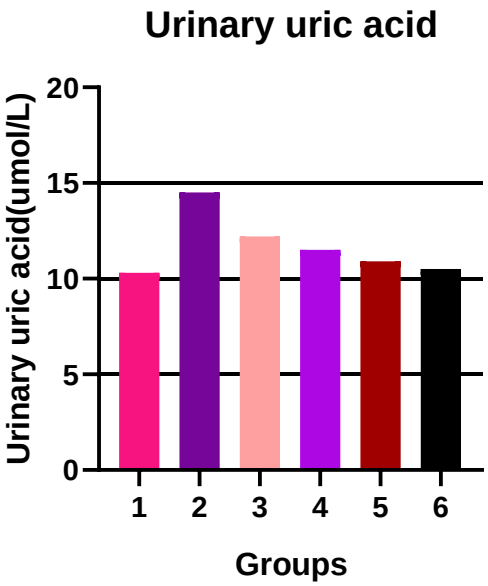


Fig 15- Urinary uric acid of various groups of rats.

5.7 Anti-oxidant studies:

5.7.1 Effect of the gentamicin, standard and test drug on super oxide dismutase:

A significant decrease (P<0.05) in super oxide dismutase was seen in gentamicin treated group when compared with the control group and dose dependent increase(P<0.05) in super oxide dismutase was observed on animals pretreated with MEAL along with gentamicin. These values are tabulated.

Table No.19- Effect of graded oral doses of MEAL on the super oxide dismutase in gentamicin induced rat.

S.N	Groups	SOD(units/mg protein)
1	I	8.963±0.023
2	II	4.512±0.011**
3	III	6.528±0.003##
4	IV	7.235±0.007##
5	V	8.361±0.223##
6	VI	8.782±0.001##

The data are expressed as Mean ±S.E.M (n=6) rats in each groups

**P<0.05 when compared with Normal Control

##P<0.05 when compared with Gentamicin (100mg/kg) control

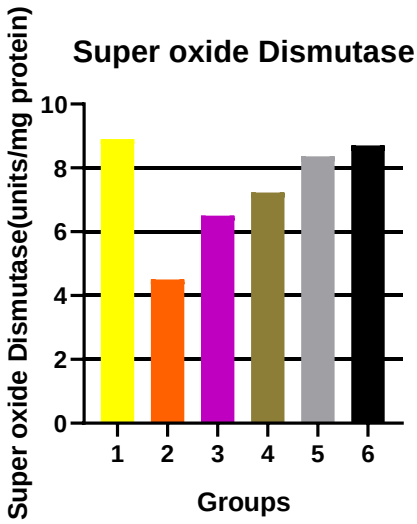


Fig 16- Super oxide Dismutase of various groups of rats.

5.7.2 Effect of the gentamicin, standard and test drug onCatalase:

A significant decrease (P<0.05) in catalase was seen in gentamicin treated group when compared with the control group and dose dependent increase(P<0.05) in catalase was observed on animals pretreated with MEAL along with gentamicin. These values are tabulated

Table No.20- Effect of graded oral doses of MEAL on the Catalase in gentamicin induced rat.

S.N	Groups	Catalase (mmol) of H_2O_2 consumed/min)
1	I	0.723±0.005
2	II	0.311±0.034**
3	III	0.405±0.004##
4	IV	0.547±0.0001##
5	V	0.781±0.0872##
6	VI	0.752±0.002##

The data are expressed as Mean ±S.E.M (n=6) rats in each group
**P<0.05 when compared with Normal Control
##P<0.05 when compared with Gentamicin (100mg/kg) control

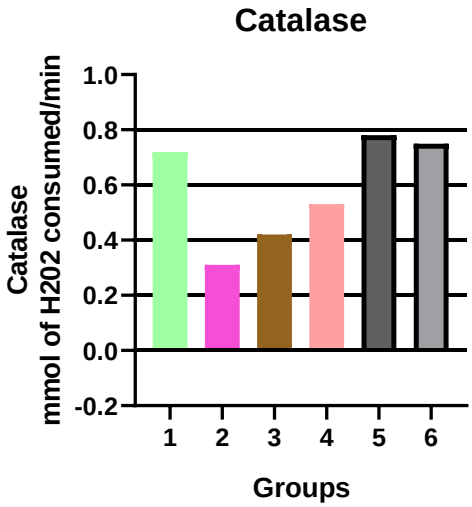


Fig 17- Catalase of various groups of rats.

5.7.3 Effect of the gentamicin, standard and test drug on lipid peroxidation:

A significant increase(P<0.05) in MDA levels was seen in gentamicin treated group when compared with the control group and dose dependent decrease(P<0.05) in MDA levels was observed on animals pretreated with MEAL along with gentamicin. These values are tabulated

Table No.21- Effect of graded oral doses of MEAL on the MDA levels in gentamicin induced rat.

S.N	Groups	MDA level (mM)
1	I	5.738±0.013
2	II	12.855±0.005**
3	III	10.236±0.002##
4	IV	7.315±0.003##
5	V	6.528±0.0011##
6	VI	5.912±0.023##

The data are expressed as Mean ±S.E.M (n=6) rats in each group
**P<0.05 when compared with Normal Control
##P<0.05 when compared with Gentamicin (100mg/kg) control

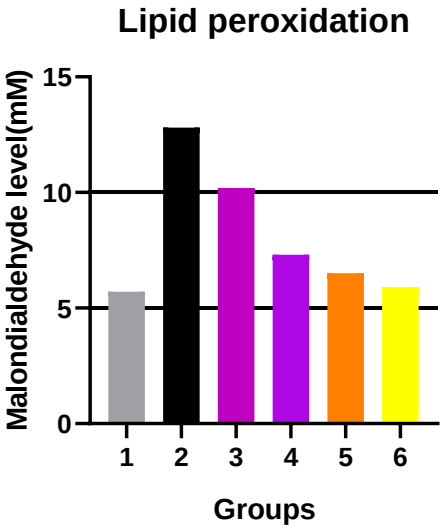


Fig 18- MDA levels of various groups

5.7.4 Effect of the gentamicin, standard and test drug on glutathione peroxidase:

A significant decrease(P<0.05) in glutathione peroxidase was seen in gentamicin treated group when compared with the control group and dose dependent increase(P<0.05) in glutathione peroxidase was observed on animals pretreated with MEAL along with gentamicin. These values are tabulated.

Table No.21- Effect of graded oral doses of MEAL on the glutathione peroxidase levels in gentamicin induced rat.

S.N	Groups	Glutathione peroxidase (mmol/min/mg protein)
1	I	4.957±0.001
2	II	2.125±0.045
3	III	2.871±0.003##
4	IV	3.541±0.055##
5	V	4.397±0.0004##
6	VI	4.667±0.015##

The data are expressed as Mean ±S.E.M (n=6) rats in each group
**P<0.05 when compared with Normal Control
##P<0.05 when compared with Gentamicin (100mg/kg) control

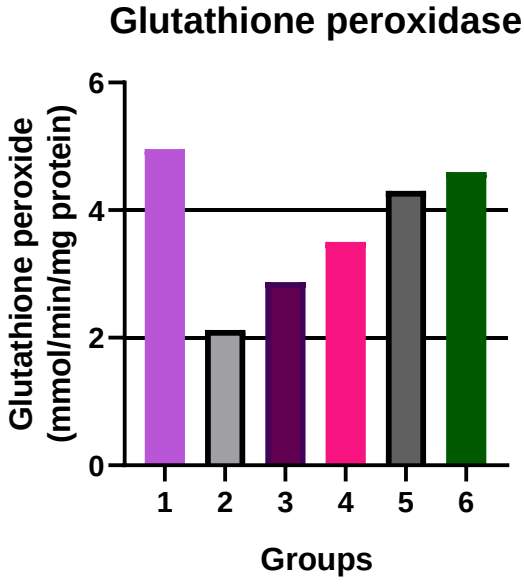
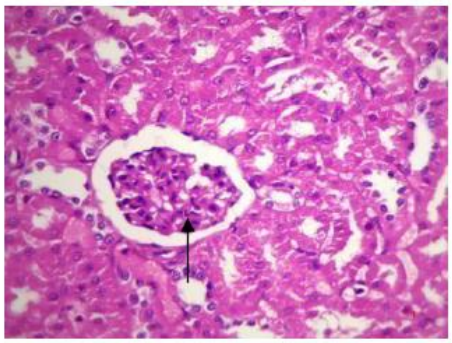


Fig 19- Glutathione peroxidase level in various groups of rats.

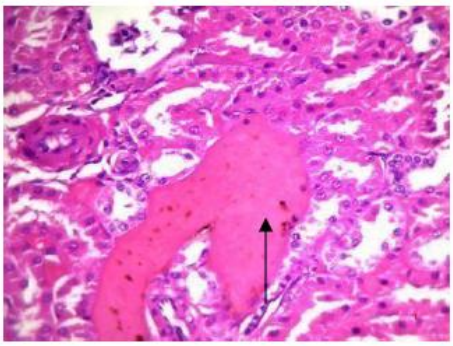
5.8 HISTOPATHOLOGICAL ANALYSIS.

5.8.1 EFFECT OF Normal saline (10ml/kg)

Architecture of kidney is Intact, Glomerulus have Normal cellularity [Fig.A, Arrow], Tubules are Intact, Blood Vessels are appearing congested [Fig.B, Arrow], Interstitium is to be Unremarkable.



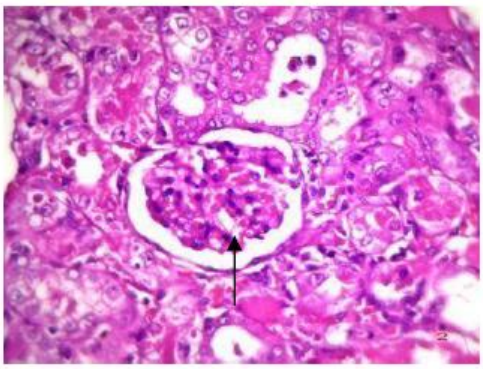
[Fig.A, H&E x400]



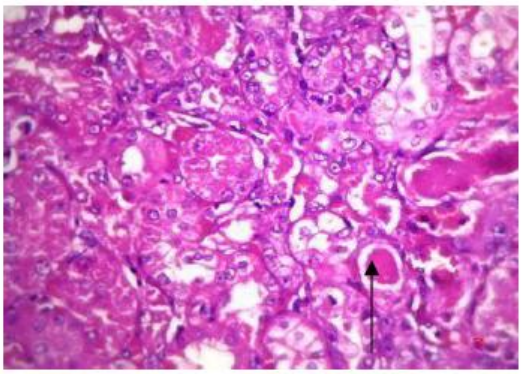
[Fig.B, H&Ex400]

5.8.2 EFFECT OF Gentamicin (100mg/kg) on kidney

Architecture of kidney is intact, Glomerulus is with Normal cellularity [Fig.A, Arrow], Tubules have marked Necrosis with tubular casts [Fig.B, Arrow],Blood Vessels are intact, Interstitium is with mild mononuclear inflammatory infiltration.



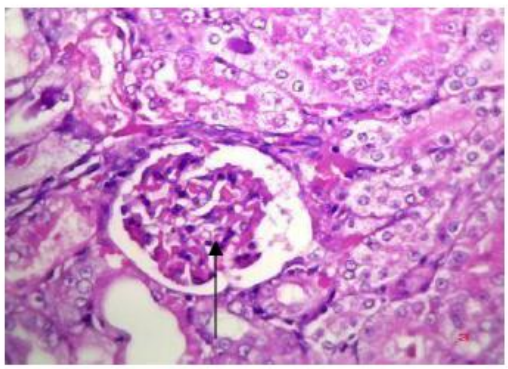
[Fig.A, H&E x400]



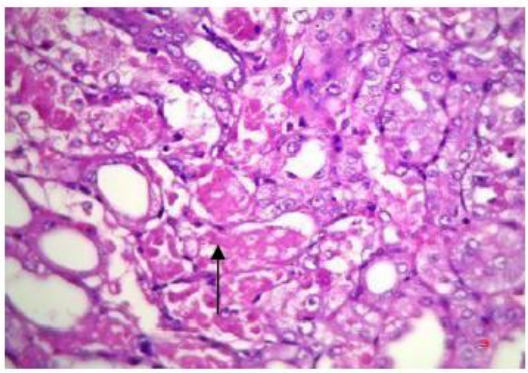
[Fig.B, H&Ex400]

5.8.3 EFFECT OF Vitamin E(200 mg/kg) + Gentamicin(100mg/kg) on kidney

Architecture of kidney was Intact, Glomerulus have Normal cellularity [Fig.A, Arrow],Tubules are found with moderate Necrosis [Fig.B, Arrow],Blood Vessels are Intact.
Interstitium is intact.



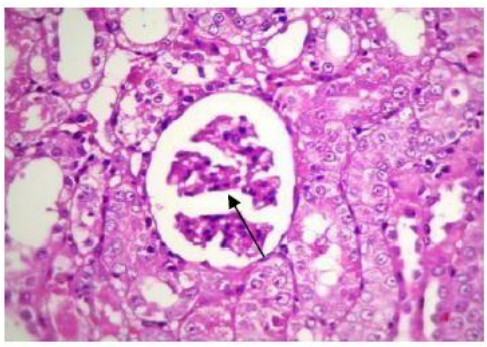
[Fig.A, H&E x400]



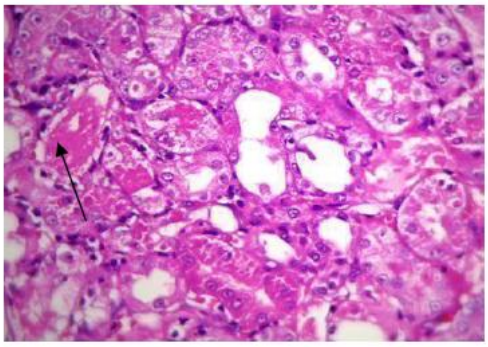
[Fig.B, H&Ex400]

5.8.3 EFFECT OF MEAL(150 mg/kg) + Gentamicin (100mg/kg) on kidney

Architecture of kidney was Intact, Glomerulus had Normal cellularity [Fig.A, Arrow],Tubules had Moderate Necrosis [Fig.B, Arrow],Blood Vessels are Intact, Interstitium is Intact.



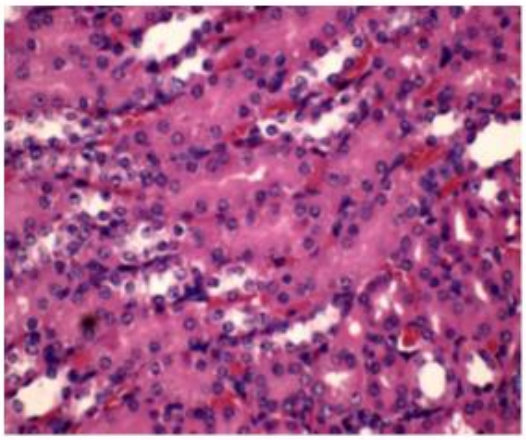
[Fig.A, H&E x400]

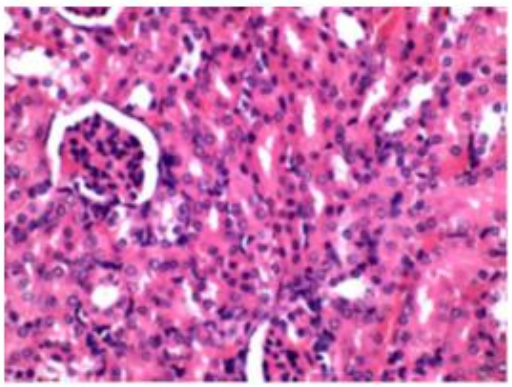


[Fig.B, H&Ex400]

5.8.4 EFFECT OF MEDL (300mg/kg)+ Gentamicin (100mg/kg) 0n kidney

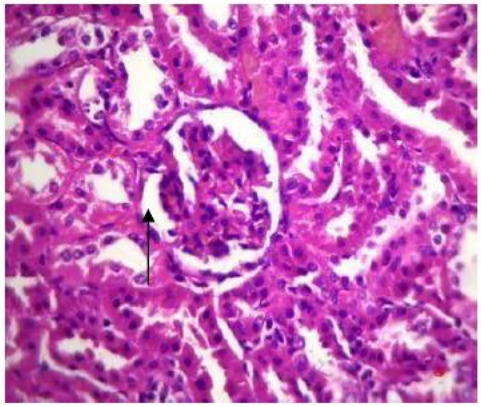
Architecture of kidney was Intact, Glomerulus had normal cellularity, Tubules were less intact and slight necrosis, Blood vessels appeared to be congested, Interstitium is slightly unremarkable.



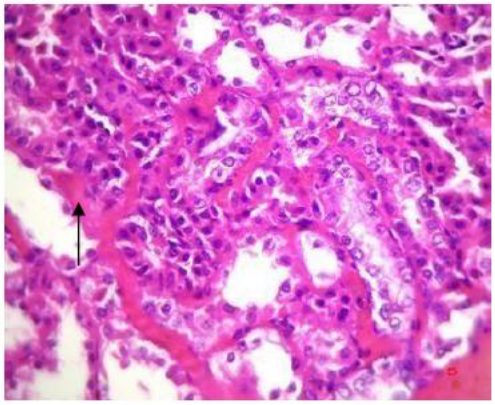


5.8.5 EFFECT OF MEAL (600 mg/kg) + Gentamicin (100mg/kg) on kidney

Architecture of kidney was Intact, Glomerulus had normal cellularity [Fig.A, Arrow], Tubules were Intact, Blood Vessels appeared congested [Fig.B Arrow], and Interstitium is Unremarkable.



[Fig.A, H&E x400]



[Fig.B, H&Ex400]

III. DISCUSSION

Acute kidney failure occurs when kidneys suddenly become unable to filter waste products from the blood. When kidneys lose their filtering ability, dangerous levels of wastes may accumulate, and blood's chemical makeup may get out of balance. Acute kidney failure — also called acute renal failure or acute kidney injury, it develops rapidly over a few hours or a few days. Acute kidney failure is most common in people who are already hospitalized, particularly in critically ill people who need intensive care. The incidence rate of acute kidney injury (AKI) around the world is not well known. Recent studies in the United States and Spain have shown incidences varying between an average of 23.8 cases per 1000 discharges with an 11% yearly increase between 1992 and 2001, to an increase from 61 to 288 per 100,000 population between 1988 and 2002 . More recently, Ali et al. reported a high incidence of 1811 cases of AKI per million population during 2003.[92] The relatively wide disparity in reported incidence rates and the increasing frequency of the condition raise concerns as to the real magnitude of the problem. In addition to a real increase in worldwide incidence, large differences in the definition of AKI and case mix likely underlie such differences. The kidneys are the significant focuses for the poisonous impacts of different chemical substances operators and thus drug-induced AKI is a frequent entity in clinical medicine. Drug induced renal toxicity is recognized as an important contributor to kidney diseases including AKI and CKD. The rate of nephrotoxic AKI is hard to gauge because of inconstancies of patient populaces and criteria of AKI. The occurrence of non - dialysis-requiring AKI is around 5000 cases per million individuals per each year and frequency of dialysis requiring AKI is 295 cases per million individuals per every year.[93]

Present was carried out to evaluate Nephroprotective and anti-oxidant activity of methanolic extract of leaves of Aerva lanata in gentamicin ind uced nephrotoxic rat. Preliminary, Phytochemical screening revealed the presence flavonoid, sterols, terpenoids, tannin, phenol, glycosides.

Acute toxicity studies were conducted according to OECD guidelines (425) up and down procedure, single dose administration of 2000mg/kg b.w. showed no mortality and so, further dose was increased to 5000mg/kg b.w., did not show any mortality and hence the dose of methanolic extract of leaves of Aerva lanata was fixed as 1/10th and 1/20th of 5000mg/kg as high dose and low dose respectively.

Gentamicin a aminoglycoside broad spectrum anti biotic obtained from Micromonospora purpurea, useful in treatment of infection caused by gram negative organism, when a dose 80mg/kg is given to rat for 10 days is sufficient to cause nephro toxicity. Gentamicin causes wide range of bio chemical changes like elevation of serum creatinine, serum urea, serum uric acid, serum total protein, urinary urea, urinary creatinine, urinary uric acid, urinary total protein and decrease in levels of anti-oxidant enzymes like super oxide dismutase, catalase, glutathione peroxidase. This might be due marked necrosis of tubules, decreased glomerular filtration and loss of interstitium architecture.

Pretreatment of rats with MEAL showed a significant reduction in elevated serum creatinine, serum urea, serum uric acid, serum total protein, urinary uric acid, urinary creatinine, urinary total protein and there was marked reduction in necrosis of tubules. Also, treatment with MEAL preserved the physiological weight gain of the animals over 10 days, as the body weight on day 11 was significantly more as compared with their baseline body weight.

In addition to bio chemical changes decrease in body weight may be due to reduced ingestion of food and increase in kidney weight may be due to marked necrosis of tubules that is distal convoluted tubules.

The present revealed that the methanolic extract of leaves of Aerva lanata possess nephroprotective action which might be due to of anti-oxidant property by extract. However, further studies are needed to identify, isolate, characterize, and screen organ protective activity.

IV. CONCLUSION

The present study was carried out to validate the nephroprotective activity of the methanolic extract of Aerva lanata leaves in gentamicin induced nephrotoxic rats.

The methanolic extract of Aerva lanata was used for dyspepsia, diarrhea, leprosy as well as immunomodulatory agent etc .,.

Preliminary phytochemical analysis reveals the presence of group of constituents which may be responsible for activity. Therefore, the methanolic extract of leaves of Aerva lanata was evaluated for nephroprotective activity and anti-oxidant activity.

The results of the study indicated that administration of methanolic extract of Aerva lanata at the dose of 250 and 500mg/kg b.w. possess nephroprotective activity in GM induced nephrotoxicity in rats. The acute toxicity study revealed that the extract was devoid of major toxic effect. The nephroprotective effect of MEDL was confirmed by its prevention over the GM induced toxicity. This MEDL reduced elevated kidney weight, serum potassium, chloride, serum urea and creatinine in GM treated rats. Histopathological studies proved that animal pre- treated with MEDL decreased the GM induced renal damage. MEDL also possess the protective effect against the oxidative induced stress which may be due anti-oxidant property of the drug

Hence, all the observations in the present study may indicate that MEDL act as a protective agent against gentamicin induced Nephrotoxicity. But experimental study should be followed by further experimental and clinical research to establish and exploit its protective role in drug-induced kidney injury.

V. SUMMARY

The present study deals with the nephroprotective and anti-oxidant activity of methanolic extract of Aerva lanata in gentamicin induced nephrotoxic rats.

Gentamicin a broad spectrum antibiotic used against gram negative organism infections. However, its use is limited because of nephrotoxicity.

In this work 30 wistar rats were divided in 6 groups each with 6 rats. group(1) received normal, group(2) received (gentamicin 100mg/kg), group(3) MEAL(150mg/kg)+ (gentamicin 100mg/kg), group(4) received MEAL(300mg/kg)+ (gentamicin 100mg/kg),group(5) received MEAL(600mg/kg) + (gentamicin 100mg/kg), group(6) received Vitamin E(200mg/kg), treatment was carried out for 10 days .

Rats treated with gentamicin alone showed significant increase in serum creatinine, serum urea, serum uric acid, urinary creatinine, urinary uric acid, urinary total protein and MDA levels, whereas rats treated with MEAL showed significant reduction in biochemical parameters and increase in levels of anti-oxidant enzymes like super oxide dismutase, catalase, glutathione peroxidase. Along, with that rats treated with gentamicin alone showed histological changes likes Tubules have marked Necrosis with tubular casts, Interstitium is with mild mononuclear inflammatory infiltration, and rats treated with MEAL showed Tubules had Moderate Necrosis, Blood Vessels are Intact, Interstitium is Intact. MEAL has protective effects against functional disturbances, oxidative stress and tissue damages induced by gentamicin.

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