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PHYTOCHEMICAL AND NEPROPROTECTIVE ACTIVITY OF ANNONA RETICULATA LEAF EXTRACT IN GENTAMICIN INDUCED NEPHROTOXICITY IN WISTAR RATS

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Abstract:

Natural products are the important source of bioactive compounds and have potential for the development of novel therapeutics. Natural products and their derivatives represent more than 50 % of all drugs in clinical use in the world. Herbal plants contain and produce a verity of chemical substance used as a remedy for treating diseases. Annona reticulata contain different chemical constituents. But it does not contain Glycosides, Alkloids, Phytosterols. Various drugs available in the market for nephro-protective activity and none of the drug is not up to mark for showing its efficacy. In the present examination treatment of rodents with ethanolic concentrate of underlying foundations of Annona reticulata significantly($p<0.05$ in 200mg/kg b.w. what's more, $p<0.01$ in 400mg/kg b.w.) diminished the degrees of SGPT in serum which means that nephrooprotective movement. SGOT is a mitochondrial catalyst discharged from heart, liver, skeletal muscle and kidney. nephro poisonous quality raised the SGOT levels in serum because of the harm to the tissues delivering intense corruption, for example, extreme viral hepatitis and intense cholestasis. Alcoholic liver harm and cirrhosis can likewise connect with gentle to direct height of transaminase.

Keywords: *Annona Reticulata, Gentamycin, Phytochemical screening, neproprotective activity.*

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INTRODUCTION:

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total. [1, 2]. Annona reticulata contain different chemical constituents. Various drugs available in the market for nephro-protective activity and none of the drug is not up to mark for showing its efficacy. From literature survey it was found that *Annona reticulata* effective in treatment of boils, abscesses and ulcers and used in dysentery and darrhoea, daibetes, insecticide, antiovulatory and abortifacient, hair tonic, malaria and syphilis [3-6]. The study period is 24 days and 15 days for gentamicin and cisplatin induced nephrotoxicity models. Animals used are male wistar rats in both models. Before performing the Nephro-protective activity of ethanolic extract of the plant aerial parts, phytochemical evaluation was done. Nephrotoxicity has been related to a selective accumulation of gentamicin in the renal cortex. Morphologic lesions of proximal tubules have been documented in optic microscopy. At the ultrastructural level, the earliest lesions observed concern lysosomes, which show an accumulation of myeloid bodies due to genearation of free oxide anions [7-15]. The cysteinyl-glycine-conjugates of Cisplatin are further metabolized to cysteine-conjugates causes the renal damage[16-18].

MATERIALS AND METHODS:

Collection, identification and Authentication of plants:

Annonareticulata utilized for the present examinations was gathered from Chitoor locale of Andhra pradesh. The plant was recognized, affirmed and verified by contrasting and voucher example accessible at Survey of restorative plants and gathering unit, Department of Botany, Sri Venkateswara University, Tirupathi by Field Botanist Dr. Madhavshetty.

Extraction procedure:

Freshly collected plant materials was dried under shade and the dried material was milled to obtain a coarse powder. To the coarse powder was packed in a soxhlet apparatus and subjected to extraction with Eathnol. The liquid extracts was collected and evaporated under reduced pressure until a soft mass obtained. The mass obtained was weighed in each case. The extracts were thoroughly air dried to remove all traces of the solvent. The percentage hield of extraction is shown in Table 1

Preliminary Phytochemical Screening:

The condensed extracts were used for preliminary screening of phytochemicals such as cholesterol, alkaloid, flavanoids, saponin, cardiac glycosides and terpenoids[19-20]. The phytochemical screening shown in table 2.

Screening Procedure:

Test for flavonoids:

Add a few drops of concentrated HCL and Mg turning to 1 ml of ethanol extract. Appearance of pink or magenta-red colour indicates the presence of flavanoids.

Test for cholesterol:

To 2 ml of the extract 2 ml of the chloroform was added in a dry test tube. Then 10 drop of acetic anhydride and 2 to 3 drops of con. H2SO4 was added. A red colour changed to blue green colour.

Test for Alkaloids:

To the extract added 1% HCl and 6 drops of Mayer's reagent and Dragendorff reagent. Any organic precipitate indicated the presence of alkaloids in the sample.

Test for terpenoids:

5ml of each extract was added to 2ml of chloroform and 3ml of con.H2SO4 to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the terpenoids.

Test for cardiac glycoside:

5mlof each extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of con.H2SO4. A brown ring of the interface indicated a deoxysugar characteristic of cardenolides. A violet ring might appear below the brown ring whereas acid layer, a greenish ring might form just gradually throughout thin Layer.

Test for steroids:

2 ml of acetic anhydride was added to 0.5 g of ethanolic extract of each sample with 2ml of H2SO4. The colour change from violet to blue or green indicated the presence of steroids

Test for Saponins:

The extract with 20 ml of distilled water was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam indicated the presence of saponins.

Pharmacological studies:
Experimental Animals Wistar rats (150-200 g) of both sexes were obtained from the animal house of from Pharmacy collage. Before and during the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, and dark/light cycle and relative humidity. Animals described as fasting were deprived of food and water for 16 h ad libitum. All animal experiments were carried out in accordance with the guidelines of CPCSEA and study was approved by the IAEC (Institutional animal ethical committee).

Induction of Nephrotoxicity:
Induction of Nephrotoxicity By Gentamicin [21].
The Nephrotoxicity in this model was induced by 80 mg/kg weight of animal by intra muscular route administration. The study period is 14 days and 24 days in preventive and curative regimen respectively.

EXPERIMENTAL PROCEDURE:
Nephroprotective examinations [22]
Impact of Annonareticulata on gentamicin-incited nephrotoxicity
Trial configuration: Rats will be separated into five gatherings, each gathering comprising of six creatures.
Gathering 1: Control with ordinary saline (5 ml/Kg)
Gathering 2: Gentamicin (80 mg/kg/body weight, i.p.), day by day for 10 days
Gathering 3: Ethanol concentrate of Annonareticulata (200 mg/kg/body weight,p.o) and all the while directed gentamicin (80 mg/kg/body weight, i.p.), day by day for 10 days.
Gathering 4: Ethanol concentrate of Annonareticulata (400mg/kg/body Weight,p.o.) and all the while managed gentamicin (80 mg/kg/body weight, i.p.), every day for 10 days.
Gathering 5: Silymarin (25mg/kg/body Weight, p.o.) and all the while managed gentamicin (80 mg/kg/body weight, i.p.), day by day for 10 days.

Toward the finish of test period, every one of the creatures will be relinquished under diethyl ether anesthesia. Blood tests will be gathered, permitted to clump. Serum was isolated by centrifuging at 2500 rpm for 15 min and dissected for different biochemical parameters. The neprotoxicity results shown in table 3-5, Graph 1-6 and Figure 1-5.

Appraisal of kidney work:
Biochemical parameters i.e., Estimation of Blood urea, Creatinine and uric corrosive were examined by the detailed techniques. The kidney was expelled, gauged and morphological changes were watched. A part of kidney was fixed in 10% formalin for histopathological examines

Statistical examination of data:
Results were communicated as mean ± S.E.M. The measurable contrast between the gatherings in the term of the mean rate of wound mending was determined as far as ANOVA mean ± S.E.M. The thing that matters was viewed as noteworthy if P< 0.05.

RESULTS:

Percentage yield of extraction

Table no1: Percentage yield

S.No	Type of extraction	Percentage yield
1.	70% Eathanol	17.54%

Quantitative phytochemical analysis of extracts

Table no2: Quantitative phytochemical analysis

S.No	Test	Ethanol extract
1.	Carbohydrates	+
2.	Glycosides	-
3.	Protins	+
4.	Fixed oils & Fats	+
5.	Alkaloids	-
6.	Phytosterols	-
7.	Flavanoides	+
8.	Phenolic compounds	+
9.	Saponins	+
10.	Tannins	+

pharmacological activity:

In gentamicin treated gathering of creatures the convergence of serum urea and creatinine were impressively expanded than the ordinary creatures (bunch 1) whichindicates serious nephrotoxicity. Treating (bunch 4 and 5) with ethanol concentrate of demonstrated critical reduction ($p<0.001$) in convergence of serum urea and creatinine contrasted with gentamicin treated gathering 2. In any case the convergence of uric corrosive less significantly expanded in the gentamicin treated gatherings (bunch 2) than control gathering (group1). Treatment with ethanol concentrate of altogether ($p<0.05$) diminishes the uric corrosive levels in gathering 4 and 5 ($p<0.01$) contrasted with gentamicin treated gathering (bunch 2).

Table 3: Effect of 80 mg/kg/day intraperitoneal gentamicin and Annonareticulata oral on serum creatinine; blood urea and serum uric corrosive in treated rodents for 10 days

Group	Drugtreatment	Serumcreatinine(mg/dl)	Bloodurea(mg/dl)	Uricacid(mg/dl)
1	5 ml/kg,i.p, NS	0.681±0.05309	22.622±1.783	4.0233±0.4233
2	80mg/kg,i.p, gentamicin	1.261±0.03701	118.76±5.981	5.136±0.273
3	80mg/kg,i.p, gentamicin+200 mg/kg	0.8566±0.0417***	54.932±6.196** *	3.933±0.2693*
4	80mg/kg,i.p, gentamicin+400 mg/kg	0.7441±0.04849** *	49.962±4.204** *	3.5733±0.1719* *
5	80mg/kg,i.p, gentamicin+Silymarin 25 mg/kg	0.7041±0.03849** *	47.762±4.204** *	3.2533±0.1719* *

Kidney weight:

In gentamicin treated gathering of creatures weight of kidneys were extensively expanded contrasted with typical creatures (group1) and treating (bunch 4 and 5) with ethanol concentrate indicated noteworthy lessening ($p<0.001$) in kidney weight.

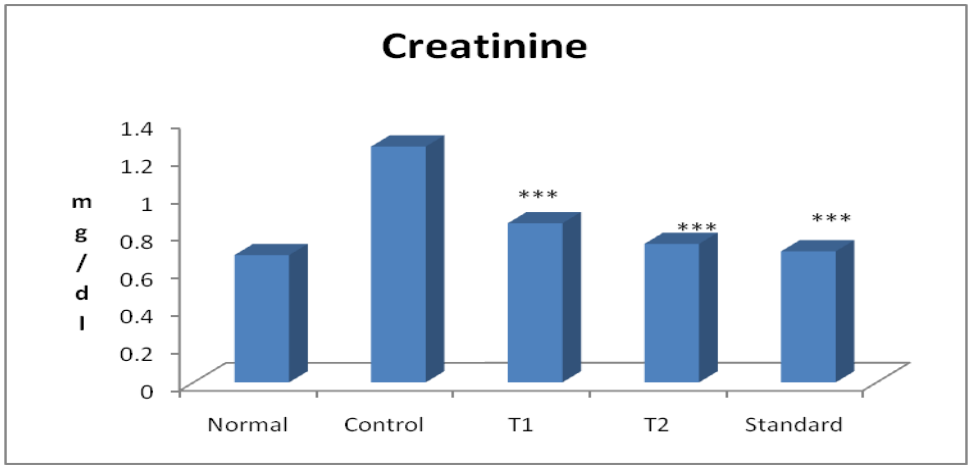
Table 4: Effect of 80 mg/kg/day intraperitoneal gentamicin and Annonareticulata oral on kidney weight in treated rodents for 10 days

Group	Drug treatment	weight(gm)
1	10 ml/kg, i.p, NS	0.567±0.0136
2	80mg/kg,i.p, gentamicin	0.712±0.0138
3	80mg/kg,i.p, gentamicin+200 mg/kg	0.6±0.0146***
4	80mg/kg,i.p, gentamicin+400 mg/kg	0.567±0.0099***
5	80mg/kg,i.p, gentamicin+silymarin mg/kg	0.546±0.0078***

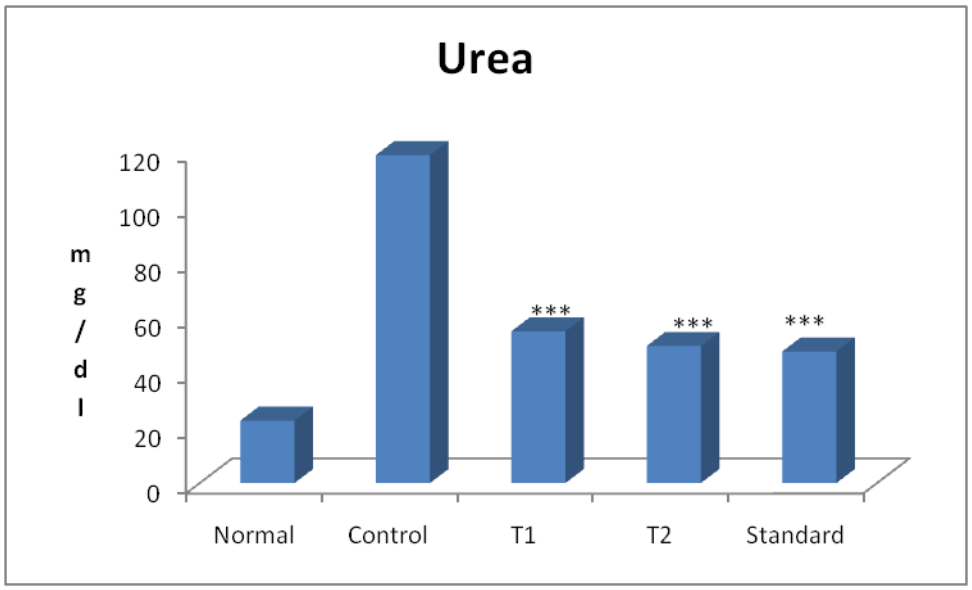
N=6 creatures in a gathering; Values are communicated as Mean ± SEM;
*: $p<0.05$, **$p<0.01$, $p<0.001$ versus Toxicant Control. ns demonstrate no noteworthy.

Table 5: Effect of 80 mg/kg/day intraperitoneal gentamicin and Annonareticulata oral on SGOT, SGPT, ALP in treated rodents for 10 days

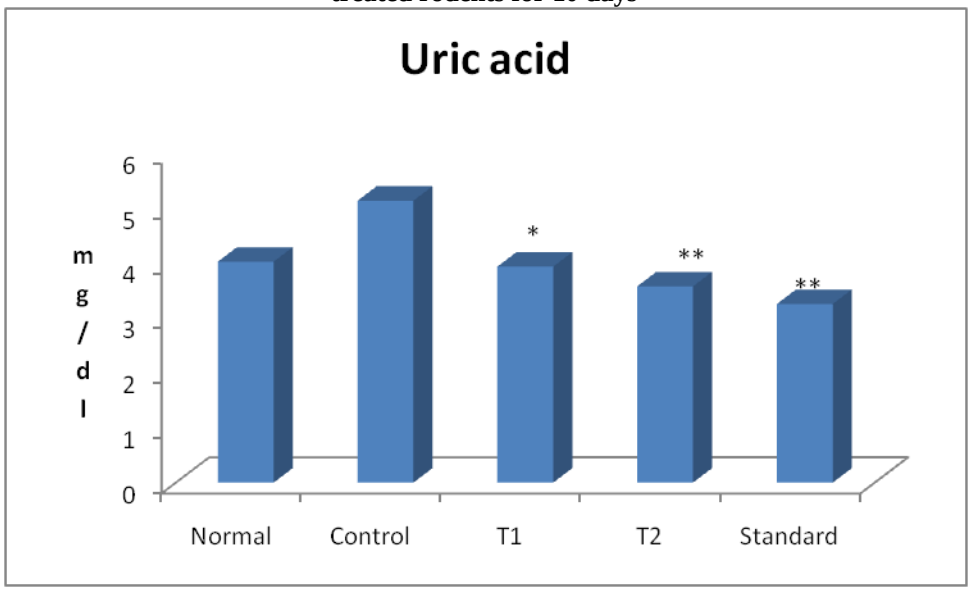
Group	Drugtreatment	SGPT levels(U/L)	SGOTlevels(U/L)	ALPlevels(U/L)
A	10 ml/kg, i.p, NS	42.6.8±1.23	45.25±1.36	34.56±1.56
B	80mg/kg,i.p, gentamicin	123.45±1.45**	136.19±3.48***	92.52±2.77***
C	80mg/kg,i.p, gentamicin+200 mg/kg	89.38±0.87**	92.45±1.76***	73.74±1.38**
D	80mg/kg,i.p, gentamicin+400 mg/kg	65.26±2.14***	55.38±1.45***	51.38±1.54**
E	80mg/kg,i.p, gentamicin+silymarin mg/kg	45.4 7±1.31***	48.18±1.57***	44.47±1.67***



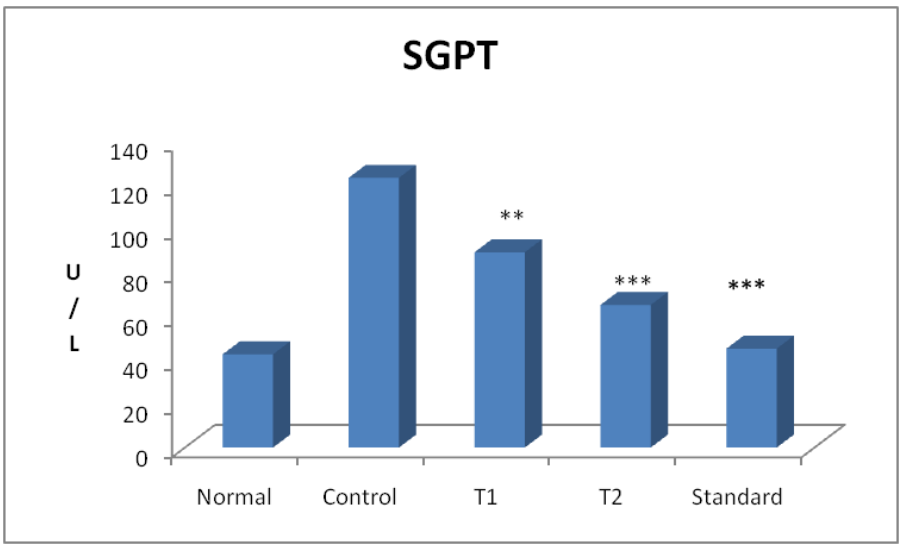
Graph 1: Effect of 80 mg/kg/day intraperitoneal gentamicin and Annonareticulataoral on serum creatinine; in treated rats for 10 days



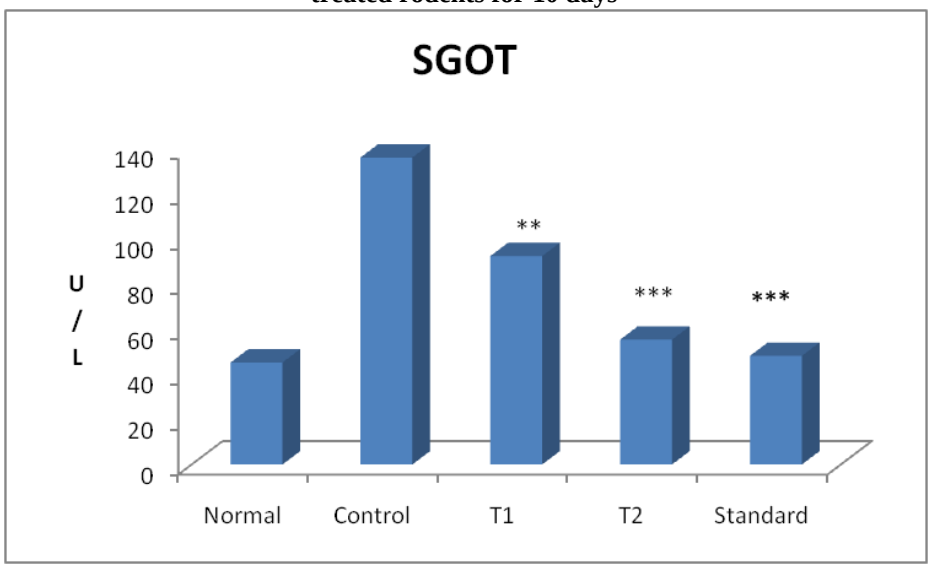
Graph 2: Effect of 80 mg/kg/day intraperitoneal gentamicin and Annonareticulata oral on blood urea in treated rodents for 10 days



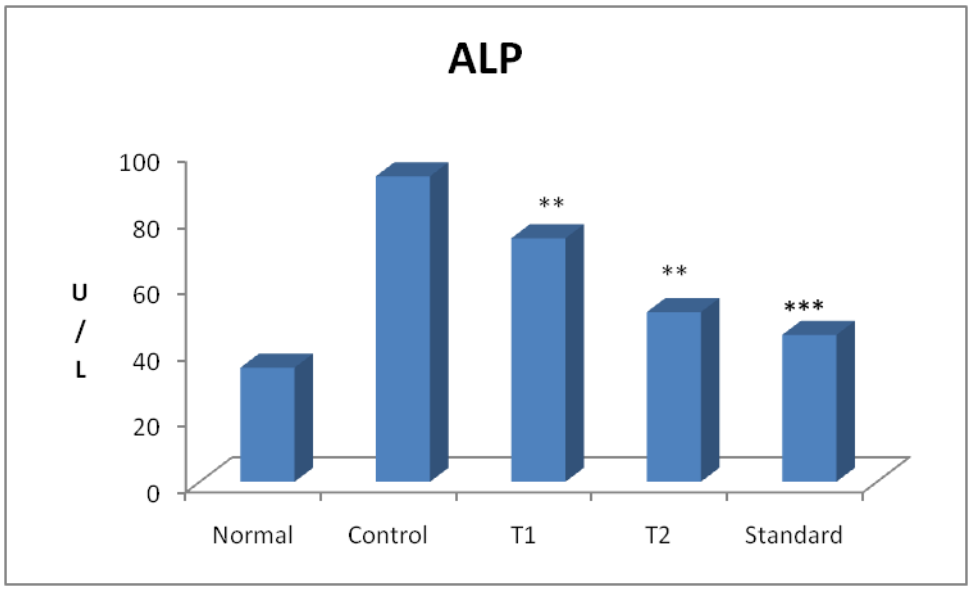
Graph 3: Effect of 80 mg/kg/day intraperitoneal gentamicin and Annonareticulata oral on serum uric corrosive in treated rodents for 10 days



Graph 4: Effect of 80 mg/kg/day intraperitoneal gentamicin and Annonareticulata oral on serum SGPT in treated rodents for 10 days



Graph 5: : Effect of 80 mg/kg/day intraperitoneal gentamicin and Annonareticulata oral on serum SGOT in treated rodents for 10 days



Graph 6: :Effectof80mg/kg/day in traperitonealgentamicin and *Annonareticulata* oralonserumALPintreatedratsfor10days

Gentamicin induced Nephrotoxicity Histopathology

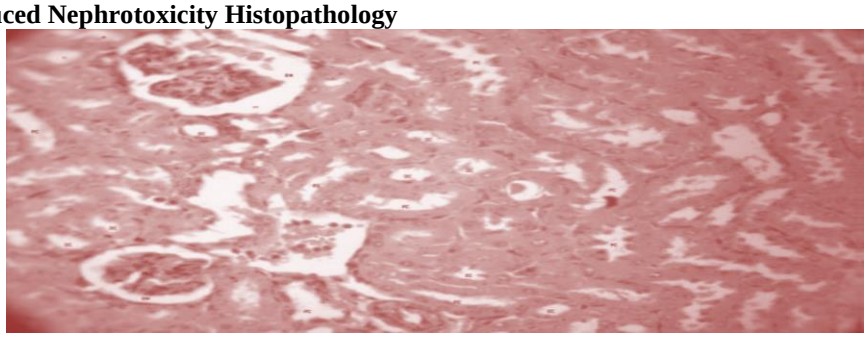


Figure 1: A sectional portrayal of ordinary rodent kidney indicating typical glomeruli with an unblemished Bowman's container , proximal tangled and distaltangledtubules

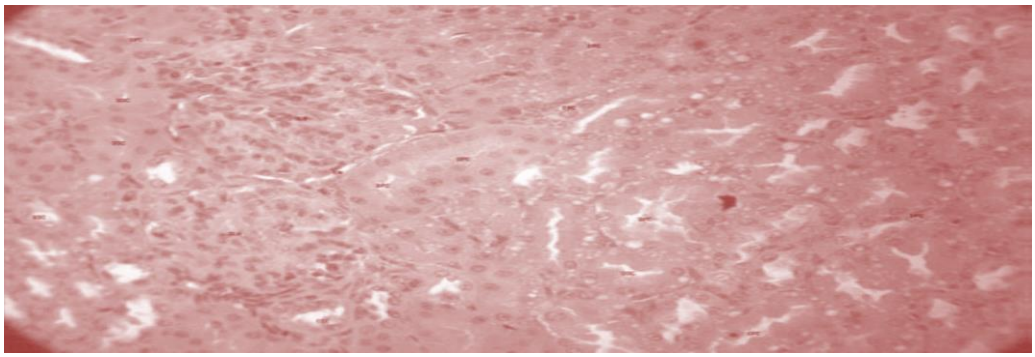


Figure 2: The cylindrical lumens were totally crushed and loaded up with liquid and throws

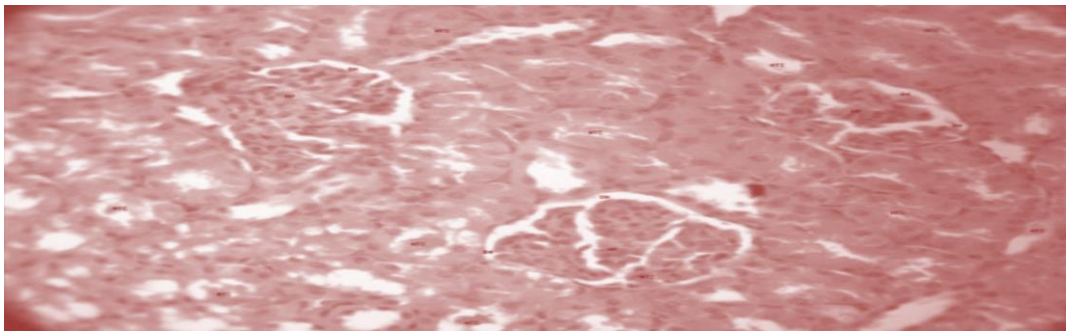


Figure 3: A sectional portrayal of 200 mg/kg/day, gentamicin–inebriated rodent kidney demonstrating mesengial expansion with dispersing of the Bowman's case. There is gentle rounded cast affidavitmediated with typical proximal tangled tubule and distal tangled tubule

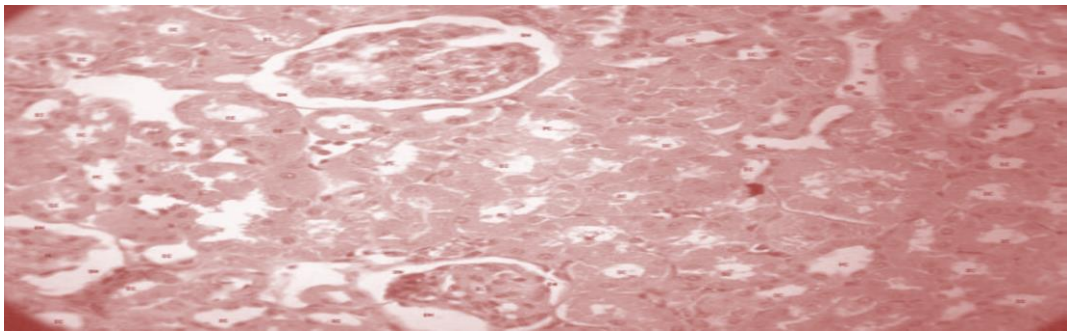


Figure 4: A sectional portrayal of 400 mg/kg/day gentamicin–inebriated rodent kidney indicating ordinary glomeruli embodied by typical Bowman's case. There is no undeniable cylindrical cast affidavit

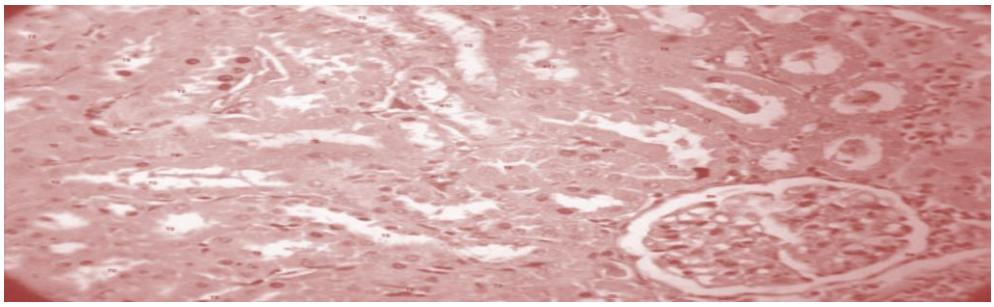


Figure 5: A sectional portrayal of 25 mg/kg/day gentamicin–inebriated rodent kidney appearing cylindrical degeneration with ordinary glomeruli and Bowman's container .

Medication incited nephrotoxicity are frequently connected with checked height in bloodurea, serum creatinine and intense cylindrical putrefaction. So these biochemical parameters have been utilized to research medication incited nephrotoxicity in creature and man. In the present investigation medication initiated nephrotoxicity were set up by single every day of the Gentamicin and Acetaminophen, for 10 days and single day by day organization intraperitoneal acetaminophen for 14 days. This poisonous quality described by stamped height in the circling levels of blood urea, serum creatinine and histological highlights of tubulonephritis in the model control(group 2) rodents when contrasted with untreated(group 1) rodents. Anyway these progressions were credited by corresponding treatment with single day by day reviewed dosages of EAR extricate for 10 days. Oral organization of plant remove altogether diminishes the urea and creatinine level in both treatment gathering contrast with toxicant gathering. Aside from the direct nephrotoxic impact of Gentamicin and Acetaminophen in gathering 2 rodents, the intense height in the deliberate biochemical parameters could likewise be ascribed to expanded catabolic condition of the

rodents due to the drag out anorexia related with Gentamicin and Acetaminophen nephrotoxicity.

In renal maladies, the serum urea aggregates on the grounds that the rate of serum urea generation surpasses the rate of freedom. Height of urea and creatinine levels in serum was taken as the list of nephrotoxicity. Creatinine gets from endogenous sources by tissue creatinine breakdown. In this way serum urea fixation is frequently viewed as a more dependable renal capacity expectation than serum creatinine. At any rate the degree of uric corrosive is nonsignificantly expanded in the toxicant gathering when contrasted with control. Oral organization of plant separate fundamentally diminishes the uric corrosive level in both treatment gathering contrast with toxicant gathering.

It was built up that Gentamicin is effectively moved into proximal tubules after glomerular filtration in a little extent where it causes proximal cylindrical damage and variations from the norm in renal course that prompts a decrease of GFR.

In histopathological investigation of Normal gathering demonstrating some veins are enlarged and clogged inside the interstitium. Likewise few dispersed mononuclear fiery penetration is seen inside the interstitium. Gentamicin treated gathering appearing glomerular blockage, Tubular throws, Peritubular clog, epithelial desquamation, Blood vessel clog. While treatment gathering show glomerularcongestion, Peritubular congestion,

Focal hydrophic degeneration of cylindrical epithelial cells and treatment group (400 mg/kg, Group IV) demonstrates just a portion of the veins are enlarged and clogged inside the interstitium. Additionally, few dissipated mononuclear provocative penetrations is seen inside the interstitium. From histopatological results we can reason that EAR remove at portion of 200 mg/kg have halfway defensive impact while EAR separate at portion of 400 mg/kg have defensive impact on Gentamicin prompted nephrotoxicity.

The discoveries propose the potential utilization of ethanol concentrate of EAR a remedially helpful nephroprotective specialist. Consequently further investigations to clarify their instruments of activity ought to be directed to help the disclosure of new restorative specialists for the treatment of renal illnesses.

During hepatic and nephro harm, cell proteins like AST, ALT and ALP present in the liver cells spill into the serum, bringing about expanded fixations. Gentamicin organization for 10 days essentially expanded all these serum compounds.

In the present examination treatment of rodents with ethanolic concentrate of underlying foundations of Annonareticulatasignificantly($p<0.05$ in 200mg/kg b.w. what's more, $p<0.01$ in 400mg/kg b.w.) diminished the degrees of SGPT in serum which means that nephrooprotective movement.

SGOT is a mitochondrial catalyst discharged from heart, liver, skeletal muscle and kidney. nephro poisonous quality raised the SGOT levels in serum because of the harm to the tissues delivering intense corruption, for example, extreme viral hepatitis and intense cholestasis. Alcoholic liver harm and cirrhosis can likewise connect with gentle to direct height of transaminase. In the present investigation treatment of creatures with ethanolic concentrate of leaves of Annonareticulatasignificantly($p<0.05$) diminished the degrees of SGOT in serum which is a characteristic of nephroprotective action.

If there should arise an occurrence of dangerous kidney, antacid phosphatase levels are high, which might be because of damaged hepatic discharge or by expanded generation of ALP by parenchymal or conduit cells.

In the present examination treatment of creatures with ethanolic concentrate of Annonareticulatasignificantly($p<0.05$ in 200mg/kg b.w. what's more, $p<0.001$ in 400mg/kg b.w) diminished the degrees of ALP in serum as a sign of nephroprotective movement

CONCLUSION:

Annona reticulata investigated for phytochemical screening. It does not contain Glycosides, Alkaloids, Phytosterols. It contains remaining other chemical constituents. In the present examination treatment of rodents with ethanolic concentrate of underlying foundations of Annona reticulata significantly ($p<0.05$ in 200mg/kg b.w. what's more, $p<0.01$ in 400mg/kg b.w.) diminished the degrees of SGPT in serum which means that nephrooprotective movement. SGOT is a mitochondrial catalyst discharged from heart, liver, skeletal muscle and kidney. nephro poisonous quality raised the SGOT levels in serum because of the harm to the tissues delivering intense corruption, for example, extreme viral hepatitis and intense cholestasis. Alcoholic liver harm and cirrhosis can likewise connect with gentle to direct height of transaminase.

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