CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.3592664

Available online at: http://www.iajps.com Research Article

PHYTOCHEMICAL SCREENING AND NEPROPROTECTIVE ACTIVITY OF *CITRUS MAXIMA*

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Abstract:
Several plants with medicinal properties are used to treat diseases. Many rely on traditional medicines, chiefly plant-based, for there major health care needs. The wide biological and medicinal properties, higher safety margins and affordability, make plant-based medicines attractive for usein developing countries for primary health care. Citrus maxima belongs to the family of Rutaceae The phytochemical screening showed the presence of alkaloids, terpenoids, sterol's,flavonoids and carbohydrates. But it does not contain tannins. In the present study, it was observed that treatment with gentamicin induced a significant elevation in the levels of serum urea, creatinine, blood urea nitrogen, serum urea and weight of kidney. However, daily treatment with Citrus maximus for 8 days conferred nephroprotection on gentamicin induced rats in a dose dependent fashion offered maximum protection.
Keywords: *Citrus Maxima, Gentamycin, Phytochemical screening, nephroprotective activity*

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Please cite this article in press ***Badhavath Ravi et al.****,* ***Phytochemical Screening And Neproprotective Activity Of Citrus Maxima .,*** *Indo Am. J. P. Sci, 2019; 06(12).*

INTRODUCTION:

Several plants with healthful properties area unit wont to treat diseases.[1] several think about ancient medicines, in the main plant-based, for there major health care desires.[2] The wide biological and healthful properties, higher safety margins and affordability, build plant based mostly medicines enticing for usein developing countries for primary health care.[3] citrus is Associate in Nursing fruit that belongs to the family of rue family. Its flesh is juicy, soft in texture and loaded in nutrients and is endemic to tropical a part of Asia.[4] Ancient texts mention its various uses and in addition describe its vital role in Ayurvedic or natural drugs practices because of its very important constituents.[5] The fruit and pulp area unit cited as nontoxic, appetizer, and viscus stimulant and abdomen tonic in ancient medical literature.[6] This plant has been used for the treatment of fatigue, diabetes, fever, insomnia, raw throat, carcinoma, coughs, and viscus disorders in Philippines and encompassing geographical region. Recently leaves of this fruit area unit according to own antitumour and system depressants activity[7,8] and its inhibitor potential is additionally used against paracetamol-evoked hepatotoxicity in rats.[9] antiseptic activity of the leaves, peel and pulp is additionally according.[10-13] whereas bark of this plant is according to own anti-diabetic activity.[11] during this study we tend to evaluated the analgesic, medicinal drug and central systema nervosum (CNS) depressant activity of methanolic extract of depart citrus fruits. this investigation was embraced to assess the Nephro defensive movement and advantageous impacts of ethanolic concentrate of citrus organic product strip in Gentamycin prompted Nephrotoxicity in rodents.

MATERIALS AND METHODS:

Collection, identification and Authentication of plants:

The plant was gathered during the long stretch of march2014, from Tekisettipalem and Anthervedipalem and Gudemellanka towns. The plant was confirmed by Mrs. P. Prasannakumari, Head of the Department of Botany, D.N.R. School of drug store, Bhimavaram.

Extraction procedure:

The plant material was collected. They are dried inshade for 20 to 30 days, when they are totally dried they are oppressed for size reduction. The dried materials are crushed to fine powder with the assistance of blender**.**The coarse powder was packed in a soxhlet apparatus and subjected to extraction with 90%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.[14,15] The phytochemical screening shown in table 2.

Screening Procedure

Test for flavanoids

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 albino rats (150-200 g) of either sex were obtained from the animal house. During and before the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd.). After randomization, the rats into various groups and before initiation of experiment, the rats were acclimatized for 7 days under standard environmental conditions of temperature, and dark/light cycle and relative humidity. Rats described as fasting, when deprived of food and water for 16 h ad libitum. All experiments on rats were carried out in accordance with the guidelines of CPCSEA and study ware approved by the IAEC (Institutional animal ethical committee).

Gentamicin induced nephrotoxicity in rats
The albino rats (150-200 g) of both sexes will be randomly divided into 5 groups of 6 each.
Thedifferent groups as described below.
Group I: Vehicle control;
Group II: Nephrotoxicity group (Gentamicin 100 mg/kg);
Group III:Citrus maxima extract (200 mg/kg, p.o.)+Gentamicin (100 mg/kg, i.p.);
Group IV: Citrus maxima extract (400 mg/kg, p.o.)+Gentamicin (100 mg/kg, i.p.);
Group V: Standard polyherbal drug cystone (5 ml/kg; p.o.)+Gentamicin (100 mg/kg, i.p.).

Experimentalprocedure
The gentamicin treated groups received 100 mg/kg/day gentamicin by the intraperitoneal (i.p.) route. Rats in the control
group I were given sterile saline solution for 8 days. Group II received 100 mg/kg gentamicin i.p. alone for 8 days.
Group III received 100 mg/kg gentamicin i.p. and Citrus maxima 200 mg/kg/ p.o. for eight days Group IV received 100 mg/kg/ gentamicin i.p. and Citrus maxima 400 mg/kg/p.o. for eight days. Group V received 100 mg/kg/ gentamicin i.p. and standard polyherbal drug cystone (5 ml/kg; p.o.) Freightways. After dosing on the 8th day, blood samples were collected via cardiac puncture method at the end of 24 h. The serum was rapidly separated and processed for determination of serum uric acid, serum creatinine, blood urea nitrogen (BUN) and serum urea using commercially available kits of Span Diagnostics. Changes in kidney weight were recorded. Three rats per group were sacrificed and both kidneys were isolated from each rat. The kidneys were weighed and processed for histopathological examinations.[16]
Histopathological examination of kidney
The kidneys were longitudinally sectioned in two halves and were kept in 10% neutral formalin solution. Both kidneys were processed, embedded in paraffin wax and sections were taken using a microtome. The sections were stained with hematoxylin and eosin and were observed under a computerized light microscope.
The neprotoxicity results shown in table 3-7and Figure 1-15.

Statistical examination of data:
The data obtained was analyzed using one-way ANOVA followed by Dunnet's multiple comparison tests. $P < 0.01$ was considered significant.

RESULTS
Percentage yield of extraction

Table no1: Percentage yield

S.No	Type of extraction	Percentage yield
1.	90% Eathanol	18.79%

Quantitative phytochemical analysis of extracts

Phytochemical screening of Citrus maximus fruit peel was done using Ethanol, the extract showed the presence of Alkaloids, glycosides, saponins, flavonoids, Fixed oils and fats and absence of tannins.

Table no2: Quantitative phytochemical analysis

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

Ppharmacological activity

Intense toxicity studies

The intense lethality investigations of Citrus maximus organic product strip was completed according to OECD rules 423. There was no gross proof of any variations from the norm saw up to a time of 4-6hrs and no mortality was seen at the most extreme endured portion (MTD) level of 2000mg/kg body weight. per oral. The most extreme tried portion was 2000mg/kg body weight. Further pharmacological screenings were done with two portion ranges for example 1/10.5 of MTD (250 mg/kg bwp.o.), 1/5 of MTD (500mg/kg bwp.o.).They were taken as Test dosages T1 and T2 separately.

***maximus* fruit peel on gentamicin induced nephrotoxicity in rats:**

Biochemical parameters:

In gentamicin treated gatherings (second and fifth) of creatures the convergence of serum urea, Creatinine , Uric corrosive and Urine Urea, uric corrosive ,creatinine impressively expanded than the ordinary creatures (bunch 1) which shows extreme nephrotoxicity. Treating (bunch 3, 4 and 6, 7) with ethanol concentrate of Citrus maximus organic product strip indicated noteworthy diminishing ($p<0.001$) in convergence of serum urea , Creatinine , Uric corrosive ,Total protein and Urine Urea, uric corrosive ,creatinine contrasted with gentamicin treated gatherings (second and fifth).

Impact of ethanolic extract of *Citrus*

Table no3: Effect of 40 mg/kg/day subcutaneous gentamicin and Citrus maximus natural product strip oral on serum creatinine, urea, uric corrosive and Total protein treated in rodents for 24 days

Group	Drug Treatment	Creatinine	Urea	Uric acid
Normal		0.53± 0.005	21.73±1.03	1.59±0.07
Control-1	40mg/kg	0.9± 0.008***	34.98±0.87***	2.39±0.04***
Pre-emptive test-1	250mg/kg	0.85±0.009***	31.29±0.74***	2.19±0.04***
Preventive test-2	500mg/kg	0.69±0.01***	29.45±0.97***	1.66±0.06
Regulator-2	40mg/kg	0.945±0.01***	37.8±1.13***	2.40±0.07***
Curative Test-1	250mg/kg	0.851±0.01***	33.8±0.91***	2.25±0.06***
Curative Test-2	500mg/kg	.725±0.01***	29.145±0.73***	1.82±0.04

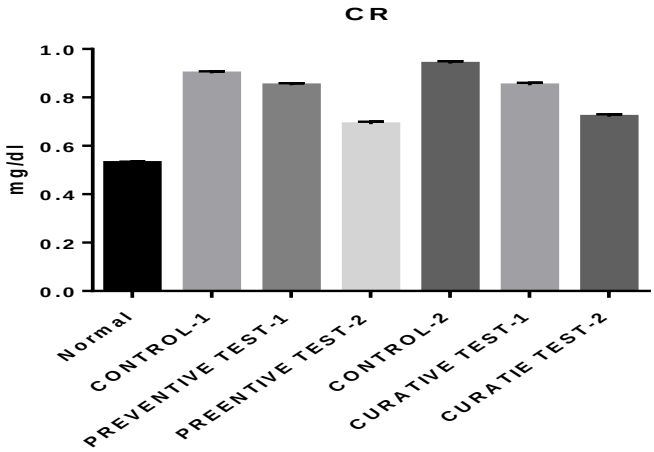

Figure no1: Effect of 40 mg/kg/day subcutaneous gentamicin and Citrus maximus natural product strip oral treated in rodents for 24 days on serum creatinine

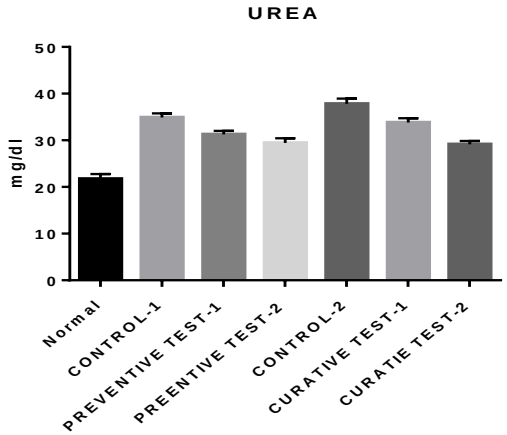

Figure no2: Effect of 40 mg/kg/day subcutaneous gentamicin and Citrus maximus fruit peel oral treated in rats for 24 days on serum Urea

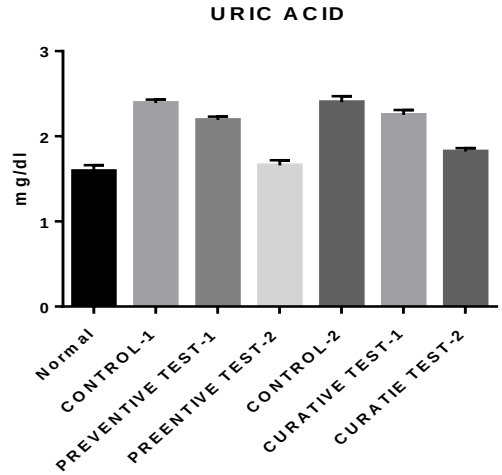

Figure no3: Effect of 40 mg/kg/day subcutaneous gentamicin and Citrus maximus fruit peel oral treated in rats for 24 days on serum uric acid

Table no4: Effect of 40 mg/kg/day subcutaneous gentamicin and Citrus maximus fruit peel oral on Urine creatinine, urea, and uric acid treated in rats for 24 days

Group	Drug Treatment	Creatinine	Urea	Uric acid
Normal		9.66± 0.06	23.59± 0.58	4.36± 0.07
Control-1	40mg/kg	14.65± 0.06***	47.02± 0.71***	7.36± 0.07***
Preventive test-1	250mg/kg	13.25± 0.08***	41.07± 0.76***	5.76± 0.06***
Pre-emptive test-2	500mg/kg	11.40± 0.08***	37.13± 0.81***	4.56± 0.09
Control-2	40mg/kg	16.72± 0.06***	51.06± 0.78**	8.69± 0.08***
Curative Test-1	250mg/kg	13.56± 0.08***	43.77± 0.80***	6.62± 0.08***
Curative Test-2	500mg/kg	12.57± 0.06***	39.05± 0.75***	6.13± 0.09**

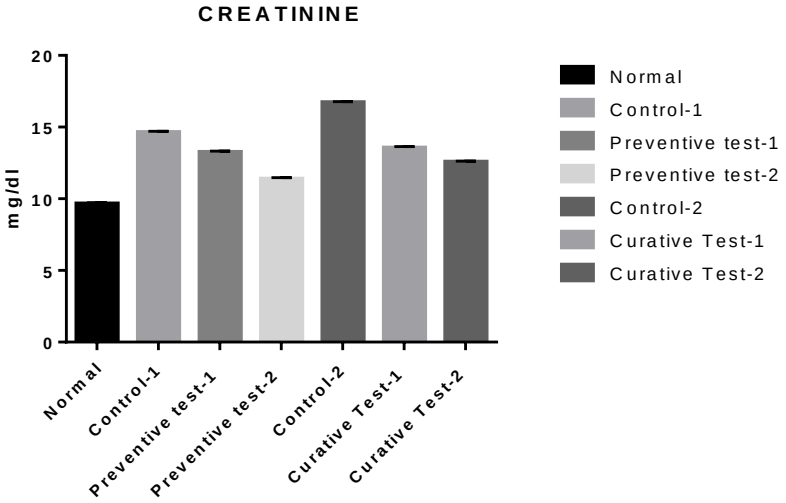

Figure no4: Effect of 40 mg/kg/day subcutaneous gentamicin and Citrus maximus natural product strip oral on Urine creatinine treated in rodents for 24 days

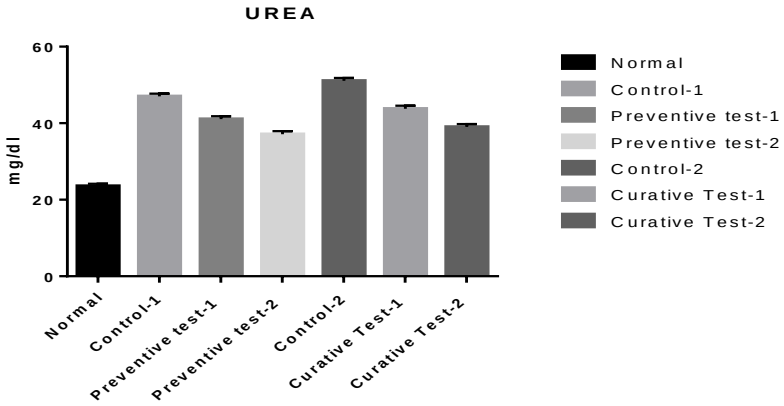

Figure no5: Effect of 40 mg/kg/day subcutaneous gentamicin and Citrus maximus natural product strip oral on urea treated in rodents for 24 days

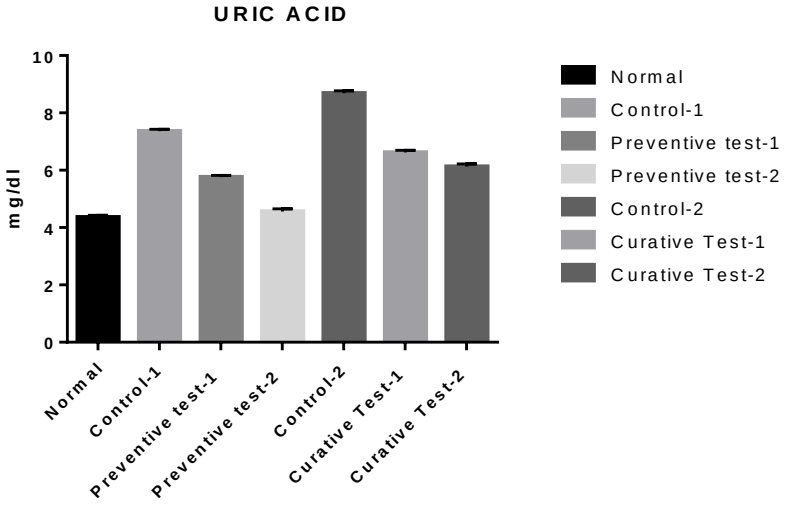

Figure no6: Effect of 40 mg/kg/day subcutaneous gentamicin and Citrus maximus organic product strip oral on Urine uric corrosive treated in rodents for 24 days

Extensively decline in movement of SOD and glutathione peroxidase in gentamicin treated creatures (second and fifth) when contrasted with typical creatures (bunch 1). Treating (bunch 3, 4 and 6, 7) with ethanol concentrate of Citrus maximus organic product strip essentially averted diminishing in the degree of SOD, GPx action contrasted with gentamicin treated rats(2nd and fifth).

In any case significant increment in action of lipid peroxidase in gentamicin treated creatures (second and fifth). Treating (bunch 3, 4 and 6, 7) with ethanol concentrate of Citrus maximus natural product strip fundamentally forestalled increment in the degree of lipid peroxidase. Consequently emphatically repress lipid peroxidation in confined tissue through its cancer prevention agent movement.

Gentamicin treated creatures (second and fifth) built up a huge harm saw as raised serum levels of explicit chemicals like SGPT, SGOT and when contrasted with ordinary control. Treating (bunch 3, 4 and 6, 7) with ethanol concentrate of Citrus maximus natural product strip demonstrated great assurance against gentamicin instigated lethality.

Table no5: Effect of 40 mg/kg/day subcutaneous gentamicin and Citrus maximus natural product strip oral on cell reinforcement parameters treated in rodents for 24 days

Group	Drug Treatment	SOD	GSH	MDA	SGPT	SGOT
Ordinary		9.29± 0.09	6.30± 0.06	76.05± 0.8	29.9± 1.34	43.63± 1.62
Control-1	40mg/kg	5.50± 0.07***	2.55± 0.05***	141.00± 0.71***	127.8± 1.42***	138.66± 1.78***
Preemptive test-1	250mg/kg	5.50± 0.07***	3.28± 0.07***	121.15± 0.71***	42.3± 1.41**	51.88± 1.50**
Preventive test-2	500mg/kg	8.72± 0.08**	4.76± 0.05**	87.08± 0.78**	33.85± 1.72	46.98± 1.56*
Control-2	40mg/kg	4.16± 0.07***	2.44± 0.19***	146.54± 0.62***	797.85± 1.45***	145.15± 1.48***
Curative Test-1	250mg/kg	5.34± 0.09***	3.20± 0.09***	133.17± 0.81***	52.21± 1.68**	62.01± 1.51***
Curative Test-2	500mg/kg	8.58± 0.05**	4.48± 0.05***	99.04± 0.76***	35.78± 1.70	48.95± 1.47**

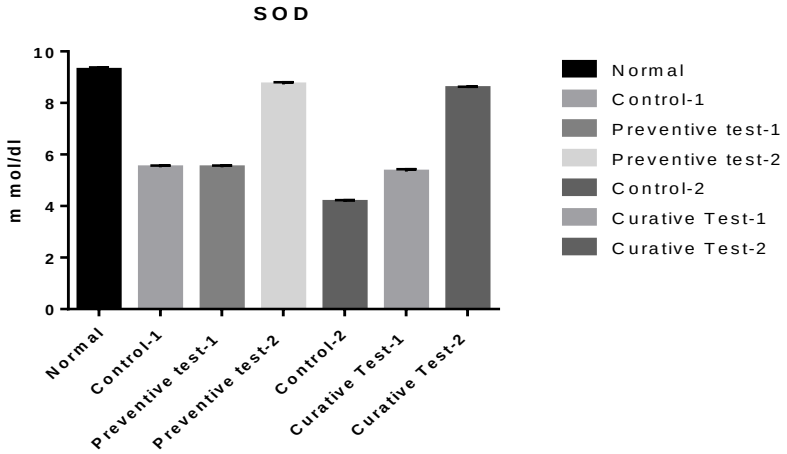

Figure no7: Effect of 40 mg/kg/day subcutaneous gentamicin and Citrus maximus natural product strip oral on SOD treated in rodents for 24 days.

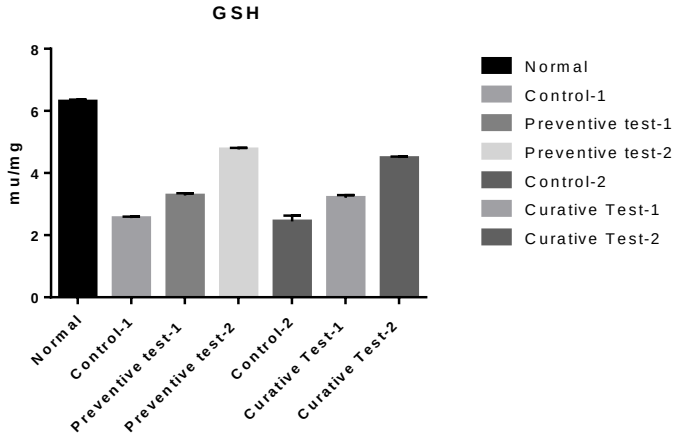

Figure no8: Effect of 40 mg/kg/day subcutaneous gentamicin and Citrus maximus natural product strip oral on GSH treated in rodents for 24 days

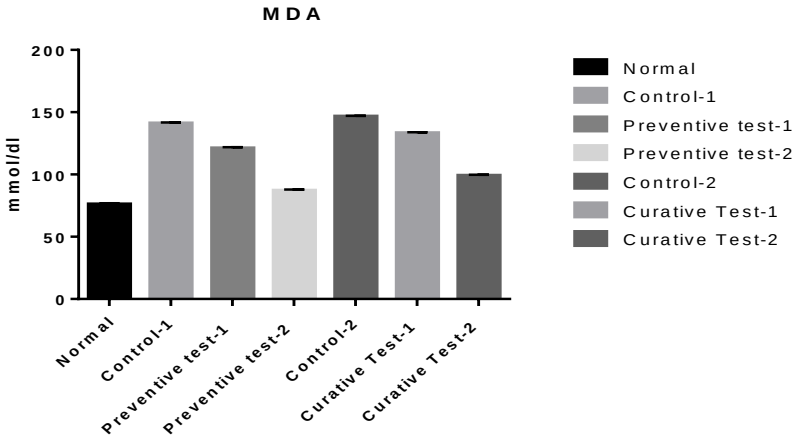

Figure no9: Effect of 40 mg/kg/day subcutaneous gentamicin and Citrus maximus organic product strip oral on MDA treated in rodents for 24 days

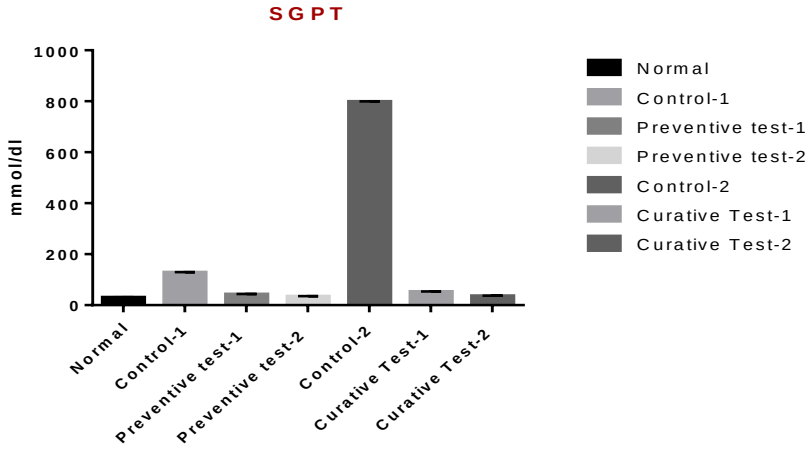

Figure no10: Effect of 40 mg/kg/day subcutaneous gentamicin and Citrus maximus natural product strip oral on SGPT treated in rodents for 24 days

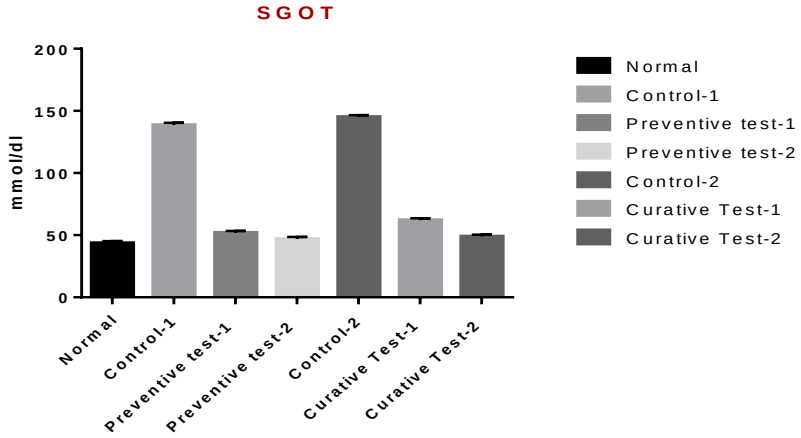

Figure no11: Effect of 40 mg/kg/day subcutaneous gentamicin and Citrus maximus organic product strip oral on SGOT treated in rodents for 24 days

Table no6: Effect of 40 mg/kg/day subcutaneous gentamicin and Citrus maximus organic product strip oral on RBC/WBC treated in rodents for 24 days

Group	Drug Treatment	WBC	RBC
Normal		8.96±0.15	5.92±0.15
Control-1	40mg/kg	8.25±0.15**	4.66±0.18**
Preventive test-1	250mg/kg	9.12±0.15	4.93±0.15
Preventive test-2	500mg/kg	9.26±0.15*	4.98±0.15*
Resistor-2	40mg/kg	7.96±0.14***	4.27±0.16***
Curative Test-1	250mg/kg	8.85±0.15	4.81±0.16
Curative Test-2	500mg/kg	9.17±0.12	4.88±0.15

There is huge ($p<0.01$) decline in the RBC and WBC in control bunches in contrast with the ordinary control gathering. Anyway in the concentrate treated gatherings there is noteworthy increment in RBC and WBC ($p<0.01$ for ethanol remove)

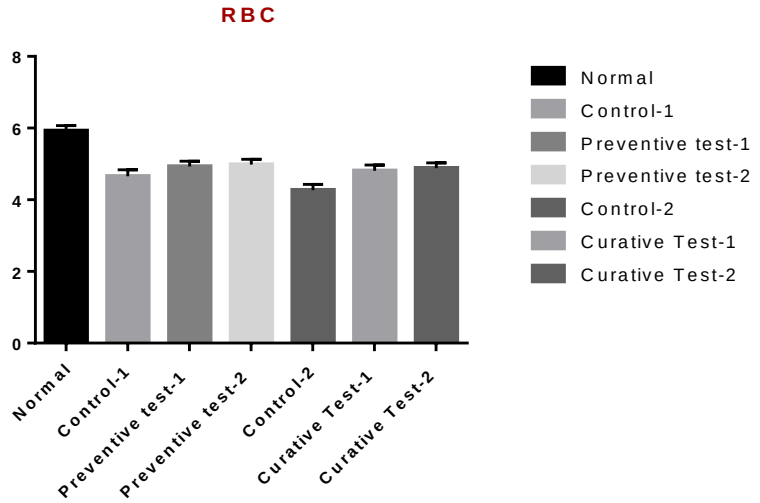

Figure no12: Effect of 40 mg/kg/day subcutaneous gentamicin and Citrus maximus organic product strip oral on RBCtreated in rodents for 24 days

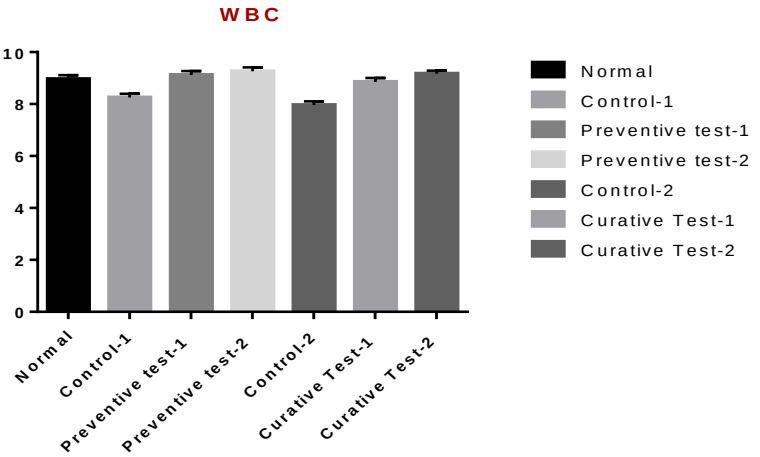

Figure no13: Effect of 40 mg/kg/day subcutaneous gentamicin and Citrus maximus organic product strip oral on WBC treated in rodents for 24 days

Table no7: Effect of 40 mg/kg/day subcutaneous gentamicin and Citrus maximus organic product strip oral on Urine volume, Urine Ph and Kidney weight treated in rodents for 24 days

Group	Drug Treatment	Urine volume	Urine pH	Kidney weight
Normal		25.1±0.45	5.9±0.22	0.95±0.00
Control-1	40mg/kg	15.39±0.22*	8.06±0.17***	1.60±0.08**
Preventive test-1	250mg/kg	20.2±0.34*	7.51±0.25*	1.51±0.09**
Preventive test-2	500mg/kg	22.04±0.27	7.43±0.22**	1.40±0.04***
Control-2	40mg/kg	13.61±0.30*	8.26±0.17***	1.75±0.05***
Curative Test-1	250mg/kg	18.54±0.27*	7.8±0.15***	1.49±0.04***
Curative Test-2	500mg/kg	20.72±0.26*	7.2±0.16***	1.29±0.07*

In gentamicin treated gathering of creatures weight of kidneys were extensively expanded contrasted with ordinary creatures (group1) and treating (bunch 3,4 and 6,7) with ethanol concentrate indicated noteworthy diminishing ($p<0.001$) in kidney weight.

There is noteworthy ($p<0.01$) increment in the pee ph and reduction in pee volume of the control bunches in contrast with the ordinary control gathering. Anyway in the concentrate treated gatherings there is noteworthy decrease in the pee ph ($p<0.05$ for ethanol concentrate) and increment in pee volume

There is critical ($p<0.01$) decline in the body loads control bunches in contrast with the ordinary control gathering. Anyway in the concentrate treated gatherings there is critical increment in body weight ($p<0.01$ for ethanol remove)

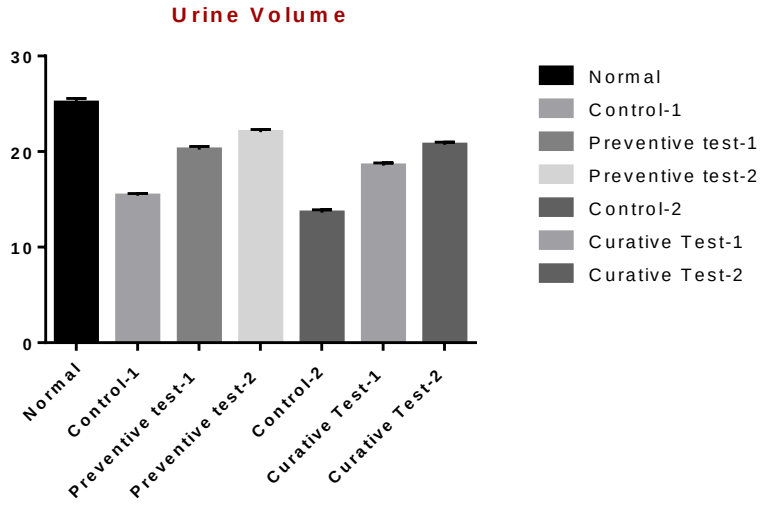

Figure no14: Effect of 40 mg/kg/day subcutaneous gentamicin and Citrus maximus natural product strip oral on Urine volume treated in rodents for 24 days Urine volume treated in rodents for 24 days

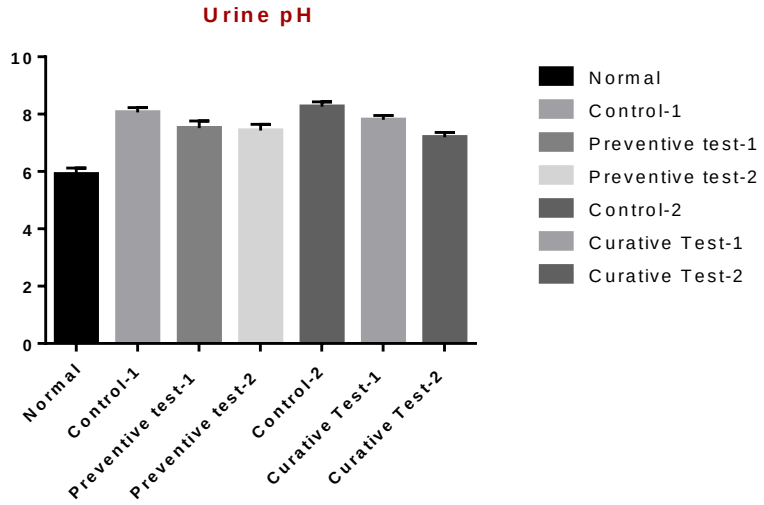

Figure no15: Effect of 40 mg/kg/day subcutaneous gentamicin and Citrus maximus natural product strip oral on Urine Ph treated in rodents for 24 days

CONCLUSION:

The phytochemical screening showed the presence of alkaloids, terpenoids, sterol's,flavonoids and carbohydrates. But it does not contain tannins. In the present study, it was observed that treatment with gentamicin induced a significant elevation in the levels of serum urea, creatinine, blood urea nitrogen, serum urea and weight of kidney. However, daily treatment with Citrus maximus for 8 days conferred nephroprotection on gentamicin induced rats in a dose dependent fashion offered maximum protection.

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