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PROTECTIVE EFFECT OF *BAUHINIA TOMENTOSA* L. EXTRACT AGAINST GENTAMICIN INDUCED NEPHROTOXICITY IN WISTAR MALE ALBINO RATS

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Keywords:

Bauhinia tomentosa, Gentamicin, Cystone, Lipid peroxidation

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ABSTRACT: The aim of the present study was to explore the effect of aerial parts of *Bauhinia tomentosa* extract against gentamicin-induced nephrotoxicity. Thirty male Wistar albino rats were divided into five groups of six animals each. Animals served as Group I (normal control 5 ml/kg b.wt, p.o), Group II (toxic control), and Group III and IV (treatment groups 200 and 400 mg/kg b.wt, p.o) and Group V (standard cystone group 5 ml/kg b.wt, i.p). All groups except group I received gentamicin (80 mg/kg b.wt, i.p) for 10 days. On the 11th day, serum profile (creatinine, blood urea nitrogen, uric acid, aspartate aminotransferases, alanine transaminase, and alkaline phosphatase) and lipid peroxidation, glutathione peroxidase, superoxide dismutase, and catalase levels were estimated in the homogenates of the kidney. Results showed that administration of extract significantly minimized elevated serum levels of biomarkers, decreased kidney lipid peroxidation, increased levels of reduced glutathione content, superoxide dismutase and catalase levels in a dose-dependent manner. In conclusion, the study revealed that ethanolic extract of *Bauhinia tomentosa* has a good protective effect against gentamicin-induced nephrotoxicity.

INTRODUCTION: Kidneys have very important tasks, specifically where they deal with excretion of unwanted and foreign substances, especially toxins. Kidneys maintain our endocrine and acid-base balance, blood pressure, erythropoiesis, *etc*. so if any damage occurs in the kidney, it reflects in the functioning of kidneys and results in failure or dysfunction of various pathophysiological systems. There is no specific therapy with pharmaceutical drugs is present in case of renal failure. Based on the usage of plant products by traditional herbal practitioners, some plants were evaluated for good kidney function and treatment of kidney disorders.

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Therapeutic success in preclinical and clinical was hundred out of thousands of plants used in kidney disorders [1]. Medicinal plants are part of human society to relive from diseases from the start of civilization [2]. There is a broad belief that green medicines are healthier and safer than synthetic drugs [3]. There exists plenty of knowledge, health benefits of herbal drugs in our ancient literature of Ayurveda (Traditional Indian Medicine), Siddha, Unani and Chinese medicine.

According to the World Health Organization, 2003 about 80 % of the population of developing countries being unable to afford pharmaceutical drugs rely on traditional medicines, mainly plant-based, to sustain their primary health care needs. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs [4, 5, 6].

Bauhinia tomentosa (Family: Fabaceae), the yellow bell orchid tree, 4 m height, the leaf is divided into two lobes with a green colored leathery texture, large bell-shaped flowers, fruits are pear-shaped and light green turns to pale brown with age. It is found along the coastal strip from Southern Kwazulu-Natal to Maputo land, Mpumalanga as well as Mozambique, Zimbabwe, tropical Africa and as far as India and Srilanka [7, 8, 9, 10, 11].

It was proved as anti-bacterial and anti-fungal [12], anti-oxidant [13], hypolipidemic and hypolipidemic [14] and immunomodulatory and anti-inflammatory [15] activities. No earlier studies have been done on the protective effect of aerial parts of *Bauhinia tomentosa* extract against gentamicin-induced nephrotoxicity. In the present study, we investigated the effect of ethanolic extract of *Bauhinia tomentosa* aerial parts against gentamicin-induced toxicity.

MATERIALS AND METHODS:

Plant Material Collection and Extraction: The plant *Bauhinia tomentosa* used for the present study was collected from the Chittoor district of Andhra Pradesh. The plant was identified, confirmed and authenticated by comparing with voucher specimen number: SVU/9-36 available at Survey of medicinal plants and amp; collection unit, Department of Botany, Sri Venkateswara University, Tirupathi by Field Botanist Dr. Madhav Shetty.

The aerial parts were cut into small pieces and shade dried. The dried material was then pulverized separately into coarse powder by a mechanical grinder. The resulting powder was then used for extraction. The extraction was done by using the process of Soxhlet extraction. 150 g of powder was suspended in 1500 ml ethanol for 7 days at 65 ºC using Soxhlet apparatus. After 7 days the extract was taken, and the residue was dried and the percentage yield of extract was calculated.

Preliminary Phytochemical Screening: The extract was subjected to qualitative phytochemical screening for the identification of phytoconstituents [16].

Experimental Animals: Male Wistar albino rats of 150-200 g weighed were used for the present study. The animals were housed in polypropylene cage (6 animals per cage), the standard conditions were maintained (12 h light and 12 h dark cycle, 20 ± 2ºC, and 40-60% humidity). The standard rat diet and water was provided *ad libitum*. All the animals were collected from the central animal house, SICRA Labs Pvt. Ltd., IDA-Kukatpally, Hyderabad, Telangana state and all experiments were conducted according to the ethical norms approved by CPCSEA (IAEC Reg. No. 1821/PO/Re/S/15/ CPCSEA).

Acute Toxicity Studies: Acute toxicity study was performed according to the OECD guidelines 425.

Experimental Procedure: Rats divided into five groups, each group consisting of six animals.

Group 1: Control with normal saline (5 ml/kg b.wt, p.o.)

Group 2: Gentamicin (80 mg/kg/b. wt, i.p.), daily for 10 days

Group 3: Ethanolic extract of *Bauhinia tomentosa* (200 mg/kg/body weight, p.o) and gentamicin (80 mg/kg/b. wt, i.p.), daily for 10 days.

Group 4: Ethanol extract of *Bauhinia tomentosa* (400mg/kg/b.wt, p.o.) and gentamicin (80 mg/kg/b. wt, i.p.), daily for 10 days.

Group 5: Standard cystone (5 ml/kg b. wt, p.o.) and gentamicin (80 mg/kg/b.wt, i.p.), daily for 10 days.

At the end of the experimental period, blood samples were collected from retro-orbital plexus. Blood samples were allowed clot for one hour at room temperature and centrifuged at 2500 rpm for 15 min to obtain the serum, used for estimation of various biochemical parameters such as creatinine, blood urea nitrogen and uric acid using coral kits and autoanalyzer. *In-vivo* antioxidant markers such as lipid peroxidation, glutathione peroxidase, catalase, and superoxide dismutase levels were also estimated.

Statistical Analysis of Data: Results were expressed as Mean ± S.E.M. The statistical difference between the groups was calculated in terms of one-way analysis of variance (ANOVA) followed by Dunnett's test. The statistical significance criterion was $p<0.05$ (95% level). $P<0.05$ is considered as significant.

RESULTS:

Percentage Yield: The Percentage yield of the extract obtained was 10.2% **Table 1**.

TABLE 1: PERCENTAGE YIELD

S. no.	Solvent used	Color and consistency	Percentage yield (%)
1	Ethanol	Dark brown sticky	10.2%

Phytochemical Screening: The phytochemical screening of plant showed the presence of phytosterols, alkaloids, glycosides, flavonoids, saponins, coumarins, triterpenoids, phenols, tannins, fixed oils and fats **Table 2**.

TABLE 2: PRELIMINARY PHYTOCHEMICAL ANALYSIS

S. no.	Phyto-constituent	Ethanol extract
1	Carbohydrates	Present
2	Proteins and Amino acids	Present
3	Phenolic compounds and tannins	Present
4	Phytosterols	Present
5	Fixed oils and fats	Present
6	Alkaloids	Present
7	Glycosides	Present
8	Flavonoids	Present
9	Saponins	Present
10	Coumarins	Present

Determination of Acute Oral Toxicity of EBT: The extract of *Bauhinia tomentosa* didn't show any mortality and toxicity at the highest dose of 2000 mg/kg.b.wt employed **Table 3**. The present research study was carried out using dose (200 and 400 mg/kg body weight) for nephroprotective activity.

Physical and Biochemical Parameters: Treatment with gentamicin reduced the normal increase of body weight whereas treatment with extract restored rise in normal body weight.

In gentamicin administered animals group, the levels of serum urea and creatinine were significantly raised compared to the normal animals (Group 1) which reveal chronic nephrotoxicity. Treating (Group 4 and 5) with ethanol extract of *Bauhinia tomentosa* exhibited significant reduction ($p<0.001$) in levels of serum urea and creatinine compared with gentamicin treated (Group 2).

The level of uric acid not significantly elevated in the gentamicin-treated groups (Group 2) as compared to the control group (Group 1).

TABLE 3: TOXICITY RECORD SHEET

S. no.	Code	Toxicity		Time of Death	Observation									
		Onset	Stop		Skin color	Eyes	Resp	CNS	Tre	Con	Sali	Diah	Sleep	Let
1	EBT	X	x	X	X	x	X	X	x	x	x	x	x	X

(TRE-Tremor, CON-Convulsions, SALI- Salivation, Diah - Diarrhea, LET-Lethargy). (X = Negetive Ø = Positive)

TABLE 4: EFFECT OF EXTRACT ON BODY WEIGHT OF RATS (g)

Group	Initial	Final
1	172.4 ± 1.15	180.46 ± 2.18
2	177.2 ± 1.15	182.29 ± 1.39
3	161.2 ± 2.15	172.2 ± 1.97**
4	172.29 ± 2.45	178.94 ± 2.39*
5	164.4 ± 1.16	174.25 ± 1.32**

n=6 animals in each group. Values are expressed as Mean ± SEM; *p<0.05, **p<0.01, ***p<0.001 *vs.* toxicant control and ns indicate non significant.

TABLE 5: EFFECT OF 80 mg/kg/day INTRAPERITONEAL GENTAMICIN AND *BAUHINIA TOMENTOSA* ORAL ON SERUM CREATININE; BLOOD UREA AND SERUM URIC ACID IN TREATED RATS FOR 10 DAYS

Group	Drug treatment	Serum creatinine (mg/dl)	Blood urea (mg/dl)	Uric acid (mg/dl)
1	5 ml/kg, p.o, Normal saline	0.681 ± 0.053	22.622 ± 1.783	4.0233 ± 0.423
2	80 mg/kg,i.p, Gentamicin	1.261 ± 0.037	118.76 ± 5.981	5.136 ± 0.273
3	80 mg/kg,i.p, Gentamicin + 200 mg/kg	0.8566 ± 0.041***	54.932 ± 6.196***	3.933 ± 0.269*
4	80 mg/kg,i.p, Gentamicin + 400 mg/kg	0.7441 ± 0.048***	49.962 ± 4.204***	3.5733 ± 0.171**
5	80 mg/kg,i.p, Gentamicin+ Cystone 5 ml/kg	0.7041 ± 0.038***	47.762 ± 4.204***	3.2533 ± 0.171**

n=6 animals in each group; Values are expressed as Mean ± SEM;*p<0.05, **p<0.01, ***p<0.001 *vs.* toxicant control and ns indicate nonsignificant

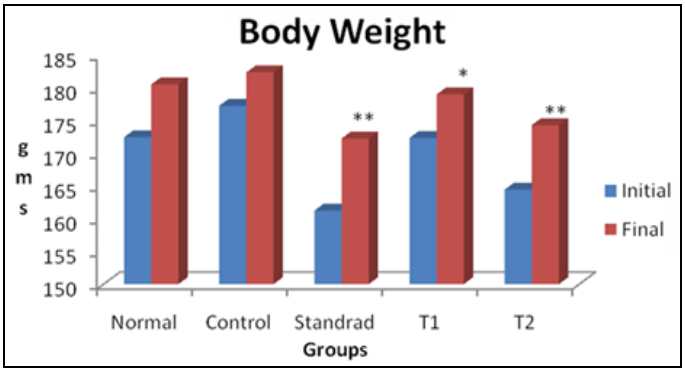

FIG. 1: EFFECT OF 80 mg/kg/day INTRA PERITONEAL GENTAMICIN AND *BAUHINIA TOMENTOSA* ORAL ON BODY WEIGHT IN TREATED RATS FOR 10 DAYS

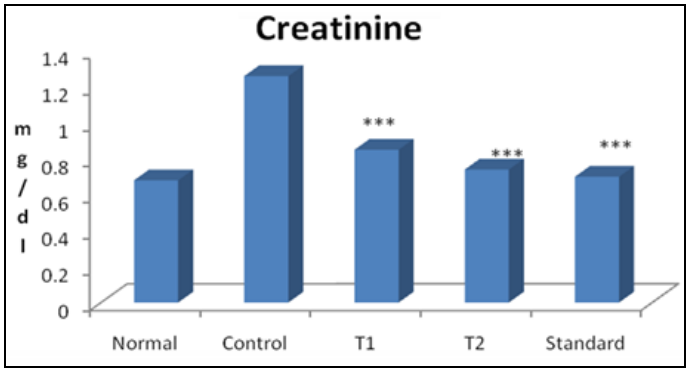

FIG. 2: EFFECT OF 80 mg/kg/day INTRAPERITONEAL GENTAMICIN *AND BAUHINIA TOMENTOSA* ORAL ON SERUM CREATININE; IN TREATED RATS FOR 10 DAYS

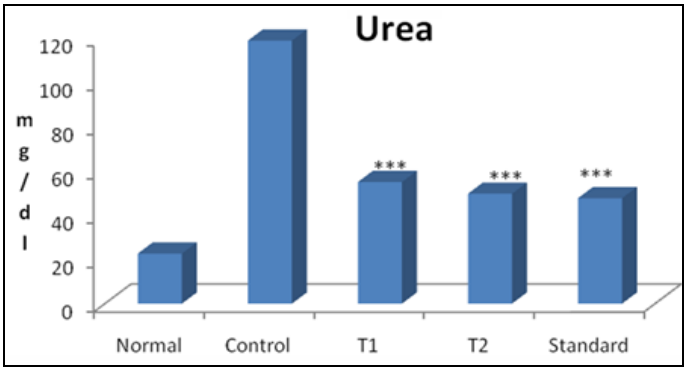

FIG. 3: EFFECT OF 80 mg/kg/day INTRAPERITONEAL GENTAMICIN AND *BAUHINIA TOMENTOSA* ORAL ON BLOOD UREA IN TREATED RATS FOR 10 DAYS

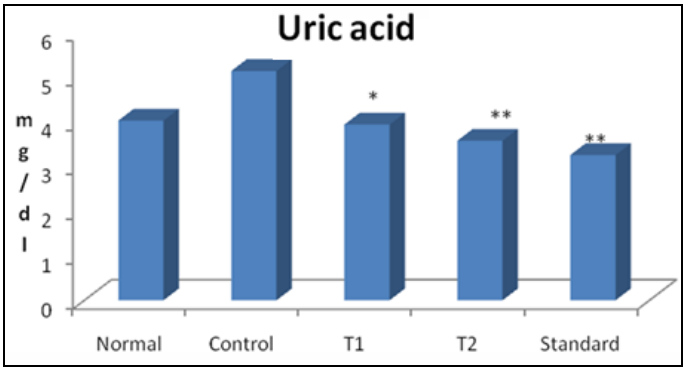

FIG. 4: EFFECT OF 80 mg/kg/day INTRAPERITONEAL GENTAMICIN AND *BAUHINIA TOMENTOSA* ORAL ON SERUM URIC ACID IN TREATED RATS FOR 10 DAYS

In gentamicin treated group of animals weight of kidneys were considerably increased compared to normal animals (Group 1) and treating (Group 3 and 4) with ethanol extract showed a significant decrease ($p<0.001$) in kidney weight **Table 6**. In the current study treatment of animals with ethanolic extract of leaves of *Bauhinia tomentosa* significantly ($p<0.05$) decreased the levels of SGOT, SGPT, and ALP in serum which is an indication of nephroprotective activity **Table 7**; **Fig. 6-8**.

TABLE 6: EFFECT OF 80 mg/kg/day INTRAPERITONEAL GENTAMICIN AND *BAUHINIA TOMENTOSA* ORAL ON KIDNEY WEIGHT IN TREATED RATS FOR 10 DAYS

Group	Drug treatment	Kidney weight(g)
1	5 ml/kg, p.o, Normal saline	0.567 ± 0.013
2	80 mg/kg,i.p, Gentamicin	0.712 ± 0.013
3	80 mg/kg,i.p, Gentamicin + 200 mg/kg	0.6 ± 0.014***
4	80 mg/kg,i.p, Gentamicin + 400 mg/kg	0.567 ± 0.009***
5	80 mg/kg,i.p, Gentamicin + Cystone 5 ml/kg	0.546 ± 0.007***

n=6 animals in each group; Values are expressed as Mean ± SEM; *p<0.05, **p<0.01, ***p<0.001 *vs.* toxicant control and ns indicate non significant.

TABLE 7: EFFECT OF 80 mg/kg/day INTRA PERITONEAL GENTAMICIN AND *BAUHINIA TOMENTOSA* ORAL ON SGPT, SGOT AND ALP LEVELS IN TREATED RATS FOR 10 DAYS

Group	Drug treatment	SGPT (U/L)	SGOT (U/L)	ALP (U/L)
1	5 ml/kg, p.o, Normal saline	42.6.8 ± 1.23	45.25 ± 1.36	34.56 ± 1.56
2	80 mg/kg,i.p, Gentamicin	123.45 ± 1.45**	136.19 ± 3.48***	92.52 ± 2.77***
3	80 mg/kg,i.p, Gentamicin + 200 mg/kg	89.38 ± 0.87**	92.45 ± 1.76***	73.74 ± 1.38**
4	80 mg/kg,i.p, Gentamicin + 400 mg/kg	65.26 ± 2.14***	55.38 ± 1.45***	51.38 ± 1.54**
5	80 mg/kg,i.p, Gentamicin + Cystone 5 ml/kg	45.47 ± 1.31***	48.18 ± 1.57***	44.47 ± 1.67***

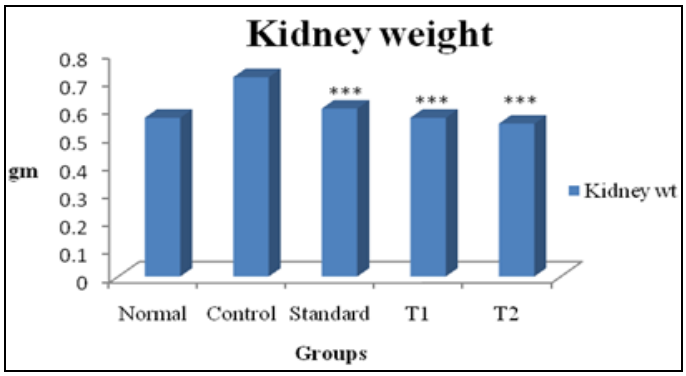

FIG. 5: EFFECT OF 80 mg/kg/day INTRAPERITONEAL GENTAMICIN AND *BAUHINIA TOMENTOSA* ORAL ON KIDNEY WEIGHT IN TREATED RATS FOR 10 DAYS.
*p<0.05, **p<0.01, ***p<0.001 *vs.* toxicant control and ns indicate non significant

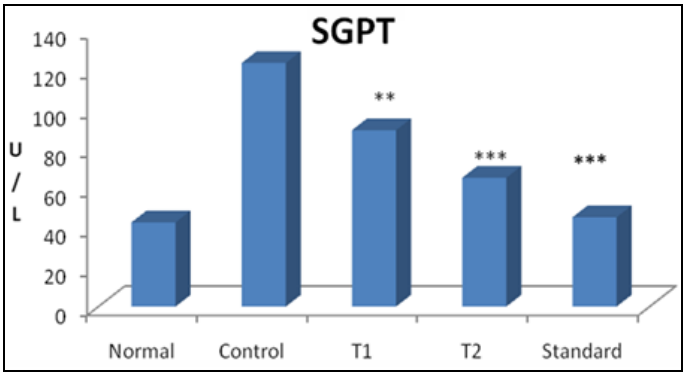

FIG. 6: EFFECT OF 80 mg/kg/day INTRA-PERITONEAL GENTAMICIN AND *BAUHINIA TOMENTOSA* ORAL ON SERUM SGPT IN TREATED RATS FOR 10 DAYS

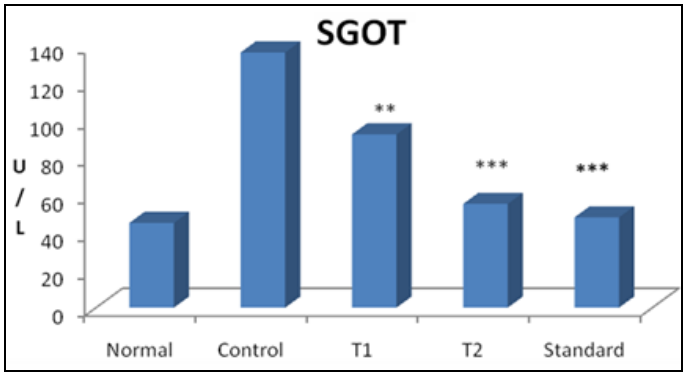

FIG. 7: EFFECT OF 80 mg/kg/day INTRAPERITONEAL GENTAMICIN AND *BAUHINIA TOMENTOSA* ORAL ON SERUM SGOT IN TREATED RATS FOR 10 DAYS

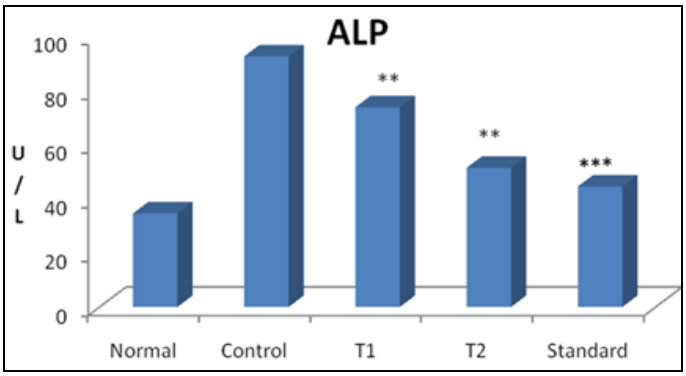

FIG. 8: EFFECT OF 80 mg/kg/day INTRAPERITONEAL GENTAMICIN AND *BAUHINIA TOMENTOSA* ORAL ON SERUM ALP IN TREATED RATS FOR 10 DAYS

Kidney Antioxidant Status: Considerably decrease in activity of catalase, SOD and glutathione peroxidase in gentamicin treated animals (Group 2) when compared to normal animals (Group 1). Treatment with ethanol extract of *Bauhinia tomentosa* significantly prevented a decrease in the level of catalase, SOD, GPx activity compared to gentamicin-treated rats (Group 2). Nevertheless, considerable increase in activity of lipid peroxidase in gentamicin treated animals (Group 2). Treatment with ethanol extract of *Bauhinia tomentosa* significantly prevented an increase in the level of lipid peroxidase. Thus strongly inhibit lipid peroxidation in isolated tissue *via* its antioxidant activity **Table 8** and **9**; **Fig. 9-12**.

TABLE 8: EFFECT OF 80 mg/kg/day INTRAPERITONEAL GENTAMICIN AND *BAUHINIA TOMENTOSA* ORAL ON LEVEL OF ANTIOXIDANT PARAMETERS CATALASE AND LIPID PEROXIDATION IN TREATED RATS FOR 10 DAYS

Group	Drug	CAT	LPO
1	5 ml/kg, p.o, Normal saline	16.34 ± 1.53	9.83 ± 0.2
2	80 mg/kg,i.p, Gentamicin	7.48 ± 0.93	20.57 ± 0.41
3	80 mg/kg,i.p, Gentamicin + 200 mg/kg	10.48 ± 0.53	18.73 ± 0.49
4	80 mg/kg,i.p, Gentamicin + 400 mg/kg	14.46 ± 1.52*	15.97 ± 0.83**
5	80 mg/kg,i.p, Gentamicin + Cystone 5 ml/kg	12.26 ± 1.32*	13.97 ± 0.68**

TABLE 9: EFFECT OF 80 mg/kg/day INTRAPERITONEAL GENTAMICIN AND *BAUHINIA TOMENTOSA* ORAL ON LEVEL OF ANTIOXIDANT PARAMETERS GP_X AND SOD IN TREATED RATS FOR 10 DAYS

Group	Drug	GPx	SOD
1	5 ml/kg, p.o, Normal saline	35.09 ± 1.96	19.94 ± 0.78
2	80 mg/kg,*i.p*, Gentamicin	20.56 ± 1.15	7.66 ± 0.31
3	80 mg/kg,i.p, Gentamicin + 200 mg/kg	24.31 ± 0.93	11.71 ± 0.66*
4	80 mg/kg,i.p, Gentamicin + 400 mg/kg	27.88 ± 0.99*	15.91 ± 0.78***
5	80 mg/kg,i.p, Gentamicin + Cystone 5 ml/kg	25.88 ± 0.79*	13.91 ± 0.58***

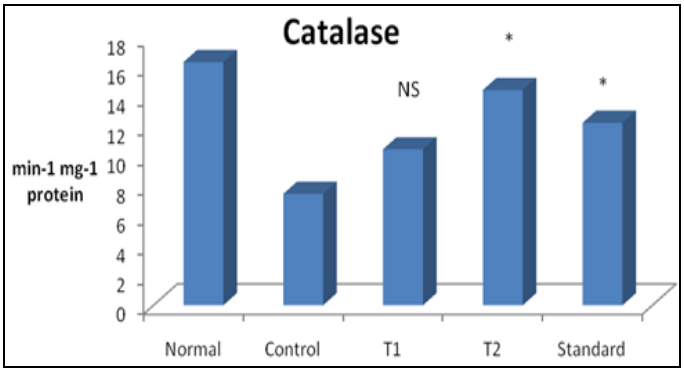

FIG. 9: EFFECT OF 80 mg/kg/day INTRAPERITONEAL GENTAMICIN AND *BAUHINIA TOMENTOSA* ORAL ON CATALASE IN TREATED RATS FOR 10 DAYS

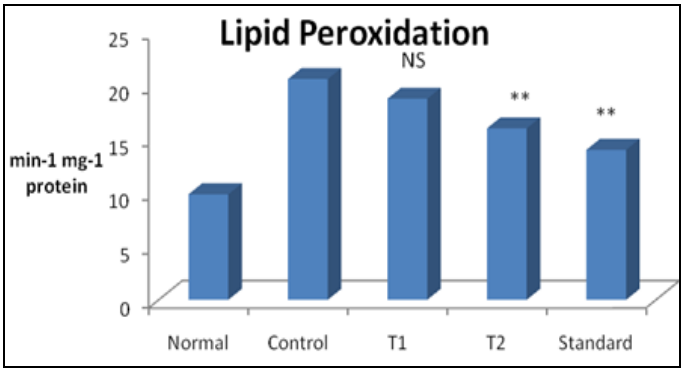

FIG. 10: EFFECT OF 80 mg/kg/day INTRAPERITONEAL GENTAMICIN AND *BAUHINIA TOMENTOSA* ORAL ON LIPID PEROXIDATION IN TREATED RATS FOR 10 DAYS

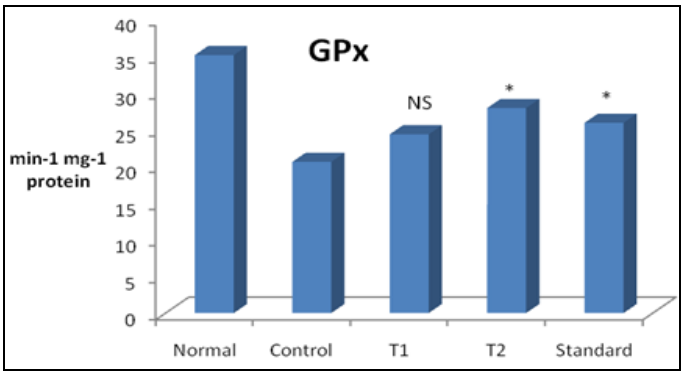

FIG. 11: EFFECT OF 80 mg/kg/day INTRA PERITONEAL GENTAMICIN AND *BAUHINIA TOMENTOSA* ORAL ON GPX IN TREATED RATS FOR 10 DAYS

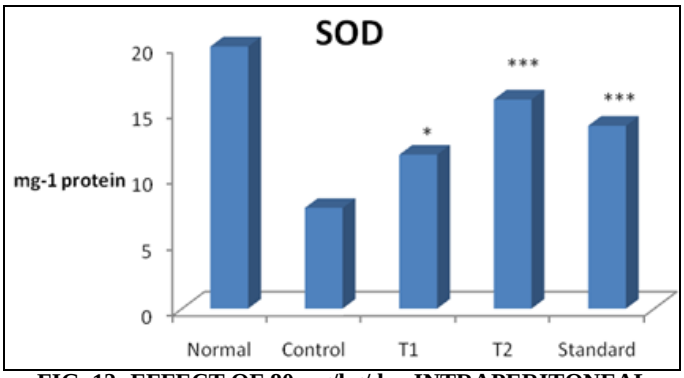

FIG. 12: EFFECT OF 80 mg/kg/day INTRAPERITONEAL GENTAMICIN AND *BAUHINIA TOMENTOSA* ORAL ON SOD IN TREATED RATS FOR 10 DAYS

DISCUSSION: Aminoglycoside antibiotic, gentamicin shows a broad spectrum of activities against both gram-positive and gram-negative bacterial infections but with a high preference for latter and its major side effect is nephrotoxicity [17, 18, 19]. One of the best models for evaluating nephroprotective activity is gentamicin drug-induced renal injury [20, 21]. Many animal experiments have demonstrated the positive correlation between oxidative stress and nephrotoxicity [22]. The accumulation of renal phospholipidosis by inhibiting lysosomal hydrolases like sphingomyelinase and phospholipases along with that it also induces oxidative stress [21, 22, 23].

Drug-induced nephrotoxicity is often linked with a significant elevation in blood urea, serum creatinine and causing acute tubular necrosis [24]. Thus biochemical parameters have been utilized for investigating chemicals and drug-induced nephrotoxicity in animals and humans [25]. In the current investigation, drug-induced nephrotoxicity was confirmed by single daily intraperitoneal injection of the gentamicin, for 10 days. This toxicity showed by a marked increase in the circulating levels of blood urea, serum creatinine, uric acid. Oral administration of plant extract marked reduction of the urea and creatinine, uric acid level in the treatment group as compared to the disease control group. Apart from the direct nephrotoxic effect of gentamicin, the acute increase in the measured biochemical parameters could also be attributed to the elevated catabolic state of the rats due to the prolong anorexia-linked with gentamicin nephrotoxicity.

In renal diseases, the serum urea accumulates because of the rate of serum urea production exceeds the rate of clearance [26]. Marked increase of urea and creatinine levels in serum was considered as the marker of nephrotoxicity [26, 27, 28]. Creatinine derives from endogenous sources by tissue creatinine breakdown. Thus serum urea concentration is often considered a more reliable renal function prediction than serum creatinine. Anyhow the level of uric acid is non-significantly increased in the toxicant group when compared to control. Oral dosing of plant extract marked reduction of the uric acid level in both treatment groups compared to the disease control group.

SGOT is a mitochondrial enzyme released from heart, liver, skeletal muscle and kidney. nephrotoxicity elevated the SGOT levels in serum due to the damage to the tissues producing acute necrosis, such as severe viral hepatitis and acute cholestasis [29]. In the case of kidney toxicity, alkaline phosphatase levels are very high, which may be due to defective hepatic excretion or by increased production of ALP by parenchyma or duct cells. Gentamicin is known to reduce the activities of catalase, glutathione peroxidase. Therefore it is no doubt to assume that the nephron protection showed by *Bauhinia tomentosa* extract in gentamicin-induced nephrotoxicity is mediated through its potent antioxidant effect. A relation between oxidative stress and nephrotoxicity has been well demonstrated in many experimental animal models.

Combinatorial administration of superoxide dismutase and vitamin E significantly decreased the nephrotoxic symptoms caused by adriamycin. In Gentamicin treated rats there was a significant rise in lipid peroxidation products (MDA) advising the role of oxidative stress. A role of lipid peroxidation in Gentamicin-induced acute renal failure has been described by evaluating the effect of diphenyl-phenylenediamine and vitamin E.

In these investigations both the agents protected from gentamicin-induced lipid peroxidation. The previous reports showed that the alkaloids could strongly inhibit lipid peroxidation induced in isolated tissues via its antioxidant activity [30]. The presence of alkaloids could be the reason of protection offered by the extract might be due to its ability to activate anti-oxidant enzymes. The findings suggest that the potential use of ethanol extract of *Bauhinia tomentosa* therapeutically used as a nephroprotective agent. Therefore further studies to explain their mechanisms of action should be conducted to aid the discovery of new therapeutic agents for the treatment of renal diseases.

CONCLUSION: On evaluating biochemical and antioxidant parameters it was found that the ethanolic extract of aerial parts of *Bauhinia tomentosa* showed nephroprotective activity in gentamicin model due to the presence of therapeutic phytoconstituents.

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CONFLICT OF INTEREST: Authors declare there is no conflict interest.

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