Bauhinia tomentosa L.: A Potential Nephroprotective Agent Against Vancomycin-Induced Nephrotoxicity

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Abstract-

Background: Vancomycin, a glycopeptide antibiotic, is widely used for the treatment of serious Gram-positive infections. However, its clinical use is often limited by nephrotoxicity, a significant adverse effect. This study investigates the nephroprotective potential of Bauhinia tomentosa, a medicinal plant traditionally used for its anti-inflammatory and antioxidant properties, against vancomycin-induced nephrotoxicity. Experimental nephrotoxicity was induced in laboratory animals through administration of vancomycin. The protective effect of Bauhinia tomentosa extract was assessed by evaluating various general parameters of kidney function and abnormal constituents in urine. Results demonstrated that Bauhinia tomentosa extract significantly ameliorated vancomycin-induced renal damage, as evidenced by improved kidney function and reduced the abnormal constituents in urine. These findings suggest that Bauhinia tomentosa possesses nephroprotective properties and may be a potential therapeutic agent to mitigate vancomycin-induced nephrotoxicity.

Aim and objectives: To evaluate the effect of Nephroprotective *Bauhinia tomentosa L.* roots on Vancomycin induced nephrotoxicity.

Materials and methods: VCM is used for inducing Nephrotoxicity and general parameters and qualitative analysis of abnormal constituents of urine was used to evaluate the results.

Result: There was statistically significant ($P<0.001$) association observed between ethanolic extract of *Bauhinia tomentosa L.* roots with nephrotoxicity induced by Vancomycin.

Conclusion: Evaluation of general parameters on nephrotoxicity in rats with *Bauhinia tomentosa L*. showed significantly increased body weight, kidney weight and urine volume significantly reduce increased general parameters which supports its Nephroprotective activity.

Keywords: Nephrotoxicity, anti-inflammatory, glycopeptide antibiotic

1.INTRODUCTION

Nephrotoxicity and Kidney failure is the one of the most common diseases in India. Nephrotoxicity can be defined as kidney disease or dysfunction caused directly or indirectly by exposure to drugs, industry or environmental chemicals. Thus, drug nephrotoxicity refers to the deterioration of kidney function with the use of drugs. Nephrotoxins are substances which displaying Nephrotoxicity. [1,2] The term renal failure primarily refers to the failure of the excretory function of the kidneys, which leads to the retention of metabolic nitrogenous waste products in the blood.[3] When the kidney is damaged, it cannot produce as much urine and waste from the body. It is high in blood electrolytes such as potassium and magnesium. Early diagnosis is important; therefore, physicians must understand the potential nephrotoxicity of the drugs they prescribe and the risk to their patients. [1,2] Nephrotoxicity can manifest as acute or chronic kidney injury, leading to a range of symptoms and complications. Understanding the causes, mechanisms, and preventive measures of nephrotoxicity is crucial for maintaining kidney health and preventing potential renal damage.[4] Many therapeutic agents can negatively affect the kidneys and cause acute renal failure, chronic interstitial nephritis, and nephritic syndrome. This is because in recent years a number of powerful therapeutic agents such as aminoglycoside antibiotics, NSAIDs and chemotherapeutic agents have been added to the therapeutic arsenal. Exposure to chemical reagents such as ethylene glycol, carbon tetrachloride, sodium oxalate, and heavy metals such as lead, mercury, cadmium, and arsenic also cause nephrotoxicity. A quick diagnosis of the disorder and discontinuation of the offending drug is required. Nephroprotectants are substances that have protective activity against nephrotoxicity. Medicinal plants have a therapeutic effect due to the presence of a

wide range of complex phytochemical substances. In early literature, various herbs were prescribed to treat kidney damage. A combination of various medicinal plants with nephroprotective properties and various renal toxins that may reduce their toxicity.

2. MATERIAL AND METHODS

2.1. Plant Material Collection, Authentication and Extraction:

The roots of plant were collected from Amravati district Maharashtra (India). The identification and authentication of plant was done at Department of Botany, Vidyabharati Mahavidyalaya, camp Road Amravati, by Prof. Kajal Apale.

The root of Bauhinia tomentosa L. was processed by washing with clean water, air-drying, pulverizing, and sieving through a 0.3 mm sieve. tool consists of several parts including a heat round bottom flask, Soxhlet extractor, and condenser. The solid coarsely powdered roots (250g) were placed in thimble and placed in an extractor. The bottom end of the extractor was connected to a round bottom flask containing a solvent (Ethanol 1500ml was chosen as the solvent), and was connected to a reflux condenser. The bottom flask was heated to boil the solvent (ethanol), the vapor rises through the branch pipe of the extractor, was condensed and drops into the thimble and the solvent (ethanol) was contacted with the solid for extraction. When the solvent (ethanol) surface exceeds the highest point of the siphon, the solvent containing the extract was return back to the round bottom flask. This cycle was repeated until the all the material extracted from the solid roots powder. The Soxhlet extractor can run continuously without any further operation, making it an excellent choice for extracting compounds over hours or even days. Filtration is not required So it save lot of time, energy and financial inputs.[5] The percentage yield of the extract was calculated.

2.2. Preliminary Phytochemical Screening:

The extract was then subjected to different phytochemical tests for identification of phytoconstituents.[6]

2.3. Experimental Animals:

Male wistar albino rats (175gm - 200gm) were provided by the animal house of Department of Pharmacology, Vidyabharati college of Pharmacy, Amravati, India (CPCSEA Registration no. 1504/PO/RE/S/11/CPCSEA). Experiment would perform in accordance with the committee for the purpose and supervision of experimental animals (CPCSEA) guidelines after the approval of the experimental protocol by the Institutional Animal Ethical committee (IAEC). Wistar albino female rats and 8-12 weeks age would be used for the study, the animal would be housed 14 per cage at temperature (22 ± 30C) with 50- 60% of relative humidity under 12h day and night cycle and fed standard rodent chow and water ad libitum.

2.4. Preparation of Doses: Vancomycin was diluted to 250mg/10ml with distilled water. Selenium was diluted to 30ml with distilled water. Two different concentrations (200 mg/kg, and 400 mg/kg) of the EEBT were prepared by dissolving the extracts in distilled water. All solutions were freshly prepared at the time of administration to the animals. Extract solution, Standard Drug and vehicle (0.9% NaCl) were given orally and Inducing drug (Vancomycin) intraperitoneally.

2.5. Treatment Protocol:

Sr.No.	Groups	No. of Animals	Treatment/Dose	Route of Administration
1.	I (Negative control)	6	Saline treatment	Oral
2.	II (Positive control)	6	VCM (150mg/kg)	IP
3.	III (Standard)	6	VCM+Sele(6mg/kg)	IP + Oral
4.	IV (Treatment 1)	6	VCM+EEBT (200mg/kg)	IP + Oral
5.	V (Treatment 2)	6	VCM+EEBT (400mg/kg)	IP + Oral

Table No. 1: Dosing, Route of administration and grouping

2.6. Protocol design: Thirty Wistar rats (175gm - 200gm) were divided randomly into 5 groups of 6 animals each.

Group I: (Normal Control) Animal were given a normal saline orally for 17 days

Group II: Vancomycin treated rats, intraperitoneally received 2 dose of Vancomycin (150mg/kg body weight) for 7 days. First dose on 1st day and second dose on 4th day.

Group III: Intraperitoneally received 2 dose of Vancomycin (150mg/kg body weight) for 7 days. first dose on 1st day and second dose on 4th day and after 7th day rats orally received standard drug Selenium (6mg/kg body weight) for 10 days

Group IV: Intraperitoneally received 2 dose of Vancomycin (150mg/kg body weight) for 7 days. first dose on 1st day and second dose on 4th day and after 7th day rats orally received treatment drug Ethanolic Extract of Roots of Bauhinia tomentosa L. (200mg/kg body weight) for 10 days

Group V: Intraperitoneally received 2 dose of Vancomycin (150mg/kg body weight) for 7 days. first dose on 1st day and second dose on 4th day and after 7th day, rats orally received treatment drug Ethanolic Extract of Roots of Bauhinia tomentosa L. (400mg/kg body weight) for 10 days

2.7. Estimation of General Parameters

1. Estimation of urine volume: The animals are kept in separate metabolic cages for 24 hours. Each rat urine volume is taken after 24 hours. The food wastes and faecal matters are removed from the urine. And the volume of urine is measured by using measuring cylinder. Estimation of urine volume was carried out two time after 7th day dose of Vancomycin and after 10th day dose of standard drug /treatment drug. After measuring urine volume, the urine sample is used for qualitative analysis of urine for abnormal constituents.

2. Estimation of Body weight: At the end of the experiment, each group of the animals were kept individually in the cages. Remove the food and water, and each animal were individually weighed and the weight were recorded as body weight.

3. Estimation of Kidney weight: At the end of the experiment, each group of the animals were kept individually in the cages. Remove the food and water, and each animal were individually euthanatized for histopathology and remove kidney of all animals and weighed and the weight were recorded.

2.8. Qualitative Analysis of Urine for Abnormal Constituent [7]

1. **Test for Proteins: Heller's test:** Take 3ml of urine in a test tube and then add few drops of concentrated Nitric acid (HNO3). formation of white ring at the junction of two fluids infers presence of protein (Albumin).
2. **Test for Bile Salt: Hay's test:** Take 2 test tubes add 2ml urine in the first test tube and 2ml of distilled water in the second test tube. A small quantity of Sulphur powder is sprinkled over the surface of each liquid in each test tube. Sulphur powder sinks in the test tube containing urine infers presence of bile salts.

2.9. Statistical Analysis: -

The results were expressed as Mean ± S.E.M. The statistical difference between the groups was calculated in terms of one – way ANOVA followed by Dunnett's test to assess the statistical significance of the results using GraphPad Prism-10.2.2 software. P- values less than 0.05 were considered as statistically significant.

3. RESULT:

3.1. Pharmacognostical examination:

% yield = (weight of the extract / weight of powder taken) × 100

Drug	**Roots of *Bauhinia tomentosa* L.**
Percentage yield	**9.3 % w/w**

Table No. 2: Percentage yield of Roots of *Bauhinia tomentosa L.*

Extract	**Colour**	**Odour**	**Solubility**

EEBT	**Brown**	**Characteristics**	**In water**

Table No. 3: Physical examination of extract

3.2. Phytochemical Investigation:

Sr. No.	Phytochemicals	Ethanolic Extract of *Bauhinia tomentosa L.* (EEBT)
1.	Alkaloids	+
2.	Glycosides	-
3.	Tannins	+
4.	Amino Acids	+
5.	Triterpenoids	+
6.	Saponins	+
7.	Proteins	-
8.	Flavonoids	+
9.	Steroids	+
10.	Fixed oils and fats	+
11.	Gums and mucilage	-
12.	Carbohydrates	+

Table No.4: Phytochemical investigation of Ethanolic Extract of *Bauhinia tomentosa L.*
Where (+) indicates Present & (-) indicates Absent

3.3. ESTIMATION OF GENERAL PARAMETERS:

1. Estimation of urine volume

Groups	Treatment	Urine volume
I	**Control**	**10.50 ± 0.7638**
II	**VCM (150mg/kg)**	**5.333 ± 0.4944**
III	**VCM + Sele (6mg/kg)**	**10.18 ± 0.6058***
IV	**VCM + BT (200mg/kg) T1**	**9.433 ± 0.2512***
V	**VCM + BT (400mg/kg) T2**	**10.23 ± 0.4876***

Table No. 5: Effect of ethanolic extract of roots of *Bauhinia tomentosa L* (EEBT) on the Urine Volume in Vancomycin induced Nephrotoxicity.
All data are expressed as mean ± SEM (n = 6) and analyzed with one-way ANOVA followed by Dunnet's test (* $p < 0.0001$) vs. VCM group.

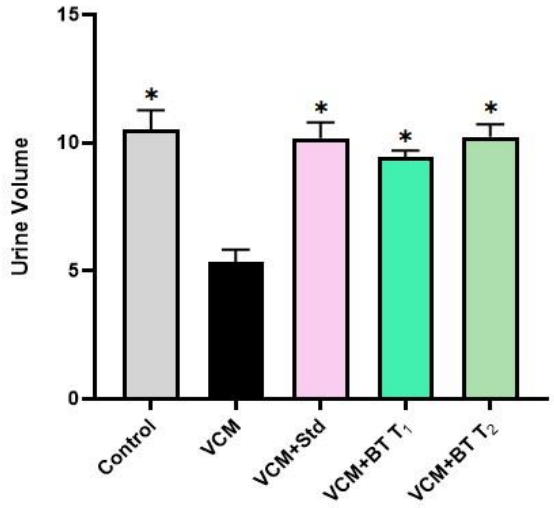

Chart No.1: Effect of ethanolic extract of roots of *Bauhinia tomentosa L* (EEBT) on the Urine Volume in Vancomycin induced Nephrotoxicity.

Table No. 5 And Chart No. 1: Treatment of Vancomycin (150mg/kg) (P<0.0001) showed significant reduction in urine volume. And decreased urine volume was significantly restored with EEBT 200mg/kg and 400mg/kg (P<0.0001) respectively.

2. Estimation of Body weight

Groups	Treatment	Body Weight
I	Control	181.0 ± 0.9661
II	VCM (150mg/kg)	187.5 ± 0.7638
III	VCM + Sele (6mg/kg)	171.5 ± 0.7638*
IV	VCM + BT (200mg/kg) T1	179.3 ± 0.8819*
V	VCM + BT (400mg/kg) T2	173.0 ± 0.9661*

Table No.6: Effect of ethanolic extract of roots of *Bauhinia tomentosa L*(EEBT) on the Body weight in Vancomycin induced Nephrotoxicity

All data are expressed as mean ± SEM (n = 6) and analyzed with one-way ANOVA followed by Dunnett's test (∗p < 0.0001) vs. VCM group.

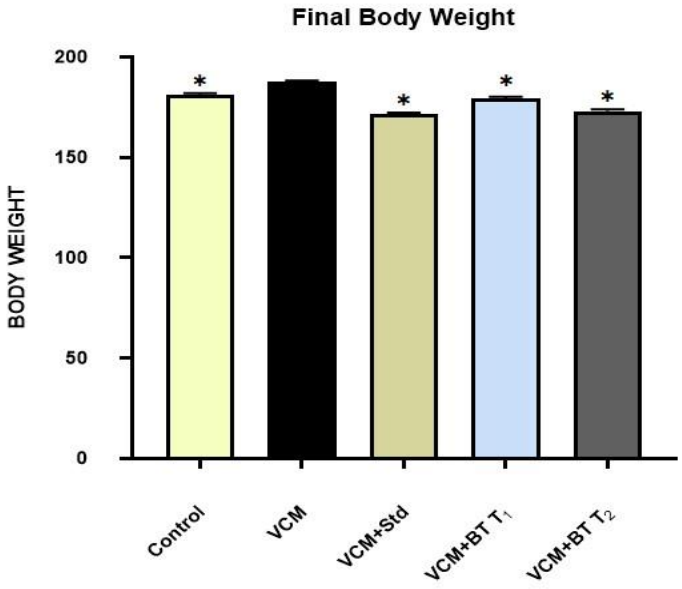

Chart No.2: Effect of ethanolic extract of roots of *Bauhinia tomentosa L* (EEBT) on the Body weight in Vancomycin induced Nephrotoxicity

Table No.6 And Chart No.2: After VCM administration, the Body weight significantly increased in rats, indicating serious toxicity that was markedly suppressed by a dose of ethanolic extract of roots of *Bauhinia tomentosa L* (200 mg/kg, 400mg/kg) and selenium (6mg/kg) ($p < 0.0001$).

3. Estimation of Kidney weight

Groups	Treatment	Kidney weight
I	Control	0.5333 ± 0.04410
II	VCM (150mg/kg)	0.9033 ± 0.05051
III	VCM + Sele (6mg/kg)	0.5733 ± 0.04264*
IV	VCM+ BT (200mg/kg)	0.6052 ± 0.02543*
V	VCM+ BT (400mg/kg)	0.5817 ± 0.02937*

Table No.7: Effect of ethanolic extract of roots of *Bauhinia tomentosa L* (EEBT) on the Kidney weight in Vancomycin induced Nephrotoxicity

All data are expressed as mean ± SEM (n = 6) and analyzed with one-way ANOVA followed by Dunnet's test ∗$p < 0.0001$ vs. VCM group.

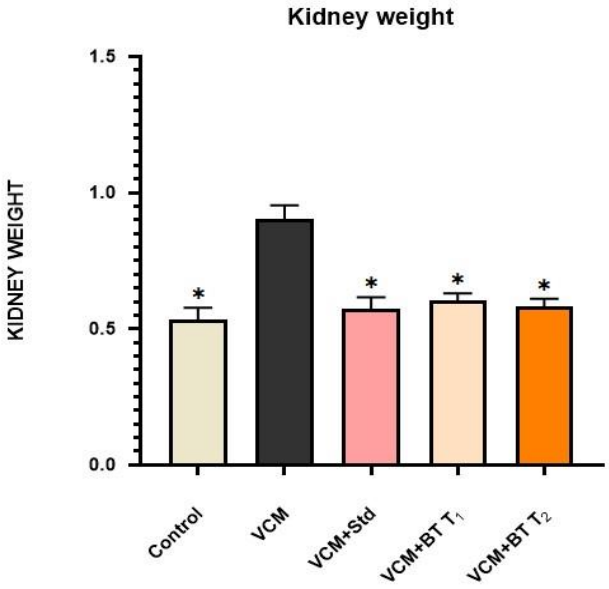

Chart No.3: Effect of ethanolic extract of roots of *Bauhinia tomentosa L* (EEBT) on the Kidney weight in Vancomycin induced Nephrotoxicity

Table No.7And Chart No.3: VCM administration, the Kidney weight significantly increased in rats ($p < 0.0001$), indicating serious toxicity that was markedly suppressed by a dose of ethanolic extract of roots of *Bauhinia tomentosa L* (200 mg/kg, 400mg/kg) and selenium (6mg/kg) ($p < 0.0001$).

3.4. Qualitative analysis of urine for Abnormal Constituent

1. Test for Proteins: Heller's test:

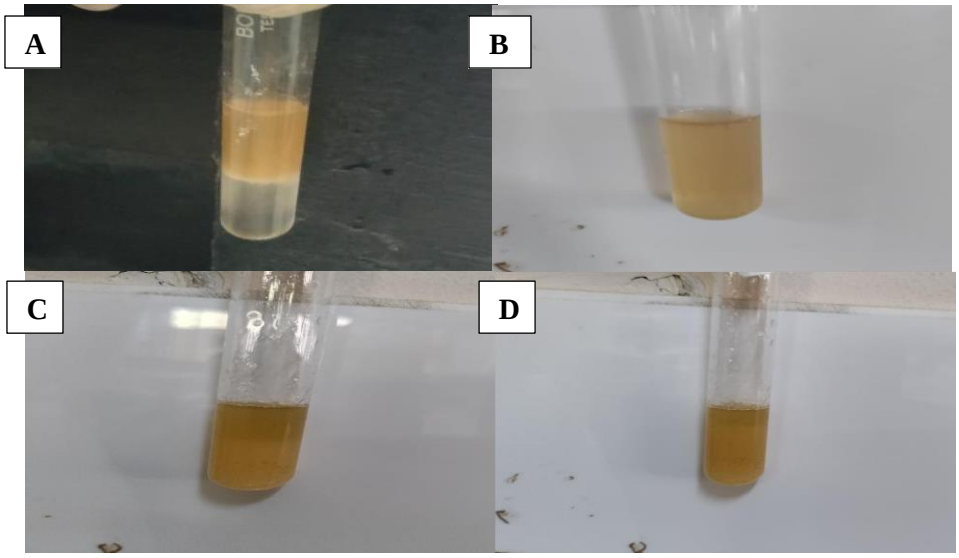

Fig. No. 1: A, B, C, D: Shows the result of Heller's test for proteins

Figure No. 1: A, B, C&D shows the result of Heller's test to investigate the proteins present or absent in the urine samples. Figure A: the urine sample of VCM (150mg/kg) induced rat group shows the white colored ring at the junction of two liquids which infers the presence of proteins in urine sample. Figure B: the urine sample of selenium (6mg/kg) treated rat group which does not show any ring at the junction of two liquids which indicates the absence of protein in urine sample. Figure (C & D): the urine sample of EEBT (200mg/kg & 400mg/kg) treated rat group which does not show any ring structure at the junction of two liquids which indicates absence of proteins in urine sample.

2. Test for Bile Salt: Hay's test

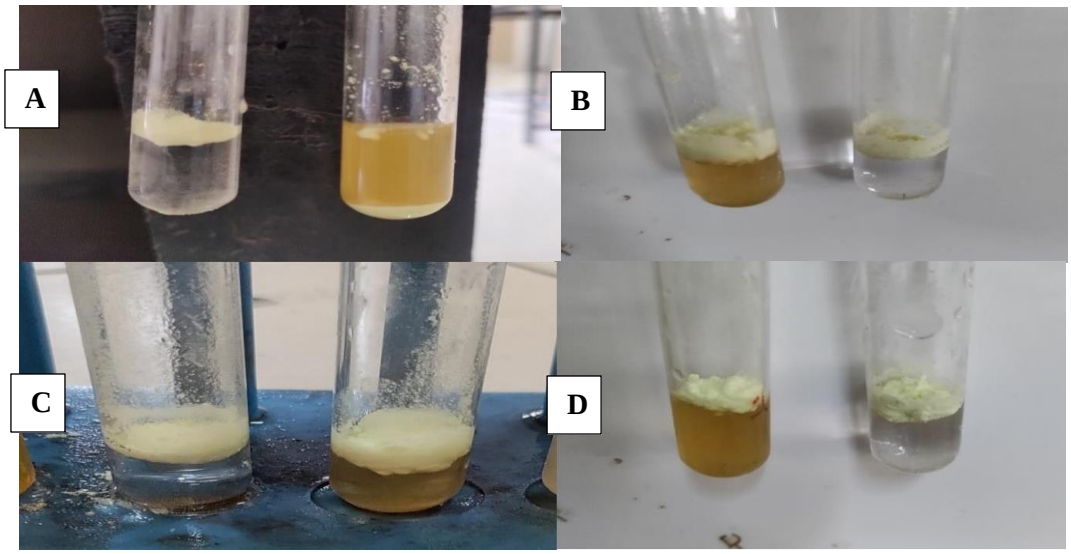

Fig. No. 2: A, B, C, D: Shows the result of Hay's test for Bile salts

Above all the figures shows the result of Hay's test to investigate the Bile salts present or absent in the urine samples. Figure A: the urine sample of VCM (150mg/kg) induced rat group shows that the Sulphur powder sinks into the urine and settle at the bottom of the test tube as compared to the water sample which infers the presence of bile salts in urine sample. Figure B: the urine sample of selenium (6mg/kg) treated rat group which shows that the Sulphur powder floats on the surface of the urine sample like water sample which indicates the absence of Bile salts in urine sample. Figure (C & D): the urine sample of EEBT (200mg/kg & 400mg/kg) treated rat group which shows that the Sulphur powder floats on the surface of the urine sample like water sample which indicates the absence of Bile salts in urine sample.

4. DISCUSSION

Nephrotoxicity is a common clinical syndrome defined as a rapid decline in renal function resulting in abnormal retention of serum creatinine and blood urea, which must be excreted. There are few chemical agents to treat acute renal failure. Studies reveal back synthetic nephroprotective agents have adverse effect besides reduce nephrotoxicity.

There is a growing interest of public in traditional medicine, particularly in the treatment of nephrotoxicity partly because of limited choice in the pharmacotherapy. Many plants have been used for the treatment of kidney failure in traditional system of medicine throughout the world. Indeed, along with the dietary measures, plant preparation formed the basis of treatment of disease until the introduction of allopathic medicine.

Ethnomedicinal plants can be used to help forestall the need of dialysis by treating the causes and effect of renal failure, as well as reducing the many adverse effect of dialysis. The phytochemicals found to be present in the roots extract of EEBT are the flavonoids, terpenoids, alkaloids, tannins, and saponins. Among them tannins, triterpenoids, flavonoids and saponins could be responsible for antioxidant property as these phytoconstituents are already reported to have antioxidant activity.

Acute toxicity studies revealed the non-toxic nature of the ethanolic extract of Bauhinia tomentosa Linn. There was no lethality or any toxic reactions found with high dose (2000 mg/kg body weight) till the end of the study. According to the OECD 420 guidelines (Acute Oral Toxicity: Fixed Dose Procedure), an LD50 dose of 2000 mg/kg and above was considered as unclassified so the ethanolic extract of Bauhinia tomentosa Linn was found to be safe.

VCM is used for treatment of serious infections, and its therapeutic effects are significantly improved by dose escalation. However, high-dose therapy with VCM is limited by its cumulative risk of nephrotoxicity.

Initially the general parameters (Urine volume, Body weight and Kidney weight) are investigated. In VCM induced rats observed that the urine output was decreased, and after treatment with selenium (6mg/kg) and ethanolic extract of Bauhinia tomentosa L.(200mg/kg,400mg/kg) the urine output was increased gradually. The rats treated with VCM shown marked Increased of body weight and kidney weight as compared to normal group also caused a marked reduction of glomerular filtration rate, which is accompanied by increase in serum

creatinine level, BUN and decline in creatinine clearance indicating induction of acute renal failure. With ethanolic extract of Bauhinia tomentosa L at the dose level of 200 and 400

mg/kg body weight and kidney weight for 15 days significantly lowered the serum level of creatinine with a significant body and kidney weight Loss, increased urine output and creatinine clearance when compared with the nephrotoxic control group.

In present study, the qualitative analysis of abnormal constituents of urine also investigated. After performing Heller's test for protein, VCM treated rat group shows the White ring at the junction of two liquids infers the presence of protein. Conversely, with selenium (6mg/kg) and EEBT (ethanolic extract of Bauhinia tomentosa L. 200mg/kg,400mg/kg) does not show any ring structure which indicates absence of protein. Similarly, after performing Hay's test for Bile salt, Shows the Sulphur powder sinks into urine sample of VCM induced rat group indicates that the presence of bile salts in urine sample. Conversely, the Sulphur powder floats on the urine sample of selenium (6mg/kg) and ethanolic extract of Bauhinia tomentosa L. (EEBT 200mg/kg,400mg/kg) which indicates the absence of bile salts.

Based on the above results, it may be concluded that Bauhinia tomentosa Linn exerted potential Nephroprotective activity against Vancomycin induced Nephrotoxicity in rats. To investigate the detailed mechanism of medicinal plant Bauhinia tomentosa Linn. Needs further studies.

5. CONCLUSION

The present study was undertaken to scientifically evaluate the nephroprotective activity of the ethanolic extract of roots of Bauhinia tomentosa Linn. The administration of VCM during experimentation is effectively induced vacuolization and necrosis, which was similar to acute renal failure in human.

Therefore, it is an effective and an ideal model for nephrotoxicity research. The estimation of abnormal constituents of urine on nephrotoxic (VCM induced) rats shows the presence of abnormal constituents (i.e. Protein and Bile salts) and with EEBT rats shows the absence of abnormal constituents. The evaluation of renal parameters on nephrotoxic rats with EEBT showed significantly elevated body weight, kidney weight and urine volume significantly reduce elevated parameters which supports its Nephroprotective activity. which proves that ethanolic extract of Bauhinia tomentosa having Nephroprotective activity.

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