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NEPHROPROTECTIVE ACTIVITY OF HYLOCEREUS UNDATUS FRUIT EXTRACT ON CISPLATININDUCED NEPHROTOXICITY IN RATS.

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ABSTRACT:

Objective: The present study was undertaken to scientifically evaluate the Nephroprotective activity of the ethanolic extract of fruit pulps of Hylocereus undatus. Method: Cisplatin administration during experimentation effectively induced apoptosis and necrosis, similar to acute renal failure in humans, making it an effective model for nephrotoxicity research. Nephrotoxic rats were treated with the ethanolic extract of Hylocereus undatus, and various renal parameters were evaluated. Results: Treatment with the ethanolic extract significantly elevated the attenuated body weight, urine volume, and creatinine clearance while significantly reducing elevated serum creatinine levels, supporting its Nephroprotective activity. Cisplatin-induced rats showed elevated levels of serum blood urea nitrogen (BUN) and lipid peroxidation parameter malondialdehyde (MDA), which were significantly decreased with treatment, demonstrating nephroprotective activity. The nephrotoxic rats also showed reduced levels of enzymatic antioxidants like superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), and non- enzymatic antioxidants like reduced glutathione (GSH), which were significantly increased with treatment, indicating antioxidant activity due to the flavonoids present in the extract. The ethanolic extract of Hylocereus undatus fruit pulp demonstrated statistically significant nephroprotective activity.

Keywords: Nephroprotective Activity, Hylocereus undatus, Cisplatin, Nephrotoxicity.

1. Introduction

The kidneys play an important role in human physiology, maintaining fluid homeostasis, regulating blood pressure, erythrocyte production and bone density, regulating hormonal balance,and filtering and removing nitrogenous and other waste products (Little and Combes, 2019; Sujana et al., 2021). Acute kidney injury (AKI) is defined as a sudden drop in kidney function, whereas chronic kidney disease (CKD) is characterized by a steady loss of functions. AKI and CKD are two of the most important public health issues that are on the rise globally. A projection of healthconcerns by 2040 ranked CKD as the fifth leading cause of death worldwide (Jager et al., 2019). In addition, kidney diseases have been recognized as risk factors for severe forms of COVID-19 (Henry and Lippi, 2020). Although a number of variables can induce this physiopathology, the rise in diabetes mellitus (DM) and hypertension is linked to an increase in their incidence as the primary causes of renal dysfunctions Factors are classified, primarily based totally at the pathway they caused kidney damage, as pre-renal, intrinsic, and post-renal factors. Pre-renal illnesses, based on clinical criteria, are associated with a reduction in renal perfusion or change in the systemic circulation, which will impair the glomerular filtration rate (GFR) and cause more severe changesto the kidney's structure. Clinical analyses reveal these dysfunctions by variations in biomarker levels, such as elevated serum creatinine, and variations in urine flow. Numerous illnesses, including haemorrhage, trauma, shock, hypertension, cirrhosis, diabetes, systemic infections, hypotension, autoimmune diseases, rhabdomyolysis, gut microbiota problems, liver injury, and intravascular volume depletion, have been recognized as pre-renal causes.The factors that directly cause kidney damage are intrinsic, whether heavy metals, trauma, Wegener's granulomatosis, proteinuria, congenital abnormalities, drug toxicity, renal atheroembolic disease, arthralgias, lupuserythematosus, or kidney cancer. Histologically, the main diagnoses are ischemic acute tubular necrosis, nephrotoxic acute tubular necrosis, and glomerulonephritis (Matuszkiewicz-Rowińska and Małyszko, 2020). Post-renal diseases are any other medical conditions that develop after the kidneys' physiological function and may indirectly cause kidney failure. These post-renal variables, which are primarily associated with problems of urine flow, include obstruction of the ureter and urethra caused by blood clots, lithiasis, or tumor growth. These conditions can raise thepressure inside tubules, which in turn can lower GFR and result in UTIs

Various approaches to kidney problems can be taken depending on the original cause's physiological process. There are already pharmaceutical treatments for both pre- and post-renal diseases; however, the majority of these medications have side effects and can occasionally result in intrinsic kidney damage. Proton pump inhibitors, antibiotics, chemotherapy, and non-steroidal anti-inflammatory medications (NSAIDs) are a few of them Nephrotoxicity of medications administered associated with risk therapies for other diseases, including pre- and post-renaldiseases, is now for both acute and chronic kidney disorders. Various approaches have been explored to treat these disorders in order to circumvent the side effects that come with medicine. Plants have been traditionally used as treatments for various diseases, among them several pathologies identified as pre-, intra-, and post-renal factors. The medicinal characteristics of plants have been attributed to their secondary metabolites, which can protect against pathogens or have important physiological benefits to prevent some diseases (Lawson et al., 2021; Isah, 2019). Numerous bioactive substances found in plants include antibacterial, diuretic, anticancer, antioxidant, and anti-inflammatory properties Further, Nephroprotective agents from plants mitigate processes such as interstitial nephritis, altered intraglomerular hemodynamics, tubular necrosis, or glomerulonephritis (Sabiu et al., 2016).

Literature survey has revealed that the plant of Hylocereus undatus is prescribed as a

nephroprotective, an anti-emetic, an antihistaminic, an antimicrobial, an antifungal, an anti-melioidosis, a defluoridation activity, an analgesic, an antipyretic, an anti-inflammatory, an anti- viral, an anti-nematodal, a molluscicidal activity, an anti-diabetic and hypolipidemic, an anti-oxidant activity, a cytotoxic, an antivenom, a hepatoprotective, an anthelmintic, a laxative[12], although information about its nephroprotective effect on fruit pulp has not been documented in any scientific study. Hence the study was undertaken to investigate the nephroprotective activity of ethanolic extractof the fruit pulp of Hylocereus undatus.

There is a growing interest of public in traditional medicine, particularly in thetreatment of nephrotoxicity partly because of limited choice in the pharmacotherapy.Certain Indian Medicinal plants have been reported to exhibit protective effect of renal tissues against injuries. The current investigation on the nephroprotective activity of Hylocereus is noteworthy due to the paucity of research conducted in this subject.

The present study was focused on the investigation of Nephroprotective activity of the ethanolic extract of fruit pulp of Hylocereus undatus on cisplatin induced nephrotoxicity in Wistar albino rats (Makris and Spanou, 2016a).

2. Materials and Methods

a. Selection of the Plant

The medicinal plant Hylocereus undatus was selected for Nephroprotective activity based on the literature survey.

b. Collection and Authentication of the Plant

The fruit pulp of Hylocereus undatus was collected from Chittor district, Andra Pradesh. The plant Hylocereus undatus was identified and authenticated by Prof. Dr. Madhava Shetti

c. Shade Drying and Cutting of the Fruit Pulp

The fruit pulp of Hylocereus undatus were collected and shade dried at the room temperature and then cut it in to small pieces, which was used for the extraction for further studies.

d. Extraction (Makris and Spanou, 2016b)

Fresh fruit pulps of Hylocereus undatus were cut into small pieces, seeds were removed and air dried. The dried pieces of Hylocereus undatus fruit pulp, weighing 100 g, were soaked in 500 ml of 95% ethanol in a round bottom flask for about 24 hours.

Extracting values of crude drug are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means Additionally, these figures show what kind of ingredients are in a crude medication.

Ethanolic extract:

Solvent-Ethanol

Reflux condensation method using Soxhlet apparatus was used to extract the material at 60–80 ºC for nine hours. Using a distillation equipment, the extract was concentrated until it reached a syrupy consistency. Finally, the extract was put in a china dish and evaporated at40-60 ºC temperature in a water bath, 22 gms of semisolid extract was obtained.

The plant extract used is Hylocereus undatus, and the part utilized is the fruit pulp.

e. Drugs and Chemicals

All the drugs, chemicals, and reagents were sourced from S.D. Fine Chemicals, Mumbai, India. All the chemicals and reagents used were of analytical reagent grade.

f. Experimental Protocol

The Nephroprotective activity was tested on five groups of Albino Wistar rats of either sex, usingcisplatin induced nephrotoxicity model (Alam, 2013; Arora and Singh, 2013) and lipoic acid as standard drug, each group consisting of six animals. Group I served as control was administered with 0.5% DMSO (Dimethyl sulphoxide) for 15 days. Group II Served as Nephrotoxic control was administered with DMSO 0.5% for 15 days, Groups III-V was also administered with DMSO. Standard drug treated group was group III (Lipoic acid 200mg/kg, p.o.). Test drug treated groupswere Group IV and V, treated with EEHU (200 and 400 mg/kg orally) (Table 1).

On the 10^{th} day 2 hours after the administration of standard Nephroprotectivedrug (Lipoic acid) and Hylocereus undatus (200 & 400 mg/kg) II-V groups received cisplatin (7.5mg/kg; i.p) using 4)

Table 1. Grouping of animals

S.No	GROUP	TREATMENT	Dose(mg/kg) p.o
1	Group I	Normal Control	0 .5% w/v
2	Group II	Negative Control (DMSO)	0 .5% w/v
3	Group III	Standard (Lipoic acid)	50
4	Group IV	EEHU (200mg/kg)	200
5	Group V	EEHU (400mg/kg)	400

g. Blood Collection Technique used in the Present Study (Yousef et al., 2009)

The blood was drawn by retro-orbital vein puncture using a fine capillary to an anticoagulant tube at the end of the study period, or on the fifteenth day. The blood was then allowed to stand for 30 minutes at 37°C before being separated from the serum using a centrifuge to assess the biochemical markers. Then, while under a light ether anesthetic, the animals were sacrificed.

h. Preparation of kidney homogenate

After the kidney was swiftly removed, ice-cold saline (0.9% NaCl) was immediately perfused. Using a homogenizer, a piece of the kidney was homogenized in cold Tris-HCl buffer (0.025 M, pH 7.4). The homogenate obtained was centrifuged at 5000 rpm for 10 minutes, supernatant was collected and used for various biochemical assays.

i. Statistical Analysis (Yousef et al., 2009)

The data was presented as Mean ± SEM. One-way analysis of variance (ANOVA) and Tukey's Multiple Comparison Test were used to assess the data. A P value of less than 0.05 was deemed statistically significant. Software called Graph Pad Prism 5.0 was used to process the data.

j. Analysis of General Parameters

Estimation of urine volume

For a full day, the animals are housed in different metabolic cages. After a day, the volume of pee from each rat is measured. Urine is treated to remove dietary wastes and feces. Additionally, a measuring cylinder is used to determine the urine's volume.

Estimation of Body weight

Upon completion of the trial, every group of animals was maintained separately within their cages.Take out the food and water, weigh each animal separately, and record the results.

k. Analysis of Serum Biochemical Parameters

Estimation of Serum Creatinine (Li et al., 2013)

A, B, C, D, and E were the labels on five test tubes. where E is considered blank, C and D are considered test, and A and B are considered standard. 2 ml of distilled water were pipetted into E(blank), 0.5 ml of serum and 1.5 ml of water into C&D (test), and 1.5 ml of water and 0.5 ml of creatinine standard (3 mg/dl) into A&B (standard). In each of the five test tubes, 6 ml of picric acid and 0.4 ml of sodium hydroxide (NaOH) (2.5M) were added (Table 2). Agents

Reagents:

1. Creatinine stock (150 mg/100 ml water),
2. Creatinine working standard for serum (3 mg/dl, dilute 10 ml stock to 500 ml),
3. Serum samples,
4. NaOH (2.5 M),
5. Picric acid.

Table 2. Determination of Serum Creatinine Levels

	Standard (A,B)	Test (C,D)	Blank (E)
Serum	-	0.5 ml	-
Distilled water	1.5 ml	1.5ml	2 ml
Standard for serum	0.5 ml	-	-
Picric acid	6 ml	6 ml	6 ml
NaOH (2.5 M)	0.4 ml	0.4 ml	0.4 ml

1. Thoroughly combine
2. Add 2.5 M NaOH (0.4 ml).
3. Give it a 20-minute rest.
4. At 520 nm, compare the absorption to the blank.

$$\text{Serum creatinine} = \frac{\text{Absorbance of test}}{\text{Absorbance of std}} \times \text{Concentration of std}$$

Estimation of Serum Blood urea nitrogen (BUN) (Slot, 1965)

The blood urea was estimated by Berthelot method (Fawcett and Scott, 1960) using the commercially available kit (Kamineni Life Sciences Pvt. Ltd. Hyderabad, India to create the test, standard, and blank, 1000 µl of working reagent-I, which contains urease reagent, and a combination of salicylate, hypochlorite, and nitroprusside were added to 10 µl of serum, 10 µl of standard urea (40 mg/dl), and 10 µl of filtered water. All the test tubes were mixed well and incubated at 37 °C for5 min. Then 1000 µl of reagent-II containing alkaline buffer, was added to all the test tubes, which were incubated at 37 °C for 5 min. The transformation of urea into ammonia and carbon dioxide is catalyzed by urease. Indo phenol, a blue-green chemical, is produced when a combination of salicylate, hypochlorite, and nitroprusside combines with the ammonia that is so released. Spectrophotometrically measured at 578 nm, the intensity of the color produced is directly proportional to the concentration of urea in the sample. The following formula was used to determine the blood urea:

Blood urea mg / dl = $\frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 40$

Blood urea nitrogen (mg/dl)=Serum urea × 0.467

l. Analysis of Urine Biochemical Parameters

Estimation of Creatinine clearance

Five tubes as A, B, C, D and E. were labelled Where A&B is taken as standard, C&D taken as testand the E where taken as blank. Into E (blank), 2 ml of distilled water, into C&D (test), 0.5 ml urine and 1.5 ml of water, into A&B (standard),1.5ml of water and 0.5 ml of creatinine standard (3mg/dl) were pipette out. 6 ml of picric acid with 0.4 ml of NaOH (2.5M) were added in all the test tubes (Table 3).

1. Creatinine stock standard: 150 mg creatinine in 100 ml water (1.5 mg/ml)
2. Creatinine working standard for urine (0.75 mg/dl): dilute 50 ml of stock andbring the volume up to 200 ml with water.
3. Urine samples.
4. NaOH (2.5 M).
5. Picric acid.

Table 3. Estimation of Serum Creatinine

	Standard A,B	Test C,D	Blank E
Urine	-	0.5 ml	-
Distilled water	1.5 ml	1.5ml	2 ml
Standard for serum	0.5 ml	-	-
Picric acid	6 ml	6 ml	6 ml

1. mix well
2. Add 0.4 ml of 2.5 M NaOH
3. Allow to stand for 20 minutes
4. Read the absorbance against the blank at 520 nm

Creatinine clearance = $\frac{\text{Urinary creatinine}}{\text{Serum creatinine}} \times$volume of urine ml/min/1.73 m^2

$$\text{Urinary creatinine} = \frac{\text{Absorbance of test}}{\text{Absorbance of std}} \times \text{Concentration of std}$$

m. Analysis of Oxidative Stress Parameters

Estimation of malondialdehyde (MDA) (Okhawa et al., 1979)
The method of measuring lipid peroxidation (LPO) used the release of malondialdehyde (MDA) as an index of LPO. One way to measure the amount of LPO in the hepatic tissue was to measure thiobarbituric acid-reactive compounds (TBARS), which are one of the end products of this process. Since malondialdehyde (MDA) makes up 99% of TBARS, the assay's foundation is the interaction of one MDA molecule with two TBARS molecules at a low pH (two to three) and 95°C for sixty minutes. Spectrophonometric analysis reveals the resulting pink chromogen at 532 nm.the method of measuring lipid peroxidation (LPO) used the release of malondialdehyde (MDA) as anindex of LPO.

Reagents
- ✓ Standard: 1, 1, 3, 3-tetra ethoxypropane (TEP).
- ✓ 8.1% Sodium dodecyl sulphate (SDS)
- ✓ 20%Acetic acid
- ✓ 0.8%Thiobarbituric acid (TBA)
- ✓ 15:1 v/v n-butanol: pyridine mixture

Procedure
1.5 ml of 20% acetic acid (pH 3.5), 0.2 ml of 8.1% SDS, and 1.5 ml of 0.8% TBA were added to
0.2 ml of tissue homogenate. Using a glass ball as a condenser, the mixture was boiled in a waterbath for 60 minutes at 95.8ºC until 4 cc of water was added. Following cooling, 5 ml of a 15:1 v/vn-butanol:pyridine combination and 1 ml of water were added, and the mixture was vigorously shaken. Following a 10-minute centrifugation at 4000 rpm, the organic layer was removed, and its absorbance at 532 nm was measured. The MDA content was given in nmoles per milligram of tissue.

2.14 Analysis of Enzymatic Antioxidants Parameters
2.14.1 Estimation of superoxide dismutase (SOD) (Misra and Fridovich, 1972)
This enzyme catalyzes the dismutation of superoxide anion (O2-) into molecular oxygen and hydrogen peroxide in the manner described below.

$H_2O + 2O_2^{-\cdot} + 2H^+ \rightarrow 2H_2O_2 + O_2$

The method developed by Misra and Fridovich (1972) was used to measure the enzyme activity.

Reagents
1. 0.1 M Carbonate-bicarbonate buffer; pH 10.2.
2. 0.6 mM EDTA solution
3. 1.8 mM Epinephrine (prepared in situ)

4. Absolute ethanol.
5. Chloroform

Procedure

The tubes holding 0.75 ml of ethanol and 0.15 ml of chloroform (cooled in ice) were filled with
0.1 ml of tissue homogenate and centrifuged. Add 0.5 ml of 0.6 mM EDTA solution and 1 ml of
0.1 M carbonate-bicarbonate (pH 10.2) buffer to 0.5 ml of supernatant. Freshly made 0.5 ml of 1.8mM epinephrine was added to start the reaction, and the increase in absorbance at 480 nm was observed. Superoxide dismutase (SOD) is measured in nmoles/mg of protein.

2.14.2 Estimation of Catalase (CAT) (Sinha, 1972)

This enzyme catalyzes conversion of hydrogen peroxide into water and molecular oxygen.
$2H_2O_2 \rightarrow 2H_2O + O_2$
The Sinha, 1972 method was used to measure the enzyme activity.

Reagents

6. Dichromate-acetic acid reagent: 5% potassium dichromate in water was mixed with glacial acetic acid in the ratio of 1:3 (v/v).
7. 0.01 M Phosphate buffer; pH 7.0.
8. 0.2M Hydrogen peroxide

Procedure

The reaction mixture contained 1 milliliter of 0.01 M phosphate buffer (pH 7.0), which had been preheated to 37°C, 0.4 milliliter of distilled water, and 0.1 milliliter of the tissue homogenate. The mixture was then incubated at 37°C. A minute was spent incubating the reaction mixture at 37°C after adding 0.5 ml of 0.2 M hydrogen peroxide to start the reaction. After 15, 30, 45, and 60 seconds, 2 cc of dichromate-acetic acid reagent was addedto stop the reaction. The same protocol was applied to standard hydrogen peroxide, which was measured and treated in the range of 4–20 µl/mol. Following ten minutes of heating ina boiling water bath, each tube was cooled, and the green color that formed was measured at 590 nm against a blank that was devoid of all components but the enzyme. U/mg proteinexpressing catalase activity.

2.14.3 Estimation of glutathione peroxidase (GPx) (Halim and Mukhopadhyay, 2006)

We measured glutathione peroxidase activity using the Hafemann et al. (1974) technique. After exposing the sample to H_2O_2 and NaN_3, the activity of GPx was ascertained by monitoring thedrop in GSH Content.
$H_2O_2 + 2\ GSH \rightarrow 2H_2O + 2\ GSSG$

Reagents

9. 5 mM GSH
10. 25 mM H_2O_2
11. 25 mM NaN_3
12. Phosphate Buffer (0.05 mM, pH 7)13. 1.65 % HPO_3^{2}
14. 0.4 M Na_2HPO_4
15. 1 mM DTNB

Procedure

Tissue homogenate (about 0.5 mg protein) was incubated in a total volume of 2.5 ml at 37oC for 10 minutes along with 0.1 ml of 5 mM GSH, 0.1 ml of 1. 25 mM H2O2, 0.1 ml of 25 mM NaN3,and phosphate buffer (0.05 mM, pH 7). Two milliliters of 1.65% HPO32-were added to halt the reaction, and the reaction mixture was centrifuged for ten minutes at 1500 rpm. 1 ml of 1 mM DTNB and 2 ml of 0.4 M Na2HPO4 were combined with 2 ml of the supernatant. The yellow- colored complex was incubated for 10 minutes at 37°C against distilled water, and the absorbanceof the mixture was measured at 412 nm. A sample that was not subjected to the same tissue homogenate processing method was retained as a non-enzymatic reaction.

2.15 Analysis of Non Enzymatic Antioxidant Parameters

2.15.1 Estimation of Reduced Glutathione (GSH) (Sharma et al., 2016)

Ellman reagent (DTNB) is the most often used method for measuring GSH in biological samples.It reacts with sulfydryl chemicals to produce a yellow color that is reasonably stable. The color that appears when this chemical dissolves in water is related to the amount of GSH present. 0.5 ml of 5% trichloroacetic acid (TCA) solution was added to 0.5 ml of citrated blood in order to precipitate the proteins. The mixture was centrifuged at 3000 rpm for 20 minutes. 0.1 ml of supernatant was combined with 0.5 ml of DTNB reagent and 1 ml of sodium phosphate buffer. The absorbance of the generated yellow color was measured at 412 nm (Ellman, 1959).

Reagents

16. TCA (5%)
17. Phosphate buffer (0.2M, pH 8.0)
18. DTNB (0.6mM in 0.2M phosphate buffer)
19. Standard GSH (10nmoles/ml of 5% TCA)

Procedure

The supernatant (0.1ml) was made up to 1.0ml with 0.2M sodium phosphate buffer (pH 8.0). Standard GSH corresponding to concentrations ranging between 2 and 10 n moles were also prepared. Two ml of freshly prepared DTNB solution was added and the intensity of the yellow colour developed was measured in a spectrophotometer (Genesys 10-S, USA) at 412nm after 10 minutes. The results are given in terms of n moles GSH/g of sample.

3. Results and Discussion

For their assessment, the extraction values of the crude medication were helpful. These figures also revealed the makeup of the ingredients in the crude medication. Using a Soxhlet device, the small bits of fruit pulp were treated to a continuous hot percolation process with a solvent of 95% ethanol. It was calculated what the extract percentage was. The final result was listed in Table 4.

Table 4. Results of the Percentage yield of EEHU

Solvent	Ethanol
Colour	Dark Brownish
Consistency	Semi-solid
Percentage yield	22%w/w

3.1 Assessment of General Biochemical Parameters

3.1.1 Assessment of urine volume

The effects of the different doses of ethanolic extract of Hylocereus undatus on urine volume (Figure 1, Table 5).

Table 5. Results of the effect of EEHU on urine volume in cisplatin induced Nephrotoxicrats

Groups	Drug Treatment &Dose	Urine Volume
I	Normal Control(0.5% DMSO)	11.85± 0.343
II	Nephrotoxic ControlCisplatin (0.75%)	4.10±0.642
III	Reference Control Cisplatin (0.75%) Lipoic acid	12.81±0.44***
IV	Cisplatin (0.75%) +EEHU (200mg/kg)	8.11±0.60*
V	Cisplatin (0.75%) +EEHU (400mg/kg)	9.90±0.24***

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

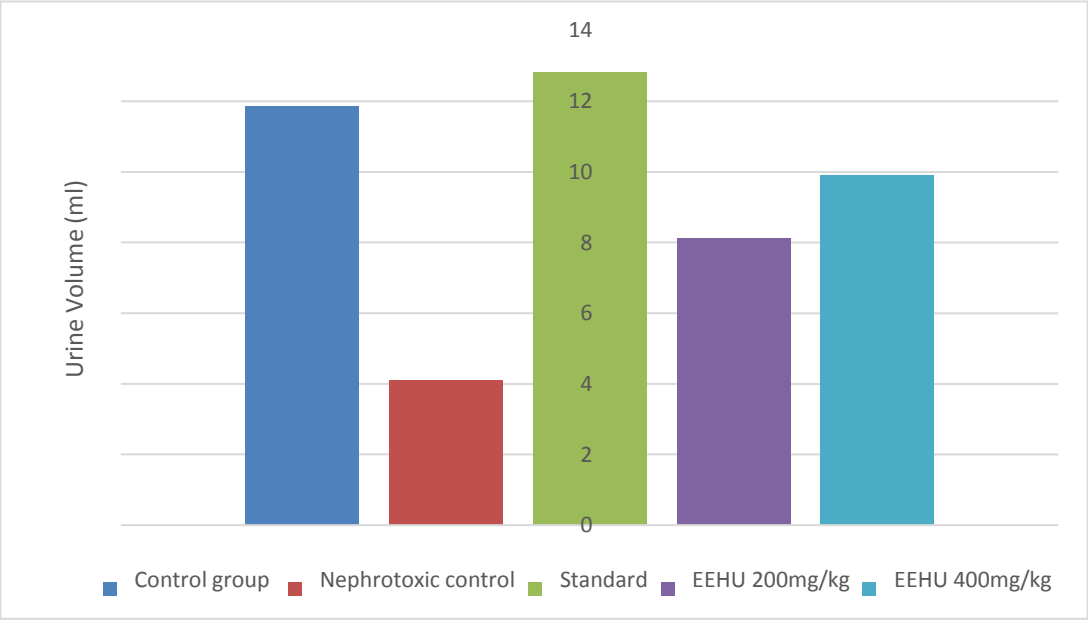

Figure 1. Diagrammatic representation of EEHU on urine volume in cisplatin inducedNephrotoxic rats

The urine volume was measured were showed in Table 5 and Figure 1.
The Nephrotoxic control (Group 2) showed significant decrease in urinevolume when compared to the normal control (Group1).Standard (Group 3) showed statistically significant increase in urine volumewhen compared to Nephrotoxic control (Group2).EEHU 200 mg/kg treated (Group 4) showed statistically significant increase inurine volume when compared to the Nephrotoxic control (Group 2).EEHU 400mg/kg treated (Group 5) showed statistically

significant increase inurine volume when compared to the Nephrotoxic control (Group 2).

3.1.2 Assessment of Body weight

The effects of the different doses of ethanolic extract of Hylocereus undatus on body weight (Table 6, Figure 2).

Table 6. Results of the effect of EEHU on Body weight in cisplatin induced Nephrotoxicrats

Groups	Drug Treatment	Body weight
I	Normal Control (0.5% DMSO)	247± 3.300
II	Nephrotoxic Control Cisplatin (0.75%)	15844± 2.612
III	Reference Control Cisplatin (0.75%) + Lipoic acid (50mg/kg)	233.83± 4.312***
IV	Cisplatin (0.75%) + EEHU (200mg/kg)	207.83± 3.49*
V	Cisplatin (0.75%) + EEHU (400mg/kg)	225± 3.784***

Values were given in Mean ±SEM;
*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

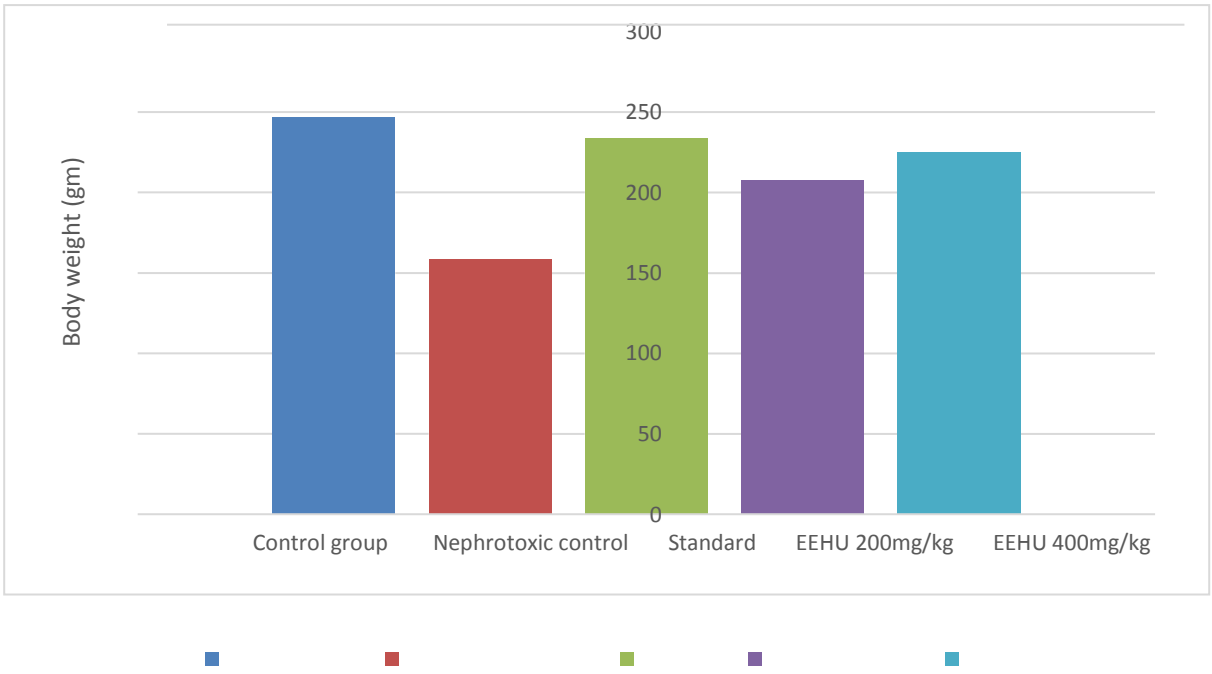

Figure 2. Diagrammatic representation of EEHU on serum creatinine level on cisplatininduced Nephrotoxicity in rats

The body weight measured were showed in Table 6 and Figure 2.
The Nephrotoxic control (Group 2) showed significant decrease in bodyweight when compared to the normal control (Group1).
Standard (Group 3) showed statistically significant increase in body weightwhen comparedto Nephrotoxic control (Group 2)
EEHU 200 mg/kg treated (Group 4) showed statistically significant increase inbodyweight when compared to the Nephrotoxic control (Group 2).
EEHU 400mg/kg treated (Group 5) showed statistically significant increase inbody weight when compared to the Nephrotoxic control (Group 2)

3.2 Assessment of Serum Biochemical Parameters

3.2.1 Serum creatinine level

The effects of the different doses of ethanolic extract of Hylocereus undatus Linn on serumcreatinine level (Table 7, Figure 3).

Table7. Results of the effect of the EEHU on serum Creatinine on Cisplatin induced Nephrotoxicity in rats

Groups	Drug Treatment	Serum creatinine
I	Normal Control(0.5% DMSO)	0.70±0.098
II	Nephrotoxic ControlCisplatin (0.75%)	4.57±0.112
III	Reference Control Cisplatin (0.75%) + Lipoic acid (50mg/kg)	0.63±0.010**
IV	Cisplatin (0.75%) +EEHU (200mg/kg)	1.45± 0.196*
V	Cisplatin (0.75%) +EEHU (400mg/kg)	0.98± 0.091**

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

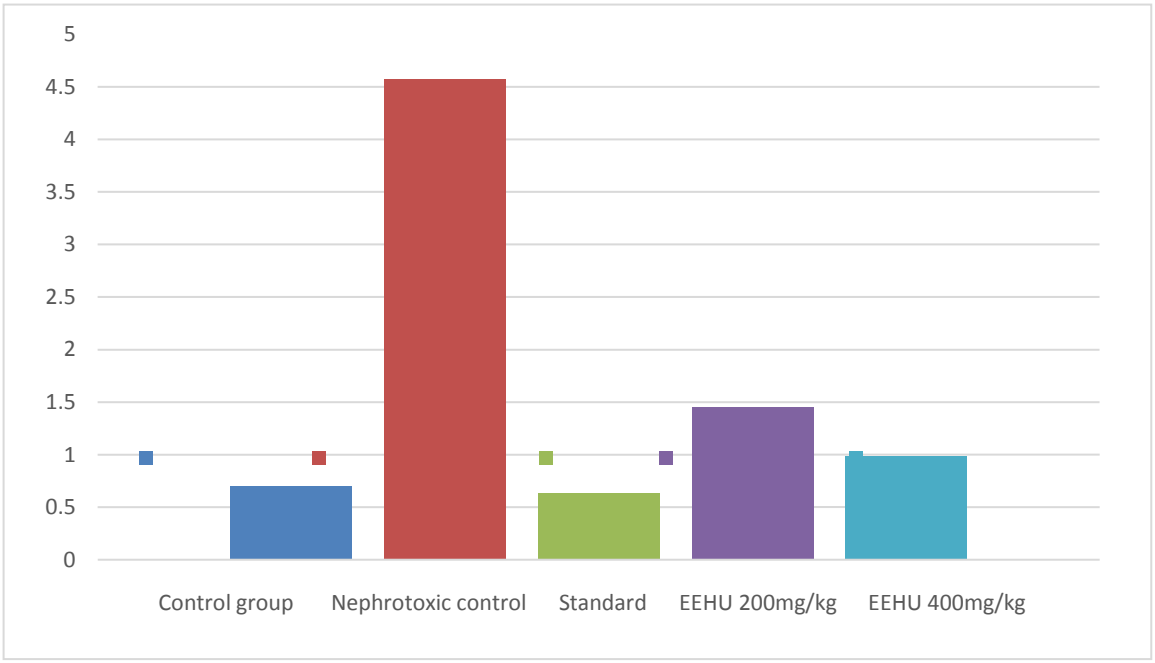

Figure 3. Diagrammatic representation of EEHU on serum creatinine levelon cisplatininduced Nephrotoxicity in rats

The Nephrotoxic control (Group 2) showed significant increase in serumcreatinine level when compared to the normal control (Group1).
Standard (Group 3) showed statistically significant decrease in serumcreatinine levelwhen compared to Nephrotoxic control (Group 2).
EEHU 200 mg/kg treated (Group 4) showed statistically significant decrease inserum creatininelevel when compared to the Nephrotoxic control (Group 2).
EEHU 400mg/kg treated (Group 5) showed statistically significant decrease inserum creatininelevel when compared to the Nephrotoxic control (Group 2)

3.2.2 Serum Blood Urea Nitrogen (BUN)

The effects of the different doses of ethanolic extract of Hylocereus undatus Linn on serumBlood urea nitrogen (BUN) level (Table 8, Figure 4).

Table 8. Results of the effect of the EEHU on serum Blood urea nitrogen on Cisplatininduced Nephrotoxicity in rats

Groups	Drug Treatment	Serum blood urea nitrogen (BUN)
I	Normal Control(0.5% DMSO)	23.57±0.501
II	Nephrotoxic ControlCisplatin (0.75%)	55.77±0.761
III	Reference Control Cisplatin (0.75%) + Lipoic acid(50mg/kg)	24.10±0.49***
IV	Cisplatin (0.75%) +EEHU (200mg/kg)	28.02±0.91*
V	Cisplatin (0.75%) +EEHU (400mg/kg)	24.67±0.57***

Values were given in Mean ±SEM;
*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

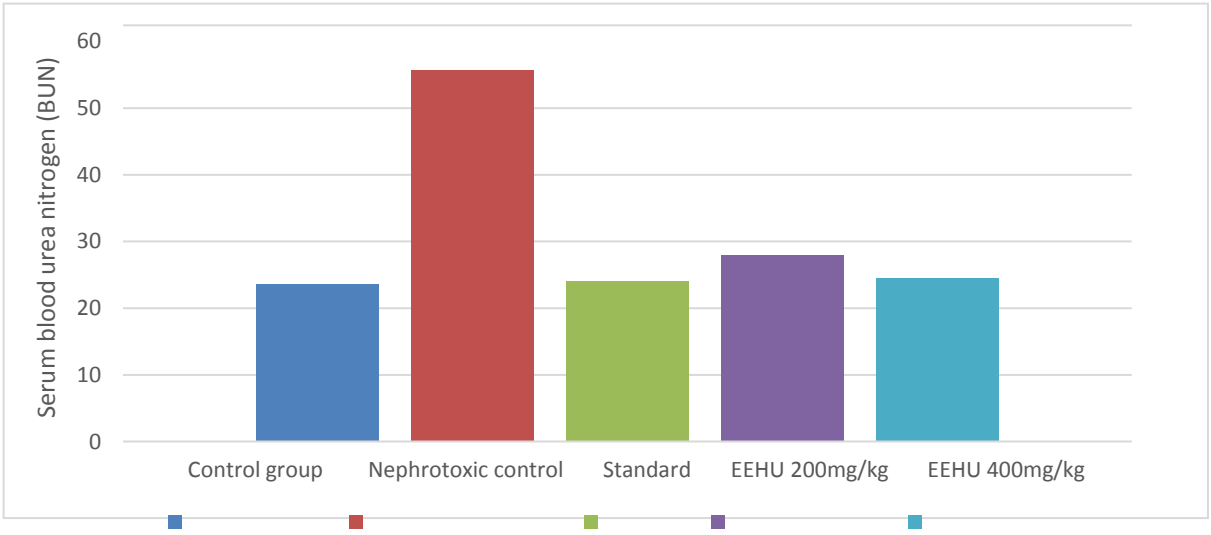

Figure 4. Diagrammatic representation of EEHU on serum Blood urea nitrogenparameters on cisplatin induced Nephrotoxicity in rats

The Nephrotoxic control (Group 2) showed significant increase in serum Blood urea

nitrogen (BUN) level when compared to the normal control (Group1).
Standard (Group 3) showed statistically significant decrease in serum Blood urea nitrogen (BUN) level when compared to Nephrotoxic control (Group 2).
EEHU 200 mg/kg treated (Group 4) showed statistically significant decrease in serum Blood urea nitrogen (BUN) level when compared to the Nephrotoxic control (Group 2).
EEHU 400mg/kg treated (Group 5) showed statistically significant decrease in serum Blood urea nitrogen (BUN) level when compared to the Nephrotoxic control (Group 2).

3.3 Assessment of Urine Biochemical Parameters

3.3.1 Assessment of creatinine clearance

The effects of the different doses of ethanolic extract of Hylocereus undatus Linn on creatinineclearance (Table 9, Figure 5).

Table 9. Results of the effect of EEHU on creatinine clearance in cisplatin induced Nephrotoxic rats

Groups	Drug Treatment	Creatinineclearance
I	Normal Control(0.5% DMSO)	20.80±1.309
II	Nephrotoxic ControlCisplatin (0.75%)	6.05±0.498
III	Reference Control Cisplatin (0.75%) +Lipoicacid(50mg/kg)	17.263±0.510***
IV	Cisplatin (0.75%) +EEHU (200mg/kg)	15.74±0.758*
V	Cisplatin (0.75%) +EEHU (400mg/kg)	16.20±1.136***

Values were given in Mean ±SEM;
*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

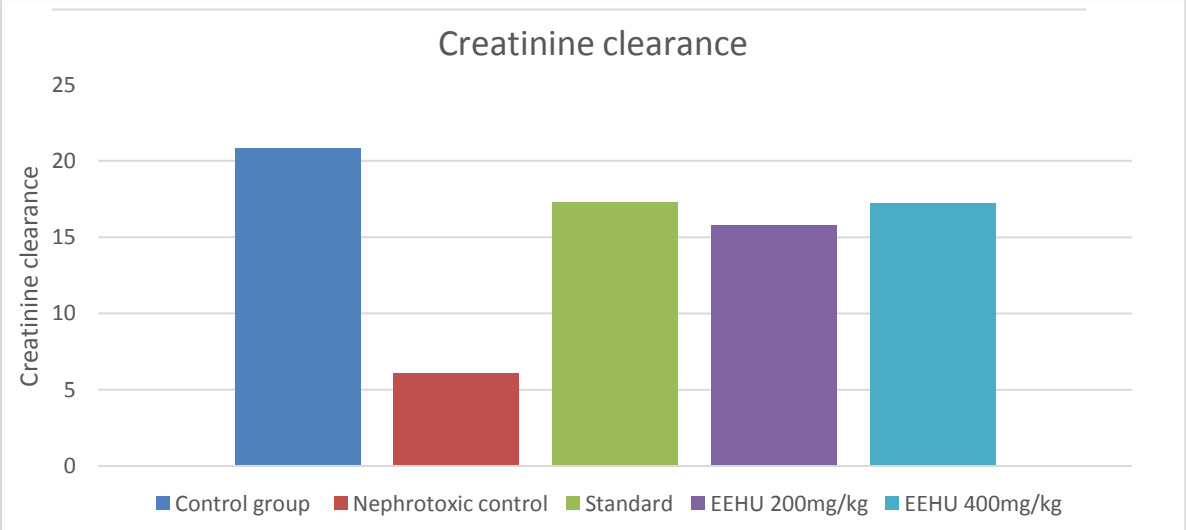

Figure 5. Diagrammatic representation of EEHU on creatinine clearance in cisplatininduced Nephrotoxic rats

The Nephrotoxic control (Group 2) showed significant decrease in creatinineclearance when compared to the normal control (Group1).
Standard (Group 3) showed statistically significant increase in creatinineclearance when compared to Nephrotoxic control (Group 2).
EEHU 200 mg/kg treated (Group 4) showed statistically significant increase increatinine clearance when compared to the Nephrotoxic control (Group 2).

EEHU 400mg/kg treated (Group 5) showed statistically significant increase increatinine clearancewhen compared to the Nephrotoxic control (Group 2).

3.4 Assessment of Oxidative Stress Parameter

3.4.1 Assessment of Malondialdehyde (MDA)

The effects of the different doses of ethanolic extract of Hylocereus undatus Linn on malondialdehyde (MDA) (Table 10, Figure 6).

Table 10. Results of the effect of EEHU on Malondialdehyde (MDA) incisplatin inducedNephrotoxic rats

Groups	Drug Treatment	Malondialdehyde(MDA)
I	Normal Control(0.5% DMSO)	7.64±0.476
II	Nephrotoxic ControlCisplatin (0.75%)	16.44±0.412
III	Reference Control Cisplatin (0.75%) + Lipoic acid (mg/kg)	7.96±0.121***
IV	Cisplatin (0.75%) +EEHU (200mg/kg)	8.88±0.436**
V	Cisplatin (0.75%) +EEHU (400mg/kg)	7.74±0.291***

Values were given in Mean ±SEM;
*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

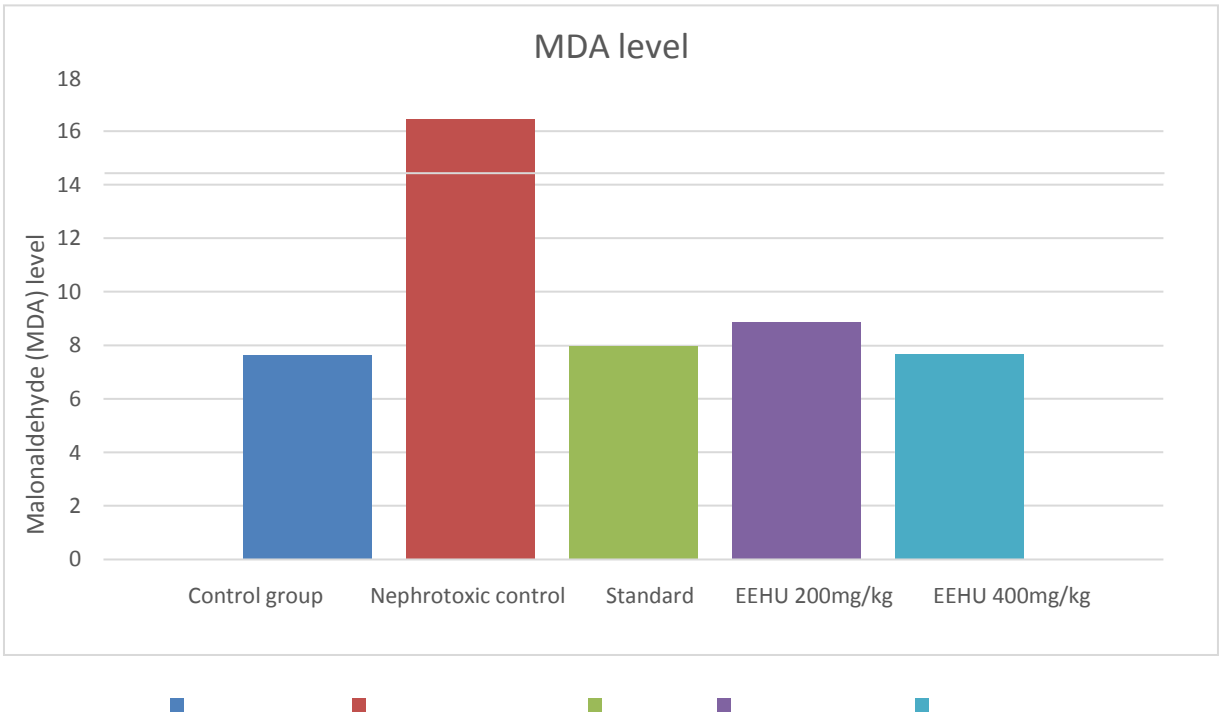

Figure 6. Diagrammatic representation of EEHU on Malondialdehyde (MDA) in cisplatininduced Nephrotoxic rats

The Nephrotoxic control (Group 2) showed significant increase in malondialdehyde (MDA) when compared to the normal control (Group1).

Standard (Group 3) showed statistically significant decrease in malondialdehyde (MDA) when compared to Nephrotoxic control (Group 2).
EEHU 200 mg/kg treated (Group 4) showed statistically significant decrease inmalondialdehyde(MDA) when compared to the Nephrotoxic control (Group 2).
EEHU 400mg/kg treated (Group 5) showed statistically significant decrease in malondialdehyde (MDA) when compared to the Nephrotoxic control (Group 2).

3.5 Assessment of Enzymatic Antioxidant Parameters

3.5.1 Assessment of superoxide dismutase (SOD)

The effects of the different doses of ethanolic extract of Hylocereus undatus Linn on superoxide dismutase (SOD) (Table 11, Figure 7).

Table 11. Results of the effect of EEHU on superoxide dismutase (SOD) in cisplatin induced Nephrotoxic rats

Groups	Drug Treatment	Superoxide dismutase (SOD)
I	Normal Control(0.5% DMSO)	19.78±0.597
II	Nephrotoxic ControlCisplatin (0.75%)	8.64±0.492
III	Reference Control Cisplatin (0.75%) + Lipoic acid(50mg/kg)	18.46±0.41***
IV	Cisplatin (0.75%) +EEHU (200mg/kg)	12.07±0.384*
V	Cisplatin (0.75%) +EEHU (400mg/kg)	14.97±0.362***

Values were given in Mean ±SEM;
*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

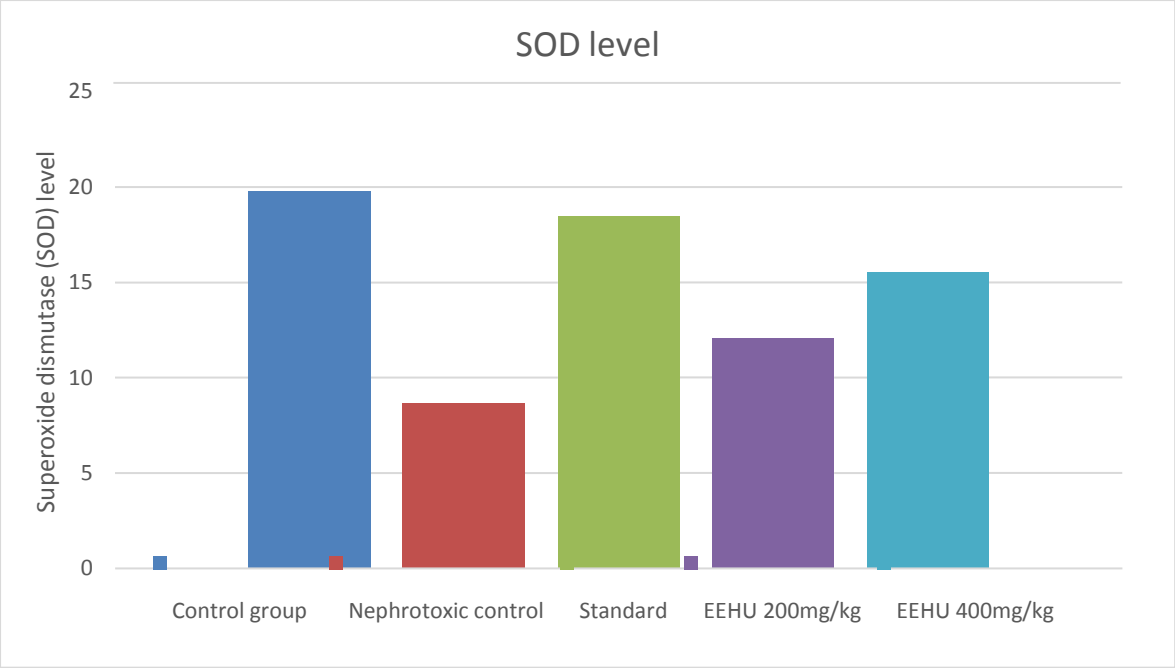

Figure 7. Diagrammatic representation of EEHU on superoxide dismutase(SOD) incisplatin induced Nephrotoxic rats

The Nephrotoxic control (Group 2) showed significant decrease in superoxidedismutase (SOD)when compared to the normal control (Group1).
Standard (Group 3) showed statistically significant increase in superoxidedismutase (SOD) whencompared to Nephrotoxic control (Group 2).
EEHU 200 mg/kg treated (Group 4) showed statistically significant increase insuperoxide dismutase (SOD) when compared to the Nephrotoxic control (Group 2).
EEHU 400mg/kg treated (Group 5) showed statistically significant increase in superoxide dismutase (SOD) when compared to the Nephrotoxic control (Group 2).

3.5.2 Assessment of Catalase (CAT)

The effects of the different doses of ethanolic extract of Hylocereus undatus Linn on Catalase(CAT) (Table 12, Figure 8).

Table 12. Results of the effect of EEHU on Catalase (CAT) in cisplatin induced Nephrotoxic rats

Groups	Drug Treatment	Catalase (CAT)
I	Normal Control(0.5% DMSO)	217.42±0.49
II	Nephrotoxic ControlCisplatin (0.75%)	103.36±0.32
III	Reference Control Cisplatin (0.75%) +Lipoic acid(50mg/kg)	198.09±0.611**
IV	Cisplatin (0.75%) +EEHU (200mg/kg)	157.46±4.061**
V	Cisplatin (0.75%) +EEHU (400mg/kg)	179±0.212***

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control.

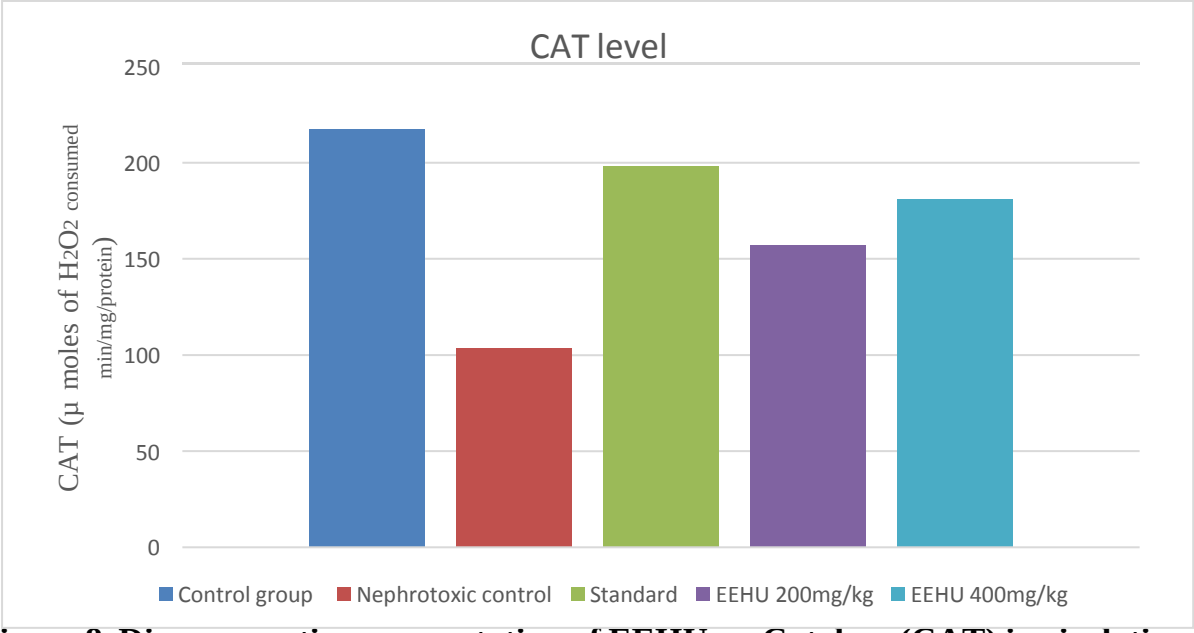

Figure 8. Diagrammatic representation of EEHU on Catalase (CAT) in cisplatin inducedNephrotoxic rats

The Nephrotoxic control (Group 2) showed significant decrease in Catalase(CAT) when compared to the normal control (Group1).

Standard (Group 3) showed statistically significant increase in Catalase (CAT)when comparedto Nephrotoxic control (Group 2).
EEHU 200 mg/kg treated (Group 4) showed statistically significant increase inCatalase (CAT)when compared to the Nephrotoxic control (Group 2).
EEHU 400mg/kg treated (Group 5) showed statistically significant increase in Catalase (CAT) when compared to the Nephrotoxic control (Group 2).

3.5.3 Assessment of Glutathione Peroxidase (GPx)

The effects of the different doses of ethanolic extract of Hylocereus undatus Linn on Glutathione peroxidase (GPx) (Table 13, Figure 9).

Table 13. Results of the effect of EEHU on Glutathione peroxidase (GPx) in cisplatininduced Nephrotoxic rats

Groups	Drug Treatment	Glutathione peroxidase (GPx)
I	Normal Control(0.5% DMSO)	24.38±0.49
II	Nephrotoxic ControlCisplatin (0.75%)	14.68±0.496
III	Reference Control Cisplatin (0.75%) + Lipoic acid(50mg/kg)	22.46±0.69***
IV	Cisplatin (0.75%) +EEHU (200mg/kg)	16.56±0.397**
V	Cisplatin (0.75%) +EEHU (400mg/kg)	20.26±0.221***

Values were given in Mean ±SEM;
*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

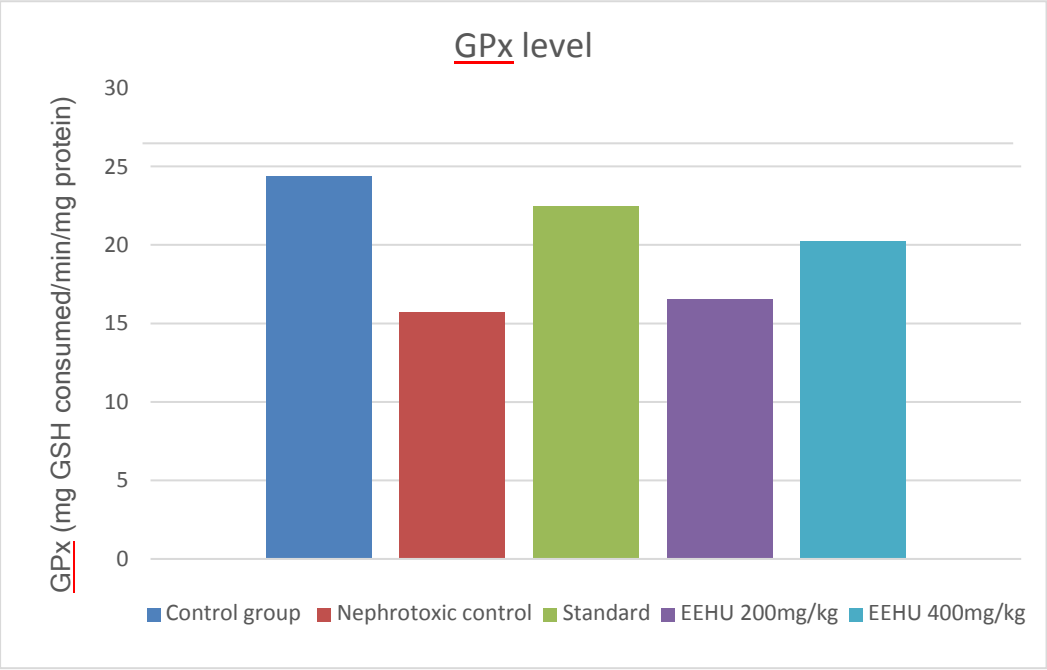

Figure 9. Diagrammatic representation of EEHU on Glutathione peroxidase (GPx) incisplatin induced Nephrotoxic rats

The Nephrotoxic control (Group 2) showed significant decrease in Glutathioneperoxidase (GPx) when compared to the normal control (Group1).

Standard (Group 3) showed statistically significant increase in Glutathioneperoxidase (GPx)when compared to Nephrotoxic control (Group 2).
EEHU 200 mg/kg treated (Group 4) showed statistically significant increase inGlutathione peroxidase (GPx) when compared to the Nephrotoxic control (Group 2).
EEHU 400mg/kg treated (Group 5) showed statistically significant increase in Glutathione peroxidase (GPx) when compared to the Nephrotoxic control (Group 2)

3.6 Assessment of Non-Enzymatic Antioxidant Parameter

3.6.1 Assessment of Reduced glutathione (GSH)

The effects of the different doses of ethanolic extract of Hylocereus undatus Linn on Reducedglutathione (GSH) (Table 14, Figure 10).

Table 14 Results of the effect of EEHU on Reduced glutathione (GSH) in cisplatininduced Nephrotoxic rats

Groups	Drug Treatment	Reduced glutathione (GSH)
I	Normal Control(0.5% DMSO)	19.14±0.712
II	Nephrotoxic ControlCisplatin (0.75%)	8.26±0.210
III	Reference Control Cisplatin (0.75%) +Lipoic acid(50mg/kg)	18.31±0.466***
IV	Cisplatin (0.75%) +EEHU (200mg/kg)	14.26±0.242**
V	Cisplatin (0.75%) +EEHU (400mg/kg)	15.11±0.545***

Values were given in Mean ±SEM;
*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

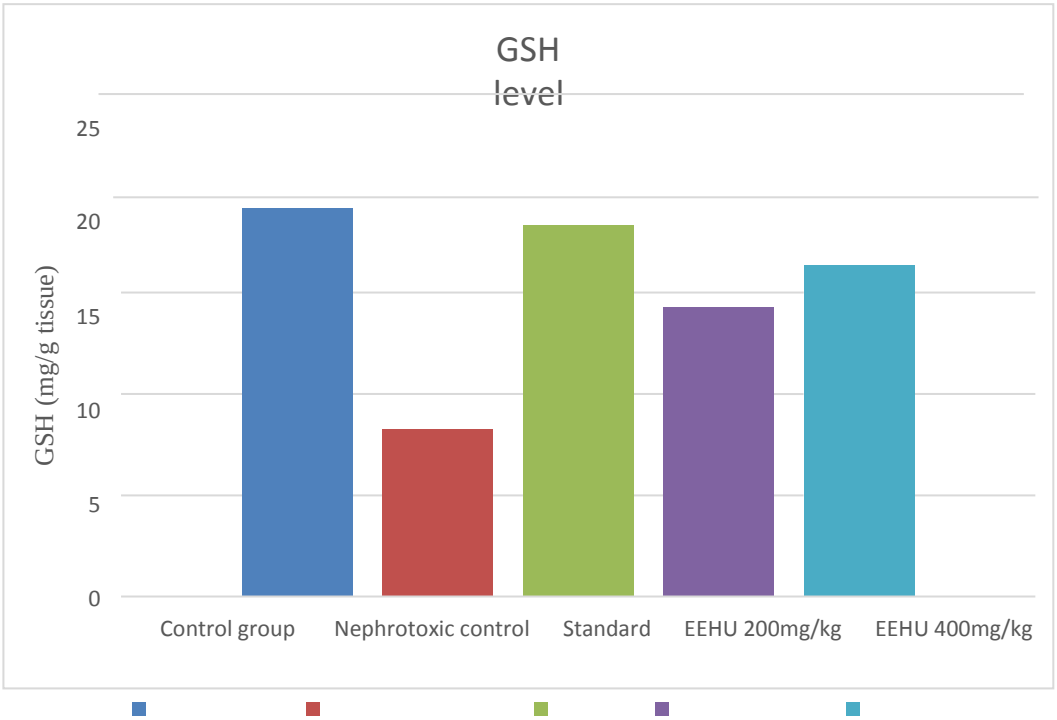

Figure 10. Diagrammatic representation of EEHU on Reduced glutathione(GSH) incisplatin induced Nephrotoxic rats

The Nephrotoxic control (Group 2) showed significant decrease in Reducedglutathione (GSH)when compared to the normal control (Group1).
Standard (Group 3) showed statistically significant increase in Reduced glutathione (GSH) when compared to Nephrotoxic control (Group 2).
EEHU 200 mg/kg treated (Group 4) showed statistically significant increase inReduced glutathione (GSH) when compared to the Nephrotoxic control (Group 2).
EEHU 400mg/kg treated (Group 5) showed statistically significant increase inReduced glutathione (GSH) when compared to the Nephrotoxic control (Group 2)

4. Discussion

Nephrotoxicity is a common clinical syndrome defined as a rapid decline inrenal function resulting in abnormal retention of serum creatinine and blood urea, which must be excreted.
There aren't many pharmacologic therapies available for 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 systemof 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 present in the fruit pulp extract are the flavonoids, terpenoids, alkaloids, tannins, saponins and anthraquinones. Among them tannins, triterpenoids, flavonoids and saponins could be responsible for antioxidant property as these phytoconstituents are already reported to have antioxidant activity.
In this reactive oxygen species (ROS) and damage to nuclear and mitochondrial DNA are caused by cisplatin, and these events trigger the induction of apoptosis and necrosis via both mitochondrial and non-mitochondrial pathways (Volarevic et al., 2019). Cisplatin also affects mitochondrial energy and may be a factor in nephrotoxicity (Peres and Cunha Júnior, 2013).
In present study, the rats treated with single dose of Cisplatin shown marked reduction of body weight as compared to normal group also caused a marked reduction of glomerular filtration rate, which is accompanied by increase in serum creatinine level and decline in creatinine clearance indicating induction of acute renal failure (Stevens and Levey, 2005). with Hylocereus undatus at the dose level of 200 and 400 mg/kg body weight for 15 days significantly lowered the serum level of creatinine with a significant weight gain, increased urine output and creatinine clearance when compared with the nephrotoxic control group.
Cisplatin administration to control rats produced a typical pattern ofnephrotoxicity which was manifested by marked increase in serum blood urea nitrogen (BUN) (Priyadarsini et al., 2012). When Hylocereus undatus was added to rats receiving cisplatin, the amounts of blood urea nitrogen (BUN) in the plasma decreased.
The elevated level of malondialdehyde (MDA), a marker of lipid peroxidation,indicates increased free radical generation in the Cisplatin induced nephrotoxicity. Cisplatin induced increment in malondialdehyde (MDA) content of plasma was significantly prevented by Hylocereus undatus treatment in the present study. Therefore, the significantly lower levels of malondialdehyde (MDA) in the kidney tissues of treated groups as compared with the Cisplatin group indicate attenuationof lipid peroxidation. This was probably due to less damage by oxygen free radicalswith Hylocereus undatus. The involvement of oxygen free radicals in tissue injuryis well established.[1]
Decrement in activity levels of renal Superoxide dismutase (SOD), Catalase (CAT) and

Reduced Glutathione (GSH) following Cisplatin treatment are in accordance with previous report on Cisplatin induced suppression of endogenous enzymatic antioxidant machinery.[1] Hylocereus undatus treatment efficiently prevented Cisplatin induced decrease in activity levels of superoxide dismutase (SOD), Catalase (CAT) and Reduced Glutathione (GSH). A correlation between oxidative stress and nephrotoxicity has been verified in numerous experimental models.

Biological systems employ a variety of defense mechanisms to shield themselves against the harmful effects of activated species. These include chain reaction terminators like the GPx system and free radical scavengers. Glutathione peroxidase (GPx) is a seleno-enzyme two third of which is present in the cytosol and one-third in the mitochondria, it catalysis the reaction of hydro-peroxides with reduced Glutathione to form Glutathione disulphide (GSSG) and the reduction product of the hydro-peroxide (Halliwell and Gutteridge, 1999). Effect of Hylocereus undatus on Glutathione peroxidase (GPx)in experimental rats' study were significantly reduced in cisplatin treated rats than inthe experimental control rats (Do Amaral et al., 2008). Decrement in the activity of renal GPx following cisplatin treatment are due to suppression of endogenous enzymatic antioxidant machinery. Supplementation with Hylocereus undatus to Cisplatin treated rats resulted in near normal activity of glutathione peroxidase (GPx). Based on the above results, it was concluded that Hylocereus undatus exerted statistically significant Nephroprotective activity against cisplatin induced Nephrotoxic rats.

5. Summary & Conclusion

Summary:

The present study was undertaken to scientifically evaluate thenephroprotective activity of the ethanolic extract of fruit pulps of Hylocereus undatus.

The phytochemical investigation revealed the presence of carbohydrate, alkaloids, flavonoids, glycosides, saponins, tannins, phenols and anthraquinone in EEHU.

The administration of cisplatin during experimentation is effectively induced apoptosis and necrosis, which was similar to acute renal failure in human. Therefore,it is an effective and an ideal model for nephrotoxicity research.

The evaluation of renal parameters on nephrotoxic rats with EEHU showed significantly elevate the attenuated body weight, urine volume, creatinine clearanceand significantly reduce in elevated serum creatinine level, which supports its Nephroprotective activity.

The cisplatin induced rats showed elevated levels of serum blood urea nitrogen (BUN) and lipid peroxidation parameter like malondialdehyde (MDA) which was significantly decreased with treatment of EEHU, which proves it having Nephroprotective activity.

The Nephrotoxic rats also showed the reduced levels of enzymatic antioxidant like sulphoxide dismutase (SOD), glutathione peroxidase (GPx) and Catalase (CAT),and non-enzymatic antioxidant like Reduced glutathione (GSH), which was significantly increased with treatment of EEHU, which showed its antioxidant activitydue to the Flavonoids which is present in the extract.

Histopathological studies on isolated kidney revealed that the EEHU, reversedthe kidney damage and also restored normal kidney architecture.

Conclusion:

In conclusion, the fruit pulp of Hylocereus undatus in an ethanolic extract showed statistically significant nephroprotective activity.

The plant extract proved to have nephroprotective potentials may because ofits known flavonoid contents and antioxidant properties.

There is a scope for further investigation on the histopathology of liver and spleen and clinical studies that are required to elucidate the active phytoconstituents with potent nephroprotective activity.

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