IJPSR (2020), Volume 11, Issue 12 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 23 November 2011; received in revised form, 20 November 2020; accepted, 24 November 2020; published 01 December 2020

EVALUATION OF *IN-VITRO* ANTIOXIDANT AND NEPHROPROTECTIVE POTENTIALS OF *CRINUM ASIATICUM* BULBS AGAINST GENTAMICIN INDUCED NEPHROTOXICITY IN ALBINO RATS

S. Suman [*1] and S. V. Suresh Kumar [2]

Jawaharlal Nehru Technological University [1], Anantapur, Ananthapuramu - 515002, Andhra Pradesh, India.
Department of Pharmacognosy [2], Creative Educational Society's College of Pharmacy, NH-7, Chinnatekur, Kurnool - 518218, Andhra Pradesh, India.

Keywords:

Crinum asiaticum bulbs, Nephroprotective, Antioxidant enzymes, Gentamicin

Correspondence to Author:
Mr. S. Suman

Research Scholar,
Jawaharlal Nehru Technological University, Anantapur, Ananthapuramu - 515002, Andhra Pradesh, India.

E-mail: kalyan.suman1985@gmail.com

ABSTRACT: Objective: To study the *in-vitro* antioxidant as well as nephroprotective potentials of *Crinum asiaticum* bulbs' ethanolic extract in contrast to gentamicin-induced nephrotoxicity in albino rats. **Materials and Methods:** The ethanolic extract of *Crinum asiaticum* bulbs subjected to preliminary phytochemical analysis to determine the phytoconstituents, and evaluation of antioxidant activity was done using *in-vitro* methods like DPPH as well as lipid peroxidation assays using ascorbic acid as standard. *The in-vivo* nephroprotective activity was estimated by utilizing Gentamicin induced nephrotoxicity in albino rats. **Results:** It was revealed by the preliminary phytochemical analysis that phenolics, tannins, steroids, terpenoids, and flavonoids components are presented. The promising results obtained by *in-vitro* antioxidant activity promoted us to evaluate Gentamicin induced nephrotoxicity in rats. Gentamicin impeded nephrotoxicity in rats as proved by extremely substantial ($p<0.001$) rise of blood urea, serum creatinine, and uric acid in Group II in comparison to Group I. Also, administration of Gentamicin in group II caused oxidative stress in rats proposed by a considerable increase in LPO level as well as a considerable decrease in GSH level. While administration of EECA in group III to V respectively caused a dose-dependent significant decrease ($p<0.01$) of uric acid, serum creatinine, as well as blood urea in comparison to group II. There is a significant increase in *in-vivo* antioxidant status ($p<0.01$) in catalase and GSH levels, however, there is a substantial reduction in LPO levels ($p<0.01$) in group III to V. **Conclusion:** The promising results suggested that *Crinum asiaticum* bulbs' ethanolic extract has nephroprotective activities in contrast to Gentamicin induced nephrotoxicity in rats.

INTRODUCTION: A variety of chemicals, medicines which includes antibiotics and environmental toxins, significantly modifies the functions and structure of various tissues as well as intestine, heart, kidney, and liver are adversely affected by these [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(12).6494-02

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(12).6494-02

Gentamicin is one of the most widely used aminoglycoside antibiotic against the urinary tract and abdomen's severe infections [2]. Although, ototoxicity and nephrotoxicity are still the biggest unfavorable reactions of the drug to effectively using it for medical purposes in the long-term [3].

Various functional, metabolic, and morphological alterations in the kidney are caused by the Gentamicin as well as gentamicin nephrotoxicity's intensity is linked with its accumulation in convoluted tubules of renal proximal that results in the tubular necrosis [4]. *Crinum asiaticum* (Common name: Asian poison bulb) belongs to the family of

Amaryllidaceae, locally known as kesaracettu, is an evergreen herb that is widely distributed throughout India along river beds and also in forest [5]. It is known as spider lily, Crinum lily, and poison bulb in English, naagadamani in Ayurveda, bakong in Malaysia, and morabau in Papua New Guinea. Various ethnomedicinal properties has been possessed by this plant as well as it is being utilised in the conventional system of medicine.

The ethno-medicinal uses of the *Crinum asiaticum* bulbs are bitter, expectorant, laxative, carminative, anthelmintic, aphrodisiac, diuretic, urinary problems, diaphoretic, nauseant, analgesic and anti-inflammatory [6], anti-obesity [7], emetic [8], anti-candidial [9]. Literature reports that the bulbs of *Crinum asiaticum* contain flavonoids, terpenoids, steroids, tannins, and phenols are the major phytoconstituents. Hence, the current study aims to evaluate the *in-vitro* antioxidant and nephro-protective potentials of *Crinum asiaticum* bulbs' ethanolic extract in contrast to gentamicin-induced nephrotoxicity in albino rats.

MATERIALS AND METHODS:

Collection of Plant Material: The *Crinum asiaticum* plant was collected from the Tirumala hills, Chittoor district, "Andhra Pradesh, India and confirmed by Assistant Professor, Prof. Dr. K. Madhava Chetty, Department of Botany, Sri Venkateshwara University, Tirupati" (Voucher Number: 2011, dated 08.08.2017). Furthermore, the drying process of the plant was done in complete shade; bulbs were separated as well as pulverized, so that coarse powder is obtained.

Extracts Preparation: Ethanol is used as a solvent, which is then used to extract the powdered material through a soxhlation process.

Preliminary Phytochemical Screening of Ethanolic extract *Crinum asiaticum* Bulbs: *Crinum asiaticum* bulb sample has been exposed to phytochemical analysis for the detection of tannins, glycosides, alkaloids, phenols, flavonoids as well as terpenoids following standard procedures.

Estimation of Phenolic Content: Folin ciocalteu process is used for measuring the *Crinum asiaticum* bulbs ethanolic extract's total phenolic content. Extract's 10 ml stock solution was organized with the respective solvent that had 2 mg/ml concentration. 1 ml extract solution was transferred into the volumetric flask of 25 ml from stock solution, then 1.5 ml Folin ciocalteu reagent and 10 ml water are added to this. After 5 min, we added 4 ml of 20% sodium carbonate solution, which resulted in a final volume of 25 ml along with distilled water's double quantity. After that, this mixture is put for 30 min, and then at 765 nm there is the development of blue color absorbance in the mixture. For calibration preparation, 50-250 µg/ml concentration range is used for preparing the standard gallic acid solution. The calibration curve of gallic acid is acquired by plotting X-axis concentration and corresponding Y-axis absorption [10].

Estimation of Total Flavonoid Content: Earlier reported methods were used to estimate the *Crinum asiaticum* bulbs ethanolic extract's total flavonoid content [11].

Aluminium Chloride Method: The earlier reported procedure was used to carry out the aluminium chloride colorimetric method. Calibration curves are developed with the Quercetin. Standard, 0.1, 0.2, 0.3, 0.4 and 0.5 ml stock solution are taken that resulted in "the 10, 20, 30, 40 and 50 µg concentrations respectively. 0.5 ml diluted standard solutions" is combined with the 2.8 ml of distilled water, "0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate", as well as 1.5 ml of 95% ethanol. After incubation, the mixture is placed for 30 min at room temperature, then at 415 nm reaction mixture's absorbance is measured. Furthermore, 10% aluminium chloride amount is replaced by the distilled water in the blank with an equivalent amount. In the same way, in accordance with the above-described method, complete flavonoid content was concluded when aluminum chloride is reacted with the *Crinum asiaticum* bulbs' ethanolic extract of 0.5 ml.

2, 4-dinitro Phenyl Hydrazine Method: This estimation utilized the method that was reported by Mohy El-Din *et al.,* for preparing the calibration curve, a standard is utilized that is known as Naringenin. Furthermore, 20 mg naringenin was dissolved in methanol, and after that, the mixture is diluted to 250, 500, 1000, 1500, and 2000 µg/ml resultant concentrations.

Furthermore, every diluted standard's 1ml is mixed separately with 1% 2,4-dinitro phenyl hydrazine reagent's 2 ml and methanol's 2 ml for 50 min at 50 °C, when the reaction mixture is cooled down at room temperature then it is mixed with the 1% potassium hydroxide's 5 ml in methanol 70% and then for 2 min at room temperature it is incubated after that 1 ml mixture is combined with the 5 ml methanol and then at 100 rpm it is centrifuged so that formed precipitate can be removed. Then, the collection of supernatants was done, which is then adjusted to 25 ml. Furthermore, at 459 nm supernatant's absorbance is measured. In the same way, as mentioned above, flavonoid content is determined when *Crinum asiaticum* bulbs ethanolic extract's every concentration of 5 ml is treated in a same way with "2, 4-dinitro phenyl hydrazine" reagent. Methanol replaced the "2, 4-dinitro phenyl hydrazine" reagent amount for the blank.

Estimation of *Crinum asiaticum* bulbs' Ethanolic Extract *In-vitro* Antioxidant Activity:

DPPH Radical Scavenging Assay: Using Sharifi-Rad (2018), the *Crinum asiaticum* bulbs' ethanolic extract effect on DPPH radical was estimated. A 0.135 mM DPPH methanol solution was formulated as well as its 1.0 ml amount was combined with 1.0 ml amount of *Crinum asiaticum* bulbs' ethanolic extract having concentrations (25-3200 µg/ml). The mixture of the reaction was carefully vortexed as well as placed in shade at 37 °C room-temperature for 30 min. Spectrophotometrically, at 517 nm solution's absorbance is measured. For instance, Ascorbic acid was used [12]. The following equation calculated the scavenge DPPH radical ability" as:

$$\text{DPPH radical scavenging activity (\%)} = [(Abs_{control} - Abs_{sample})] / (Abscontrol)] \times 100$$

Where, $Abs_{control}$ represents DPPH radical + methanol absorbance, Abs sample represents DPPH radical + sample extract /standard absorbance.

Lipid Peroxidation Assay Method: Ademosun *et al.,* described" a method that was used for determining the lipid peroxidation' inhibition. Further, homogenization of 10 gm of rat liver tissue was donein phosphate buffer solution along with a polytron homogenizer (Remi) having 7.4 pH, which produced homogenate of 25% w/v. Next, for 10minutes, centrifugation of homogenization was done at 4000 rpm. Supernatant's 0.1 ml was combined with 0.1 ml of different concentrations of ethanolic extract of *Crinum asiaticum* bulbs (25-3200 µg/ml) and 0.1 ml Kcl (30 mM), 0.1ml "ascorbic acid" (0.06 mM), 0.1ml "ammonium ferrous sulphate" was added as well as at 37°C it was incubated for 1 h. After that, 1.5ml TBA (0.8%), 1.5 ml of 20% glacial acetic acid, as well as sodium dodecyl sulphate 0.2 ml (8%) were used to treat the reaction mixture. Distilled water was used so that the total mixture volume is attained was 4ml, and then for 1 h at 100 °C it was placed in an oil bath. After the mixture was cooled down, 5 ml 15:1v/v butanol-pyridine mixture, as well as 1 ml distilled water is added to it. Furthermore, for 10 min, tubes were centrifuged at 4000 rpm after vigorous shaking. At 532 nm, the organic layer's absorbance is measured that contains TBARS (Thiobarbituric Acid Reactive Substance). Instead of a test compound, 0.1 ml respective vehicle was used for preparing the control sample. It resulted in the 50% inhibition concentration (IC_{50}) and percentage inhibition [13].

Acute Toxicity Studies: OECD-423 procedures were used to carry out the acute oral toxicity study [14]. For the effective dosage, non-median lethal dose's (LD50) 1/10th part is used [15].

Nephroprotective activity of *Crinum asiaticum* bulbs ethanolic extract, in contrast, to Gentamicin induced nephrotoxicity in albino rats [16]. This study used the albino rats whose weight was 150-180g. The dose of is lower 100 mg/kg, medium 200mg/kg, higher dose 400 mg/kg was used. Five groups of animals were formed; each had 6animals in it, which is started prior to gentamicin injection and continued with the eight days gentamicin treatment. The protocol was approved by the IAEC, Number: DEC /IAEC /CESCOP /2017-16

Group 1: Normal

Group 2: Disease control treated with (Gentamicin, 80 mg/kg, bd. wt).

Group 3: Rats were treated with EECA (100 mg/kg, bd. wt) for consequence 8 days along with gentamicin (80 mg/kg, bd. wt).

Group 4: Rats were treated with EECA (200 mg/kg, bd. wt) for consequence 8 days along with gentamicin (80 mg/kg, bd. wt).

Group 5: Rats were treated with EECA (400 mg/kg, bd. wt) for consequence 8 days along with gentamicin (80 mg/kg, bd. wt)

Assessment of Nephroprotective Activity:

Biochemical Estimation:

Serum Analysis: The collected blood samples were centrifuged for 10 min at 10,000rpm as well as the serum samples were subjected to estimation of biochemical parameters such as BUN, uric acid, creatinine, and electrolytes (chlorides, potassium and sodium).

Kidney Homogenate Analysis: The isolated kidneys were homogenized with a homogenizer. The kidney homogenates were subjected to an *in-vivo* antioxidant study using lipid peroxidation [LPO] and glutathione estimation, respectively.

Histopathological Studies: The animals from all the respective groups were euthanized by using CO_2 chamber at the end of the study followed by the isolation of kidneys. The slides were prepared by staining with eosin and hematoxylin and observed under an electron microscope.

Assessment of Oxidative Stress:

Lipid Peroxidation [LPO]: The lipid peroxidation estimation was done according to (Samuel Okwudili Onoja *et al.,* 2014). 2 ml of 10% tissue homogenate was pipetted out. To this, added 2 ml of 30% of "Tri-chloro acetic acid" followed by 2 ml of 0.8%TBA ("Thio-Barbituric acid") reagent.

Cotton was used to cover the test tubes, and further, they were put at 80 ˚C for 30 min in a shaking water bath. After removing test tubes from the shaking water bath, they were put in ice-cold water for the next 30 min.

Furthermore, for the next 15 min, they were centrifuged at 3000 rpm. Against appropriate blank, at 37 ˚C at 535 nm supernatant's absorbance is calculated (excluding homogenate), which was described as the formation of n protein moles/milligram in tissues, as well as following formula is used for "its calculation [17]

$$\text{Concentration} = A \times (V/E) \times P$$

Where A represents the volume of solution, E represents the extinction coefficient ($1.56 \times 10^5 m^{-1} cm^{-1}$), and P represents the tissue's protein content calculated as microgram of tissue's protein/milligram."

Glutathione Estimation: Glutathione estimate has been carried out as per (Atieh Modares *et al.,* 2015). In sodium chloride solution, 2 ml of 10% homogeneous was prepared, 2.5 ml of 0.02 m EDTA was added to homogenate as well as shaken forcefully. 2 ml of such combination was taken and cold distilled water's 4ml, as well as 1 ml of 50% trichloroacetic acid, was mixed as well as for 10 min it was shaken. After this, for 15 min at 3000 rpm, the contents were centrifuged. Supernatant's 2 ml was mixed with TRIS buffer's 0.4M, which has 8.9 pH. The complete solution was nicely blended, as well "as 0.1 ml of 0.01M DTNB was mixed. The reagent absorbance except homogenate was read at 412 nm [18].

$$\text{Micro mol/mg wet tissue: } [A/13600] \times \text{Dilution factor} \times 1000$$

Statistical Analysis: The statistical data was established with the help of graph pad prism 5 software version 5.3. Each value is described "as mean ±S.E.M for 6 rats in every group. One-way ANOVA followed by Dunnett's t-test, Disease Control *vs*. Normal, Disease Control *vs.* Treatment, and ***p<0.001, **p<0.01, *p<0.05 were compared."

RESULTS:

Preliminary Phytochemical Screening of Ethanolic Extract *Crinum asiaticum* Bulbs: *Crinum asiaticum* bulbs ethanolic extract's phytochemical screening disclose the phenolic, tannins, steroids, terpenoids, and flavonoids phyto-constituents' presence.

Phenolic Content Estimation: Folin ciocalteu process is used for measuring the *Crinum asiaticum* bulbs ethanolic extract's total phenolic content through a standard gallic acid. The phenolic content was found to be 43.95 µg/ml.

Estimation of Total Flavonoid Content: *Crinum asiaticum* bulbs ethanolic extract's total flavonoid content was discovered to be 28.77 µg/ml from quercetin and naringenin calibration curve.

***In-vitro* Antioxidant Activity:**

DPPH Assay: The effect of ascorbic acid and ethanolic extract of *Crinum asiaticum* bulbs was

showed significant DPPH activity for radical scavenging. The antioxidants effect on DPPH radical scavenging was considered due to the ability to donate hydrogen. Also, antioxidant activity was discovered in a dose-dependent way as well as they showed an IC_{50} value of 105.49 µg/ml and 238.3 µg/ml, respectively.

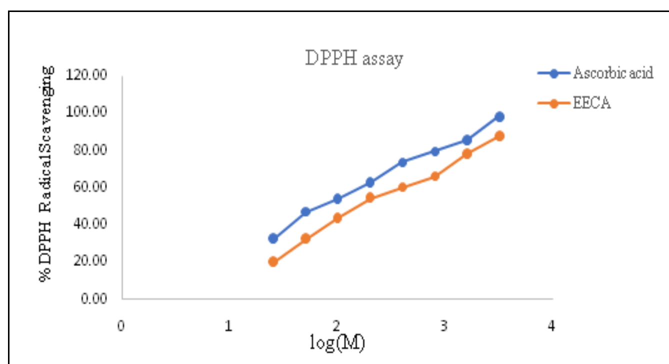

FIG. 1: EFFECT OF DISTINCT CONCENTRATIONS OF ASCORBIC ACID AND *CRINUM ASIATICUM* BULBS' ETHANOLIC EXTRACT ON DPPH* RADICALS

LPO Inhibition Assay: The lipid peroxidation is inhibited by the *Crinum asiaticum* bulbs ethanolic extract as well as ascorbic acid's effect that was induced through Fe^{2+}, which is rat liver homogenate's ascorbate system.

The MDA (malon-dialdehyde) as well as associated substances generation which reacts with TBA which is discovered to be inhibited by *Crinum asiaticum* bulbs ethanolic extract as well as ascorbic acid. *Crinum asiaticum* bulbs ethanolic extract and ascorbic acid IC_{50} value are discovered to be 495.68 µg/ml and 210.45 µg/ml, respectively.

Acute Toxicity Studies: The *Crinum asiaticum* bulbs' ethanolic extract LD_{50} was found to be 2000 mg/kg after a study that is based on acute oral toxicity in accordance with 423 guidelines of OECD. 1/5th, 1/10th as well as 1/20th of the same "dose (100 mg/kg, 200 mg/kg and 400 mg/kg respectively") were selected as well as further studies were conducted.

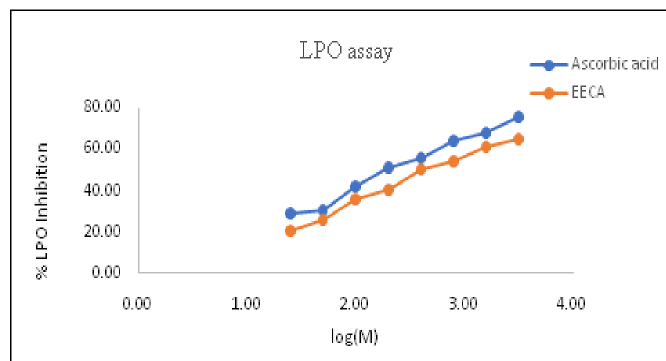

FIG. 2: EFFECT OF DIFFERENT CONCENTRATIONS OF ASCORBIC ACID AND *CRINUM ASIATICUM* BULBS' ETHANOLIC EXTRACT ON LPO INHIBITION

Nephroprotective Activity of *Crinum asiaticum* bulbs' Ethanolic Extract in Contrast to Gentamicin Induced Nephrotoxicity in Albino Rats:

Serum Analysis: The current study treatment with gentamicin (80 mg/kg, i.p) results in a substantial (###p<0.001) elevation in a higher level of (43.36 ± 1.048, 6.938 ± 0.700, 6.741 ± 0.50) serum BUN, creatinine and uric acid respectively when the comparison is made with the normal group.

These changes were restored significantly in the rats treated with EECA (100 mg/kg, p.o) pointedly decreases in BUN(*p<0.05; 40.54 ± 2.629), uric acid (**p<0.01; 4.370 ± 0.504) and creatinine (***p<0.001; 5.350 ± 0.30), at the dose of EECA (200 mg/kg, p.o) substantially (*p<0.05) lowers the BUN (37.85 ± 2.86) and (***p<0.001; 3..43±0.730, 4.580 ± 0.54) creatinine and uric acid respectively, at the dose of EECA (400 mg/kg, p.o) substantially (**p<0.01; 32.45 ± 2.79) lowers the BUN (31.76 ± 2.89) and (***p<0.001; 3.105 ± 1.040, 4.20 ± 0.21) creatinine and uric acid respectively in comparison to disease control **Table 1**.

TABLE 1: *CRINUM ASIATICUM* (BULBS)ETHANOLIC EXTRACT'S EFFECT ON SERUM LEVELS OF URIC ACID, CREATININE AND BUN IN GENTAMICIN (80 mg/kg, I.P) INDUCED NEPHROTOXICITY IN RATS

S. no	Groups	BUN (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
1	Normal	28.02 ± 2.207	2.626 ± 0.190	3.790 ± 0.52
2	Disease Control	43.36 ± 1.048###	6.938 ± 0.700###	6.741 ± 0.50###
3	EECA (100 mg/kg, BW)	40.54 ± 2.629*	4.370 ± 0.504**	5.350 ± 0.30***
4	EECA (200 mg/kg BW)	37.85 ± 2.86*	3..43 ± 0.730***	4.580 ± 0.54***
5	EECA (400 mg/kg BW)	32.45 ± 2.79**	3.105 ± 1.040***	4.20 ± 0.21***

"Every value is expressed as mean ±S.E.M for 6 rats in every group. Comparisons made between ###p<0.001, ##p<0.01, #p<0.05; Normal *vs.* Disease control, ***p<0.001, **p<0.01, *p<0.05; Disease control *vs.* Treatment: One-way ANOVA followed by Dunnett's -t test."

Effect of Ethanolic Extract of *Crinum asiaticum* Bulbs on Renal Electrolytes: Intra-peritoneal gentamicin injection (80 mg/kg, i.p) to rat's results in substantial growth in serum levels of sodium, (###p<0.001; 6.308 ± 0.640) and decrease in potassium and chloride levels (###p<0.001; 2.633 ± 0.526, 86.2 ± 5.904) in comparison to untreated rats group. However, treatment with EECA at the dose of (100 mg/kg, p.o) lowers the sodium levels (*p<0.05; 5.163 ± 0.389) and increases the potassium and chloride levels (***p<0.001; 2.934 ± 0.293, **p<0.01; 95.54 ± 5.918) respectively, at the doses of (200 mg/kg & 400 mg/kg) sodium levels were decreased (**p<0.01; 4.880 ± 0.367, ***p<0.001; 3.960 ± 0.501), potassium (***p<0.001; 3.160 ± 0.387, 3.286 ± 0.419) and chloride (***p<0.001; 98.08 ± 4.710, 102.1 ± 4.870) levels were increased **Table 2**.

TABLE 2: *CRINUM ASIATICUM* (BULBS) ETHANOLIC EXTRACT'S EFFECT ON RENAL LEVELS OF SODIUM, POTASSIUM, CHLORIDE IN GENTAMICIN (80MG/KG, I.P) INDUCED NEPHROTOXICITY IN RATS

S. no	Groups	Sodium (mg/dl)	Potassium (mg/dl)	Chloride (mg/dl)
1	Normal	3.726 ± 0.261	3.537 ± 0.591	109.3 ± 2.000
2	Disease Control	6.308 ± 0.640###	2.633 ± 0.526###	86.2 ± 5.904###
3	EECA (100 mg/kg, BW)	5.163 ± 0.389*	2.934 ± 0.293***	95.54 ± 5.918**
4	EECA (200 mg/kg BW)	4.880 ± 0.367**	3.160 ± 0.387***	98.08 ± 4.710***
5	EECA (400 mg/kg BW)	3.960 ± 0.501***	3.286 ± 0.419***	102.1 ± 4.870***

Every value is expressed as mean ± S.E.M for 6 rats in every group. Comparisons made between ###p<0.001, ##p<0.01,#p<0.05; Normal *vs.* Disease control, ***p<0.001, **p<0.01, *p<0.05;Disease control *vs.* Treatment: One-way ANOVA followed by Dunnett's -t test."

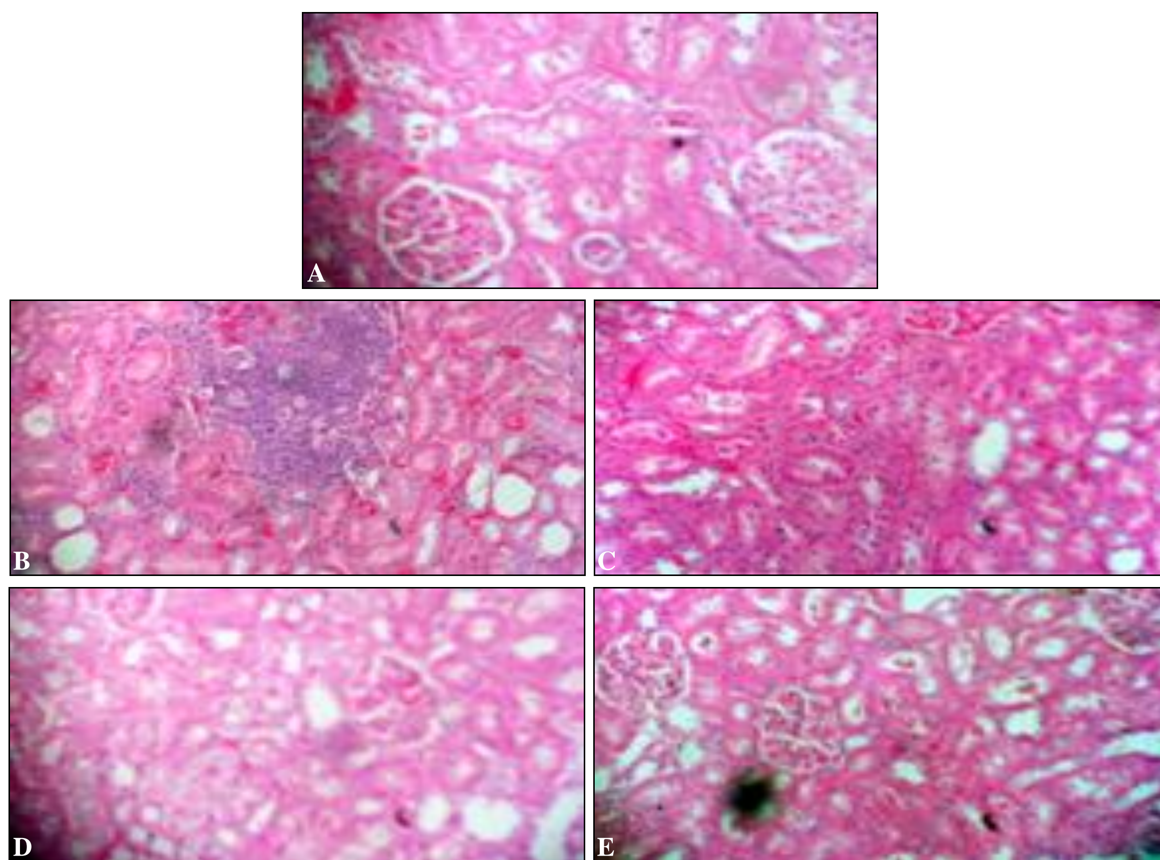

FIG. 3: HISTOLOGICAL SECTIONS OF RAT KIDNEYS 3A: NORMAL CONTROL, 3(B): DISEASE CONTROL TREATED WITH GENTAMICIN (80 mg/kg, BD. WT), 3C: RATS TREATED WITH EECA (100 mg/kg, BD. WT) +GENTAMICIN (80 mg/kg, BD. WT), 3D: RATS TREATED WITH EECA (200 mg/kg, BD. WT) +GENTAMICIN (80 mg/kg, BD. WT), 3E: RATS TREATED WITH EECA (400 mg/kg, BD. WT) +GENTAMICIN (80 mg/kg, BD. WT)

***Crinum asiaticum* (bulbs) Ethanolic Extract's Effect on Renal Tissue Enzymes:** *Crinum asiaticum* bulbs ethanolic extract's effect on gentamicin-induced changes in glutathione (GSH), lipid peroxides (LPO) kidney tissue as represented in **Table 3**. Gentamicin treatment are discovered with substantial (#p< 0.05) rise in lipid peroxide concentration, whereas there is a significant (#p<

0.05) reduction in the GSH levels in comparison to the normal rats. *Crinum asiaticum* bulbs ethanolic extract's administration substantially (*p< 0.05) undermined the Gentamicin's nephrotoxic effects by increasing GSH and reducing LPO concentrations in dose-dependently **Table 3**.

TABLE 3: *CRINUM ASIATICUM* (BULBS) ETHANOLIC EXTRACT'S EFFECT ON RENAL TISSUE ENZYME LEVELS LPO AND GSH IN GENTAMICIN (80 mg/kg, I.P) INDUCED NEPHROTOXICITY IN RATS

S. no	Groups	LPO nM MDA g^{-1} of tissue	GSH μM GSH g^{-1} of tissue
1	Normal	72.68 ± 1.26	80.65 ± 1.59
2	Disease Control	143.08 ± 2.54$^{###}$	38.97 ± 1.52$^{###}$
3	EECA (100 mg/kg, BW)	105.45 ± 1.38*	43.56 ± 0.35*
4	EECA (200 mg/kg BW)	94.65 ± 2.67**	57.75 ± 1.78**
5	EECA (400 mg/kg BW)	89.75 ± 0.50***	64.53 ± .0.85***

"Every value is expressed as mean ± S.E.M for 6 rats in every group. Comparisons made between ###p<0.001, ##p<0.01,#p<0.05; Normal *vs.* Disease control, ***p<0.001, **p<0.01, *p<0.05; Disease control *vs.* Treatment: One-way ANOVA followed by Dunnett's -t test."

***Crinum asiaticum* (Bulbs) Ethanolic Extract's Effect on Renal Histopathological Changes:** All parts of the kidney showed normal appearance in the control group. Treatment with GM caused acute renal damage, which was marked by tubular desquamation, marked peritubular inflammation. The kidney sections of rats treated with EECA showed moderate to mild (Peritubular inflammation, intestinal lumen, tubular desquamation), indicating protective effect of EECA in a dose-dependent manner.

DISCUSSION: The main objective of this research work was to evaluate *in-vitro* antioxidant and nephroprotective potentials of *Crinum asiaticum* bulbs against nephrotoxicity produced in rats through Gentamicin. This is a bacterial antibiotic with a broad-spectrum that is widely utilized in veterinary practice for treating serious acute infections. Even with this application of therapeutic, due to its extreme acute nephrotoxicity, its use is limited [19]. Many researches have documented that increase the generation of superoxide anions, hydroxyl radicals, hydrogen peroxide and reactive nitrogen species in the kidney are major perpetrators of acute renal failure caused by gentamicin [20]. The gentamicin mechanism where renal damage is induces however, elusive in nature.

The therapeutic antioxidants such as diethyl dithiocarbamate, l-histidinol, deferoxamine, methimazole, vitamin C, vitamin E, and thymoquinone were extensively studied in the protection of renal physiological function [16, 20, 21]. BUN and Serum creatinine are recognized biomarkers in the early stages of chronic renal damage as well as renal damage, ALT, AST, ALP high levels, and total protein are exceptional [22, 23, 24]. In this research work, Gentamicin (80 mg kg^{-1} b.wt., i.p) "increased uric acid levels, creatine levels, and blood urea nitrogen levels significantly. Mysteriously, the concentration of serum biochemical parameters decreased significantly in *Crinum asiaticum* bulbs treatment. This may be because of the antioxidant properties of" phytoconstituents found in it. These changes in biochemical parameters have been associated with renal histopathological lesions.

Gentamicin, irrespective of cell damage, inhibits many cell membrane transporters of basolateral membranes and brush boundaries, contributing to electrolyte effects. Transport inhibition decreases cell viability as well as affects tubular reabsorption that ultimately leads to apoptosis and necrosis [25, 26]. A significant reduction in potassium and calcium levels along with, at the same time, non-significant increases in sodium ions found in gentamicin therapy. Nephroprotective effects of ethanolic extract of *Crinum asiaticum* bulbs demonstrate enhanced ameliorative activities on kidneys.

Several researchers stated "that aminoglycoside is a classic antibiotic capable of causing nephrotoxicity by inducing reactive oxygen" species [27, 28]. The elevated MDA level (in tissues lipid peroxidation as an indicator) outcomes in decrease of material like polyunsaturated fatty acid that acts as a free radical substrate, in particular hydrogen superoxide and peroxide. These exacerbated free radicals damage the antioxidant role of GPx, GSH, catalase, and SOD [29]. As a consequence, there is a mismatch between the antioxidant and oxidant status leads to

cell damage. Certain studies indicate that the cationic association of aminoglycosides with anionic phospholipids (kidneys) induces nephrotoxicity [30]. By following the ion interaction cycle, as a catalyst, Gentamicin can procedure a "chelation complex iron (iron-GM complex)" for inducing oxidative free radicals in the renal tissues [31]. This also demonstrates that there has been a rise in lipid peroxidation products (MDAs) along with a decline in non-enzyme (GSH) antioxidants in the renal tissues of gentamicin therapy. Nonetheless, a surprisingly ethanolic extract of *Crinum asiaticum* bulbs maintains MDA and GSH levels in EECA-treated groups as well as likely because of (1) detoxification of possible reactive species of oxygen along with (2) enzymatic as well as non-enzymatic antioxidant functions restoration.

CONCLUSION: It is determined that the *Crinum asiaticum* bulbs' ethanolic extract contains flavonoids, terpenoids, steroids, tannins, and phenolic compounds with good antioxidants and nephroprotective properties. It is highly recommended that the Crinum plant be regarded as a possible herbal medicinal plant and encouraged to carry out detailed research to identify the specific phytoconstituents responsible for the activities referred to above.

ACKNOWLEDGEMENT: I would like to thank the management and Principal of Creative Educational Society's College of Pharmacy for providing unconditional support for carrying out this work.

CONFLICTS OF INTEREST: The authors declare that there is no conflicts of interest.

REFERENCES:

1. Al-Ghamdi M, Ahmed A, El-Zohri M and Shahat AA: Hepatoprotective, nephroprotective, anti-amylase, and antiglucosidase effects of *Ziziphus spina-christi* (L.) against carbon tetrachloride-induced toxicity in rats. Trop J Pharm Res 2019; 18(5): 781-90.
2. Nagai J and Takano M: Molecular aspects of renal handling of aminoglycosides and strategies for preventing the nephrotoxicity. Drug Metab Pharma 2004; 19: 159-70.
3. Khan SA, Priyamvada S, Farooq N, Khan S, Wasim KM and Yusufi ANK: Protective effect of green tea extract on gentamicin-induced nephrotoxicity and oxidative damage in rat kidney. Pharmacol Res 2009; 59: 254-62.
4. Pedraza-Chaverri J, Maldonado PD, Medina-Campos ON, Olivares-Corichi IM, Grandos-Silvestre M and Hernandez-Pando R: Garlic ameliorates gentamicin nephrotoxicity: relation to antioxidants. Free Radic Biol Med 2000; 29: 602-11.
5. Warrier PK: Indian Medicinal Plants; a compendium of 500 Species. Orient longman 2006; 2: 209-211.
6. Rahman MA, Hossain SA, Ahmed NU and Islam MS: Analgesic and anti-inflammatory effects of *Crinum asiaticum* leaf alcoholic extract in animal models. Afr J Biotechnol 2013; 12(2): 212-18.
7. Jeong YJ, Sohn EH, Jung YH, Yoon WJ, Cho YM, Kim I, Lee SR and Kang SC: Anti-obesity effect of *Crinum asiaticum* var. japonicum baker extract in high-fat diet-induced and monogenic obese mice . Biomed. Pharmacother 2016; 82: 35-43.
8. Haque M, Jahan S and Rahmatullah M: Ethnomedicinal uses of *crinum asiaticum*: A review. World J Pharm Pharm Sci 2014; 3: 119-28.
9. Surain P and Aneja KR: Anticandidal potential of *Crinum asiaticum* leaves extract against selected oral and vaginal Candida pathogens. J of Innovative Bio 2014; 1: 27-30.
10. Hmid I, Elothmani D, Hanine H, Oukabli A and Mehinagic E: Comparative study of phenolic compounds and their antioxidant attributes of eighteen pomegranate (Punica granatum L.) cultivars grown in Morocco. Arab J Chem 2017; 10: S2675-84.
11. Mohy El-Din SM and El-Ahwany AM: Bioactivity and phytochemical constituents of marine red seaweeds (*Jania rubens*, *Corallina mediterranea* and *Pterocladia capillacea*). J Taibah Univ Sci 2016; 10(4): 471-84.
12. Sharifi-Rad J, Sharifi-Rad M, Salehi B, Iriti M, Roointan A, Mnayer D, Soltani-Nejad A, Afshari A: *In-vitro* and *in-vivo* assessment of free radical scavenging and antioxidant activities of Veronica persica Poir. Cell Mol Biol 2018; 64(8): 57-64.
13. Ademosun AO, Oboh G, Bello F and Ayeni PO: Antioxidative properties and effect of quercetin and its glycosylated form (Rutin) on acetylcholinesterase and butyrylcholinesterase activities. Evid-Based Compl Alt. 2016; 21(4): NP11-7.
14. Organization for Economic Cooperation and Development. Guidelines for testing of chemicals (OECD-423).
15. Handa SS and Sharma A: Hepatoprotective activity of andrographolide from Andrographis paniculata against carbontetrachloride. Indian J Med Res 1990; 92: 276-83.
16. Aiswarya N, Chandran V, Teerthanath S and Rakesh KB: Nephroprotective effect of aqueous extract of Pimpinella anisum in Gentamicin induced nephrotoxicity in wistar rats. Phcog J 2018; 10(3).
17. Onoja SO, Omeh YN, Ezeja MI and Chukwu MN: Evaluation of the *in-vitro* and *in-vivo* antioxidant potentials of *Aframomum melegueta* methanolic seed extract. J Trop Med 2014; 1-6.
18. Modaresi A, Nafar M and Sahraei Z: Oxidative stress in chronic kidney disease. Iran J Kidney Dis 2015; 9(3): 165.
19. Ahn JM, You SJ, Lee YM, Oh SW, Ahn SY, Kim S, Chin HJ, Chae DW and Na KY: Hypoxia-inducible factor activation protects the kidney from gentamicin-induced acute injury. PLOS One 2012; 7(11): e48952.
20. Azab AE, Fetouh FA and Albasha MO: Nephro-protective effects of curcumin, rosemary and propolis against Gentamicin induced toxicity in guinea pigs: Morphological and biochemical study. American Journal of Clinical and Experimental Medicine 2014; 2(2): 28-35.
21. Mostafa-Hedeab G, Sati LM, Elnaggar HM, Elgatlawey ZO, Eltwab AA, Elsaghayer WA and Ali H: Ameliorating effect of olive leaf extract on cyclosporine induced Nephrotoxicity in Rats. Iran J Kidney Dis 2015; 9(5): 361.

22. Lopez-Giacoman S and Madero M: Biomarkers in chronic kidney disease, from kidney function to kidney damage. World J Nephrol 2015; 4(1): 57.
23. Abuelezz SA, Hendawy N and Gawad AS: Alleviation of renal mitochondrial dysfunction and apoptosis underlies the protective effect of sitagliptin in gentamicin-induced nephrotoxicity. Journal of Pharmacy and Pharmacology 2016; 68(4): 523-32.
24. Teo SH and Endre ZH: Biomarkers in acute kidney injury (AKI). Best Pract Res Clin Anaesthes 2017; 31(3): 331-44.
25. Govindappa PK, Gautam V, Tripathi SM, Sahni YP and Raghavendra HL: Effect of *Withania somnifera* on Gentamicin induced renal lesions in rats. Rev Bras Farmacogn 2019; 29(2): 234-40.
26. Raghavan V and Weisz OA: Discerning the role of mechanosensors in regulating proximal tubule function. American Journal of Physiology - Renal Physiology 2015; 310(1): F1-5.
27. Randjelovic P, Veljkovic S, Stojiljkovic N, Sokolovic D and Ilic I: Gentamicin nephrotoxicity in animals: current knowledge & future perspectives. EXCLI J 2017; 16: 388.
28. Perazella MA: Pharmacology behind common drug nephrotoxicities. Clinical Journal of the American Society of Nephrology 2001; 13(12): 1897-08.
29. Niki E, Omata Y, Fukuhara A, Saito Y and Yoshida Y: Assessment of radical scavenging capacity and lipid peroxidation inhibiting capacity of antioxidant. Journal of Agricultural and Food Chemistry 2008; 56(18): 8255-60.
30. Wiland P and Szechcinski J: Proximal tubule damage in patients treated with Gentamicin or amikacin. Polish Journal of Pharmacology 2003; 55(4): 631-7.
31. Ghaznavi H, Fatemi I, Kalantari H, Hosseini Tabatabaei SM, Mehrabani M, Gholamine B, Kalantar M, Mehrzadi S and Goudarzi M: Ameliorative effects of gallic acid on gentamicin-induced nephrotoxicity in rats. J Asian Nat 2018; 20(12): 1182-93.

How to cite this article:
Suman S and Kumar SVS: Evaluation of *in-vitro* antioxidant and nephroprotective potentials of *Crinum asiaticum* bulbs against gentamicin induced nephrotoxicity in albino rats. Int J Pharm Sci & Res 2020; 11(12): 6494-02. doi: 10.13040/IJPSR.0975-8232.11(12).6494-02.

All © 2013 are reserved by the International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **Android OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)