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"Evaluation of Nephroprotective activity of Canna indica flower extract on rats"

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ABSTRACT
Objective: The present study was designed to evaluate the nephroprotective activity of flowers extract of Canna indica L in rats.
Methods: Canna indica flowers extract (CIFE) was evaluated for protection against rifampicin (1 g/kg body wt.,p.o) and gentamicin (20 mg/kg body wt., p.o.) induced nephrotoxicity in albino rats. The degree of nephroprotection was determined by measuring serum creatinine, BUN, uric acid, sodium ion, potassium ion and kidney weight index. Histopathological studies were also carried out on kidney of experimental animals.
Result: The Canna indica flowers extract found to contain cardiac glycoside, carbohydrate, proteins, alkaloids, glycosides, saponins, phytosterol, flavonoid, and trepenoids, steroids, tannins, phylobatinins, phenols as chemical constituents. The nephroprotective effect of the extract was found to be significant in toxicity produced by rifampicin and gentamicin. The histopathological studies on kidneys were in agreement with biomarkers estimation.The nephroprotective action may be due to antioxidant action of the extract.
Conclusion: The Canna indica flowers extract has significant nephroprotective activity when tested in albino rats.

INTRODUCTION:

The term acute renal failure (ARF) is currently substituted by acute kidney injury (AKI). AKI is a reversible condition in which there is a sudden decline in renal function, manifested by hourly/daily/weekly elevation in serum creatinine and blood urea nitrogen[1]. Several drugs causes nephrotoxicity which includes antibiotics, primarily Aminoglycosides like Gentamicin[2] ,Sulphonamides, Amphotericin B, Polymyxin, Neomycin, Bacitracin, Rifampin, Trimethoprim, Cephaloridine, Methicillin, Oxy- and Chlorotetracyclines, Analgesics, including NSAIDS [3].

There are many drugs available for nephroprotective activities. Flavonoids and phenolic compound (Antioxidants) are one of the agents which are extensively used for Cardioprotective and nephroprotective activities. Antioxidants are the substances which chemically react with free radicals and render them harmless and at the same time break the viscous circle, which involve in the decomposition of fatty acids and proteins, the creation of new free radicals and leads to eventual cell death [4].The antioxidant defense system includes both endogenously and exogenously derived compounds, dietary plants based antioxidant have recently received a great attention. Hence many studies have been performed to identify antioxidant compounds with pharmacological activity and a limited toxicity from medicinal plants[6].Antioxidants may play an important role in the chronic disease prevention by arresting oxidative damage caused by reactive oxygen species (ROS) to vital molecule such as DNA, lipids and proteins[89].

However, for a number of reasons, complimentary medicine has grown popularity in recent years. Dietary measures and traditional plant therapies as prescribed by Ayurvedic and other indigenous systems of medicine are commonly used in India. Many indigenous Indian medicinal plants have been found to be useful to successfully manage Nephroprotection some of them have been tested and their active ingredients isolated. The World Health Organization (WHO) has also recommended the evaluation of the plants.

Digitalis purpurea, Cheiranthus cheiri, Apocynum cannabinum plants have evaluated and reported that they have significant nephoroprotective activity which contain cardiac glycoside, carbohydrate, proteins, flavonoids, terpenoids, alkaloids, steroids, tannins and saponis.

Canna indica flower extract also containing cardic glycosides, carbohydrate, proteins, flavonoids, terpenoids, alkaloids, steroids , tannins, saponins, and phylobatinins. So, I intended to caary out neproprotective activity of ethanolic extract of canna indica flower againt Rifampicin and gentamicin induced toxicities.

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MATERIALS AND METHODS

1. Material

1.1 Experimental animals

Species: Albino rats
Strain: Wistar
Sex: Male
Source: Invivo Biosciences, Kamath Layout, Bengaluru-91, Karnataka
Body weight: 150-200 g
Identification: By cage card and body markings.
Number of animals: 6 in each group.
Acclimation: One week in experimental room.

1.2 Selection of animals:

After acclimatization the animals were subjected to a gross observation to ensure that the selected rats were in good state of health. Rats were randomly selected for final allotment to the study.

1.3. Environmental condition:

Air conditioned rooms with optimal air changes per hour, relative humidity, temperature and elimination cycle set to 12 hour light and 12 hour in dark. The animals were maintained under standard condition in an animal house approved by the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Mallige College Of Pharmacy, Bangalore. Reg No.1432/ PO/ RE/S/11/CPCSEA & 27/05/2017.

1.4 Accommodation : Group housed in polypropylene cages with stainless steel grill top. Facilities for food and water bottle and bedding of clean paddy husk. The husks in the cages were renewed thrice a week to ensure hygiene and maximum comfort for animals.

❖ **Diet:** "Amrut" brand pelleted feed was provided ad libitum.

❖ **Water:** UV purified and filtered water was provided ad libitum in polypropylene bottles with stainless steel sipper tubes.

Determination of acute toxicity of various fractions :

Acute toxicity study will be conducted to determine median lethal dose (LD50) of the methanol extract. Acute toxicity study of extract. Acute toxicity study will be carried out in albino Mice by "Up and Down method" (OECD guidelines 425).

Different dose levels (Up to 2000 mg/kg body weight) of the extract will be administered orally to overnight fasted Mice to different groups consisting of three animals in each group. Following the administration of the extract, animals will be observed continuously for 2-3 h.

Hence in the present study 125 mg/kg and 250 mg /kg was taken as dose for cardioprotective and nephroprotective activity.

Evaluation of Nephroprotective activity of Canna indica flower extract on rats.

Animals:

Healthy male albino rats of weighing 180-220g will be included for the study. The animals will be obtained from animal house of Mallige College of Pharmacy. The protocol for animal clearance is approved from Institutional Animal Ethical Committee for experimental purpose. They will be maintained under laboratory condition with controlled environment of temperature, humidity as per Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA) guidelines. They will be provided with standard diet and water ad libitum.

A. **Evaluation of Nephroprotective activity :**

1. Rifampicin induced nephrotoxicity

Table 5: Effect of Canna indica flower against Rifampicin induced nephrotoxicity.

Sl.No.	Group	Treatment	Duration of treatment
1.	Normal Control	Normal Saline	Daily for 14 days
2.	Positive control	Rifampicin	Rifampicin (1g/kg p.o) Every 72 h.
3.	Canna indica flower extract Control	Rifampicin + Canna indica flower extract	125 mg/kg p.o after 30 min. Rifampicin (1g/kg p.o) every 72 h.

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4.	Canna indica flower extract Pretreated	Rifampicin + Canna indica flower extract	250 mg/kg p.o after 30 min. Rifampicin (1g/kg p.o) every 72 h.

2. Gentamicin induced nephrotoxicity :

Table 6: Effect of Canna indica flower against gentamicin induced nephrotoxicity

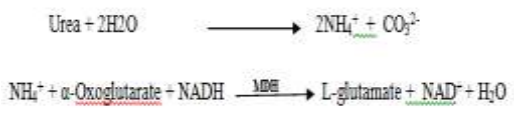
Sl.No.	Group	Treatment	Duration of treatment
1.	Normal Control	Normal Saline	Daily for 14 days
2.	Positive control	Gentamicin	Gentamicin (20 mg/kg p.o) For 8 days
3.	Canna indica flower extract Control	Gentamicin + Canna indica flower extract	Gentamicin (20mg/kg p.o) for 8 days+ Canna indica flower extract (125mg/kg p.o)
4.	Canna indica flower extract Pretreated	Gentamicin + Canna indica flower extract	Gentamicin (20mg/kg p.o) for 8 days+ Canna indica flower extract (250mg/kg p.o)

The study was carried out for 14 days. On 15th day all the animals will be anaesthetized by thiopental sodium. The blood samples will be collected by retro orbital plexus method for biochemical screening.

I. Biochemical Estimation

a) Blood urea nitrogen

Principle: Urea is hydrolysed in presence of urease to produce ammonia and CO_2 the ammonia produced combines with α-oxoglutarate and NADH in presence of GLDH to yield glutamate and NAD^+.



Procedure: Pipetted 1.0ml of reagent into test tubes and allow reagent to attain 37°C. Added 0.01ml (10ul) of sample to test tube and immediately placed in the spectrophotometer. After thirty seconds absorbance was recorded. Sixty seconds after the first reading another reading was taken. The absorbance was recorded at 340 nm.

Calculation: Urea (mg/dl) = Abs. of test / Abs. of Std. X Conc. of Std.

b) Serum creatinine

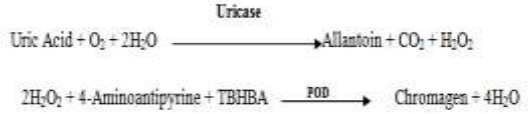
Principle: Creatinine reacts with picric acid in alkaline conditions to form a color complex that absorbs at 510 nm. The rate of formation of color is proportional to the creatinine in the sample.

Procedure: Pipetted 1.0ml of reagent into test tubes and allowed reagent to attain 37°C. Added 0.05ml (50ul) of sample to test tube and immediately placed in the spectrophotometer. After thirty seconds absorbance was recorded. Sixty seconds after the first reading another reading was taken. The absorbance was recorded at 510 nm.

Calculation: Serum creatinine (mg/dl) = Abs. of test / Abs. of Std. X Conc. of Std.

c) Uric acid

Principle: Uric Acid is oxidized by Uricase to allantoin and hydrogen peroxide. TBHBA + 4-aminoantipyrine + hydrogen peroxide, in the presence of peroxidase, produces a coloured chromagen that is measured at 520nm. The color intensity at 520nm is proportional to the concentration of uric acid in the sample.



Procedure: Pipetted 1.0 ml of reagent into tubes, warmed at 37°C for five minutes. The absorbance

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was recorded at 520 nm against water blank. Added 0.025 ml (25 ul) of serum to the tubes containing reagent, mixed and incubated at 37°C for one minute. After one minute, absorbance was recorded.

Calculations: Uric Acid (mg/dl) = Abs. of test / Abs. of Std. X Conc. of Std.

SI Units (mM/L): Multiply the result (mg/dl) by 10 to convert dl to L and divide by 168 (the molecular weight of Uric Acid).

mg/dl x .0595 = mMol/L

d) Estimation of Serum Potassium

Principle: Sodium tetraphenylboron reacts with potassium ions in a protein free alkaline medium to produce a turbid suspension of K-tetraphenylboron. The amount of turbidity produced is proportional to the Potassium concentration at 578 nm.

Procedure: Pipetted 1.0 ml of reagent into tubes, warmed at 37°C for five minutes. The absorbance was recorded at 578 nm against water blank. Added 0.2 ml (200 ul) of serum to the tubes was recorded.

Calculations:

Potassium concentration (mMol/L) = Abs. of test / Abs. of Std. X Conc. of Std

e) Estimation of Serum Sodium

Principle: β-galactosidase acts on ONPG (O-nitrophenyl) as substrate in the presence of sodium. The breakdown of substrate results in decline of OD at 405 nm.

Procedure: Pipetted 1.0 ml of reagent into tubes, warmed at 37°C for five minutes. The absorbance was recorded at 405 nm against water blank. Added 0.04 ml (40 µl) of serum to the tubes containing reagent, mixed and incubated at 37°C for one minute. After one minute, absorbance was recorded.

Calculations:

Sodium concentration (mMol/L) = Abs. of test / Abs. of Std X Conc. of Std.

II. Kidney weight index (KWI)

After blood withdrawal, all the rats were sacrificed by cervical dislocation; the right kidney was dissected out, washed in ice cold saline, weighed after blotting with filter paper and kidney weight index (KWI) was computed table 9 and graphically in figure 17.

$$\text{Kidney weight index (KWI)} = \frac{\text{Kidney weight (mg)}}{\text{Body weight (g)}}$$

III. Statistical Analysis

The values were expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Bunferroni comparison tests. P values < 0.05 were considered as significant.

IV.**Histopathological studies**

The animals were sacrificed on the day of blood withdrawal and kidneys were isolated. It was washed with ice cold saline, weighed and preserved in 10% formalin solution. The kidneys were processed and embedded in paraffin wax. The sections were stained with Hematoxylin and Eosin and observed under light microscope. The results were depicted as figure .

Table 7: Chemical constituents of Canna indica flowers extract:

Sl. No.	Particulars	Results
1.	Carbohydrates	+
2.	Cholesterol	+
3.	Proteins	+
4.	Amino Acids	+
5.	Alkaloids	+
6.	Flavonoids	+
7.	Terpenoids	+
8.	Cardiac Glycosides	+
9.	Steroids	+
10.	Tannins	+
11.	Sapponins	+
12.	Phlobatininis	+

Note: Present (+), Absent (-)

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Table 14: Effect ofCanna indica flowersextract on renal markers in the serum of control and Rifampicin induced nephrotoxicity in rats

N=6, values are expressed as mean ± SEM, #P<0.001 in comparison with healthy control and *P<0.01, **P<0.001 in comparison with gentamicin

Group	Creatinine (mg/dl)	BUN (mg/dl)	Uric acid (mg/dl)	K^+ (mmol/l)	Na^+ (mmol/l)
I. Normal control	0.567 ±0.0333	15.0 ±0.447	0.467±0.0760	11.3 ±0.212	7.39 ±0.174
II. Rifampicin	4.52±0.0543#	35.7 ±0.715#	8.45 ±0.0671#	6.81 ±0.209#	4.87±0.106#
III. CIFE 125mg + Rifampicin	2.55± 0.043***	23.8±1. 74***	5.85±0.062***	9.26±0.228 ***	5.92±0.10***
IV. CIFE250mg + Rifampicin	0.533±0.0211	15.0±0. 365	0.483±0.0654	11.0±0.207	7.43±0.124

Table 15: Effect of Canna indica flowers extract on kidney weight indices (KWI) of control andRifampicin induced nephrotoxicity in rats

Group	KWI (mg/g)
I. Normal control	2.62 ±0.0218
II. Rifampicin	2.95 ±0.0877
III. CIFE 125mg + Rifampicin	2.88±0.131
IV. CIFE250mg + Rifampicin	2.65 ±0.0251

N=6, values are expressed as mean ± SEM, #P<0.01 in comparison with healthy control, * P<0.05 in comparison with gentamicin.

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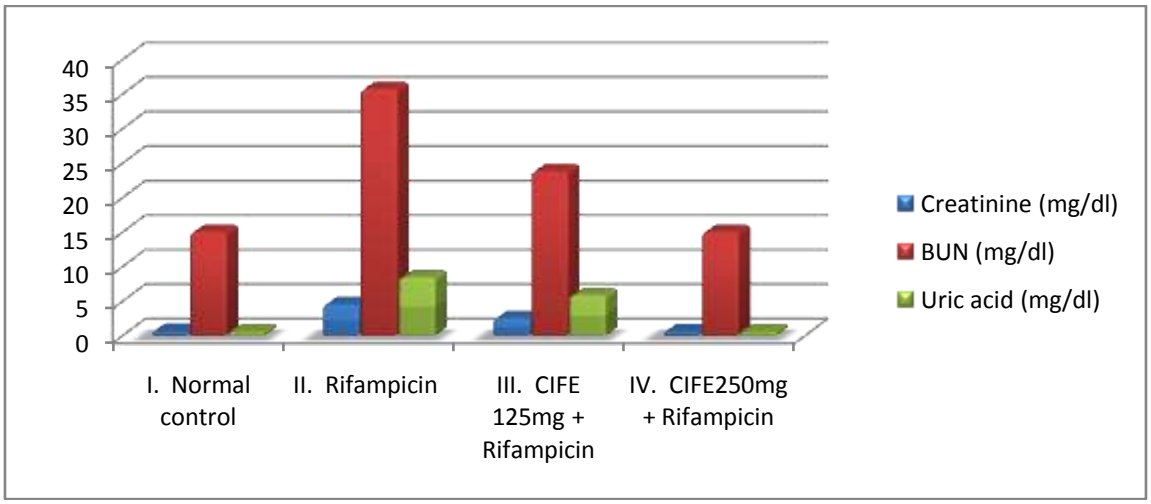

Figure 23: Effect of Canna indica flowers extract on creatinine, BUN and uric acid in the serum of control and Rifampicin induced nephrotoxicity in rats

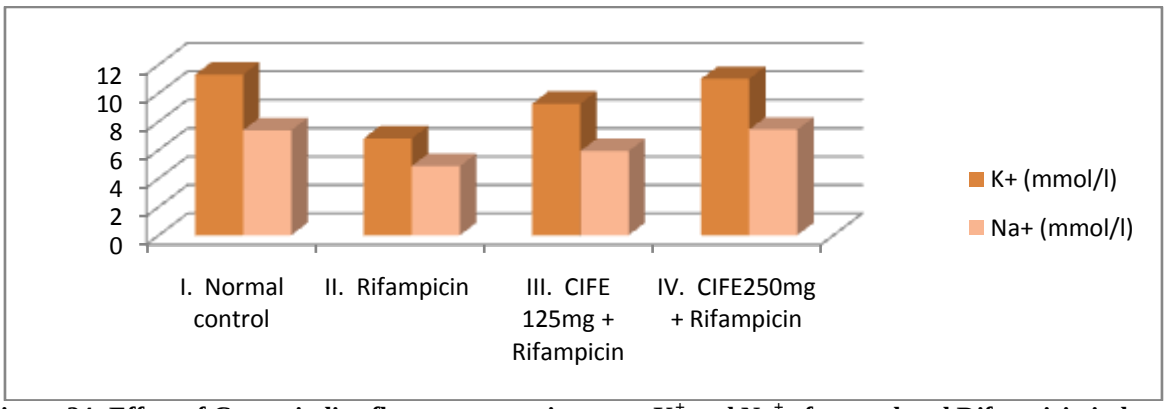

Figure 24: Effect of Canna indica flowers extract in serum K^+ and Na^+ of control and Rifampicin induced nephrotoxicity in rats

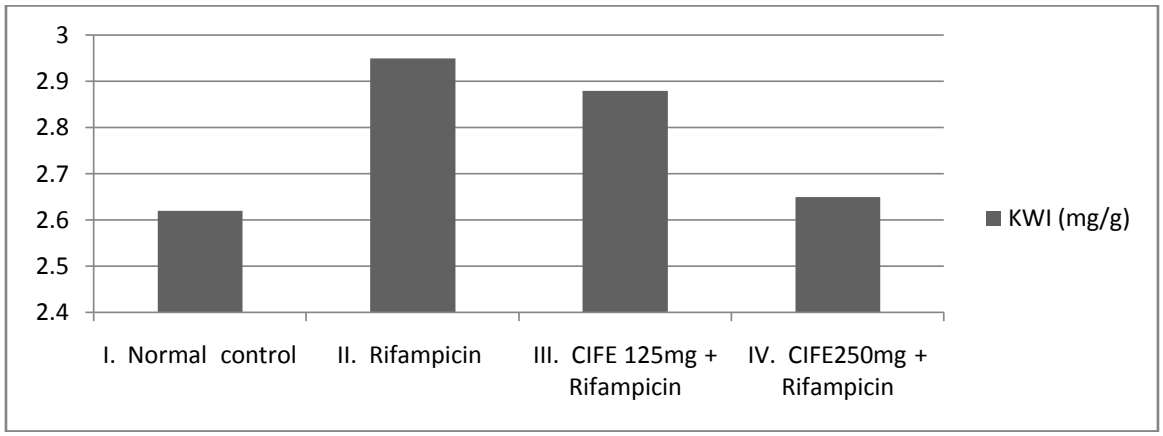

Figure 25: Effect of Canna indica flowers extract on kidney weight index (KWI) of control and Rifampicin induced nephrotoxicity in rats

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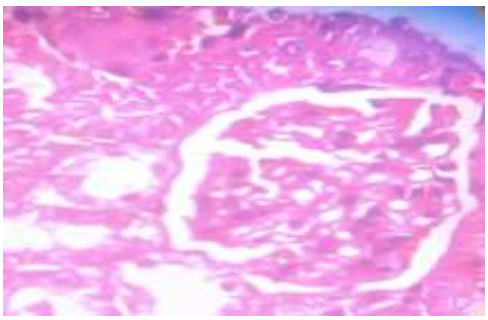
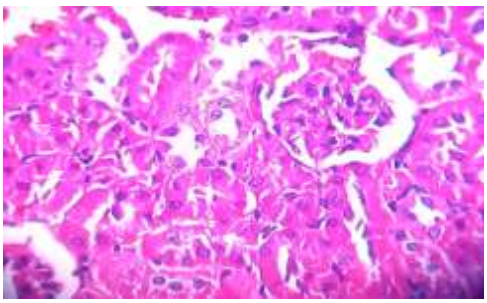
Table 16: Effect of Canna indica flowers extract on kidney architectures of control and Rifampicin induced nephrotoxicity in rats.

Observation	Normal control	Rifampicin	CIFE125mg + Rifampicin	CIFE250mg + Rifampicin
Necrosis of nephron	-	+ + +	+ +	-
Tubular necrosis	-	+ + +	+ +	-
Glomerular damage	-	+ +	+	-
Tubular cast hemorrhage	-	+ +	+	-
Blood vessels congestion	-	+ +	+ +	-
Peritubular congestion	-	+ + +	+ +	-
Glomerular congestion	-	+ + +	+	-
Infilteration	-	+ + +	+ +	-

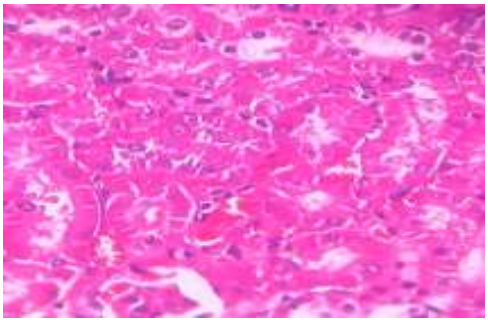
Note: Nil (-), Present (+), Moderate (+ +), Severe (+ + +)



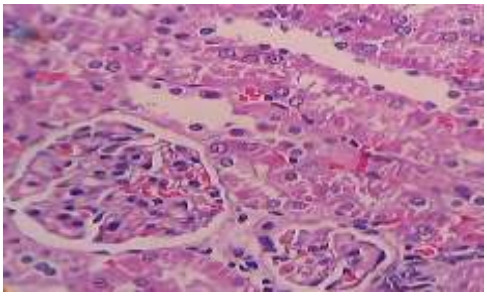
(A) Normal control



(B) CIFE 250 mg+ Rifampicin



(C) Rifampicin



(D)) CIFE 125 mg+ Rifampicin

Figure 26: Kidney's architectures of rats in rifampicin induced nephrotoxicity model.

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Table 17: Effect of Canna indica flowersextract on renal markers in the serum of control and gemtamicin induced nephrotoxicity in rats

Group	Creatinine (mg/dl)	BUN (mg/dl)	Uric acid (mg/dl)	K^+ (mmol/l)	Na^+ (mmol/l)
I. Normal control	0.583±0.0307	15.2±0.543	0.467±0.0760	11.1±0.336	7.12±0.155
II. Gentamicin	4.48±0.168#	33.3±0.333#	8.62±0.0946#	7.21±0.223#	4.44±0.106#
III. CIFE125mg + Gentamicin	2.63±0.230**	26.2±1.74*	5.65±0.494**	8.98±0.092**	5.69±0.101 **
IV. CIFE250mg+ Gentamicin	0.533±0.0422	14.8±0.477	0.483±0.0654	11.0±0.209	7.20±0.124

N=6, values are expressed as mean ± SEM, #P<0.001 in comparison with healthy control and *P<0.01, **P<0.001 in comparison with gentamicin

Table 18: Effect of Canna indica flowers extract on kidney weight indices (KWI) of control and gemtamicin induced nephrotoxicity in rats

Group	KWI (mg/g)
I. Normal control	2.66±0.0630
II. Gentamicin	3.13±0.0844#
III. CIFE125mg + Gentamicin	2.89±0.0534*
IV. CIFE250mg+ Gentamicin	2.66±0.0981

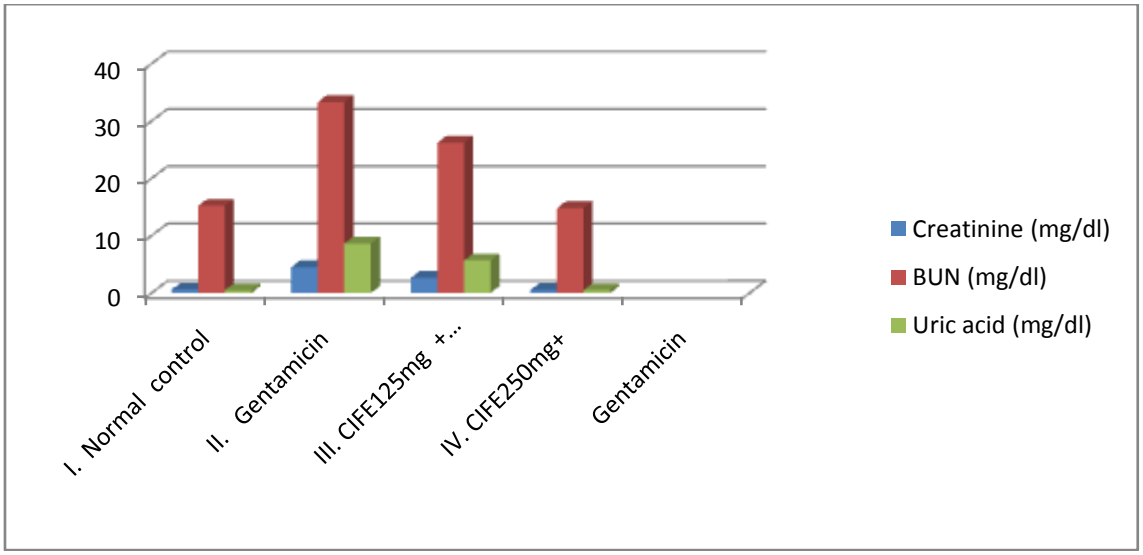

Figure 27: Effect of Canna indica flowers extract on creatinine, BUN and uric acid in the serum of control and gentamycin induced nephrotoxicity in rats

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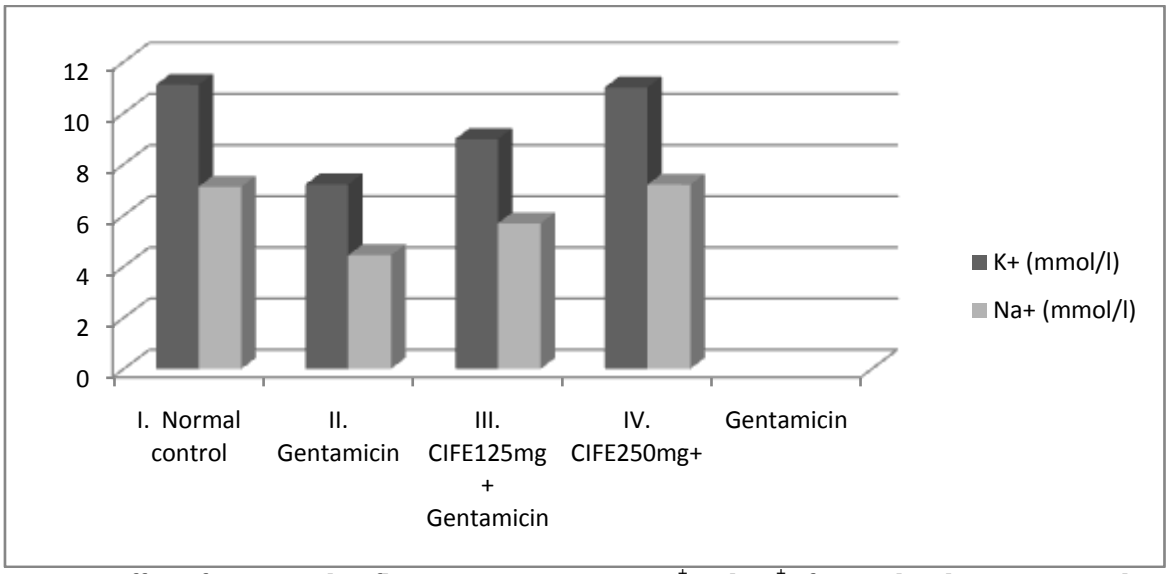

Figure 28: Effect of Canna indica flowers extract in serum K^+ and Na^+ of control and gentamicin induced nephrotoxicity in rats

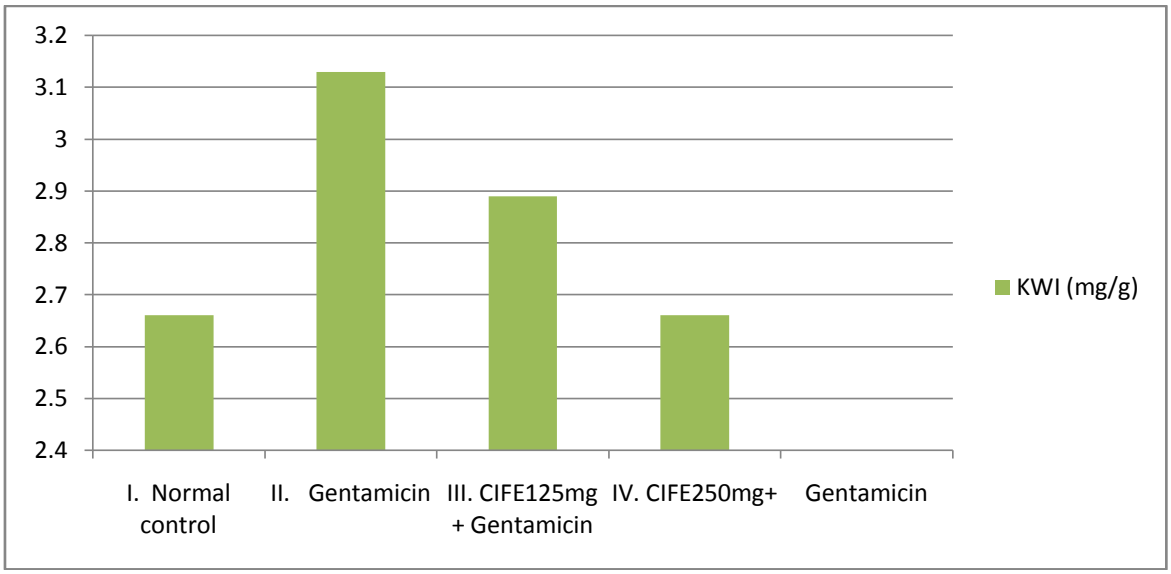

Figure 29: Effect of Canna indica flowers extract on kidney weight index (KWI) of control and gentamicin induced nephrotoxicity in rats

Table 19: Effect of Canna indica flowers extract on kidney architectures of control and gentamicin induced nephrotoxicity in rats.

Observation	Normal control	Gemtamycin	CIFE 125mg + Gemtamycin	CIFE 250mg + Gemtamycin
Necrosis of nephron	-	+ + +	+ +	-
Tubular necrosis	-	+ +	+	-

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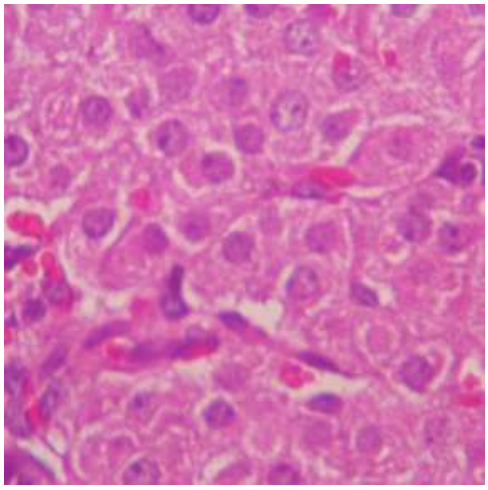
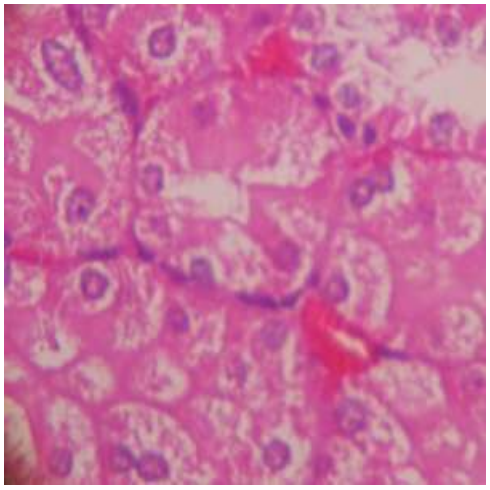
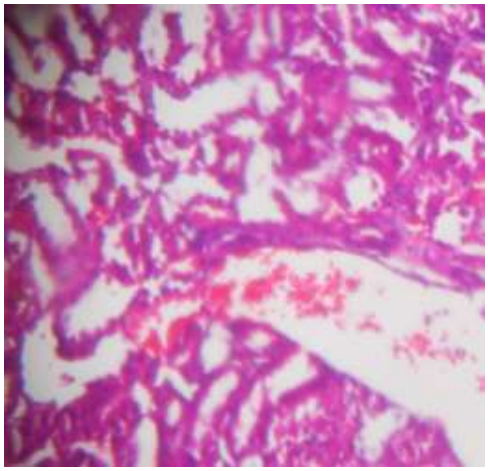
Glomerular damage	-	+ + +	+	-
Tubular cast hemorrhage	-	+ +	+ +	-
Blood vessels congestion	-	+ + +	+ +	-
Peritubular congestion	-	+ + +	+ +	-
Glomerular congestion	-	+ + +	+ +	-
Infilteration	-	+ +	+	-



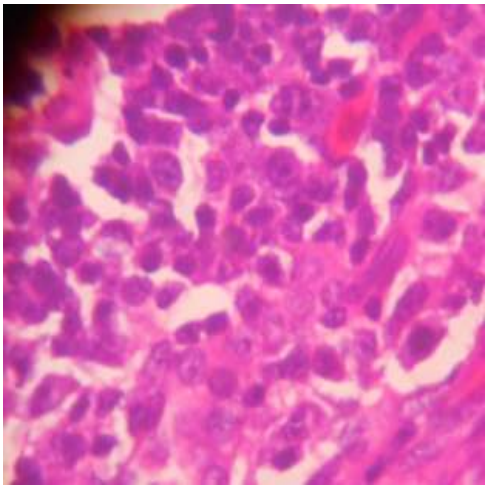
(A) Normal control



(B) CIFE 125mg + Gentamicin



(C) Gentamicin



(D) CIFE 250mg + Gentamicin

Figure 30: Kidney's architectures of rats in gemtamicin induced nephrotoxicity model

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5.1 Extraction and phytochemical analysis of Canna indica L. flowers extract

Successive soxhlet extraction process of Canna indica L. flowersyielded 9.8% of extract. The extract powder was deep brown in colour and hygroscopic in nature. The extract wassubjected to qualitative test to identify the presence of phytoconstituents. The result is depicted in Table 7. It was observed from the preliminary phytochemical screening of ethanolic extract ofCanna indica L. flowers, cardiac glycoside, carbohydrate, proteins, alkaloids, glycosides, saponins, phytosterol, flavonoid, and trepenoids, steroids, tannins, phylobatinins, phenols as chemical constituents. However the principal components like flavonoids, phenols, glycosides were present in the extract.

Evaluation of nephroprtective activity of Canna idica L. in albino rats

5.3.1 Nephroprotective activity of Canna indica flowers extract against Rifampicin-induced nephrotoxicity in albino rats.

In this study, nephroprotective activity of Canna indica flowers extract was evaluated against rifampicin-induced nephropathy. The changes in the biomarkers in different treatment group are reported in table 14 and graphically illustrated in figure 23 and 24. The result have shown the significant increase of serum creatinine, BUN and uric acid and decreased levels of potassium and sodium ions in group II which received single dose of Rifampicin 1 g/kg. The increased creatinine, BUN and uric acid were decreased to 2.55, 23.8 and 5.85 from 4.52, 35.7 and 8.45 respectively in the group where in rodents were treated with CIFE 250mg/kg. Moreover, in group IV, serum potassium and sodium ions concentration were also increased near to that of healthy control animals. Rats treated with CIFE 250mg/kg alone did not show much of difference in the measured biomarkers.

Significant increasese in KWI of rats in group III was observed in comparison with healthy control as shown in table 15. Whereas, in group IV, where in rats were treated with CIFE 250mg/kg along with rifampicin, KWI has reduced showing nephroprotectant property of the extract. CIFE 250 mg/kg + rifampicin for 14 days did not make much difference in KWI of experimental rats.

Histopathological study as shown in table 16 has revealed the severity of necrosis of nephron and tubules, perotubular congestion and glomerular congestion by rifampicin treatment. The damage to glomerular blood vessel congestion and tubular hemorrhage were found to be moderate. Blood vessels, glomerular and peritubular congestion have reduced in the CIFE 250 mg/kg and paracetamol treated group. Tubular cast hemorrhages has recovered in the extract treated group. Healthy control rats and rats received CIFE 250 mg/kg has not shown any significant changes in architectural damage in kidney. Microscopical photographs of kidney's architecture of rats of each group are shown at figure 26.

5.3.1 Nephroprotective activity of Canna idica L. flowers extract against gentamicin-induced nephrotoxicity in albino rats.

The evaluation of nephroprotective activity was carried out by measuring renal biomarkers like creatinine, BUN, uric acid, potassium ion and sodium ion along with kidney weight index (KWI) in experimental rats against gentamicin induced toxicity. From the reports of biomarkers as mentioned in table 17 and graphically presented in figure 27 and figure 28, it was observed that in the group injected with gentamicin 20mg/kg body weight, creatinine, BUN and uric acid increased where as potassium ions and sodium ions concentration decreased significantly than the healthy control rats indicating the induction of nephropathy by gentamicin. In the group which received CIFE 250mg/kg p.o. + gentamicin 20mg/kg for the experimental period did not make any significant changes in the renal biomarker observed in healthy control. Group treated CIFE 125mg/kg along with gentamicin 20mg/kg significantly, decreased the elevated creatinine, uric acid and BUN and also rised the declined potassium and sodium ions when compared to group II.

KWI, whose rise is an indication of nephropathy, The KWI increased significantly in group received gentamicin 20mg/kg body weight alone for 8 days. The elevated KWI decreased significantly in the group III where in rats were treated with CIFE 125mg/kg +gentamicin 20 mg /kg in addition to gentamicin injection. CIFE 250mg /kg for 8 days did not make any significant changes in KWI of the rats. The results of KWI of all the groups are presented in table 18 and figure 29.

Further, histopathological investigation of kidney section of rats has confirmed the induction of nephrotoxicity by gentamicin and the nephroprotective activity of the extract against gentamicin. Report of histopathological findings

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are illustrated in table 19 in detail and pictures in figure 30, where the nephropathy is severe in rats received gentamicin alone. The severity of nephropathy is less in the rat treated with CIFE250 mg/kg along with gentamicin in comparison with the rat received gentamicin alone.

5.3.2 Nephroprotective activity of Canna indica flowers extract against Rifampicin-induced nephrotoxicity in albino rats.

In this study, nephroprotective activity of Canna indica flowers extract was evaluated against rifampicin-induced nephropathy. The changes in the biomarkers in different treatment group are reported in table 14 and graphically illustrated in figure 23 and 24. The result have shown the significant increase of serum creatinine, BUN and uric acid and decreased levels of potassium and sodium ions in group II which received single dose of Rifampicin 1 g/kg. The increased creatinine, BUN and uric acid were decreased to 2.55, 23.8 and 5.85 from 4.52, 35.7 and 8.45 respectively in the group where in rodents were treated with CIFE 250mg/kg. Moreover, in group IV, serum potassium and sodium ions concentration were also increased near to that of healthy control animals. Rats treated with CIFE 250mg/kg alone did not show much of difference in the measured biomarkers.

Significant increasese in KWI of rats in group III was observed in comparison with healthy control as shown in table 15. Whereas, in group IV, where in rats were treated with CIFE 250mg/kg along with rifampicin, KWI has reduced showing nephroprotectant property of the extract. CIFE 250 mg/kg + rifampicin for 14 days did not make much difference in KWI of experimental rats.

Histopathological study as shown in table 16 has revealed the severity of necrosis of nephron and tubules, perotubular congestion and glomerular congestion by rifampicin treatment. The damage to glomerular blood vessel congestion and tubular hemorrhage were found to be moderate. Blood vessels, glomerular and peritubular congestion have reduced in the CIFE 250 mg/kg and paracetamol treated group. Tubular cast hemorrhages has recovered in the extract treated group. Healthy control rats and rats received CIFE 250 mg/kg has not shown any significant changes in architectural damage in kidney. Microscopical photographs of kidney's architecture of rats of each group are shown at figure 26.

DISCUSSION

The leaf extract of Canna indica flowers yielded the good percentage of yield of 9.8%. The alcoholic extract contained chemical constituent such as cardiac glycoside, carbohydrate, proteins, alkaloids, glycosides, saponins, phytosterol, flavonoid, and trepenoids, steroids, tannins, phylobatinins, phenols as chemical constituents. These results are in agreement with results published in standard books and research papers.

Myocardial infarction is one of the most common manifestations of cardiovascular disease. The pathogenesis of acute myocardial infarction has not yet been fully understood, but studies on ISO or DOX induced cardio-toxicity provide a good insight into this pathology and clearly indicate the involvement of oxidative stress 94, 95. In the present study, we found that canna indica a strong cardio-protective effect against ISO and DOX – induced myocardial necrosis in rats.

Myocardium contains n abundant amount of diagnostic marker enzymes for MI and once metabolically damaged, it released its intercellular contents into the extracellular fluid 96. Hence the serum levels of these marker enzymes reflect the alterations in membrane integrity and/or permeability. Cytosolic enzymes CPK, LDH, AST, ALT and SGPT which serve as the diagnostic markers, leak out from the damaged tissue to blood stream when cell membrane becomes permeable or rupture 97,98. Assay of the activity of CPK in serum in an important diagnostic, because of the marked abundance of this enzyme in myocardial tissue and virtual absence from most of other tissues and its consequent sensitivity. CPK iso-enzyme activity is useful not only as an index of early diagnosis of MI, but also any type of myocardial injury. ISO or DOX administration to rats showed elevated levels of serum CPK 99, which was found to be significantly low in the bioactive fractions pretreated rats. Lactate dehydrogenase (LDH) is an intracellular enzymes, which catalyzes the readily reversible reaction involving the oxidation of lactate to pyruvate with nicotinamide adenine dinucleotide (NAD) serving as coenzyme. This clinically significant enzyme rises within 24-48 hours after a heart attack and peaks in two to three days in the serum 100. Consistent with the above clinical observations, in the present study we observed a significant rise in the LDH levels of rats treated with DOX or ISO after 48 or 72h of the respective treatment. Canna indica pretreated significantly reduced the elevated

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levels of LDH indicating the reduction in the severity of MI.

It has been reported that infarction of as little as 10% of the total myocardium produces a significant rise in the serum levels of AST, ALT and is linear with the amount of infarction 101. In accordance to the above reports, ISO or DOX treatment elevated these enzyme levels in serum to a significant extent. Pretreatment with Canna indica flower extract (125and 250 mg/kg) significantly lowered the ISO or DOX-induced elevation of serum levels of these diagnostic marker enzymes. It demonstrates that Canna indica could protect membrane integrity thereby restricting the leakage of these enzymes.

Nephrotoxicity is one of the notable side effects and therapeutic limitations of analgesic and antipyretic drugs, especially acetaminophen and also anti-tubercular regimen such as rifampicin and isoniazid. Oxidative stress related renal disease, induction of renal tubular necrosis; glomerular damage and renal inflammation are some of the major incident implicated in drugs induced nephrotoxicity 75. A relationship between oxidative stress and nephrotoxicity has been well demonstrate in many experimental animal models. In recent times, there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidant in reducing tissue injuries caused by free radicals.

In the present study, rifampicin administration for 14 days significantly increased serum enzymes such as blood urea nitrogen, serum creatinine and serum uric acid. Canna indic treated group showed significant decrease in the serum enzymes level in contrast to that of the treated groups. Treated groups had significant prevented the elevation of these enzymes levels compared to the control group and hence indicating their nephroprotective effect against rifampicin-induced renal damage.

Histopathological changes such as cortical glomerular, bowman space, nephron tubules, medulla structure, calyx structure and granulated materials were observed in the refampicin-treated control group. The various fractions treated animals had significantly prevented these histopathological changes, future indicating their nephroprotective activity.

Here in the current investigation, ethanolic extract of Canna indica the significant reduction of the serum enzymes and least inflammatory filtrate, necrotic tubules and structural damage showing marked nephroprotection which may be due to antioxidant potential of Canna indica. Phytochemical screening of ethanoic extract of choreospondias axillaries fractions has revealed polyphenolic compounds like flavonoids, tannins, and phenolic acids as major components. These are very vital for the free-radical scavenging and antioxidant activities of plants as they act as hydrogen donors and thus neutralize the free-radicals. This radicals scavenging potential of Ethanolic extract of Canna indica can also be supported by significantly decreased lipid peroxidation in the in vitro models. Moreover, its antioxidant activity has also been partially held responsible for its preventive and therapeutic effects on acute hepatic injury in rats. On the other hand ethyl acetate and water fraction exhibits reduction of the serum enzymes compared to that of the positive control group of nephrotoxic rats. Therefore further studies to explain their mechanisms of action should be conducted to aid the discovery of new therapeutic agents for the treatment of renal diseases.

The evaluation of nephroprotective effect of Canna indica flowerswas performed against rifampicin and gentamicin induced toxicity in albino rats.

The gentamicin plays a role in reactivation of oxygen metabolites leading to variety of renal disease and renal toxicity. Gentamicin has been shown both in in-vitro and in-vivo studies to enhance the generation of reactive oxygen metabolites. Iron is important in models of tissue injury, presumably because it is capable of catalyzing free-radical formation. Gentamicin has been shown to cause release of iron from renal cortical mitochondria[79]. The lysosomal phospholipidosis appear to be the key event in the development of nephrotoxicity due to aminoglycosides. The other mechanism attributed to gentamicin induced renal impairment is free radical generation. The kidney provides the principal excretory route for elimination of aminoglycoside antibiotics from the body. Pharmacokinetic studies in both experimental animals and humans have demonstrated that these are not metabolized and excreted primarily by glomerular filtration[80-81].

The nephrotoxicity observed in experimental rats confirms the elevated level of creatinine, BUN and uric acid whereas K^+ and Na^+ level are decreased. The CIFE at the dose of 250 mg/kg injected for 8 days reversed the nephtotoxicity produced by gentamicin. The probable mechanism may be due to the antioxidant

property which might have neutralized oxygen metabolite.

CONCLUSION

The Canna indica flowers extract has significant cardioprotective and nephroprotective activity when tested in albino rats.

SUMMARY

The aim of this study was to evaluate the cardio and nephro protective properties of Canna indica flowersextract in albino rats.

- The ethanolic extract of Canna indica flowers extract found to contain cardiac glycoside, carbohydrate, proteins, alkaloids, glycosides, saponins, phytosterol, flavonoid, and trepenoids, steroids, tannins, phylobatinins, phenols as chemical constituents
- Treatment with Canna indica flowers extract has protected the kidney from rifampicin and gentamicin induced nephrotoxicity in albino rats. This was demonstrated by reducing the elevated levels of biomarkers like creatinine, BUN, and uric acid. In addition depletion of serum Na^+ and K^+ were reduced in rifampicin and gentamycin induced nephrotoxicity in rodents by Canna indica flowers extract. Histopathological studies has supported the results.

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