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Ethnopharmacological Investigation of Some E Medicinal Plants for the Management of Nephrotoxicity

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Abstract

Traditional systems of medicine, especially Ayurveda contains number of preparations for treating liver & GIT disorders. In the present study deals with investigation of some medicinal plants for the management of nephrotoxicity of plant. For study we used leaves extract of *A. cordifolia* , leaves extract of *Cyathea gigantea* and seed extract *Persea Americana* seed in 1:1:1 ratio. We have evaluated the nephroprotective activity of combination of extracts by selecting experimental models which included gentamicin-induced nephrotoxicty in rats. Our study proved that this combination of plant extract possess considerable amount of flavonoid and phenolic compounds. *In-vivo* nephroprotective activity results obtained also provided sufficient scientific evidences about the use of CE combination of plant extract *(*The leaves of *A. cordifolia* , leaves of *Cyathea gigantea* and seed *Persea Americana* seed) for the treatment of drug induced kidney injury.

Keyword - *Cyathea gigantean,* GIT disorders, nephrotoxicty, phenolic compounds ect.

Introduction

Modern herbal therapy has only recently developed and commercialized old techniques. Many patients went to herbal medicine because they were unsatisfied with conventional treatment options like surgery or drugs. Due in large part to the perception that they are safe since they are natural, herbal medicines are still widely used today.[1-3]

Cyathea gigantea (*C. gigantea*) (Wall. ex. Hook.) (Cyatheaceae) is a tree fern found extensively in moist open areas of Northeastern to Southern India, Thailand, Srilanka, Nepal and Western Java. The Cyatheaceae is the scaly tree fern family and includes the world's tallest tree ferns, which reach heights up to 20 m. Traditionally the fresh rhizome of *C. gigantea* mixed with black pepper seeds powdered and taken orally with milk twice a day for one week in stomach against white discharges. *C. gigantea* have several active constituents like triterpenes, sterols, saponins, flavonoids, hentriacontane, β-sitostenone, β-sitostanone, diploterol, sitosterol, hopan-29-ol and whole plant contains oleanolic acid. The first investigation on flavonoids constituents in the genus *Cyathea* was carried out by Harada *et al*. Oleanolic acid is a triterpenoid having antitumor, hepatoprotective and antiviral activity. Oleanolic acid is found to exhibit strong anti-HIV activity. Dietary phytosterols like β-sitosterol is having anticancer activity.

Herbal drugs play a major role in the treatment of hepatic disorders. In the absence of reliable liver protective drugs in modern medicine, in India, a number of medicinal plants and their formulations

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are used to cure hepatic disorders in traditional systems of medicine. Several studies were conducted in the field of drug discovery and development but due to the side effects of modern medicine, natural remedies are considered to be effective and safe alternate treatments for hepatotoxicity.

The *Cyathea gigantea* as a Source of Natural Antioxidant The antioxidant activity of hexane, chloroform, hydro-alcoholic and aqueous extract of whole plant of *Cyathea gigantea* (family *Malvaceae*) was evaluated using in-vitro models, DPPH free radical scavenging,scavenging of hydrogen peroxide and reducing power method **.**[4-5]

Adina cordifolia L has been used in oriental medicine since ancient times as an essential component of various antiseptic and febrifuge prescriptions **.** The bark is acrid and bitter and is used in biliousness. The roots are used as an astringent in dysentery **.**The *A. cordifolia* stem has been evaluated for its antiulcer potential. It is also used as Febrifuge, Antiseptic, Anti-fertility, Anti-inflammatory, Anti-rheumatoid, Bitter tonic, Anti-cancer, Anti-microbial.

It contains 10-deoxyadifoline, 10-deoxycordifoline indole alkaloid, cordifoline, adifoline. Di-OH-tetra-OMe flavone has been isolated from defatted heartwood. Oleoresin obtained from incision of trunk yields essential oil (5.2- 6.8 %). Stem contains yellow coloring matter, napthaquinone and adinin. The leaves contain ursolic acid and quercetin. It also contains 7-hydroxycoumarin (umbelliferone), D-glucosylcoumarin (skimmin). [6-7]

Avocado (*Persea americana Mill*) belongs to the Lauraceae family and represents one of the four most important tropical fruits with global production and trade in expansion across Europe[1]. The avocado nutritional and sensorial quality has influenced the increase of its consumption in many countries. The above is reflected in the cultivated areas increase and therefore in world production.

The avocado fruit has a lot of nutrients. This includes its high content of essential minerals, potassium, vitamin E and B complex. The avocado seed also contains various classes of natural products such as phytosterols and triterpenes, fatty acids with olefinic, acetylenic bonds, furanoic acid, dimmers of flavonols and oligomeric proanthocyanidins, β-D-glucoside of 8-hydroxyabscisic acid and epi- dihydrophaseic acid β-d-glucoside . [8-9]

2. MATERIAL & METHODS

Collection of plant material and Preparation of plant powder

The leaves of *A. cordifolia* and Cyathea gigantea plant were collected from natural habitat. The plant leaves was used for the preparation of the extract. The plants leaves was collected and dried under shade and then coarsely powdered with the help of mechanical grinder. After passing through filter No. 40, the powder was placed in an airtight container for later use. [10-11]

Preparation of extracts:

The collected, cleaned and powdered leaves of *A. cordifolia, and Cyathea gigantea* were used for the extraction purpose. 500 gm of powdered material was evenly packed in the soxhlet apparatus. It was then extracted with various solvents from non-polar to polar such as petroleum ether, chloroform, acetone and ethanol. The solvents used were purified before use. The extraction method used was continuous hot percolation and carried out with various solvents, for 72 hrs. The

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aqueous extraction was carried out by cold-maceration process. The extracts were concentrated by vacuum distillation to reduce the volume to 1/10; the concentrated extracts were transferred to 100ml beaker and the remaining solvent was evaporated on a water bath. Then they were cooled and placed in a dessicator to remove the excessive moisture. The dried extracts were packed in airtight containers and used for further studies.

Mature and healthy fruits of avocado pear were obtained from local market and kept at room temperature for 4days in order for them to ripe. The fruits were washed with distilled water, cut open with a knife and the seeds as well as the seed coverings were manually removed. The seeds were then chopped into smaller sizes, oven dried at 50ºC for 48 hours and then pulverized with the use of laboratory blender . The pulverized samples were packaged in waterproof polyethylene bags and stored at 4ºC until required for analysis and extraction. Solvent extraction was carried out on 100 g of pulverized sample with soxhlet apparatus for a period of 8 hours using with various solvents from non-polar to polar such as petroleum ether, chloroform, acetone and ethanol. The solvents used were purified before use. The extraction method used was continuous hot percolation and carried out with various solvents, for 72 hrs [12-14]

Combination of Plant Extract with ultrasound treatment (CE):

The combination of all three plants in 1:1:1 ratio used in for further study. The 2 gm of *A. cordifolia* leaves exract , 2 gm of *Cyathea gigantean* leave extract and 2 gm of *Persea Americana* seed extract are dissolved in minimum quantity of organic solvent like alcohol and were kept in a beaker and sonicated at 30 mint at room temperature.The solvent surafe in the beaker was kept at the same level of water in the ultrasonic bath. Whenever temperature increase in the ultrasonic bath was observed , fresh water was circulated to maintain the temperature. The beaker of extract also covered with aluminum foil to avoid loss of solvent by evaporation. After sonicate the combination extract was then dried and kept in air tight container until further use.

PHARMACOLOGICAL SCREENING[15-18]

Assessment of *in vitro* antioxidant activity of CE

DPPH (1,1-Diphenyl-2-picryl hydrazine) radical scavenging activity

DPPH free radical scavenging activity was evaluated using the method described by Blois.[127] Various concentrations of CE and ascorbic acid (standard) ranging (10- 100 µg/mL) were mixed with 1 mL of freshly prepared 0.3 mM DPPH ethanol solution and 2 mL of 0.1M acetate buffer. Incubated at room temperature for 30 mins and the Absorbance of resulting solutions were then measured colorimetrically at 517 nm. DPPH solution (1.0 mL, 0.3 mM) treated with 1mL ethanol, served as negative control. Ascorbic acid was used as positive control under the same assay condition. Samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The antioxidant activity of the extract was expressed as IC_{50}. Higher absorbance indicates lower free radical scavenging activity. The percentage of DPPH radical scavenging activity of extract was calculated from decrease in absorbance in comparison with control by using formula:

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Percentage inhibition (%) = [(Abs $_{control}$ – Abs $_{test}$) / Abs $_{control}$] X 100

Assessment of *in vivo* antioxidant activity of CE

Evaluation of Nephroprotective Activity -

Thirty albino rats were used in each drug induced toxicity models. Animals were divided into five groups with 6 animals in each group, designated as Group I, II, III, IV and V. Animals in group I served as control and group II served as toxic control. Rats in Group II to V were treated with drugs which induce toxicity. Animals in group III, IV and V also received standard drug, low dose of CE (250 mg/kg) and high dose CE of (500 mg/kg) respectively.

S.No	Group	Treatment
1	Group-I	Normal Saline 1ml /Day, For 8 days
2	Group –II	GM 100mg/Kg for 8 days,i.p.
3	Group-III	GM +Quercetin 50 mg/Kg for 8 days
4	Group-IV	GM + Extract 250 mg/Kg for 8 days
5	Group-V	GM + Extract 500 mg/Kg for 8 days

Gentamicin (GM) induced nephrotoxicity[106-107]

Table : Grouping of Animal in gentamicin (GM) induced nephrotoxicity model

RESULTS AND DISCUSSION

Determination of extractive value

Extractive values determined were showed in table 6.2 The extractive values indicate the presence of considerable amount of constituents in solvents.

Table 1: Ash Values

Plant Name	Part Used	Types of Ash	Percentage of Ash(w/w)
Cyathea gigantea	Leaves	Total ash	11.34
		Acid Insoluble	0.85
		Water soluble	4.12

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Determination of extractive value

Extractive values determined were showed in table 2 The extractive values indicate the presence of considerable amount of constituents in solvents.

Table 2: Extractive Values of *Adina cordfolia* ,*Cyathea gigantean and Persea Americana*

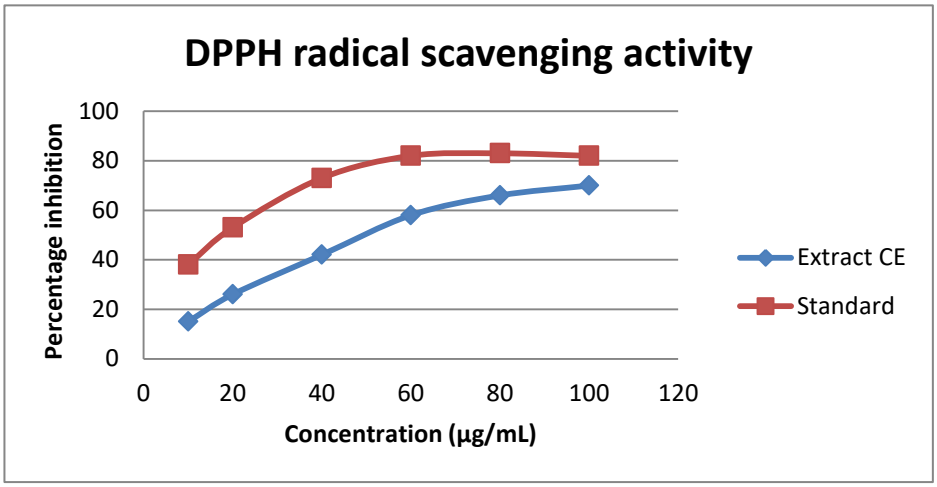
Solvent	% Yield		
	Adina cordfolia	*Cyathea gigantean*	*Persea Americana*
Pet. Ether	1.67	2.13	1.89
Chloroform	2.23	3.86	1.54
Acetone	14.1	9.6	15.8
Ethanol	9.21	14.2	10.5
Aqueous	16.2	27.7	19.2

***In vitro* pharmacological studies**

DPPH radical scavenging Activity

Radical scavenging activity of extract was observed from decrease in absorbance of DPPH with increase in concentration. Absorbance value of CE had shown as 1.53, 1.20, 0.99, 0.73, 0.46 and 0.35 and standard ascorbic acid exhibited as 1.29, 0.79, 0.31, 0.08, 0.05 and 0.04 at 10, 20, 40, 60, 80 and 100 µg/mL respectively extract CE at a concentration of 10-100 µg/mL inhibited production of DPPH radical by 17-81% and showed significant ($P<0.05$) scavenging effects on DPPH radical compared to standard ascorbic acid which exhibited 41-99% of inhibition. IC_{50} value of CE was found to be 46.34 µg/mL and that of ascorbic acid (standard) was 14.12 µ g/mL. This result indicated that CE contained sufficient phytochemical constituents capable to donate 'H' for the conversion of free radical DPPH to non-free radical DPPH-H

Table: Percentage inhibition of DPPH radical scavenging activity of CE, in comparison to standard ascorbic acid



***In vivo* pharmacological studies**

Nephroprotective activity of combination of plant extract at concentration of 250 mg/kg (low dose)

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and 500 mg/kg (high dose) were investigated using various general, urinary, blood, serum and kidney homogenate parameters against gentamicin-induced nephrotoxicity in Wistar albino rats.

Gentamicin (GM)-induced nephrotoxicity

Table 15 illustrated the effect of CE on general parameters of GM-induced nephrotoxicity in control and experimental animals that were obtained at the end of the experiment in each group. Body weight was recorded before commencing the experiment. This results revealed that body weight of animals in group II were significantly ($p<0.01$) reduced after treatment, compared to control group, where as body weight of animals in group III ($p<0.001$), group IV ($p<0.01$) and group V ($p<0.01$) were significantly increased compared to animas in toxic control group. In GM-treated group, the kidney weight was found to be significantly increased ($p<0.001$) compared to normal control group. Significant reduction in kidney weight were observed in animals co-treated with low dose of CE ($p<0.01$), high dose of CE ($p<0.001$) and quercetin ($p<0.001$). 24-hr urine volume in the GM-treated group were found to be significantly ($p<0.01$) reduced in comparison to control group. Supplementation of quercetin, low dose of CE ($p<0.01$) and high dose of CE ($p<0.001$) to GM-treated rats increased urine output. However, water intake was significantly ($p<0.05$) increased only in animals co- administered with 500 mg/kg of CE. Urinary pH in GM-treated group was found to be 6.6±0.05 which revealed no significant ($p>0.05$) changes in urinary pH compared to animals co-treated with extract/standard.

Table 15: Effect of CE on general parameters in GM-induced nephrotoxicity

Parameters Studied (Unit)	Group I Normal control	Group II GM 100 mg/kg	Group III GM+QTN 50 mg/kg	Group IV GM+CE 250 mg/kg	Group V GM+CE 500 mg/kg
Change in body wt. (g)	2.96±0.43	1.48±0.11^{**a}	3.26± 0.14^{***b}	2.53± 0.17^{**b}	2.50± 0.22^{**b}
Kidney wt. (g)	0.64±0.00	0.97±0.05^{***a}	0.70±0.08^{***b}	0.73±0.00^{**b}	0.71±0.00^{***b}
Water intake (mL/24 hr)	12.36±0.57	14.54±0.76^{ns}	17.36±0.82^{ns}	17.36± 0.82^{ns}	17.88±0.86^{*b}
Urine volume (mL/24 hr)	7.74±0.15	5.73± 0.22^{**a}	7.63±0.30^{**b}	7.26±0.16^{**b}	7.84± 0.09^{***b}
Urine pH	6.8±0.04	6.6±0.05^{ns}	6.8±0.03^{ns}	6.7±0.05 ^{ns}	6.8±0.05 ^{ns}

GM: gentamicin, QTN: quercetin, CE: Combination of Plant Extract. Values are expressed in mean ± standard error of mean (n=6), *p<0.05, **p<0.01, ***p<0.001 ^{a} significant compared o control group (group I), ^{b}significant compared with GM-induced group (group II), ^{ns} not significant.

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Effect of CE on urinary total protein and albumin levels in experimental Animal

GM-treated rats showed a marked increase in urinary protein and albumin excretion ($p<0.01$). Animals co-treated with CE/quercetin significantly ($P<0.01$) reduced the incidence of proteinuria, however, significant ($P<0.01$) reduction in albuminuria were observed only in rats co-treated with quercetin or high dose of CE, compared to group II rats .

Table 15: Effect of CE on urinary total protein and albumin levels in GM-induced nephrotoxicity

Urinary parameters (Unit)	Group I Normal control	Group II GM 100 mg/kg	Group III GM+QTN 50 mg/kg	Group IV GM+CE 250 mg/kg	Group V GM+CE 500 mg/kg
Total protein (g/dL)	**3.66 ±0.01**	**4.51±0.02^{**a}**	**3.56±0.01^{**b}**	**4.08±0.04^{**b}**	**3.58±0.05^{**b}**
Albumin (g/dL)	**0.76±0.02**	**0.83±0.06^{**a}**	**0.63±0.01^{**b}**	**0.81±0.07^{ns}**	**0.72±0.01^{**b}**

GM: gentamicin, QTN: quercetin, CE: Combination of Plant Extract. Values are expressed in mean ± standard error of mean (n=6), *p<0.05, **p<0.01, ***p<0.001 ^{a} significant compared o control group (group I), ^{b}significant compared with GM-induced group (group II), ^{ns} not significant.

Effect of CE on urinary electrolyte levels in experimental animal

Urinary excretion of calcium and magnesium were found to be much higher ($p<0.01$) in group II animals, compared to control group. Co-treatment with CE at 250 mg/kg ($p<0.01$), quercetin ($p<0.001$) and CE at 500 mg/kg ($p<0.001$) significantly decreased urinary calcium excretion compared to GM-treated animals. Urinary magnesium excretion were significantly ($p<0.01$) lowered in animals co-administered with standard/ extract. However, significant differences were not observed in sodium and potassium levels among animals in different groups

Table 15: Effect of CE on urinary electrolyte levels **in GM-induced nephrotoxicity**

Urinary parameters (Unit)	Group I Normal control	Group II GM 100 mg/kg	Group III GM+QTN 50 mg/kg	Group IV GM+CE 250 mg/kg	Group V GM+CE 500 mg/kg
Sodium (mmol/L)	74.16±1.86	75.17±1.86^{ns}	71.64±0.33^{ns}	74.31±1.92^{ns}	74.31±1.49 ^{ns}
Potassium (mmol/L)	3.21±0.02	3.21±0.02^{ns}	3.20±0.02^{ns}	3.15±0.01^{ns}	3.21±0.02^{ns}
Calcium (mg/dL)	8.90±0.01	10.89± 0.01^{**a}	6.48±0.02^{***b}	8.03±0.05^{**b}	7.83±0.06^{***b}
Magnesium (mg/dL)	0.77±0.01	0.82±0.07^{**a}	0.74±0.04^{**b}	0.78±0.05^{**b}	0.77±0.06^{**b}

GM: gentamicin, QTN: quercetin, CE: Combination of Plant Extract. Values are expressed in mean ± standard error of mean (n=6), *p<0.05, **p<0.01, ***p<0.001 ^{a} significant compared o control group (group I), ^{b}significant compared with GM-induced group (group II), ^{ns} not significant

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Effect of CE on Hematological parameter in experimental animal

Table demonstrated that hemoglobin (Hb), packed cell volume ($p<0.001$), red blood cells (RBCs) and mean corpuscular hemoglobin (MCH) levels were significantly ($p<0.01$) lowered in rats treated with GM than those in the control group. White blood corpuscles ($p<0.01$) and polymorphs ($p<0.001$) were found to be significantly elevated in rats in group II, compared to animals in group I. Results revealed that the animals co- treated with standard/extract caused a significant ($p<0.01$) increase in RBCs, haemoglobin and MCH level. Packed cell volume (PCV) were found to be increased only in animals treated with quercetin/high dose of CE ($p<0.01$). Total WBC and polymorphs were found to be significantly reduced in animals co-treated with standard/ extract ($p<0.01$)

Table 15: Effect of CE on Hematological parameter in GM-induced nephrotoxicity

Parameters studied (Unit)	Group I Normal control	Group II GM 100 mg/kg	Group III GM+QTN 50 mg/kg	Group IV GM+CE 250 mg/kg	Group V GM+CE 500 mg/kg
RBC (million/mm^3)	7.35±0.14	6.81±0.31**a	7.22±0.21**^{b}	6.85±0.31**^{b}	7.10±0.43**^{b}
HB (g/dL)	14.11± 0.28	11.76± 0.58***^{a}	14.02±0.13**^{b}	13.96±0.21**^{b}	13.83±0.31**^{b}
PCV (%)	43.66±0.52	38.27±0.81***a	39.58±0.61**^{b}	38.29±0.42^{ns}	38.53±0.20**^{b}
MCH (pg)	20.67±0.12	20.19±0.69**a	20.84±0.27**^{b}	20.45±0.49**^{b}	20.67±0.51**^{b}
WBC (1X10^3/mm^3)	8.22± 0.41	8.43±0.33**a	8.21±0.41**^{b}	8.24±0.21**^{b}	8.15±0.10**^{b}
Lymphocytes (%)	60.05± 0.01	59.96 ±0.08 ^{ns}	59.97±0.01 ^{ns}	59.96±0.02^{ns}	59. 96±0.05 ^{ns}
Monocytes (%)	4.97±0.00	5.00±0. 01 ^{ns}	4.96±0.01 ^{ns}	4.97± 0.06 ^{ns}	4.96±0.06 ^{ns}
Polymorphs (%)	12.22±0.21	15.84 ±0.11***^{a}	14.65±0.32**^{b}	14.19±0.20**^{b}	14.05±0.11**^{b}
Eosinophils (%)	1.93±0.01	2.02±0.01 ^{ns}	1.97±0.08 ^{ns}	1.97±0.02 ^{ns}	1.96±0.01 ^{ns}

GM: gentamicin, QTN: quercetin, CE: Combination of Plant Extract. Values are expressed in mean ± standard error of mean (n=6), *$p<0.05$, **$p<0.01$, ***$p<0.001$, ^{a}significant compared with control group, ^{b}significant compared with GM-induced group, ^{ns}not significant

Effect of CE on Serum total protein and albumin in experimental animal

Serum total protein and albumin level ($p<0.05$), were found to be significantly decreased in GM-treated animals, compared to rats in group I. However, significant differences ($p>0.05$), were not observed on *p.o.* administration of 250 and 500 mg/kg of CE in group IV & V, whereas rats in group III showed significant change in total protein ($p<0.05$) and albumin ($p<0.01$) level, compared to the animals in group II

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Table 15: Effect of CE on Serum total protein and albumin **in GM-induced nephrotoxicity**

Serum parameters (Unit)	Group I Normal Control	Group II GM 100 mg/kg	Group III GM+QTN 50 mg/kg	Group IV GM+CE 250 mg/kg	Group V GM+CE 500 mg/kg
Total protein (g/dL)	7.17±0.01	7.13±0.05^{*a}	7.17±0. 09^{*b}	7.15±0.09^{ns}	7.17±0.07^{ns}
Albumin (g/dL)	4.34±0.00	4.31±0.04^{*a}	4.35±0.00^{**b}	4.32±0.00^{ns}	4.34±0.03^{ns}

GM: gentamicin, QTN: quercetin, CE: Combination of Plant Extract. Values are expressed in mean ± standard error of mean (n=6), *p<0.05, **p<0.01, ***p<0.001, ^{a}significant compared with control group, ^{b}significant compared with GM-induced group, ^{ns}not significant

Effect of CE on Serum electrolyte in experimental animal

Sodium ,magnesium (p<0.01) and calcium (p<0.001) levels were found to be significantly decreased in GM-treated animals, compared to group 1 animals. Co- administration of standard/ extract significantly attenuated (p<0.01) changes in calcium and magnesium levels, however, no significant differences (p>0.05) were observed in sodium and potassium levels, compared to group II rats

Table : Effect of CE on Serum electrolyte in experimental animal in GM-induced nephrotoxicity

Serum parameters (Unit)	Group I Normal Control	Group II GM 100 mg/kg	Group III GM+QTN 50 mg/kg	Group IV GM+CE 250 mg/kg	Group V GM+CE 500 mg/kg
Sodium (mmol/L)	137.8±0.07	137.3±0.05^{**a}	137.6±0.15^{ns}	137.4±0.11^{ns}	137.6±0.14^{ns}
Potassium(mmol/L)	5.75±0.00	5.75±0.07	5.73±0.07^{ns}	5.76±0.01^{ns}	5.78±0.01^{ns}
Calcium (mg/dL)	10.60±0.13	8.17±0.00^{***a}	9.04±0.00^{**b}	9.25±0.08^{**b}	9.43±0.06^{**b}
Magnesium(mg/dL)	2.44±0.01	2.23±0.02^{**a}	2.54±0.01^{**b}	2.34±0.09^{**b}	2.44±0.01^{**b}

Effect of CE on Serum BUN, creatinine and uric acid level in experimental animal

Group II animals treated with GM demonstrated a significant (p<0.001) elevation in the serum levels of creatinine, uric acid and blood urea nitrogen (BUN). Co-treatment with quercetin significantly (p<0.001) decreased creatinine, uric acid and BUN, compared to group II rats. Animals co-administered with extract significantly (p<0.01) decreased creatinine, and uric acid. BUN levels were significantly lowered in animals co- treated with extract at low concentration (p<0.01) and high concentration (P<0.001), compared to toxic control group.

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Table :Effect of CE on Serum BUN, creatinine and uric acid level in GM-induced nephrotoxicity

Serum Parameters (Unit)	Group I Normal control	Group II GM 100 mg/kg	Group III GM+QTN 50 mg/kg	Group IV GM+CE 250 mg/kg	Group V GM+CE 500 mg/kg
BUN (mg/dL)	15.83±0.19	28.89±0.02^{***a}	12.13±0.02^{***b}	18.92±0.01^{**b}	14.74±0.04^{***b}
Creatinine (mg/dL)	0.68±0.00	1.98±0.02^{***a}	0.81±0.00^{***b}	1.51±0.01^{**b}	0.94±0.01^{**b}
Uric acid (mg/dL)	2.13±0.08	3.22±0.01^{***a}	2.03±0.01^{***b}	3.04±0.08^{**b}	2.95±0.05^{**b}

: gentamicin, QTN: quercetin, CE: Combination of Plant Extract, BUN : blood urea nitrogen. Values are expressed in mean ± standard error of mean (n=6), *p<0.05, **p<0.01, ***p<0.001, ^{a}significant compared with control group, ^{b}significant compared with GM-induced group, ^{ns}not significant

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