International Journal of Scientific Research and Engineering Development-– Volume 5 Issue 2, Mar-Apr 2022

Available at www.ijsred.com

RESEARCH ARTICLE OPEN ACCESS

Nephroprotective Effect of Polyherbal Extract Containing Punica granatum and Clerodendrum Inerme Leaves Against Paracetamol Induced Renal Damage in Rats

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ABSTRACT

Various ethnomedicinal plants from customary arrangement of medication viz Ayurveda and Unani, which are acclaimed by the Ayurvedic and Unani doctors to have nephroprotective properties and ordinarily being utilized to treat the different renal problem have broadly researched for their nephroprotective impact which showed huge outcomes in different examinations. *Punica granatum*and*Clerodendrum inerme* Leaves have been usually used in Heart diseases, Stomach disorders, Dental care, cancer, osteoarthritis, diabetes, Anemia, liver ailments and kidney disorders. The current research was designed to explore the Nephroprotectiveactivity of a Polyherbal extract containing *Punica granatum*and*Clerodendrum inerme* leaves employing Paracetamol induced Nephrotoxic models.Rats were isolated into five sets each group comprising of six rats. Methanolic extract of *Punica granatum* leaves (MEPGL), aqueous extract of *Clerodendrum inerme*leaves (AECIL) and Polyherbal extract (MEPGL+AECIL)were prepared and Nephroprotective activity was determined against Paracetamol intoxicated rats.Blood urea level was increased in the Paracetamoltreated group. Polyherbal extract, (400 mg/kg, p.o)restored the elevated level of BUN. Serum creatinine level was also increased in the Paracetamoltreated group.Polyherbal extract, (400 mg/kg, p.o) of the leaves reduced the elevated level of Creatinine to near normal producing significant Nephroprotective activity than the individual extracts. A histopathological examination of kidney fractions has further augmented the biochemical evidences of nephroprotective function of the *Punica granatum*and*Clerodendrum inerme* Leaf extract by preserving the renal architecture of the kidney tissue to near normal. However further research is essential to find out the exact mechanism responsible for the synergistic nephroprotective effect of the Polyherbal extract.

Key words: Punica granatumleaves, Clerodendrum inerme leaves, Polyherbal extract,Paracetamol , nephroprotective activity, Blood urea nitrogen, Serum creatinine.

INTRODUCTION

Studies reveal that synthetic nephroprotective agents have adverse effect besides reducenephrotoxicity, various environmental toxicant and clinically usefuldrugs,acetaminophenandgentamicin,can causes evereorgantoxicities through the metabolic activation to highly reactive free radical[1].Right from its beginning, the documentation of tradition alknowledge, especially medicinal uses of plants, has provided many important drugs of modern day. Several herbal drugs act as good non-specific cyto protective. Plant and plant products have been utilized with varying success to cure and prevent diseases throughout the world[2]. The rapeutically important drugs can be developed from plant sources which are used intraditional systems of medicines. Indian traditional system of medicine is based on the empirical knowledge of observations and the experience and more than 5000 plants are used by different ethnic communities in India[3]. However,despite recognition of drug induced nephrotoxicity and concerted scientific efforts directed into developing therapeutic orprophylactic agents to induce protection against chemically and drug induced nephrotoxicity, conventional chemo the rapeuticoptions available to either treat or prevent its development, are still limited.In the absence of reliable and effective modern nephroprotective drugs and available traditional medicines employed for the diseasetreatment,concertedeffortsarecurrentlychanneledtowardsexploringcomplementaryormeasuretotre atorpreventthe disease. Nephroprotectives are the substances which have defensive movement against nephrotoxicity. Therapeutic plants have healing properties because of the presence of different active constituents in them[4]. Ancient literature has illustrated various herbs for the cure of kidney disease[5]. Organization of different therapeutic plants having nephroprotective movement alongside various nephrotoxic agents may lessen its harmfulness.[6]Free radicals use oxygen and are consolidated in the mitochondria of mammalian cells and abilities to begin the biochemical responses[7]. Ordinary cell assimilation yields nitrogen species and responsive oxygen (RNS and ROS) which cytotoxically affect microorganisms and other unsafe animals. In any case a couple of proteins are accessible that produce ROS/RNS, for instance, NADPH oxidases, NOS and myeloperoxidases. In this way an equibrium is essential to be kept up between positive for oxidants and malignant growth anticipation specialists in cells for their common working. However, by and large there is high creation of favorable to oxidants in cells prompting oxidative harm. Oxidative harm is the chief figure included numerous illnesses including kidney sicknesses [8].

Cancer prevention agent's acts by ending free radicals and their intermediates that causes oxidation by starting a succession of responses that at long last causes cell harm[9]. Antioxidants are lipophilic (Retinoids, flavonoids, tocopherol, ubiquinols and carotenoids) and furthermore water solvent (ascorbic corrosive and urate) substances. These demonstration by extinguishing the oxidative interaction and there by decline the centralizations of hurtful free radicals[10].The leaves of *Punica granatum*plant is accounted for to have various helpful properties, for example, mitigating cardiovascular diseases, anti-anemic,antidiabetic, antipyretic and pain relieving, antifungal, antimicrobial, antibacterial and antiparasitic, against malignant growth and Nephroprotective activity.[11] The leaves of the *Clerodendrum inerme*plant is accounted for to have antidiabetic, antiarthritic, expectorant, emetic, hepatoprotective, Nephroprotective, temperament raising and abortificiant impacts.[12]

Antioxidants acts by terminating free radicals and their intermediates that causes oxidation by initiating a sequence of reactions that finally causes cellular damage[13].Antioxidants are lipophilic (Retinoids, flavonoids, tocopherol, ubiquinols and carotenoids) and also water soluble (ascorbic acid and urate) substances. These act by quenching the oxidative process and there by decrease the concentrations of harmful free radicals [14]. Research study on herbal drug shows that they have multidimensional mechanism of action against kidney diseases and are also safer than the allopathic drug.However there is no existing pharmacological data that indicate the usefulness of Polyherbal extract of *Punica*

*granatum*and*Clerodendrum inerme* Leavesagainst hepatic damage. Therefore, this investigation was carried out to evaluate the nephro-protective action of Polyherbal extract of *Punica granatum* and Clerodendrum inerme Leaves and comparison of nephro-protective efficacy of Polyherbal extract with individual extracts.

MATERIALS AND METHODS

Assortment and Confirmation of Plant Material

For the current assesment, the Punica granatum andClerodendrum inerme Leaveswere gathered from the region of the Meerpet, Saroornagar, Hyderabad. Sample specimens of Punica granatum and Clerodendrum inerme Leaves were kept in a polythene sack. The specimens were kept in new condition by adding 2% formalin. The scientific name of the plant has been checked with http://www.theplantlist.org on fifteenth, March, 2020. Plant materials were recognized and verified by Senior Scientist (Eco Botany), Dr. N. Sivaraj, NBPGR, Hyderabad.

Extraction

The Punica granatum and Clerodendrum inerme Leaves were dried in shade light independently and precisely pulverised to a coarse powder. The coarse powders weights of Punica granatum and Clerodendrum inerme Leaves were found to be 1365 g and 1438 g. The powders were presented to hot constant progressive extraction in a Soxhlet extraction with solvents in the increasing order of polarity utilizing petroleum ether, ethyl acetic acetate, acetone, methanol and water under controlled temperature (50-60 °C).The extracts thus obtained were concentrated in vacuum rotary evaporator and extracts were kept in dessicator for further utilization.

Preparation of Polyherbal extract (PHE)

Preparation of Polyherbal extract of Punica granatum andClerodendrum inerme Leaves was done by mixing the two extracts in equal ratios of 1:1.

Phytochemical investigation

Phytochemical subjective investigation was performed by oppressing the crude extracts for recognizable proof tests to distinguish the presence of flavonoids, glycosides, alkaloids, sugars, fixed oils, tannoids, phytosterols, proteins, aminoacids, lignins, phenolic mixes, saponins, gums and mucilages.[15]

Methanolic extract of *Punica granatum* leaves (MEPGL) & aqueous extract of *Clerodendrum inerme*leaves (AECIL) were found to possess significant number of active constituents and are selected for antioxidant activity. The PHE was prepared by mixing the extracts of *Punica granatum* and *Clerodendrum inerme*leaves in equal ratio (1:1).[16]

Animals

Albino Rats (180-200 g) and Mice (20-25 g) were acquired from Sainath agencies, Musheerabad, Hyderabad (282/99/CPCSEA) and housed in creature facility of the organization. After haphazardly isolating the animals in to various sets, the rodents were acclimated for a time of one month before commencement of test. Rats were confined in polypropylene cages and protected under standard natural conditions, for example, temperature (26 ± 2ºc), relative stickiness (45-55%) and 12hr dull/light

cycle. The rats were taken care of with rodent pellet diet (Golden Mohur Lipton India Ltd.) and water *ad libitum*.The study protocol was approved from the institutional animal ethical committee with no: 1447/PO/Re/S/11/CPCSEA-36/A.

Single dose oral intense toxicity for multi week with net behavioral study

The Acute toxicity assessment of MEPGL and AECIL were performed based on OECD Guidelines No. 423 by utilizing mice and fixed dose studies were chosen where the limit dosage is 2000 mg/kg.

Experimental methods

Evaluation of nephroprotective activity in Paracetamol induced nephrotoxicity[17, 18]:

Procedure:

In the dose response experiment, albino rats were randomly assigned into 5 groups of 6 individuals each.

Group-I: Animals (-ve control) were administered normal saline 1ml/kg p.o., for 7 days

Group-II: Animals (+ve control) were administered normal saline 1ml/kg p.o., for 7 days

Group-III: Animals were administered with MEPGL 400 mg/kg p.o., for 7 days.

Group-IV: Animals were administered with AECIL400 mg/kg p.o., for 7 days.

Group V: Animals were administered with PHE (MEPGL+AECIL)400 mg/kg p.o., for 7 days

On 5th day, 30 min after the administration of normal saline, MEPGL 400 mg/kg, AECIL 400 mg/kg and PHE 400mg/kg to Group-II, III, IV& V respectively, Paracetamol 2g/kg was given orally. After 48 hours of Paracetamol feeding, rats were sacrificed under mild ether anesthesia. Blood samples were collected for evaluating the serum biochemical parameters. The kidney was dissected out, blotted off blood and washed with saline to estimate the tissue GSH level and LPO status and stored in 10% formalin and proceeded for histopathology to evaluate the details of renal architecture in each group microscopically. The blood so collected was centrifuged immediately to get clear serum and was subjected to various biochemical studies.

Parameter Assessed for the Renal Functions:

Blood urea[19,20,21]:

Principle:

The estimation of Urea in serum involves the following enzyme catalyzed reactions:

Urea + H_2O $2NH_3 + CO_2$

NH3 + α - KG + NADH Glutamate + NAD

α - KG : α - Ketoglutarate

GLDH : Glutamate dehydrogenase

The rate of decrease in absorbance is monitored at 340 nm and is directly proportional to urea concentration in the sample.

Serum creatinine[22]:

Principle:

Creatinine in alkaline solution reacts with picrate to form a coloured complex which absorbs at 500-520 nm. The amount of complex formed is directly proportional to the creatinine concentration.

Histopathological examination:

Two animals from each group were sacrificed on the day of blood withdrawal and kidneys were isolated. It was washed with saline and preserved in 10% formaldehyde solution. The kidneys were processed and embedded in paraffin wax. The sections were stained with Hematoxylin and Eosin and observed under light microscope[23].

Statistical analysis

Entire values were declared as Mean± SEM for all the trial models.

Findings were deciphered by following assay:

i. Analysis of variance test (one way)continued by Dunnett's test.
ii. The findings were considered to be statistically remarkable when $p<0.05$

RESULTS AND DISCUSSION

Phytochemical investigations

Preliminary phytochemical screening of MEPGL and AECIL indicates the presence of lignins, flavonoids, alkaloids, tannins, glycosides, fixed oils, fats, carbohydrates and saponins. Methanolic extract of *Punica granatum*leaves and aqueous extract of *Clerodendrum inerme*leaves were selected for *in vivo* Nephroprotective activity. Flavonoids are detected more in methanolic extract of *Punica granatum*leaves and flavonoids are reported to have Nephroprotective properties. Saponins are detected with greater clarity in aqueous extract of *Clerodendrum inerme*leaves and saponins are accounted for to be answerable for antioxidant and nephroprotective activity.

Preparation of Polyherbal extract of *Punica granatum* and *Clerodendrum inerme*Leaves

Both the leaf extracts were combined in equal ratios (1:1) i.e 10 gms of *Punica granatum*leaf extract with 10 gms of *Clerodendrum inerme*leaf extract.

Acute toxicity studies of MEPNL and AESMF

The methanolic extract of *Punica granatum*leaves (MEPGL) and aqueous extract of *Clerodendrum inerme*leaves (AECIL) were administered to mice at dosages 5, 50, 300 and 2000 mg/kg with oral needle didn't show any side effects of poisonousness. The rodents were analyzed for about fourteen days, twice in a day has not shown harmful signs. Thus oral LD50 of MEPGL and AECIL was finished to outperform 2000 mg/kg. Therefore 2000 mg/kg was viewed as most secure higher portion for methanolic extract of *Punica granatum*leaves and aqueous extract of *Clerodendrum inerme*leavesand one fifth of 2000 mg/kg i.e 400 mg/kg (higher portion) of MEPGL, AECIL and Polyherbal extract (MEPGL+AECIL) were preferred for the further Nephroprotective action.

Effects of Methanolic extract of *Punica granatum*leaves (MEPGL), aqueous extract of *Clerodendrum inerme*leaves(AECIL) and Polyherbal extract (MEPGL+AECIL)against Paracetamol induced Nephrotoxicity in rats

Blood urea nitrogen level was increased in the Paracetamoltreated group to 48.33 mg/dl. 400 mg/kg Polyherbal extract of the leaves restored the elevated level to 24.32 mg/dl displaying significant Nephroprotective activity than individual extracts.(BUN with MEPGL=36.75 mg/dl, AECIL=29.03 mg/dl).

Serum creatinine level was also increased in the Paracetamoltreated group to 1.82 mg/dl. However 400 mg/kg Polyherbal extract of the leaves reduced the elevated level to 0.59 mg/dl which was more significant than the individual leaf extracts (Serum Creatinine with MEPGL= 1.23 mg/dl, AECIL=0.79 mg/dl).

The results are shown in the table no.1 and figure No. 1

Histopathological Studies in Paracetamol induced Nephrotoxicity:

Group A: Normal control (-ve control) showed structure of kidney with normal glomeruli, proximal and distal tubules and with normal interstitium and blood vessels.
Suggestive: **Normal kidney**

Group B: Paracetamol treated group (+ve control) showed structure of kidney with glomerular congestion. Interstitium showed infilteration with inflammatory cells, tubular necrosis, peritubular necrosis and presence of casts.
Suggestive: **Massive total necrosis**

Group C: Treatment done with 400 mg/kg of Methanolic extract of *Punica granatum* leavesshowed structure of kidney with normal glomeruli, proximal and distal tubles with interstitium showing scant lymphocytic infiltration.
Suggestive: **Interstitial Nephritis**

Group D: Treatment done with 400 mg/kg of aqueous extract of *Clerodendrum inerme* leavesshowed structure of kidney with normal glomeruli, proximal and distal tubles with interstitium showing scant lymphocytic infiltration.
Suggestive: **Interstitial Nephritis**

Group E: Treatment done with 40 mg/kg Polyherbal extract showed structure of kidney with normal glomeruli, proximal and distal tubules with interstitium showing few lymphocytes.
Suggestive: **Residual Interstitial Nephritis.**

Table No.1: Effect of MEPGL, AECIL and Polyherbal extract (MEPGL+AECIL) in Paracetamol induced renal damage in rats

Gr. (n=6)	**Treatment regimen**	**Blood urea Nitrogen (mg/dl)**	**Serum creatinine (mg/dl)**
I	Negative Control (1ml vehicle)	21.40 ± 0.384	0.54 ± 0.010
II	Positive Control Paracetamol (2 g/kg p.o.)	48.33 ± 1.078	1.82 ± 0.053
III	Paracetamol +MEPGL (2 g/kg p.o. +400 mg/kg p.o.)	36.75 ± 0.669*	1.23 ± 0.027*

IV	Paracetamol + AECIL (2 g/kg p.o. +400 mg/kg p.o.)	29.03 ± 0.887**	0.79 ± 0.017**
V	Paracetamol + PHE (MEPGL+AECIL) (2 g/kg p.o. +400 mg/kg p.o.)	24.32± 0.753***	0.59± 0.325***

Findings are represented as mean ± SEM (n=6), ANOVA (one way) continued by Dunnett test, *P<0.05, **P<0.01, ***P<0.001 as equated to control.

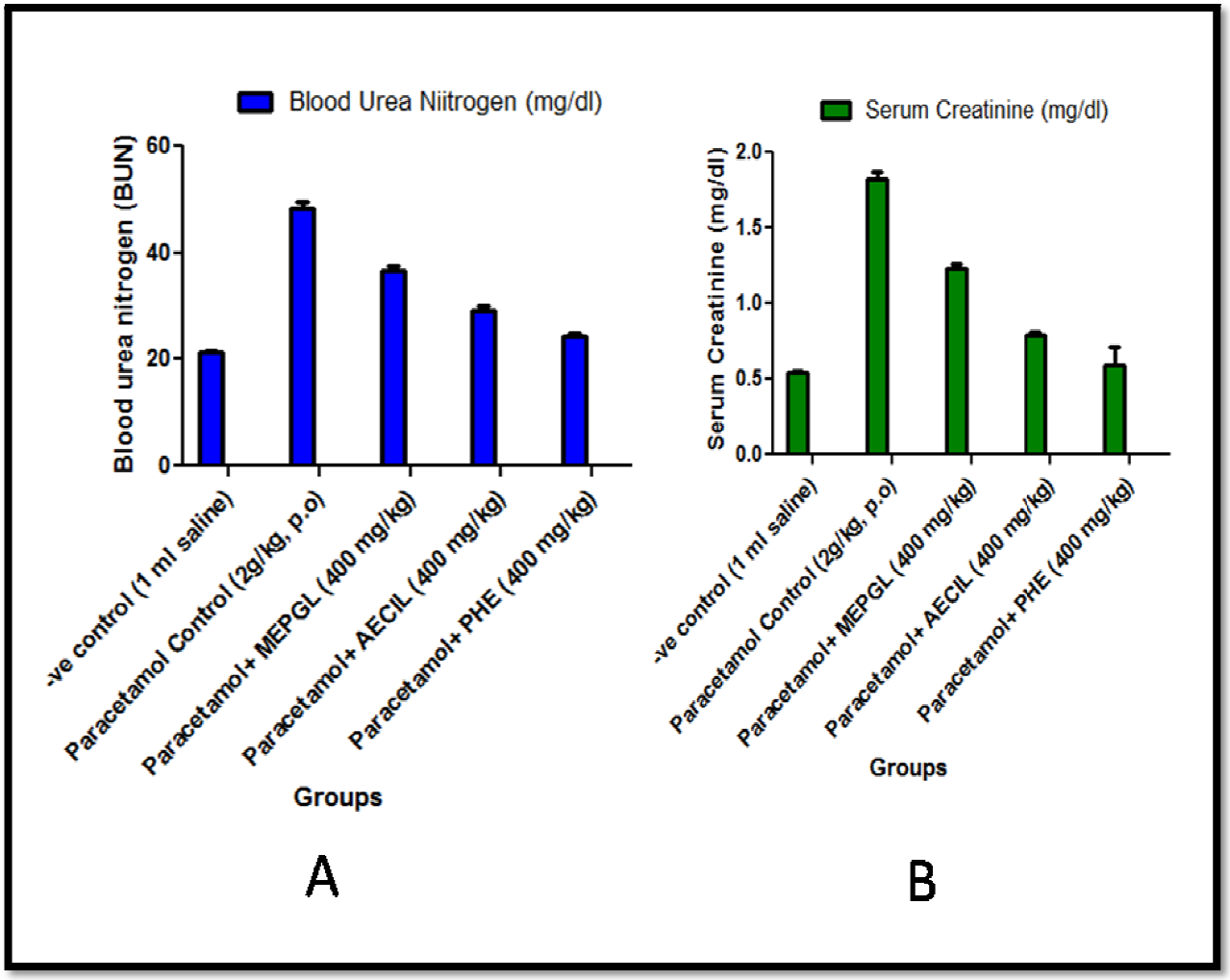

Figure No. 1: Effect of MEPGL, AECIL and Polyherbal extract (MEPGL+AECIL)on A. Blood urea Nitrogen B. Serum Creatinine in Paracetamol induced Nephrotoxicity.

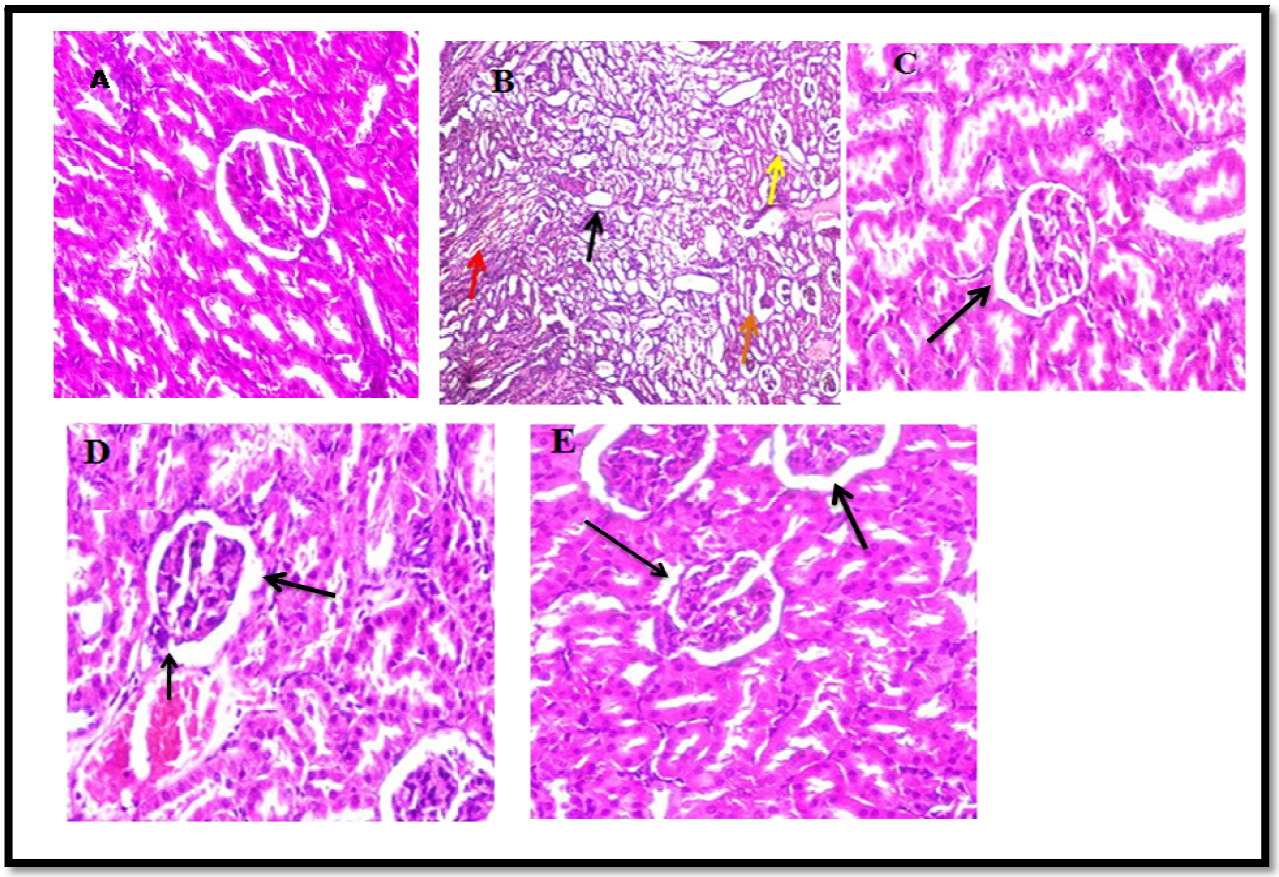

Figure No. 2: Histopathological photographs showing the effect of MEPGL, AECIL and Polyherbal extract (MEPGL+AECIL) on renal architecture in Paracetamol induced Nephrotoxicity. a) Negative control b) Paracetamol control c) MEPGL 400 mg/kg d) AECIL 400 mg/kg e) Polyherbal extract 400 mg/kg.

CONCLUSION

Phytochemical screening of methanolic extract of *Punica granatum* leaves and aqueous extract of *Clerodendrum inerme*leaves has confirmed the presence of flavonoids, Saponins, tannins, alkaloids, glycosides and lignans. With acute toxicity studies of MEPGL & AECIL on rats, therapeutically safest dose is 400 mg/kg. Further Nephroprotective activity reveals that the Polyherbal extract of *Punica granatum* leaves and aqueous *Clerodendrum inerme*leaves was found to be more effective in reversing the interstitial nephritis induced by Paracetamol when compared to individual extracts. Hence it can be hypothesized that the above mentioned chemical constituents in the leaves of the plants may have synergistic Nephroprotective potentials. However further studies are essential to ascertain the exact mode of Nephroprotective action of the polyherbal extract.

Acknowledgements

The authors are also thankful to Senior Scientist (Eco Botany), Dr. N. Sivaraj, NBPGR, Rajendranagar, Hyderabad for kind co-operation in authentication of plants. We would like to thanks to Institutional

Animal Ethics Committee of Pharmacy College for providing ethical clearance of *in vivo* study for the present research.

SOURCE OF FUNDING

No Funding

CONFLICT OF INTERESTS

This is to inform that the authors declare that they have no conflict of interest with respect to this article.

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