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Research Article

Nephroprotective and Anti-Inflammatory Activity of Combination of Punica Granatum (Fruit Peel) And Tectona Grandis (Bark)

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ABSTRACT

Herbal medication in addition referred to as herbal treatment or phytoconstuients refers to employ a plant's seed, berries, roots, leaves, bark or flowers for healthful functions. The procedure of herbs to treat a variety of different ailments is universal and exists in every human culture on Earth. The plants Punica granatum and Tectona grandis is important medicinally and claimed to be helpful in the treatment of different ailments like diabetes, inflammations, eruptions and bites of poisonous animals etc., and contains different classes of compounds of pharmacological importance such as, alkaloids, glycosides, saponins, flavonoids and triterpines and have been screen biologically for Nephroprotective and anti-inflammatory actions.

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INTRODUCTION

Herbal medication in addition referred to as herbal treatment or phytomedicine refers to, berries, roots, leaves, bark or flowers for healthful functions[1-5]. Herbalist contains a long tradition of use outside of typical medication. Ancient Chinese and Egyptian papyrus writings describe healthy use for plants at the same as early as 3000 BC[3]. Native cultures (such as African and Native American) use herbs in their heal rituals, whereas others developed ancient healthy systems (such as written material and ancient Chinese medicine) during which seasoned therapy were used. Researchers start that folks in numerous elements of the globe attend uses of similar plants for a similar purpose[6-9]. The procedure of herbs to treat a variety of different ailments is universal and exists in every human culture on Earth. Despite this, the major use of medical herbs still occurs in societies which are not fully developed Because of the high costs involved with industrialized modern medicines, many people living in increasing nations simply do not have the financial resources to give for them, and as a result, they are enforced to use normal herbs as an affordable alternative.[10-14].

Fig.No.1 Herbs

The plants Punica granatum and Tectona grandis is important medicinally and claimed to be helpful in the treatment of different ailments like diabetes, inflammations, eruptions and bites of poisonous animals etc., and contains different classes of compounds of pharmacological importance such as, alkaloids, glycosides, saponins, flavonoids and triterpines and have been screen biologically for Nephroprotective and anti-inflammatory actions[15-18].

Fig. No.: 2 Fruit peel of *Punica granatum*

Fig. No.: 3 Tectona grandis plant

METERIALS AND METHOD

Table No: 1 List of materials and equipment's used during experiment.

Sr. No	Name of the materials and equipment
1	Anesthetic ether (Sigma Solvents and Pharmaceuticals, Mumbai)
2	Formalin (Hi-Media Laboratory, Mumbai.)
3	Ethanol (Nice-Cochin)
4	Carrageenin (Nice-Cochin)
5	Diclofenac sodium (Cipla, Mumbai)
6	Distilled water.
7	Evans blue (S.D. Fine chemicals, Mumbai)
8	Lead Mercury (S.D. Fine chemicals, Mumbai)
9	Restraint cages (Shri. Venkateswara Enterprises, Bangalore)
10	Chloroform (Nice-Cochin)
11	Refrigerator (Whirlpool-Kelvinator India Ltd, India)

Collection and authentification of plant materials:

The stem of *Punica granatum and Tectona grandis* were collected from limited area and authentified by botanist Dr. Narasingham BSI.

Preparation of special extracts (petrolium ether, chloroform, ethanolic and aqueous extracts):

The fruit peel of *Punica granatum* and bark of *Tectona grandis* were dried in shade at room high temperature then Subjected to size decrease to a fine powder with the help of mixer grinder. Then the powder was extract with petroleum ether, chloroform, ethanol and water consecutively by simple extraction. The extract was move into the before weighed clear beaker and evaporate to a thick paste on the water bath, maintained at 50ºC to get extracts. The extract be lastly air dry thoroughly to remove all traces of the solvent and the percentage yield was planned each time before extracting with next solvent, marc will be dried in hot air oven below 50ºC and each extract will be concentrated by distilling off the solvent and then evaporated to dryness on a water bath to get the extracts.

Pharmacological evaluation:

Depending upon the occurrence of phytoconstituent in the different extracts, the ethanolic and aqueous extracts are selected for the following pharmacological actions.

- Anti-inflammatory activity
- Nephroprotective activity

Anti-Inflammatory Activity By Carrageenan Induced Paw Oedema[60, 61,74]

Group A: Control (Carrageenin 1%)
Group B: Standard (Diclofenac 30 mg/ kg)
Group C: EEPG+EETG (200 mg/kg p.o)
Group D: EEPG+EETG (400 mg/kg p.o)
Group E: AEPG+AETG (200 mg/kg p.o)
Group F: AEPG+AETG (400 mg/kg p.o)

Experimental Procedure:

Albino rats (150-200 g) be separated into 6 groups each containing 6 animals they be fast overnight prior to and through the test but include free access to water. Group A be served as toxicant control treated among toxicant Carrageenan, group B with Diclofenac (40 mg/kg p.o.) that serve because normal Groups C, D, E and F be administer with EEPG, EETG and AEPG, AETG (200 mg/kg and 400 mg/kg dose p.o) respectively. The rats of groups B, C, D, E and F be administer with 1% of Carrageenin into sub plantar part of right hind paw of rats 1h after administration of Diclofenac/extracts. directly here later than the oedema volumes of the injected paws be considered plethysmographically at prefixed time interval. The variation among paw volume of the treat animals is considered and the mean oedema quantity be calculated19-28. Percentage decrease in oedema volume was calculated by use the formula.

$$\text{Percentage reduction} = \frac{Vo - Vt}{Vo} \times 100$$

Where,

Vo = Volume of the paw of control at time 't'.

Vt = Volume of the paw of drug treated at time 't'.

Statistical analysis:

All consequences will be expressed as mean ± SEM from 6 animals. Statistical variation in mean will be analyze use one-way ANOVA (analysis of variance) follow by position hoc test (Dunnett's't' test). P< 0.05*, 0.01** and 0.001*** will be measured as statistically important[29-37].

Nephroprotective activity by Gentamicin Induced Nephrotoxicity in Rats[47, 52]

The evaluation of the ethanolic extract for nephroprotective action be done according to the process known in the literature with minor modification.

Total 36 animals are in use and 6 rats were selected in each of the follow groups:
Group I: Control group
Group II: Gentamicin control group (60mg/kg)
Group III: Gentamicin + Ethanolic extract (400mg/kg)
Group IV: Gentamicin + ethanolic extract (200mg/kg)
Group V: Gentamicin + Aqueous extract (400mg/kg)
Group IV: Gentamicin + Aqueous extract (200mg/kg).

Activity profile of the test formulations in gentamicin induced changes in different parameters:

Serum uric acid is the ending product of purine catabolism. So, any fault in the glomerular filtration rate causes the rise into the stage of uric acid in the blood. The raise later than gentamicin can be credited to the GFR impairment. The exchange of the elevation by any substance may be analytic of the exchange of the GFR impairment53-59.

RESULTS AND DISCUSSION

The nature and percentage yield of different extract be given below:

Table No.: 2 Nature and Percentage yield of the extracts

Sr. No.	Name of the Extract	Nature	Colour	%Yield (w/w) g
1.	Pet.ether	Sticky	Dark green	2.00
2.	Chloroform	Sticky	Dark green	6.50
3	Ethanol	Sticky	Dark green	9.50
4	Aqueous	Sticky	Dark brown	11.00

Preliminary phytochemical screening:

EEPG+EETG and AEPG+AETG were subjected for phytochemical selection and start to include tannins, sterols, flavonoids, glycosides, and alkaloids triterpines in ethanolic and aqueous extracts. The phytochemical constituents of different extract of Punica granatum and Tectona grandis are shown below:

Table No.:3. Phytochemical evaluation of combined extracts of *Punica granatum* and *Tectona grandis*

Sr. No.	Tests	Petroleu mether	Chloro form	Etha nol	Water
01	Alkaloids	-ve	-ve	+ve	+ve
02	Carbohydrates	-ve	-ve	+ve	+ve
03	Flavonoids	-ve	-ve	+ve	+ve
04	Saponins	-ve	-ve	+ve	+ve
05	Sterols	+ve	+ve	+ve	+ve
06	Tannins	-ve	-ve	+ve	+ve
07	Glycosides	-ve	-ve	+ve	+ve

Pharmacological activities:

Acute oral toxicity study:

The mice treated with EEPG+EETG and AEPG+AETG at a dose of 200 mg/kg, p.o. exhibited usual behaviour, without any symbols of passivity, stereotypy and vocalization. Their motor movement and secretory symbols were also usual and no sign of depression. EEPG+EETG and AEPG+AETG even up to the dose level of 2000 mg/kg body weight did not create any behavioural symptoms or mortality. So 1/10th and 1/5th doses of (maximum dose tested for each extract) be chosen as medium and high dose and were used in the current study to investigate nephroprotective and anti- inflammatory actions

Anti-inflammatory activity by Carrageenin induced paw oedema model in rats:

The EEPG+EETG and AEPG+AETG with the selected doses i.e. 200 and 400 mg/kg consist of show a important drop in paw oedema volume in

Carrageenin induced paw oedema in rats at different time interval. Results are tabulated in Table No. 4.7. Diclofenac sodium (30 mg/kg) was used as typical situation and it has considerably reduced paw oedema volume by 17.81% at 1st h, 27.58% at 2nd h, 50.09% at 3rd h and74.13 % at 4th h, which was created to be a moment dependent result.

During 1st h of study EEPG+EETG and AEPG+AETG with 200mg/kg and 400 mg/kg doses have extensively reduced oedema volume by 3.43, 10.72, 3.43, 7.72 % respectively noted as time dependent result.

During 2nd h of study EEPG+EETG and AEPG+AETG with 200mg/kg and 400 mg/kg dose have significantly reduced oedema volume by 13.9, 24.28, 15.11, 17.29.

% respectively noted as time reliant result.

During 3rd h of study EEPG+EETG and AEPG+AETG with 200mg/kg and 400 mg/kg doses have considerably reduced oedema volume by 32.07, 34.33, 38.08,

47.46 % correspondingly noted as time dependent result.

During 4th h of study EEPG+EETG and AEPG+AETG with medium and high doses have significantly reduced oedema volume 51.72, 56.89, 55.17, 60.34 % respectively which was record as time dependent effect and result be graphically represent in Fig No.:4.5.

Table No. 4. Anti-inflammatory effect of EEPG+EETG and AEPG+AETG on paw volume in Carrageenin induced paw edema in rats

Groups	Treatment	Paw oedema volume			
		60 min	120 min	180 min	240 min
Control	Carragenin	0.466±0.042	0.4833±0.047	0.533±0.04	0.58±0.030
Standard	Diclofenac sodium (30mg/kg)	0.383±0.030	0.35±0.02236	0.266±0.033	0.15±0.02236
EEPG+EETG	200mg/kg	0.45±0.0428	0.4166±0.047	0.366±0.033	0.2833±0.030
EEPG+EETG	400mg/kg	0.416±0.047	0.366±0.033	0.35±0.022	0.25±0.022
AEPG+AETG	200mg/kg	0.45±0.0428	0.41±0.036	0.33±0.0421	0.26±0.049
AEPG+AETG	400mg/kg	0.43±0.0210	0.4±0.042	0.28±0.030	0.233±0.033

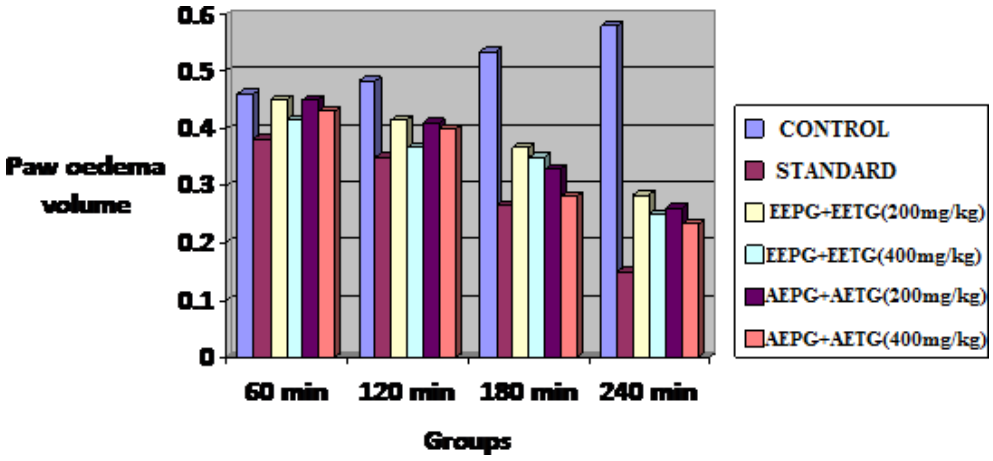

Fig. No. 4 Anti-inflammatory effect of EEPG+EETG and AEPG+AETG on pawvolume in Carrageenin induced paw oedema in rats

Table No. 5. Percentage reduction of paw volume in Carrageenin induced paw oedema

Groups	Treatment	% Reduction in paw volume			
		60 min	120 min	180 min	240 min
Control	Carragenin	-	-	-	-
Standard	Diclofenac sodium (30mg/kg)	17.81	27.58	50.09	74.13
EEPG+EETG	200mg/kg	3.43	13.9	32.07	51.72
EEPG+EETG	400mg/kg	10.72	24.28	34.33	56.89
AEPG+AETG	200mg/kg	3.43	15.11	38.08	55.17
AEPG+AETG	400mg/kg	7.72	17.29	47.46	60.34

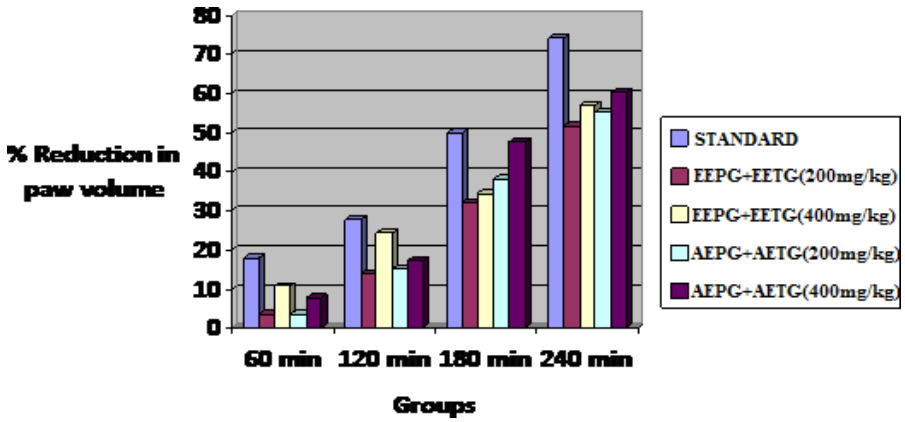

Fig. No. 5 Percentage reduction of Paw oedema volume in different groups

Nephroprotective activity by Gentamicin induced nephrotoxicity in albino rat's model:

Gentamicin like other aminoglycoside antibiotics causes nephrotoxicity by inhibiting protein synthesis in renal cells. This alters the body weight and elevates the usual levels of blood urea and serum creatinine.

The administration of ethanolic and aqueous extracts of combined doses (200mg/kg and 400 mg/kg) of Punica granatum and Tectona grandis has shown a significant reduction in blood urea and serum creatinine levels as shown in the tables below.

Table No: 6 % of Body weight change in gentamicin induced Nephrotoxicity in albino rats.

Groups	% of body weight change
Normal control	3.44 ± 0.190
Toxic control	9.926± 0.448***
EEPG+EETG (200mg/kg)	8.216 ± 0.410***
EEPG+EETG (400mg/kg)	5.776 ± 0.463**
AEPG+AETG (200mg/kg)	6.3± 0.44
AEPG+AETG (400mg/kg)	4.4 ± 0.411

Values are expressed in mean ±SEM where n = 6, Significant at P < 0.05*, 0.01** and 0.001***, compared to control group.

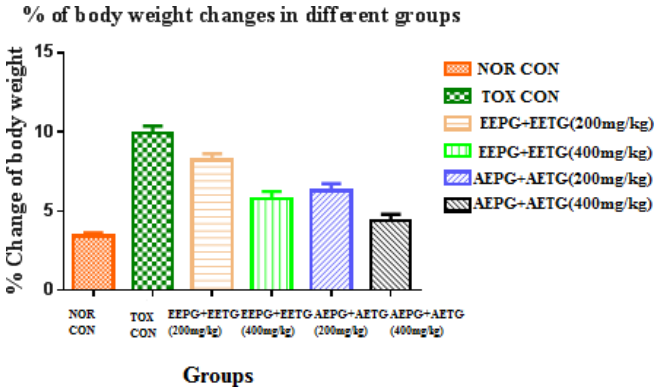

Fig. No: 6 % of Body weight changes in special groups by Gentamycin inducedNephrotoxicity model.

Table No.: 7 Blood urea and serum creatinine levels of different groups by gentamicin induced Nephrotoxicity

Groups	Blood urea	Serum creatinine
Normal control	31.03± 5.018	1.023± 0.053
Toxic control	70.591 ± 4.064***	2.05± 0.081***
EEPG+EETG (200mg/kg)	57.385 ± 11.724**	1.918± 0.07***
EEPG+EETG (400mg/kg)	45.916 ± 11.65*	1.53 ± 0.11***
AEPG+AETG (200mg/kg)	51.948 ± 3.673***	1.668± 0.080***
AEPG+AETG (400mg/kg)	38.046 ± 5.280	1.191 ± 0.053

Values are expressed in mean ±SEM where n = 6, Significant at P < 0.05*, 0.01** and 0.001***, compared to control group.

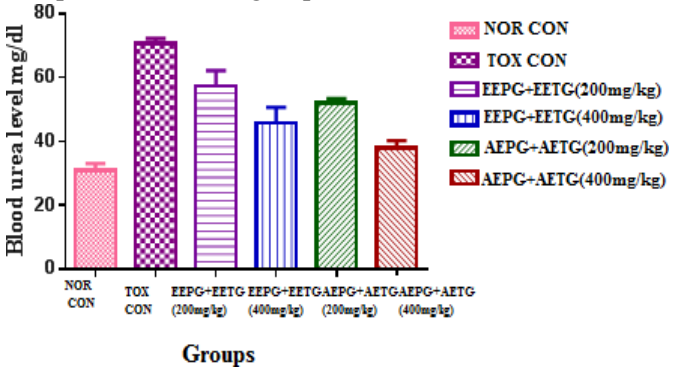

Fig. No:7. Blood urea level of different groups in gentamicin induced Nephrotoxicity model

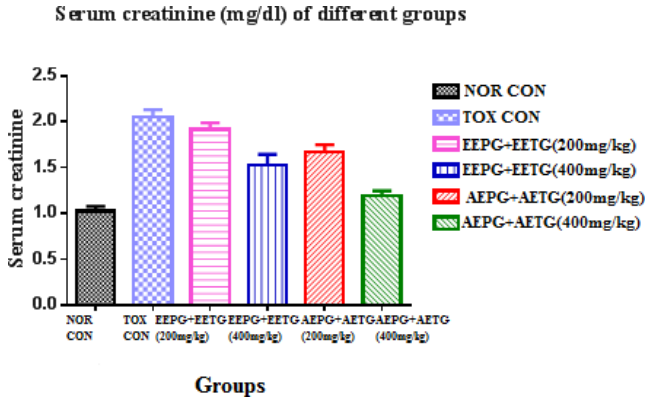

Fig. No: 8. Serum creatinine level of special groups in gentamicin induced Nephrotoxicitymodel

DISCUSSION

In the current study the fruit peel of Punica granatum and bark of Tectona grandis were chosen for the anti-inflammatory activity. It was previously reported that the individuals of the Punica granatum and Tectona grandis have potent anti-inflammatory activity. The preliminary phytochemical research reveals the presence of alkaloids, flavonoids, saponins, tannins and glycosides in both ethanolic and aqueous extracts. Inflammation is a state which is cause by chemical mediators like prostaglandins and cytokinin's. It is report that the phytochemicals like flavonoids, saponins and tannins decrease the inflammation. In the current study the above-mentioned phytochemicals are current in both the extracts this can be recognized for its anti- inflammatory movement.

The fruit peel Punica granatum be reported to contain phenolic compounds like flavonoids, which possess antioxidant property, The fruit peel of Punica granatum were previously report for its nephroprotective movement on diabetes-induced renal damage. Nephrotoxicity is a regular cause of human being which is mainly caused due to oxidative stress. In the current study both the extracts containing polyphenolic compounds have antioxidant property, so the nephroprotective activity of the above-mentioned plants Punica

granatum and Tectona grandis is appropriate to its antioxidant property.

CONCLUSION

In the present study Punica granatum and Tectona grandis both the extracts significantly reduced the nephrotoxicity and inflammation. Punica granatum and Tectona grandis both nephrotoxicity and inflammation may be due to creation of inflammatory mediators by oxidative stress. In the present study is observed that due to the presence of phytoconstituents like alkaloids, flavonoids, saponins and tannins which also decrease the oxidative stress and inflammatory mediators. The anti-inflammatory and nephroprotective activity may be dependable for above phytoconstituents.

Further studies are essential to begin the exact mechanism of phytoconstituents responsible for the above actions.

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CONFLICT OF INTEREST

The authors state that there is NO conflict of interest.

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