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Nephroprotective and Anti-Inflammatory Activity of Stems of *Murraya Koenigii*

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ABSTRACT

The plant Murrraya koenigii, family Rutaceae, commonly known as curry leaf, basically known for its' aroma and medicinal properties. Different extracts like petrolium ether, chloroform, ethanolic and aqueous extracts of murraya koenigii and studied for anti-inflammatory and nephroprotective activities, based on results, concluded that murraya koenigii shows significant anti-inflammatory and nephroprotective activities.

Key Words: *Murraya koenigii, anti-inflammatory activity and nephroprotective activity.*

INTRODUCTION

The plant *Murrraya koenigii,* family *Rutaceae*, commonly known as curry leaf, is a medicinal plant that has been widely used in India as Ayurvedic herbal medicine, basically known for its' aroma and medicinal properties [1]. The part of plant is covered with fine down and has a strong peculiar smell. It is more or less deciduous shrub or tree up to 6m in height and 15-40cm in diameter with short trunk [2]. It is popularly known for the activities like antibacterial, antifungal, anti-diarrheal, anti-inflammatory and cytotoxic activities. Major chemical constituents *Murrraya koenigii are* carbazole alkaloids, identified as mahanimbine, girinimbine, murrayanine murrayafoline-A and one triterpene [3].

Fig. 1: Stems of *Murraya koenigii*

MATERIALS AND METHODS

The stems of *Murraya koengii* were collected from local areas of Hyderabad and Authentified by botanist Dr. Rasingham, BSI, Hyderabad.

Preparation of different extracts (petrolium ether, chloroform, ethanolic and aqueous extracts): The stems of *Murraya koengii* were dried in shade at room temperature then subjected to size reduction to a fine powder with the help of mixer grinder. Then the powder was extracted with petroleum ether, chloroform, ethanol and water successively by simple extraction. The extract was transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50^{0}C to get extracts. The extracts were finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated each time before extracting with next solvent, marc will be dried in hot air oven below 50^{0}C and each extract will be concentrated by distilling off the solvent and then evaporated to dryness on a water bath to get the extracts. These extracts were stored in airtight containers in a refrigerator below 10^{0}C. The extracts were examined for their color and consistency. Their percentage yield was calculated with reference to air-dried powder sample used for the extraction.

Preliminary phytochemical screening: The preliminary phytochemical investigations will be carried out with the petroleum ether, chloroform, ethanolic and aqueous extracts of stems of *Murraya koengii* for qualitative identification of phytoconstituents present with each extract and test were carried out by following standard methods. All the chemicals and reagents used were of analytical grade.

Determination of acute toxicity:
Experimental animals: Albino mice 18-25gm were procured from Shri Venkateswara Enterprises – Bengaluru, for experimental purpose and the animals were acclimatized for 8 days under standard husbandry conditions

Method:
The acute toxicity of stem extracts of *Murraya koengii* was determined in albino mice of either sex weighing between 18-22gm those maintained under standard husbandry conditions. The animals were fasted 3hrs prior to the experiment and "up and down" (OECD Guideline No. 420) were adopted for toxicity studies. Animals were administered with single dose of extracts and observed for its mortality during 48hrs study period (short term) toxicity. Based on the short-term toxicity profile of the extracts the doses of the next animals were determined as per as OECD Guidelines No: 420. All the animals were observed for long term toxicity (14 days).

Pharmacological evaluation: Depending up on the presence of active constituents in the various extracts the ethanolic and aqueous extracts were selected for the following pharmacological activities.

a) Anti inflammatory activity and
b) Nephroprotective activity.

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Anti-inflammatory activity by carrageenan induced paw oedema: [4, 5]

Group A : Control (Carrageenin 1%)
Group B : Standard (Diclofenac 30mg/kg)
Group C : EEMK (200mg/kg *p.o*)
Group D : EEMK (400mg/kg *p.o*)
Group E : AEMK (200mg/kg *p.o*)
Group F : AEMK (400mg/kg *p.o*)

Experimental Procedure: Albino rats (150-200gm) were divided into 6 groups each containing 6 animals they were fasted overnight prior to and during the experiment but have free access to water. Group A was served as toxicant control treated with toxicant Carrageenan; group B with Diclofenac (40mg/kg *p.o.*) that served as standard. Groups C, D, E and F were administered with EEMK and AEMK (200mg/kg and 400mg/kg dose *p.o*) respectively. The rats of groups B, C, D, E and F were administered with 1% of carrageenan into sub plantar region of right hind paw of rats 1hr after administration of diclofenac/extracts. Immediately thereafter the oedema volumes of the injected paws were measured plethysmographically at prefixed time intervals. The difference between paw volumes of the treated animals was measured and the mean oedema volume was calculated. Percentage reduction in oedema volume was calculated by using 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 results will be expressed as mean ± SEM from 6 animals. Statistical difference in mean will be analyzed using one-way ANOVA (analysis of variance) followed by Post hoc test (Dunnett's't' test).

Nephrpotective activity by gentamicin induced nephrotoxicity in Rats: The evaluation of the ethanolic and aqueous extracts for nephroprotective activity was done according to the procedure given in the literature with minor modifications.

Total 36 animals were taken and 6 rats were allotted in each of the following groups;

Group I : Control group
Group II : Gentamicin control group (60mg/kg)
Group III: Gentamicin + Ethanolic extract (200mg/kg)
Group IV: Gentamicin + Ethanolic extract (400mg/kg)
Group V: Gentamicin + Aqueous extract (200mg/kg)
Group VI: Gentamicin + Aqueous extract (400mg/kg)

Activity profile of the test formulations in gentamicin induced changes in different parameters: Serum uric acid is the end product of purine catabolism. So, any defect in the glomerular filtration rate causes the rise in the level of uric acid in the blood .The raise after gentamicin can be attributed to the GFR impairment. The decrease in the elevated uric acid by any substance may be due to the antagonism of gentamicin induced disturbance in the glomerulus. Creatinine clearance gives the glomerular filtration rate. Administration of gentamicin leads to significant elevation of serum creatinine level indicating injury to the glomerular apparatus. The reversal of the elevation by any substance may be indicative of the reversal of the GFR impairment.

RESULTS

The nature and percentage yield of various extracts was shown below:

Table No. 1: Nature and Percentage yield of the extracts

S. No	Name of the Extract	Nature	Colour	%Yield (w/w) gm
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: EEMK and AEMK were subjected for phytochemical screening and found to contain tannins, sterols, flavonoids, glycosides and alkaloids triterpines in ethanolic and aqueous extracts. The phytochemical constituents of various extracts of *Murraya koenigii* was shown below:

Table No. 2: Phytochemical evaluation of different extracts of stems of *Murraya koenigii*

S.No.	Tests	Petroleum ether	Chloroform	Ethanol	Water
1	Alkaloids	-Ve	-Ve	+Ve	+Ve
2	Carbohydrates	-Ve	-Ve	+Ve	+Ve
3	Flavonoids	-Ve	-Ve	+Ve	+Ve
4	Saponins	-Ve	-Ve	+Ve	+Ve
5	Sterols	+Ve	+Ve	+Ve	+Ve
6	Tannins	-Ve	-Ve	+Ve	+Ve
7	Glycosides	-Ve	-Ve	+Ve	+Ve

Pharmacological activities:

Acute oral toxicity study: The mice treated with EEMK and AEMK at a dose of 2000mg/kg, *p.o.* exhibited normal behaviour, without any signs of passivity, stereotypy and vocalization. Their motor activity and secretory signs were also normal and no sign of depression. EEMK and AEMK even up to the dose level of 2000mg/kg body weight did not produce any behavioural symptoms or mortality. So 1/10th and 1/5th doses of (maximum dose tested for each extract) were selected as medium and high doses and were used in the present study to explore nephroprotective and anti-inflammatory activities.

Anti-inflammatory activity by Carrageenan induced paw oedema model in rats: The EEMK and AEMK with the selected doses *i.e.* 200 and 400mg/kg have exhibited a significant reduction in paw oedema volume in Carrageenan induced paw oedema in rats at different time intervals. Diclofenac sodium (30mg/kg) was used as standard reference and it has significantly reduced paw oedema volume by 17.81% at 1st hr, 27.58% at 2nd hr, 50.09% at 3rd hr and 74.13 % at 4th hr, which was found to be a time dependent effect. During 1st hr of study EEMK and AEMK with 200mg/kg and 400mg/kg doses have significantly reduced oedema volume by 3.43, 10.72, 3.43, 7.72% respectively noted as time dependent effect.

During 2nd hr of study EEMK and AEMK with 200mg/kg and 400mg/kg doses have significantly reduced oedema volume by 13.9, 24.28, 15.11, 17.29% respectively noted as time dependent effect. During 3rd hr of study EEMK and AEMK with 200mg/kg and 400mg/kg doses have significantly reduced oedema volume by 32.07, 34.33, 38.08, 47.46 % respectively noted as time dependent effect. During 4th hr of study EEMK and AEMK with medium and high doses have significantly reduced oedema volume 51.72, 56.89, 55.17, 60.34% respectively which was recorded as time dependent effect and result are graphically represented.

Table No. 3: Anti-inflammatory effect of EEMK and AEMK on paw volume in Carrageenan induced paw edema in rats

Groups	Treatment	Paw oedema volume			
		60min	120min	180min	240min
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

EEMK	**200mg/kg**	0.45±0.0428	0.4166±0.047	0.366±0.033	0.2833±0.030
EEMK	**400mg/kg**	0.416±0.047	0.366±0.033	0.35±0.022	0.25±0.022
AEMK	**200mg/kg**	0.45±0.0428	0.41±0.036	0.33±0.0421	0.26±0.049
AEMK	**400mg/kg**	0.43±0.0210	0.4±0.042	0.28±0.030	0.233±0.033

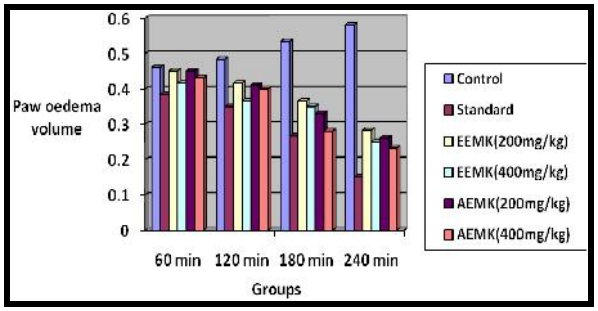

Fig. 2: Anti-inflammatory effect of EEMK and AEMK on paw volume in Carrageenan induced paw oedema in rats

Table No. 4: Percentage reduction of paw volume in Carrageenan induced paw oedema

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

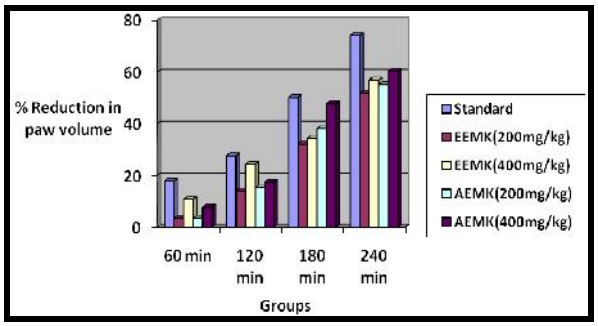

Fig. 3: Percentage reduction of Paw oedema volume in different groups

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. The serum creatinine and blood urea nitrogen (BUN) were found to be significantly increased in rats treated with only gentamicin, whereas treatment with the ethanolic and aqueous extracts of stems of *Murraya koenigii reversed* the effect of gentamicin indicating nephroprotective activity. Among various doses, the aqueous extract of dose 400mg/kg has shown good nephroprotective activity.

Table No. 5: % 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***
EEMK(200mg/kg)	8.216 ± 0.410***
EEMK(400mg/kg)	5.776 ± 0.463**
AEMK(200mg/kg)	6.3± 0.44
AEMK(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.

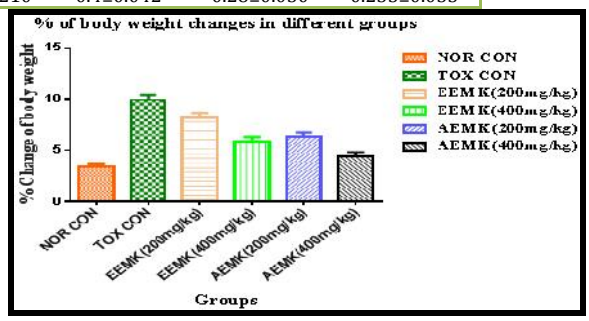

Fig. 4: % of Body weight changes in different groups by Gentamycin induced Nephrotoxicity model

Table No. 6: Blood urea and serum creatinine levels of dfferent 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***
EEMK(200mg/kg)	57.385 ± 11.724**	1.918± 0.07***
EEMK(400mg/kg)	45.916 ± 11.65*	1.53 ± 0.11***
AEMK(200mg/kg)	51.948 ± 3.673***	1.668± 0.080***
AEMK(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.

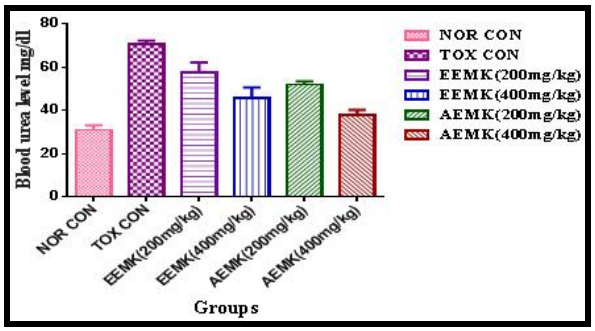

Fig. 5: Blood urea level of different groups in gentamicin induced Nephrotoxicity model

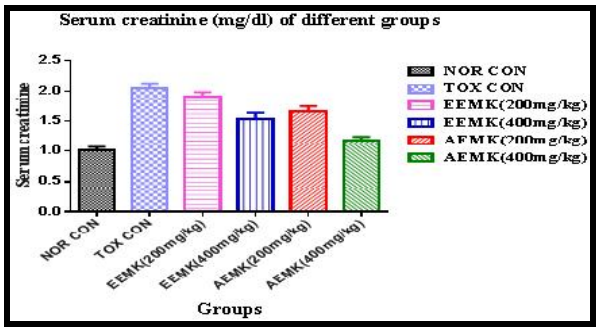

Fig. 6: Serum creatinine level of different groups in gentamicin induced Nephrotoxicity model

DISCUSSION

In the present study the stems of *Murraya koenigii* were selected for the anti-inflammatory activity. It was already reported that the leaves of *Murraya koenigii* having potent anti-inflammatory activity [6]. The preliminary phytochemical investigation reveals the prescence of alkaloids, flavonoids, saponins, tannins and glycosides in both ethanolic and aqueous extracts. Inflammation is a state

which is caused by chemical mediators like prostaglandins and cytokinins. It is reported that the phytochemicals like flavoinoids, saponons and tanins reduce the inflamation. In the present study the above mentioned phytochemicals are present in both the extracts this may be attributed for its anti- inflammatory activity.

The stems of *Murraya koenigii* were reported to contain phenolic compounds like flavonoids, which possess antioxidant property [7, 8]. The leaves of *Murraya koenigii* were already reported for its nephroprotective activity on diabetes-induced renal damage. Nephrotoxicty is a common cause of human being which is mainly caused due to oxidative stress. In the present study both the extracts contaning polyphenolic compounds having anti oxidant property, so the nephroprotective activity of the above mentioned plant *Murraya koenigii* is due to its anti oxidant property.

CONCLUSION

In the present study, both the extracts significantly reduced the nephrotoxicity and inflammation. Both nephrotoxicity and inflammation may be due to generation of inflammatory mediators by oxidative stress. It is observed that due to the presence of phytoconstituents like alkaloids, flavonoids, saponins and tannins which also reduce the oxidative stress and inflammatory mediators. The anti inflammatory and nephroprotective activity may be responsible for above phytoconstituents. Further studies are required to establish the exact mechanism of phytoconstituents responsible for the above activities.

REFERENCES:

1. Vandana Jain, Munira Momin and Kirti Laddha. International Journal of Ayurvedic & Herbal Medicine, **2012**; 2(4): 607-627.
2. Shah Rajesh Kumar, Das Loveleena and Sangma Godwin. Medicinal Property of Murraya Koenigii-A Review, *Int. Res. J. Biological Sci., **2013**;* 2(9): 80-83.
3. Haslizawati abu BN, Aspollah S, Mawardi R, Sharif1 A, Khalid K and Umi KY. Chemical constituents from stem barks and roots of *Murraya koenigii* (*Rutaceae*), *The Malaysian Journal of Analytical Sciences*, **2007**; 11(1): 173-176.
4. Thilahgavani N, Ramasamy P, Mohd Effendy A, Thirukanthan CS and Charles SV. Biological activity of carbazole alkaloids and essential oil of *Murraya koenigii* against antibiotic resistant microbes and cancer cell lines, *Molecules*, **2011**; 16: 9651-9664.
5. Rani U, Verma N and Batra A; Total phenolic (flavonoid) contents and antioxidant capacity of *Murraya koenigii* extracts, International *Journal of Medicinal Plant Research*, **2012**; 1(7): 124-128.
6. Debosree, Ghosh, Syed benazir F, Elina M, Monalisa D and Debasish B. Protective effect of aqueous leaf extract of *Murraya koenigi* against lead induced oxidative stress in rat liver, heart and kidney: a dose response study, *Asian Journal of Pharmaceutical and Clinical Research*, **2012**; 5(4): 54-58.
7. Mohammed U and Barhate SD. Investigation and study of anti-inflammatory activity of *Murraya koenigii spreng.* Leaves, *International Journal of Pharmacognosy and Phytochemical Research*, **2012**; 4(1): 12-16.
8. Juhi M, Asia Y, Rattan deep S and Aradhana. phytochemical investigation and in-vitro antioxidant potential of leaves of *Murraya koenigii, International journal of integrative biology*, **2009**; 7(3): 171-174.

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