ejpmr, 2017,4(4), 595-599

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

STUDY OF ACUTE TOXICITY AND ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF *GREWIA FLAVESCENS JUSS* WHOLE PLANT USING RATS.

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Article Received on 11/02/2017 **Article Revised on 05/03/2017** **Article Accepted on 26/03/2017**

ABSTRACT

The study was designed to investigate the acute toxicity and anti-inflammatory effect of *Grewia flavescens* juss, Ethanolic extract. The effect of *Grewia flavescens* juss, Ethanolic extract was studied for oral acute toxicity using type of Examinations like Clinical symptoms, Mortality, Body weight, Pathological examination and carrageenan induced paw edema, method for studying anti-inflammatory activity respectively. The results of the acute toxicity studies indicate that the LD50 of Ethanolic Extract of Grewia Flavescens juss (EECF) is more than 2000mg/kg. mortality was not observed in any group. Effect of various drugs on anti- inflammatory activity was evaluated by using carrageenan induced paw edema method. Percentage of anti-inflammatory increased in EEGF groups when compared to carrageenan induced animals. The different concentration of *Grewia flavescens* juss showed prompt anti-inflammatory activity.

KEY WORDS: *Grewia flavescens* juss, Ethanolic extract, acute toxicity, anti-inflammatory.

INTRODUCTION

Inflammation and pain are common nonspecific manifestations of many diseases. Although opiates and non-steroidal anti-inflammatory drugs (NSAIDs) have been used commonly in these conditions, but some adverse reactions occur with these drugs such as renal damage, gastrointestinal disturbances, respiratory depression, and possible dependence.[1-2] In recent years, there has been an increasing interest to find new anti-inflammatory drugs with possibly fewer side effects from natural sources and medicinal plants. Grewia flavescens popularly known as "donkeys berry", is a shrub or small tree, often seen in groups along the edges of roads, river banks and dry rivers, growing in large uniform groups. The plant parts are being used in Indian folk medicine. The leaves were reported to be useful in ulcerated tongue, colic pain, wounds, cholera and dysentery. Grewia flavescens a multi-stemmed shrub or small tree, up to 5 m high. Its bark is dark grey-brown belongs to Tiliaceae family. The plant is used traditionally as Anthelmentic, CNS depressant[3], anti-inflammatory, antimalarial. Nearly 40 species of this genus are found in India, some of which are well known for their medicinal value.[4-5] The berries of *Grewia flavescens* are soaked in water for two or three days to make a refreshing drink.[6] Hence the present study was designed to investigate the acute toxicity and anti-inflammatory effect of *Grewia flavescens* juss, Ethanolic extract.

MATERIALS AND METHODS

Plant material: The crude Grewia flavescens juss whole plant was collected from Sri Venkateswara University, Tirupati, Andhra Pradesh, India, in the month of January, 2016. The plant was authenticated with Plant voucher specimen no. 1397, by plant taxonomist Dr. K. Madhava chetty, Assistant professor, Department of botany, Sri venkateswara University, Tirupati, A.P, India. The Grewia flavescens juss whole plants were cut into proper Size and washed 3 times with drinking water then dried in shade with proper care. The dried plant materials were blended in to coarse powder and passed through sieve 60.

Preparation of extract: The coarse powder 500gm was subjected to maceration and transferred to stopper flask, and treated with pure ethanol until the powder is fully immersed at room temperature. The flask was shaken every hour for the first six hours and then it was kept aside and again shaken after 24 hours from time to time to ensure better extraction. This process was repeated for 7 days, followed by exhaustive maceration for 5 days by using solvent ethanol. The solvent was decanted and filtered with filter paper and recovered with help of

rotary vacuum evaporator. The extract was dried under desiccator and stored in an air tight container.[7] The final extract was then subjected to investigate the acute toxicity and anti-inflammatory effect.

Animals: Wistar Rats both males and females of 8 to 10 weeks old weighing 142–245 g were taken, and individually housed in polypropylene cages lined with husk renewed every 24 h in well-ventilated rooms at 22±3ºC and relative humidity between 30 to 80%, under artificial lighting12:12 hour light period per day and dark cycle in hygienic condition. The rats were fed with standard laboratory pellet diet and drinking water ad libitum. The commercial diet and drinking water were provided. The experimental protocols were duly approved by the Institutional Animal Ethics Committee (I/IAEC/LCP/014/2016WR♀ + ♂).

Acute oral toxicity studies

OECD Guideline 423: Pre-specified fixed doses of 5, 50, 300 or 2000 mg/kg are used. There is an option to use an additional dose level of 5000 mg/kg, but only when justified by a specific regulatory need. Groups of animals are dosed in a stepwise procedure, with the initial dose being selected as the dose expected to produce mortality in some animals. Further groups of animals may be dosed at higher or lower fixed doses, depending on the presence of mortality, until the study objective is achieved; that is, the classification of the test substance based on the identification of the dose(s) causing mortality, except when there are no effects at the highest fixed dose.

Experimentation

Number of Test Animals: 4 groups each group with 6 animal totally 24
Mode Of Application: oral
Duration And Frequency Of Treatment: single dose
Dosage Level: 50, 300, 2000 mg/kg
Applied Volume: 1 ml/100 g
Vehicle: Distilled water
Post Treatment Examination Period: 14 days
Type of Examinations: Clinical symptoms, Mortality, Body weight, Pathological examination.

Grouping

Table 1. Grouping of animals.

Groups	Dose EEGF (mg/kg)	No. Of Animals Male	Female
Control	0	3	3
Low Dose	50	3	3
Medium Dose	300	3	3
High Dose	2000	3	3

Measuring of body weights: The weights of the rats were measure at receiving and at randomization of animals, as well as before and after (24 hour) treatment for every 2 days.

General status, behavior, clinical symptoms: During the first 6 hours of the treatment each rat observes for clinical symptoms. Special attention was paid - besides of the general status, and behavior - on alterations of skin and fur, mucous membranes, eyes, circulation, breath, functions of vegetative nervous system, salivation, diarrhea, convulsions. The type and intensity of symptoms and local lesions were recorded individually.

Mortality: The mortality was recorded during the first 6 hours and thereafter twice a day for 14 days.

Anti-inflammatory activity on carrageenan induced paw edema: 7 groups of 6 rats in each were treated with vehicle, EEGF (100, 200 & 400mg/kg p.o), aspirin (50&100mg/kg p.o) and combination of aspirin 50mg/kg & EEGF 100mg/kg p.o using gastric canula. Paw edema was induced by injecting 0.1 ml of 1% w/v carrageenan suspended in 1% CMC into sub-plantar tissues of the left hind paw of each rat. The paw thickness was measured before injecting the carrageenan and after 60, 120, 180, 240 min. using vernier caliper. The anti-inflammatory activity was calculated as percentage inhibition of edema in the animals treated with extract under test in comparison to the carrageenan control group.

The percentage (%) inhibition of edema is calculated using the formula

$$\% \text{ inhibition} = To - Tt/To \times 100$$

Where T_t is the thickness of paw of rats given test extract at corresponding time and T_o is the paw thickness of rats of control group at the same time.

RESULTS AND DISCUSSION

Parameters

Body weights

Table 2. Measured body weight of all animals from 0 to 14th day.

Groups	0 day	1 day	3rd day	5th day	7th day	9th day	11th day	13th day	14th day
Group 1 CONTROL	F219gm	221	225	231	230	236	239	241	245
	F230	233	234	239	241	247	245	249	253

	F227	228	228	231	234	237	239	242	245
	M232	235	236	239	245	246	250	254	255
	M216	214	219	223	225	227	232	236	237
	M236	239	243	247	249	250	253	254	257
Group2 5mg/kg	F160gm	162	163	165	169	172	173	176	177
	F150	149	152	155	159	160	162	163	165
	F142	142	145	146	145	147	149	152	156
	M159	160	162	164	163	169	169	172	175
	M175	176	178	178	182	181	186	185	187
	M166	168	169	174	176	178	179	181	183
Group3 300mg/kg	F218gm	219	221	224	223	226	229	231	234
	F210	212	215	214	217	220	222	223	224
	F202	201	205	204	209	215	217	220	223
	M240	240	242	243	245	250	252	258	259
	M234	235	234	237	239	237	240	243	245
	M245	246	247	251	253	257	257	260	261
Group4 2000mg/kg	F185gm	187	186	191	193	197	199	202	205
	F220	223	225	228	226	233	236	234	239
	F214	213	215	217	219	224	225	227	231
	M196	197	203	205	207	207	211	214	217
	M200	203	206	204	207	209	215	217	220
	M223	227	228	232	235	239	243	245	247

Behavioral changes

Table 3. Observation of behavioral changes in all groups.

Signs	**Group 1**	**2**	**3**	**4**
Skin and Fur	Normal	Normal	Normal	Normal
Eyes And mucous membranes	Normal	Normal	Normal	Normal
Behavior	Normal	Normal	Normal	Normal
Lethargic	Absent	Absent	Absent	Absent
Sleep	Absent	Absent	Absent	Absent
Tremors/ convulsions	Absent	Absent	Absent	Absent
Salivation	Absent	Absent	Absent	Absent
Diarrhea	Absent	Absent	Absent	Absent
Excitability	No	No	No	No
Death	No	No	No	No
Other symptoms	Nil	Nil	Nil	Nil

The results of the acute toxicity studies indicates that the LD50 of Ethanolic Extract of Grewia Flavescens juss (EECF) is more than 2000mg/kg. mortality was not observed in any group. After single dose of treatment EEGF to all groups persistently increases body weight during 14 days observation period. General appearance and behavioral observations The appearance and behavioral parameters of animals after drug administration is indicator of the toxicity of the test drug the behavioral patterns of animals were observed first 6 h and followed by 14 h after the administration. No significant changes were observed in wellness parameters used for evaluation of toxicity. Skin, fur, eyes, mucous membrane, behavioral pattern, salivation and sleep pattern parameters of the treated animals were found to be normal. No toxic symptom or mortality was observed in any animal. All treated animals lived up to 14 days after the administration of EEGF.

Anti inflammatory activity: All values were shown as mean±SDM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey test. $P<0.05$ was considered statistically significant.

Table 4: Anti-Inflammatory Activity of EEGF on Carrageenan Induced Paw Edema

Treatments	**60 min (mean ± sd)**	**120min(mean ± sd)**	**180 min(mean ± sd)**
Carrageenan Control	1.45 ± 0.12	2.25 ± 0.12	3.625 ± 0.17
EEGF 100mg/kg	1.125 ± 0.20*	1.75 ± 0.20***	2.25 ± 0.12***

EEGF 200mg/kg	1.050 ± 0.12**	1.65 ± 0.12***	2.0 ± 0.11***
EEGF 400mg/kg	0.95 ± 0.12***	1.40 ± 0.081***	1.6 ± 0.081***
Aspirin 50mg/kg	1.2 ± 0.08ns	1.65 ± 0.12***	2.07 ± 0.12***
Aspirin 100mg/kg	0.8 ± 0.08***	1.35 ± 0.057***	1.65 ± 0.12***
Aspirin 50mg/kg +EEGF 100mg/kg	0.7 ± 0.081***	0.95 ± 0.12***	1.16 ± 0.11***

Significance done by ANOVA Tukey test. *P<0.05, **P<0.01, ***P<0.001 Compared with carrageenan control, ns = Not significant Compared with carrageenan control.

Table 5: Percentage inhibition of paw edema

Treatments	Percentage of inhibition at 1st hour	Percentage of inhibition at 2nd hour	Percentage of inhibition at 3rd hour
EEGF 100mg/kg	22.41%	22.22%	37.93%
EEGF 200mg/kg	27.58%	26.66%	44.82%
EEGF 400mg/kg	34.48%	37.77%	55.86%
Aspirin 50mg/kg	17.24%	26.66%	42.89%
Aspirin 100mg/kg	44.82%	40%	54.48%
Aspirin 50mg/kg +EEGF 100mg/kg	51.72%	57.77%	68%

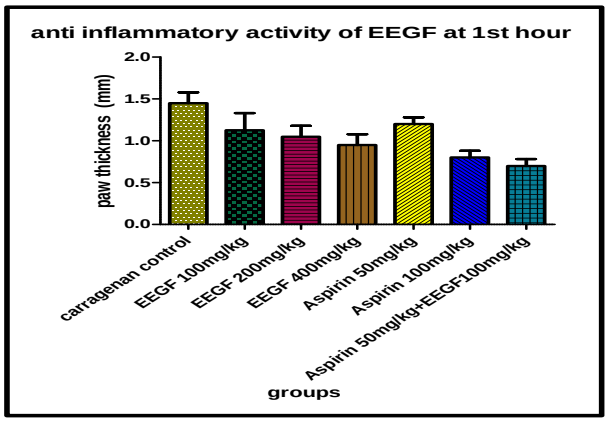

Figure 1. Anti- inflammatory activity of EEGF at 1st hour.

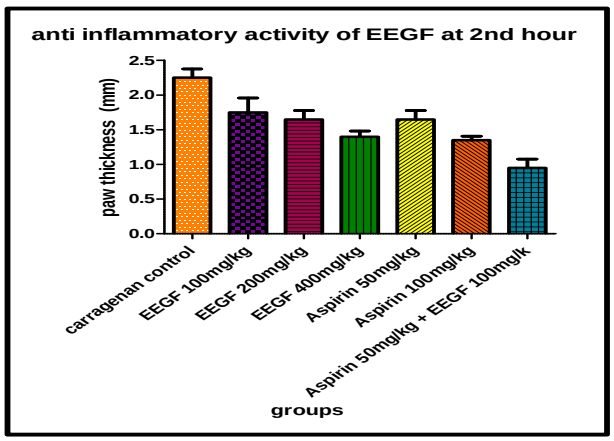

Figure 2. Anti- inflammatory activity of EEGF at 2nd hour.

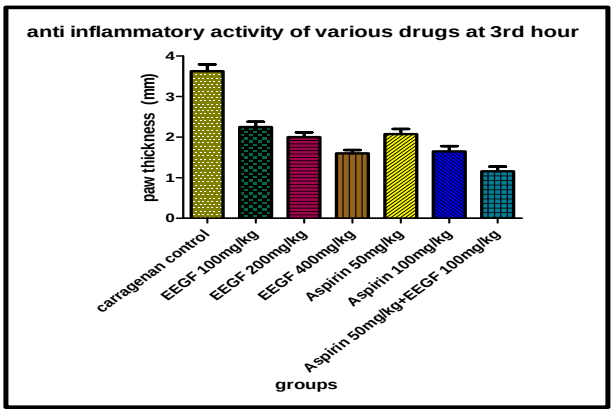

Figure 3. Anti- inflammatory activity of EEGF at 3rd hour.

CONCLUSION

All the animals survived by the end of the study; Clinical signs & symptoms did not reveal any major findings of toxicity. The LD50 of the EEGF was greater than 2000mg/kg (Category 5 as per OECD guidelines 420, 423 & 425 for acute Toxicity Studies) and hence it is practically nontoxic. Effect of various drugs on anti-inflammatory activity was evaluated by using carrageenan induced paw edema method. EEGF effectively decreased the Percentage of inhibition of paw edema in EEGF groups when compared to carrageenan induced animals.

REFERENCES

1. Domaj MI, Glassco W, Aceto MD, Martin BR. Antinociceptive and pharmacological effects of metanicotina, a selective nicotine agonist. J. Pharmacol. Exp. Ther. 1999; 291: 390–398.
2. Farshchi A, Ghiasi G, Malek Khatabi P, Farzaei Hossein NA. Antinociceptive Effect of Promethazine in Mice. Iran. J. Basic Med. Sci. 2009; 12: 140–145.

3. Prakash L and Singh R. Chemical examination of Grewia flavescens, Pharmazie. 1981; 36(8): 576.
4. Akhila S Et al. An ethno pharmacological survey on medicinal plants from idamalayar, ernakulam (dist), kerala. World journal of pharmacy and pharmaceutical sciences. 2015; 4(8): 1254-1260.
5. Praveen Kumar Goyal, University of Rajasthan, Jaipur, India, Phytochemical and Pharmacological Properties of the Genus Grewia: A Review, International Journal of Pharmacy and Pharmaceutical Sciences, 2012; 4(4): ISSN-0975-1491.
6. http://natureswow2.blogspot.in/2012/08/sandpap er-raisingrewia-flavescens.html
7. Harborne J B. Phytochemical Methods: A Guide to Modern Technique of Plant Analysis. Chapman and Hall, London.1984.