GSC Advanced Research and Reviews

eISSN: 2582-4597 CODEN (USA): GARRC2

Cross Ref DOI: 10.30574/gscarr

Journal homepage: https://gsconlinepress.com/journals/gscarr/

(RESEARCH ARTICLE)

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Evaluation of analgesic and anti-inflammatory activities of ethanolic fruit extract of *Terminalia chebula*

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GSC Advanced Research and Reviews, 2024, 18(02), 160–166

Publication history: Received on 30 December 2023; revised on 07 February 2024; accepted on 09 February 2024

Article DOI: https://doi.org/10.30574/gscarr.2024.18.2.0051

Abstract

Analgesic and Anti-inflammatory activities of ethanolic fruit extract of *Terminalia chebula* was evaluated using tail-flick method, eddy's hot plate method, acetic acid induced writhing responses and *in-vivo* anti-inflammatory property was determined using carrageenan rat paw edema. The ethanolic fruit extract of *Terminalia chebula* at different concentrations significantly inhibited the nociception in comparision with different standard drugs that showed anti-nociception. The test extract also reduced the edema induced in rats using carrageenan paw edema method as compared with the standard drug Diclofenac sodium 10mg/kg. The results obtained in this research study found to be very significant when compared to standard reference drug.

Keywords: *Terminalia chebula*; Analgesic; Anti-inflammatory activity; Standard Reference Drugs

1. Introduction

The medicinal plant *Terminalia chebula* [1-6], belonging to the family Combretaceae commonly known as Black or Chebulic Myrobalan also termed as King of Medicines is used to cure all kind of the diseases. This plant is abundantly available in remote areas of Yeleswaram and Rampachodavaram forest areas. The local natives are using the decoction of *Terminalia chebula* for the treatment of arthritic, anti-parasitic, anti-bacterial and as wound healing agent. Based on the available sources the researcher made a sincere attempt to explore the biological activities.

2. Materials and methods

2.1. Collection of Plant

The medicinal plant *Terminalia chebula* was collected from interior parts of Yeleswaram and Rampachodavaram forest sources of East Godavari District and the plant was authenticated by Taxonamist Prof Dr. S B Padhal.

2.2. Preparation of the Extract

The fruit of the plant was dried under shade, coarsely powdered and was subjected to extraction process using soxlet apparatus with ethyl alcohol for 72 hours. The solvent was evaporated and the crude extract powder was used for few days and this extract powder was used for the evaluation of analgesic and anti-inflammatory activities.

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2.3. *In-vivo* analgesic activity

2.3.1. Tail-flick Method: [7-9]

In this method adult Albino rats of either sex weighing 150-200 g, were selected for the present study. The animal experiments were performed based on the Institutional Ethics Committee (IEC) approval and guidelines REG. NO. 1269/PO/E/S/08/CPCSEA. The animals were housed in polypropylene cages and were allowed to acclimatize to the environment. The basal reaction time to the radiant heat was recorded using stop watch by placing the tip of the tail on the heat source. The basal reaction time was observed at 0, 15, 30, 60 and 120 minutes. The analgesic effect of ethanolic fruit of *Terminalia chebula* was assessed using this method.

In tail-flick method, was rats were treated with the fruit extract of *Terminalia chebula* with 150mg/kg and 300mg/kg orally and the nociception is significantly inhibited in the test group rats.

2.3.2. Eddy's Hot Plate Method: [9-11]

The Eddy's hot plate method is the most versatile method for evaluation of analgesic activity. The animals were placed on the hot plate maintained at 55°C. The animals basal reaction time was recorded by observing the time taken for paw licking or jumping using a stopwatch. The reaction time was observed at 0, 15, 30, 60 and 120 minutes. A cutoff period of 10 seconds was maintained to avoid any damage to the paws of the rat. The percentage inhibition and the reaction time at each time interval was calculated. The anti-nociceptive effect of the fruit extract of *Terminalia chebula* was assessed with 150mg/kg and 300mg/kg using this method.

$$\textbf{Percentage inhibition} = \frac{\boldsymbol{RT}}{\boldsymbol{RC}} \times \mathbf{100}$$

where RT= Reaction time in treated group; RC= Reaction time in control group

2.3.3. Acetic Acid Induced Writhing Responses: [7, 11]

Intraperitoneally acetic acid 0.1ml of 1% solution is injected and the number of writhes were recorded for each animal. For scoring purpose a writhe is indicated by stretching of abdomen and stretching of hind limbs. These were observed for 20-30 minutes and the change in number of writhing's in test group compared with standard treated and control treated groups.

The percentage inhibition of the fruit extract of *Terminalia chebula* of 150mg/kg and 300mg/kg was calculated using the formula:

$$\textbf{Percentage inhibition} = \mathbf{1} - \frac{\boldsymbol{nT}}{\boldsymbol{nC}} \times \mathbf{100}$$

where nT= Average number of writhing in treated group; nC= Average number of writhing in control group

2.4. *In-vivo* Anti-inflammatory activity:

2.4.1. Carrageenan Induced Paw Edema in Rats:

Carrageenan was used to induce edema in rats. The animals were pretreated with fruit extract of *Terminalia chebula* of 150mg/kg and 300mg/kg suspended in 2% acacia mucilage. After 30 minutes, 0.1ml of 1% w/v suspension of carrageenan in distilled water was injected subcutaneously into the sub-plantar region of left hind paw of the animals. Measurement of paw size was detected using plethysmometer. Paw sizes were measured immediately before and after carrageenan injection. Edema inhibitory activity was calculated using the formula:

$$\textbf{Percentage inhibition} = \mathbf{1} - \frac{\boldsymbol{VT}}{\boldsymbol{VC}} \times \mathbf{100}$$

where VT= Edema volume of treated group; VC= Edema volume of control group

The *in-vivo* activity in carrageenan induced paw edema was evaluated and compared with the standard drug Diclofenac Sodium.

3. Results and discussion

3.1. *In-vivo* analgesic activity

3.1.1. Tail-flick Method

In this method, rats were treated with ethanolic fruit extract of *Terminalia chebula* 150mg/kg and 300mg/kg orally. The extract significantly inhibited the perception by 38.75% at 30 mins with 150mg/kg body weight and the test extract at 30 minutes with 300mg/kg produced 45.60% inhibition of pain. And the results were compared with standard drug Diclofenac sodium 10mg/kg significantly reduced the pain to 55% at 30 minutes time interval. The results were tabulated in Table-1 and Figure-1.

Table 1 Effect of ethanolic fruit extract of *Terminalia chebula* in rats using Tail-flick method.

S.No	Treated Group	0 mins		15 mins		30 mins		60 mins		120 mins	
		BRT	% Inhibition	BRT	% Inhibition	BRT	% Inhibition	BRT	% Inhibition	BRT	% Inhibition
1	Control	5.2±0.50	-	6.0±0.15	-	6.5±0.60	-	7.2±0.10	-	4.8±0.50	-
2	Std-Diclofenac Sodium 10mg/kg	5.5±0.20	-	7.2±0.04	32.50	7.8±0.20	55	6.5±0.40	30.20	5.8±0.66	18.50
3	Extract of *Terminalia chebula* 150mg/kg	6.0±0.30	-	6.7±0.30	24.40	8.80±0.05	38.75	7.50±0.02	25.20	7.10±0.02	10.50
4	Extract of *Terminalia chebula* 300mg/kg	5.6±0.40	-	7±0.05	27.50	7.80±0.04	45.60	6.90±0.06	28.65	6.45±0.05	15.50

All values are expressed in Mean ± SEM, n=3, p<0.001 when compared with standard values.

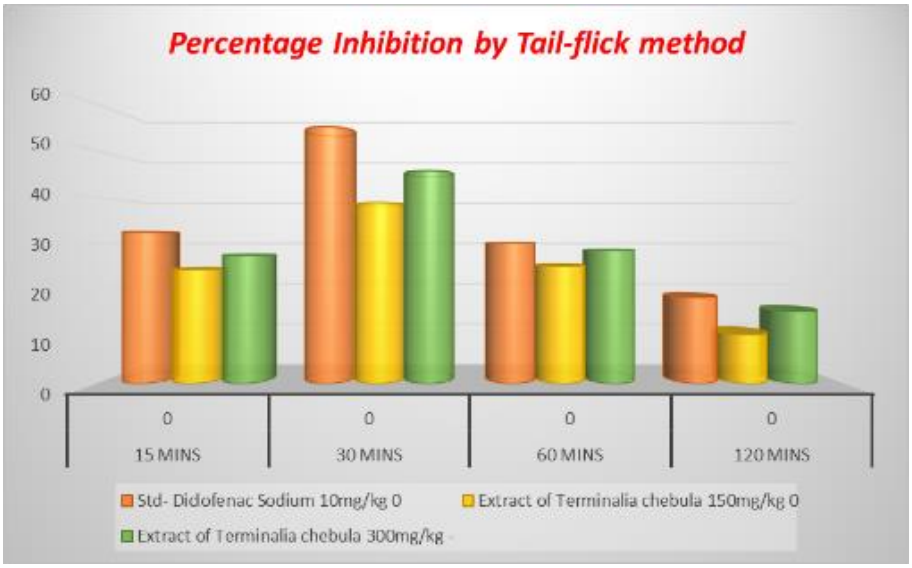

Figure 1 Percentage Inhibition of ethanolic fruit extract of *Terminalia chebula* in rats using Tail-flick method.

3.1.2. Eddy's Hot Plate Method

In Eddy's hot plate method, the selected rats were treated with the ethanolic fruit extract of *Terminalia chebula* with doses of 150 mg/ml and 300 mg/ml orally. The extract 150 mg/kg at 30 minutes inhibited the pain perception by 65.50% and with 300mg/kg body weight at 30 minutes significantly inhibited 72.40%, whereas the standard drug Tramadol 5mg/kg body weight significantly inhibited pain perception at 30 minutes by 82%.The results were tabulated in Table-2 and Figure-2.

Table 2 Effect of Ethanolic fruit extract of *Terminalia chebula* in rats using Eddy's Hot Plate method.

S.No	Treated Group	0 mins		15 mins		30 mins		60 mins		120 mins	
		BRT	% Inhibition	BRT	% Inhibition	BRT	% Inhibition	BRT	% Inhibition	BRT	% Inhibition
1	Control	0.0±0.04	-	6.5±0.20	-	7.2±0.13	-	8.0±0.20	-	7.5±0.30	-
2	Std-Tramadol 5mg/kg	7.0±0.05	-	9.8±0.80	45.0	10.20±0.50	82	12.50±0.30	75	10±0.2	45
3	Extract of *Terminalia chebula* 150mg/kg	6.0±0.30	-	7.8±0.05	37	10.5±0.20	65.50	10 9±0.40	60	10.10±0.20	30
4	Extract of *Terminalia chebula* 300mg/kg	6.0±0.15	-	9.10±0.02	40	11.50±0.04	72.40	11.10±0.04	67	8.5±0.02	37

All values are expressed in Mean ± SEM, n=3, p<0.001 when compared with standard values.

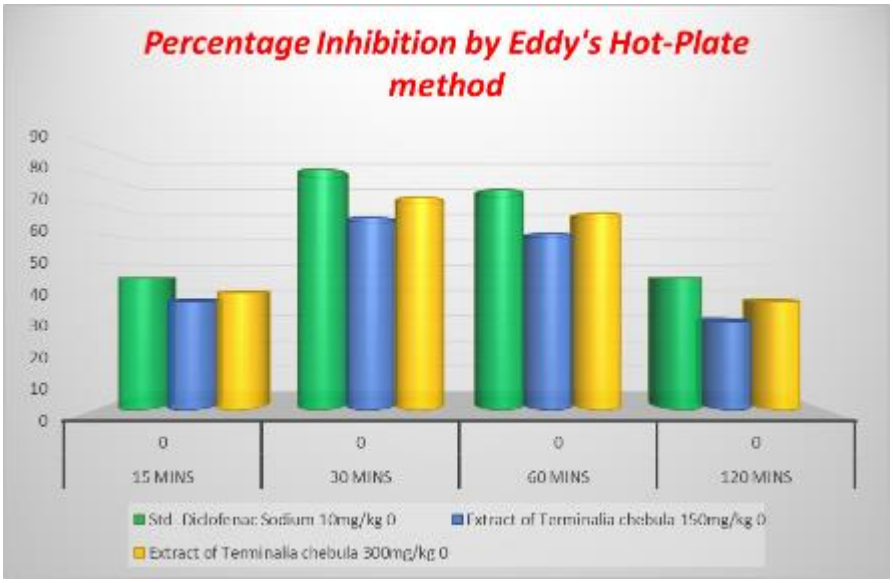

Figure 2 Percentage Inhibition of ethanolic fruit extract of *Terminalia chebula* in rats using Eddy's Hot Plate method.

3.1.3. Acetic Acid Induced Writhing Responses

Intraperitoneal injection of low dose of Acetic acid produce painful reaction which is characterized as constriction of abdomen, turning of trunk and extension of hind legs are taken as reaction time to chemically induce pain. The fruit extract of *Terminalia chebula* at a dose of 150 mg/ml body weight reduced the writhing responses to 20 and percentage

inhibition was found to be 60.50% and at a dose of 300mg/kg, the writhing response were reduced to 12 and percentage inhibition was found to be 74%. The Pentazocin 5mg/ml as a reference standard reduced the writhing response to 2 and the percentage inhibition was found to be 92%. The results were tabulated in Table-3 and Figure-3.

Table 3 Effect of *Terminalia chebula* fruit extract in Acetic Acid Induced Writhing Responses in rats.

S.No	Treatment	Number of Writhings	Percentage of Inhibition
1	Control	75.0±1.50	-
2	Std- Pentazocin 5mg/kg	2.0±0.10	92
3	Extract of *Terminalia chebula* 150mg/kg	20±3	60.50
4	Extract of *Terminalia chebula* 300mg/kg	12±1.50	74

Values are Mean ± SEM, n=3.

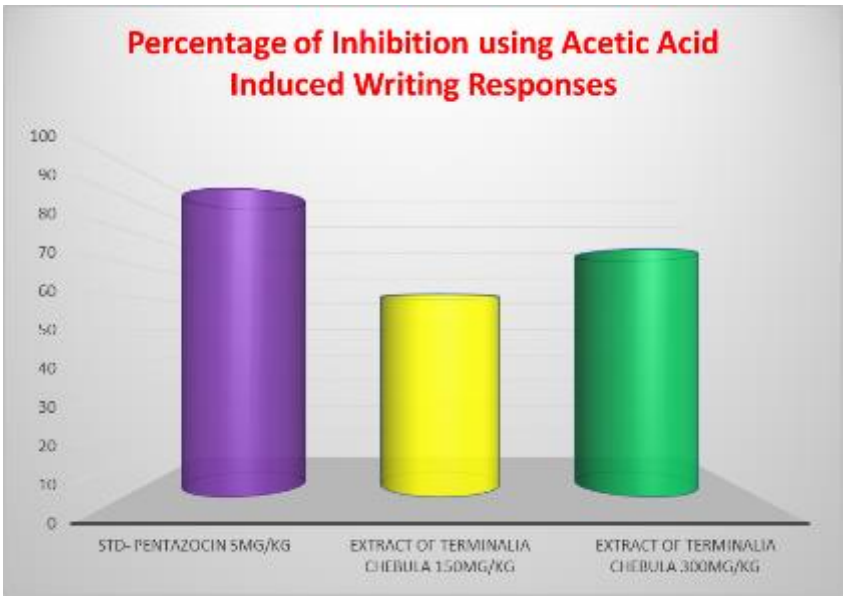

Figure 3 Effect of *Terminalia chebula* fruit extract in Acetic Acid Induced Writhing Responses in rats.

3.2. *In-vivo* Anti-inflammatory activity

3.2.1. Carrageenan Induced Paw Edema in Rat

Carrageenan induced paw edema model is versatile and sensitive method to evaluate the effect of Non-steroidal anti-inflammatory agents. The anti-inflammatory effect of *Terminalia chebula* fruit extract in carrageenan induced paw edema in rats was observed and the extract at a dose of 150 mg/kg and 300mg/kg showed significant anti-inflammatory activity by inhibiting the percentage increase in carrageenan induced rat paw edema. The percentage inhibition of Protein denaturation was calculated. The results were tabulated in Table-4 and Figure-4.

Table 4 Anti-inflammatory effect of *Terminalia chebula* fruit extract by Carrageenan induced paw edema model.

S.No	Treated Group	Mean inhibition in Paw Diameter (in mm)				
		0 Hour	1st Hour	2nd Hour	3rd Hour	4th Hour
1	Control	0.32±0.02	0.48±0.04	0.80±0.06	0.96±0.15	0.84±0.03
2	Std- Diclofenac Sodium 10mg/kg	0.24±0.11	0.3±0.01 40	0.45±0.02 48	0.51±0.03 53.50	0.29±0.04 64.50

3	Extract of *Terminalia chebula* 150mg/kg	0.28±0.01	0.58±0.05 23	0.50±0.04 34.40	0.58±0.05 44.30	0.40±0.03 51.50
4	Extract of *Terminalia chebula* 300mg/kg	0.27±0.03	0.60±0.04 25	0.50±0.02 39.50	0.5±0.03 48	0.32±0.02 56.50

All values are expressed in Mean ± SEM, n=3, p<0.001 when compared with standard values.

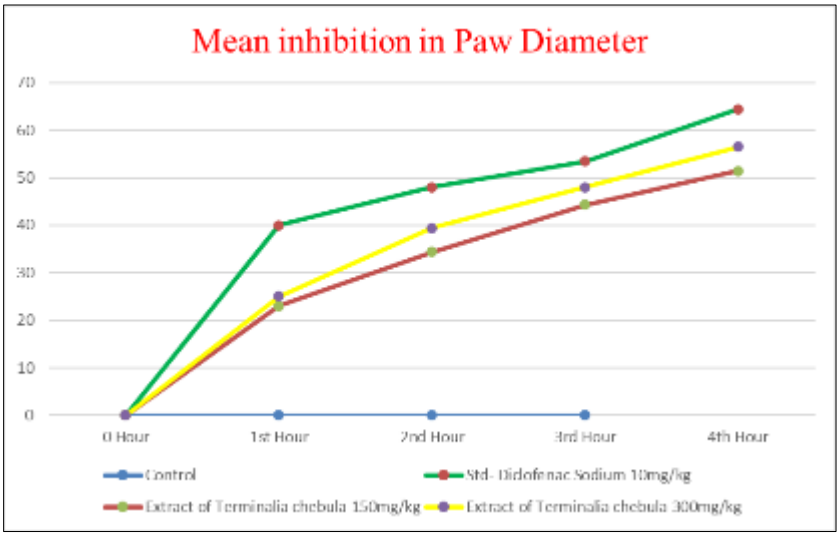

Figure 4 Anti-inflammatory effect of *Terminalia chebula* fruit extract by Carrageenan induced paw edema model.

4. Conclusion

The detailed research study and the results obtained indicates that the ethanolic fruit extract of *Terminalia chebula* possessed decent Analgesic and Anti-inflammatory activities. This research can be considered as an indicative for the folkloric usage of this medicinal plant possessing significant biological activities. Further research in detail need to be carried out in the light of modern sciences.

Compliance with ethical standards

Acknowledgments

The authors would like to express their thanks to the management of Aditya College of Pharmacy for providing necessary facilities to carry out this research work.

Disclosure of conflict of interest

The authors have not discussed any Conflict of interest.

Statement of ethical approval: The ethical approval for this work was carried out as per the guidelines laid down by IAEC as per the provisions made by CPCSEA of Aditya College of Pharmacy.

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