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Research Article

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Analgesic potential of *Calotropis procera* (Ait.) R. Br. leaves

Yogesh Murti*, Saurabh Sharma and Pradeep Mishra

Institute of Pharmaceutical Research, GLA University, Mathura (U.P.), India

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ABSTRACT

Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. In the present study n-butanol fraction and chromatographic elutes of n-butanol fraction (n-hexane, chloroform and chloroform: methanol (9:1) elutes) of ethanolic extract of Calotropis procera (Ait.) R. Br. leaves were evaluated for analgesic activity by tail immersion and hot plate method. The chloroform elute of n-butanol fraction significantly increased the time taken to withdraw the tail to the nociceptive stimuli (44.30 %) as well as increased the latency period of jumping or paw licking (69.62 %) at 100 mg/kg dose when compared with standard group animals. In conclusion, this study provides evidences for the analgesic activity of leaves of Calotropis procera which could partly contribute to its ethnomedical use.

Key words: *Calotropis procera*, analgesic activity, chromatographic elutes

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INTRODUCTION

An analgesic is any member of the diverse group of drugs used to relieve pain. The word *analgesic* derives from Greek *an-* ("without") and *algos* ("pain"). Analgesic drugs act in various ways on the peripheral and central nervous systems; they include paracetamol (acetaminophen), the non-steroidal anti-inflammatory drugs (NSAIDs) such as the salicylates, narcotic drugs such as morphine, synthetic drugs with narcotic properties such as tramadol, and various others.

Herbal medicine is becoming popular all over the world than the Allopathic medicine for medication. Several medicinal plants have been screened based on the integrative approach on drug development from Ayurveda[1]. The use of the plants, plant extracts and pure compounds isolated from natural sources has always provided a foundation for modern pharmaceutical compounds. *Calotropis procera* (Ait.) R. Br., belongs to family Asclepiadaceae is an Asian shrub and its different parts are used for the treatment of many diseases. The presence of a number of phytoconstituents, its wide variety of pharmacological actions *Calotropis procera* (Ait.) R. Br. plant has a good sign for future biopharmaceutical prospect[2,3]. Different extracts of *Calotropis procera* (Ait) R.Br. leaves have been found to possess following diverse biological activities like antidiarrhoeal[4], antioxidant[5-7], antipyretic[8], analgesic[9], antimicrobial[10,11], spasmolytic[12], schizonticidal[13], cytotoxic[14,15], hepatoprotective[16], hypoglycemic[17] and abortifacient[18]. The literature survey revealed that different parts of *Calotropis procera* (Ait.) R. Br plants showed anti-inflammatory activity[19-27] but there is no analgesic activity of *Calotropis procera* (Ait.) R. Br. leaves reported. In continuation of phytochemical and biological investigation on *Calotropis procera* leaves [5,28-31], the authors have set forth the objective of evaluating the analgesic potential of the *Calotropis procera* (Ait.) R. Br. leaves.

EXPERIMENTAL SECTION

Collection and preparation of plant material

The leaves of *Calotropis procera* (Ait) R. Br. were collected in the month of February from the local field of Mathura (Uttar Pradesh), India. The leaves were cleaned by washing with running water and shade dried, then powdered to pass through 100 mesh size. Powdered leaves were extracted with ethanol by maceration for seven days at room temperature. The solvent was recovered under reduced pressure and ethanolic extract was obtained as brownish green viscous residue. The dried residue was suspended in water and further extracted with *n*-butanol to obtained water and *n*-butanol fraction. The *n*-butanol fraction and water fraction was concentrated under reduced pressure and vacuum dried. The *n*-butanol fraction was subjected to column chromatography. The column was run with different solvents and ethanolic extract, *n*-butanol fraction, water fraction and chromatographic elutes of *n*-butanol fraction (*n*-hexane, chloroform, chloroform: methanol;9:1) were investigated for their *in vivo* analgesic activity.

Phytochemical screening

Qualitative assay, for the presence of plant phytoconstituents such as carbohydrates, alkaloids, glycosides, flavonoids, tannins and saponins were carried out on the ethanolic extract, *n*-butanol fraction and water fraction of *Calotropis procera* (Ait) R.Br leaves following standard procedure[32,33].

Experimental animals

The *in vivo* analgesic activity of ethanolic extract, different fractions and chromatographic elutes of *n*-butanol fraction was conducted on at Animal house, GLA University, Mathura. Wistar rats weighing between 140-150 gm were used for the present investigation. The animals were maintained under standard hygienic conditions with 12 hr day and night cycle with food and water *ab libitum*. All procedures described were reviewed and approved by the Institutional animal ethical committee (GLAIPR/IAEC/03/12/PharmChem/R7).

Analgesic activity

Analgesic activity was carried out by two methods as Eddy's hot plate method and tail immersion method. The animals were divided in to eight groups with three animals in each group. In both methods all the samples were prepared in 1.0 % tween 80 solution in water. As group I received 100 mg/kg ethanolic extract, group II received 100 mg/kg water fraction, group III received 100 mg/kg *n*-butanol fraction, group IV, V, VI received *n*-hexane, chloroform and chloroform: methanol (9:1) chromatographic elutes of *n*-butanol fraction (100 mg/kg), group VII served as control and received 10 mL/kg tween 80 (1.0 %) solution in water and group VIII received 100 mg/kg diclofenac sodium and served as standard.

Eddy's hot plate method

The hot plate test was used to measure analgesic activity by following the method, described by Eddy and Leimbark [34] with minor modifications. The animals were positioned on Eddy's hot plate and kept the temperature 55ºC and measure the response time (either paw licking or jump whichever appear first). The cut of time for the reaction was 15 sec. The same procedure was repeated after 30, 60, 90, 120, 150 and 180 min and values were noted down as given in table 1.

Tail immersion method

In this method tail of animal was dipped up to 5 cm in hot water maintained at 55±0.5ºC [35]. The response time was noted down as sudden withdrawal of tail from the water. The cut off time was 15 sec. The same procedure was followed after 30, 60, 90, 120, 150 and 180 min and reading were noted as shown in table 2.

Table 1: Analgesic activity result of *Calotropis procera* (Ait) R.Br. leaves by Hot plate method

Name of Drug/Extract	Dose (mg/kg)	Reaction Time in Sec.					
		30 min	60 min	90 min	120 min	150 min	180 min
Ethanolic	100	1.03±0.049	1.11±0.015	1.05±0.016	1.06±0.024	1.22±0.075	1.52±0.017
Water	100	1.15±0.038	1.17±0.030	1.15±0.027	1.18±0.092	1.28±0.046	1.44±0.037
n-butanol	100	2.11±0.034**	2.52±0.020**	3.22±0.127**	4.22±0.037**	4.52±0.023**	4.89±0.049**
n-hexane	100	2.50±0.137**	4.10±0.061**	4.22±0.124**	4.25±0.070**	4.85±0.353**	4.83±0.051**
Chloroform	100	2.41±0.141**	3.84±0.056**	3.74±0.032**	4.46±0.048**	4.79±0.030**	2.42±0.123**
Chloroform: Methanol (9:1)	100	1.03±0.030	1.18±0.085	1.28±0.050	1.05±0.207	1.24±0.075	1.56±0.226
Control (1.0 % Tween 80, 10 mL/kg)	-	1.20±0.784	1.25±0.020	1.36±0.045	1.18±0.037	1.39±0.011	1.84±0.020
Diclofenac sodium	100	2.67±0.085	4.56±0.005	6.92±0.049	8.11±0.026	11.53±0.072	6.28±0.030

*All the values are given as Mean ± SEM; N=6; *P<0.01 & **P<0.001 as compared to control*

Table 2: Analgesic activity result of *Calotropis procera* leaves (Ait) R.Br. by Tail immersion method

Name of Drug/Extract	Dose (mg/kg)	Reaction Time in Sec.					
		30 min	60 min	90 min	120 min	150 min	180 min
Alcoholic	100	1.71±0.048	1.14±0.035	1.26±0.044	1.88±0.044	1.36±0.008	1.45±0.122
Water	100	1.63±0.046	1.13±0.030	1.12±0.103	1.46±0.125	1.13±0.062	1.32±0.031
n-butanol	100	3.16±0.020**	4.11±0.037**	5.84±0.043**	5.02±0.046**	5.28±0.896**	5.42±0.130**
n-hexane	100	3.22±0.012**	4.10±0.061**	5.17±0.088**	4.21±0.085**	3.56±0.028**	3.30±0.029**
Chloroform	100	3.12±0.017**	4.07±0.071**	4.58±0.044**	5.04±0.160**	4.70±0.075**	4.49±0.018**
Chloroform: Methanol (9:1)	100	1.70±0.048	1.12±0.017	1.24±0.023	1.56±0.023	1.54±0.015	1.45±0.026
Control (1.0 % Tween 80, 10 mL/kg)	-	1.77±0.029	1.32±0.071	1.38±0.017	2.20±0.096	2.25±0.031	1.77±0.078
Diclofenac sodium	100	3.91±0.015	4.88±0.011	6.77±0.011	9.26±0.029	12.45±0.0057	7.15±0.023

*All the values are given as Mean ±SEM; N=6; *P<0.01 & **P<0.001 as compared to control*

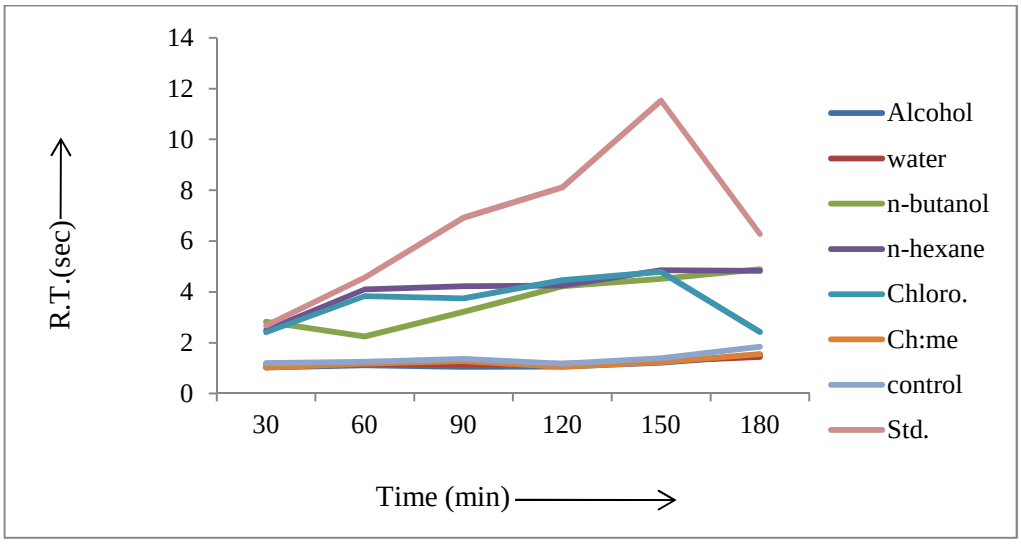

Figure 1: Analgesic activity result of *Calotropis procera* (Ait) R.Br. leaves by Hot plate method

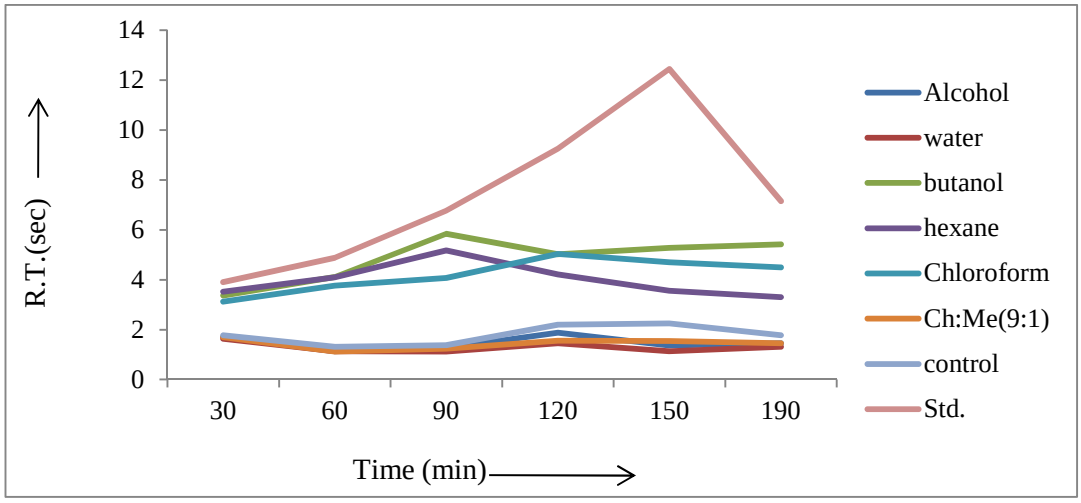

Figure 2: Analgesic activity result of *Calotropis procera* (Ait) R.Br. leaves by Tail immersion method

Table 3: Percentage inhibition result of *n*-butanol fraction and its chromatographic elutes of *Calotropis procera* (Ait) R.Br. leaves (Hot plate method)

Name of Drug/Extract	Dose (mg/kg)	% Inhibition					
		30 min	60 min	90 min	120 min	150 min	180 min
n-Butanol	100	43.12	50.39	57.76	72.03	69.24	62.37
n-Hexane	100	52.71	69.51	67.77	72.23	71.34	61.90
Chloroform	100	50.80	67.44	63.34	73.54	70.98	58.00
Diclofenac sodium	100	55.05	72.58	80.34	85.45	87.94	70.70

Table 4: Percentage inhibition result of *n*-butanol fraction and its chromatographic elutes of *Calotropis procera* (Ait) R.Br. leaves (Tail immersion method)

Name of Drug/Extract	Dose (mg/kg)	% Inhibition					
		30 min	60 min	90 min	120 min	150 min	180 min
n-Butanol	100	43.39	67.88	76.88	56.17	57.38	67.34
n-Hexane	100	45.03	67.80	73.30	47.73	36.79	46.36
Chloroform	100	43.26	67.56	69.80	56.34	52.12	60.57
Diclofenac sodium	100	54.73	72.95	79.61	76.24	81.92	79.44

RESULTS AND DISCUSSION

Preliminary phytochemical screening of ethanolic extract revealed the presence of carbohydrates, glycosides, alkaloids saponins, tannins, flavanoids and steroids. Water fraction showed the presence of carbohydrates, glycosides, alkaloids and tannins while *n*-butanol fraction showed presence of glycosides, alkaloids, saponins and flavanoids.

The result of analgesic activity by hot plate method showed that *n*-butanol fraction, *n*-hexane and chloroform elutes of *n*-butanol fraction of ethanolic extract of *Calotropis procera* (Ait) R.Br. leaves showed marked increase in response time as compared to control with percentage inhibition at time 90 min as 57.76, 67.77 and 63.63 respectively. Sandard drug diclofenac sodium (100 mg/kg) showed percentage inhibiton 80.34 at time 90 min (table 3). The *n*-butanol fraction, *n*-hexane and chloroform elute showed significant ($P<0.001$) analgesic action while ethanolic extract, water fraction and chloroform:methanol (9:1) elute showed insignificant activity. Similarly result of tail immersion method showed that *n*-butanol fraction, *n*-hexane and chloroform elutes of *n*-butanol fraction of ethanolic extract of *Calotropis procera* (Ait) R.Br. leaves showed significant ($P<0.001$) increase in response time as compared to control with percentage inhibiton at time 90 min as 76.88, 73.30 and 69.80 respectively. Sandard drug diclofenac sodium (100 mg/kg) showed percentage inhibiton 79.61 at time 90 min (table 4). The effect was increase with time up to 180 min.

Analgesic effect of *Calotropis procera* (Ait) R.Br. had a rapid onset and a fairly long duration of action (upto 180 min) (Figure 1 & 2). Several flavonoids isolated from medicinal plants have been reported to possess analgesic activity [36,37]. As the leaves of *Calotropis procera* (Ait) R.Br. contain flavonoidal compounds[38,39]; the analgesic activity of this plant may be due to them. This is an interesting and therapeutically important finding which also provides scientific evidence in support of the claim that this plant is effective in analgesia[40].

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