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PHYTOCHEMICAL & ANALGESIC INVESTIGATION OF CALOTROPIS PROCERA LEAVES

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ABSTRACT :

Preliminary phytochemical study of the petroleum ether, ethanolic and ethanolic extract of *Calotropis procera*, showed the presence of saponins, terpens, phenols, alkaloids, tannins and flavonoids. Different solvents (Petroleum ether, chloroform and ethanolic) are used for extraction of leaves of C. procera & were subjected for phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, cardiac glycosides, tri-terpenoids, phenolic compounds and tannins in the C. procera extract. The acute toxicity study revealed safely of the extract up to a dose of 2000 mg/kg in mice. The ethanolic, petroleum ether and chloroform extract of leaves of C. procera at a dose of 100, 200 and 500 mg/kg was selected for analgesic activity. Different extracts (Petroleum ether, chloroform and ethanolic) of leaves of C. procera were investigated for analgesic activity by using different models. The analgesic properties were studied on acetic acid induced writhing and tail flick latent period in rats. The result shows that the ethanolic extract of the leaves of *C. procera* was found to be effective.

Keywords: *Calotropis procera*, Analgesic Activity, Ethanolic Extract, Acetic Acid- Induced Writhing.

INTRODUCTION

Since time immemorial, nature has played a cardinal role in discovery of modern drugs, including centrally acting medicines. Several researchers have demonstrated benefits of herbal remedies in refractory patients of anxiety, depression and epilepsy [1-4].

C. procera or sweet akand belonging to family: Asclepiadaceae is native to India and grow well in lower hills at 900m altitude [5,6].

Different part of *Calotropis procera* are reported to be used for treatment of toothache and earache, sprain, anxiety, pain, epilepsy and in mental disorders, diarrhoea, analgesic activity and pregnancy interceptive properties [7-10]. The stem bark of *Calotropis procera* yields resin and wax.

The active constituent isolated from plant include b-amyrin and its isovalerate, a and b-calotropeols, mixture

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The leaves of *C. Procera* are reported to contain taraxasterly acetate, pinoresinol, medioresinol, uzarigenin, calotropin, calactin, calacitnic acid, calacitnic acid methyl of tetracyclic triterpene, traces of sterols, C_{31} and C_{33} hydrocarbons, fatty acids, giganteol, cardiac glycosides, calotropin, uscharin, calotoxin, uscharidin and gigantin [11]. Ester, 19-carboxyl-calacitnic methyl ester, drummondol, 15b-hydroxycalotrin, the C_{11} bicylic lactone norisopenoid, the rare diphenyl furfuran lignan, salicifoliol and 19-nor- and 18,20-epoxy-cardenolides [12].

Different parts of the plant have been used in Indian traditional system of medicine for the treatment of leprosy, ulcers, tumours, piles and diseases of spleen, liver and abdomen. The root of the plant is used as a carminative in the treatment of dyspepsia [13]. Further, the root bark and leaves of *Calotropis procera* are used by various tribes of central India as a curative agent for jaundice [14]. The chloroform extract of the root has been shown to exhibit protective activity against carbon tetrachloride induced liver damage [15]. The milky white latex of this plant has been reported to exhibit potent anti-inflammatory, analgesic and weak antipyretic activity in various experimental

models [16-18]. The latex also inhibits inflammatory response elicited by various inflammatory mediators [19]. [19] Besides, it has also been demonstrated to possess antioxidant and anti-hyperglycaemic property [20]. Recently, the aqueous extract of the latex has been shown to inhibit cellular infiltration and afford protection against development of neoplastic changes in the transgenic mouse model of hepatocellular carcinoma [21].

Ethanolic extract of *C. procera* has been shown antipyretic, analgesic, anti-inflammatory and neuromuscular blocking activity [22].

Thus in review of potential use of plant in folklore for the treatment of CNS diseases and isolation of centrally active substances it was that important to systematically evaluate the analgesic activity of *Calotropis procera.* The literature survey revealed that *Calotropis procera* have been studied extensively because of their ready accessibility, diverse biological activity like analgesic, anti-HIV, and anti-inflammatory, anti-oxidants and analgesic activities.

Thus, it was decided to phyto-chemical investigation of *Calotropis procera* and evaluated them for anti-diarrheal activities.

MATERIALS AND METHODS

Plant Material

C. procera were obtained locally from sub-urban hills of District Chhatarpur of Madhya Pradesh. They were authenticated by the **Department of Botany, Saifia College, Bhopal (M.P.) (India) and were given a specimen no. (Specimen/05/2019)**.

Leaves were carefully and mechanically separated washed with water. After drying in shade they were powered and stored. The leaves powder was extracted using soxhelation successively with petroleum ether, chloroform and ethanol for 24 hours. The extract obtained was store in airtight container in desiccators. The yield of ethanolic extract was found to be 9.27%. Preliminary phyto-chemical analysis was carried out on all the 3 extracts of *C. procera* to assess the presence of alkaloids, glycosides, saponins, flavanoids and steroids.

Drugs and Chemicals

Pentazocine and Aspirin were procured from gift sample of Cadila Pharmaceuticals from Ahmadabad.

Instruments and Equipment Used

Animal weighing balance (Ramon surgical corporation, Delhi), Analytical weighing Balance, (Inco, India), Rota Rod Apparatus (VJ Instruments, Amaravati, India), Actophotometer (Inco, India), Analgesiometer (Inco, Ambala).

Animals

Wistar rats weight 150-200 g and albino mice weight 18-30 g were used. The animals were obtained from **Animal House Facility, College of Pharmacy, SSSUTMS, Sehore.** Animals were randomized and allocated to different treatment groups (5 per group). Animals were kept at a temperature of 24 ± 2°C and relative humidity of 30-70%. A day with 12:12 light: dark cycle with free access to rodent chow and tap water. An Institutional Animal Ethics Committee (IAEC) approved all procedure and guidelines given by CPCSEA were followed **(Protocol no. CPCSEA/46/2019).**

Preliminary Phytochemical Screening

The preliminary Phytochemical Screening was carried out on the petroleum ether, chloroform and ethanolic extracts of *C. procera* for qualitative identification. Tests for common phytochemicals were carried out by standard methods described in practical pharmacognosy.

Acute Toxicity Study

The acute toxicity studies were carried out for ethanolic extract of *C. procera* using fix dose method according to OECD guidelines no. 425. Healthy adult female Swiss albino mice weighing between 20 to 30 g were used for study. [131]

ANALGESIC ACTIVITY

Analgesic activity of ethanolic extract was tested as anti-nociceptive effect against chemical and thermal noxious stimuli in mice.

Acetic acid- induced writhing (Chemical method)

This was carried out in groups of mice (n=5) by nothing the writhing responses produced by intraperitoneal administration of 1% acetic acid (0.1 ml/ 10g) 15min after intraperitoneal injection of either control vehicle or ethanolic extract of *C. procera* (in different doses) were compare against the standard analgesic aspirin (200mg/kg). The number of writhes produced in these animals was counted for 30 min.

Tail flick method (Thermal method)

Analgesic activity was recorded by using analgesiometer. The rats were placed in rat holder, with its tail coming out through a slot in the lid. The tail was kept on bride of analgesiometer called jacket with an electrically heated nichrome wire, underneath. The tail received radient heat from wire, heated by passing current of 6 mA. The time taken for with drawal of tail after switching on the current was taken as latent period, in scene of tail flicking response and was consider as index nociception. The cut off time for determination of latent period was taken as 30s to avoid injury to skin. Three tail flick latencies were measured (Basal reaction time) per rat at each time interval and he means of tail-flick latencies were used for statistical analysis. After recording the basal reaction time in group of

rats (n=5) at least 3 consecutive trial were selected for further experimentation and were administrated i.p. either control vehicle *C. procera* extract (in different doses) or pentazocin was used as reference standard and were tested 30 min later.

Statistical Analysis

The data were expressed as mean ± SD, statistical significance was analyse using one way analysis of variance (ANOVA) followed by Tukey's multiple comparisons. $P < 0.05$ was considered as statistically significance.

Material

The fruit of *Terminalia chebula* was collected from Regional Research Institute (RRI), Bhopal, and were authenticated by the Botany Department, Saifia College Bhopal. A voucher specimen (RRI/BNG/SMP/Drug Authentication/2019-286) of fruit has been deposited in museum of Department of pharmacology, College of Pharmacy, Sri Satya Sai University of Technology & Medical Sciences.

Animals

Wistar albino rats of either sex weighing between 150-250 gm were used. Institutional Animal Ethics Committee approved the experimental protocol; animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). Albino rats were used in this thesis was obtained from the Bioneeds Animal House Dhavas Pet, Tumkur. The animals were housed in Poly propylene cages and maintained at 24˚C ± 2˚C under 12h light/ dark cycle and were feed *ad libitum* with standard pellet diet and had free access to water. The animals were given standard diet supplied by Pranav Agro Industries Ltd. Sangli. The composition of the diet are protein 10%, Arachis oil 4%, Fibers 1%, Calcium1%, Vitamin A 1000 IU/gm and Vitamin D 500 IU/gm.

RESULTS AND DISCUSSION

Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, cardiac glycosides, triterpenoids, phenolic compounds and tannins in the *C. procera* extract (Table 1).

Analgesic Activity

One way ANOVA revealed a significant effect of all treatment groups in acetic acid induced writhing test as well as tail flick method. The post-hoc analysis by Tukey's test revealed significant effect of all dose of *C. procera* ($P<0.001$) on analgesic activity in mice.

Acetic acid-induced writhing response

The first study showed that the application of different doses of extracts had significant analgesic effects in the animals under investigation. The results of doses 200 and 500 mg/Kg were significant and comparable with the effect of aspirin in analgesic activity (Table 2).

Tail Immersion Method

The result of tail immersion test in mice is presented in Table 3. The result shows that the extract at the dose of 500mg/kg and the reference drug Pentazocin significantly ($P = 0.0001$) increased the PRT when compared to the negative group (Group 1). At the doses of 100 and 200 mg/kg, the extract did not show any significant increase in PRT, although there was a marginal increase in the mean PRT from 7.80±1.92 to 13.40±1.14*** for petroleum ether extract of *Calotropis procera* (Table 3).

Table 1: Estimation of Phytochemical Analysis of Different Extract of *C. procera.*

Sr. No	Chemical test		PEE	CE	EE
1	Test for Alkaloids	Hager's Test	-ve	-ve	-ve
		Mayer's Test	-ve	-ve	-ve
		Dragendroff's Test	-ve	-ve	+ve
		Wagner's Test	-ve	-ve	-ve
2	Test for carbohydrates	Molisch's Test	+ve	+ve	-ve
		Fehling's Test	-ve	-ve	+ve
		Barford’s Test	-ve	-ve	-ve
		Benedict's Test	-ve	-ve	+ve
3	Test for cardiac glycosides	Baljet test	-ve	-ve	+ve
		Legal test	-ve	-ve	+ve
4	Test for Anthraquinone glycosides	Modified Borntrager’s test	-ve	-ve	-ve
		Borntrager’s test	-ve	-ve	-ve
5	Test for saponins glycosides	Foam test	-ve	-ve	+ve
		Hemolytic test	-ve	-ve	-ve

Sr. No	Chemical test		PEE	CE	EE
6	Test for fixed oil	Stain Test	+ve	+ve	-ve
7	Test for Proteins and Amino acids	Millons's Test	-ve	-ve	-ve
		Biuret Test	-ve	-ve	-ve
		Ninhydrin Test	-ve	-ve	-ve
8	Test for Phytosterols and triterpenoids	Liebermann-Burchard Test	-ve	-ve	+ve
		Salkowski Test	-ve	-ve	-ve
9	Test for flavonoids	Shinoda test	-ve	-ve	+ve
10	Test for tannin	Lead acetate solution	-ve	-ve	-ve
		5% $Fecl_3$ solution	-ve	-ve	-ve

PEE= Petroleum Ether Extract, **CE =** Chloroform Extract, **EE =** Ethanol Extract

Table 2. Effect of *C. procera* Leaves Extract on Acetic Acid Induced Writhing in Mice

Group	Treatment	Dose	Mean No. of writhing
I	Control	0.1 ml/10g	76.40±7.86
II	Aspirin	200mg/kg	10.40±3.21***
III	*Calotropis procera* (PEE)	100 mg/kg	65.20±4.60*
IV	*Calotropis procera* (PEE)	200mg/kg	46.20±3.42***
V	*Calotropis procera* (PEE)	500mg/kg	30.60±2.88***
VI	*Calotropis procera* (CE)	100 mg/kg	54.35±2.65*
VII	*Calotropis procera* (CE)	200mg/kg	39.22±2.22***
VIII	*Calotropis procera* (CE)	500mg/kg	27.55±1.75***
IX	*Calotropis procera* (EE)	100mg/kg	61.33±3.83*
X	*Calotropis procera* (EE)	200mg/kg	45.15±2.45***
XI	*Calotropis procera* (EE)	500mg/kg	23.45±2.65***

PEE = Petroleum Ether Extract; **CE =**Chloroform Extract; **EE =**Ethanolic Extract.

Data represents mean ±SD; one-way of analysis of variance, ANOVA followed by Tukey's multiple Comparison Test (*n*=5), values are compared with control animals, $p<0.05$. *P<0.01, **P<0.001, ***P<0.0001.

Table 3. Effect of C. procera Leaves Extract on Tail Flick Latent Period in Rats

Group	Treatment	Dose (mg/kg)	Mean latent period in rats		
			Initial	After 30 min	After 60 min
I	Control	0.1 ml/10g	8.00±0.71	8.2±0.84	8.40±0.55
II	Pentazocin	10mg/kg	8.20±0.84	16.60±1.34***	18.60±1.14***
III	*Calotropis procera***(PEE)**	100mg/kg	8.00±0.71	9.80±0.84	11.60±1.14***
IV	*Calotropis procera***(PEE)**	200mg/kg	7.80±1.92	11.60±1.14**	13.40±1.14***
V	*Calotropis procera***(PEE)**	500mg/kg	7.60±1.14	15.40±1.14***	16.40±1.14***
VI	*Calotropis procera***(CE)**	100mg/kg	7.95±0.72	8.90±0.80	10.55±1.24***
VII	*Calotropis procera***(CE)**	200mg/kg	7.55±1.05	10.45±1.25**	15.25±1.25***
VIII	*Calotropis procera***(CE)**	500mg/kg	7.40±1.11	14.74±1.03***	15.23±1.79***
IX	*Calotropis procera***(EE)**	100mg/kg	8.25±0.92	10.05±0.55	12.00±1.25***
X	*Calotropis procera***(EE)**	200mg/kg	7.25±1.20	10.25±1.20**	12.04±1.36***
XI	*Calotropis procera***(EE)**	500mg/kg	8.74±1.12	16.35±1.20***	15.35±1.25***

Data represents mean±SD; one-way of analysis of variance, ANOVA followed by Tukey's multiple Comparison Test (*n*=5), values are compared with control animals, $p<0.05$. *P<0.01, **P<0.001, ***P<0.0001.

PEE = Petroleum Ether Extract, CE =Chloroform Extract, EE =Ethanolic Extract

DISCUSSIONS

The acute toxicity study revealed safely of the extract up to a dose of 2000 mg/kg in mice. The ethanolic, petroleum ether and chloroform extract of leaves of *C. procera* at a dose of 100, 200 and 500 mg/kg was selected for analgesic activity.

Different extracts (Petroleum ether, chloroform and ethanolic) of leaves of *C. procera* were investigated for analgesic activity by using different different models. The analgesic properties were studied on acetic acid induced writhing and tail flick latent period in rats. The result shows that the ethanolic extract of the leaves of *C. procera* was found to be effective.

Preliminary phytochemical study of the petroleum ether, ethanolic and ethanolic extract of *Calotropis procera*, showed the presence of saponins, terpens, phenols, alkaloids, tannins and flavonoids.

Different solvents (Petroleum ether, chloroform and ethanolic) are used for extraction of leaves of *C. procera* & were subjected for phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, cardiac glycosides, tri-terpenoids, phenolic compounds and tannins in the *C. procera* extract.

REFERENCES

1. Bhutada P, Mundhada Y, Bansod K, Dixit P, Umathe S, Mundhada D. Anticonvulsant activity of berberine, an isoquinoline alkaloid in mice. *Epilepsy Behav*, 3, 2010, 207-210.
2. Bum EN, Taiwe GS, Nkainsa LA, Moto FC, Seke Etet PF, Hiana IR, Bailabar T, Rouyatou Seyni P, Rakotonirina A, Rakotonirina SV. Validation of anticonvulsant and sedative activity of six medicinal plants. *Epilepsy Behav*, 14, 2009, 454–458.
3. Carlini EA, Plants and the central nervous system. *Pharmacol. Biochem. Behav*, 3, 2003, 501–512.
4. Chen Y, Wang HD, Xia X, Kung HF, Pan Y, Kong LD. Behavioral and biochemical studies of total furocoumarins from seeds of Psoralea corylifolia in the chronic mild stress model of depression in mice. *Phytomedicine*, 14, 2007, 523–529.
5. Anonymous, The Wealth of India, New Delhi, India: *National Institute of Scientific and Industrial Research*, 3, 1998.
6. Kirtikar K, Basu BD. Indian Medicinal Plants. 7th ed. Dehradun: International book distributors; 2001.
7. Pathak K and Argal A. CNS activity of Calotropis gigantea roots. *J. Ethnopharmacol*, 106, 2006, 142–145.
8. Chitme HR, Chandra R, Kaushik S. Studies on anti-diarrhoeal activity of Calotropis gigantean r.br. in experimental animals. *J. Pharm. Pharmaceut. Sci*, 7, 2004, 70–75.
9. Pathak AK, Argal A. Analgesic activity of Calotropis gigantea flower. *Fitoterapia*, 78, 2007, 40-42.
10. Srivastava SR, Keshri G, Bhargavan B, Singh C, Singh MM. Pregnancy interceptive activity of the roots of Calotropis gigantea Linn. in rats. *Contraception*, 75, 2007, 318–322.
11. The Wealth of India, A Dictionary of Indian Raw Materials and Industrial Products, Publication and Information Directorate, Council of Scientific and Industrial Research Publication (CSIR), New Delhi 1992.
12. Lhinhatrakool T, Sutthivaiyakit S. 19-nor- and 18, 20-epoxycardenolides from the leaves of *Calotropis gigantea. J. Nat. Prod*, 69, 2006, 1249–1251.
13. Kumar VL, Arya S. Medicinal uses and pharmacological properties of Calotropis procera. In: Govil, J.N. (Ed.), *Recent Progress in Medicinal Plants*, 11, 2006, 373-388.
14. Samvatsar S, Diwanji VB. Plant sources for the treatment of jaundice in the tribals of Western Madhya Pradesh of India. *Journal of Ethnopharmacology*, 73, 2000, 313–316.
15. Basu A, Sen T, Ray RN, Nag Chaudhuri AK. Hepatoprotective effects of Calotropis procera root extract on experimental liver damage in animals. *Fitoterapia*, 63, 1992, 507–514.
16. Kumar VL, Basu N. Anti-inflammatory activity of the latex of *Calotropis procera*. *Journal of Ethnopharmacology*, 44, 1994, 123-125.
17. Dewan S, Sangraula H, Kumar VL. Preliminary studies on the analgesic activity of latex of Calotropis procera. *Journal of Ethnopharmacology*, 73, 2000a, 307–311.
18. Dewan S, Kumar S, Kumar VL. Antipyretic effect of latex of Calotropis procera. *Indian Journal of Pharmacology*, 32, 2000b, 252.
19. Arya S, Kumar VL. Antiinflammatory efficacy of extracts of latex of Calotropis procera against different mediators of inflammation. *Mediators of Inflammation*, 2005, 228–232.
20. Roy S, Sehgal R, Padhy BM, Kumar VL. Antioxidant and protective effect of latex of Calotropis procera against alloxan-induced diabetes in rats. *Journal of Ethnopharmacology*, 102, 2005, 470–473.
21. Choedon T, Mathan G, Arya S, Kumar VL, Kumar V. Anticancer and cytotoxic properties of the latex of Calotropis procera in a transgenic mouse model of hepatocellular carcinoma. *World Journal of Gastroenterology*, 2 (12), 2006, 2517–2522.
22. Mossa JS, Tariq M, Mohsin A, Aqeel A, Al-Yahya MA, Al-Said MS, Rafatullah S. Pharmacological studies on aerial part of Calotropis procera. *Am J Chin Med*, 19, 1991, 223–231.