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Phytochemical Investigation and Analgesic Activity of *Calotropis Procera*

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Abstract

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*, Pain, Analgesic Activity, Ethanolic Extract, Pentazocine.

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1. Introduction

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

C. procera or sweet akand, belonging to the family Asclepiadaceae, is native to India and grows well in the lower hills at 900m altitude [5,6]. Different parts of *Calotropis procera* are reported to be used for the 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 constituents isolated from the plant include β-amyrin and its Isovalerate, a and b-calotropeols, a mixture of tetra cyclic triterpene, traces of sterols, C31 and C33 hydrocarbons, fatty acids, giganteol, cardiac glycosides, calotropin, uscharin, calotoxin, uscharidin and gigantin [11]. The leaves of *C. Procera* are reported to contain taraxasterly acetate, pinoresinol, medioresinol, uzarigenin, calotropin, calactin, calacitnic acid, calacitnic acid methyl ester, 19-carboxyl-calacitnic methylester, drummondol, 15b-hydroxycalotrin, the C11 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 the Indian traditional system of medicine for the treatment of leprosy, ulcers, tumours, piles and diseases of the spleen, liver and abdomen. [6] The root of the plant is used as a carminative in the treatment of dyspepsia [13]. Further, the rootbark 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 the inflammatory response elicited by various inflammatory mediators [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 the 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*.

Medicinal plants have been used in traditional healthcare system throughout human history and are considered as a source of healthy human life. Different parts of the plants, like roots, leaves, stem, bark, fruits and seeds have been used in combating infection and strengthening the immune system. *Calotropis procera* is a potential medicinal plant highly valued for its characteristic aroma and bioactive compounds.

The literature survey revealed that *Calotropis procera* have been studied extensively because of its ready accessibility, diverse biological activities like analgesic, anti-HIV, and anti-inflammatory, anti-oxidants and analgesic activities. Thus, it was decided to phyto-chemical investigation of *Calotropis procera* and evaluate them for anti-diarrheal activities.

2. Materials and Methods:

2.1 Plant materials:

C. procera were obtained locally from sub-urban hills of District Bhopal of Madhya Pradesh. They were authenticated by the Department of Botany, Saifia College, Bhopal (M.P.) (India) and were given a specimen no. (Specimen/05/2022).

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.

2.2 Drugs and Chemicals

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

2.3 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).

2.4 Animals

Wistar rats weight 150-200 g and albino mice weight 18-30 g were used. The animals were obtained from A**nimal** House Facility, College of Pharmacy, RKDF College of Pharmacy, Bhopal. Animals were randomized and allocated to different treatment groups (5 per group). Animals were kept at a temperature of 24 ± 2^{o}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/89/2022).

2.5 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.

2.6 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.

2.7 Analgesic Activity

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

2.8 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* (indifferent doses) were compare against the standard analgesic aspirin (200mg/kg). The number of writhes produced in these animals was counted for 30 min.

2.9 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 withdrawal 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 as30s 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 administered i.p. either control vehicle *C. procera* extract (in different doses) or pentazocin was used as a reference standard and were tested 30 min later.

2.10 Statistical Analysis:

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

3. Results and Discussion

In this work, a methodology for the validation of medicinal plants with analgesic activity has been applied, specifically one used to investigate chronic pain activity, as the search for new compounds of natural origin with analgesic activity is important in the Western world.

3.1 Extraction of Plant Materials

The percentage yield of the successive extraction of *Calotropis procera* leaves are presented in Table 1.

3.2 Phytochemical Screening:

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

3.3 Acute Toxicity Study:

The acute toxicity study revealed safely of the extract up to a dose of 2000 mg/kg dose level in mice. So that $1/20^{th}$, $1/10^{th}$ and $1/4^{th}$ (i.e. 100 mg/kg, 200 mg/kg and 500 mg/kg orally) was selected for analgesic activity. For analgesic activity of petroleum ether extract, chloroform extract and ethanolic extract of leaves of *C. procera* was prepared in distilled water for oral route of administration.

3.4 Analgesic Activity:

One way ANOVA revealed a significant effect of all treatment groups in acetic acid induced writhing testas 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.

3.5 Acetic acid-induced writhing response

The first study showed that the application of different doses of extracts had significant analgesic effects in the animal study. The results of doses 200 and 500 mg/Kg were significant and comparable with the effect of aspirin in analgesic activity (Table 4).

3.6 Tail Immersion Method

The results of the tail immersion test in mice are presented in Table 6.6. 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 1: Description of different extracts of *Calotropis procera* leaves.

Extracts	Colour	Odour	Consistency	%Yield(w/w)
Petroleum Ether Extract	Faint Yellow	Characteristic	Dry	3.5
Chloroform Extract	Dark Yellow	Characteristic	Dry	3.8
Ethanol Extract	Faint Yellow	Characteristic	Sticky	9.27

Table 2: Estimation of Phytochemical analysis of different extract of *C. procera*

Sr. No	Chemical Test		PEE	CE	EE
1	Test for Alkaloids	Hager'sTest	-ve	-ve	-ve
		Mayer'sTest	-ve	-ve	-ve
		Dragendroff'sTest	-ve	-ve	+ve
		Wagner'sTest	-ve	-ve	-ve
2	Test for carbohydrates	Molisch'sTest	+ve	+ve	-ve
		Fehling'sTest	-ve	-ve	+ve
		Barford'sTest	-ve	-ve	-ve
		Benedict'sTest	-ve	-ve	+ve
3	Test for cardiac glycosides	Baljettest	-ve	-ve	+ve
		Legaltest	-ve	-ve	+ve
4	Test for Anthraquinone glycosides	Modified Borntrager's test	-ve	-ve	-ve
		Borntrager'stest	-ve	-ve	-ve
5	Test for saponins glycosides	Foamtest	-ve	-ve	+ve
		Hemolytic test	-ve	-ve	-ve
6	Test for fixed oil	StainTest	+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	Leadacetate solution	-ve	-ve	-ve
		5% Fecl3 solution	-ve	-ve	-ve

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

Table 3: Preliminary acute toxicity levels of crude extracts.

Group	Extracts Dose Levels (mg/kg)			N	N^0dead PEE	N^0dead CE	N^0dead EE
	PEE	CE	EE				
Group1	100	100	100	6	0	0	0
Group2	200	200	200	6	0	0	0
Group3	500	500	500	6	0	0	0
Group4	1000	1000	1000	6	0	0	0
Group5	2000	2000	2000	6	1	2	1
Control	1mldH2O	1mldH2O	1mldH2O	6	0	0	0

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

Table 4: 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**=Ethanol Extract

Data represent 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 5: Effect of *C. procera* leaves extract on tail flick latent period in rats

Group	Treatment	Dose (mg/kg)	Mean latent period in rats		
			Initial	After30min	After60min
I	Control	0.1ml/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***

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.

5. Conclusion:

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.

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