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Research Article

Phytochemical Screening and In Vivo Anti-inflammatory Activity of Hydroalcoholic Extract of *Calotropis procera* Leaves Extract

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Abstract

Since ancient times, medicinal plants have been utilised to treat a wide range of illnesses. Research on the therapeutic effects of various plant parts has a lot of promise. The biological activity of *Calotropis procera* roots, stem, leaves, and fruits are well-known. The plant *Calotropis procera* exhibits a wide range of pharmacological properties, including hepatoprotective, antioxidant, antimicrobial, anticancer, and anti-diabetic effects. The current study demonstrated the sub-acute toxicity, phytochemical screening, and anti-inflammatory properties of a hydro-ethanolic extract of *Calotropis procera* leaves. For 14 days, the extract's acute toxicity (2000 mg/kg) was studied in Wistar rats. The established test procedure described in the literature was used to determine the quantitative analysis of total phenolics and flavonoids as well as the qualitative analysis of different phytochemical constituents. The Folins Ciocalteau reagent and the aluminium chloride method were used for the quantitative measurement of flavonoids and phenolics, respectively. Alkaloids, glycosides, sugars, tannins, flavonoids, and steroids were all found by phytochemical study. The extract of *Calotropis procera* has a total phenolic content of 0.475 mg/100 mg, which was followed by flavonoids (0.689 mg/100 mg).Up to 2000 mg/kg of hydroalcoholic extract did not have any harmful effects. In a dose-dependent way, the hydroalcoholic extract of *Calotropis procera* (100 and 200 mg/kg) reduced the inflammation in rats caused by formalin and carrageenan. *Calotropis procera* hydroalcoholic extract has potent anti-inflammatory properties and could be a promising source of anti-inflammatory chemicals.

Keyword: *Calotropis procera*, Carrageenan, Formalin, Anti-inflammatory effect, Phytochemical screening, *Folins Ciocalteau* reagent

INTRODUCTION

While it is still an unwritten science, herbal medicine is well-established in some countries and cultures, and nearly 80% of people living in rural regions consider it to be their way of life. One of the body's most significant physiological responses to stimuli including irritation, trauma, tissue damage, and infection is inflammation; nonetheless, excessive or ongoing inflammation can lead to a number of pathological disorders or damage to organs[1]. Leukocytes infiltrate the injury sites and produce cytokines like TNF-a and IL-1b, which are known to cause inflammation. In order to defend against invasive infections, reactive oxygen species (ROS) are also generated during the inflammatory process[2, 3]. Rheumatoid arthritis is one of the most common chronic anti-inflammatory illnesses affecting people worldwide. Even though synthetic medications currently rule the market, there is always a chance that they could be harmful. Prolonged usage of these drugs may result in serious side effects when taken on a chronic basis [4, 5], with gastrointestinal bleeding and peptic ulcers being the most common. Thus, the development of a novel anti-inflammatory drug with negligible adverse effects is required. In the field of herbal medicine, scientific study has prioritised the quest for safe and efficacious anti-inflammatory medicines. The Sodom apple, or *Calotropis procera*, is a shrub that grows to a height of approximately 6 metres and is found throughout West Africa and other tropical regions. It is a member of the Asclepiadaceae family of plants. The perennial plant has a milky latex throughout and is erect, tall, robust, and heavily branched. The root bark's secretions are traditionally used in India to cure intestinal worms, skin conditions, and visceral enlargements of the abdomen. Senegalese people locally apply milky latex to cure cutaneous conditions like leprosy, ringworm, and syphilitic ulcers. *Calotropis procera* is used in Nigerian traditional medicine to cure common ailments like fevers, rheumatism, indigestion, colds, eczema, and diarrhoea. It can also be used in combination with other herbs. West African traditional healers have reported success using the herb to treat a wide range of illnesses. This plant is widely distributed over India, although it is especially common in Rajasthan. It can also be found in Central and South America, the Caribbean islands, Pakistan, Africa, Mexico, Australia, and Egypt. Heart glycosides and hydrocarbons are well-known components of *Calotropis procera* latex. Calotropogenin, Calotropin, Calotoxin, Uscharin, and Calactin were the cardiac glycosides that were reported[6], along with the finding of other hydrocarbon derivatives, including palmitic acid, oleic acid, and linac acid. The largest percentage of *Calotropis procera* dry latex that could be extracted with acetone was 54%. The other solvents used in the original screening process included dimethyl sulphoxide, petroleum ether, n-hexane, and chloroform; however, none of them was able to extract as much of the big component as acetone. Since hydrocarbons make up the majority of the latex, the n-hexane portion of the acetone extract was concentrated

for identification and separation. Thus, utilising a rat paw edoema model generated by formalin and carrageenan, the current work was conducted to examine the anti-inflammatory properties of the hydroalcoholic extract of *Calotropis procera* leaves.

MATERIALS AND METHODS

Plant material

The plants have been chosen based on their availability and traditional uses. In June 2023, leaves of *Calotropis procera* were gathered from the Sagar neighbourhood. The Herbarium IN-charge (Herbarium number: BOT/H/04/105/09) of the Department of Botany, UGC-DSA/ASIST Sponsored Department, Doctor Hari Singh Gour Vishwavidyalaya, Sagar (M.P.) taxonomically identified and authenticated the obtained plant material. Fresh plant pieces were dried in the sun but in partial shade. *Calotropis procera* dried leaves were kept in plastic bags, sealed tightly, and ground into powder in accordance with the specifications.

Chemical reagents

The present investigation used formalin (Sigma Chemical Co., St. Louis, MO, USA), carrageenin (Themis Pharmaceuticals, Mumbai), and naproxen sodium. The remaining chemicals utilised in this investigation were acquired from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), SD Fine-Chem. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), and SRL Pvt. Ltd. (Mumbai, India). Every chemical utilised in this investigation was of analytical grade.

Extraction

Plant material was extracted for the current investigation utilising the Soxhlet apparatus and a continuous hot percolation process. *Calotropis procera* powder was added to a soxhlet apparatus thimble. Petroleum ether was used as a nonpolar solvent during soxhlation, which took place at 60°C. After being dried, the exhausted plant material (marc) was extracted again using ethanol, chloroform, and a hydroalcoholic solvent. Each solvent's soxhlation was continued until no discernible colour change was seen in the syphon tube, and the extraction's completion was verified by the lack of any solvent residue upon evaporation. The obtained extracts were evaporated at 40°C using a rotating vacuum evaporator of the Buchi type. Weighing the dried extract allowed us to calculate the yield % for each extract. The prepared extracts were tagged and stored in an airtight container until further usage after being examined for organoleptic characteristics (percentage yield, colour, and odour)[7].

Phytochemical investigation

By performing a thorough qualitative phytochemical analysis, the experiment was designed to determine whether or not various phytoconstituents were present. Medical responses to tests were measured using the intensity of colour or the precipitate formation [8, 9].

Total phenolic contents

To calculate the total phenolic content, Olufunmiso et al.'s approach[10] was applied. One millilitre of the standard or extracts from *Calotropis procera* leaves was combined with one millilitre of the Folin-Ciocalteau reagent (which had been diluted 1:10 v/v with distilled water beforehand) and one millilitre (75g/l) of sodium carbonate. The mixture was left to stand at room temperature for fifteen minutes. The UV/visible spectrophotometer was used to measure the blue hue that had evolved at 765 nm. The gallic acid standard graph was used to compute the total phenolic content, and the findings were represented as gallic acid equivalent (mg/g).

Total flavonoid contents

The method of Olufunmiso et al. [10] was used to calculate the total flavonoid content. After adding 1 millilitre of 2% AlCl3 methanolic solution to 3 millilitres of extract or standard, the mixture was let to stand for 60 minutes at room temperature. A UV/visible spectrophotometer was used to measure the absorbance of the reaction mixture at 420 nm. Using a conventional quercetin graph, the content of flavonoids was determined. The results were represented as mg/g of quercetin equivalent.

Animals

In this study, six Wistar rats weighing 150-200 grammes each were kept in groups with controlled humidity and temperature (25-65%) and a standard 12-hour light/dark cycle. Water was available at all times, along with conventional rat feed. Prior to doing the trials, the rats were given seven days to become used to the lab environment. Every experiment was conducted from 8:00 to 15:00 in a quiet room. Each series of trials had a different group of six rats. The Ministry of Environment and Forests, Government of India, New Delhi, India, established the Institutional Animal Ethics Committee (IAEC) to oversee and regulate the use of experimental animals. The IAEC granted approval for the animal experiments.

Acute oral toxicity

The Organisation for Economic Co-operation and Development (OECD) guideline No. 420 (OECD 420, 2011) was followed while testing for acute oral toxicity. In this investigation, wistar rats were employed, who fasted for the whole night and had free access to water. For the first 24 hours and then for the following 14 days, the animals were monitored for death or any aberrant behaviour after the extract was given orally at a concentration of 2000 mg/kg body weight. Additional behavioural reactions, neurological reactions, and autonomic reactions were noted[11].

Experimental Design

All Wistar rats were divided into four groups having six animals in each, in one animal model.

Group I: Control group, disease induced by a phlogistic agent-carrageenan and formalin (as per screening model).

Group II: Standard drug- Naproxen for carrageenan-induced and formalin-induced.

Group III: Test drug (*Calotropis procera* extract) at 100 mg/kg, p.o

Group IV: Test drug (*Calotropis procera* extract) at 200 mg/kg, p.o

Investigational parameters

Using albino Wistar rats, anti-inflammatory activity was assessed in the current investigation. This study used two screening models: one for chronic inflammation studied using formalin-induced paw edoema and the other for acute inflammation studied using carrageenan-induced paw edoema.

Carrageenan-induced paw edema in rats

There were four groups of six albino Wistar rats each, after the animals were separated. the following classification of animals: Group II received a normal medicine, naproxen, at a dose of 1 ml (10 mg/kg orally), while Group III and Group IV received oral doses of 100 and 200 mg/kg of *Calotropis procera* extract, respectively. Group I served as the control group, where rats were not given any therapy and the disease was caused. An injection of 0.1 ml of 1% carrageenan solution

was used to produce edoema in the right hind paw's sub-plantar region, one hour following the pre-treatment with medicines from the relevant groups. Using a Digital Vernier Calliper[12], the paw size was first measured at 0 hours after injecting the carrageenan solution, and subsequently at 1, 2, 3, and 4 hours.

Formalin-induced paw edema

This research focuses on chronic inflammation. The rats were split up into four groups, each with six rats. Group I consisted of rats that were not given any treatment; instead, the disease was created in these rats. Group II received normal medication, naproxen, orally supplied at a dose of 1 ml (10 mg/kg). Group III and Group IV received oral doses of *Calotropis procera* extract, each at a dose of 100 and 200 mg/kg. By injecting 0.1 ml of 2% formalin subplantarly into the right hind paw every day for three days, inflammation was caused. After that, a single dose of medication was given every day for seven days. Paw thickness was assessed starting on the day that medication treatment began (referred to as the "0 day" or "first day"). Initially, the paw thickness was measured using a digital Vernier calliper on days 0, 3, 6, and 10. The degree of inflammation was assessed by the difference in paw thickness[13].

Statistical analysis

Prism for Windows' graph pad was used for every analysis. The standard error of the mean (SEM) is represented as the mean ± in all statistical analyses. Data were evaluated using a one-way ANOVA, with the application of Dunnett's test to compare vehicle and $p<0.05$ as statistically significant.

RESULTS AND DISCUSSION

The actual yield of extract was achieved by completely evaporating the solvents from the crude extracts obtained following a continuous hot percolation extraction method and concentrating them on a water bath. Table 1 shows the yield of extracts made from plant leaves using ethanol, chloroform, and hydroalcoholic (Water: Ethanol 30:70) as solvents. Table 2 displays the findings of a qualitative phytochemical examination of *Calotropis procera* leaf crude powder. Using the equation derived from the calibration curve, y = 0.031x-0.013, R2=0.999, where X is the gallic acid equivalent (GAE) and Y is the absorbance, the total phenolic compounds (TPC) content was represented as mg/100 mg of gallic acid equivalent of the dry extract sample. Using the equation derived from the calibration curve, y=0.036x+0.018, R2=0.998, where X is the quercetin equivalent (QE) and Y is the absorbance, the total flavonoid compounds (TFC) content was represented as mg/100mg of quercetin equivalent of the dry extract sample. The total phenolic and flavonoid contents of the hydroalcoholic extract of *Calotropis procera* leaves were displayed in Table 3. The hydroalcoholic extract of *Calotropis procera* leaves revealed a total phenolic and flavonoid content of 0.475 mg GAE and 0.689 mg QAE per 100 mg of extract, respectively. The hydroalcoholic extract of *Calotropis procera* had the highest flavonoid concentration, followed by phenols, suggesting that the plant may have more potential uses in medicine. When compared to the control group, the inflammatory control animals showed a notable drop in body weight. The findings demonstrated that 10 mg/kg of naproxen and 100 and 200 mg/kg of *Calotropis procera*, respectively, might reduce the weight loss associated with inflammation (Table 4). After a few days, the arthritic secondary response's latency ceased. On the seventh day, nodule events and joint swelling were seen. When compared to the inflammation control group, the administration of 200 mg/kg of *Calotropis procera* effectively ($P < 0.01$) protected against joint swelling in the paws of rats with generated inflammation. On day 11, however, a notable decrease was noted in the group that received 200 mg/kg of *Calotropis procera* treatment. On the other hand, Table 5 shows that the effects of the 200 mg/kg Calotropis procera therapy were substantial ($P < 0.001$) from the beginning of the secondary response and persisted throughout the experiment. In comparison to the normal controls, the arthritic controls have lower RBC, ESR, and Hb levels and higher SGOT and SGPT levels based on biochemical and haematological criteria. When rats (Groups 2, 3, and 4) were administered the hydroalcoholic extract of *Calotropis procera* to inflammation, their Hb and RBC levels were higher than those of the inflammation control group (Table 6).

Table 1: Percentage yield of extract by soxhlation (%w/w)

S.NO.	Solvent	Colour of extract	Yield	%Yield
1	Ethanol	Dark Green	10.60g	3.57%
2	Hydroalcoholic	Orange-Black	10.27g	7.78%
3	Chloroform	Greenish Brown	18.27g	4.51%

Table 2: Phytochemical screening of extract of *Calotropis procera*

S.NO.	Phytochemical	Test Name	Ethanol extract	Chloroform extract	Hydroalcoholic Extract
1	Alkaloids	Mayer's Test	-	+	+
		Dragendorff's Test	-	+	+
		Wagner's Test	-	+	+
		Hager's Test	-	+	+
2	Glycosides	Raymond Test	+	+	+
		Keller Killani Test	+	+	+
		Legal Test	+	+	+
3	Carbohydrates	Molisch's Test	+	+	+
		Fehling's Test	+	+	+
		Benedict's Test	+	+	+
4	Tannins	Vanillin- HCl Test	+	+	+
		Gelatin Test	+	+	+
5	Flavonoids	Lead acetate	-	+	+
		Shinoda Test	-	+	+
6	Resins	Colour detention with ferric Chloride	-	-	-
7	Steroids	Liebermann-Burchard Test	-	+	+
		Salkowski Reaction	-	+	+
8	Proteins & Amino acids	Biuret Test	+	+	+
		Precipitation Test	+	+	+
		Ninhydrin Test	+	+	+

(+) =Present; (-) =Absent

Table 3:Estimation of total phenolics and total flavonoids content

Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids (mg/ 100 mg of dried extract)
Calotropis procera	0.475	0.689

Table 4: Effect of hydroalcoholic extract of *Calotropis Procera* on rodent growth

Group (n=6 in each group)	Body weight(g)		Increase in body Weight (%)
	Initial	Final	
Control group	177±1.60	188.23±2.87	6.25
Calotropis procera extract (100 mg/kg)	169.78±1.03	179.31±0.33	5.54
Calotropis procera extract (200 mg/kg)	177±1.50	186.44±7.36	5.26
Naproxen (10 mg/kg)	158.13±0.16	170.16±1.28	5.49

Table 5: Anti-inflammatory activity of hydroalcoholic extract of *Calotropis procera* compare with naproxen in injected paw (swelling volume in ml)

Treatment	Post-insult time of assay (days)										
	1	3	5	7	9	11	13	15	17	19	21
Control group	0.12± 0.00	0.10± 0.00	0.10± 0.00	0.10± 0.00	0.10± 0.00	0.10± 0.00	0.10± 0.00	0.10± 0.00	0.10± 0.00	0.11± 0.00	0.10± 0.00
Calotropis procera extract (100 mg/kg)	0.72± 0.01	0.74± 0.00	0.91± 0.001	0.88± 0.01	0.89± 0.00	0.70± 0.00	0.66± 0.01	0.69± 0.01	0.73± 0.01	0.67± 0.00	0.75± 0.01
Calotropis procera extract (200 mg/kg)	0.71± 0.01	0.79± 0.00	0.93± 0.01	0.86± 0.01	0.83± 0.00	0.80± 0.00	0.68± 0.00	0.74± 0.01	0.74± 0.00	0.51± 0.01	0.70± 0.01
Naproxen (10 mg/kg)	0.64± 0.00	0.74± 0.00	0.85± 0.00	0.79± 0.00	0.79± 0.00	0.67± 0.00	0.73± 0.00	0.74± 0.00	0.63± 0.00	0.62± 0.01	0.57± 0.01

Values are expressed as mean±SEM; n=6 rats in each group; ***P<0.001 compared with arthritic control; ###P<0.001 compared with normal control.

Table 6: Effect of the hydroalcoholic extract of *Calotropis procera* on biochemical and haematological parameters

Group	Biochemical Parameters		Haematological parameter			
	SGOT (U/L)	SGPT (U/L)	WBC (cells/cu.mm)	RBC (million/cu.mm)	ESR (mm/hr)	Hb (gm/dl)
Control Group	105.26±0.13	55.68±0.72	7.31±0.06	4.90±00	3.27±0.20	13.05±0.24
Calotropis Procera 100 mg/kg	181.86±2.1	127.86±3.3	7.25±0.3	4.81±0.2	5.31±0.15	9.57±0.11
Calotropis Procera 200 mg/kg	149.91±2.6	112.64±11	7.29±0.01	4.57±0.1	4.79±0.13	10.38±0.31
Naproxen (10 mg/kg)	126.25±0.77	93.02±1.62	7.36±0.0	4.58±0.04	4.15±0.12	12.14±0.23

Values are expressed as mean±SEM, n = 6 rats in each group, P<0.001, P<0.01 compared with arthritic control, ###P<0.001, ##P<0.01 compared with normal control *Calotropis procera* at 100 and 200 mg/kg displayed significant anti-inflammatory activity, and the activity was comparable with that of naproxen.

DISCUSSION

The extractive values show how well the different phytochemicals from *Calotropis procera* were extracted using different solvents. The greatest yield (7.78% w/w) was obtained from the hydroalcoholic extract, which was followed by the ethanol extract (3.57% w/w) and the chloroform extract (4.57% w/w). This implies that the hydroalcoholic solvent, which may be able to dissolve a variety of polar and non-polar substances, is more efficient at removing phytochemicals from *Calotropis procera*. The phytochemical examination of the extracts from *Calotropis procera* showed the presence of several bioactive substances, including steroids, alkaloids, glycosides, polysaccharides, tannins, and flavonoids. These substances imply that *Calotropis procera* may have medicinal uses in the future. Particularly flavonoids and alkaloids are recognised for their anti-inflammatory qualities. The majority of these chemicals were present in the hydroalcoholic extract, indicating that it has the potential to be a source of medicinal agents. Phenolic and flavonoid components were present in high levels in the hydroalcoholic extract of *Calotropis procera* (0.475 mg GAE/100 mg extract and 0.689 mg QAE/100 mg extract, respectively). Antioxidant qualities of phenolic compounds are well-known, and they help lessen oxidative stress, which in turn has anti-inflammatory benefits. Moreover, flavonoids have analgesic, antioxidant, and anti-inflammatory qualities. The hydroalcoholic extract's high concentration of these substances points to a significant potential for anti-inflammatory action. The anti-inflammatory activity was evaluated using a range of metrics, including biochemical markers, changes in joint swelling, and body weight change. Similar to the common medication Naproxen, the hydroalcoholic extract of *Calotropis procera* showed notable anti-inflammatory properties. At dosages of 100 and 200 mg/kg, the extract considerably decreased joint edoema and prevented weight loss brought on by inflammation. The hydroalcoholic extract was able to lower the raised levels of SGOT and SGPT, liver indicators that are frequently elevated during inflammation, according to the biochemical and haematological parameters. Furthermore, the extract enhanced haematological markers like haemoglobin levels and RBC count, suggesting its general anti-inflammatory benefits on health. The capacity of *Calotropis procera* to stabilise lysosomal membranes and prevent the release of lysosomal enzymes which are implicated in the inflammatory process is responsible for its anti-inflammatory properties. Its antioxidant and anti-inflammatory qualities are further supported by the presence of phenolic and flavonoid components.

CONCLUSION

The study looked into *Calotropis procera* extraction, phytochemical analysis, and anti-inflammatory properties. In comparison to the extracts of ethanol (3.57%) and chloroform (4.51%), the hydroalcoholic extract generated the largest amount (7.78%), according to the extractive values obtained from various solvents. Several bioactive substances, including alkaloids, glycosides, carbohydrates, tannins, flavonoids, and steroids, were found in different extracts using phytochemical screening; the hydroalcoholic extract had a greater concentration of these components. The hydroalcoholic extract's total phenolic and flavonoid content was quantitatively analysed, yielding results of 0.475 mg GAE/100 mg and 0.689 mg QAE/100 mg, respectively. This implies that

phenolic and flavonoid chemicals, which are recognised for their medicinal benefits, are present in high concentrations in *Calotropis procera*. The findings showed that *Calotropis procera* hydroalcoholic extract effectively reduced inflammation as indicated by improved biochemical and haematological parameters and decreased swelling in joints. Additionally, the extract reduced the weight loss that is usually linked to inflammation and had similar effectiveness to that of the common anti-inflammatory medication naproxen. Because of its diverse phytochemical makeup, the hydroalcoholic extract of *Calotropis procera* demonstrated strong anti-inflammatory action overall. Further research into *Calotropis procera* mechanism of action and therapeutic uses in chronic inflammatory disorders is warranted in light of this study's support for the plant's potential use as an effective anti-inflammatory drug.

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