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ORIGINAL RESEARCH ARTICLE

Analgesic and Anti-inflammatory Properties of *Salix alba* Linn and *Calotropis procera* (Aiton) Dryand

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ABSTRACT

The methanolic extracts of *Salix alba* Linn and *Calotropis procera* (Aiton) Dryand were tested for the analgesic and anti-inflammatory effects in white albino mice. The acetic acid writhing response in mice was used to assess analgesic activity and plethysmometric measurement of formalin-induced paw edema was used to evaluate the anti-inflammatory activity of both the plant extracts. The extracts exhibited a dose-dependent analgesic action. *S. alba* extracts showed more potency than the standard drug aspirin in all the doses tested and *C. procera* extract showed prominent response in comparison to aspirin at doses as high as 62.5 mg/kg of plant extracts. The result also indicates that the extracts inhibited the paw edema size and shows inhibition of the inflammation (*$p<0.05$) well above the inhibitory effect of the aspirin (100mg/kg i.p). Acute toxicity tests showed that both of the plant extracts had no significant toxicity up to the dose of 125 mg/kg body weight. Phytochemical screening showed the presence of alkaloids, tannins and glycosides in both the plants. The results indicated that both of the extracts possesses significant analgesic as well as anti-inflammatory properties.

Key words: *Salix alba, Calotropis procera,* analgesic, anti-inflammatory, plethysmometr.

INTRODUCTION

The plant constituents have been the most successful source of medicine for their implementation in the discovery of drugs. The thousands of species of plants with counter less number of such natural substances have found their way as potential drugs into market. The plants have been proven very rich sources of structurally and biologically interesting metabolites. Since less than 10% of the world's biodiversity has been evaluated for potential biological activity, many more useful natural lead compounds await discovery. *Salix alba* Linn and *Calotropis procera* (Aiton) Dryand are plants with proven therapeutic activity and used in Nepal in the traditional medicines [1]. *C. procera* has been reported to have anti-inflammatory, anti-diarrheal, analgesic [2], anti-fertility and anti-ulcer [3], properties. *S. alba* has been reported to have anti-pyretic and analgesic effects. Herbal formulations of traditional medicines are available either in single drug or in combined form with multi-drugs which are being popular in comparison to modern drugs. Synergism plays a major role in therapeutic efficacy of herbs or herbal formulation. The effective concentration of ingredients in combination can be significantly reduced or the effects of ingredients in combination are significantly increased with respect to that of each individual ingredient in a particular therapy. This cannot be easily distinguished from polyherbal additive effects and usually rely on high margins of variation [4]. Synergy between different herbs in polyherbal formulation is shown pharmacological activity by a combination of *Urtica dioica*, and *Pygeum africanum*, which is taken for benign prostate hyperplasia and combination of both inhibits 5-reductase and aromatase more significantly than the sum of either alone [5]. A randomized, placebo-controlled trial of the efficacy of a standardized single herbal extract of *Salix alba*

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bark for osteo-arthritis of the hip and knee, was carried out recently. The study, in mainly elderly patients, was carried out in a hospital environment and measurement of pain assessed by both the patient and physician. The efficacy was confirmed, but the results went beyond that and synergy was implicated. Interestingly, the gastrointestinal side effects commonly encountered with non-steroidal anti-inflammatory drugs such as aspirin were not seen at the doses used, although it is usually assumed that willow bark is effective due to its salicin content [6]. Thus, some herbal medications may produce a more favorable response when an extract is given versus an isolated single constituent. However, the advantages of single constituents versus extracts should be considered on a case-by-case basis. There is no report on their synergistic effects especially on analgesic and anti-inflammatory properties of the Nepalese plants. The objective of the present study is to evaluate the analgesic and anti-inflammatory properties of *Salix alba* Linn and *Calotropis procera* (Aiton) Dryand from Nepal and their combining effects on mice.

MATERIALS AND METHOD

Plant materials

The plants were collected from Dhulikhel and Panchkhal in March 2013. The authentication of the plant samples have been confirmed by the Tirtha Maya Shrestha full time faculty of the Department of Pharmacy, Kathmandu University. The herbarium of both of the plants is deposited at Kathmandu University, Department of Pharmacy. The plant sample was dried in shade and then leaf and stem of *Calotropis procera* (Aiton) Dryand and bark of *Salix alba* Linn. were ground in coarse powder. The powders were then preserved in air tight plastic bags.

Fig 1: Barks of *S. alba*

Fig 2: Leaves and young shoots of *C. procera*

Extraction

The dried spices were washed thoroughly with sterile double distilled water to make these spices completely free from any possible contamination. Twenty gram of each dry sample was crushed and cold extraction using methanol was carried out for 7 days. The residues of two more extraction were again subjected to the extraction following exactly the same procedure for the complete extraction of metabolites. Phytochemical screening was also performed from these extracts by using the established method.

Experimental animals

Male Swiss mice (25–35 g) were kept in an automatically controlled temperature room (23±2 °C) in 12 h light–dark cycles, with freely available water and food. Animals were acclimatized to the laboratory for at least 2 h before testing and were used only once for experiments. These were performed after following the protocol by the Institutional Ethics Committee of the Kathmandu University and were carried out in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigation of experimental pain in conscious animals [7] . The number of animals and intensity of noxious stimuli used were the minimum necessary to demonstrate consistent effects of the drug treatments.

Chemicals and drugs

Tween-80 from Merck Specialities Private Limited (Germany) as suspending agent, Aspirin from Reckitt Benckiser (India) as standard drug and 0.5 % acetic acid were used as writhing inducer.

Preparation of test materials and standard

Different weights of crude extracts of *S. alba* and *C. procera* were taken and diluted with distilled water and tween-80 (1%) respectively to make

different doses. Aspirin tablet (350 mg) was dissolved in tween -80 (1%) and distilled water separately and volume was made to 35 ml by dilution. Distilled water for *Salix* extract and tween-80 (1%) for *Calotropis* extract were taken as control.

Analgesic and anti-inflammatory activities

Study was carried out by using standard published methods; acetic acid induced writhing method was used to investigate analgesic activity[8] and formalin induced hind paw edema was used for anti-inflammatory activity[9]. The experimental animals were randomly divided into five and three groups consisting of 5 mice each for analgesic and anti-inflammatory activity respectively. Prior administering the drugs, each mouse was weighed properly and the doses were adjusted accordingly. The test samples, control, and standard aspirin were administered 0.2 ml, i.p at the beginning of the experiment. After 30 minutes 0.7% acetic acid was injected i.p to each of the animals of all the groups. Immediately after injection of acetic acid, the animals were observed for a wave of contraction and elongation of abdominal musculature referred to as 'writhing and the number of writhing for the next 10 minutes were counted for each mouse. For anti-inflammatory test each mice were marked in their left hind paw up to a common level. After 30 minutes the hind paw volume was measured up to the marked level using plethysmometer (Medicaid PM-707 Delhi, E-16/17 Sector 8, Rohini, Delhi-110085). Immediately after that in the left hind paw of the mice 25 µl 1% formalin was injected in sub plantar region using micro syringe.

Statistical analysis

Data were expressed as mean±SEM. Statistical significance was determined via Dunnet test. $p<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Analgesic activity

C. procera extract was used to reuce the acetic acid-induced writhing responses to observe the extract's analgesic property in mice. Treatment with extract (62.50, 31.25, 15.62mg/kg) or Aspirin (100 mg/kg) resulted in an inhibition of the writhing number compared to the control. Similar pattern of analgesic activity has been also observed in the groups treated by *S. alba*.

Table 1: Effect of different extracts of *Calotropis procera* and drug groups on the writhing response in mice

Group	Total no of writhing	Mean ± SD	Percentage of protection
Control (1% Tween 80)	64	12.8 ± 2.16	-
62.50 mg/kg	4	0.8 ± 0.83*	93.75
31.25 mg/kg	32	6.4 ± 1.51*	50
15.62 mg/kg	60	12 ± 3.16*	6.25
Aspirin (100 mg/kg)	25	5 ± 1.87*	60.93

* $p < 0.05$, Dunnet test as compared to the control

Table 2: Effect of different extracts of *Salix alba* and drug groups on the writhing response in mice

Group	Total no of writhing	Mean ± SD	Percentage of protection
Control (Distilled Water)	137	27.4 ± 7.33	-
93.50mg/kg	3	0.6 ± 0.54	97.81
62.5 mg/kg	6	1.2 ± 0.89	95.62
46.87mg/kg	12	2.4 ± 1.51	91.24
Aspirin (100 mg/kg)	24	4.8 ± 0.83	82.48

** $p < 0.05$, Dunnet test as compared to the control

Table 3: Summary of effect of different combination of extracts of *Salix alba* L. and *Calotropis procera*,(Ait.) R. Br. extracts on the writhing response in mice.

Group	Total no of writhing	Mean ± SD	Percentage of protection
Control	98	19.6 ± 3.50	-
C1	5	1 ± 1	94.89
C2	8	1.6 ± 1.51	91.83
C3	26	5.2 ± 1.64	73.46

** $p < 0.05$, Dunnet test as compared to the control. C1= (31.25mg/kg *Calotropis procera* + 31.25 mg/kg *Salix alba)*, C2= (41.6mg/kg *Calotropis procera* + 20.9 mg/kg *Salix alba*), C3= (20.9 mg/kg *Calotropis procera* + 41.6 mg/kg *Salix alba*).

Anti-inflammatory activity

Formalin induced hind paw edema method was used for anti-inflammatory activity. Extracts from both *C. procera* and *S. alba* were given in various doses as given in table 5 and result was found significantly positive. The results have been obtained in carefully controlled experiments where the volume of inflammated paw was decreased due to treatment of plant extracts.

Table 4: Summary of Mice Paw Edema Test of *Calotropis procera,(Ait.) R. Br.* extract

Group	0 min	30 mins	60 mins	120 mins	180 mins	240 mins	300 mins
Control	0.172±0.019	0.206±0.025	0.244±0.054	0.254±0.036	0.25±0.054	0.266±0.039	0.312±0.019
Calotropis (62.5 mg/kg)	0.1688±0.021	0.20±0.037	0.218±0.04	0.18±0.017	0.19±0.015	0.218±0.010	0.256±0.015
Aspirin (100mg/kg)	0.174±0.018	0.192±0.026	0.214±0.015	0.214±0.011	0.234±0.041	0.252±0.0356	0.294±0.0167

** $p < 0.05$, Dunnet test as compared to the control

Table 5: Summary of Mice Paw Edema Test of *Salix alba* L. extract

Group	0 min	30 mins	60 mins	120 mins	180 mins	240 mins	300 mins
Control	0.134±0.023	0.17±0.023	0.216±0.013	0.21±0.018	0.248±0.044	0.27±0.049	0.27±0.086
Salix (93.5 mg/kg)	0.134±0.015	0.16±0.020	0.16±0.020	0.17±0.015	0.18±0.016	0.18±0.021	0.18±0.023
Aspirin (100mg/kg)	0.134±0.011	0.168±0.021	0.20±0.012	0.18±0.025	0.24±0.030	0.266±0.030	0.264±0.018

** $p < 0.05$, Dunnet test as compared to the control

Both the extracts showed dose dependent analgesic property and were more potent than Aspirin. The maximum % of inhibition for *C. procera* and *S. alba* were found at the doses of 62.5 and 93.5 mg/kg respectively. Formalin assay for anti-inflammatory activity shows the effect of drugs potency to utilize the drug as medicinal importance [10]. Formalin test has two phases; early or first phase (neurogenic pain) and delayed or second phase (inflammatory pain). It seems that early phase is induced by C-fiber activation and peripheral tissues and action change in dorsal root of spine are the main reasons of delay. The main reason of pain in the second phase is the inflammatory reactions [11] . The first phase occurs within an hour of injection and also due to the serotonin component [12]. Prostaglandins play a major role in the development of the second phase of reaction which is measured around 3 h time [13]. The presence of prostaglandin in the inflammatory exudates form the injected foot has been well demonstrated previously by other workers [14] . Results show that the extract of *Calotropis procera*, has maximum inhibitory effect during the second phase i.e after 3rd hour of injection of formalin. Aspirin also showed significant inhibition of edema towards the later phase which might be due to inhibition of prostaglandin mediated inflammation. The extract of *Salix alba* showed maximum inhibition of edema three hours after the injection of formalin. However significant inhibition is seen even one hour later. We may assume that *Salix alba* has effect upon both the phases.

Combining herbs creates a synergistic effect, which enhances the therapeutic properties of each herb, and also minimizes any possible negative properties. The synergestic effect on analgesic activity of both of the herbal formulatins, the formulation C1 is the most effective and showed synergestic response for analgesic effect. Similar results were also obtained in previous studies when the formulations of two herbs used [5].

Table 6: Determination of LD50 of plant extracts

Concentration (mg/kg)	% Mortality *of Salix alba*	% Mortality of *Calotropis procera*
62.5		0
125	0	0
250	0	100
500	100	100
1000	100	100

Calotropis procera LD_{50} 187.5 mg/kg , *Salix alba* LD50 = 375 mg/kg.

LD50 is a standard measurement of acute toxicity that is stated in milligrams (mg) of pesticide per kilogram (kg) of body weight. LD50 represents the individual dose required to kill 50 percent of a population of test animals. Therefore the values for inhalation toxicity are based on tests in laboratory animals which are given in the different doses of both of the plant extracts. The dose at which the analgesic and anti-inflammatory effect shown has been found is safe as found in the study of *Calotropis procera* (LD50 187.5 mg/kg) and *Salix alba* (LD50 = 375 mg/kg).

CONCLUSION

The methanolic extracts of *Salix alba* Linn. and *Calotropis procera* (Aiton) Dryand exhibited a dose-dependent analgesic property. *S. alba* extracts showed more potency than the standard drug aspirin in all the doses tested and *C. procera* extracts overcame aspirin at doses as high as 62.5 mg/kg. The result also indicates that the extracts inhibited the paw edema by interruption of the arachidonic acid metabolism and shows inhibition of the inflammation (*p<0.05) well above the inhibitory effect of the aspirin (100mg/kg i.p). Further acute toxicity tests were carried out which showed both the plant extracts showed no signs of toxicity up to the dose level of 125 mg/kg body weight. The results indicated that both the extracts rich in alkaloids, tannins and glycosides had significant analgesic as well as anti-inflammatory effects.

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