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A study on analgesic activity of *Holarrhena antidysenterica* leaves

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Abstract

The plant *Holarrhena antidysenterica* commonly known as 'Kutaja' belongs to the family Apocynaceae, is a small shrub or small tree evergreen in nature. The plant is medicinally important which is used to cure various diseases like diarrhea, dysentery, piles, biliousness and also with potent antioxidant activity. The analgesic activity of plant *Holarrhena antidysenterica* was evaluated by using the petroleum ether, chloroform and ethanolic extracts at the dose of 300, 300 and 250 mg/kg body weight respectively in the albino mice. The study was conducted as per the acetic acid induced writhing method. The ethanolic extract showed significant analgesic activity when compared to the standard Aspirin at the dose of 150 mg/kg of body weight. The findings indicated that the ethanolic extract exhibited potent analgesic activity comparable to that of the standard drug.

Keywords: *Holarrhena antidysenterica*, analgesic, albino mice, aspirin.

1. Introduction

Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments. The demand for plant based medicines, health products, pharmaceuticals, food supplement, cosmetics etc are increasing in both developing and developed countries, due to the growing recognition that the natural products are non-toxic, have less side effects and easily available at affordable prices^[1]. Drugs which are used presently for the management of pain and inflammatory conditions are either steroidal like corticosteroids or nonsteroidal like aspirin. All of these drugs possess more or less side and toxic effects like renal failure, allergic reactions, hearing loss or they may increase the risk of haemorrhage by affecting platelet function^[2]. On the contrary many medicines of plant origin had been used since ages without any adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop more effective and cheaper drugs. Plants represent a large natural source of useful compounds that might serve as lead for the development of novel drugs^[3]. Pain is the common phenomenon in all animals, atleast in vertebral animals, similar to that felt by man. Analgesic effects of animals are comparable with the therapeutic effects in man. *Holarrhena antidysenterica* commonly known as kurchi, is a rasayana herb used in Indian system of medicine^[4]. Bark of *Holarrhena antidysenterica* is used in Ayurveda as an anti- microbial, anti-inflammatory & analgesics^[5,6]. The study was undertaken to evaluate the analgesic properties of leaves extract of *Holarrhena antidysenterica* in either sex of swiss albino mice.

2. Materials and Methods

2.1 Plant material

The fresh leaves of *Holarrhena antidysenterica* were collected from surrounding places of Shivamogga, Karnataka. The botanical identity was confirmed using the standard herbaria maintained at Bangalore having the voucher specimen number No-biomed/V.U/H.A/16/08. The plant was dried under shade and the dried leaves of the plant were grounded with a blender. The powdered part was kept in nylon bags in a deep freezer until the time of use.

2.2 Preparation of plant extract

The powdered leaves were subjected to soxhlet extraction using petroleum ether (60- 80°C), chloroform and ethanol in successive mode respectively for 48 hours. The solvent was then recovered using Rotary Vacuum Evaporator and the concentrated extract was preserved in an airtight bottle.

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2.3 Animals

Adult Wistar albino mice of either sex, weighing 18-22 g were procured from National College of Pharmacy, Shivamogga, Karnataka. The animals were housed in polypropylene cages in standard environmental conditions of temperature (21 ± 2 °C), humidity ($55\pm 10\%$) and a 12-hour light-dark cycle. They were supplied with commercial diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee of National College of Pharmacy, Shivamogga (Registration No. 144/1999/CPCSEA/ dt: 10/04/2000).

2.4 Drugs

The pet ether, chloroform and ethanolic extracts of *Holarrhena antidysenterica* at a dose of 300 mg/kg, 300 mg/kg and 250 mg/kg body weight was used respectively. The animals received the standard drug Aspirin at the dose of 150 mg/Kg body weight.

2.5 Analgesic activity by acetic acid-induced writhing method

The analgesic activity of the extracts was evaluated using the acetic acid induced writhing method in mice^[7]. In this method, acetic acid was administered intraperitoneally to the experimental animals to create pain sensation. As a positive control, any standard NSAID drug can be used. In the present study, diclofenac sodium was used to serve the purpose. The animals were divided into five groups (I to V) of six animals in each. Group I served as the positive control with no protection. Group II animals received the standard drug whereas group III to V animals were orally administered the various plant extracts viz., pet ether, chloroform and ethanolic extracts at the dose of 300, 300 and 250 mg/kg body weight respectively. Test samples and vehicle were administered orally 30 mins prior to intraperitoneally administration of 0.6% v/v acetic acid solution (10 ml/Kg). Aspirin was administered 15 mins prior to acetic acid injection. Then the animals were placed on an observation table. Each mouse of all groups were observed individually for counting the number of writhing they made in

15 mins commencing just 5 mins after the intraperitoneal administration of acetic acid solution. The number of writhes in each treated group was compared to that of the control group. Aspirin was used as a reference standard.

2.6 Statistical analysis

The results were expressed as mean \pm SEM and were evaluated by ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the vehicle, control and standard. The $p < 0.01$ compared to control and $p < 0.05$ compared to standard were considered to be statistically significant.

3. Results & discussion

The results of the present investigation shows that the ethanolic extract of *Holarrhena antidysenterica* exhibits maximum analgesic activity at 20 min at the given dose 250 mg/kg (i.p.), ($p < 0.01$) and it was significant when compared with control and standard group. The chloroform extract showed a considerable activity whereas the pet ether extract activity was seen to be insignificant as compared to the standard (Fig 2). The photograph in Fig 1 depicts the abdominal constriction (writhing) method of analgesic activity. The results are tabulated in the table 1.



Fig 1: Acetic acid-induced writhing in animals showing the abdominal constriction

Table 1: Assessment of peripheral analgesic action of extracts of *Holarrhena antidysenterica* by glacial acetic acid-induced writhing test in Albino mice

Sl. No	Group	Number of writhing movements (mean \pm SEM) 15 mins	% Protection
1	Control	50.50 \pm 3.112.97	
2	Standard drug	15.50 \pm 0.86 ^a	69.30
3	Pet ether extract	22.50 \pm 4.330.99 ^a	55.44
4	Chloroform extract	9.33 \pm 1.730.83 ^a	81.52
5	Ethanol extract	8.17 \pm 1.450.71 ^a	83.82
	P value	<0.01	

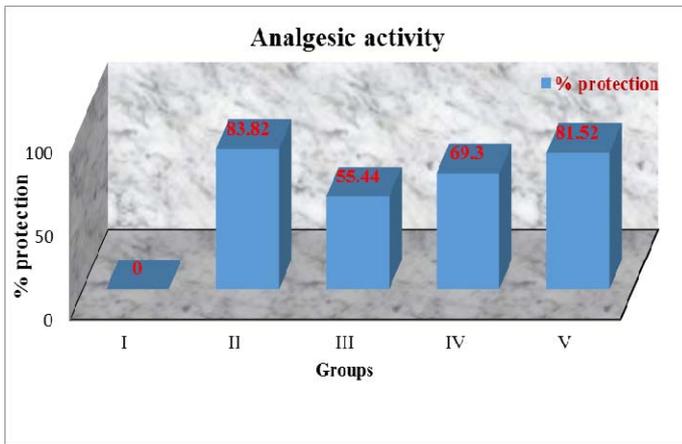


Fig 2: Effect of leaves extracts of *Holarrhena antidysenterica* as an analgesic when compared with the standard drug

4. Conclusion

Any injury or tissue damage is associated with pain and inflammation. Analgesics can act on peripheral or central nervous system. Peripherally acting analgesics act by blocking the generation of impulses at chemoreceptor site of pain, while centrally acting analgesics not only raise the threshold for pain, but also alter the physiological response to pain and suppress the patient's anxiety and apprehension. Green plants represent a reservoir of effective chemicals and can provide natural source of natural pesticides^[8]. Herbal extracts contain different phytochemicals with biological activity and can be of valuable therapeutic index. The ethanolic extract of *Holarrhena antidysenterica* exhibited potent analgesic effect against thermal noxious stimuli indicating the extract acts as a peripheral analgesic agent. The analgesic activity may be attributed to the reported phytoconstituents present in the ethanol extract like alkaloids, flavonoids, steroids, tannins and phenolics.

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