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EVALUATION OF ANALGESIC ACTIVITY OF A POLYHERBAL FORMULATION IN ALBINO RATS

K. Hari Kamesh Kiran[1]*, Dr. A. Lakshman Rao[2] and A. Tirupathi Rao[3]

[1,2]V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, A.P., India.
[3]Aditya Pharmacy College, Surampalem, A.P., India.

***Corresponding Author: K. Hari Kamesh Kiran**
V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, A.P., India.

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ABSTRACT
The present study was aimed to investigate the analgesic activity of the ethanolic extract of a polyherbal formulation in rats. The polyherbal formulation is made of leaves of Datura stramonium, rhizome of Zingiber officianale & fruits of Piper longum and it were taken as two doses i.e., 200, 300 mg/kg to evaluate the analgesic activity by Tail flick method and Acetic acid induced writhing test. The results of present study show that polyherbal formulation possesses significant analgesic activity in all tested experimental animals. The study may conclude that Polyherbal formulation possesses central analgesic activity which was evaluated using Tail flick method and peripheral activity in Acetic acid induced writhing test.

KEYWORDS: Analgesic activity, peripheral activity, central analgesic, writhing, Tail flick.

INTRODUCTION
Pain is an unpleasant sensory feeling triggered in the nervous system by intense or damaging stimuli that may be sharp or dull. This is a common symptom that may be seen in many of the diseased conditions like Rheumatoid arthritis, gout, pancreatitis, migraine, ulcer, Sciatica, Appendicitis, Trigeminal neuralgia, fractures, ulcerative colitis, Spondylitis etc. Currently available remedies for pain are Nonsteroidal anti-inflammatory drugs. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the agents reduce pain by suppressing the formation of prostaglandins, by inhibiting the enzyme Cyclooxygenase (COX-1 and COX-2). An investigation on pain relieving activity of plant based drugs used in traditional medicine has been paid attention as they are cheap. The polyherbal formulation contains leaves of Datura stramonium, rhizome of Zingiber officianale & fruits of Piper longum. All these plant parts have analgesic activity individually[1-6] and they were used in traditional system of medicine. The present study explores the synergistic effect of analgesic activity of all the three plant parts in the polyherbal formulation and it has a potent activity than the standard drug diclofenac sodium.

MATERIALS AND METHODS
Plant material
In this polyherbal formulation we have used three plant parts. They are leaves of Datura stramonium, rhizome of Zingiber officianale & fruits of Piper longum. The Mature fresh leaves of Datura stramonium and rhizome of Zingiber officianale were collected from the local region of gudlavalleru and fruits of Piper longum were purchase from local vendor. The plant materials were identified and authenticated taxonomically at Botanical Survey of India.

Preparation of extracts
The leaves of Datura stramonium, rhizome of Zingiber officianale & fruits of Piper longum were washed, cleaned, dried under shade and cutted into small pieces. Coarse powder of all the plant materials, which is taken in the ratio 1:1:1, was made and extracted by maceration with 95% Ethyl alcohol for 72 h at room temperature. Then the marc is again extracted with 95% alcohol using soxhlet apparatus. The whole extract of individual plants was collected in conical flasks, filtered and the solvents were evaporated to dryness under reduced pressure. The polyherbal formulation extract was then analyzed by qualitative tests and was found to contain alkaloids, flavonoids, glycosides, sterols and tannins.[7]

Animals
The Albino rats weighing 150–180 g of either sex were used for the study. The animals were housed in solid-bottomed polypropylene cages and acclimatized to animal house conditions. The rats were fed with commercial rat's diet and water. The experiments were designed and conducted in accordance with ethical norms approved by Committee for the Purpose of

Control and Supervision on Experiments on Animals (CPSCEA) and Institutional Animal Ethical Committee (IAEC).

Drugs and dosage

The poly-herbal formulation was administered orally at doses of 200 mg/kg and 300 mg/kg in the form of suspension prepared in double distilled water containing acacia (10%, w/v). Diclofenac sodium (5mg/kg) and acetic acid (0.6%) were purchased from local market.

Acute toxicity study

Acute toxicity study was performed in accordance with OECD guidelines 425.[8] No adverse effect or mortality was detected in albino rats up to 3 gm/kg, *p.o* of poly-herbal formulation during the 24 to 72 hrs observation periods. For this period the rats were continuously observed for 5 hrs for any gross behavioral, neurological or autonomic toxic effect and lethally after 24 to 72 hrs.

Drug treatment

Albino rats were divided into four groups of 4 animals each. Group I served as Control were given Acacia (10% (w/v) in double distilled water, *p.o*). Group II was administered standard drug Diclofenac sodium (5mg/kg,*p.o*). Group III & IV served as test groups and treated with poly-herbal formulation (200 & 300 mg/kg, *p.o)* in double distilled water containing Acacia (10%, w/v) respectively. The prepared extract was administered once for analgesic activity.

EVALUATION OF ANALGESIC ACTIVITY

1. Tail flick method

Analgesic activity was also evaluated using tail flick method. In this method, 1 to 2 cm of the tail of mice was immersed in warm water kept constant at 55^{o}C. The reaction time was the time taken by the mice to withdraw their tails. To evaluate the central analgesic effects of the polyherbal formulation, tail flick test was performed by time taken for rats to withdraw the tail when immersed in water maintained at 55±0.5^{o}C was measured. The animals were divided into three groups of 4 animals each.

Group I- Control received 2ml of 10 % gum acacia suspension I.P.
Group II – 10% Gum, acacia suspension with Diclofenac sodium 5 mg/kg oral.
Group III – 10% Gum, acacia suspension of PHF 200 mg/kg oral.
Group IV- 10% Gum acacia suspension of PHF 300 mg/kg body oral respectively.

The animals are allowed to adapt to the cages for 30 min before testing. The lower 5cm portion of the tail is marked. This part of the tail is immersed in a cup of freshly filled water of extract 55^{o}C. Within a few seconds the rat reacts by withdrawing the tail. The reaction time is recorded in seconds by a stopwatch. After each determination the tail is carefully dried. The reaction time is determined before and periodically after I.P administration of the control, standard drug and the test substance, e.g., after 0, 1, 2 and 3 hour. The cut off time of the immersion is 15 sec. The withdrawal time of untreated animals is between 1 and 5.5 sec. A withdrawal time of more than 6 sec therefore is regarded as a positive response.

2. Acetic acid induced writhing in mice[9, 10]

Albino rats of either sex were used for the study. Test animals were administered orally with the drugs 1 hour prior to acetic acid (0.6% v/v in water, 0.1ml/10g, i.p.) administration. The mice were placed individually in glass beakers 5 min after acetic acid injection and were then observed for 45 min and the number of writhing was recorded for each animal.

Statistical analysis

The observations are represented as Mean ± S.E.M. The data were processed by one-way analysis of variance (ANOVA) followed by *Dunnett's* post hoch test. *$P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Results

Tail Flick Method

In the tail flick method, latency to flick the tail increased significantly from 1st hr to 3rd hrs after single oral administration of polyherbal formulation, and the highest nociception inhibition of stimulus exhibited by polyherbal formulation (300 mg/kg) was observed at 3rd hr indicating dose and time dependant analgesic activity of polyherbal formulation compared to control group. The observations are given in table-1.

Table-1:

Treatment (mg/kg)	Time (seconds)			
	0 hr	1 hr	2 hr	3hr
Control	2±0.50	2.66±0.28	2.5±0.50	2.33±0.57
Standard	2.16±0.28	4.67±0.57	10.33±0.57	11±0.50
PHF 200	1.83±0.28	5.33±0.57	6.83±0.29	8.16±0.28
PHF 300	1.83±0.28	5.16±0.29	10.5±0.50	13.3±0.57

Values are expressed as mean ± SEM; n =4.

Writhing Method

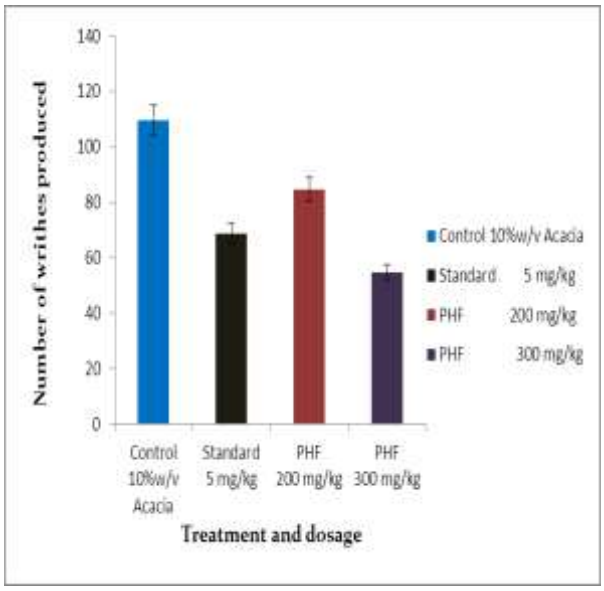
Acetic acid induced writhing in mice

Diclofenac sodium (5 mg/kg, p.o.) significantly ($p<0.01$) inhibited the acetic acid induced writhing episode as compared with control. Pretreatment of mice with polyherbal formulation (200 and 300 mg/kg, p.o.) significantly inhibited the acetic acid induced writhing episodes in a dose dependent fashion with an inhibition of 22.79% and 50.15% respectively when compared against control group. Hence, the polyherbal formulation 2 (300 mg/kg) showed synergistic inhibition of writhing effect as compared to the standard, Diclofenac sodium (5 mg/kg, p.o.). The observations are given in table-2.

Table-2:

Treatment	Dose (mg/kg)	Number of writhes observed	Percentage inhibition
Control	10%w/v Acacia	109.66±0.58	0
Standard	5 mg/kg	68.67±0.58	37.37
PHF 200	200 mg/kg	84.66±0.58	22.79
PHF 300	300 mg/kg	54.66±0.58	50.15

Values are expressed as mean ± SEM; n =4.



Graph-1: Number of writhes produced by different treatments of Control, Standard and Polyherbal Formulation doses.

DISCUSSION

The results of present study show that polyherbal formulation possesses significant analgesic activity in all tested experimental animals. Analgesia is produced by endogeneous substances and many other excite pain at nerve endings. The poly herbal formulation possesses central analgesic activity by increasing the latency to flick the tail in tail immersion method and elevated mean basal reaction time in hot plate method and Acetic acid causes analgesia by liberating endogenous substances and many others that excite pain at nerve endings. According to the percentage inhibition on the number of writhes obtained with the various doses of the polyherbal formulation, it was found that the intensity of the analgesic effect was similar to that of Diclofenac sodium and the polyherbal formulation dose 300 mg/kg is more potent than the diclofenac sodium and Inhibit cyclooxygenase in peripheral tissues, thus interfering with the mechanism of transduction in primary afferent nociceptors. As mentioned earlier PHF has three different constituents. A survey on the activity of constituents is used individually in folk medicine for treatment of pain. The analgesic activity of these 3 plants mentioned in literature. Hence it was concluded that polyherbal formulation possess good analgesic activity than individual consistuents.

CONCLUSION

The results of the present study shows that the Polyherbal formulation possesses significant analgesic activity in all the tested experimental animal models indicating inhibition of all phases of inflammation. The Polyherbal formulation possesses central analgesic activity which was evaluated using Tail flick method and peripheral activity in Acetic acid induced writhing test. Acetic acid causes analgesia by liberating endogenous substances and many others that excite pain at nerve endings.[11, 12] According to the percentage inhibition on the number of writhes obtained with the various doses of the polyherbal formulation, it was found that the intensity of the analgesic effect was significantly higher than that of Diclofenac sodium. Diclofenac sodium inhibit cyclooxygenase in peripheral tissues, thus interfering with the mechanism of transduction in primary afferent nociceptors.[13] Further there is a scope to determine its mechanism of action.

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