World Journal of Pharmaceutical research

Volume 2, Issue 6, 2525-2534. Research Article ISSN 2277 – 7105

EVALUATION OF ANALGESIC ACTIVITY OF POLYHERBAL LEAVES EXTRACT OF ALOE VERA AND CANNABIS SATIVA IN EXPERIMENTAL ANIMALS

Saurabh Doshi*, Paresh Mohan, Mahaveer Prasad Kabra, Sandeep Singh

Department of Pharmaceutical Chemistry, Kota College of Pharmacy, Kota, Rajasthan.

Article Received on
24 August 2013,

Revised on 28 Sept. 2013,
Accepted on 30 October 2013

***Correspondence for Author:**

Saurabh Doshi
Department of Pharmaceutical Chemistry,
Kota College of Pharmacy,
Kota, Rajasthan, India
saurabh.doshi08@gmail.com

ABSTRACT

The purpose of this investigation was to study the analgesic property of the polyherbal leaves extract of aloe vera and cannabis sativa combination in rats. Anti-inflammatory activity of this combination was reported, so that extract combination may be have analgesic activity. Here, we have taken polyherbal leaves extract of aloe vera and cannabis sativa combination to determine analgesic activity. Dose of the polyherbal leaves extract of aloe vera and cannabis sativa combination were selected 100mg/kg and 200mg/kg. The analgesic activity of that extract combination was studied using hot-plate method, tail clip method and tail-immersion method in mice and rat respectively. The extract combination at all the doses used and the diclofenac sodium (100 mg/kg, i.p.) significantly increases the analgesic activity for hot plate, tail clip and tail immersion method. The analgesic activity in the extract combination treated animals was found to be significantly higher in all the models compared to vehicle control animals. diclofenac sodium (100 mg/kg, i.p.) produced a significant analgesic activity when compared with the control group. The analgesic activity of polyherbal leaves extract of aloe vera and cannabis sativa combination was however, less than that of diclofenac sodium.

Keywords- Analgesic, diclofenac sodium, hot plate, tail clip, tail Immersion.

INTRODUCTION

The plant Aloe vera and Cannabis sativa is native of India. Aloe vera has marvelous medicinal properties. The ten main areas of chemical constituents of Aloe vera include: amino acids, anthraquinones, enzymes, minerals, vitamins, lignins, monosaccharide,

polysaccharides, salicylic acid, saponins, and sterols. [1] Aloe vera contains salicylic acid which is an aspirin like compound with anti-inflammatory, analgesic and anti bacterial properties.

Aloe vera is a species of succulent plant belonging to the family Asphodelaceae. The mucilaginous gel from the parenchymatous cells in the leaf pulp of Aloe vera has been used since early times for a host of curative purposes. It has been found to possess wound healing, anti-inflammatory, anti-oxidant, anti-atherogenic, anti-diabetic, anti-hypertensive and antibiotic properties. [2-3]

Every one of the essential amino acids are available in Aloe vera and they include isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine, and tryptophan. Some of the other non-essential amino acids found in Aloe vera include alanine, arginine, asparagine, cysteine, glutamic acid, glycine, histidine, proline, serine, tyrosine, glutamine, And aspartic acid.

Another constituent of Aloe vera includes saponins. These are soapy substances from the gel that is capable of cleansing and having antiseptic properties. The saponins perform strongly as anti-microbial against bacteria, viruses, fungi, and yeasts. [4] The plant sterols or phyto-steroids in Aloe vera include Cholesterol, Campesterol, Lupeol, and B (Beta sign) Sitosterol. [5] The plant steroids have fatty acids in them that have antiseptic, analgesic, and anti-inflammatory properties. [6] The basic literature survey exposed that the combination of both polyherbs has no significant data on the analgesic activity of has been reported. Hence the present study was undertaken to evaluate scientifically traditionally used medicinal plants for the claimed activity. [7]

The Cannabis plant and its products consist of an enormous variety of chemicals. Some of the 483 compounds identified are unique to Cannabis, for example, the more than 60 cannabinoids, whereas the terpenes, with about 140 members forming the most abundant class, are widespread in the plant kingdom. The term “cannabinoids” represents a group of C21 terpenophenolic compounds found until now uniquely in Cannabis sativa L. THC (Tetrahydrocannabinol) is the pharmacologically and toxicologically most relevant and best studied constituent of the Cannabis L. plant, responsible for most of the effects of natural Cannabis preparations. [8]

MATERIALS AND METHODS

Plant material

The leaves of Aloe vera and Cannabis sativa were collected from local area of Kota, Rajasthan and were authenticated by Department of Botany, Government college of Kota, Rajasthan.

Extraction

The Aloe Vera leaves are cut at the bottom about 2-3 cms. Above the bottom and fed to the juicing machine. Residual juice from the squeezed leaves is drained and collected. The viscous clear transparent juice is then passed through a filtration-cum-homogenizing unit to get clear, water white, transparent juice. Adequate preservative, sodium benzoate plus potassium sorbate, for example are added. The leaves are dried under aseptic conditions and ground. The freeze dried powder is packed under nitrogen. [9]

Cannabis leaves should soak for 1 to 10 days. Some folks soak it for up to four weeks, following that up with a secondary five day soak in fresh ethanol just to ensure all cannabinoids have been leached.

Animals

The study was conducted on Albino rats (Wistar) of 200-250 g and swiss albino mice (22-25 gm) from Animal House of Kota College of Pharmacy, Kota, Rajasthan and maintained under standard conditions (room temperature 24- 27^{o}C and humidity 60-65%) with 12 h light and dark cycle. The animals were kept in polypropylene cages and fed with standard pellet diet and water ad libitum. The animal experiments were approved by the ethics committee of the institute.

Drugs and chemicals

The standard drug diclofenac sodium was collected from MARTIN & BROWN BIOSCIENCES, Baddi, Dist.- Solan (H.P.), India. The other drugs and fine chemicals were purchased from R. S. ENTERPRISES, Jaipur (Raj.), India. All other chemicals and solvents were obtained from local firm (India) and were of highest purity and analytical grade.

Acute oral toxicity studies

The acute toxicity study was done as per the OECD guidelines (423). The combination of plant extract was administered parentally in different doses, where 24 h toxicity was recorded

to identify the toxic dose. No mortality and no signs of toxicity were found at the dose of 2000 mg/kg body weight of combination of plant extract. Therefore, it meight be considered that combination of plant extract have an LD50 value above 2000 mg/kg. Two doses 100 mg/kg and 200 mg/kg were selected for present study. The toxicity study also revealed its safeness, thus the combination of plant extract can be hypothesized it is nontoxic. [10]

Analgesic Activity

The activity was evaluated by using Eddy's hot plate method, Tail immersion method and Haffner's tail clip method in respected animal. Albino mice of either sex were divided into four groups of six animals each.

Group I served as normal control received acetic acid (1ml/100gm of 1% v/v solution), group II was served as positive control, received diclofenac sodium (100mg/ kg i.p.), group III and IV were pretreated with Polyherbal leaves extract of Aloe Vera and Cannabis sativa 100 mg / kg and 200 mg / kg, p.o. respectively . All the respective grouped animals were injected with acetic acid (1ml/100gm of 1% v/v solution) after 60 min administration of drugs.

1. Eddy's hot plate method

The study was conducted on swiss albino mice (22-25 gm). The animals were divided into four groups having six animals in each as follows:

GROUP	DRUG	DOSE OF DRUG
I	Normal control (acetic acid)	1ml/100gm of 1% v/v solution
II	Diclofenac sodium	100 mg/ kg i.p.
III	Polyherbal leaves extract of Aloe Vera and Cannabis sativa	100 mg / kg i.p.
IV	Polyherbal leaves extract of Aloe Vera and Cannabis sativa	200 mg / kg i.p.

In this hot plate method, animals from the each group were placed on the hot plate, which is commercially available, consists of an electrically heated surface. Temperature of this hot plate is maintained at 55-56^{o}C and observation is done up to the time until either paw licking or jumping was noted. Then the average reaction time was noted at the interval of 30 min. i.e.

after 30, 60, 90, and 120 minutes following oral administration of the std. drug and test compounds. [11-12]

2. Tail immersion method

Tail immersion method was used to determine the analgesic activity. Rats of wistar strain were randomly divided into a four groups having six animals in each as follows:

GROUP	DRUG	DOSE OF DRUG
I	Normal control (acetic acid)	1ml/100gm of 1% v/v solution
II	Diclofenac sodium	100 mg/ kg i.p.
III	Polyherbal leaves extract of Aloe Vera and Cannabis sativa	100 mg / kg i.p.
IV	Polyherbal leaves extract of Aloe Vera and Cannabis sativa	200 mg / kg i.p.

They were fasted overnight but during the experiment had free access to water. Both the doses of Polyherbal leaves extract of Aloe Vera and Cannabis sativa (100 mg/kg, 200 mg/kg) were administered orally 60 minutes prior to the commencement of the estimation of reaction time. The temperature of the water in the organ bath was set at 55 ± 0.5 ^{o}c with the help of thermostat. The reaction time was determined by immersing tail in hot water and the time taken by the rat to withdraw its tail clearly out of water was noted. Observations were repeated and noted at an interval of 30 minutes up to 120 minutes i.e. after 30, 60, 90, and 120 minutes following oral administration of the std. drug and test compounds. [13-14]

3. Haffner's tail clip method

In this study, the male mice (18-25 gm) were used. The test compound is administered orally to fasted mice. The animals were divided into four groups having six animals in each as follows:

GROUP	DRUG	DOSE OF DRUG
I	Normal control (acetic acid)	1ml/100gm of 1% v/v solution
II	Diclofenac sodium	100 mg/ kg i.p.

III	Polyherbal leaves extract of Aloe Vera and Cannabis sativa	100 mg / kg i.p.
IV	Polyherbal leaves extract of Aloe Vera and Cannabis sativa	200 mg / kg i.p.

The drug is administered 60 min. prior testing and artery clip was applied to the root of the tail (approximately 1 cm from the body) to induce pain. The animal quickly response to this noxious stimuli by biting the clip. The time between stimulation onset and response is measured by a stop-watch in 1/10 seconds increments. [15]

Statistical analysis

The Statistical analysis was performed by using One Way ANOVA followed by Dunnet's comparision test. The values are expressed as mean ± SEM and the $P<0.01$ was taken as significant.

RESULT

In analgesic studies, Polyherbal leaves extract of Aloe Vera and Cannabis sativa showed significant analgesic activity at 200 mg/kg tested dose level. In hot plate method, Polyherbal leaves extract of Aloe Vera and Cannabis sativa at a dose of 100mg/kg show significant activity where as at a dose of 200mg/kg showed more significant analgesic activity (8.31±0.41**) after 60 minutes (Table no.1).

In tail immersion method, Polyherbal leaves extract of Aloe Vera and Cannabis sativa at a dose of 100mg/kg showed significant effect at 30 minutes(3.37 ± 0.27*) and more significant analgesic activity (6.65 ± 0.54**) after 120 minutes whereas at a dose of 200mg/kg showed more significant analgesic activity (4.10 ± 0.12**) after 30 minutes (Table. no. 2).

In tail clip method, Polyherbal leaves extract of Aloe Vera and Cannabis sativa at a dose of 100mg/kg show significant activity where as at a dose of 200mg/kg showed more significant analgesic activity (4.88± 0.19**) after 60 minutes (Table. no. 3).

The analgesic activity in the extract combination treated animals was found to be significantly higher in all the models compared to vehicle control animals. Diclofenac sodium treated animals showed significant analgesic activity after 30 min. in every model. Diclofenac

sodium (100 mg/kg, i.p.) produced a significant analgesic activity when compared with the control group. The analgesic activity of polyherbal leaves extract of aloe vera and cannabis sativa combination was however, less than that of diclofenac sodium. The results showed significant analgesic activity against thermal stimuli.

Table-1 Effect of Polyherbal leaves extract on latency to hotplate test in mice.

Sr. No.	Treatment (mg/kg)	Mean latency (s) before and after drug administration			
		Before drug treatment(0 min)	After30 min.	After 60min.	After 90 min.
1	Normal control (acetic acid)	4.32 ± 0.19	5.73 ± 0.21	6.03 ± 0.11	6.07 ± 0.32
2	Diclofenac sodium (100mg/kg,i.p.)	3.91 ± 0.28	8.13±0.31**	9.29±0.27**	9.76±0.41**
3	Polyherbal leaves extract (100 mg/kg, i.p.)	4.59 ± 0.57	6.92 ± 0.43*	7.12 ± 0.36*	7.4 ± 0.61*
4	Polyherbal leaves extract (200 mg/kg, i.p.)	4.22 ± 0.32	7.09 ± 0.21*	8.31±0.41**	8.73±0.51**

Values are expressed as mean ± S.E.M., n=6, Diclofenac sodium 100 mg/kg, Polyherbal leaves extract 100,200 mg/kg, *p<0.05, **p<0.01, as compared with normal control using one way ANNOVA followed by Dunnet test.

Table-2 Effect of Polyherbal leaves extract to tail immersion test in rats

Sr. No.	Treatment (mg/kg)	Reaction time (sec)			
		After 30 min.	After 60 min.	After 90 min.	After 120 min
1	Normal control (acetic acid)	2.27 ± 0.32	2.63 ± 0.35	3.17 ± 0.23	3.72 ± 0.29
2	Diclofenac sodium (100mg/kg,i.p.)	4.84± 0.19**	5.20 ± 0.14**	6.50±0.09**	8.08 ± 0.12**
3	Polyherbal leaves extract (100 mg/kg, i.p.)	3.37 ± 0.27*	4.10 ± 0.25*	5.50 ± 0.39*	6.65 ± 0.54**
4	Polyherbal leaves extract (200mg/kg, i.p.)	4.10 ± 0.12**	4.60 ± 0.17**	6.10±0.15**	7.46 ± 0.21**

Values are expressed as mean ± S.E.M., n=6, Diclofenac sodium 100 mg/kg, Polyherbal leaves extract 100,200 mg/kg, *p<0.05, **p<0.01, as compared with normal control using one way ANNOVA followed by Dunnet test.

Table-3 Effect of Polyherbal leaves extract to Haffner's tail clip test in mice

Sr. No.	Treatment (mg/kg)	Reaction time (sec)			
		After 30 min.	After 60 min.	After 90 min.	After 120 min.
1	Normal control	2.70 ± 0.12	3.10 ± 0.31	3.40 ± 0.22	3.72 ± 0.21
2	Diclofenac sodium (100 mg/kg, i.p.)	4.22±0.27**	5.54± 0.17**	6.41±0.09**	7.12± 0.12**
3	Polyherbal leaves extract (100 mg/kg, i.p.)	2.79 ± 0.55	3.60 ± 0.25*	4.58 ± 0.39*	5.10 ± 0.57*
4	Polyherbal leaves extract (200mg/kg, i.p.)	3.50 ± 0.31*	4.88± 0.19**	5.70±0.15**	6.85± 0.23**

Values are expressed as mean ± S.E.M., n=6, Diclofenac sodium 100 mg/kg, Polyherbal leaves extract 100,200 mg/kg, *p<0.05, **p<0.01, as compared with normal control using one way ANNOVA followed by Dunnet test.

DISCUSSION

Results of present study indicated that Polyherbal leaves extract of Aloe Vera and Cannabis sativa possesses significant analgesic activity. The hot plate, tail immersion and tail clip method elucidate central analgesic activity of castor oil. Drugs that act primarily on the central nervous system inhibit both phases equally while peripherally acting drugs inhibit the late phase. The mechanism of analgesic effect of Polyherbal leaves extract of Aloe Vera and Cannabis sativa could probably be due to inhibition of the effect or release of endogenous substances that induces pain nerve endings similar to that of NSAIDs. Polyherbal leaves extract of Aloe Vera and Cannabis sativa possesses analgesic potential that may be due to saponin, steroids and alkaloids in it. Here Aloe vera and Cannabis sativa being an indigenous drug used by different communities. The result obtained in this study suggested that Polyherbal leaves extract of Aloe vera and Cannabis sativa possess significant analgesic activity against acetic acid induced writhing in mice. [16]

CONCLUSION

It is to be quite apparent by above investigations that Polyherbal leaves extract of Aloe Vera and Cannabis sativa possesses potent analgesic effect against different stimuli. Our results suggest that polyherbal leaves extract of aloe vera and cannabis sativa combination possesses

significant analgesic property and this is evidenced by significant increase in the reaction time by stimuli in different experimental models.

ACKNOWLEDGEMENT

We are very thankful to Kota College of Pharmacy, Kota and also all staff members for providing facilities.

REFERENCES

1. Barcroft A. Aloe Vera Healer, www.JoJaffa.com,http://www.AloeVeraHealer.com.
2. Davis RH Kabbani JM, Moro NP. Aloe Vera and wound Healing. J Am Podiatr Med Assoc, 1987; 77(4):165-9.
3. Shelton MS. Aloe Vera, its chemical and therapeutic properties. Int J Dermatol, 1991; 30: 679-3.
4. Atherton, Dr. Peter, Aloe Vera Myth or Medicine, Positive Health Publications http://www.positivehealth.com/permit/Articles/Aloe%20Vera/atherton.htm.
5. Properties of Aloe Barbadensis Miller Constituents, http://www.forevershop.nl/aloe%20barbadensis.htm.
6. Urch D. Aloe Vera Nature's Gift, Great Britain: Blackdown Publications, 1999, pp.15.
7. Kirtikar KR , Basu BD. Indian Medicinal Plants, Text volume –II,1999, pp.1130.
8. Grotenhermen F. Effects of Cannabis and the cannabinoids, in Cannabis and Cannabinoids—Pharmacology, Toxicology, and Therapeutic Potential (Grotenhermen F. and Russo E., eds.), Haworth Press, New York; 2002, pp.55–5.
9. Devaraj A, Karpagam T. Int J Pharm 2011; 2(3): 103-10.
10. OECD guideline for testing of chemical, Acute Oral Toxicity- acute toxic class method no.-423, dec. 2001, CPCSEA guidelines, section 5, of the prevention of cruelty to Animals Act,1960, ministry of environment and forest (AWD) government of india.
11. Vogel G H. Drug discovery and evaluation, Pharmacological Assays 2nd ed., Springer Publication: 2002, pp.697.
12. Ghosh MN. Fundamentals of Experimental Pharmacology. 4th ed. Hilton and Company: 2008, pp.163.
13. Chandra D. Analgesic effect of aqueous and alcoholic extract of Madhuka longifolia, Indian J. Pharmacol 2001; 33: 108-11.
14. Vogel G H. Drug discovery and evaluation, Pharmacological Assays 2nd ed., Springer Publication: 2002, pp.694.

15. Elisabetsky E, Amador TA, Albuquerque RR, Nunes DS, Cavalho AT. Analgesic activity of psychotria colorata (Wild ex R and S). muell arg. Alkaloids. J. Ethnopharmacol 1995; 48, 77- 3.
16. Dhara AK, Suba VT Sen, S Pal, chaudhuri AKN. Preliminary studies on the anti-inflammatory and analgesic activity of Tragia involucrate Linn. J Ethnopharmacol 2000; 72, 265-68.