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Short Communication

Evaluation of Analgesic Activity of Some Polyherbal Extracts against Acetic Acid Induced Writhing in Experimental Animals

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

Department of Pharmaceutical Chemistry, Kota College of Pharmacy, SP-1, RIICO Industrial Area, Ranpur, Jhalawar road, Kota, Rajasthan, India – 324009

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ABSTRACT

Aloe vera contains salicylic acid which is an aspirin like compound with anti-inflammatory, analgesic and anti bacterial properties. Cannabis sativa is an annual herbaceous plant in the cannabaceae family. The aim of present study was to evaluate analgesic activity of that combination extract against acetic acid induced writhing in mice. All animals were divided into four groups of six mice each. Group I was treated as toxicant control to observe writhing and group II was pretreated with diclofenac sodium (100 mg/kg, i.p.) Group III and IV were pretreated with polyherbal leaves extract of Aloe vera and Cannabis sativa at two doses 100mg/kg and 200mg/kg p.o. respectively. Polyherbal extract and Diclofenac sodium were given before 60 minute of acetic acid administration. The results showed that polyherbal extract significantly reduced number of writhing when compared with group I (Toxicant control) mice and the results were dose dependent. The toxicity study also revealed its safeness, thus the combination of plant extract can be hypothesized it is nontoxic. It is concluded that polyherbal extract can offer protection against acetic acid induced writhing in mice.

Keywords: **A**cute toxicity, aloe vera and cannabis sativa leave extract, analgesic, diclofenac sodium, and writhing response.

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. 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 [2]. The plant sterols or phyto-steroids in Aloe vera include Cholesterol, Campesterol, Lupeol, and B (Beta sign) Sitosterol [3]. The plant steroids have fatty acids in them that have antiseptic, analgesic, and anti-inflammatory properties [4]. 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 [5]. 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 [6]. As a consequence of the development of synthetic cannabinoids (e.g., nabilone [7], HU-211

***Corresponding Author Address:** Saurabh Doshi, Department of Pharmaceutical Chemistry, Kota College of Pharmacy, Kota, Rajasthan, India; E-mail: saurabh.doshi08@gmail.com*

{dexanabinol [8], or ajulemic acid [9]}) and the discovery of the chemically different endogenous cannabinoid receptor ligands ("endocannabinoids," e.g., anandamide, 2-arachidonoylglycerol) [10,11], the term "phytocannabinoids" was proposed for these particular Cannabis constituents [12]. 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 preparation [13].

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 cm. 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 [14].
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 mice of either sex weighing 20-25gm were procured from Animal House of Kota College of Pharmacy, Kota, Rajasthan. The animals were kept in polypropylene cages and fed with standard pellet diet and water ad libitum, maintained at an ambient temperature of $25^0C \pm 2^0C$ and relative humidity 50%±15%. The study was conducted after approval of Institutional Animal Ethical Committee.

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 LD_{50} 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 [15].

Analgesic Activity: The activity was evaluated by using acetic acid induced writhing in mice. Albino mice of either sex were divided into four groups of six animals each. Group I served as toxicant 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 minute administration of drugs [16].

Statistical Analysis: The results were expressed as mean ± S.E.M. and statically analyzed by one-way ANOVA followed by Dunnett's test. $P<0.01$ was regarded as statistically significant.

RESULTS

Polyherbal leaves extract of Aloe vera and Cannabis sativa (100mg/kg and 200mg/kg, p.o.) significantly reduced the number of writhing induced by acetic acid when compared with group I mice (toxicant control). The test for acute toxicity was found to be non-toxic at the dose of 100mg/kg and 200mg/kg and did not cause death to animals tested. The result was dose dependent.

DISCUSSION

The study was conducted on due to increase in frequency of intake of synthetic drug and their side effects, there is needed to focus on herbal drug having fewer side effects. The analgesic activity of the plant is attributed to the presence of the enzyme carboxypeptidases and bradykinase that tend to relieve pain [17]. The plants are known to contain some alkaloids and steroidal substances responsible for the release of pain with immunemodulatory and antioxidative properties have been reported in earlier studies [18]. These tend to assist in the reduction of pain through the stimulation of the immune system and the reduction of prostaglandins

that are responsible for the pain. Acetic acid induced writhing response in mice is simple, rapid and reliable model to evaluate peripheral type of analgesic action of herbal and other drugs. 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 [19].

CONCLUSION

On the basis of these findings, it may be inferred that Polyherbal leaves extract of Aloe vera and Cannabis sativa is an effective for analgesic activity .This activity may be due to presence of active constituents like flavonoids, saponins, steroids.

Table 1: Effect of polyherbal leaves extract of Aloe vera and Cannabis sativa to writhing in mice

Sr. No.	Groups	Drug Treatment	Onset of action(min.)	No. of writhing (10 min)
1)	I	Toxicant control (acetic acid 1ml/100gm of 1%v/v.)	5 ± 1.23	45±2.5
2)	II	Acetic acid + Diclofenac sodium (100mg/kg, i.p.)	7± 0.75	22±2.66
3)	III	Acetic acid + Polyherbal leaves extract of Aloe vera and Cannabis sativa (100mg/kg, p.o.)	7± 0.80	25±3.50
4)	IV	Acetic acid + Polyherbal leaves extract of Aloe vera and Cannabis sativa (200mg/kg, p.o.)	10± 0.33	15±2.66

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