A Study on Analgesic Efficacy and Adverse Effects of Aloe Vera in Wistar Rats.

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Short title for running head: Analgesic efficacy and adverse effects of Aloe vera

Summary

Study was conducted to find out the analgesic efficacy and adverse effects of aqueous extract of Aloe vera (*Aloe barbadensis*) in Wistar rats. The study was carried out using male Wistar rats (150-200gm). The animals were divided into 5 groups (n=6) receiving different treatments. Both visceral and somatic pain in animals was assayed using radiant heat method, hot plate method and writhing test. The first group of animals was taken as control, the second group was given the reference standard drug and the other groups received indigenous drug Aloe vera gel at different doses. For sub-acute toxicity study, Aloe vera was administered daily for 14 days at the dose level of 300 mg/kg dose. Biochemical analysis of blood and histopathological study of GI mucosa was done after 14 days. Aqueous extract of Aloe vera gel showed significant analgesia compared to control. The results were significant (p<0.001) in radiant heat method and also in hot plate method (p<0.05) at the dose of 300 mg/kg. Writhing test showed maximum inhibition (51.17 %) at the dose of 300 mg/kg. No adverse effects on renal and hepatic functions were found with Aloe vera. Histopathological study of GI mucosa showed preservation of normal architecture with Aloe vera. The aqueous extract of Aloe vera gel showed significant analgesia and to be safe in respect of the renal and hepatic functions along with no adverse effects on GI mucosa.

Keywords: adverse effects, Aloe vera, analgesic efficacy, sub-acute toxicity

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the mainstay of treatment in pain and inflammation. They act by inhibiting the enzyme cyclooxy-genase (COX), the enzyme responsible for biosynthesis of prostaglandins and certain related autacoids. These drugs usually are effective against pain of low-to-moderate intensity. Gastrointestinal erosions and ulcerations, disturbances of platelet activation, changes in renal function etc.[1] are the known adverse effects of different NSAIDs though they are widely used in patients.

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]. Chemical analysis has shown the gel to contain various carbohydrate polymers, notably either glucomannans or pectic acid, along with a range of other organic and inorganic components[4]. The aqueous extract of Aloe vera gel has been reported to reduce prostaglandin E_2 production from arachidonic acid by inhibiting cyclooxygenase[5]. It thus exerts anti-inflammatory and analgesic properties. It has been utilized for reducing pain during dental treatments, mouth ulcers, sores, blisters, hemorrhoids and for wound healing[6,7].

In order to overcome the adverse effects of the conventional NSAIDs, there has been a search for analgesic activity in indigenous drugs for years together. Keeping India's rich biodiversity in mind, the present research work has been undertaken to study the efficacy and safety of Aloe vera gel extract on analgesia in rats.

Materials and methods:

Preparation of aqueous extract of aloe vera

The leaf of Aloe vera plant was obtained from National Medicinal Plant Board, Govt of West Bengal. The collected leaves were washed in cold water. The lower 1 inch of the leaf base and the tapering 2-4 inch of the leaf top and the spines around the leaves were removed using a knife. Then the knife is introduced into the mucilage layer below the green rind and the mucilage is collected. Aloe constituents are found in this mucilage layer. 100 gram of gel was mixed with 100 ml of distilled water and blended to obtain 100% (w/v) extract. The blended material was squeezed through a muslin cloth. The filtrate was freeze-dried under vacuum using a lyophiliser. Different dose levels of Aloe vera gel were made by reconstituting the extract at a concentration of 6% (w/v) in order to keep the volume of drug administered to each animal within 1 ml.

Animals

They were housed in polypropylene cages and kept under controlled room temperature (24 ± 2^0 C) having relative humidity of 60 -70% in a 12 h light-dark cycle. The rats were given free access to standard laboratory diet and water. Animals were deprived of food but not water for four hours before the experiment. They were divided into 5 groups each containing 6 rats. The first group served as a control group which received distilled water. The second group received the reference standard drug. The third, fourth and fifth groups were given aqueous extract of Aloe vera at the dose level of 100, 200 and 300 mg/kg[8] orally respectively for study of analgesic effect. The study protocol was approved by the Institute animal ethics committee of West Bengal University of Animal & Fishery Sciences.

Drugs

Aspirin (Zydus Medicus) and pethidine (Dey's Medical, Kolkata) were used for the study. Aspirin was given orally after suspending in distilled water by gastric canula fitted with a syringe. Aqueous extract of Aloe vera at doses of 100, 200 and 300 mg/kg were similarly administered orally. Pethidine was mixed in water for injection before intra-peritoneal administration.

Radiant Heat method

The animals were held in suitable restrainer with the tail protruding out. Radiant heat was applied over the tail on a single spot over the proximal one-third with the help of analgesiometer (tail flick type). The time taken by the animal to withdraw (flick) the tail was taken as the reaction time (latency period). Before administration of the test compound or the standard drug, the normal reaction time was recorded. Animals were subjected to a preliminary screening and rats showing tail-flick response in 10-12 seconds were selected. The animals are submitted to the same testing procedure after 30, 60, 90 and eventually 120 minutes after administration of the drug and test compound. For each individual animal, the reaction time was noted. Pethidine (30 mg/Kg s.c.) was given as reference standard[10].

Hot Plate method

The hot plate was maintained at 55.0 ± 1^0C. The time taken to cause a discomfort reaction (licking paws or jumping) was recorded as response latency or reaction time. Before administration of the test compound or the standard, the normal reaction time was determined. The animals are submitted to the same testing procedure after 30, 60, 90 and eventually 120 minutes after administration of the drug and test compound. For each individual animal the reaction time was recorded. Pethidine (30 mg/Kg s.c.) was given as reference standard. A cut-off time of 30 seconds was followed to avoid any thermal injury to the paws[11].

Writhing induced with 4% Sodium Chloride

The animals were pretreated with drugs 45 minutes before induction of writhing. The animals received the standard drug aspirin (20 mg / Kg p.o.) which served as reference

standard. Analgesic activity of Aloe vera gel (100, 200, 300 mg/kg p.o.) was assessed by counting the number of writhes induced by intraperitoneal injection of 1ml/kg of 4% Nacl. The rats are placed individually into glass beakers and five minutes were allowed to elapse. The rats were then observed for a period of ten minutes and the number of writhes was recorded for each animal[9]. Percentage protection against abdominal constriction was taken as an index of analgesia. It was calculated as:

$$\frac{\text{No. of writhing in control group} - \text{No. of writhing in treated group}}{\text{No. of writhing in control group}} \times 100$$

Testing of sub-acute toxicity

Blood sample was taken prior to experiment for estimation of liver function test, urea, creatinine in the control group of animals. For testing sub-acute toxicity, 3 rats receiving aspirin and 3 rats receiving Aloe vera in the dose 300mg/kg were to be given the drug daily for 14 days. The biochemical tests were repeated after 14 days in those receiving Aloe vera. Among those receiving aspirin one animal died after 7 days. Immediate post-mortem was done and on macroscopical examination of the stomach mucosa, thickening and paleness with presence of greyish-white necrotic foci were detected. GI tissue was taken in 10% formal saline for histopathological examination. Aspirin was discontinued in the other 2 animals. After 14 days, 3 rats which received 300 mg/kg of Aloe vera were sacrificed by euthanasia with ketamine (200 mg/kg body weight). Macroscopical examination of gastro- intestinal mucosa did not show any obvious abnormality. Specimen of stomach and intestine were collected for histopathological study.

Statistical analysis

The results of all the three methods were expressed as mean± SEM. Statistical analysis was determined using one-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test.

Results

Pain induced by application of radiant heat in rats. The aqueous extract of Aloe vera gel in dose of 100 mg/kg showed significant increase in latency of tail flick response compared to control at 30 minutes onwards following administration of drug. Highly significant result was seen after 90 minutes. The doses of 200 and 300 mg/kg increased the latency of tail flick response which highly significant at all time points ($p<0.001$). [Table 1]

Hot plate method

The aqueous extract of Aloe vera gel the dose of 100 and 200mg/kg did not show any significant increase in the mean basal reaction time in hot plate method compared to control. The dose of 300mg/kg significantly increased basal reaction time ($p<0.05$). The highest inhibition to nociceptive stimulus was observed at 60 mins. at 300mg/kg of the extract ($p< 0.01$). [Table 2]

Writhing induced by 4% NaCl

The aqueous extract of Aloe vera at doses of 200 and 300 mg/kg reduced significantly (P<0.001) the number of abdominal constrictions induced by 4% NaCl compared to control group. Maximum inhibition of writhing response with aqueous extract Aloe vera was 51.17% with 300 mg/kg, which was comparable to aspirin [Table 3]. For subacute toxicity study, 9 rats were equally divided into 3 groups containing 3 animals each. First group was kept for control and received distilled water daily for 14 days, while animals of second group were administered aspirin daily at 20mg/kg for 14 days. Aloe vera extract was given at 300 mg/kg daily for 14 days to animals of group 3. Blood samples were collected from orbital plexus prior to experiment and after 14 days post dosing of drugs for AST, ALT, BUN and CRT analysis.

Tests for sub-acute toxicity

Histopathological examination of G.I. tract mucosa of rat showed preservation of normal architecture with Aloe vera at a dose of 300 mg/kg [Figure1] whilst the rat G.I. tract mucosa showed proliferation, desquamation and coagulative necrosis of the lining mucosal epithelium with cellular infiltration and atrophy of secretary glands which received aspirin.[Figure 2]. Estimation of blood for AST, ALT, urea and creatinine showed no significant difference between the control group of animals and those receiving Aloe vera at 300 mg/kg. [Table 4]

Table 1. Effect of aqueous extract of Aloe vera on radiant heat induced tail flick response in rats

	Pre-treatment response	30 mins	60 mins	90 mins	120 mins
Control	10 ± 0.36	10 ± 0.5	10.33 ± 0.49	9.66 ± 0.55	10.33 ± 0.49
Pethidine (30 mg/kg)	10 ± 0.57	19.5± 0.61***	20 ± 0.85***	18.33± 0.55***	16.66± 0.42***
Aloe vera (100 mg/kg)	9.83 ± 0.47	12.5 ± 0.42*	11.25± 0.36*	13.33± 0.42***	12.83 ± 0.3**
Aloe vera (200 mg/kg)	9.83 ± 0.6	13.66± 0.33***	15.16± 0.3***	15 ± 0.36***	14.33 ± 0.49***
Aloe vera (300 mg/kg)	10.5 ± 0.42	14 ± 0.36***	15.33± 0.55***	14.33± 0.33***	14.33± 0.33***

Data was analyzed by ANOVA test followed by Dunnett's *post hoc* test. Each value is the mean ± SEM; n=6, *P < 0.05, **P < 0.01, ***P < 0.001 when compared with control group.

Table 2. Analgesic effect of Aqueous extract of Aloe vera on hot plate method in Rats

	Pre-treatment response	30 mins	60 mins	90 mins	120 mins
Control	10.16 ± 0.47	9.5 ± 0.43	9.5 ± 0.42	9.66 ± 0.49	9.33 ± 0.33
Pethidine (30mg/kg)	10.66±0.88	15.33± 0.42***	16.66 ± 0.66***	16.16± 0.47***	15.16 ±1.3***
Aloe vera (100mg/kg)	10.5±0.42	9.66 ± 0.49	9.66 ± 0.42	10 ± 0.57	10 ± 0.57
Aloe vera (200mg/kg)	11±0.36	10.5 ± 0.76	10.83 ± 0.7	10.5 ± 0.43	11.33 ± 0.55
Aloe vera (300mg/kg)	10.3±0.49	12.83 ± 0.6*	12.5 ± 0.42**	12.66 ± 0.49*	12. 5± 0.42*

Data was analyzed by ANOVA Test followed by Dunnett's *post hoc* test. Each value is mean ± SEM; n==6, *P<0.05, **P<0.01, ***P<0.001 when compared with control group

Table 3. Effect of aqueous extract of Aloe vera gel on writhing induced by 4% NaCl IP in rats

	Number of writhes ± SEM	% of inhibition	Significant
Control	5.83 ± 0.47	0	NS
Aspirin	1.8 ± 0.3	68.96	P< 0.001
Aloe vera (100mg)	4.8 ± 0.30	17.24	P>0.05
Aloe vera (200mg)	3.66 ± 0.21	37	P<0.001
Aloe vera (300mg)	2.83 ± 0.3	51.17	P<0.001

Table 4

Biochemical parameters in rats receiving Aloe vera at 300 mg/kg.

Values expressed as mean ± SEM

	Control	Aloe vera (300 mg/kg)
SGOT (AST) IU/L	59.83 ± 2.104	60.33 ± 1.85
SGPT (ALT) IU/L	34.33 ± 1.9	36.66 ± 2.07
BUN gm/dl	10.66 ± 0.66	11.66 ± 0.55
Creatinine gm/dl	1.09 ± 0.08	1.11 ± 0.04

Figure 1: Architecture of mucosa of G.I. tract of rat was preserved with aqueous extract of Aloe vera at 300 mg/kg. [H &E X100]

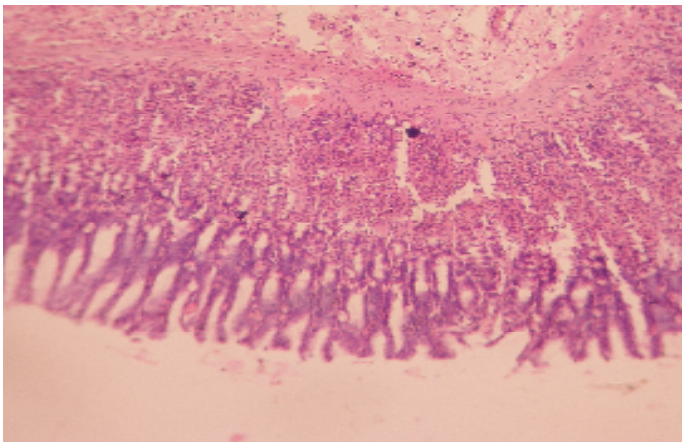
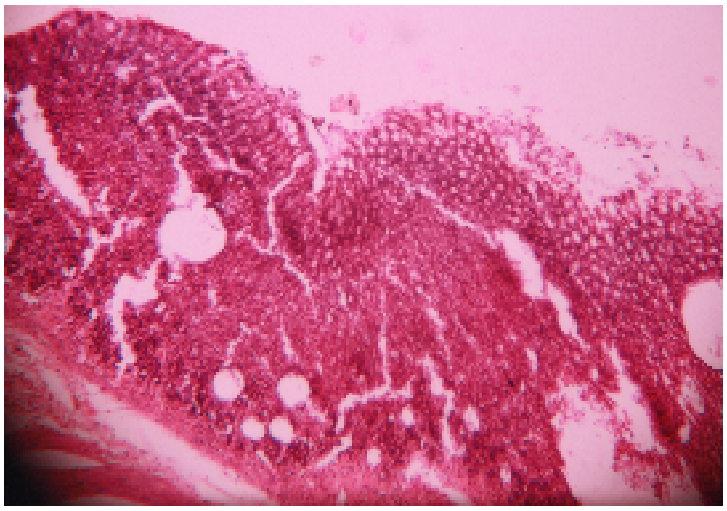


Figure 2: Necrosis and sloughing of epithelium of the intestinal mucosa with cellular infiltrations and atrophy of secretory glands seen in rat receiving aspirin. [H&E X 100]



Discussion

A study of analgesic efficacy and adverse effects of Aoe vera gel in wistar rats has been done in various models of pain for study of both visceral and somatic pain. The stimulus may be thermal (tail flick, tail immersion, hot plate tests), mechanical (tail or paw pressure tests), electrical stimulation of (paw, tail) or chemical (writhing test)[12].

Pain is an unpleasant sensation localized to a part of the body. The perception of pain is supported by a system of sensory neurons and neural afferent pathways that specifically respond to potentially noxious, tissue-damaging stimuli. The small diameter myelinated A-delta and the unmyelinated C fibres, present in nerves to skin and to deep somatic and visceral structures, respond maximally to painful stimuli. These are the primary afferent nociceptors (pain receptors)[13]. Inflammation produced by mild tissue damage or infection causes afferent C and A-delta fibres to be activated by low intensity stimuli and pain occurs[14]. Substances like kinins, prostanoids, serotonin, histamine etc. are released upon damage to cells. These are potent algogenic substances and induce pain by directly stimulating nociceptors in skin, joints, muscles, as well as by sensitizing them to heat and mechanical stimuli[15].

NSAIDs act primarily on peripheral pain mechanisms but also in CNS to raise pain threshold[16]. They are the most commonly used anti-inflammatory, antipyretic, analgesic drugs. Most NSAIDs block prostaglandin synthesis by inhibiting COX-1 and COX-2 nonselectively, but now some selective COX-2 inhibitors has been developed. Of the common toxicities caused by NSAIDs due to inhibition of prostaglandin synthesis, gastric mucosal damage is most troublesome. This sometimes limits the use of this group of drugs in patients with chronic pain.

Aloe Vera (Aloe barbadensis Miller) traditionally known as *Ghrita-kumari,* is a perennial plant having thick fleshy leaves from which a thick juice flows when transversely cut[17]. The inner parenchymal cells below the cuticle contain a transparent mucilaginous jelly which is referred to as Aloe vera gel. Multiple ingredients have been found in Aloe leaf gel[18]. Some of them are ligin, saponin, anthraquinones, minerals, vitamins, amino acids, enzymes, sugars and sterols. Studies have been conducted on the different uses of this plant for its anti-inflammatory, antidiabetic, wound healing properties [5,19,20]. The biological activity of Aloe vera gel is suggested to be due synergistic action between the polysaccharide base and other components. Mannose-6-phosphate, a major polysaccharide in Aloe gel has a role in wound healing and anti-inflammatory activity[8]. The gel polysaccharides, especially the acetylated mannans have been seen to possess immunomodulatory properties[21]. Many scientific studies on the use of Aloe vera have been undertaking to determine its beneficial and toxic potentials[22,23] . Although claims have been made of the potential of Aloe vera as analgesic, limited data is available so far to establish its analgesic use[24]. There are studies which have elicited gastro-protective effects of Aloe vera, which may be further beneficial to patients who need analgesics[25]. This study was thus done with the aim to assess the efficacy of Aloe vera gel as an analgesic and also to determine its adverse effects if any.

The hot plate and radiant heat methods are suitable for evaluation of somatic pain. Centrally acting analgesics like pethidine is used as reference standard for this purpose[10]. In the method of pain induction by application of radiant heat on rat tail, the aqueous extract of Aloe vera gel at the dose of 200 and 300 mg/kg showed highly increased significantly in latency of tail flick at all time points.

The aqueous extract of Aloe vera gel at a dose of 300mg/kg showed significant increase in basal reaction time in hot plate method. It was thus found to be effective as an analgesic in the models applied for study of somatic pain at higher doses.

Writhing was induced by intra-peritoneal injection of 4% sodium chloride for study of visceral pain. The reference drug (aspirin) offers relief from inflammatory pain by inhibiting the formation of pain mediators in the peripheral tissues. On intra-peritoneal injection of 4% Nacl, the nociceptive response is due to release of endogenous substances such as bradykinin and prostaglandins, which stimulate the nociceptive endings. A highly significant reduction in the number of abdominal constrictions with Aloe vera was observed at 200 and 300mg/kg compared to control indicating good analgesic activity in visceral pain.

The histopathological examination of rat G.I. tract mucosa showed preservation of normal architecture with Aloe vera at a dose of 300 mg/kg while desquamation and coagulative necrosis of the lining mucosal epithelium with cellular infiltration and atrophy of secretary glands were observed with the use of aspirin. Aloe vera gel was thus found to have no detrimental effects on mucosa of the gastro-intestinal tract even on prolonged use at a dose of 300 mg/kg.

Hence, it can be concluded that the aqueous extract of Aloe vera gel has efficacy as analgesic on both the somatic and visceral components of pain at a dose of 300 mg/kg having no adverse effects as well as on hepatic and renal functions on the gastro-

intestinal mucosa. In the light of previous studies on gastro-protective effects of Aloe vera gel, it may be used as an analgesic. The potential of Aloe vera gel as an additive analgesic to conventional drugs may further be explored as it is seen to have less adverse effects.

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