Original Research Article

To evaluate anti-nociceptive activity of Aloe vera gel extract in graded doses in Albino rats by using radiant heat induced pain by using Analgesiometer

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Abstract

Background: Pain is a reaction of the body to harmful stimuli and is therefore a protective early warning system, the sensation of pain in postoperative patients, cancer patients, and other chronic pain patients has little positive effect. **Objective:** To evaluate anti-nociceptive activity of Aloe vera gel extract in graded doses in Albino rats by using radiant heat induced pain by using Analgesiometer. **Materials and methods:**The present study is undertaken to evaluate the anti-nociceptive effect of Aloe vera in animal models of pain in Albino rats. It was conducted in the Department of Pharmacology, Kamineni Institute of Medical Sciences (KIMS) from October 2012 - September 2014.**Results:**Aloe vera leaf gel extract powder dissolved in distilled water was evaluated for anti-nociceptive activity in tail flick method in albino rats. Tramadol, the known synthetic opioid analgesic is used as standard drug for comparing the effects of Aloe vera. Aloe vera in the doses of 200mg/kg and 400mg/kg orally produced significant anti-nociceptive effect in tail flick method in comparison to control group (DW). The standard drug tramadol in the dose of 20mg/kg orally also produced significant anti-nociceptive effect in tail flick method in comparison to control group (DW).**Conclusion:** Aloe vera leaf gel extract powder (dissolved in distilled water) possesses anti-nociceptive activity at 200 mg/kg and 400 mg/kg dosage at 60, 90 and 120 minutes when given orally in tail flick, model in albino rats.

Keywords- anti-nociceptive activity, Aloe vera gel extract, Albino rats, analgesiometer

Introduction

Pain is the most common symptom of any illness. Pain has been described by the International Association for the Study of Pain as an "unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage". [1]

The term pain, derived from the Latin word "poena" for punishment, reflects thedeleterious effects that can be inflicted upon the body. [1] The nature of pain is highly subjective. Pain has both sensory (somatic) and psychological (affective) components. One person may feel pain in response to noxious stimuli, while another person may disregard the stimuli. The affective (psychological) aspects of pain play a critical role in pain perception. The principal objective of alleviating pain is to remove or abolish the cause of pain. But it is not always possible to do so. Hence, analgesics are used for the symptomatic treatment of pain. The analgesics are of two types: Opioids and Non-Opioids.The primary clinical use of opioids is relieving pain. The term opiate refers to compounds structurally related to products found in opium, a word derived from opos, the Greek word for "juice," natural opiates being derived from the resin of the opium poppy, Papaver somniferum. Opiates include the natural plant alkaloids, such as Morphine, Codeine, Thebaine, and many semisynthetic derivatives. An opioid is any agent, regardless of structure, that has the functional and pharmacological properties of an opiate. [2] Opioids produce a wide spectrum of unwanted effects, including respiratory depression, nausea, vomiting, dizziness, mental clouding, euphoria/dysphoria, pruritis, constipation, increased pressure in the biliary tract, urinary retention and hypotension.

Because of these unwanted effects, there is a continuous and ongoing research to identify more effective and safer agents. Plant extracts have been used for a long time as a traditional remedy for several medical conditions. Many plant extracts are tested for their medicinal activity. Aloe vera has been used in folk medicine as a remedy for various diseases.

Aloe barbadensis Miller (family: Liliaceae) is asucculent plant having fleshy, thick, serrated, lanceolate shaped green to grayish leaves and isstrongly considered a beneficial agent for wound healing, protecting mucous membrane, treating ulcer,preventing diabetes, and being effective in skin care. [3-6] Aloe vera iswidely found in subtropical and tropical areas of theworld. Among all 400 species of Aloe, Aloe barbadensis Miller [syn. Aloe vera (L.)] is considered to be the mostbiologically active plant. [7] Besides acting as a therapeutic agent, it has long been used in dietary supplements and for cosmetic purposes. [5]

It also has been reported to possess pharmacological properties like antioxidant, wound healing, antibacterial, antifungal, and immunomodulatory effects. [8] The healing of burn wounds is one of the main indications for Aloeveragel use in both animal and human clinical Studies. [9] Recently, it has been shown that Aloe veracan facilitate wound healing in post haemorroidectomypatients. [10] Aloe vera decreased chronic anal fissure pain. [11]

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Several studies have been done to know the different effects of Aloe vera. But only a few studies were conducted to know the analgesic effect. So the present study has been planned to evaluate the anti-nociceptive activity of Aloe vera gel extract in Albino rats in different experimental models.

Materials & Methods: The present study is undertaken to evaluate the anti-nociceptive effect of Aloe vera in animal models of pain in Albino rats. It was conducted in the Department of Pharmacology, Kamineni Institute of Medical Sciences (KIMS) from October 2012 - September 2014.It was a randomized controlled study conducted in Albino rats with prior permission of Institutional Animal Ethics Committee (IAEC).

Animals: Albino rats (150-200 gm) of both sexes procured from National Institute of Nutrition, Hyderabad were used. The animals housed in central animal house, KIMS under standard laboratory conditions, maintained on 12:12 light-dark cycle and had free access to food and water.

Models for pain:

1. Pain induced by generating Radiant type of heat by using Analgesiometer in Albino rats.
2. Pain induced by direct heat of Thermal type by Hot Water Bath method in Albino rats.
3. Pain induced by mechanical stimulus by Haffner's tail clip method in Albino rats.

The animals were acclimatized to laboratory conditions fifteen days before the tests. Each animal was used only once in the experiment.

Drugs and chemicals:

Aloe vera leaf gel dried powder: Bhaskara Biotech.
Tramadol: Zydus Cadila.
Distilled water.

Drug administration: All drugs were administered orally using oral feeding tube. For control rats, distilled water was given orally. The standard drug Tramadol (10 mg/kg, 20 mg/kg) was dissolved in distilled water and given orally to standard group rats. Aloe vera leaf gel dried powder (100 mg/kg, 200 mg/kg and 400 mg/kg) was dissolved in distilled water and given orally to test group rats.

Tail flick test

Instruments and apparatus for tail flick test: Analgesiometer

Principle: Rat tail is exposed to Radiant heat produced by Nichrome wire (Indirect heat).

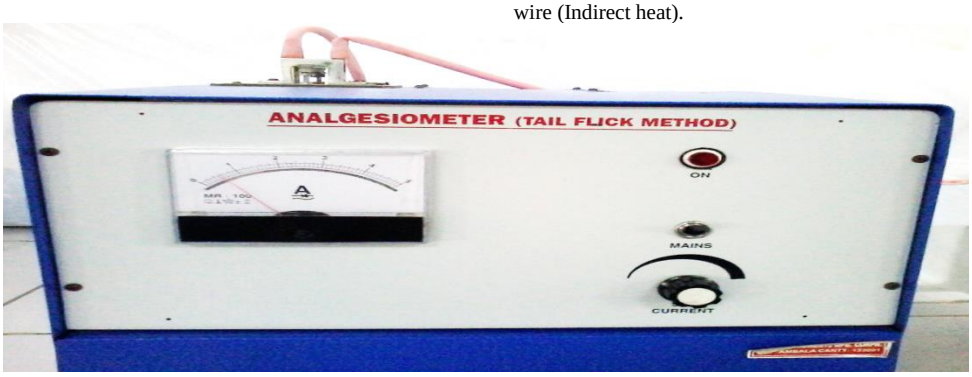

Fig 1: Analgesiometer

Description

The instrument is equipped with rat holder to hold the rat in position. It has opening through which the tail hangs out so that it can be placed over the nichrome wire. There is continuous flow of water around the wire to avoid heating of the surroundings so that the radiant heat is emitted only from the nichrome wire.This instrument operates on 220/230 V and 50Hz. The distal 2-3cms of tail placed over the nichrome wire. Tail flick latency i.e. the time duration from switching on the analgesiometer after placing the tail over the nichrome wire, till the flicking of the tail is noted.

Procedure:

Tail flick test by analgesiometer[12]**:**Albino rats of either sex were selected by the process of randomization and those showing reaction time of less than 6 sec were used for experimental purpose. They were weighed and divided into 7 groups containing 6 animals in each group. The tail flick latency was measured at basal level i.e. at 0 minute, i.e. immediately after giving the drug, and then successively at 30 min, 60 min, 90 min, 120min of duration after drug administration. Tail flick latency is the time duration from switching on the analgesiometer after placing the tail over the nichrome wire till the flicking of the tail. Distilled water was used as control. The anti-nociceptive activity was considered as positive when reaction time is more than 6 sec. Cut-off time is taken as 10 sec in order to prevent the damage to the rat tail.

Fig 2:Tail flick test procedure

Statistical analysis: ANOVA test was used for calculation for statistical significance in between groups. p value < 0.05 is considered as statistically significant.Maximum possible effect in percentage (MPE%) at 120 minutes is calculated in each group for calculating statistical significance.

$$\text{MPE\%} = \frac{\text{(Post Drug Latency – Pre Drug Latency)}}{\text{(Cut-Off Time – Pre Drug Latency)}} \times 100$$

Results

Tail flick latency (sec) was recorded at 0 min, 30 min, 60 min, 90 min and 120 min after drug administration. Oral administration of Aloe vera increased the tail flick latency period (sec) (Mean ± SE) in the doses of 200 mg/kg and 400 mg/kg at 60 min (6.08±0.15, 7.16±0.10 respectively), 90 min (6.75±0.11, 7.66±0.10 respectively), and 120 min (7.33±0.10, 8.58±0.15 respectively) interval in comparison to control (DW) treatment group (2.83±0.17, 3.08±0.08, 3.25±0.11 respectively) (Table no. 3,4,5), indicating Aloe vera can produce anti-nociceptive effect in tail flick test. However, there is not much increase in the tail flick latency in the Aloe vera 100 mg/kg (oral) treatment at 60, 90 and 120 min (2.92±0.15, 3.17±0.10, 3.17±0.10 respectively) in comparison to control (DW) treatment group (Table no. 2,5).Oral (p.o) administration of known analgesic drug Tramadol in the anti-nociceptive dose of 20 mg/kg produced increase in the tail flick latency at 60, 90 and 120 min (7.91±0.08, 8.41±0.08, 9.33±0.16 respectively) in comparison to control (DW) treatment group (Table no. 1.5). However, there is not much increase in the tail flick latency in the Tramadol 10 mg/kg (p.o) treatment at 60, 90 and 120 min (2.91±0.08, 3.08±0.20, 3.25±0.11 respectively) in comparison to control (DW) treatment group. (Table no. 5).Maximal possible effect (MPE) in tail flick latency in percentage (%) at 120 min was calculated (Mean±SE) in Aloe vera 200 mg/kg, Aloe vera 400 mg/kg, and Tramadol 20 mg/kg (62.30±1.67, 80.16±2.27, 90.63.±2.32 respectively) which is more and statistically significant in comparison to control group (6.74±2.43) (Table no. 6). These results suggest that Aloe vera 200 mg/kg, Aloe vera 400 mg/kg and Tramadol 20 mg/kg can produce significant anti-nociceptive effect in the tail flick test model in Albino rats. Further intergroup comparison of MPE (%) shown that Aloe vera 400 mg/kg (80.16±2.27) is not comparable with Tramadol 20 mg/kg (90.63±2.32) (Table no. 6). MPE (%) of combination group Aloe vera 100 mg/kg + Tramadol 10 mg/kg (6.47±2.29) did not show any significant change with Aloe vera 100 mg/kg (5.57±1.97) alone or Tramadol 10 mg/kg (8.70±2.58) alone indicating Aloe vera cannot potentiate anti-nociceptive effect of Tramadol (Table no.6).

Table 1: Tail Flick Latency in seconds of Tramadol 20 mg/kg (Analgesic dose) (p.o)

SL NO	Tail Flick Latency in seconds				
	0 min	30 min	60min	90 min	120 min
1	3.00	3.00	8.00	8.50	9.00
2	2.50	3.50	7.50	8.00	9.00
3	3.00	3.00	8.00	8.50	9.00
4	3.00	3.00	8.00	8.50	9.50
5	2.50	3.50	8.00	8.50	10.00
6	3.00	3.50	8.00	8.50	9.50
Total	17.00	19.50	47.50	50.50	56.00
Mean	2.83	3.25	7.91	8.41	9.33
SD	0.25	0.27	0.20	0.20	0.40
SE	0.10	0.11	0.08	0.08	0.16

Table 2: Tail Flick Latency in seconds of Aloe vera 100 mg/kg (p.o.)

SL NO	Tail Flick Latency in seconds				
	0 min	30 min	60 min	90 min	120 min
1	2.00	3.00	3.00	3.00	3.00
2	3.00	3.00	3.00	3.50	3.50
3	2.50	2.50	2.50	3.00	3.00
4	3.00	3.00	2.50	3.00	3.00

5	3.00	3.50	3.00	3.50	3.50
6	3.00	3.00	3.50	3.00	3.00
Total	16.50	18.00	17.50	19.00	19.00
Mean	2.75	3.00	2.92	3.17	3.17
SD	0.42	0.32	0.38	0.26	0.26
SE	0.17	0.13	0.15	0.10	0.10

Table 3: Tail Flick Latency in seconds of Aloevera 200 mg/kg (p.o.)

SL NO	Tail Flick Latency in seconds				
	0 min	30 min	60 min	90 min	120 min
1	3.00	3.50	5.50	6.50	7.00
2	3.00	3.00	6.00	7.00	7.50
3	3.00	3.50	6.50	7.00	7.50
4	2.50	3.00	6.50	7.00	7.50
5	3.00	3.00	6.00	6.50	7.00
6	3.00	3.50	6.00	6.50	7.50
Total	17.50	19.50	36.50	40.50	44.00
Mean	2.91	3.25	6.08	6.75	7.33
SD	0.20	0.27	0.37	0.27	0.25
SE	0.08	0.11	0.15	0.11	0.10

Table 4: 8 Tail Flick Latency in seconds of Aloe vera 400 mg/kg (p.o.)

SL NO	Tail Flick Latency in seconds				
	0 min	30 min	60 min	90 min	120 min
1	3.00	3.50	7.50	8.00	8.50
2	2.50	3.00	7.00	7.50	8.50
3	3.00	3.00	7.50	8.00	8.50
4	3.00	3.50	7.00	7.50	9.00
5	3.00	3.50	7.00	7.50	8.00
6	2.50	3.00	7.00	7.50	9.00
Total	17.00	19.50	43.00	46.00	51.50
Mean	2.83	3.25	7.16	7.66	8.58
SD	0.25	0.27	0.25	0.25	0.37
SE	0.10	0.11	0.10	0.10	0.15

Table 5:Comparison of tail flick latency (in sec) in with different groups (Mean ± SE)

	0 min	30 min	60 min	90 min	120min
Control (DW)	2.75±0.11	3.00±0.18	2.83±0.17	3.08±0.08	3.25±0.11
Tramadol (10 mg/kg)	2.58±0.20	3.00±0.13	2.91±0.08	3.08±0.20	3.25±0.11
Tramadol (20 mg/kg)	2.83±0.10	3.25±0.11	7.91±0.08	8.41±0.08	9.33±0.16
Aloe vera (100 mg/kg)	2.75±0.17	3.00±0.13	2.92±0.15	3.17±0.10	3.17±0.10
Aloe vera (200 mg/kg)	2.91±0.08	3.25±0.11	6.08±0.15	6.75±0.11	7.33±0.10
Aloe vera (400 mg/kg)	2.83±0.10	3.25±0.11	7.16±0.10	7.66±0.10	8.58±0.15
Aloe vera 100 mg/kg + Tramadol 10 mg/kg	2.58±0.20	2.92±0.24	3.00±0.18	2.92±0.15	3.08±0.08

Aloe vera 200 mg/kg and 400mg/kg (p.o.) produced dose dependent increase in tail flick latency (sec) at 60 min, 90min, and 120 min in comparison to Distilled water (control) 0.5 ml p.o.Tramadol 20 mg /kg p.o. produced increase in tail flick latency (sec) at 60 min, 90 min, and 120 min in comparison to Distilled water (control) 0.5 ml p.o.Combination group Aloe vera 100 mg/kg + Tramadol 10 mg /kg did not show much increase response in tail flick latency (sec) at 60min, 90 min, 120 min in comparison to Distilled water (control) 0.5 ml (p.o), Aloe vera 100 mg/kg alone and Tramadol 10 mg /kg alone.

Table 6:MPE (in %) of tail flick latency in various drug pre-treatments

SL NO	CTRL	T 10	T 20	AV 100	AV 200	AV 400	AV 100 + T 10
1	0.00	7.14	85.71	12.50	57.14	78.57	0.00
2	6.67	12.50	86.67	7.14	64.28	80.00	6.67
3	13.33	7.14	85.71	6.67	64.28	78.57	0.00
4	0.00	0.00	92.86	0.00	66.67	85.71	7.14
5	7.14	6.67	100.00	7.14	57.14	71.43	12.50
6	13.33	18.75	92.86	0.00	64.28	86.67	12.50
Total	40.47	52.2	543.81	33.45	373.79	480.95	38.81
Mean	6.74	8.70	90.63	5.57	62.30	80.16	6.47
SD	5.96	6.33	5.69	4.82	4.10	5.56	5.60
SE	2.43	2.58	2.32	1.97	1.67	2.27	2.29

CTRL – Control, T 10 – Tramadol 10 mg/kg, T 20 – Tramadol 20 mg/kg, AV 100 – Aloe vera 100mg/kg, AV 200 – Aloe vera 200mg/kg, AV 400 – Aloe vera 400mg/kg, AV 100 + T 10 – Aloe vera 100mg/kg + Tramadol 10 mg/kg

MPE in % of increased tail flick latency is more in Tramadol 20 mg/kg, Aloe vera 200 mg/kg and Aloe vera 400 mg/kg in comparison to control group. Further comparison showed there is no much increase in MPE in % of tail flick latency of combination group of Aloe vera 100 mg/kg + Tramadol 10 mg/kg in comparison to Aloe vera 100 mg/kg alone and Tramadol 10mg/kg alone.

Discussion

Pain is an ill-defined, unpleasant sensation usually evoked by an external or internal noxious stimulus. The principal objective of the treatment of pain is to remove or abolish the cause of pain. But it is not always possible to do so. Hence, analgesics are used for the

symptomatic treatment of pain. The analgesics are of two types a) opioids and b) non-opioids (NSAIDs).

Opioids produce a wide spectrum of unwanted effects, including respiratory depression, nausea, vomiting, dizziness, mental clouding, euphoria/ dysphoria, pruritis, constipation, increased pressure in the biliary tract, urinary retention, hypotension and dependance. NSAIDs are known to produce unwanted effects like cardiac toxicities, peptic ulceration, perforation of stomach, blood dyscrasias and renal toxicities. Because of these unwanted effects, there is a continuous and ongoing research to identify more effective and safer agents. Plant extracts have been used for a long time as a traditional remedy for several medical conditions. Many plant extracts are tested for their medicinal activity. Aloe verahas been used in folk medicine as a remedy for various diseases. Hence, Aloe vera leaf gel dried powder dissolved in distilled water was evaluated for the anti-nociceptive activity in three experimental models conducted in albino rats i.e. tail flick method, tail immersion method, tail clip method. The known opioid analgesic Tramadol is used as standard drug.

Tail flick test: Tail flick latency (sec) was recorded at 0 min, 30 min, 60 min, 90 min and 120 min after drug administration. Oral administration of Aloe vera increased the tail flick latency period (sec) (Mean ± SE) in the doses of 200 mg/kg and 400 mg/kg at 60 min (6.08±0.15, 7.16±0.10 respectively), 90 min (6.75±0.11, 7.66±0.10 respectively), and 120 min (7.33±0.10, 8.58±0.15 respectively) interval in comparison to control (DW) treatment group (2.83±0.17, 3.08±0.08, 3.25±0.11 respectively), indicating Aloe vera 200 g/kg and 400mg/kg can produce anti-nociceptive effect in tail flick test.The known analgesic drug Tramadol in the anti-nociceptive dose of 20 mg/kg produced increase in the tail flick latency at 60, 90 and 120 min (7.91±0.08, 8.41±0.08, 9.33±0.16 respectively) in comparison to control (DW) treatment group. Further Maximum possible effect (MPE%) of Tramadol 20 mg/kg (90.63±2.32) is significantly more than MPE effect of Aloe vera 400 mg/kg (80.16± 2.27) suggesting that Tramadol is more potent anti-nociceptive than Aloe vera in tail flick test model. MPE (%) of combination group (low doses) Aloe vera 100 mg/kg + Tramadol 10 mg/kg (6.47±2.29) did not show any significant change in comparison with Aloe vera 100 mg/kg (5.57±1.97) alone or Tramadol 10 mg/kg (8.70±2.58) alone indicating Aloe vera cannot potentiate anti-nociceptive effect of Tramadol.

Conclusion

Aloe vera leaf gel extract powder (dissolved in distilled water) possesses anti-nociceptive activity at 200 mg/kg and 400 mg/kg dosage at 60, 90 and 120 minutes when given orally in tail flick, model in albino rats.

Anti-nociceptive effect of Tramadol 20 mg/kg in terms of maximum possible effect in percentage (MPE%) at 120 minutes is significantly more than Aloe vera 400 mg/kg orally suggesting Tramadol is more potent anti-nociceptive than Aloe vera in tail flick method.

Combination of sub-antinociceptive doses of Aloe vera 100 mg/kg and Tramadol 10mg/kg did not show significant anti-nociceptive activity at 60, 90 and 120 minutes suggesting that Aloe vera had no synergistic action with Tramadol.

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