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Research Article

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY AND ANALGESIC EFFECT OF *ALOE VERA* LEAF EXTRACT IN RATS

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ABSTRACT
Clinical evaluation of analgesic and anti-inflammatory drugs envisages the development of side effects that makes efficacy of a drug arguable. Alternatively, indigenous drug with fewer side effects is the major thrust area of research in the management of pain and inflammation. In the present study aqueous extract of whole leaf of *Aloe vera* at various concentrations was investigated for its anti-inflammatory and analgesic activities in albino wistar rats. Carrageenan and formaldehyde-induced rat paw oedema was used to evaluate the anti-inflammatory activity and tail flick, hot plate and acetic acid tests were used to assess the analgesic activity of *A. vera* leaf aqueous extracts. Whole leaf aqueous extracts at various concentrations (100, 200, 400, and 600 mg/kg of bw) significantly reduced formation of oedema induced by carrageenan and formaldehyde and granuloma formation in a dose dependent manner. Further, acetic acid-induced writhing model exhibited significant analgesic effect characterized by reduction in writhes. Whole leaf aqueous extract showed dose-dependent increase in tolerance to thermal stimulus comparable to indomethacin. No mortality was observed during the acute toxicity test at a dosage of 600mg/kg. Thus whole leaf aqueous extract of *Aloe vera* can be exploited as non toxic drug for the treatment and clinical management of inflammation and pain.
KEYWORDS: Analgesic, Anti-inflammation, *Aloe vera*, Indomethacin

INTRODUCTION

Despite the progress made in medical research during the past decades, treatment of many serious diseases is still problematic. India has a severe shortage of human resources for health. It has a shortage of qualified health workers and the workforce is concentrated in urban areas. Bringing qualified health workers to rural, remote, and underserved areas is very challenging. Many Indians, especially those living in rural areas, receive care from unqualified providers who use plants as source of raw drug in an unscientific manner. Chronic inflammatory diseases remain one of the major health problems[1]. Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, and extravasations of fluid, cell migration, tissue breakdown and repair[2]. Inflammation is one among them, conventional drugs used to ameliorate this phenomenon are either too expensive or toxic and not commonly available to rural folks that constitute the major populace of the world, this study therefore seek to assess *Aloe vera* for anti-inflammatory activity and analgesic effects in experimental animal models.

Aloe vera (syn *Aloe barbadensis* Miller) belongs to the Liliaceae family. *Aloe vera* is a succulent plant that grows in hot, dry climate. Cosmetic and some medicinal products are made from the mucilaginous tissue in the centre of the *Aloe vera* leaf and called *Aloe vera* gel. The peripheral bundle sheath cells of *Aloe vera* pro-duce intensely bitter, yellow latex, commonly termed *Aloe* that has laxative effects. However, total leaf extracts may contain anthraquinones. Studied Pharmacological effects of *Aloe* as in vitro or in animals include anti-inflammatory and anti-arthritic activity, and antibacterial and hypoglycaemic effects. *Aloe vera* has been used for medicinal purposes in several cultures for millennia. *Aloe vera* contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids, and amino acids. There are over 400 species of *Aloe*[3], with perennial, strong and fibrous roots and numerous, persistent, fleshy leaves, proceeding from the upper part of the root, narrow, tapering, thick and fleshy, usually beset at the edges with spiny teeth. *Aloe vera* (*Aloe barbadensis* Miller) is a specific species of *Aloe* and is classified as a succulent plant. Flowers are erect, terminal spikes without calyx, corolla tubular, yellow or purplish color. Capsules contain numerous angular seeds. *Aloe vera* is one of the most widely used healing plants in the history of mankind. The most important constituents of *Aloe*s are the two Aloins, namely Barbaloin and Isobarbaloin. *Aloe* leaf exudates

also possess antidiabetic[4] and cardiac stimulatory activity[5]. *Aloe vera* is one of the few substances known to effectively decrease inflammation and promote wound healing[6]. *Aloe vera* gel could promote the healing of burns and other cutaneous injuries and ulcer[7].

MATERIALS AND METHODS

Chemicals and drugs

The chemicals used in the study carrageenan, formaldehyde, acetic acid and indomethacin were purchased from Sigma-chemicals, Bangalore, India

Plant material collection

Fresh leaves of *Aloe vera* were collected from Tiruchirappalli district, Tamilnadu, India in October 2010. The material was identified by Rev. Bro. John Britto, The Rapinat Herbarium at St Joseph College, Tiruchirappalli district, Tamilnadu, India. A voucher specimen (KA001 2011/01) was deposited in the St Joseph College, Tiruchirappalli, Tamilnadu, India.

Aqueous extraction of *Aloe vera*

Leaves were collected, washed in cold water; spines around the leaves were removed using a knife after which the leaves were sliced. Two hundred grams of the sliced material were mixed with 100 ml of distilled water and blended in an electric blender for 3 min[8] to obtain 200% (w/v) extract. The blended material was squeezed through a muslin cloth. The filtrate was freeze-dried at -50°C under vacuum using a lyophiliser and kept in a freezer at −20°C until use. Various dose levels of the *Aloe vera* were made by reconstituting the extract at a concentration of 1% (w/v).

Animals and experimental design

Thirty Wistar rats, of either sex, weighing 120±35g were used. The rats were purchased from animal house, Bangalore, India. The experiments were designed as per ethical guidelines of Madurai Kamaraj University, Madurai, Tamilnadu under standardized environmental conditions (ambient room temperature 25±2°C and 12h LD cycle). For each of the experiments conducted and explained below, a completely randomised design was used in which the rats were randomly grouped into six groups of five rats each. The rats were allowed free access to standard commercial rat pellets (Hindustan Lever Limited, India). Clean water was provided *ad libitum* throughout the experimental periods.

Preliminary phytochemical screening

Preliminary phyto chemical screening of the extracts was carried out as per the methods and tests given by Dey and Raman[9].

Anti-inflammatory activities

Carrageenan-induced rat paw oedema

Distilled water (DW) and indomethacin were administered intraperitoneally (i.p) to rats in group I (negative control; 5 ml/kg b.w) and group II (positive control; 10 mg/kg b.w) respectively. The extract of *Aloe vera* (100, 200, 400 and 600 mg/kg b.w i.p) was administered to rats in group III – VI respectively[11]. An hour later, rats were injected with 0.05 ml of 1% carrageenan suspension into the foot pads of the left hind paws[4]. Linear diameters of the injected paws were measured using a micrometer screw gauge (Sterling Manufacturing Co., India) for four hours at one hour intervals. Increases in the paw diameter were taken as an indication of paw oedema. The percentage inhibition of inflammation was computed using the formula[12].

$$\%\ inhibition = \frac{Do - Dt}{Do} X\ 100$$

where: D0 = the a*vera*ge inflammation (hind paw oedema) of the negative control group at a given time period; Dt = the a*vera*ge inflammation (hind paw oedema) of the treated group at a given time period[10].

Cotton pellet granuloma test in rats

The cotton pellet granuloma model test in rats was performed according to Winter and Porter was used with slight modification to evaluate the effect of *Aloe vera* on chronic inflammation. The animals were anaesthetized with chloroform. The back skin was shaved and disinfected with 70% ethanol. An incision was made in the lumbar region. Subcutaneous tunnels were formed with a blunted forceps. Pre-weighed autoclaved cotton pellets (10 ± 1 mg) were aseptically placed on neck region of previously depilated back of the rats. On day 1, the rats in group III-VI received the extract of *Aloe vera* 100, 200, 400 and 600 mg/kg b.w i.p respectively. The negative control (group I) received only equivalent volume of vehicle (3% (v/v) Tween 85), while the positive control (group II) received indomethacin (10 mg/kg b.w) as anti-inflammatory agent. All samples were administered once daily for the next seven days. On day 8, the animals were killed, then the pellets were dissected out, freed of tissue attachments, and dried in the oven overnight at 60°C until the weight remained constant. The dried pellets were weighed and the mean weight of the granuloma tissue formed around each pellet was determined. The level of inhibition of granuloma tissue development was calculated using the relation[13]:

$$Inhibition\ of\ Granuloma\ Tissue\ (\%) = 100\ X\ \frac{Tc\ X\ Tr}{Tc}$$

where T_C is weight of granuloma tissue of control group and Tr is weight of granuloma tissue of treated group.

Formaldehyde-induced paw oedema

The experimental rats in group I and groups III - VI orally received 5 ml/kg b.w of DW and graded levels *Aloe vera* extract (100, 200, 400 and 600 mg/kg b.w i.p) respectively, for 7 consecutive days. Rats in group II was administered with indomethacin (10 mg/kg b.w s.c. (sub-cutaneous)). After one hour, on the first and the third day of the experimental period, the rats were injected with 0.1 ml of 2% formaldehyde into the foot pad of the left hind paw[14]. On the first day, and third day paw oedema was measured using a micrometer screw gauge (Sterling Manufacturing Co., SMC 20326, India) an hour before and after formaldehyde injection. On other days paw oedema was measured daily an hour after the treatment with the test extracts. The percentage inhibition of inflammation was calculated as in the Carrageenan-induced rat paw oedema.

Analgesic activity

Hot Plate Method

Rats were placed on an Ugo Basile hot plate at 55°C ± 1°C. Response time was recorded as the time elapsed before the mouse responded (by licking, flicking of a hind limb or jumping). Only rats with a control response time of 4–9s were included in the study. 0.9 % Saline (5 ml/kg b.w), indomethacin (10 mg/kg b.w) and graded dosage levels of *Aloe vera* extracts (100, 200, 400 and 600 mg/kg b.w i.p) were administered to rats in group I, group II and groups III - VI respectively. The reaction time of animal was noted down at 0, 30, 60, 90, 120, 150 and 180 minutes after the treatment[15].

Tail Immersion Method

0.9 % Saline (5 ml/kg b.w), indomethacin (10 mg/kg b.w) and graded dosage levels of *Aloe vera* extracts (100, 200, 400 and 600 mg/kg b.w i.p) were administered to rats in group I, group II and groups III - VI respectively. The rats were held in position in a suitable restrainer with the tail extending out. The tail of the rat 4 - 5 cm from its tip was dipped into a water bath maintained at 55 ± 0.5°C. The time in seconds taken to flick or withdraw the tail out of the water was recorded as the reaction time due to the analgesia in the extracts and commercial drug. This was recorded after every 30 minutes for 3 hours[15].

Acetic acid-induced writhing response in rats

Distilled water at 5 ml/kg body weight (group I), indomethacin at 10 mg/kg body weight (group II) and graded dosage levels of *Aloe vera* extracts (100, 200, 400 and 600 mg/kg b.w i.p) were administered to the experimental animals. Thirty minutes later, 0.6% acetic acid solution was administered intraperitoneally to all the experimental rats. The number of writhes occurring was counted for 30 min after a latency period of 5 min. A significant reduction of writhes in tested animals compared to those in the control group was considered as an antinociceptic response (reducing sensitivity to painful stimuli) and was calculated using the formula:

$$\frac{C - D}{C} \times 100$$

where C is the a*vera*ge number of writhings for the control group of rats and *D* is the a*vera*ge writhings of the extract treated rats[16]

Toxicity studies

For toxicity studies the extracts of *Aloe vera* in the dosage of 200, 400, and 600mg/kg were administered in two groups of rats respectively. The mortality rates were observed after 72 hours. The LD_{50} was determined using the graphical methods[17].

Statistical analysis

The experimental results were expressed as the mean ± S.D

RESULTS

In the present study, preliminary qualitative phytochemicals screening of aqueous extract of *Aloe vera* was carried out. The screening revealed the presence of phytochemicals like tannins, Phlobatannins, saponnin, Flavonoides, terpenoids, cardiac glycosides. The anti-inflammatory effect of the aqueous extract of *Aloe vera* and indomethacin in carrageenan induced paw edema model in rats has been shown in Table I. The aqueous extract of *Aloe vera* produced dose-dependent and significant inhibition of carrageenan-induced paw edema. The inhibition was significant at the doses of 100 mg/kg (+ 9.20%), 200 mg/kg (+4.84%), 400 mg/kg (-9.44%), 600 mg/kg of b.w (-23.24%) and was comparable to that of the standard drug indomethacin (-7.39%). The 600 mg/kg b.w of *Aloe vera* exhibited most significant anti- inflammatory activity in Carrageenan induced paw oedema.

The anti-inflammatory effect of *Aloe vera* was calculated depending on the wet and dry weight of cotton pellets. The continuous oral treatment (7 days) with plant extract (100, 200, 400 and 600 mg/kg b.w) remarkably reduced the formation of granuloma which was indicated by the significant reduction in weight of cotton pellets (wet minus dry).The different dosage of plant extracts showed significant inhibition in wet and dry weights of cotton pellet granuloma. *Aloe vera* exhibited 14.33%, 27.01% 35.37% and 43.53% of inhibition in wet and dry weights of granuloma, respectively. The inhibition exhibited by 400 mg/kg b.w of *Aloe vera* was comparable with

standard drug indomethacin (38.51%). The 600 mg/kg b.w exhibited most significant inhibition of granuloma formation. Table II. The anti-inflammatory effect aqueous extract of *Aloe vera* was identified by the method of formalin induced oedema can be used to clarify the possible mechanism of the acute and chronic anti-inflammatory effect of the *Aloe vera* Table III

The aqueous extract of *Aloe vera* produced dose-dependent and significant inhibition of formalin induced paw edema both at acute and chronic inflammation. On third day the inhibition was significant at the doses of 100 mg/kg (-5.43%), 200 mg/kg (-7.1%), 400 mg/kg (-18.6%) and 600 mg/kg (-35.1%) of b.w. At the end of the experiment, on seventh day the inhibition was significant at the dosage of 100 mg/kg (16.09%), 200 mg/kg (21.15%), 400 mg/kg (26%), and 600 mg/kg (33%) of b.w. The results of 400 mg/kg b.w of aqueous extract of *Aloe vera* was comparable with standard drug indomethacin which exhibited 25.25% in inhibition on the other hand 600 mg/kg b.w of aqueous extract of *Aloe vera* exhibited the most significant anti-inflammatory activity in formalin induced paw oedema both in acute and chronic inflammation. *Aloe vera* produced significant ($P < 0.001$) analgesic activity in hot plate and tail flick methods at all tested doses when compared to that of control. (Table IV and V) In Acetic acid induced writhing model the number of writhes produced in animals treated with extract of *Aloe vera* was significantly lower than control group (Table VI). Test for acute toxicity was found to be non-toxic at the dosage of 200, 400, and 600mg/kg b.w and did not cause death of the animals tested.

DISCUSSION

Carrageenan is widely used to induce acute inflammation[18].Thus Carrageenan-induced rat paw oedema is a suitable test for determining anti-inflammatory action of drugs and has been frequently used to assess the anti oedematous effect of natural products. The development of oedema in the paw of the rat after the injection of carrageenan is a biphasic response. The initial phase is due to the release of histamine and serotonin and the maintenance of the oedema during the plateau is caused by kinin like substances[19]. The second phase of oedema is due to the release of prostaglandins, protease and lysozyme, that is mediated by bradykinin, leucotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages[20]. Histamine and serotonin are important inflammatory mediators and they are potent vasodilatory substances as well as they increase the vascular permeability.

The result of the carrageenan induced paw oedema indicates that *Aloe vera* plays a crucial role against inflammation. It could be argued that the suppression of the first phase may be due to inhibition in the release of mediators, such as histamine and serotonin and the action in the second phase may be explained by an inhibition of cyclooxygenase. Thus, it may be suggested that treatment of *Aloe vera* may prevent the inflammatory action of carrageenan by decreasing PGE_2 level, which may be also be due to the presence of vitamin B1, B2, B6, C, β-carotene, choline, folic acid, α-tocopherol in the flesh of *Aloe vera*[21]. Reduction in the level of prostaglandins might be due to the phenolic compound present in the C*ucurbita pepo Linn* which resulted in the reduction of paw oedema[22].

The cotton pellet model is an indication for the proliferative phase of inflammation. It has been widely employed to assess the transudative, exudative and proliferative components of chronic inflammation[23]. Chronic inflammation is a reaction arising when the acute response is insufficient to eliminate proinflammatory agents. The inflammation starts as proliferation of fibroblasts, the infiltration of neutrophils, the proliferating cells penetrate the exudates producing a highly vascularised reddened mass known as granuloma tissue[24]. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma and the dry weight correlates well with the amount of granulomatous tissue formed. In our study the granulomatous tissue formation was significantly reduced with the administration of *Aloe vera*. Presence of phenolic compounds in aqueous extract of *Aloe vera* may be responsible for the anti-inflammatory activities in sub-acute inflammatory models.

Efficacy of anti-inflammatory agents in sub-acute inflammatory condition is indicated by their ability to inhibit the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation[25] Phenolic compounds from *Emblica officinalis* were able to suppress cotton pellet induced acute and chronic inflammation in animal model[26]. The inhibitory action of *Pholiota nameko* polysaccharide (PNPS_1) against granuloma formation in rats induced by cotton implantation was found to significantly ($P<0.05$) reduced the granuloma formation[13]. Formalin causes peripheral tissue inflammation. Acute inflammation lasts a relatively short duration, only for few minutes to few days where as chronic for longer period. Acute inflammation induced by formalin results from cell damage, which provokes the production of endogenous mediators such as

histamine, serotonin, prostaglandins and bradykinin and results in the exudation of fluid, plasma proteins and the emigration of leukocytes, predominantly neutrophils. Neutrophils stimulation also causes increased vascular permeability and produces edema which causes inflammation[27].

The results of the present study, the anti inflammatory activity of *Aloe vera* may be attributed to inhibition of inflammatory mediators. The presence of phenolic compounds in aqueous extract of *Aloe vera* may be responsible for the anti-inflammatory activities in both acute and chronic inflammation models. Anti-inflammatory activity of *A. vera* is attributed to the inhibition of arachidonic acid pathway through cyclo-oxygenase. Also, the aqueous extract of *Aloe* gel is reported to have inhibited the production of prostaglandins E_2 from arachidonic acid *in vitro*[29]. Prostaglandins tend to stimulate nerves that signal pain to the brain and are involved in the swelling of the blood vessels at the injured site, opening space in the capillary walls for the white blood cells to enter. Therefore, reduction in the level of prostaglandins might be due to the phenolic compound present in the *Aloe vera* which resulted in the reduction of paw oedema.

The hot plate method has been found to be suitable for the evaluation of centrally but not of peripherally acting analgesics. The validity of this test has been shown even in the presence of substantial impairment of motor performance[30]. In hot plate test, nociceptive reaction towards thermal stimuli in rat is a well validated model for detection of opiate like analgesic drugs where in pain response is from spinal origin[31]. In our present study the four doses of the extracts had increased the reaction time in dose dependent manner 600mg/ kg bw of *Aloe vera* had exhibited the highest anti-nociceptive effect to the thermal stimulus at 180 min, which is comparable to the effect of standard drug of indomethacin. The present findings of the study indicate that the methanolic extract of *Aloe vera* may be centrally acting.

MECB (methanolic extract of *Caesalpinia bonducella*) produced significant ($P < 0.01$) analgesic activity at all test doses when compared to that of control. Additionally, MECB at different doses potentiated the analgesic activity of the standard drug (Morphine 5 mg/kg)[32]. In tail flick method, the aqueous extract of *Aloe vera* (100, 200,400 and 600mg/kg) produced significant increase in the mean latency of biting of the tail-clip and was dose dependent. 600 mg/ kg b.w of aqueous extract of *Aloe vera* had exhibited the highest anti-nociceptive effect to the thermal stimulus at 180 minutes, which is comparable to the effect of standard drug of indomethacin. The aqueous extract of *Aloe vera* exhibited antinociceptive activity, which indicates both central and peripherally mediated anti-nociceptive properties. The analgesic activity of the plant is attributed to the presence of the enzymes carboxypeptidases and bradykinase that tend to relieve pain[33]. The plants are known to contain some alkaloids and steroidal substances responsible for the release of pain with immunomodulatory and antioxidative properties have been reported in earlier studies[34]. 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[35]. *Cucurbita pepo* contains polysaccharides, para-aminobenzoic acid, fixed oils, sterol, proteins and peptides and the fruits are source of carotenoids and γ-aminobutyric acid responsible for the inflammatory action. The abdominal constriction is related to the sensitization of nociceptive receptors by prostaglandins[36]. In our present study acetic acid induced writhing model the extract of *Aloe vera* reduced the writhes significantly in dose dependent manner at $p<0.001$.The plant *Wattakaka voludilis* possessed potent analgesic property in their acetic acid induced writhing model in animals[37]. Further investigations are needed to substantiate this effect which may throw more light on its mechanism.

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Table 1: Inhibition of inflammation (%) by the aqueous extract of *Aloe vera* and indomethacin on carrageenan induced left hind rat paw oedema

Treatment	Dosage mg/kg	Reaction Time				
		0h	1h	2h	3h	4h
Group-I	-	3.75±0.023	5.22±0.017*	5.39±0.063**	4.92±0.02**	4.46±0.015**
Group-II	10	3.52±0.0310 (-5.3%)	5.125±0.023* (-1.9%)	5.22±0.017NS (-3.15%)	4.62±0.008** (-6.09%)	4.13±0.02** (-7.39%)
Group-III	100	3.63±0.017 (+3.12%)	5.39±0.06* (+5.2%)	5.27±0.04** (+0.95%)	4.81±0.012** (+4.11%)	4.51±0.018** (+9.20%)
Group-IV	200	3.16±0.035 (-10.22%)	4.33±0.030* (-15.42%)	5.13±0.012** (-1.72%)	4.64±0.025** (+0.43%)	4.33±0.014** (+4.84%)
Group V	400	2.54±0.021 (-27.84%)	3.17±0.018* (-38.08%)	4.27±0.012** (-18.19%)	3.82±0.020** (-17.3%)	3.74±0.026** (-9.44%)
Group VI	600	2.32±0.015 (-34.09%)	3.05±0.012* (-40.42%)	4.17±0.012** (-20.11%)	3.72±0.012** (-19.48%)	3.175±0.012** (-23.24%)

Group-I: Negative control, Group-II: Positive control, Group- III, IV, V: Extract of *Aloe vera* Values are means ± S.D n = 5, * Group I Vs Group II, ** Group II Vs Group III, IV, V $P < 0.05$.

Table 2: Anti-inflammatory effect of aqueous extract of *Aloe vera* on cotton pellet granuloma model in rats

Control	Dose Mg/Kg	Granuloma Tissue Weight (mg)	Inhibition %
Saline	-	24.525 ± 1.899	-
Indomethacin	10	15.06 ± 0.428	38.59
Aloe vera	100	21.01 ± 0.704	14.33
Aloe vera	200	17.9 ± 1.507	27.01
Aloe vera	400	15.85 ± 1.008	35.37
Aloe vera	600	13.85 ± 0.802	43.53

Table 3: Inhibition of acute and chronic inflammation (%) by the aqueous extract of *Aloe vera* and indomethacin on formaldehyde induced left hind rat paw oedema

Treatment Group	Dosage mg/kg	Reaction time						
		1	2	3	4	5	6	7
Group-I		4.93±0.03	5.06±0.02	6.07±0.01	5.54±0.03	5.16±0.03	4.65±0.03	4.35±0.02
Group-II	10	4.64±0.03 (-5.88%)	4.44±0.03 (-2.25%)	5.44±0.02 (-0.37%)	4.64±0.02 (-16.24%)	3.75±0.03 (-27.32%)	3.35±0.04 (-27.95%)	3.25±0.26 -25.28%
Group-III	100	4.83±0.04 (+4.09%)	4.65±0.02 (+4.72%)	5.74±0.04 (+5.51%)	5.16±0.03 (+11.20%)	4.64±0.02 (+23.73%)	4.24±0.03 (+26.56%)	3.65±0.02 (+12.30%)
Group-IV	200	4.76±0.02 (+2.58%)	4.66±0.03 (+4.95%)	5.64±0.03 (+3.67%)	5.06±0.02 (+9.05%)	4.54±0.03 (+21%)	3.95±0.03 (+17.91%)	3.43±0.03 (+5.53%)
Group V	400	4.45±0.03 (-4.09%)	4.25±0.03 (-4.27%)	4.94±0.03 (-9.19%)	3.84±0.02 (-17.24%)	3.73±0.02 (-0.53%)	3.54±0.03 (+5.67%)	3.22±0.02 (+0.92%)
Group VI	600	4.36±0.02 (-6.03%)	4.14±0.03 (-6.75%)	3.94±0.04 (-7.57%)	3.54±0.03 (-23.70%)	3.33±0.03 (-11.2%)	3.17±0.17 (+5.37%)	2.92±0.02 (-10.15%)

Group-I: Negative control, Group-II: Positive control, Group- III, IV, V: Extract of *Aloe vera* Values are means ± S.D n = 5, * Group I Vs Group II, ** Group II Vs Group III, IV, V $P < 0.05$.

Table 4: Analgesic Activity of *Aloe Vera* Using Hot Plate Method

Experimental Groups	Latency time in seconds at						
	0min	30mins	60mins	90mins	120mins	150mins	180mins
Group I	4.3±0.294	3.6±0.40	3.4±0.29	3.37±0.26	2.47±0.34	1.52±0.35	1.38 ±0.26
Group II	4.55±0.40 (+5.81%)	4.85±0.20 (+26.94%)	5.45±0.31 (+60.29%)	5.57±0.40 (+65.28%)	5.6±0.18 (+120.6%)	5.55±0.05 (+253.2%)	5.4± 0.08 (302.17%)
Group III	4.25±0.26 (-6.59%)	4.6±0.40 (+0.65%)	5.3 ± 0.40 (+1.28%)	5.49 ± 0.40 (+2.87%)	5.78±0.29 (-4.22%)	5.15±0.129 (-4.09%)	4.9 ± 0.21 (-11.71%)
Group IV	4.35±0.34 (-4.39%)	5.22±0.17 (+14.22%)	5.6±0.40 (+2.75%)	6.27±0.22 (+12.56%)	6.35±0.12 (+14.67%)	5.43±0.330 (-5.21%)	5.1 ± 0. 082 (-8.10%)
Group V	4.5±0.374 (-1.09%)	5.4±0.31 (+18.16%)	5.65±0.43 (+3.66%)	6.35±0.26 (+14%)	6.22±0.26 (+14.12%)	5.032±0.02 (-6.33%)	4.9 ± 0.21 (-5.40%)
Group VI	4.6±0.40 (+2%)	5.47±0.34 (+19.69%)	5.71±0.47 (+4.77%)	6.52±0.35 (+17.05%)	6.15±0.12 (+12.84%)	5.017±0.01 (-6.70%)	4.65± 0.31 (-4.5 %)

Group I – negative; Group II – positive control Indomethacin; Group III – *Aloe Vera* (100mg/kg); Group IV – *Aloe Vera* (200mg/kg); Group V – *Aloe Vera* (400mg/kg) ; Group VI - *Aloe Vera* (600mg/kg)

Table 5: Analgesic Activity of *Aloe Vera* Using Tail flick Method

Experimental Groups	Latency Time						
	0 min	30 mins	60 mins	90 mins	120 mins	150 mins	180 mins
Group I	3.2±0.21	3.03±0.03	2.92±0.21	2.82±0.02	2.6±0.18	2.5±0.01	1.85±0.20
Group II	4.18±0.12 (+5.81%)	5.1±0.06 (+26.94%)	5.4±0.28 (+60.29%)	5.57±0.40 (+65.28%)	5.7±0.12 (+120.6%)	5.87±0.45 (+253.2%)	5.92±0.39 (+253.2%)
Group III	3.5±0.06 (-6.59%)	3.9±0.02 (+0.65%)	4.1 ± 0.18 (+1.28%)	4.5 ± 0.21 (+2.87%)	4.8±0.77 (-4.22%)	5.1±0.43 (-4.09%)	5.3±0.39 (-4.09%)
Group IV	3.62±0.01 (-4.39%)	4.34±0.02 (+14.22%)	5.04±0.03 (+2.75%)	5.2±0.08 (+12.56%)	5.45±0.31 (+14.67%)	5.9±0.21 (-5.21%)	6.1±0.21 (-5.21%)
Group V	3.72±0.02 (-1.09%)	4.9±0.06 (+18.16%)	5.12±0.01 (+3.66%)	5.4±0.02 (+14%)	5.63±0.03 (+14.12%)	6.1±0.22 (-6.33%)	6.35±0.44 (-6.33%)
Group VI	4.24±0.03 (+2%)	5.04±0.35 (+19.69%)	5.37±0.51 (+4.77%)	5.65±0.19 (+17.05%)	5.85±0.21 (+12.84%)	6.2±0.43 (-6.70%)	6.5±0.31 (-6.70%)

Group I – negative; Group II – positive control Indomethacin; Group III – *Aloe Vera* (100mg/kg); Group IV – *Aloe Vera* (200mg/kg); Group V – *Aloe Vera* (400mg/kg); Group VI - *Aloe Vera* (600mg/kg)

Table 6: Acetic Acid Induced –Writhing Test

Treatment	Dosage (mg/kg)	No. of times writhing within 30mins	Inhibition percentage
Control	0	9.17±0.22	-
Indomethacin	10	0.55±0.26	-94.00%
Extract	100	4.35±0.36	-52.56%
Extract	200	3.45±0.20	-62.37%
Extract	400	2.25±0.23	-75.46%
Extract	600	0.61±0.29	-93.34%

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