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Original Research Article

ANTI-INFLAMMATORY, ANTIPYRETIC AND ANALGESIC ACTIVITIES OF CHLOROFORM EXTRACT OF *ALOE BARBADENSIS* MILLER IN ALBINO RATS

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Abstract: *The pharmacological activities of the leaf gel of Aloe vera have been extensively evaluated. Gel and latex are two basic products of aloe leaves. Latex and gel contain biologically active components. Polysaccharides contained in the leaf gel attribute most of the health benefits like anti-inflammatory, pain and fever, associated with aloe vera. In the present study, chloroform extract of A. barbadensis at various concentrations was investigated for its anti-inflammatory, antipyretic and analgesic activities in albino rats. Twenty-four albino rats were randomly divided into three groups (control, standard and experimental group). Division of groups was the same for all activities. Control and standard groups contain 4 rats in each group whereas experimental group contains 16. All the rats in three groups were treated with carrageenan to induce oedema, Brewer's yeast to induce pyrexia and acetic acid to induce pain. The control group was treated with normal saline for all the activities. Standard group rats were treated with the reference drug diclofenac for anti-inflammatory and analgesic activities and paracetamol for antipyretic activity. Experimental group rats were given chloroform extract of A. barbadensis with 50, 100, 200 and 400mg/kg concentration. The result showed a significant inhibition (98%) in oedema at 3rd hour at the dose of 400mg/kg as compared to control group. For antipyretic activity, there was a significant reduction (66%) in pyrexia at 4th hour at the dose of 50mg/kg as compared to control group. And in analgesic model a significant reduction (64%) in the writhing at the dose of 400mg/kg as compared to control group. These results demonstrated that the chloroform extract of Aloe barbadensis miller have anti-inflammatory, antipyretic and analgesic activity and suggested its inhibitory actions on inflammation, fever and pain.*

Keywords: Aloe barbadensis miller leaf, chloroform, anti-inflammatory, antipyretic , analgesic, paracetamol, diclofenac

Introduction

Recently, there has been a growth in the use of natural products for the prevention and treatment of oral problems, which maygbe advantageous for low socioeconomic-level individuals of both urban and rural areas. Aloe vera, one of the many natural remedies already on the market, is the most well-liked and is currently attracting lot of scientific interest (Alyas et al., 2022; Zhou *et al*., 2018). The Arabic term Aloe vera, which means "shining bitter material," is derived from the Latin word vera, which means "truth." Aloe vera plants have been used since biblical times. It is a perennial succulent xerophyte that forms water-storing tissue in the leaves in order to live in arid regions with little orgin consistent rainfall (Tiwari and Upadhayay, 2018). The plant has stiff lance-shaped grey-green leaves with a centre pulp of mucilaginous material that contains transparent gel. The polysaccharides found in the leaf gel of aloe vera have been linked to the herb's health benefits (Shekhawat *et al*., 2021). Monoecious perennial plants with shallow roots make up the Aloe genus. Aloe species are commonly found in Africa, India, and other dry regions and are typically found in arid conditions. There are over 140 different species of aloe, with the majority being found in South Africa (Salehi et al., 2018). They might, however, also be cultivated in areas with subtropical winter and summer precipitation. The main variables

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limiting genus spread are temperature, soil moisture, fire tolerance, and rainfall (Alyas et al., 2020; Abihudi et al., 2020).

Since they were first used to cure diseases thousands of years ago, phytotherapeutics have seen a sharp rise in popularity in recent years. By the 20th century's close, 170 natural medications are given formal recognition. According to the World Health Organization (WHO), 11% of all critical therapeutic pharmaceuticals are derived from plants, and 80% of people on earth use medicinal flora to get a range of medications to address their fundamental health care needs (Paul *et al*., 2018). Aloes are perennial succulents, also known as xerophytes, that have the ability to retain significant amounts of water in their tissue. They may also utilize crassulacean acid metabolism, an adaptation to the photosynthetic pathway that results in the production of malic acid (Boudreau *et al*., 2013). Technically speaking "gel" or "mucilage" refers to the viscous transparent liquid found inside the parenchyma cells, while "pulp" or "parenchyma tissue" refers to the complete fleshy interior section of the leaf, including the cell walls and organelle (Tesfaye *et al*., 2020).

Methodology

Healthy and fully ripped leaves of Aloe vera were collected from village fields of Chichawatni, Pakistan. Firstly, Aloe vera leaves were washed with fresh water and dried with clean towel. Then leaves were peeled off with a knife and the white gel of Aloe vera was collected in a container. The pulp was liquefied by using a blender. The 200 ml liquid gel was then added in the 700 ml chloroform for 15 days. Jar with Aloe vera gel and chloroform was shaken every day. After 15 days, extract was filtered from chloroform with the help of funnel and filter paper. 250mL extract was obtained after chloroform treatment. The extract was dried using a flash rotary evaporator. Evaporated the obtained extract on rotatory vacuum evaporator at temperature 40-45^{o}C and atmospheric pressure was 100 mm Hg. The extract obtained after rotatory evaporation was placed in 1 petri dish and placed for 24hrs at room temperature for solidification of extract. Female albino rats weighed 140 g to 160 g (4-6weeks old) were obtained. Rats were housed in polypropylene cages at University of Lahore animal house. Rats were fasted before being used in the experiments. After that they were given distilled water and balanced feed.

Anti-inflammatory activity

24 rats were divided into 3 groups: control group, standard group and experimental groups. Control group and standard group consisted of 4 rats in each group. Experimental group consisted of 16 rats which were further divided on the basis of different doses.Control group was named as group I. In control group, rats were treated with normal saline.Standard group was considered as group II. In standard group, rats were treated with diclofenac drug. Experimental group or group III was treated with different doses (50, 100, 200 and 400mg/kg) of *Aloe barbadensis miller* extract. Firstly, all rat groups were treated with carrageenan to induce inflammation. 0.1ml of 1 % carrageenan solution in normal saline was injected into sub planter region of paw. Albino rats were catagorized into 3 sets, group I/control group and group II/standard group consisted of 4 rats in each group, whereas group III/experimental group consisted of 16 rats. After one-hour induction of carrageenan (post inflammation), the animals received treatments Paw sizes were measured before carrageenan induction (pre-inflammation), after one hour induction of carrageenan/post inflammation and then at hourly intervals after administration of different treatments for three hours.

Anti-pyretic activity

24 rats were divided into 3 groups, 8 rats in control and standard groups and 16 rats in experimental group which were further divided on the basis of doses. In group I group II and group III (standard and experimental design groups), all rats were treated with brewer's yeast with normal saline (mixture) which was injected below the nape of neck (50 mg/kg, 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight). The catagorization of animals was same as in the anti-inflammatory model. Temperature was measured before yeast induction (pre-pyrexia). After the interval of 21 hours of Brewer's yeast induction, pyrexia developed and temperature was noted (post- pyrexia). The maximum rise in temperature was 101.48. Standard group was treated with referance drug paracetamol injection (50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg) in intraperitoneal tissues while the experimental group was treated with leaves extract of *A. barbadensis miller* (50 mg/kg, 100mg/kg, 200 mg/kg, and 400 mg/kg) injections. After the interval of 1, 2, 3, 4 hours, temperature was measured with help of thermometer.

Analgesic activity

24 rats divided into 3 groups, 8 rats in control and standard groups and 16 rats in experimental group. The rats in experimental group were further divided on the basis of different doses. Acetic acid induces writing which is used to analyze the potential of chloroform extract in pain. Albino rats were categorized similar as the previous two activity models. In group I (control group), all the rats were given normal saline before one hour of acetic acid induction. Standard drug Dicleofenac (50, 100, 200 and 400mg/kg) and extract of Aloe vera (50, 100, 200 and 400 mg/kg) were injected to group II and group III respectively, 1 hour before the

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administration of acetic acid (intraperitoneal). After that, all rats were treated with acetic acid (50, 100, 200 and 400 mg/kg body weight) and immediately placed in separate boxes. A writhe was recorded with the help of stopwatch. A number of abdominal constrictions were counted in the period of 35 minutes.

Statistical analysis

Measurable examination was performed utilizing ANOVA; the centrality of distinction was acknowledged at $P < 0.005$. The after effects of the investigation were communicated as mean±S.E.M. The factual centrality of distinction was assessed by Student's t-test for unpaired correlation. When $P < 0.05$ contrast was viewed as huge.

Table I: Anti-inflammatory effect of chloroform extract of *A. barbadensis* on carrageenan-induced oedema in albino rats

Groups	Treatment	Pre-Inflammation	Post-Inflammation	1-hour	2-hour	3-hour
Group I	**Control group**	3.32±0.19	4.03^{b}±0.14	3.76^{b}±0.12	3.59^{c}±0.1	3.52^{b}±0.11
Group II	**Standard group**	3.88±0.12	5.55^{a}±0.12	6.1±0.51	6.18^{b}±0.12	5.47^{a}±0.29
Group III	**Treated at 50mg/kg**	3.61±0.02	5.57^{a}±0.53	6.17^{a}±1.1	6.01^{b}±0.38	6.08^{a}±0.41
	Treated at 100mg/kg	3.72±0.16	6.01^{a}±0.94	6.79^{a}±0.57	6.39^{b}±0.13	5.36^{a}±0.87
	Treated at 200mg/kg	3.21±0.17	5.59^{a}±0.11	6.26^{a}±0.2	6.44^{b}±0.21	5.51^{a}±0.42
	Treated at 400mg/kg	3.52±0.05	5.86^{a}±0.9	7.11^{a}±0.18	7.13^{a}±0.03	5.91^{a}±0.27
	p-value	0.0887	0.0304	0.0044	< 0.0001	0.0026

Transcripts on the different means within column differ significantly at $p \leq 0.05$

Results

Effect of Aloe barbadensis Extract on Oedema

Carrageenan is a phlogistic intermediary and edema is its pathophysiological reaction. The leave extract of *A. barbadensis* and the standard drug diclofenac showed significant ($p < 0.05$) % inhibition in induced paw oedema of rats after 1-3hrs compared to the control group (Table: I, Figure: I). The result showed a significant inhibition in oedema at 2nd hour at the dose of 400mg/kg as compared to control group (Table: I, II.)

Table II: Percentage inhibition of inflammation in different groups of albino rats.

	Percentage inhibition		
Treatment	**1-hour**	**2-hour**	**3-hour**
Control group	0 %	0 %	0 %
Standard group	37 %	62 %	84 %
Treated at 50mg/kg	27 %	39 %	43 %
Treated at 100mg/kg	49 %	80 %	77 %
Treated at 200mg/kg	38 %	66 %	79 %
Treated at 400mg/kg	89 %	98 %	67 %

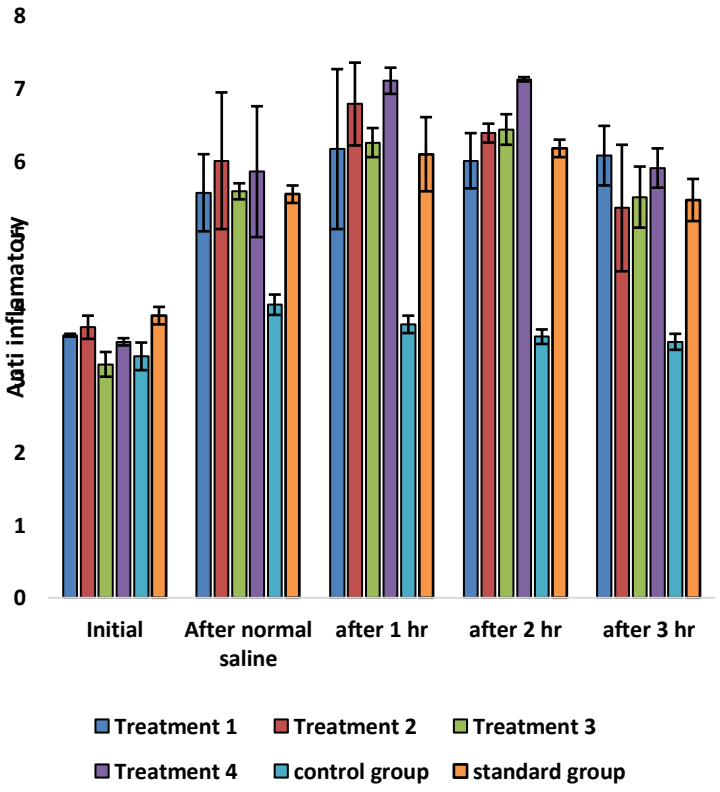

Figure I: Paw size of rats (mm); in control, standard and treated groups of albino rats

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Table III: Antipyretic effect of chloroform extract of *A. barbadensis* on yeast-induced pyrexia in albino rats.

Groups	Treatment	Initial temperature	0-hour	1-hour	2-hour	3-hour	4-hour
Group I	**Control group**	98.10±0.18	101.±0.47	100.4±0.52	100.1^{a}±0.5	99.9±0.48	99.4±0.42
Group II	**Standard group**	97.93±0.17	100.9±0.51	99.3±0.36	98.5^{ab}±0.3	98.3±0.21	97.9± 0.19
	Treated at 50mg/kg	98.05±0.75	99.8±0.05	98.9±0.45	96.5^{b}±0.35	94.1±2.45	96.8± 0.35
	Treated at 100mg/kg	97.55±0.75	100.5±0.15	100.5±0.15	92.9^{d}±0.8	91.8± 1.8	92.90± 0.8
Group III	**Treated at 200mg/kg**	97.70±0.2	99.8 ±0.75	99.7±0.3	96.2^{bc}±2.65	95.5±0.55	96.2± 2.65
	Treated at 400mg/kg	97.25±0.55	100.4±0.3	99.2±0.35	93.5^{cd}±0.45	94.6±1.05	93.8± 0.45
	p-value	0.5906	0.473	0.2658	0.0005	0.0006	0.0006

Transcripts on the different means within column differ significantly at p≤0.05

Effect of A. barbadensis Extract on Pyrexia

Fever was treated with *A. barbadensis* extracts at various doses (50, 100, 200 and 400 mg/kg). The results showed that there was a significant inhibition in pyrexia at 4th hour at the dose of 50mg/kg which was 63% as compared to control group whereas at 1st, 2nd and 3rd hrs it showed 36%, 45% and 57% inhibition respectively (Table:III, IV) (Figure II).

Effects of A. barbadensis extract in Analgesic model

The significant analgesic activity of extract was (p< 0.0001). The result analysis showed a significant reduction in the writhing at the dose of 400mg/kg which is 64% (Table: V) (Figure III).

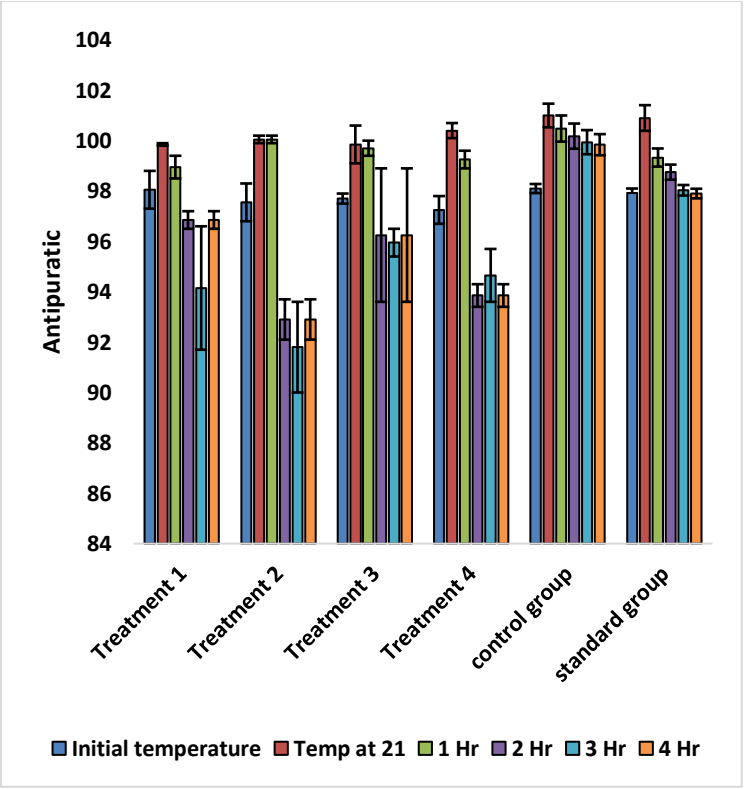

Figure II: Reduction of pyrexia in different groups of albino rats at 1-4hrs.

Table IV : Percentage inhibition of pyrexia in different groups of albino rats.

	Percentage inhibition			
Treatment	**1-hour**	**2-hour**	**3-hour**	**4-hour**
Control group	17 %	28 %	36 %	40%
Standard group	52 %	72 %	96 %	100 %
Treated at 50mg/kg	50 %	66 %	31 %	66 %
Treated at 100mg/kg	0 %	28 %	33 %	40 %
Treated at 200mg/kg	6 %	16 %	18 5	23 %
Treated at 400mg/kg	36 %	45 %	57 %	63 %

Table V: Analgesic effect of chloroform extract of *A. barbadensis* on acetic acid- induced writhing in albino rats.

Treatment	Control group	Standard group	Treated at 50mg/kg	Treated at 100mg/kg	Treated at 200mg/kg	Treated at 400mg/kg	p-value
Analgesic time	18.50^{a}±0.65	6.50^{d}±1.04	12.00^{c}±1	15.00^{bc}±1	16.00^{ab}±1	18.50^{a} ±0.5	< 0.0001
Percentage inhibition	0 %	10 %	13 %	18 %	35 %	64 %	

Transcripts on the different means within column differ significantly at p≤0.05

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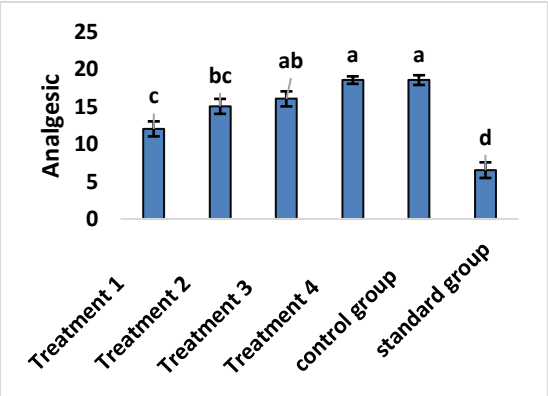

Figure III: Writhing mean in different groups of rats.

Discussion

According to our study the Aloe vera gel and chloroform extracts both contain substances that may lessen edoema caused by carrageenan. Our studies correlated with Higgs *et al*, (1979) who described that diclofenac prevents the production of prostaglandins, similar to most non-steroidal anti-inflammatory drugs, which may account for its anti-inflammatory effects in carrageenan-induced rat paw edoema. Previous studies showed that *A. vera* boosts humoral immunity and lowers cell-mediated immunity (Halder *et al*., 2012). Numerous studies demonstrated the anti-inflammatory effects of A. vera using various modes of action (Prabjone *et al*., 2006). The anti-inflammatory effects of *A. barbadensis* are attributed to their constituent phytochemicals like terpenoids, flavonoids etc. In current studies, the standard group showed 64% inhibition in the pain after injection of drug diclofenac. Prostaglandins are among the inflammatory mediators released by the powerful oedematous agent formalin, which can cause inflammation. In comparison to the control group, the chloroform extract of Aloe barbadensis considerably (P>0.05) decreased the carrageenan-induced oedema after of 3 hours. The present investigation showed that the chloroform extract of *A. barbadensis* showed the dose-dependent significant 66% inhibition in fever. The standard group was found to reduce pyrexia better than that of the plant extract. The antipyretic action of plant extract was similar to that of paracetamol, but it started working 1 hour after the treatment. The chloroform extract caused considerable temperature drops, indicating that it may have antagonised prostaglandin at doses of 50 and 400mg/kg. It was established that the administration of chloroform extract of Aloe vera at 50 and 400mg/kg body weight resulted in considerable antipyretic action. However, as compared to the control group, the chloroform extract of Aloe vera at 50 and 400mg/kg significantly reduced the rat's brewer's yeast-induced pyrexia. Joseph and Raj, (2010) discovered similar outcomes, demonstrating that aloe vera also reduces fever. Although no antipyretic activity has developed with reference to alcoholic extracts. The present results confirmed and extended previous traditional methods of routinely using Aloe vera for analgesic and antipyretic aliments. We observed significant reduction in the writhing (64%) as compared to control group. The polysaccharide in aloe gel, has analgesic properties. For the purpose of studying visceral pain, writhing was elicited by intraperitoneal injection of acetic acid. Diclofenac, the reference medication (Alyas et al., 2020), reduced pain by preventing the synthesis of pain mediators in peripheral tissues. When acetic acid is injected intraperitoneally, endogenous chemicals such as bradykinin and prostaglandins are released, stimulating the nociceptive terminals and causing the nociceptive response. At 200 and 400mg/kg compared to control, a highly significant decrease in the number of abdominal constrictions was seen, demonstrating strong analgesic action in visceral discomfort. The analgesic property we have found in Aloe vera may be due to the presences of anthraquonone in it (Amrit and Samir, 2008).

Conclusion

Finding of the present study have demonstrated that *Aloe vera* has potent analgesic, anti-pyretic and anti-inflammatory activity and its use could be justifiable in traditional medicine to treat inflammation, fever and painful conditions. The administration of aloe vera gel extract showed preventive responses in above mentioned activities in albino rats, as evidenced through experiments, performed on albino rats. Furthermore, there is a need to explore hidden effects of aloe vera on the basis of preclinical and clinical trial sources and need of further investigation on cellular and molecular base level for safety and therapeutic efficacy.

Conflict of interest

The authors declared absence of conflict of interest.

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