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ANTI-INFLAMMATORY AND IMMUNOMODULATORY ACTIVITY OF ETHANOL EXTRACT OF *ALOE VERA* GEL

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ABSTRACT: The current treatment of inflammation includes NSAIDs, selective COX-2 inhibitors, glucocorticoids and anti-TNFα has peptic ulcer, renal and hematological toxic side effects as well as some of these drugs are more expensive. Hence it is necessary to evaluate the new cost effective as well as less toxic drug. *Aloe vera* showing a lots of promise and hence researcher all over are trying to harness the anti-inflammatory potential of *Aloe vera.* **Objective:** To study the anti-inflammatory and immunomodulatory activity of *Aloe vera* gel extract. **Methodology:** The anti-inflammatory activity was evaluated by using carrageenan induced rat paw edema model for acute inflammation and cotton pellet granuloma for chronic inflammation and to evaluate the ulcer potential. The immunomodulatory activity was evaluated by phagocytic activity of Polymorphonuclear cells (PMNs) on Candida cells. **Results:** The *Aloe vera* gel extract in the doses of 100mg/kg, 200mg/kg, show significant anti-inflammatory activity while 400mg/kg show equal anti-inflammatory activity as that of Aspirin on acute model of inflammation. *Aloe vera* gel is not having ulcer potential as that of Aspirin. *Aloe vera* gel extract at large doses *i.e.* 200mg/kg and 400mg/kg show significant immune stimulant activity. But *Aloe vera* gel did not show chronic anti-inflammatory activity. **Conclusion:** *Aloe vera* gel extract has better acute anti-inflammatory activity with good immune stimulant activity. But has no ulcer potential like that of NSAIDs and also have no chronic anti-inflammatory activity.

INTRODUCTION: The current pain treatment options include nonsteroidal anti-inflammatory drugs, selective COX-2 inhibitors, glucorticoids and anti-TNFα. A cursory look at the respective side effects *i.e.*

- Non-steroidal Anti-inflammatory drugs -Peptic ulcer, gut bleeding
- Selective COX-2 and Anti-TNFα - Renal toxicity, hematological and cardiac toxicity.

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Thus it is obvious that there is need of a cost effective and much safer alternative, *Aloe vera* is showing a lot of promise and hence chemists all over the world are trying to harness the anti-inflammatory potential of an *Aloe vera*.

Aloe vera has been described as a "magic bullet" in various literatures but when examined closely and thoroughly it is not one but many magic bullets that combine to create the magic of the *Aloe vera* [1]. It is the unique composition of *Aloe vera* that gives this plant an innumerable biological activities leading to a creation of a myth of it being a miracle plant.

MATERIALS AND METHODS:

Plant materials/Chemical analysis: Commercial *Aloe vera* was obtained as a solid residue by

evaporating the yellow exudates, which drains from the leaves cut from *Aloe vera* plants. This solid residue of *Aloe vera* leaves weighed around 620g. This dry powder was subjected to double maceration process with ethanol (50%). The obtained yield positively responded to the test for anthraquinones, glycosides such as modified Borntrager test, borax test, bromine test *etc* [4]. These *Aloe vera* gel extract was diluted in water for easy administration in rats and mice for oral route.

Toxicity study: This was carried out on male and female mice weighing around 25 - 30g. LD_{50} value was determined by "Staircase or up and down" method. Five groups of 2 mice each were taken and were given single dose of *Aloe vera* extract, doses were started with 50mg/kg, the subsequent doses were then increased by factor 1.5, if the dose was tolerated or decreased by factor 0.7, if the dose was lethal and observed for a period of 24hrs for any mortality [5].

Animals: Experimental studies were performed on albino rats of either sex, weighing about 150-200g, divided into 5 groups in acute and chronic inflammation study while 4 groups in ulcer potential and immunomodulatory study. Aspirin (100mg/kg in rats) was used as standard drug. All the animal experiments were approved by the Institutional animal ethics committee (IAEC) approval number is BJMC/EC-01/09/2002.

Experimental Methods:

1. Acute Inflammation Study:

Carrageenan Induced Rat Paw Edema: Edema was induced by injecting 0.2ml of carrageenan (1%w/v) into the sub plantar region of the right hind paw. After 1h, *Aloe vera* gel extract was given by oral route. The dosage details are given in **Table 1**. One group served as control and was given only vehicle. The other group received aspirin as a standard control drug. The volume of the paw was measured with plethysmograph before and after drug given upto the 6h. Results were determined as the increase in percentage edema upto 6h as compared to both control groups (i.e. +ve and –ve control group) [6, 7, 8, 9].

2. Chronic inflammation:

Cotton Pellet Induced Granuloma in Rats: Autoclaved cotton pellets (10mg each) were implanted subcutaneously by incision on the back under ether anaesthesia. Drugs were administered orally for 5 days. Animals were sacrificed on the day 5 and the granuloma was dissected out, dried in an oven at 60 ^{o}C. The weight of granuloma was calculated by measuring the difference between this weight and initial weight. This difference in weight was taken as the parameter for the comparison of the anti-inflammatory activity of the test drug [10].

3. Ulcer potential: Gastric irritation properties of orally administered compound were evaluated in fasted rats. After treatment, with test extract, the animal were sacrificed at 5h and 7h from each test group and compared with aspirin group [11].

The stomach was removed and inspected for signs of irritation and ulcer [11].

4. Immunomodulatory Study:

Phagocytic Activity of Polymorphonuclear Cells: Rats received test extract orally daily for 28 days. On the 29th day, the animals were subjected to the immunological screening. At the end of the drug treatment phase, drop of blood collected on clear, dry glass slide and placed in a moist chamber to permit the adherence of PMNs [12].

After which the clot was gently removed without disturbing the adherent PMNs that covered with a suspension of *Candida albicans* cells and incubated for 1 hour. The slide the stained with Giemsa stain and the effect of *Aloe vera* on phagocytic activity expressed as "percentage of PMNs showing phagocytosis and average number conditional Candida cells per PMNs [12].

Data analysis: The data are expressed as mean ± SEM. Statistical analysis was performed by unpaired and paired "t" test [13].

RESULTS: *Aloe vera* gel extract in the dose 100mg/kg and 200mg/kg exhibited significant ($p<0.001$) anti-inflammatory activity as compared to control group while at a dose of 400mg/kg show good anti-inflammatory activity in acute model **Table 1**. In chronic model of inflammation, *Aloe Vera* gel extract did not show any significant reduction in weight of granuloma with any of 3 doses, when compared to both control and aspirin groups **Table 2**.

TABLE 1: ACUTE ANTI-INFLAMMATORY ACTIVITY

Group (dose/kg)	Increase in percentage (%) edema					
	1hr	2hr	3hr	4hr	5hr	6hr
D/W - I	27.1± 1.47	48.1±5.41	101.8±3.90	170.6±7.53	269.3±27.49	346.5±16.2
Aspirin- II 100mg/kg	27.5±2.53	4.5±2.18	101.1±10.5	90.16±11.28$^{\bullet\bullet\bullet}$	66.16±9.30$^{\bullet\bullet\bullet}$	42.16±2.92$^{\bullet\bullet\bullet}$
Aloe vera – III 100mg/kg	27.5±0.88	55.6±2.8$^{\#\#}$	114.5±4.16$^{\bullet}$	165±1.59$^{\#\#\#}$	175.8±6.30$^{\bullet\bullet\#\#\#}$	161.6±5.61$^{\bullet\bullet\bullet\#\#\#}$
Aloe vera – IV 200mg/kg	43.1±4.00$^{\bullet\bullet\bullet\#\#}$	60.1±2.02$^{\bullet\bullet\#\#}$	159.1±7.43$^{\bullet\bullet\bullet\#\#}$	150.1±2.83$^{\bullet\#\#\#}$	132.6±12.66$^{\bullet\bullet\bullet\#\#\#}$	76.1±7.04$^{\bullet\bullet\bullet\#\#}$
Aloe vera -V 400mg/kg	45.5±3.07$^{\bullet\bullet\#\#}$	76.1±6.62$^{\bullet\bullet\#\#}$	147.5±7.58$^{\bullet\bullet\bullet\#\#}$	128.6±12.79$^{\bullet\bullet\#\#}$	95.1±2.38$^{\bullet\bullet\bullet\#\#}$	51.83±1.46$^{\bullet\bullet\bullet\#}$

Values: Mean ± SEM, Each group: 6 animals.
Comparison with group I: $^{\bullet}$p < 0.05, $^{\bullet\bullet}$p< 0.01, $^{\bullet\bullet\bullet}$p <0.001
Comparison with group II: $^{\#}$p <0.05, $^{\#\#}$p < 0.01, $^{\#\#\#}$p <0.001

TABLE 2: CHRONIC ANTI-INFLAMMATORY ACTIVITY

Groups	D/WI	Aspirin 100mg/kg II	*Aloe vera* 100mg/kg III	*Aloe vera* 200mg/kg IV	*Aloe vera* 400mg/kg V
Weight of pellets	23.65±0.675	12.28±0.522$^{\bullet\bullet\bullet}$	23.5±0.708	22.25±0783	22.1±0.921

Value: Mean ± SEM, Each group: 6 animals.
Comparison with group I: $^{\bullet\bullet\bullet}$p <0.001

In ulcer potential study, the ulcerogenic activity of aspirin was compared with that of *Aloe vera* gel extract. It was seen that *Aloe vera* gel extract did not produce ulcer at 100mg, 200mg and 400mg/kg. **(Fig. 1)**

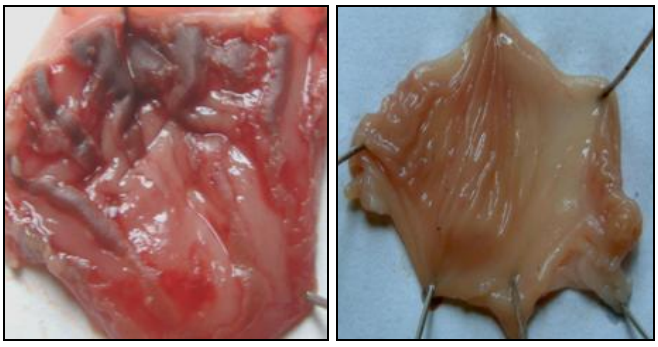

FIG. 1.a: ASPIRIN TREATED RATS SHOW ULCER WITH NECROSIS b: *ALOE VERA* TREATED RATS SHOW NO ULCER POTENTIAL

In immunomodulatory study, *Aloe vera* gel extract in the dose of 100mg/kg did not show phagocytic activity as compared to control group. *Aloe vera* gel extract in the dose of 200mg and 400mg/kg showed significant phagocytic activity as compared to control group **Table 3**.

TABLE 3: IMMUNOMODULATORY ACTIVITY

Groups (dose/kg)	Percentage (%) of Candida cells engulfed by Polymorphonuclear leucocytes (PMNs)
D/W I	1.58 ± 0.0962
Aloe vera II 100mg/kg	1.51± 0.120
Aloe vera III 200mg/kg	2.22 ± 0.278$^{\bullet}$
Aloe vera IV 400mg/kg	2.83 ± 0.175$^{\bullet\bullet\bullet}$

Value: Mean ± SEM, Each group: 6 animals.
Comparison with group I: $^{\bullet}$p < 0.05, $^{\bullet\bullet}$p< 0.01, $^{\bullet\bullet\bullet}$p< 0.001

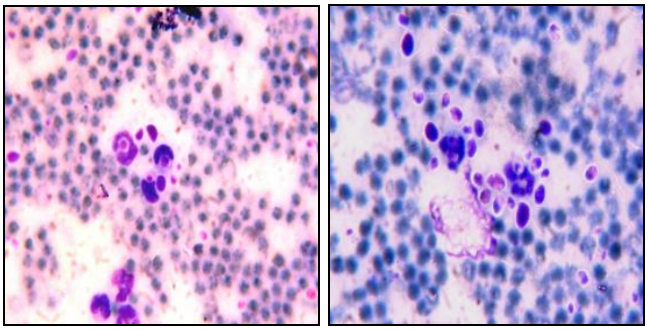

FIG. 2.a: VEHICLE TREATED RATS WITH PMN CELLS b: *ALOE VERA* TREATED RATS SHOW INCREASED NUMBER OF PMN CELLS

DISCUSSION: The results of the present investigation suggest that *Aloe vera* gel extract produced acute anti-inflammatory effect. Carrageenan induced inflammatory process is believed to be biphasic. The initial phase seen at the 1st 3h is attributed to release of histamine and Serotonin. The 2nd accelerating phase of swelling is due to the release of PGs, bradykinin, and lysozyme. It has been reported that the 2nd phase of edema is sensitive to both clinically useful steroidal and nonsteroidal anti-inflammatory agents [14].

The anti-inflammatory activity of *Aloe vera* gel extract suggests that they could have acted by affecting kinin, PGs, bradykinin and lysozyme synthesis. In cotton pellet granuloma *Aloe vera* gel extract did not show chronic anti-inflammatory effect.

Major problem of use of NSAIDs is gastric ulcer side effects. But use *Aloe vera* gel extract, even at large dose did not produce ulcer. This is the major advantage of *Aloe vera* gel extract over aspirin.

In immunomodulatory study, *Aloe vera* gel extract produce immunostimulant activity. It could be due to chronic use of *Aloe vera* gel extract produce immunostimulation.

The *Aloe vera* gel extract from leaves of *Aloe vera* showed good acute anti-inflammatory effect on carrageenan induced edema with good immunostimulant effect but no ulcer potential. It didn't show chronic anti-inflammatory effect.

These results provide a scientific basis for the utilization of this herb in traditional medicine for the treatment of inflammatory disease. The test drugs have been found to be effective in acute models further tests are needed to explore the extract active principles responsible for the anti-inflammatory and immunostimulatory activity.

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CONFLICTS OF INTEREST: Nil

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