AN EVALUATION OF ALOE VERA FOR ITS ACUTE ANTI-INFLAMMATORY ACTIVITY IN RAT PERITONITIS MODEL

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ABSTRACT

Objectives: The purpose of the study was to evaluate the anti-inflammatory activity of aloe vera in albino rats and to compare the anti-inflammatory activity of aloe vera with a standard Anti-inflammatory drug indomethacin in formalin induced peritonitis model. Methods: Anti inflammatory model used in the present study was formalin induced peritonitis model. The animals were randomly divided into 3 groups of 6 each. One group would serve as control and would receive 2% gum acacia suspension orally (without drug). Other two groups would receive drug indomethacin and Aloe vera extract orally. Each rat was fed with respective drug, Anti-inflammatory activity was expressed as Percent inhibition (PI). Statistical analysis was performed using One-way analysis of variance (ANOVA) followed by Scheffe’s post hoc test. P < 0.05 was considered statistically significant. Results: The PI with indomethacin and Aloe vera in formalin induced peritonitis were 63.809% and 47.619%. Indomethacin and Aloe vera showed significant anti-inflammatory activity. After four hours of induction of inflammation, with the peritoneal fluid analysis of control groups showed increased leukocyte and neutrophil count where as group which was treated with Indomethacin and Aloe vera showed reduced number of total leukocytes and neutrophils. Conclusion: The result in this study suggests that aqueous extract of Aloe vera has anti-inflammatory activity comparable to standard drug indomethacin. Aloe vera, thus can be a promising anti-inflammatory agent.

Key Words: Anti-inflammatory; Aloe vera; Indomethacin ; Formalin ; Peritonitis.
INTRODUCTION
Inflammation is the response of living tissue to mechanical injuries, burns, microbial infections, and other noxious stimuli that involve changes in blood flow, increased vascular permeability, activation and migration of leucocytes and the synthesis of local inflammatory mediators\textsuperscript{1}. The term anti-inflammatory agent refers to an agent which reduces one or more components of the inflammatory process. The anti-inflammatory agent should not only have the ability to control the inflammation, but also mitigate the consequences of chronic inflammation (like the formation of the fibrous adhesion bands following peritonitis and joint deformities in Rheumatoid arthritis). In view of the complex process involved in the process of inflammation, the drugs employed will be heterogeneous in structure, diverse in mechanism of action and variable in effectiveness. They may also possess antipyretic and analgesic activity along with their anti-inflammatory activity, which makes them useful in the management of inflammatory disorders associated with pain and fever and thus minimize the suffering in inflammatory diseases.

There has been an explosion of scientific information concerning plants, crude plant extracts and various substances from plants as medicinal agents during the last 20 to 30 years. Although herbal medicine has existed since the dawn of time, our knowledge of how plants actually affect human physiology remains largely unexplored.\textsuperscript{2} Some medicinal plants used in traditional medicine to treat inflammatory diseases like Wild plum (Harpephyllum caffrum), Pineapple lily (Eucomis autumnalis), Woolly nightshade (Solanum mauritianum) and Dead man’s tree (Synadenium cupulare) have been screened.\textsuperscript{3}

The plant of Aloe vera and its usage as a drug dates back to 6000 years B.C. One prescription that belong to 1550 BC shows Aloe vera plant used for different illnesses. It was known to people in Egypt and also Greece, for example Aristotle explained the special characteristics of Aloe vera. Aloe was used by Hippocrates and Arab physicians, and was carried to the Western Hemisphere by Spanish explorers. Legend has it that Alexander the Great, captured the island of Socotra in the Indian Ocean to secure its aloe supplies to treat his wounded soldiers.\textsuperscript{4} Today aloe vera gel is an active ingredient in hundreds of skin lotions, sun blocks and cosmetics. Aloe first gained popularity in the United States in the 1930's with reports of its success in treating X-ray burns. Recently, aloe extracts have been used to treat cancer sores, stomach ulcers and even AIDS.\textsuperscript{4} Here an attempt has been made to explore its anti-inflammatory activity.
MATERIALS AND METHODS

MATERIALS

1. Animals: Albino rats, of either sex, weighing 150-250 g, were used for the study. The rats were inbred in the central animal house of the Department of Pharmacology, Mysore Medical College & Research Institute, Mysore

Inclusion criteria: Healthy animals with normal behavior and activity.

Exclusion criteria: Pregnant and Diseased animals.

2. Chemicals: Aloe Vera aqueous extract (25mg/kg), Indomethacin (10mg/kg)

1% Formalin, 2% Gum acacia.


CHEMICALS

a. Gum acacia: Gum acacia is a dried exudate from Acacia Senegal (a small tree) and certain other species of Acacia. It comes as a white powder. It is a suspending agent. Here it is used in the concentration of 2% as a suspending agent for the administration of standard and test compounds.

b. Formalin: It is a 37% solution of formaldehyde gas in water. This Phlogistic agent is irritant and pungent to mucous membrane, and strength of the solution used in this study is 1% for intraperitoneal injection.

c. Indomethacin: This is one of the known potent and established anti-inflammatory agent used in therapeutics. Chemically it is 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H indole -3-acetic acid.
d. Aqueous extract of Aloe vera: The Aqueous extract of Aloe vera was obtained from Himalaya drug company. The leaves of Aloe vera were washed to remove dirt and impurities. Some of the leaves were dried for two weeks under low sun intensity, crushed in a mortar and further ground into a coarse powder. Then four times demineralised water was transferred with respect to quantity of the Raw Herb (twice). Extraction was done at 85-95°C for 3 hrs in a Soxhlet’s apparatus. It was then filtered and was concentrated at 65-75°C up to 25% solids. It was later spray dried (inlet temperature 180°C and outlet temperature 100°C) and was packed in a double lined poly bag.

METHOD
FORMALIN INDUCED PERITONITIS IN ALBINO RATS:
The method of Teotino et al, 1963 with some modifications was adopted here to study the acute inflammatory reaction induced by formalin injected into the peritoneal cavity of the rats.

Albino rats of either sex weighing between 150-250 gms of similar characteristics were used. Animals were randomly divided into 3 groups of 6 rats each;

I group: Control (1ml of Vehicle, 2% Gum acacia suspension);
II group: Standard drug (Indomethacin 10mg/kg);
III group: Test drug (Aloe vera 25mg/kg).

All the drugs were administered orally. One hour later peritonitis was induced by intraperitoneal administration of 1ml of 1% formalin and the animals were maintained in cages. The animals were sacrificed at the end of 4 hours and the abdominal cavity was opened, the exudates found was collected and measured by placing the animal on a glass funnel. The anti-inflammatory activity of the drugs was calculated by the following formula,

Percent anti-exudate activity= 100 (1- Vt/Vc)

Where, Vc= mean volume of exudates in the control group,
Vt = mean volume of exudates in the test group.

The observed results are displayed in Table 1.

After four hours peritoneal fluid which were collected and measured were subjected to peritoneal fluid analysis, which was processed by centrifugation at 2000rpm for 10min and RBCs were destroyed. The total leukocyte count was determined in a Neubauer chamber and differential cell count was performed using light microscope.
Statistical methods applied

Descriptive statistics

The Descriptive procedure displays univariate summary statistics for several variables in a single table and calculates standardized values (z scores). Variables can be ordered by the size of their means (in ascending or descending order), alphabetically, or by the order in which you select the variables.

The One-way ANOVA procedure produces a one-way analysis of variance for a quantitative dependent variable by a single factor (independent) variable. Analysis of variance is used to test the hypothesis that several means are equal. This technique is an extension of the two-sample t test.

Scheffe’s Post hoc test

Once it is determined that differences exist among the means, post hoc range tests and pairwise multiple comparisons can determine which means differ. Range tests identify homogeneous subsets of means that are not different from each other. Pairwise multiple comparisons test the difference between each pair of means, and yield a matrix where asterisks indicate significantly different group means at an alpha level of 0.05. Scheffe’s post hoc test is one of the widely used post hoc tests in medical sciences.

All the statistical methods were carried out through the SPSS for Windows (version 16.0)

Table 1: Showing the mean peritoneal exudate volume (ml) and PI of different groups in formalin induced peritonitis model.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Mean peritoneal exudates (ml) ±SD</th>
<th>Percent inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1ml of vehicle)</td>
<td>6</td>
<td>2.100±0.414</td>
<td>0.0%</td>
</tr>
<tr>
<td>Indomethacin (10mg/kg)</td>
<td>6</td>
<td>0.766±0.233</td>
<td>63.809%</td>
</tr>
<tr>
<td>Aloe vera (25mg/kg)</td>
<td>6</td>
<td>1.100±0.433</td>
<td>47.619%</td>
</tr>
</tbody>
</table>

Table 1 - Shows the mean peritoneal exudate volume (ml) collected after 4 hrs of intraperitoneal formalin injection in different groups of drugs.

- Control group showed a maximal exudate volume of 2.100ml indicating a maximal peritoneal inflammation.
- Standard group showed the minimal exudate volume of 0.766ml indicating a very less peritoneal inflammation than control and a maximal anti-inflammatory activity.
• The test group Aloe vera showed the exudate volume of 1.100ml indicating a less peritoneal inflammation than control.

• Standard group showed a better anti-exudate activity than the test drug under the present experimental conditions.

• Table also shows the Percent inhibition of anti-exudate activity in different drug groups.

Table 2: ANOVA ANALYSIS- Mean peritoneal exudate volume (ml)

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>5.778</td>
<td>2</td>
<td>2.889</td>
<td>20.900</td>
<td>.000</td>
</tr>
<tr>
<td>Within Groups</td>
<td>2.073</td>
<td>15</td>
<td>0.138</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>7.851</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2- Showing One-way ANOVA conducted for the data obtained in Formalin induced peritonitis model.

It is observed that p value was <0.05, indicating that there is significant difference among the groups included in the study with respect to the variable (mean peritoneal exudates volume) considered in the study. Scheffe’s post-hoc test was carried to see whether any two groups showed significant difference. The results showed that there is no statistically significant difference among the Standard and Aloe vera groups indicating homogeneity with respect to the variable (mean peritoneal exudates volume) considered in the study.

Table 3: Showing Total leukocyte count in peritoneal exudate volume (cells/µl) and PI of different groups in formalin induced peritonitis model.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Total leukocyte count (mean cells/µl ± SE)</th>
<th>Percent inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1ml of vehicle)</td>
<td>6</td>
<td>85.666±3.595</td>
<td>0.0%</td>
</tr>
<tr>
<td>Indomethacin (10mg/kg)</td>
<td>6</td>
<td>63.666±6.253</td>
<td>74.318%</td>
</tr>
<tr>
<td>Aloe vera (25mg/kg)</td>
<td>6</td>
<td>66.000±4.795</td>
<td>77.043%</td>
</tr>
</tbody>
</table>

The percentage of leukocyte inhibition was calculated by equation

Percentage = 100 (1 - \frac{t}{c})

Where, \( c \) = leukocyte count in the control group,

\( t \) = leukocyte count in the test group.

The observed results are displayed in Table 3.
Table 4: Showing neutrophils in peritoneal exudate volume (cells/µl) and PI of different groups in formalin induced peritonitis model.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Neutrophils(mean cells/µl ± SE)</th>
<th>Percent inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1ml of vehicle)</td>
<td>6</td>
<td>72.166±4.657</td>
<td>0.0%</td>
</tr>
<tr>
<td>Indomethacin (10mg/kg)</td>
<td>6</td>
<td>33.333±4.279</td>
<td>53.810%</td>
</tr>
<tr>
<td>Aloe vera (25mg/kg)</td>
<td>6</td>
<td>38.000±5.144</td>
<td>47.343%</td>
</tr>
</tbody>
</table>

The percentage of leukocyte inhibition was calculated by equation

\[
\text{Percentage} = 100 \times \left(1 - \frac{t}{c}\right)
\]

Where, \( c \) = neutrophil count in the control group,
\( t \) = neutrophil count in the test group.

The observed results are displayed in Table 4.

Table 5: Showing the total count, neutrophils and lymphocytes in the peritoneal exudates of different groups in formalin induced peritonitis model.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total count(mean cells/µl ± SE)</td>
</tr>
<tr>
<td>Control</td>
<td>85.666±3.595</td>
</tr>
<tr>
<td>Standard</td>
<td>63.666±6.253</td>
</tr>
<tr>
<td>Aloe vera</td>
<td>66.000±4.795</td>
</tr>
</tbody>
</table>

Table 6: ANOVA ANALYSIS- Total count, Neutrophils and Lymphocytes in the peritoneal exudates

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total count</td>
<td>Between Groups</td>
<td>1752.444</td>
<td>2</td>
<td>876.222</td>
<td>5.840</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>2250.667</td>
<td>15</td>
<td>150.044</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>4003.111</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Between Groups</td>
<td>5071.444</td>
<td>2</td>
<td>2535.722</td>
<td>18.705</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>2033.500</td>
<td>15</td>
<td>135.567</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>7104.944</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Between Groups</td>
<td>5394.333</td>
<td>2</td>
<td>2697.167</td>
<td>20.288</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>1994.167</td>
<td>15</td>
<td>132.944</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>7388.500</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesothelial cells</td>
<td>Between Groups</td>
<td>33.444</td>
<td>2</td>
<td>16.722</td>
<td>2.451</td>
</tr>
<tr>
<td>-------------------</td>
<td>----------------</td>
<td>--------</td>
<td>---</td>
<td>--------</td>
<td>-------</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>102.333</td>
<td>15</td>
<td>6.822</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>135.778</td>
<td>17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

After four hours of induction of inflammation, control groups showed increased leukocyte and neutrophil count where as group which was treated with Indomethacin and Aloe vera showed reduced number of total leukocytes and neutrophils.

Table 3 and 4 shows total leucocyte count and neutrophils respectively in peritoneal exudate volume (cells/µl) and PI of different groups in formalin induced peritonitis model.

Table 5 shows the total count, neutrophils and lymphocytes in the peritoneal exudates of different groups in formalin induced peritonitis model. Control groups showed increased leukocyte count where as group which was treated with Indomethacin and Aloe vera showed reduced number of total leukocyte count and neutrophils but did not reduce lymphocytes and mesothelial cells. This result was confirmed by the statistical analysis that indicated significant differences (p< 0.05) among the control group with Indomethacin and Aloe vera group as shown in table 6.

Graph 1: Bar diagram showing Mean peritoneal fluid volume (ml) - Formalin induced peritonitis model.
Graph 2: Bar diagram showing the total count, neutrophils and lymphocytes in the peritoneal exudates of different groups in formalin induced peritonitis model.

DISCUSSION

Inflammation is a complex and dynamic condition in which many changes take place at the site of inflammation as well as systemically. It involves a complex array of enzymes activation, release of mediators, extravasation of fluid, migration of cells, tissue breakdown and repair.\textsuperscript{10} It is known that the acute inflammatory response consists of three main vascular effects viz. vasodilatation and increased vascular flow, increased vascular permeability and leucocytes migration to the injured tissues.\textsuperscript{11}

The inflammatory response represents a complex biological and biochemical process involving cells of the immune system and a plethora of biological mediators. Cell-to-cell communication molecules known collectively as cytokines play an extremely important role in mediating the process of inflammation.\textsuperscript{12} During an inflammatory response, both the level and the profile of PG production change dramatically. PG production is generally very low in uninflamed tissues but increases immediately in acute inflammation before the recruitment of leukocytes and the infiltration of immune cells.\textsuperscript{13}

The acute induced-inflammatory is a fast response to a harmful agent responsible to induce the defense mechanism of the body (leukocytes and antibody) to the lesion place. The defense mechanisms are present and move according to the bloodstream. In an inflammatory process the blood vessels suffer some alterations to facilitate the movement of the leukocytes and the antibody to the lesion place, whose rate depends on the level of inflammation. Leukocyte recruitment to the site of inflammation is a fundamental event in the inflammatory
process. Cell migration occurs as a result of much different process including adhesion and cell mobility.14

Formalin when injected into peritoneal cavity irritates the peritoneum and bowel and induces acute inflammatory reaction producing aseptic peritonitis. According to a previous study, formalin administered at a low dose of 1.75% induces an edema which mainly results from a neurogenic inflammation mediated by neuropeptides such as substance P. At higher dose of 5%, formalin induces edema which mainly depends on the release of substance P, prostanoids, 5-hydroxytryptamine and histamine.15 The PI of peritonitis by Indomethacin was 63.809% while that of Aloe vera was 47.619%. Hence Aloe vera showed 74.627% anti-exudate activity as that of standard Indomethacin under the present experimental conditions. Therefore Aloe vera showed a comparable anti-exudate activity as that of the reference drug. Extravasated neutrophils become activated once in the inflammatory sites, secreting a variety of substances such as growth factors, chemokines and cytokines, complement components, proteases, NO, reactive oxygen metabolites, and peroxynitrite, all important mediators of tissue injury.16,17,18 Prevention of neutrophil dependent inflammatory pathways is likely to contribute to the reduced fluid extravasation.

The data in our study showed that Indomethacin and Aloe vera were effective in reducing peritonitis induced by formalin. The probable mechanism for the acute anti-inflammatory action could be due to anti-bradykinin activity and inhibition of synthesis of prostaglandins and other inflammatory mediators like histamine and serotonin in early hours of inflammation.

CONCLUSION
At the end of the study it can be concluded that Aqueous extract of Aloe vera has shown anti-inflammatory effect in albino rats and the anti-inflammatory activity of Aloe vera is comparable to Indomethacin. However, Aloe vera also has antioxidant property, which may also be useful in various inflammatory disorders, as a secondary effect.

The anti-inflammatory property of Aloe vera may be due its ability to prevent the production of pro-inflammatory mediators like PG, histamine and serotonin, and may also be due to its anti-oxidant effect. These studies are valuable for identifying lead compounds for anti-inflammatory drugs, keeping in mind the side effects of NSAIDs and corticosteroids. Since animal studies cannot be directly compared with effects on humans, further extensive studies...
need to be done to isolate the active substance/ substances responsible for its anti-inflammatory activity and confirm this activity in animal models as well as in clinical trials.

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