COMPARISON OF EFFECT OF ALOE VERA GEL WITH ASPIRIN AND CELECOXIB ON PLATELET AGGREGATION.

Sidra Mushtaq1, Zobia Mushtaq2, Javeria Sarfraz3, Mufakhara Fatimah4, Sadida Bahawal5, Qura-tul-Ain6, Sadia Chiragh7

ABSTRACT… Objective: This study was designed to compare the effect of Aloe vera gel with aspirin and celecoxib on platelet aggregation. Study Design: Comparative Study. Setting: Post graduate Medical Institute Lahore, Children Hospital, Lahore. Period: September 2015 to September 2016. Material & Methods: Blood was withdrawn from anti-cubital vein, complete blood count was checked, platelet rich plasma was prepared by centrifuging citrated whole blood and then incubated with Aloe vera low (AVL), Aloe vera high (AVH), aspirin and celecoxib for 30 minutes at 37C. After adding the agonist arachidonic acid, reading was then taken for 3 minutes and percentage aggregation was recorded. Results: Platelet aggregation with aspirin, AVH and AVL was statistically significantly lower as compared to control and celecoxib groups. Conclusion: This study has demonstrated a dose dependent anti-platelet effect of Aloe vera gel which is comparable to aspirin.

Key words: Anti-Platelet, Aloe Vera, Aspirin, Celecoxib.

INTRODUCTION

The use of Aloe vera dates back to biblical times for treating skin problems, burns and infections.1 Reported pharmacological actions of Aloe vera include anti-inflammatory, antibacterial, antioxidant, anti-cancer, antifungal as well as anti-diabetic.2 These diverse activities shown are known to be due to synergistic action of the compounds present, rather than a single chemical substance.3

Chronic inflammatory diseases remain one of the major health problems. NSAID use as anti-inflammatory agents is spread over centuries, in conditions like rheumatoid arthritis and osteoarthritis. Aspirin is the prototype of this group. Long term use of non-selective NSAIDs like aspirin carry the drawback of causing gastritis, ulcers and even perforations.4 The anti-inflammatory benefits of these drugs are primarily due to COX-2 inhibition, while inhibition of COX-1 leads to GIT complications. These complications led to the introduction of selective COX-2 inhibitors like celecoxib. Many studies have shown lower incidence of GIT complications with COX-2 inhibitors.5 However COX-2 inhibitors have the adverse effect of cardiovascular thrombotic episodes.6

It is well established now that aspirin irreversibly inhibits Cyclo-oxygenase-1 in platelets, blocking TXA-2 synthesis, resulting in inhibition of platelet aggregation and decreased risk of thrombosis, leading to its use as antiplatelet drug.7 Gastric adverse effects with aspirin and pro-thrombotic adverse effects encountered with selective COX-2 inhibitors have emphasized the need to develop such anti-inflammatory agents, with both decreased GIT and cardiovascular adverse effects.8

Aloe vera has been reported to have potent anti-inflammatory activity in addition to ulcer healing properties.9 Thus when prospect of anti-
inflammatory use of Aloe vera is considered, it seems to have an advantage over aspirin and other non-specific NSAIDs, but it’s effect on platelet aggregation is yet to be determined.

Study of Aloe vera’s effect on platelet aggregation is important from many ways like decreased adverse effects, potential herb-drug interactions and from the perspective of new anti-platelet and anti-inflammatory drug development.

MATERIAL & METHODS

Eighteen normal healthy volunteers of both sexes, with age ranging from 18-35 years and baseline platelet count within normal limits (150-400 × 10⁹/L) were selected for the study. Subjects with history of bleeding disorders, Hb < 10g/dl, pregnancy and intake of NSAIDs/any drug (clopidogrel, heparin) affecting platelet aggregation in past 2 weeks were excluded.

Concentration of Test Compounds Prepared were; Aspirin – 100 μmol/l, Celecoxib – 3 μmol/l, AVL – 10 microgram/ml, AVH – 100 microgram/ml, Arachidonic acid – 0.5 mmol /L (Arachidonic acid leaflet by Chrono-log corporation).

Preparation of Aloe Vera Gel

Aloe vera plant was taken from garden at home and was identified from Botany Department of Punjab University Lahore. Age of the plant was approximately 2 years. Leaves were washed, and skin was peeled off. Aloe vera gel was blended in a blender and then filtered through Whitman filter paper. This contained 10,000 μg/ml according to composition of solids in Aloe vera gel. Informed consent was taken. Eight ml of blood was withdrawn from the ante-cubital vein. Two ml blood was put in EDTA vacutainers, rested for 20 minutes and processed within 1 hour after collection. Baseline haemoglobin and platelet count was checked through hematology analyzer (Sysmex corporation, model no KX_21, serial no B 3483). Six ml blood was put in citrated vials. Platelet rich plasma (PRP) was prepared by centrifuging citrated whole blood at 500 rpm for 15 minutes at 37°C. Aim was to concentrate platelets on values recommended in literature between 150-400 × 10⁹/L. Platelet count was checked through hematology analyzer. Samples which were clotted or haemolysed were discarded (2 such PRP samples were discarded).

Samples with platelet count higher than 450 x 10⁹/L were recent refuge at 4000 rpm for 20 minutes. This was used to adjust PRP samples to standard platelet count. Samples were rested for 30 minutes before further testing. 245 μl PRP was then taken in 5 microcuvettes labelled as control, aspirin, Aloe vera high (AVH), Aloe vera Low (AVL) and celecoxib. 2.5 μl of test compound solutions were then put in respective micro cuvettes. All micro cuvettes were incubated for 30 minutes at 37°C. Magnetic stir bar was then added. Aggregation was induced by adding 2.5 μl of arachidonic acid solution making final concentration 0.5mM. Reading was taken for 3 minutes by recording percentage aggregation using light transmission aggregometer (LTA chronolog corporation USA, model no 490-2DR, serial no 980131).

The data was analyzed using SPSS (Statistical Package for Social Sciences) version 20. Mean ± SD and median with inter-quartile range was given for quantitative variables. Shapiro Wilk test was used to check the normality of data. Data was not normally distributed so non parametric Kruskal Wallis H test was used to observe group mean difference in platelets aggregation among treatments. For multiple comparisons, Mann Whitney U test with Bonferroni adjustment were used. A p-value of ≤ 0.05 was considered statistically significant.

RESULTS

In this study, mean age of the 18 subjects studied was 26.04 ± 3.08 years, ranging from 18 to 35 years. Mean haemoglobin level was 12.97 ± 1.74 g/dl and mean platelet count was 333.12 ± 57.03 x 10³ /μl. Table-I shows mean values of platelet aggregation in %, along with standard deviation, median, interquartile range, minimum and maximum values.
Comparison of Platelet Aggregation among Five Treatments

Platelet aggregation with aspirin, AVH, and AVL was statistically significantly lower as compared to control and celecoxib groups.

Platelet aggregation with aspirin was low as compared to AVH and AVL. The difference in platelets aggregation between aspirin and AVH was not significant whereas in AVL, platelets aggregation was significantly higher as compared to aspirin. The platelets aggregation in AVL was higher as compared to AVH but difference was not statistically significant.

Platelet aggregation with celecoxib was significantly higher as compared to aspirin, AVH and AVL but compared to control, difference was not significant (Figure-1).

It was calculated by subtracting value of platelet aggregation with each treatment from control value. Table-II shows mean values of inhibition of platelet aggregation in %.

### Table-I. Platelet aggregation with aspirin, Aloe vera and celecoxib (n=18).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
<th>Median (Inter-quartile Range)</th>
<th>Minimum</th>
<th>Maximum</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>87.88 ± 18.23</td>
<td>98.0 (70.1 – 99.0)</td>
<td>54</td>
<td>99</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Aspirin</td>
<td>3.98 ± 3.01</td>
<td>5.0 (1.0 – 6.0)</td>
<td>1</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>AVH</td>
<td>16.29 ± 4.12</td>
<td>17.0 (11.5 – 18.5)</td>
<td>11</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>AVL</td>
<td>25.01 ± 4.78</td>
<td>25.0 (19.0 - 28.0)</td>
<td>19</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Celecoxib</td>
<td>89.45 ± 16.70</td>
<td>99(73.5 – 99.0)</td>
<td>54</td>
<td>99</td>
<td></td>
</tr>
</tbody>
</table>

AVH= Aloe vera high concentration
AVL= Aloe vera low concentration

### Table-II. % inhibition of platelet aggregation with aspirin, Aloe vera, celecoxib (n=18).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
<th>Median (Inter-quartile Range)</th>
<th>Minimum</th>
<th>Maximum</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>95.39 ± 2.85</td>
<td>95.0 (92.0 – 97.0)</td>
<td>87</td>
<td>97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AVH</td>
<td>81.5 ± 2.55</td>
<td>80.0 (78.5 – 81.0)</td>
<td>74</td>
<td>86</td>
<td></td>
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<tr>
<td>AVL</td>
<td>71.54 ± 4.98</td>
<td>69.0 (66.5 – 73.5)</td>
<td>60</td>
<td>80</td>
<td></td>
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<tr>
<td>Celecoxib</td>
<td>-1.78 ± 4.89</td>
<td>0.01 (-1.7 – 0.01)</td>
<td>-14.9</td>
<td>00</td>
<td></td>
</tr>
</tbody>
</table>

AVH= Aloe vera high concentration
AVL= Aloe vera low concentration

Comparison of the Percentage Platelet Inhibition among Treatments

Inhibition of platelet aggregation was calculated by subtracting value of platelet aggregation with each treatment from control. Inhibition of platelet with AVH, AVL and celecoxib was statistically significantly lower as compared to aspirin.
The difference in platelets inhibition between AVH and AVL was not significant whereas in both group’s platelets inhibition was significantly higher as compared to celecoxib. Celecoxib was rather found to have a slight prothrombotic effect, which was not statistically significant (Fig-2).

![Mean % Platelet Inhibition](image)

<table>
<thead>
<tr>
<th>Mean % Platelet Inhibition</th>
<th>Aspirin</th>
<th>AVH</th>
<th>AVL</th>
<th>Celecoxib</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>95.39</td>
<td>81.5</td>
<td>71.54</td>
<td>-1.78</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Atherothrombotic diseases leading to MI, stroke and other embolic events are leading cause of death all over the world. Increased platelet aggregation plays an important role in the development and progression of atherosclerotic plaques.\(^\text{14}\) NTI-platelet agents have both prophylactic and therapeutic role. Ever increasing requirement for new and safer anti-platelet agents has compelled researchers to embark upon this field. Aloe vera is being used as anti-inflammatory agent for centuries. In this study we have compared the effect of Aloe vera gel with aspirin and celecoxib on platelet aggregation.

Mean platelet aggregation with aspirin was 3.98% and mean platelet inhibition was calculated to be 95.39%. Thus our study has demonstrated less than 10% aggregation with aspirin on LTA.

In a study\(^\text{15}\), similar results were found with aspirin on LTA, showing less than 10% aggregation of platelets. Mean platelet aggregation observed with celecoxib was 89.45% and mean platelet inhibition was calculated to be -1.78%. Celecoxib has shown statistically insignificant prothrombotic effect. Inhibition of vascular PGI-2 production in the absence of platelet TXA-2 inhibition, favors vasoconstriction and thrombosis\(^\text{16}\), but this effect is dependent on production of PGI-2 from endothelium and is therefore not so prominent in vitro.

In our study, mean platelet aggregation with AVL was 25.01% and mean inhibition of platelet fell around 71.54%. Mean platelet aggregation with AVH was 16.29% and mean platelet inhibition 81.5%. Aloe vera thus showed dose dependent inhibition of platelet aggregation. Platelet aggregation with aspirin, AVH, and AVL was statistically significantly lower as compared to control and celecoxib. The difference in platelet aggregation with aspirin and AVH was not significant whereas in AVL platelet aggregation was significantly higher as compared to aspirin.

This is the first study to demonstrate effect of crude Aloe vera gel on platelet aggregation in vitro. Other herbs with anti-platelet effect have demonstrated similar results.\(^\text{17}\)

Besides our research, only one in vivo study has been conducted on Aloevera so far by Singh and Fahim\(^\text{18}\), for demonstrating its anti-platelet effect. It was an in vivo study requiring expertise and expensive instruments. In contrast, the study we have conducted was performed on human blood, which is the only study so far. It is also a comparative study in which we have compared anti-platelet effect with traditional NSAID like aspirin and celecoxib. We used LTA (light transmission aggregometer) which is still the best and cost effective method available for measuring platelet aggregation. Additionally Aloe vera gel, the main active ingredient known for the proposed effect was used in our study compared to whole leaf approach used by Singh and Fahim.

Kishore administered crude aloe vera gel to mice for 5 and 29 consecutive days and observed the effect on thrombosis by measuring bleeding time which was significantly prolonged as compared to control.\(^\text{19}\) This reflects the anti-platelet activity
of Aloe vera, as bleeding time is indicator of platelet function.

Possible mechanism of anti-platelet effect seems to be through arachidonic acid pathway as in this study we used arachidonic acid as platelet aggregating agent with marked inhibition of aggregation with aloevera gel. Studies have demonstrated cyclooxygenase inhibitory activity of aloevera extracts. Platelet COX-1inhibitory activity is also demonstrated by quercetin which is an important constituent of aloe vera.

Further studies should be conducted to isolate active constituents responsible for anti-platelet activity of aloe vera for new drug development. Reversibility of COX-1 inhibition also needs to be investigated. Human studies will be required to establish the optimum dose and duration of action.

CONCLUSION
This in vitro study has established a significant dose dependent anti-platelet effect of Aloe vera gel, which is comparable with aspirin at higher dose. Caution must be taken with concurrent use of aloe vera gel and other anti-thrombotic agents.

REFERENCES
ALOE VERA GEL


AUTHORSHIP AND CONTRIBUTION DECLARATION

<table>
<thead>
<tr>
<th>Sr. #</th>
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