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PLATELET AGGREGATION;
EFFECT OF ALOE VERA GEL

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ABSTRACT: The herbal use for medical purposes is increasing world over. Aloe vera is one such herb with established anti-inflammatory action. It has great prospect in terms of replacing traditional NSAIDS due to better side effect profile on gastrointestinal tract but its effect on platelet aggregation is what needs to be determined. **Objectives:** This study was designed to see the concentration dependent action of Aloe vera gel on platelet aggregation. **Study Design:** Comparative study. **Setting:** Post Graduate Medical Institute Lahore**,** Children Hospital Lahore. **Duration of Study:** One Year. **Methodology:** This study was conducted on healthy volunteers selected from staff and students of Ameer-ud-din Medical College. After determining baseline Hb and platelet counts, PRP was prepared and then incubated with 2 different concentrations of Aloe vera low (AVL) and Aloe vera high (AVH) for 30 minutes. Aggregation was stimulated by adding the agonist arachidonic acid. Light transmission aggregometer was used to record platelet aggregation activity graphically for 3 minutes. **Results:** The data was analyzed using SPSS version 20. Kruskal Wallis H test was performed to compare the platelet aggregation, which revealed that platelet aggregation with AVL and AVH were statistically significantly lower, amounting to 25.89% and 16.72% respectively as compared to 88.28% observed with control. **Conclusion:** This study has established in vitro anti-platelet effect of Aloe vera which is dose dependent.

Key words: Aloe Vera, Anti-platelet.

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INTRODUCTION

Various herbal and natural products have found widespread use in recent years in the field of alternative medicine. Aloe vera is one such plant which has been used in traditional medicine for centuries, for treating skin problems, burns and infections.[1] Aloe vera is a shrub like plant with 2 major parts: central parenchyma and vascular bundles that surround the parenchyma.[2] Aloe vera gel obtained from central parenchymal cells is the main source of compounds with diverse structures and functions like vitamins, minerals, polysaccharides, phenols and enzymes.[3]

Aloe vera use dates back centuries with diverse pharmacological actions being reported especially marked anti-inflammatory action.[4] Chronic inflammation is the hallmark of multiple diseases encountered nowadays.[5] Contemporary anti-inflammatory drugs which are available over the counter are NSAIDS: Selective COX-2 and non-selective COX inhibitors. Aspirin is the prototype of non-selective group. Dozens of other NSAIDs and NSAID formulations are available, which are used widespreadly in most countries. This vast use is one reason for the interest in tolerability and efficacy issues regarding these drugs.[6] Aspirin has potent anti-inflammatory effects, but GIT toxicity resulting in gastritis, ulcers and perforations can limit long term use of non-specific NSAIDS for this purpose.[7]

Selective COX-2 inhibitors were developed solely to overcome this GIT adverse effect[8], as it is COX-2 enzyme that is upregulated in chronic inflammation and needs to be inhibited, while COX-1 by generating prostaglandins has housekeeping role for organs esp GIT.[9] However, COX-2 inhibition proved pro-thrombotic and caused concern over increasing cardiovascular

thrombotic episodes.[10]

This has paved the way for development of new anti-inflammatory agents with better GIT and cardiovascular adverse profile. This is where Aloe vera has shown prospect: it has well documented anti-inflammatory[11], gastro protective and ulcer healing properties[12], which gives it edge over non-specific NSAIDS like COX-2. What remains to be established is its effect on platelet aggregation. This effect on platelet aggregation will also determine it's drug interactions, adverse effects and what prospect lies in developing new anti-inflammatory and anti-platelet drug.

MATERIAL AND METHODS

Subjects aged between 18-31 years (n=18) were selected from staff and students of PGMI and Ameer-ud-din Medical College. After taking informed consent, blood sample was taken from each to determine baseline Hb and platelet count.

Concentration of Test Compounds

AVL – 10 μg/ml
AVH – 100 μg/ml
Arachidonic acid – 0.5 mmol/L

Aloe vera gel preparation

Aloe vera plant aged 2 years was taken. After peeling skin, gel was extracted and then put in a blender. It was later filtered through whattman filter paper. Aloe vera gel contains 1% solids, so this extract amounted to 10,000 μg/ ml. This was labeled as stock solution. This was then diluted 100 and 1000 times respectively to obtain the desired concentrations of 100 μg/ ml and 10 μg/ ml.

Sampling

Blood was withdrawn from ante-cubital vein. To check complete blood count, 2ml was put in EDTA vacutainers. To concentrate platelets between value of 150- 400 x 10^9 / L, platelet rich plasma (PRP) was prepared. For this purpose 6 ml was taken in sodium citrate vials and centrifuged at 500 rpm for 15 minutes. For samples where platelet count was greater than 450 x 10^9 / L, platelet poor plasma (PPP) was prepared by centrifuging at 4000 rpm for 20 minutes.

LTA procedure

- 3 microcuvettes were taken and labeled as:
  Control
  Aloe vera High (AVH)
  Aloe vera Low (AVL)
- In children hospital hematology lab, platelet aggregation studies on lumi aggregometer are performed on total volume of 250 μl.
- 245 μl PRP was then taken in 2 microcuvettes according to Chronolog Arachidonic acid leaflet
  AVH
  AVL
- 247.5 μl PRP was taken in microcuvette labelled Control. 2.5 μl extra PRP was taken to account for same volume of test compounds in other microcuvettes.
- 2.5 μl of test compound solutions were then put in respective microcuvettes except control.
- All microcuvettes were then incubated for 30 minutes at 37° C.
- After adding magnetic stir bar, aggregation was induced by adding arachidonic acid 2.5 μl, making final concentration 0.5mM, according to chronolog USA arachidonic acid kit leaflet.
- Percentage aggregation was recorded graphically for 3 minutes.

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 18 subjects were studied. Out of 18, 12 (66.7%) were male (Figure-1).

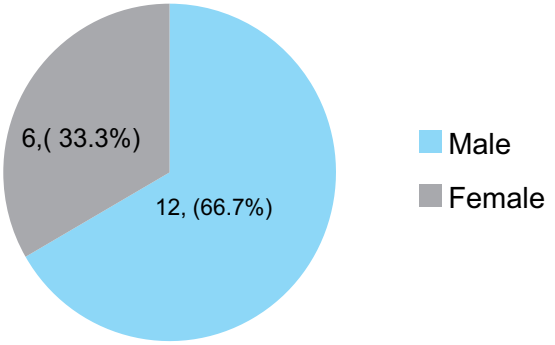

Figure-1. Frequency of patients according to gender

Mean age of the subjects was 24.06 ± 4.06 years with age range of 18 to 31 years. Mean haemoglobin level was 13.67 ± 1.53 g/dl and mean platelet count was 325.44 ± 65.08 x 10^3 / μl (Table-I).

	Mean ± S.D	Minimum	Maximum
Age (Years)	24.06 ± 4.06	18	31
Haemoglobin level (g/dl)	13.67 ± 1.53	11	16
Platelets count in PRP x 10^3 / μl.	325.44 ± 65.08	247	477

Table-I. Descriptive statistics for age, haemoglobin and platelets

Platelets Aggregation

Table-II shows mean values of platelet aggregation in %, along with standard deviation, median, interquartile range, minimum and maximum values. There was no difference in platelet aggregation between 2 genders.

Comparison of Platelet Aggregation among Three Treatments

As data was not normally distributed, Kruskal Wallis H test was performed to compare the platelets aggregation of three treatments i.e. Control, Aloe vera high dose (AVH) and Aloe vera low dose (AVL). Kruskal Wallis H test revealed that there was a statistically significant difference in mean platelet aggregation among groups.

For multiple comparisons, Mann Whitney U test with Bonferroni adjustment was used which showed that platelet aggregation with AVH, and AVL was statistically significantly lower as compared to control. The platelets aggregation in AVL was higher as compared to AVH but difference was not statistically significant (Table-III, Figure-2).

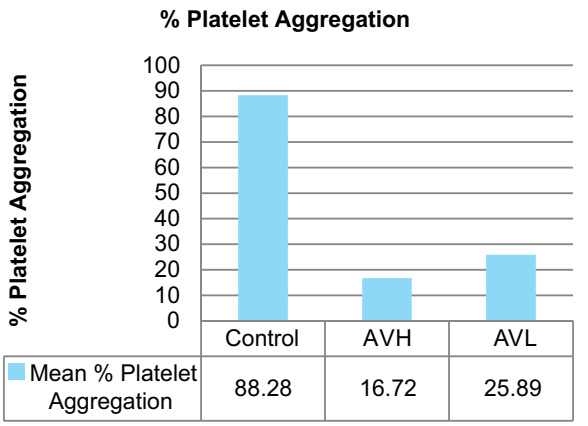

Figure-2. Bar chart showing comparison of % platelet aggregation (mean ± sd) among groups
*** p value < 0.001 * p value < 0.05 versus control group.
AVH= Aloe vera high concentration AVL= Aloe vera low concentration

Groups	Platelets Aggregation (%)				
	Mean ± SD	Median (Inter-quartile Range)	Minimum	Maximum	p- value
Control	88.28 ± 17.70	100 (71.7 – 100.0)	50	100	< 0.001
AVH	16.72 ± 4.08	18.0 (12.7 – 20.0)	10	24	
AVL	25.89 ± 5.12	25.5 (20.0 - 30.0)	19	34	

Table-II. Platelet aggregation with control, Aloe vera high and Aloe vera low (n=18)

Multiple Comparison					
Sr. No.	Groups		Mean Difference	Std. Error	p-value
1	Control	AVH	71.556***	8.63	< 0.001
		AVL	62.389*	8.63	0.013
2	AVH	AVL	-9.167	8.63	0.741

Table-III. Pair wise comparison of platelet aggregation among groups (n=18)
*** p value < 0.001 ** p value < 0.01 * p value < 0.05
AVH= Aloe vera high concentration AVL= Aloe vera low concentration

Inhibition of Platelet Aggregation

Table-IV shows mean values of inhibition of platelet aggregation in %, along with standard deviation, median, inter quartile range, minimum and maximum values.

Comparison of the Percentage Platelet Inhibition among Treatments

As data was not normally distributed, Kruskal Wallis H test was performed to compare the platelets inhibition of AVH and AVL vs control. Kruskal Wallis H test revealed that there was a statistically significant difference in mean platelets inhibition of Aloe vera treated groups vs control.

For multiple comparisons, Mann Whitney U test with Bonferroni adjustment was used which showed that platelets inhibition with AVH and AVL was statistically significantly higher as compared to control. Platelet inhibition with AVH was higher as compared to AVL but the difference was not statistically significant (Table-V, Figure-3).

Groups	Platelets Inhibition (%)				
	Mean ± SD	Median (Inter-quartile Range)	Minimum	Maximum	p- value
AVH	80.94 ± 2.77	81.0 (79.5 – 82.0)	76	88	< 0.001
AVL	70.11 ± 5.14	70.0 (67.0 – 74.3)	60	80	

Table-IV. % inhibition of platelet aggregation of aloe vera high, aloe vera low (n=18)

Groups	Platelets Inhibition (%)				
	Mean ± SD	Median (Inter-quartile Range)	Minimum	Maximum	p- value
AVH	80.94 ± 2.77	81.0 (79.5 – 82.0)	76	88	< 0.001
AVL	70.11 ± 5.14	70.0 (67.0 – 74.3)	60	80	

Table-IV. % inhibition of platelet aggregation of aloe vera high, aloe vera low (n=18)

Comparison					
Sr. No.	Groups		Mean Difference	Std. Error	p-value
1	AVH	AVL	10.83	1.57	0.093

Table-V. Pair wise comparison of % inhibition of platelet aggregation among groups (n=18)
*** p value < 0.001 ** p value < 0.01 * p value < 0.05
AVH= Aloe vera high concentration AVL= Aloe vera low concentration

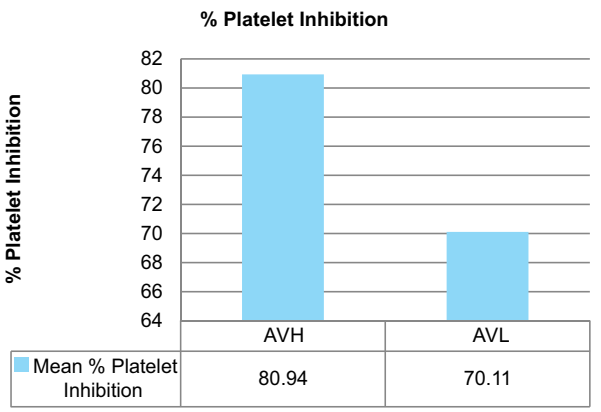

Figure-3. Bar chart showing comparison of % inhibition of platelet aggregation among groups (n=18)
*** p value < 0.001 * p value < 0.05 versus control group.
AVH= Aloe vera high concentration AVL= Aloe vera low concentration

DISCUSSION

Platelets are known to cause thrombi in body, both physiological and pathological. Increased platelet aggregation results in pathological thrombi that manifest as atherothrombotic diseases, leading to cardiovascular events, which remain one of the leading cause of death worldwide. Excessive platelet aggregation and atherosclerosis are essential culprits for its onset and development.[13] Therefore this area is of keen interest for researchers to develop new anti-platelet agents with improved efficacy and fewer side-effects.

Aloe vera, a well known medicinal plant for ages has been known to elicit various beneficial effects including anti-inflammatory. This anti-inflammatory effect is used clinically as well.[14] In contrast to traditional NSAIDs like aspirin, Aloe vera has an edge that it has no ulcerogenic effect

rather ulcer healing property.[15] Selective COX-2 inhibitors also have less GIT side effects but have documented pro-thrombotic effect, which discourages their long term use. So Aloe vera has important prospect in this regard in terms of replacing these traditional anti-inflammatory drugs. Additionally anti-platelet effect can pave way for development of new anti-platelet agent.

The present study demonstrates the concentration dependent effect of Aloe vera gel on platelet aggregation. Subjects aged between 18-35 years from both genders were selected. After drawing blood sample, baseline haemoglobin and platelet count were determined. Platelet rich plasma was prepared and then incubated with respective drugs for 30 minutes. Aggregation was induced by adding arachidonic acid. Percentage aggregation was recorded for 3 minutes.
The data was analyzed using SPSS (Statistical Package for Social Sciences) version 20. Kruskal Wallis H test was performed to compare the platelets aggregation, which revealed Aloe vera's dose dependent effect on platelet aggregation with AVH and AVL showing statistically significantly lower platelet aggregation as compared to control.

In our study, mean platelet aggregation with AVL was 25.89 % and mean platelet inhibition fell around 70 %. Mean platelet aggregation with AVH was 16.72 % and mean platelet inhibition 81 %. Aloe vera thus showed dose dependent inhibition of platelet aggregation. Platelet aggregation with AVH and AVL was statistically significantly lower as compared to control.

As no similar study is available regarding action of Aloe vera on platelet aggregation, we used herbs with documented anti-platelet effect as reference for dosage selection. Many compounds of herbal origin, such as Pycnogenol (PYC), a French maritime pine bark extract, has documented antiplatelet effect. It was found that under in vitro conditions, ethanol-dissolved PYC showed anti-platelet activity as well as enhanced the efficacy of aspirin to inhibit platelet function.[16] PYC was used in concentrations of 10, 50, and 100 μg/ml, so we selected 10 and 100 μg/ml as concentration of AVL and AVH respectively.

An in vivo study, conducted on mice by Singh and Fahim[17], demonstrated anti-platelet effect of Aloe vera. Study by Singh and Fahim used a very complicated in vivo method for demonstrating anti-platelet effect, which required intubation, craniotomy, slicing open cerebral vessels both arterial and venous, washing brain surface with ACSF solution and measuring aggregated platelet size, thus requiring specialized personnel and instruments.

Our study in contrast, was an in vitro study, conducted on human blood, 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. Additionally we used only Aloe vera gel, the main component of Aloe vera to demonstrate effect on platelets, excluding the juice or the exudate portion. Our study has studied effect on platelets by traditional aggregometer (LTA), which is still the gold standard test for measuring platelet aggregation and is cost-effective and easier comparatively. We also checked effect of 2 different concentrations of Aloe vera gel on platelets and found that its antiplatelet effect is concentration dependent.

CONCLUSION

This study demonstrates a potent in vitro anti-platelet effect of Aloe vera gel, which is dose dependent and comparable in potency with aspirin. This paves way for number of prospects for new drug development.

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PREVIOUS RELATED STUDY

Tahira Tasneem, Seema Mazhar, Zafar Iqbal. Platelet aggregation studies in diabetic retino-pathy (Original) Prof Med Jour 9(1) 29-35 Jan, Feb, Mar 2002.

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