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 Material & Methods: Blood was withdrawn from anti-cubital vein, complete blood count was checked, platelet rich plasma was prepared by centrifuging citrated whole blood and then incubated with Aloe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 its 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/l¹⁰ Celecoxib – 3 µmol/l¹¹ AVL – 10 microgram/ml¹², AVH – 100 microgram/ml¹², 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.¹³ 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 5microcuvetteslabelled 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 chrono-log 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 ± 3.08years, 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 %.

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

Table-I. Platelet aggregation with aspirin, Aloe vera and celecoxib (n=18).
AVH= Aloe vera high concentration  AVL= Aloe vera low concentration

| Groups   | Mean ± SD (Inter-quartile Range) | Minimum | Maximum | P-Value |
|----------|----------------------------------|---------|---------|---------|
| Aspirin  | 95.39 ± 2.85 (95.0 (92.0 – 97.0)) | 87      | 97      | < 0.001 |
| AVH      | 81.5 ± 2.55 (80.0 (78.5 – 81.0))   | 74      | 86      |         |
| AVL      | 71.54 ± 4.98 (69.0 (66.5 – 73.5)) | 60      | 80      |         |
| Celecoxib| -1.78 ± 4.89 (0.01 (-1.7 – 0.01)) | -14.9   | 00      |         |

Table-II. % inhibition of platelet aggregation with aspirin, Aloe vera, celecoxib (n=18).
AVH= Aloe vera high concentration AVL= Aloe vera low concentration.
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).

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.17

Besides our research, only one in vivo study has been conducted on Aloevera so far by Singh and Fahim18, 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.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-1 inhibitory 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 ant-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.

Copyright© 15 Nov, 2019.

REFERENCES
1. Moghaddasi S, Verma, SK. Aloe vera their chemical composition and applications: A review. Int J Biol Med Res. 2011; 2(1): 466-71.

2. Radha MH, Laxmi Priya NP. Evaluation of biological properties and clinical effectiveness of Aloe vera: A systematic review. Journal of Traditional and Complementary Medicine. 2015; 5: 21-6.

3. Ombito JO, Salano EN, Yegon PK, Ngetich WK, Mwangi EM, Kibet Koech GK. A review of the chemistry of some species of genus Aloe (Xanthorrhoeaceae family). JSIR.2015; 4(1): 49-53.

4. Kwok CS, Loke YK. Critical Overview on the Benefits and Harms of Aspirin: A review. Pharmaceuticals 2010; 3: 1491-1506.

5. Furst DE, Ulrich RW, Prakash S. Non-steroidal anti-inflammatory drugs, disease modifying anti-rheumatic drugs, non-opioid analgesics and drugs used in gout. In: Katzung BG, Masters SB, Trevor AJ, eds. Basic and Clinical Pharmacology.13th ed. New York: Mc-Graw hill; 2016. p. 635-57.

6. Mathew ST, Devi G, Prasanth VV, Vinod B. Efficacy and safety of COX-2 inhibitors in the clinical management of arthritis. ISRN Pharmacol.2011; 480291.

7. Rao PPN, Knaus EE. Evolution of non-steroidal anti-inflammatory drugs (NSAIDs): cyclooxygenase (COX) inhibition and beyond. J Pharm Pharmaceut Sci. 2008; 11(2): 81-110.

8. Day RO, Graham GG. The vascular effects of COX-2 selective inhibitors. Aust Prescr. 2004; 27(6): 142-5.

9. Paul S, Dutta S, Chaudhuri TK, Bhattacharjee S. Anti-inflammatory and protective properties of aloe vera leaf crude gel in carrageenan induced acute inflammatory rat models. Int J Pharm Pharm Sci. 2014; 6(9): 368-71.

10. Hosseinzadegan H, Tafti DK. Mechanisms of platelet activation, adhesion and Aggregation. Thromb Haemost Res. 2017; 1(2).

11. Agarwal S, Coakeley M, Reddy K, Riddell A, Mallett S. Quantifying the effect antiplatelet therapy: A comparison of the platelet function analyzer (PFA-100) and modified thromboelastography (m TEG) with light transmission platelet aggregometry. Anaesthesia. 2006; 105(4): 676-83.

12. Rubio-Senent F, De-Roos B, Duthieb G, Fernández-Bolañosa J, Rodríguez-Gutiérreza G. Inhibitory and synergistic effects of natural olive phenols on human platelet aggregation and lipid peroxidation of microsomes from vitamin E deficient rats. Post print of European Journal of Nutrition. 2015; 54(8): 1287-95.

13. Ime AU, Ani EJ, Nna VU, ObetenCE. Aloe Vera and garlic ameliorate thermoxidized palm oil-induced haemostatic derangement in albino Wistar rats. Micro Med. 2017; 5(2): 53-9.

14. Patzelt J, Verschoor A, Langer HF. Platelets and the complement cascade in atherosclerosis. Frontiers in Physiology.2015; 6(49):1-9.

15. Agarwal S, Coakeley M, Reddy K, Riddell A, MallettS. Quantifying the effect of antplatelet therapy: A comparison of the platelet function analyzer (PFA-100) and modified thromboelastography (m TEG) with light transmission platelet aggregometry. Anaesthesia. 2006; 105(4): 676-83
16. Patrono C. Cardiovascular effects of cyclooxygenase-2 inhibitors: a mechanistic and clinical perspective. Br J Clin Pharmacol. 2016; 82: 957–64.

17. Alkadi KAA, Adam A, Taha M, Hasan MH, Shah SAA. Prenylated Xanthone and Rubraxanthone with Antiplatelet Aggregation Activity in Human Whole Blood Isolated from Garciniagriphithii. Orient J chem.2013; 29(4): 1291-95.

18. Singh S, Fahim MA. In vivo antiplatelet aggregatory activity of Aloe vera juice on mice cerebral micro vessels. IJPR.2004; 3(2): 70.

19. Kishore K. Effect of Aloe Vera (Aloe barbadensis) on Thrombosis in Mice. Pharmacologia. 2015; 6(8): 347-54.

20. Lindsey KL, Jäger AK, Viljoen AM, van Wyk BE. Cyclooxygenase inhibitory activity of Aloe species. South African journal of botany. 2002; 68(1):47-50.

21. Jäger AK, Van Staden J. Cyclooxygenase inhibitory activity of South African plants used against inflammation. Phytochemistry Reviews. 2005; 4(1):39-46.

22. Nworo CS, Akah PA. Anti-inflammatory medicinal plants and the molecular mechanisms underlying their activities. Afr J Tradit Complement Altern Med. 2015; 12:52-6.

23. Lucini L, Pellizzoni M, Pellegrino R, Molinari GP, Colla G. Phytochemical constituents and in vitro radical scavenging activity of different Aloe species. Food Chemistry. 2015 Mar 1;170:501-7.

**AUTHORSHIP AND CONTRIBUTION DECLARATION**

| Sr. # | Author(s) Full Name | Contribution to the paper | Author(s) Signature |
|-------|---------------------|---------------------------|---------------------|
| 1     | Sidra Mushtaq       | Data collection, Analysis, Main author. | Sidra |
| 2     | Zobia Mushtaq       | Edition and Finalization of manuscript. | Zobia |
| 3     | Javeria Sarfraz     | Article writing and edition. | Javeria |
| 4     | Mufakhara Fatimah   | Article writing edition and finalization. | Mufakhara |
| 5     | Sadida Bahawal      | Edition and review + guidance. | Sadida |
| 6     | Qura-tul-Ain        | Edition and review. | Qura-tul-Ain |
| 7     | Sadia Chiragh       | Supervised research edited + finalized draft of manuscript. | Sadia |