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Effect of reconstituted, lyophilized cold aqueous extract of *Aloe vera* on human whole blood clotting time - A pilot study

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1. Introduction

Aloe vera is a pea-green coloured succulent plant that belongs to the Liliacea family and is found globally [1]. The gel and latex have healing effects, and thus the plant is well identified for its therapeutic role in the field of cosmetology [2]. Studies have also demonstrated the role of *Aloe vera*, in the treatment of conditions such as skin burns, pressure ulcers, stomatitis, periodontitis, indigestion and constipation, in addition to its known antioxidant, immunomodulatory and antidiabetic properties [1–3] (as noted in Table 1).

With regard to hemostatic effect, studies have shown mixed results wherein *Aloe vera* extract used intraperitoneally in Wistar rats caused prolongation of clotting time, while another study demonstrated reduction in clotting time when the extract was used with rat blood in an in-vitro set-up [4,5]. Although the anti-hemostatic effects seen with its use have been attributed to the presence of either salicylate mediated inhibition of prostaglandin synthesis, astringent zinc producing anti-platelet effect or polysaccharide mediated thrombo/fibrinolysis [4, 6,7]; the discrepancy with regard to its effect on whole blood clotting time remains untested, with no literature reported in human subjects. In order to clarify the influence of the herb on human blood, this pilot was designed to analyse the effect of reconstituted lyophilized cold aqueous extract of *Aloe vera* on whole blood clotting time in human volunteers (*in-vitro*) to dissect the specifics of its hemostatic effect.

2. Materials and methods

2.1. Pilot particulars

The study was conducted in accordance with guidelines laid down in the declaration of Helsinki and Institutional Ethics Committee. The purpose of this in-vitro study was explained to all participants in detail, and after obtaining written, informed consent, 6 healthy, adult volunteers were recruited (Age: 30 ± 10 years; male = 3 and female = 3). Any volunteers with history of bleeding dyscrasias/coagulopathies or intake of medication causing deranged bleeding or clotting were excluded. Seven ml of venous blood was drawn from each individual to perform the whole blood clotting time.

2.2. Extract preparation

Around 250 g of fresh leaves of Aloe vera *chinensis* were taken and cleaned with 70% ethanol. Following removal of rind, clear parenchyma or gel within individual leaves was filleted into sections approximately 5 cm in dimension. Gel fillets were washed thoroughly with deionized water to remove latex and then ground into a liquid. This liquid obtained was then centrifuged at 2500 rpm for 5 min to remove unwanted fibers. Clear supernatant (approximately 150 ml) was added at a volume of 4ml/well to non-treated, sterile, 6-well culture plates and lyophilized at −72 °C for 48 h. The concentrated, freeze-dried extract was refrigerated at 4 °C until further use (Fig. 1).

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Table 1
Showing literature demonstrating far-reaching actions and therapeutic roles of Aloe vera extracts and constituents.

S. No.	Study Reference	Study Type	Composition	Primary Findings
1. Probiotic/Antimicrobial action				
	Sun et al. [14]	Animal: Mice	Aloe polysaccharides	Improvement in clinical symptoms of influenza
	Panahi et al. [15]	Human: Randomized trial	Aloe syrup	Reduced frequency of gastric reflux
2. Anti-inflammatory action				
	Li et al. [16]	Animal: Mice	Aloe emodin and rhein	Reduced levels of Tumor necrosis factor, Nitric oxide and inflammatory interleukins
	Werawatganon et al. [17]	Animal: Mice	Lyophilized Aloe gel	Reduced levels of hepatic injury and malondialdehyde levels
3. Wound healing/Dermal Protection				
	Wahedi et al. [18]	Animal: Mice	Aloesin	Inducing granulation tissue formation and accelerated wound closure
	Hekmatpou et al. [19]	Human: Randomized trial	Pure Aloe gel	Reduced incidence of pressure ulcers
4. Anti-cancer/Immunomodulatory action				
	Im et al. [20]	Animal: Mice	Processed Aloe gel	Reduction in cyclophosphamide induced immunotoxicity
	Liu et al. [21]	Animal: Mice	Aloe emodin	Increased survival time and reduced cell migration
5. Anti-diabetic action				
	Noor et al. [22]	Animal: Rats	Crude Aloe extract	Increase in pancreatic islet volume and Insulin secretion
6. Cardio-protective action				
	Sahin et al. [23]	Animal: Rats	Whole Aloe extract	Reduced renal and pulmonary reperfusion ischemia injury

2.3. Reconstitution

To prepare the final dose, 15 mg freeze-dried powder of *Aloe gel* was resuspended with 200 μL deionized water for each experiment (minimum volume required for complete reconstitution of powder). Deionized water (200 μL) also served as vehicular control in the experiments.

2.4. Assessment of clotting time

Clotting time assessment was performed as per description provided by Ochei et al. [8] as a modification of Lee and White technique for estimation. In brief, two sterile syringes were used to draw blood. 1 ml of initially drawn blood in the first syringe was discarded to minimize the influence of tissue thromboplastin on the reading. Time was noted once the second syringe was introduced to collect blood. After collection of 6 ml of blood; 1 ml each was added to 6 glass test tubes (3 tubes per arm of study), which were pre-filled with control and test extract. [a. Reconstituted lyophilized *Aloe vera*, and b. Deionized water (Control)]. All tubes were kept in a 37 °C water-bath and left undisturbed for 5 min after collection of blood. At the end of 5 min, the first tube from each set was gently tipped to 45° to assess for clot formation. This was repeated at 30 s intervals till clotting was observed. After clotting in the first tube, time was noted and similar tilting was performed for the second and, consequently the third tube. The time recorded for clotting of the third tube was considered as the whole blood clotting time of the individual. (Additional purpose of the first two tubes was to intimate the clotting interval in the third tube, as agitation could accelerate the clotting process).

2.5. Statistical analysis

Clotting time data for each arm was taken, and results were expressed as Mean ± SD. Wavemetrics IgorPro software was utilized for graphical representation of data. Comparison between groups was performed using Mann Whitney *U* test, as stated for comparison of non-parametric sample data, by running the data on SPSS version 17.0. A P value of <0.05 was regarded as significant.

3. Results

It was observed that the mean clotting time with a) Control was 10.67 ± 0.98 min and b) *Aloe vera* was 18.67 ± 1.47 min. Clotting time observed with control was within the normal range (5–15 min), while with *Aloe vera,* there was an abnormal prolongation of clotting time which was also significantly longer when compared to values obtained with vehicular control (**P=0.0052**, Fig. 2). An additional assessment of the pH of reconstituted extract revealed a range of 6.7 ± 0.2, while assessment of blood sample pH revealed that addition of reconstituted extract did not acidify the blood sample.

4. Discussion

Multiple studies have advocated healing properties of *Aloe vera* over the course of the past few decades [9–11]. Phytochemical analysis of aloe vera whole extract has yielded data on its constituents which include active molecules of high research interest, generally implicated for the therapeutic activity of aloe vera. These primarily include anthraquinones such as aloin, emodin and acemannan, which have documented effects on cellular proliferation, turnover and signalling(2). With regard to treatment of the gel extract, aloe vera extract preparation in the current study included lyophilization or freeze drying, which is a common methodology utilized to obtain quantifiable dry powder, which has a significantly longer shelf-life as compared to fresh whole extract or its constituents. This methodology was specifically selected as lyophilization results in relatively unaltered extraction of the product as opposed to plain evaporation or heat induced drying methods. It is important to note that any treatment of whole extract may have implications on the composition. Such alterations are also noted for lyophilization, with a study evidencing that, it is specifically the polysaccharides (namely acemannan and aloin), and to some extent flavonoids, that show partial distraction while the mineral content remains largely unchanged [12]. Since the plant is available globally, it has also found its way into multiple home remedies for common ailments. Literature has also hinted at the anticoagulant and anti-platelet nature of the gel as seen in animal studies or isolated case reports [4,5,7,13]. At present, no restrictions exist to sale and oral consumption of the juice or concentrate, therefore it is crucial to assess the safety of the extract at doses presently being consumed. In this pilot study, we observed a significant increase in the mean clotting time beyond the normal range, when *Aloe vera* extract was used. This increase was validated, as the vehicular control demonstrated a significantly lower time for clotting than *Aloe vera*. These results corroborate the findings of the study by Dapper et al. who also demonstrated similar effects while negating the report by Ramesh H. et al. who showed pro-coagulant effects in Wistar rats [4,5]. This is the first study where the effect of the lyophilized extract has been assessed utilizing human blood in vitro. With respect to the mechanism of action observed with the effects seen in this pilot, it may be speculated that variable presence of anthraquinones may be responsible for the change in clotting time. It has been

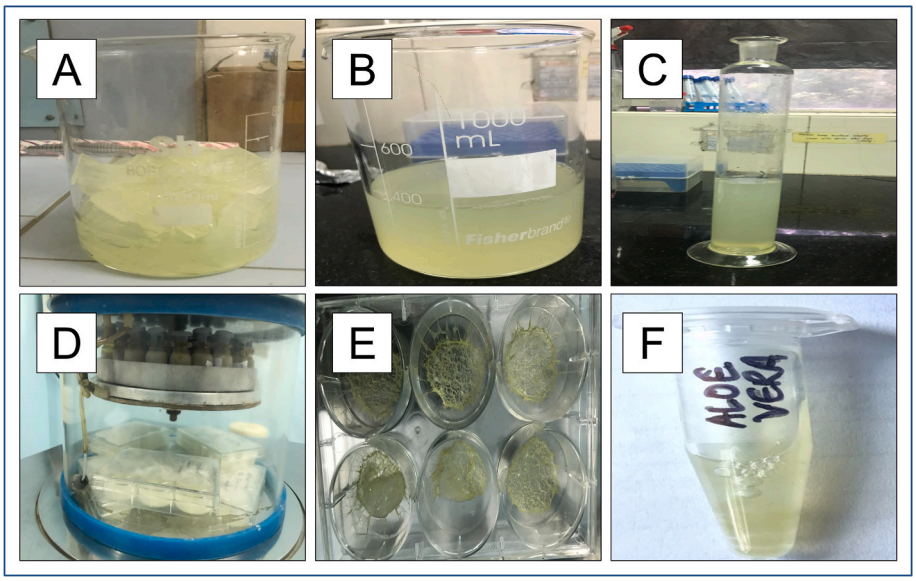

Fig. 1. Shows the sequence of aqueous extract preparation ie. isolation of gel, centrifugation and obtaining supernatant of Aloe vera (A–C) D: Culture plates filled with extract being lyophilized; E: Lyophilized extract; F: Extract reconstituted with 200 μL of deionized water.

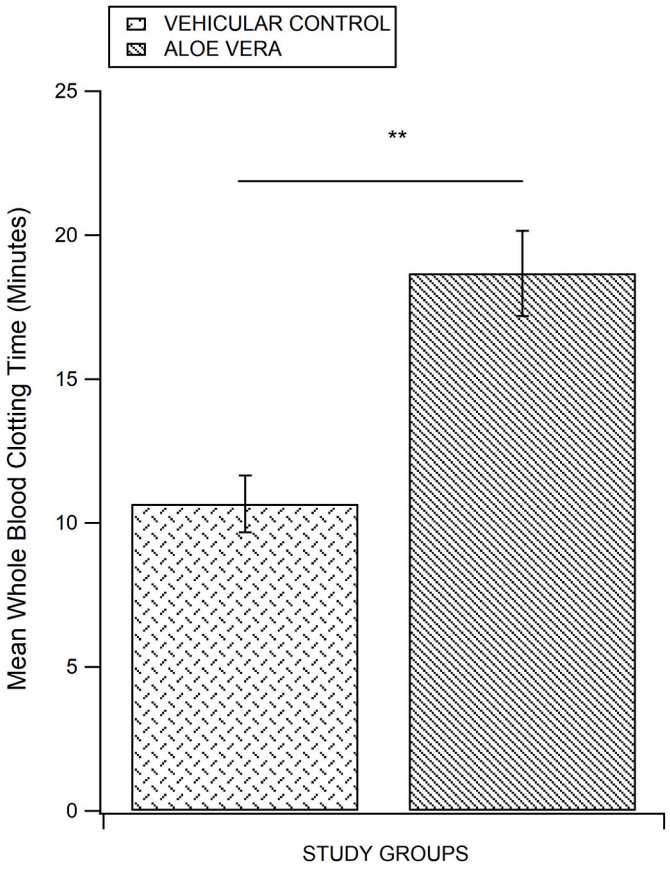

Fig. 2. Shows graphical representation of Mean clotting time (in minutes) of human blood, as seen with addition ofAloe vera extract vs. vehicular control. Statistical analysis done using Mann Whitney *U* Test. (**P < 0.01).

observed that aloin and emodin cause a decrease in the levels of Interleukin (IL)- 1, 6 and Tumor Necrosis Factor (TNF). Both TNF and IL-1, 6 are pro-inflammatory cytokines and present a pro-coagulant picture [2, 13]. Their reduction could be the possible mechanism by which prolongation of clotting time occurs. It requires mention that with respect to the study findings, although Lee and White's test serves as a good screening method, an in-depth analysis of clotting time would require other parameters such as PT, aPTT estimation on samples where the pH of blood has been normalized before addition of the extracts. In addition to this, stringent analysis of herbal sub-components is required to identify active molecules responsible for the specific therapeutic action, in addition to investigating the pathway through which they predominantly exert their effect in vivo.

5. Conclusion

The observations of this pilot sound caution to the consumption of *Aloe vera* concentrate, especially in individuals with known bleeding tendencies or coagulopathies. Another important consideration could be the potential benefit that *Aloe vera* may provide in patients suffering from pro-coagulable states, by working as an adjunct to regular anti-coagulation therapy, an implication which warrants further exploration.

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CRediT authorship contribution statement

Deena Susie Melenshia: Methodology, Formal analysis, Data Curation, Writing – original draft. **Soosai Manickam Amirtham:** Methodology, Writing – review & editing. **Grace Rebekah:** Formal analysis, Writing – review & editing. **Elizabeth Vinod:** Methodology, Writing – review & editing. **Upasana Kachroo:** Conceptualization, Methodology, Formal analysis, Data curation, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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