

Effect of Aloe Vera (*Aloe barbadensis*) on Thrombosis in Mice

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ABSTRACT

Background and Objective: Herbal substances are served as the source of traditional and the modern drugs. Most of these entities are derived from the higher plants. The antioxidant plants that have been clinically documented to be effective in thrombosis. Aloe vera, commonly known as guar patha and is well documented as antioxidant herb. Therefore, it appears worthy to investigate the effect of aqueous extract of aloe vera (10, 20 and 30 mg kg⁻¹, i.p.) on thrombosis in mice, using bleeding time methods. **Materials and Methods:** All the agents were administered on each day and repeated for 5 and 29 consecutive days and on 6th and 30th day, 30 min before the determination of bleeding time and λ_{\max} . **Results:** The aloe extract significantly decreased the bleeding time and λ_{\max} as compared to vehicle (normal saline and distilled water) treated control groups. The results suggest that aqueous extract of aloe vera is a promising antithrombotic plant based agent. **Conclusion:** The antithrombotic effect of aloe may be due to its antioxidant property, because oxidants play a significant role in hypercoagulation of blood.

Key words: Thrombolytics, fibrinolytics, coagulation, thrombosis, antioxidant, bleeding time, λ_{\max}

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INTRODUCTION

Thrombosis is responsible for many cardiac problems including unstable angina, myocardial infarction, postangioplasty occlusion and stroke. To form a thrombus, three steps take place, (1) Exposure of the circulating blood to a thrombogenic surface, such as damaged vascular endothelium, (2) sequence of platelet related events, involving first platelet adhesion, aggregation and release of agents which further promoting aggregation and causing vasoconstriction and (3) Activation of the clotting mechanism plays important role in the formation of fibrin. At the site of vascular injury and in the presence of fluid shear stress, platelets get activated and they secrete adenosine diphosphate from cellular storage granules. Activated platelets also cause hydrolysis of free arachidonic acid to prostaglandin endoperoxides. The released adenosine diphosphate and prostaglandins further amplify platelet activation process, finally the activated and degranulated platelets attach to an occlusive thrombus at the site of vascular damage. Atherosclerotic plaques result from the organization of thrombi apart from atherosclerosis¹. These events are precipitated by plaque rupture which cause exposure of thrombogenic material to the flowing blood. To reduce mortality due to plaque rupture or thrombus related acute coronary syndromes, plaque stabilization is one of the important intervention. Lipid

lowering and antithrombotic treatment can stabilize plaque. Currently available antithrombotic agents used to treat coronary artery thrombosis are aspirin, heparin and plasminogen activators. Aspirin blocks one of the several pathways of platelet activation. However, it does not prevent shear induced platelet activation. Heparin has limited effectiveness due to bleeding strokes. Plasminogen activators like streptokinase and tissue plasminogen activator (t-PA) are highly effective thrombolytic agents². Platelet glycoprotein IIb/IIIa and fibrinogen inhibitors are most promising in preventing thrombotic complications. No drug is yet well established or known which has been derived from plants.

Aloe barbadensis Miller, commonly referred to as aloe vera, is one of approximately 420 species^{3,4}. *Aloe barbadensis* (family: Aloeaceae or Xanthorrhoeaceae) is a natural herb frequently used in cosmetics and many other health products including drugs and medicines. It has been known and used for centuries for its health, beauty, medicinal and skin care properties. Some 2000 years ago, the Greek scientists regarded aloe vera as the universal panacea.

The leaves are thick with serrated and spiny edges. The leaves are cut open and the flesh scooped out. The scooped out flesh is gel like substance used for topical and internal medicine. Aloe vera is comprised of

approximately 99% water; all of other chemicals are contained in the remaining 1% of the plant. Many compounds with diverse structures have been isolated from both the central parenchyma tissue and the exudate arising from the cells adjacent to the vascular bundles. The chemicals found in aloe are anthraquinones, lignin, saponins, minerals, vitamins, amino acids, enzymes, sterols, sugars and polysaccharides^{5,6}. It is an oldest medicine reported for the treatment of burns, blisters, frost bite, allergic skin rash, herpes, warts, inflammatory skin disorders, psoriasis, dandruff and tooth and gum diseases. Aloe vera finds its usage in numerable face packs, moisturizers, soaps, bath gels, shampoos, conditioners, tooth pastes, face and skin creams, cleansers, sunscreens, shaving gels, baby lotions and wipes. Local application of aloe prevents aging, wrinkles, scars, stretch marks and black circles around the eyes. It is also used to treat sunburn, dry skin, cuts, skin eruptions, insect bites, eczema and acne⁷. Internally, aloe used as aphrodisiac, diuretic and mild laxative. To treat peptic ulcers, regulate menstruation cycle and help in menstruation cramps. To regulate digestion, flush out toxins; help with piles and hemorrhoids and to improve liver and kidney functions^{8,9}. It has been claimed that the polysaccharides in aloe vera gel have therapeutic properties such as immunostimulation, anti-inflammatory effects, wound healing, promotion of radiation damage repair, anti-bacterial, anti-viral, anti-fungal, anti-diabetic, anti-neoplastic and stimulation of hematopoiesis and antioxidant activities^{6,10}. Therefore, it appeared worth to investigate the effect of aqueous extract of *Aloe barbadensis* on thrombosis in mice, using bleeding time methods.

MATERIALS AND METHODS

Swiss albino mice (30–40 g) of either sex, kept in an animal house provided with 12 h light and dark cycle, free access to water and standard diet, were employed in the present study. The experiments were conducted in a semi-sound proof laboratory between 10.00 am to 5.30 pm. Animals were procured from Indian Veterinary Research Institute (IVRI) Izatnagar, Bareilly. The research was conducted as per the guidelines of “Committee for the Purpose of Control and Supervision of Experiments on Animals” (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, New Delhi.

Experimental protocol: Twelve groups of mice ($n = 12$) were employed. All pharmacological agents, normal saline (10 mL kg^{-1}), distilled water (10 mL kg^{-1})

and aqueous extract of aloe vera (10, 20 and 30 mg kg^{-1}) were administered intraperitoneally (i.p.). The vitamin-K (10 mg kg^{-1} , i.p.) and heparin sodium (100 IU kg^{-1} , i.v.) were administered for 5 and 29 consecutive days and on 6th and 30th day, 30 min before the determination of bleeding time and λ_{max} . The bleeding time in mice ($n = 6$) by filter paper method, in seconds and λ_{max} in mice ($n = 6$) by spectrophotometer, at 540 nm, were noted down. The spectrophotometer (Systronics, 89-92, Naroda industrial area, Ahmedabad) was used to determine the absorbance by the blood contents.

Bleeding time methods: The bleeding time is defined as the time required for bleeding to stop from a standard incision. Bleeding time measurements in animals are used to evaluate the haemorrhagic properties of antithrombotic drugs. Bleeding time in mice was evaluated by the method of Dejana *et al.*¹¹ to assess the bleeding time in comparison to heparin and vitamin-K. Mice were anaesthetized by sodium pentobarbital (70 mg kg^{-1} , i.p.) and placed on a pad at room temperature¹². Bleeding time in mice was measured by two ways: via filter paper method and λ_{max} method.

Filter paper method: The tail 3 mm from tip of mice was cut and the blood oozed was soaked on a filter paper (Whatman number 1 filter paper discs, Whatman International Ltd., Maidstone, England) which was monitored at an interval of 30 sec till the bleeding stopped. Any blood dripping during the 30 sec intervals was allowed to drop freely onto the filter paper. The time elapsed from the tail tip incision to the stoppage of bleeding was recorded as the bleeding time^{11,13}. If bleeding continued after 20 min, bleeding was stopped by cauterization to prevent hypovolemic shock.

Lambda max (λ_{max}) method: The tail was cut 3 mm from the tip with a number 10 surgical razor blade. The tail was carefully immersed in 40 mL of distilled water at room temperature. The time until bleeding stops is determined within a maximum observation time of 20 min. Blood loss was evaluated as a function of absorbance at 540 nm due to haemoglobin content in water.

Aloe vera (*Aloe barbadensis*) extract: The fresh leaves of aloe vera were collected and washed with distilled water and then cut open to collect the gel. The gel is then evaporated at 60°C in a hot-air oven for four days to get a solid dry mass. This was then converted into powder by grinding and stored under refrigeration^{14,15}.

Drugs and chemicals: All the drug solutions were freshly prepared before use. The aqueous extract of aloe vera was used to prepare from refrigerated powder of aloe vera gel previously prepared from fresh leaves of the plants. Vitamin-K (Samarth Life Sciences Pvt Ltd., Ram Mandir Road, Goregaon (W), Mumbai-400104) and heparin sodium (Biologicals E. Limited, at Rampur Ghat Road, Paonta Sahib, Dist. Sirmour, Himachal Pradesh-173025) were purchased from market.

Statistical analysis: All the results were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. A value of $p < 0.05$ was considered statistically significant. The statistical analysis was done by using Statistics Calculator, a software from StatPac Inc¹⁶.

RESULTS

Effect of vehicle on thrombosis (Group I to II):

Mice of group I and group II were treated with normal saline (10 mL kg⁻¹, i.p.) and distilled water (10 mL kg⁻¹, i.p.) respectively. Distilled water produced no marked effect on bleeding time and λ_{\max} as compared to normal saline treated control mice (Fig. 1). It indicates that vehicle treatment produced no marked effect on normal blood flow in tail vein of mice. This suggests that the normal saline and distilled water *per se* did not affect the normal bleeding time of animals atleast in mice species.

Effect of vitamin-K, heparin and vitamin-K+heparin on thrombosis (Group III-V):

Mice of group III, IV and V were treated with vitamin-K (10 mg kg⁻¹, i.p.), heparin sodium (100 IU kg⁻¹, i.v.) and vitamin-K (10 mg kg⁻¹, i.p.)+heparin sodium

(100 IU kg⁻¹, i.v.), respectively. The vitamin-K *per se* significantly reduced bleeding time and mean λ_{\max} as compared to control groups animals, but heparin *per se* significantly enhanced bleeding time and λ_{\max} as compared to control groups mice. The vitamin-K as alone and when given in combination with heparin significantly decreased the bleeding time and mean λ_{\max} in the animals previously treated with heparin sodium (100 IU kg⁻¹, i.v.) as compared to *per se* effect of heparin sodium (Fig. 2). This exhibit that vitamin-K reduces the blood flow from the tail vein of mice. It suggests that vitamin-K enhances the coagulation of blood as well as suppresses the heparin induced fluidity of the blood in mice.

Effect of aqueous gel of *Aloe barbadensis* (aloe vera) on thrombosis (Groups VI-XII)

Effect of aqueous gel of aloe vera on thrombosis:

Aloe vera gel (10 mg and 20 mg kg⁻¹, i.p.) did not produce any significant effect on thrombosis in mice of groups VI-VII but aloe gel (30 mg kg⁻¹, i.p.) significantly enhanced the bleeding time and mean λ_{\max} in mice of group-VIII treated with aloe vera aqueous gel for 6 consecutive days and mice of group-IX were treated with aloe vera aqueous gel for 30 consecutive days, as compared to normal saline treated mice (Fig. 3). Results revealed that aloe vera aqueous gel reduce the viscosity of blood of mice in dose dependent manner.

Effect of aqueous gel of aloe vera+vitamin-K on thrombosis:

The treatment of aloe vera gel (30 mg kg⁻¹, i.p.), significantly enhanced the bleeding time and mean λ_{\max} in mice of group-X, previously treated with vitamin-K (10 mg kg⁻¹, i.p.) as compared to *per se* effect

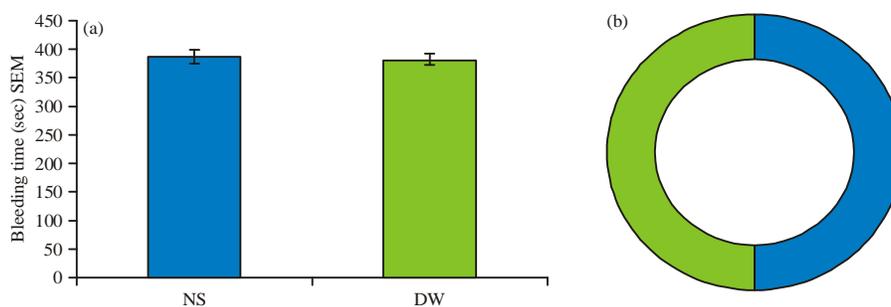


Fig. 1(a-b): Effect of vehicle on thrombosis against, (a) Bleeding time and (b) Mean λ_{\max} . NS: Normal saline (10 mL kg⁻¹, i.p.), DW: Distilled water (10 mL kg⁻¹, i.p.). Dose was administered for 5 consecutive days and 30 min before the determination of bleeding time and λ_{\max} on 6th day. Green represents mean value of bleeding time (sec) and λ_{\max} recorded on day 6th vs. mean value of bleeding time (sec) and λ_{\max} in normal saline (blue) treated control group

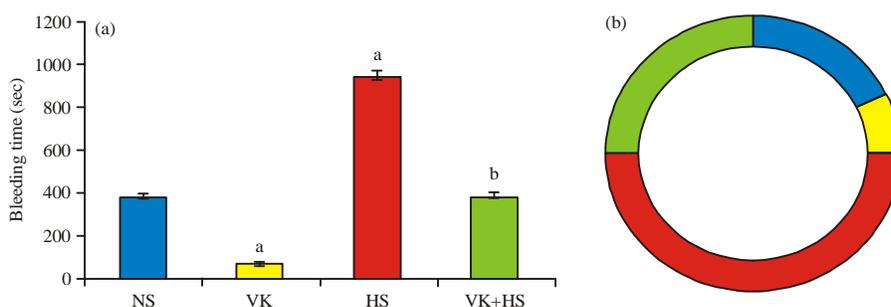


Fig. 2(a-b): Effect of vitamin-K, heparin and vitamin-K+heparin on thrombosis against, (a) Bleeding time and (b) Mean λ_{\max} . NS: Normal saline (10 mL kg^{-1} , i.p.), VK: Vitamin-K (10 mg kg^{-1} , i.p.), HS: Heparin sodium (100 IU kg^{-1} , i.v.), VK+HS: Vitamin-K (10 mg kg^{-1} , i.p.)+heparin sodium (100 IU kg^{-1} , i.v.). Dose was administered for 5 consecutive days and 30 min before the determination of bleeding time and λ_{\max} on 6th day. Green represents mean value of bleeding time (sec) and λ_{\max} recorded on day 6th. b: $p < 0.05$ vs. mean value of bleeding time (sec) and λ_{\max} in vitamin-K (orange) treated groups. a: $p < 0.05$ vs. mean value of bleeding time (sec) and λ_{\max} in normal saline (blue) treated control group

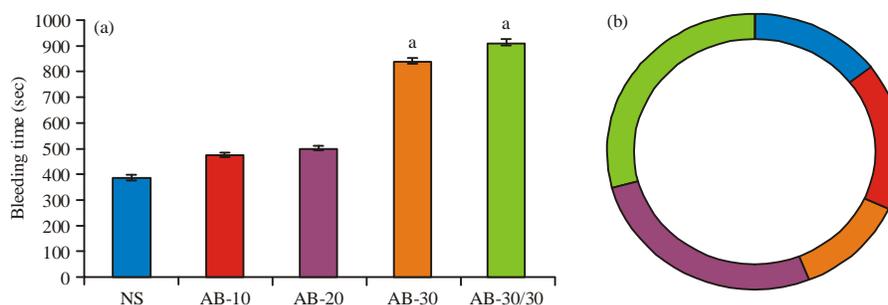


Fig. 3(a-b): Effect of aqueous gel of *Aloe barbadensis* (aloe vera) on thrombosis against (a) Bleeding time and (b) Mean λ_{\max} . NS: Normal saline (10 mL kg^{-1} , i.p.), AB-10, AB-20, AB-30: Aqueous gel of *Aloe barbadensis* 10, 20 and 30 mg kg^{-1} , respectively. Dose administered intraperitoneally for 5 consecutive days and 30 min before the determination of bleeding time and λ_{\max} on 6th day. AB-30/30: Aqueous gel of *Aloe barbadensis* 30 mg kg^{-1} for 30 days administered intraperitoneally for 29 consecutive days and 30 min before the determination of bleeding time and λ_{\max} on 30th day. Purple, orange and red represents mean values of bleeding time (sec) and λ_{\max} recorded on day 6th and green represents mean value of bleeding time (sec) and λ_{\max} recorded on day 30th. a: $p < 0.05$ vs. mean value of bleeding time (sec) and λ_{\max} in normal saline (blue) treated control group

of vitamin-K (Fig. 4). It indicates that aloe vera gel attenuated vitamin-K induced reduction in blood flow from the tail vein of mice. It also suggests that aloe vera aqueous gel suppressed the vitamin-K induced coagulation of blood in mice.

Effect of aqueous gel of aloe vera+heparin on thrombosis: The mice of group-XI were treatment with aloe vera gel (30 mg kg^{-1} , i.p.) and heparin sodium (100 IU kg^{-1} , i.v.), did not produce any significant effect on bleeding time and mean λ_{\max} as compared to *per se* effect of heparin sodium (Fig. 5). It indicates that aloe

vera aqueous gel did not potentiate the effect of heparin sodium.

Effect of aqueous gel of aloe vera+vitamin-K+heparin on thrombosis: Aloe vera gel (30 mg kg^{-1} , i.p) enhanced the bleeding time and mean λ_{\max} in mice of group XII, previously treated with vitamin-K (10 mg kg^{-1} , i.p.) and heparin (100 IU kg^{-1} , i.v.) (Fig. 6). It indicates that aloe vera aqueous gel increased the flow of blood in tail vein of mice. This suggests that aloe vera

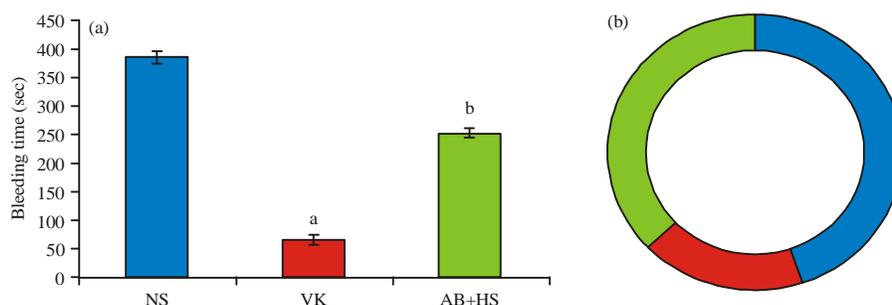


Fig. 4(a-b): Effect of aqueous gel of *Aloe barbadensis* + vitamin-K on thrombosis against (a) Bleeding time and (b) Mean λ_{\max} . NS: Normal saline (10 mL kg^{-1} , i.p.), VK: Vitamin-K (10 mg kg^{-1} , i.p.) and AB+VK: Aqueous gel of *Aloe barbadensis* (30 mg kg^{-1} , i.p.)+vitamin-K (10 mg kg^{-1} , i.p.). Dose was administered for 5 consecutive days and 30 min before the determination of bleeding time and λ_{\max} on 6th day. Green represents mean value of bleeding time (sec) and λ_{\max} recorded on day 6th. b: $p < 0.05$ vs. mean value of bleeding time (sec) and λ_{\max} in vitamin-K (red) treated group. a: $p < 0.05$ vs. mean value of bleeding time (sec) and λ_{\max} in normal saline (blue) treated control group

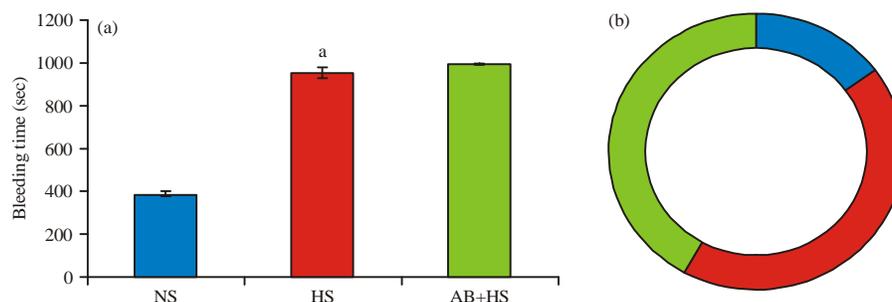


Fig. 5(a-b): Effect of aqueous gel of *Aloe barbadensis* + heparin on thrombosis against (a) Bleeding time and (b) Mean λ_{\max} . NS: Normal saline (10 mL kg^{-1} , i.p.), HS: Heparin sodium (100 IU kg^{-1} , i.v.), AB+HP: Aqueous gel of *Aloe barbadensis* (30 mg kg^{-1} , i.p.)+heparin sodium (100 IU kg^{-1} , i.v.). Dose was administered for 5 consecutive days and 30 min before the determination of bleeding time and λ_{\max} on 6th day. Green represents mean value of bleeding time (sec) and λ_{\max} recorded on day 6th vs. mean value of bleeding time (sec) and λ_{\max} in heparin (red) treated group. a: $p < 0.05$ vs. mean value of bleeding time (sec) and λ_{\max} in normal saline (blue) treated control group

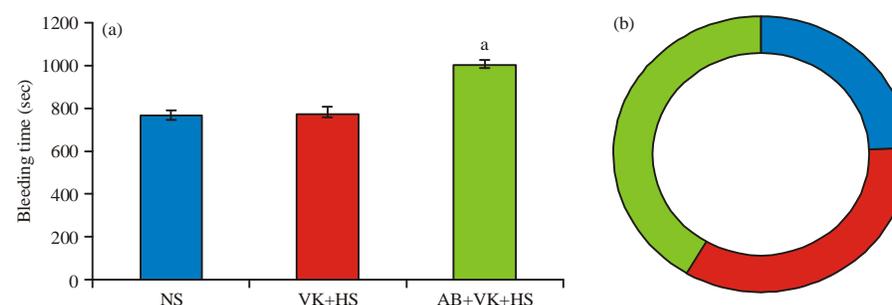


Fig. 6(a-b): Effect of aqueous gel of *Aloe barbadensis* + vitamin-K + heparin on thrombosis against (a) Bleeding time and (b) Mean λ_{\max} . NS: Normal saline (10 mL kg^{-1} , i.p.), VK+HS: Vitamin-K (10 mg kg^{-1} , i.p.)+heparin sodium (100 IU kg^{-1} , i.v.), AB+VK+HS: Aqueous gel of *Aloe barbadensis* (30 mg kg^{-1} , i.p.)+vitamin-K (10 mg kg^{-1} , i.p.)+heparin sodium (100 IU kg^{-1} , i.v.). Dose was administered for 5 consecutive days and 30 min before the determination of bleeding time and λ_{\max} on 6th day. Green represents mean value of bleeding time (sec) and λ_{\max} recorded on day 6th. a: $p < 0.05$ vs. mean value of bleeding time (sec) and λ_{\max} in normal saline (blue) and vitamin-K+heparin (red) treated groups

aqueous gel reduced the clotting effect of vitamin-K only, because aloe did not potentiate the action of heparin as when given in combination with heparin sodium as in mice of group-XI.

DISCUSSION

The vitamin-K reduces the bleeding time and mean λ_{\max} in mice possibly because of raising the level of prothrombin in blood. Prothrombin is very essential component of blood that initiates blood coagulation by converting itself into thrombin. The present observations are in support of various other scientific studies which exhibit that vitamin-K enhances the coagulability of blood¹⁷. Heparin sodium enhanced the bleeding time and mean λ_{\max} in mice by reducing the coagulability of blood. The present data supported by earlier reports which states that the heparin binds to enzyme inhibitor antithrombin-III and inactivates thrombin and other proteases involved in blood clotting. It is also reported by many scientists that heparin and its low molecular weight derivatives prevent thrombosis¹⁸⁻²¹.

In the present study, it is found that the aqueous gel of aloe vera significantly enhanced the bleeding time and mean λ_{\max} in mice. This indicates that the aloe vera gel have the potential antithrombotic activity. The present observations are supported by the earlier research reports which indicates that the aloe vera gel play a significant role in prevention of platelet aggregation²², accumulation of blood or clot lysis. Aloe vera plant such as *Aloe vulgari* and *Aloe barbadensis* has being used traditionally as a medicinal plant for centuries. Gel from the inner central zone of the leaves and latex from pericyclic cells are used for numerous medicinal purposes²³. A study, elucidated the beneficial effects of aloe vera juice on the platelet aggregation in cerebral micro-vessels of mice. Male mice were injected with saline (control) or aloe vera juice 1 h before the experiment. The results show that in the animals treated with aloe vera juice, venule as well as arteriole platelet aggregation timings were significantly delayed in comparison to the controls. Data shows the beneficial influence of aloe vera by delaying thrombus formation in the cerebral micro-vessels, *in vivo*. It is also found that aloe vera juice is beneficial in delaying the platelet formation in the blood vessels of the brain. The delay in thrombus formation may be attributed to the presence of superior antioxidants present in the aloe vera juice by protecting cells in the body against destruction by free radicals thus reducing the risk of arteriosclerosis²². The

study also found that the rats had a less incidence of thrombosis or blood clots in the heart²⁴. Several studies in animal models as well as in human have suggested that the ingestion of aloe gel may have beneficial effect by lowering serum cholesterol, serum triglycerides and serum phospholipids which when elevated, seem to accelerate the deposition of fatty material in the large and medium sized arteries, including the coronary arteries of the heart²⁵. It is further reported that the rats were fed high cholesterol diets with the experimental group fed the aloe polysaccharide i.e., glucomannan. The group fed glucomannan showed, decreased total cholesterol levels, decreased triglyceride levels, decreased phospholipid levels, decreased non-esterified fatty acid levels, increased HDL cholesterol and markedly increased HDL as compared to control animals. The research have shown that the use of aloe gel may have a salubrious effect on fat metabolism which if active in human would tend to decreased the risk of coronary artery disease²⁶. Another study exhibit that aloe attenuated lipid raising effect of triton in monkeys. Like aloe vera an antioxidant plant *Hericum erinaceus* also reported to reduce lipid level in diabetic animals²⁷. It is also reported that the husks of isabgol and aloe gel produce a remarkable effects on lipid metabolism²⁶.

The aqueous extract of *Aloe barbadensis* when administered intraperitoneally, produced dose dependent antithrombotic activity in mice. Aqueous extract of *Aloe barbadensis* in the dose 30 mg kg⁻¹ exhibited increased bleeding time and mean λ_{\max} as compared to vehicles (normal saline and distilled water) treated control groups of mice. This effect was also compared with the standard drug heparin which increased bleeding time and λ_{\max} ; and against thrombosis or coagulation induced by vitamin-K. The results indicates that aqueous extract of *Aloe barbadensis* significantly reduced viscosity of blood; inhibited the platelet adhesion and aggregation and enhanced the blood flow from the tail of mice. The present observations suggests that aqueous extract of *Aloe barbadensis* possess promising antithrombotic activities. The antithrombotic effect of *Aloe barbadensis* may be due to its antioxidant property, because oxidants play a significant role in hypercoagulation of blood. The present results are in support with a recent *in vitro* antioxidant and thrombolytic study of green tea or *Camellia sinensis*²⁸.

CONCLUSION

In *in vivo* antithrombotic screening studies done in mice, showed that the aqueous extract of *Aloe barbadensis*

possessed good antithrombotic activity in *per se* as well as in presence of heparin sodium (an anticoagulating agent). The aqueous extract of aloe also reversed vitamin-K (a coagulating agent) induced blood viscosity. Therefore, the extract of *Aloe barbadensis* can be considered as a potential source of natural antithrombotic agents. This is only a preliminary study conducted on thrombosis and to make final comment the extract of *Aloe barbadensis* should be thoroughly investigated phytochemically and pharmacologically to exploit its medicinal and pharmaceutical potentialities.

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