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SCREENING OF ANTITHROMBOTIC EFFECT OF ETHANOLIC EXTRACT OF BRAHMI (BACOPA MONNIERA) IN MICE

[1]Kamal Kishore ,[2]Saurabh Mishra*

Department of Pharmacy, M.J.P. Rohilkhand University, Bareilly-243006, (Uttar Pradesh), India.

***Corresponding author Email: saurabhmcm2002@gmail.com

Abstract

Natural 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 clinically documented to be effective in thrombosis. Bacopa monniera is a small creeper, commonly known as brahmi or jalanimba is well documented as antioxidant herb. Therefore for it appears worthy to investigate the effect of ethanolic extract of bacopa monniera (10mg, 20mg& 30 mg kg^{-1}, i.p.) on thrombosis in mice, using bleeding time methods, all the agents were administered on each day and repeated for 5 & 29 consecutive days and on 6^{th} & 30^{th} day, 30 min before the determination of bleeding time & λmax. The Brahmi extract significantly decreased the bleeding time and λmax as compared to vehicle (normal saline & distilled water) treated control groups. The results suggests that ethanolic extract of bacopa monniera (Brahmi) is a promising antithrombotic plant based agent. The antithrombotic effect of brahmi 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, λmax.

Introduction

The incidences of cardiac problems are increasing day-by-day. Therefore, it is very much essential to continue investigate and develop new therapies for their prevention. Hypercholesterolemia and thrombosis are the major risk factors for cardiac disorders. It is reported that atherosclerotic plaques result from the organization of thrombi (Rokitansky, 1852). Thrombosis is the formation of a clot or thrombus inside a blood vessel due to hypercoagubility, endothelial injury or obstruction of flow of blood. When a blood vessel is injured, the body uses platelets and fibrin to form a clot, to prevent loss of blood. If the formation of blood clot is too much, and the clot breaks free, an embolus is formed (Handin, 2005; Furie and Furie, 2008). While haemostasis is essential for the survival, the pathological formation of a blood clot or thrombosis plays a role in angina, myocardial infarction, heart attack, ischaemic stroke, cardioembolic stroke in patients with atrial fibrillation and venous thromboembolic disease, including deep vein thrombosis (Fuster et al., 2005; Tapson, 2008; Waldo, 2008). The blood supply blocks towards heart may causes heart attacks and if the blood supply reduces towards the brain that may causes brain stroke, memory impairment, paralysis and behavioural dysfunctions. The development and propagation of a thrombus depend on the presence of abnormalities in blood flow, the blood vessel wall and blood clotting components (Virchow, 1856) such as hypercoagubility may occurs due to malignancy, pregnancy and peri-partum period, oestrogen therapy, trauma or surgery of lower extremity, hip, abdomen or pelvis, inflammatory bowel disease, nephritic syndrome, sepsis and thrombophilia. The vascular wall injury may be due to trauma or surgery, venepuncture, chemical irritation, heart valve disease or replacement, atherosclerosis and indwelling catheters. The stasis may be due to atrial fibrillation, left ventricular dysfunction, immobility or paralysis, venous insufficiency or varicose veins, venous obstruction from tumour and obesity or pregnancy (Turpie, 2002). The conditions causing hypercoagulation, vascular wall injury and stasis are related to free radical generation (Akiba et al., 1998).

Antioxidants are essential for good health and are found naturally in wide variety of foods and plants including vegetables and fruits. More than 175 medicinal plants are listed to have antioxidant property (Veersham and Asres, 2005). They protecting against oxidative damage of cells by act as radical scavengers, hydrogen donors, enzyme inhibitors, singlet oxygen quenchers, synergist and metal chelating agents. They play a vital role in controlling numerous pathophysiological conditions in human being. Oxygen is essential for aerobic life process. It is used by human body cells to break down carbohydrates, proteins and fats that give energy. However, cells under aerobic conditions are threatened with the insult of reactive oxygen molecules that are efficiently taken care of by the powerful antioxidant system in human body (Rice-Evans and Diplock, 1993). Atoms or group of atoms that have at least one unpaired electron, which make them highly reactive, are known as free radicals. Free radicals are the by-products, produced by metabolically active cells, fried foods, cigarette smoke, air and water pollution as well as toxins also creates free radicals. Excess free radicals (oxidative stress) implicated in the etiopathogenesis of a variety of human diseases (Treitinger et al., 2000; Beck, 2000). Free radical attacks DNA and blood vessels cause cancer, cardiovascular diseases and stimulate platelet aggregation that initiates thrombosis (Akiba et al., 1998). They are also involved in arthritis, stroke, cataracts and degenerative problems such as diabetes (Lipinski, 2001), Alzheimer's disease, retinal degeneration, ischemic degeneration and aging (Barja, 2004). It is clear from above studies free radicals or oxidative stress implicated in causation and progression of various diseases including thrombosis (Havel and Rapaport, 1995).

Thrombin is a serine amino acid protease that cleaves peptide bonds in selective (Bode and Stubbs, 1993) substrate including fibrinogen, factor-V, VIII & XIII. Thrombin also participates in platelet,

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endothelial cell & leukocyte activation (Becker, 2005). Beside this it acts as a catalyst for converting fibrinogen to fibrin, which subsequently cross-links to from the mesh that creates a thrombus (Gurm and Bhatt, 2005). Thrombin surface possess two positively charged regions named exosites that play key roles in the specificity of thrombin towards macromolecules substrate such as fibrinogen, cofactors and some inhibitors such as hirudin peptide or heparin. Therefore the pivotal role of thrombin in the pathogenesis of these diseases makes this enzyme the main target for antithrombotic agents. Antithrombin is one of the most important physiological inhibitors of serine proteases involved in blood coagulation. While unfractionated heparin remains the most commonly used antithrombotic agent. Vitamin-K is a fat-soluble vitamin which occurs naturally in plants as phylloquinone (vitamin K1) and is produced by gram-negative bacteria in the human gastrointestinal tract as menaquinone (vitamin K2). This vitamin was found to be essential for normal functioning of hemostasis. In addition, a number of clinical conditions in which vitamin K deficiency was found, include hemorrhagic disease of the newborn, obstructive jaundice, and malabsorption syndromes. A variety of thrombogenic conditions such as atrial fibrillation, deep vein thrombosis, pulmonary embolism, and prosthetic cardiac valves, Can be treated with a potent anticoagulant warfarin which is a vitamin K antagonist. The wide use of this narrow therapeutic index drug has resulted in significant risk for major bleeding. Vitamin K serves as one of the major reversing agent for patients over-anticoagulated with warfarin (Merli and Fink, 2008). Heparin is a highly sulfated glycosaminoglycan, is widely used as an injectable anticoagulant, and has the highest negative charge density of any known biological molecule. It is derived from endothelial cells and stored in mast cells and released only into the vasculature at sites of tissue injury. Heparin and its low-molecular-weight derivatives are effective at preventing thrombosis. Heparin binds to the enzyme inhibitor antithrombin III (AT), causing a conformational change that result in its activation. The activated AT then inactivates thrombin and other proteases involved in blood clotting (Ahuja et al., 1946; Nahas et al., 1975; Bjork and Lindahl, 1982). It is exhibited from the earlier studies that vitamin-K & heparin play a significant role in blood haemostasis.

Natural substances are served as the source of traditional and the modern drugs and also medicinal agents. Most of these entities are derived from the higher plants. The Ocimum sanctum, Vasaka, Glycyrrhiza glabra, etc. are some of the antioxidant plants that have clinically documented to be effective in thrombosis. Bacopa monniera is documented as antioxidant memory enhancing agent. Brahmi is a small creeper, commonly known as brahmi or jalanimba (Kirtikar and Basu, 2001), distributed mainly in warm parts of the world. Phytochemical studies have shown that Bacopa monniera contains many active constituents including alkaloids, saponins, brahmi A - F and nicotine; brahmi A and B being the major constituents (Bose and Bose, 1931; Basu and Pabari, 1947; Chopra et al., 1956; Chopra, 1958; Malhotra and Das, 1959; Bone, 1996). The plant is used in the indigenous system of medicine for the treatment of cardiac, respiratory (Chatterji et al., 1963) and neuropharmacological disorders like insomnia, insanity, depression (Chatterji et al., 1965), anxiety (Basu et al., 1967; Garia et al., 1996a), psychosis, epilepsy (Garia et al., 1996b) and stress (Rastogi and Dhar, 1960; Shashi et al., 2000). It also possesses, anti-inflammatory, analgesic (Singh and Singh, 1980), antipyretic (Kapoor, 1990), spasmolytic, antirheumatic, anticancer, antiulcer (Mahato et al., 2000; Hou et al., 2002; Chakravarty et al., 2003), astringent, bitter, cooling and anti-diarrhoeal properties (Chakravarty et al., 2001). An alcoholic extract of the plant was found to improve learning and memory in several test models (Sairam et al., 2001; Cerevenka and Jabodar, 2006; Holcomb, 2006) and to some extent corrected the abnormal behavior (Goel et al., 2003). Bacopa monniera improves performance, information processing, learning and acquisition in animals and humans (Ganguly and Malhotra, 1967; Holcomb et al., 2006). Brahmi prevents the rate of depletion of acetylcholine level in aged humans population (Kulkarni, 2000; Channa et al., 2003) by inducing choline acetylase activity in the frontal cortex and hippocampus (Samiulla et al., 2001). There are reports indicating that bacopa monniera increased level of antioxidant enzymes such as superoxide dismutase, catalase amylase, glutathione peroxidase (Shashi et al., 2000) and lipid peroxidase. It is also reported that bacopa monniera enhanced the concentration of glutamate, serotonin, protein kinase-c and protein synthesis, especially in brain cells and decreased nor-epinephrine concentration. Therefore, it appeared worth to evaluate pharmacological basis for antithrombotic activity of antioxidants of plant origin. The present study has been designed to investigate the effect of ethanolic extract of bacopa monniera on thrombosis in mice using bleeding time methods.

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Bacopa monniera plant with flowers

Materials and Methods

Swiss albino mice (30-40gm) of either sex, kept in an animal house provided with 12 hours 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 (10ml/kg), distilled water (10ml/kg) and ethanolic extract of brahmi (30mg/kg) were administered intraperitoneally (i.p.). The vitamin-K (10mg/kg, i.p.) and heparin sodium (100 IU/kg, i.v.) were administered for 5 & 29 consecutive days and on 6^{th} & 30^{th} day, 30 min before the determination of bleeding time λmax. The bleeding time in mice (n=6) by filter paper method, in seconds and λmax 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. (1979) to assess the bleeding time in comparison to heparin and vitamin-K. Mice were anaesthetized by sodium pentobarbital (70mg/kg, i.p.) and placed on a pad at room temperature (Baumgartner et al., 2010). Bleeding time in mice was measured by two ways: via filter paper method and λmax 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 (Dottl and Ripke, 1936; Dejana et al., 1979). If bleeding continued after 20 min, bleeding was stopped by cauterization to prevent hypovolemic shock.

Lambda max (λmax) 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

Drugs and chemicals

All the drug solutions were freshly prepared before use. The ethanolic extract of brahmi (Nivaran Herbal Pvt. Ltd. Chennai, India), Vitamin-K (Samarth Life Sciences Pvt Ltd., Ram Mandir Road, Goregaon (W), Mumbai-400104) and heparin sodium (Bioloogicals E. Limited, at Rampur Ghat Road, Paonta Sahib, Dist. Sirmour, Himachal Pradesh-173025) were purchased from market.

Statistical analysis

The tests were used for determining whether there were significant differences in effects between mice that received plant extracts, vitamin-K, heparin & their combinations and vehicle (normal saline or distilled water) treated control groups. 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 Inc (Walonick, 2010).

Results

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

Mice of group-I & group-II were treated with normal saline (10ml/kg, i.p.) and distilled water (10ml/kg, i.p.) respectively. Distilled water produced no marked effect on bleeding time and λmax as compared to normal saline treated control mice (Figure-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.

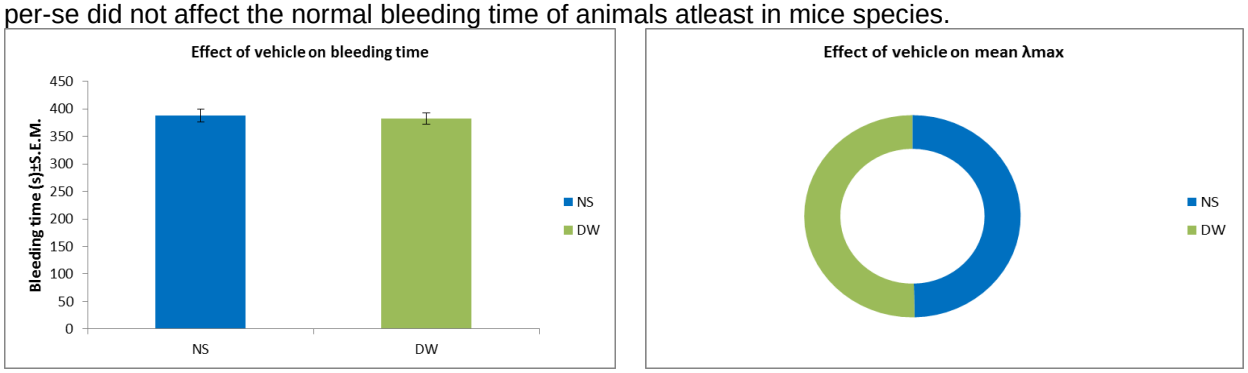

Figure-1: Effect of vehicle on thrombosis

NS represents normal saline (10ml/kg, i.p.) and DW represents distilled water (10ml/kg, i.p.) administered for 5 consecutive days and 30 min before the determination of bleeding time and λmax on 6th day. (Green represents mean value of bleeding time (s) and λmax recorded on day 6th Vs mean value of bleeding time (s) and λmax in normal saline (blue) treated control group).

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

Mice of group-III, IV & V were treated with vitamin-K (10mg/kg, i.p.), heparin sodium (100 IU/ kg, i.v.) and vitamin-K (10mg/kg, i.p.)+heparin sodium (100 IU/kg, i.v.) respectively. The vitamin-K per-se significantly reduced bleeding time and mean λmax as compared to control groups animals, but heparin per-se significantly enhanced bleeding time & λmax as compared to control groups mice. The vitamin-K as and when given in combination with heparin significantly decreased the bleeding time and mean λmax in the animals previously treated with heparin sodium (100 IU/kg, i.v.) as compared to per-se effect of heparin sodium (Figure-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.

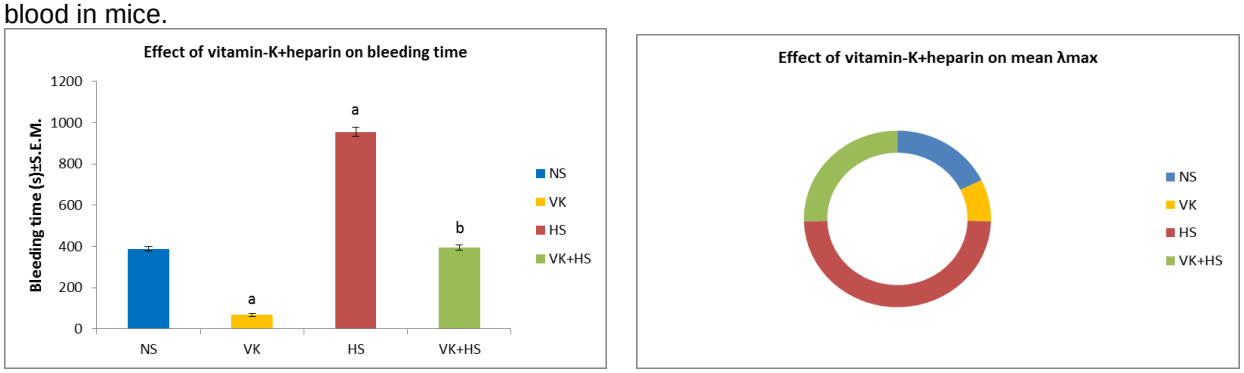

Figure-2: Effect of vitamin-K, heparin & vitamin-K+heparin on thrombosis

NS represents normal saline (10ml/kg, i.p.) VK represents vitamin-K (10mg/kg, i.p.), HS represents heparin sodium (100 IU/kg, i.v.) and VK+HS represent vitamin-K (10mg/kg, i.p.)+heparin sodium (100 IU/kg, i.v.) administered for 5 consecutive days and 30 min before the determination of bleeding time and λmax on 6th day. (Green represents mean value of bleeding time (s) and λmax recorded on day 6th. b=P<0.05 Vs mean value of bleeding time (s) and λmax in vitamin-K (orange) treated groups. a=P<0.05 Vs mean value of bleeding time (s) and λmax in normal saline (blue) treated control group).

Effect of ethanolic extract of Bacopa monniera (brahmi) on thrombosis (Groups-VI-XII)

Effect of ethanolic extract of brahmi on thrombosis: The ethanolic extract of brahmi (10mg and 20mg/kg, i.p.) did not produce any significant effect on thrombosis in mice of groups-VI-VII, but brahmi (30mg/kg, i.p.) significantly enhanced the bleeding time and mean λmax in mice of group-VIII-IX, as compared to normal saline (10ml/kg, i.p.) treated control mice (Figure-3). Results suggest that ethanolic extract of brahmi enhances blood flow from the tail of mice in dose dependent manner, because ethanolic extract of brahmi in dose of 10mg and 20mg/kg, i.p. raise blood flow as compared to normal saline but not

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in significant manner. Only 30mg/kg, i.p. dose of ethanolic extract of brahmi significantly enhanced blood flow in mice or increased the bleeding time.

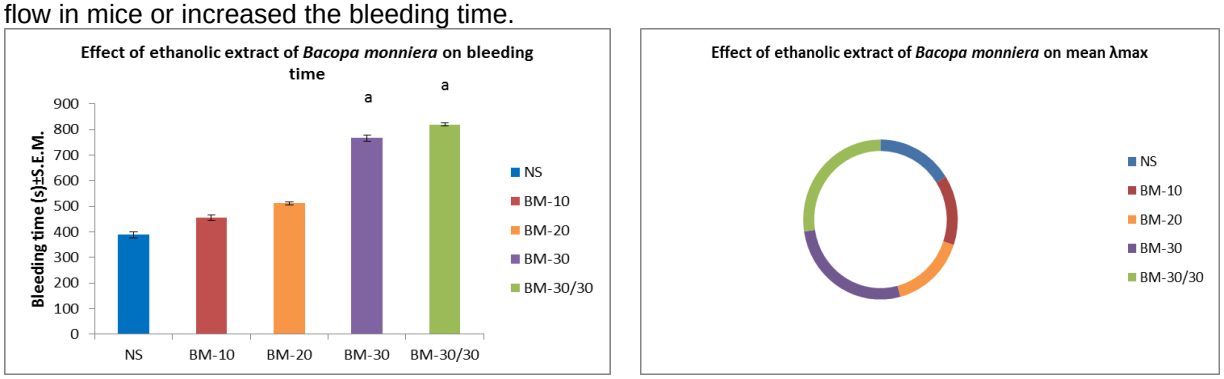

Figure-3: Effect of ethanolic extract of Bacopa monniera (brahmi) on thrombosis

NS represents normal saline (10ml/kg, i.p.) and BM-10, BM-20, BM-30 represents ethanolic extract of Bacopa monniera 10mg, 20mg & 30mg/kg respectively, administered intraperitoneally for 5 consecutive days and 30 min before the determination of bleeding time and λmax on 6th day. BM-30/30 represents ethanolic extract of Bacopa monniera 30mg/kg for 30 days administered intraperitoneally for 29 consecutive days and 30 min before the determination of bleeding time and λmax on 30th day. (Purple, orange & red represents mean values of bleeding time (s) and λmax recorded on day 6th and green represents mean value of bleeding time (s) and λmax recorded on day 30th. a=P<0.05 Vs mean value of bleeding time (s) and λmax in normal saline (blue) treated control group).

Effect of ethanolic extract of brahmi+vitamin-K on thrombosis: The treatment of ethanolic extract of brahmi (30mg/kg, i.p.), significantly enhanced the bleeding time and mean λmax in mice of group-X, previously treated with vitamin-K (10mg/kg, i.p.) (Figure-4). It indicates that ethanolic extract of brahmi attenuated vitamin-K induced reduction in blood flow from the tail vein of mice. This suggests that brahmi interferes with coagulating factors which are introduced by vitamin-K.

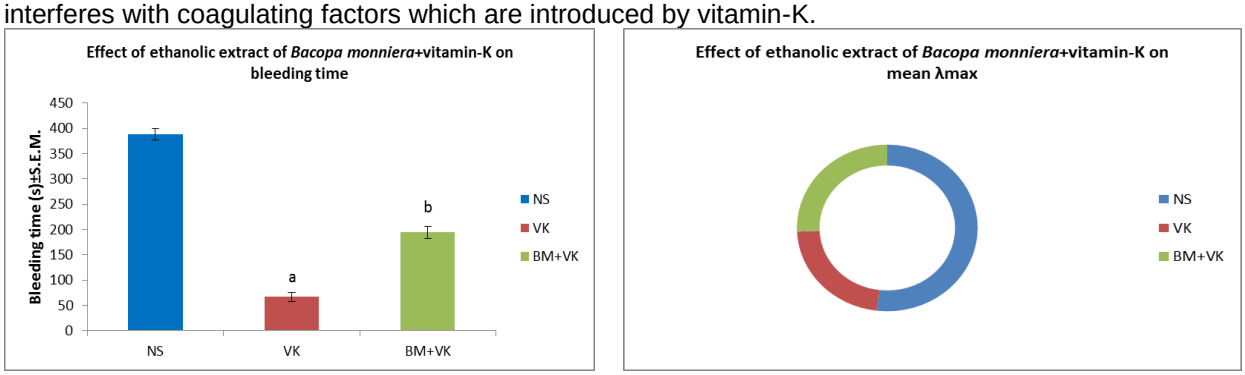

Figure-4: Effect of ethanolic extract of Bacopa monniera+vitamin-K on thrombosis

NS represents normal saline (10ml/kg, i.p.), VK represents vitamin-K (10mg/kg, i.p.) and BM+VK represents ethanolic extract of Bacopa monniera (30mg/kg, i.p)+vitamin-K (10mg/kg, i.p.) administered for 5 consecutive days and 30 min before the determination of bleeding time and λmax on 6th day. (Green represents mean value of bleeding time (s) and λmax recorded on day 6th. b=P<0.05 Vs mean value of bleeding time (s) and λmax in vitamin-K (red) treated group. a=P<0.05 Vs mean value of bleeding time (s) and λmax in normal saline (blue) treated control group).

Effect of ethanolic extract of brahmi+heparin on thrombosis: The mice of group-XI treated with ethanolic extract of brahmi (30mg/kg, i.p.) did not produce any significant effect on bleeding time and mean λmax in mice that were previously treated with heparin sodium (100 IU/kg, i.v.), as compared to per-se effect of heparin (Figure-5). This suggests that ethanolic extract of brahmi did not suppressed coagulating factors as heparin sodium when given in combination.

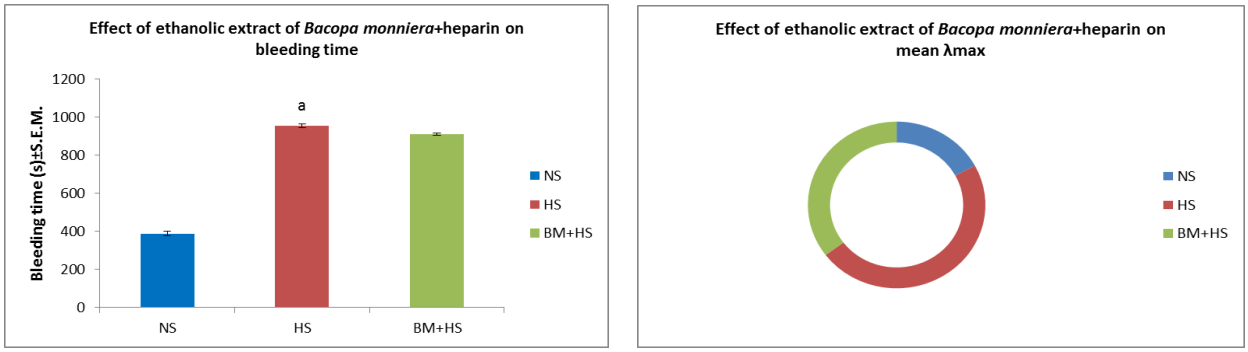

Figure-5: Effect of ethanolic extract of Bacopa monniera+heparin on thrombosis

NS represents normal saline (10ml/kg, i.p.), HS represents heparin sodium (100 IU/kg, i.v.) and BM+HS represents ethanolic extract of Bacopa monniera (30mg/kg, i.p)+heparin sodium (100 IU/kg, i.v.) administered for 5 consecutive days and 30 min before the determination of bleeding time and λmax on 6th day. (Green represents mean value of bleeding time (s) and λmax recorded on day 6th. Vs mean value of bleeding time (s) and λmax in heparin (red) treated group. a=P<0.05 Vs mean value of bleeding time (s) and λmax in normal saline (blue) treated control group).

Effect of ethanolic extract of brahmi+vitamin-K+heparin on thrombosis: The ethanolic extract of brahmi (30mg/kg, i.p.) enhanced the bleeding time and mean λmax in mice of group-XII, previously treated with vitamin-K (10mg/kg, i.p.) and heparin sodium (100 IU/kg, i.v.) (Figure-6). It exhibits that ethanolic extract of brahmi potentiate the effect of heparin sodium and also reduces clotting effect of vitamin-K in mice.

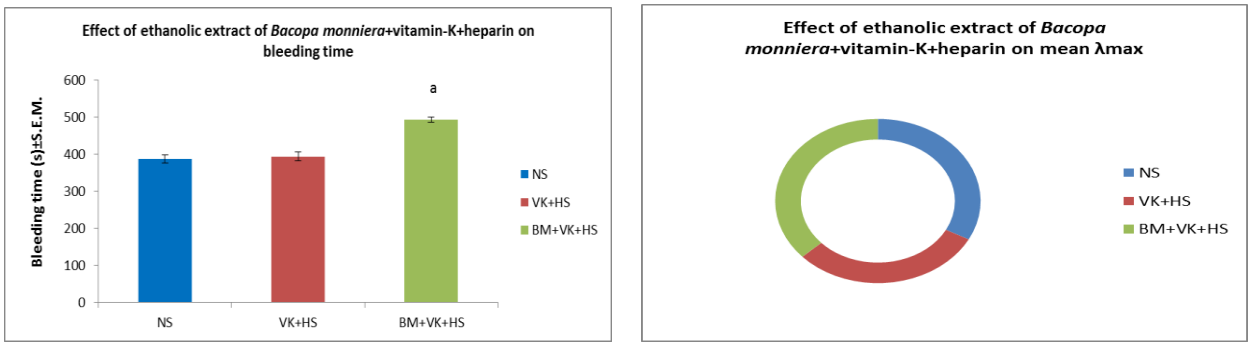

Figure-6: Effect of ethanolic extract of Bacopa monniera+vitamin-K+heparin on thrombosis

NS represents normal saline (10ml/kg, i.p.), VK+HS represents vitamin-K (10mg/kg, i.p.)+heparin sodium (100 IU/kg, i.v.) and BM+VK+HS represents ethanolic extract of Bacopa monniera (30mg/kg, i.p)+vitamin-K (10mg/kg, i.p.)+heparin sodium (100 IU/kg, i.v.) administered for 5 consecutive days and 30 min before the determination of bleeding time and λmax on 6th day. (Green represents mean value of bleeding time (s) and λmax recorded on day 6th. a=P<0.05 Vs mean value of bleeding time (s) and λmax in normal saline (blue) and vitamin-K+heparin (red) treated groups).

Discussion

Thrombosis is responsible for many cardiac problems including unstable angina, myocardial infarction, postangioplasty occlusion and stroke. To form a thrombus, three steps take place, (i) Exposure of the circulating blood to a thrombogenic surface, such as damaged vascular endothelium (ii) sequence of platelet related events, involving first platelet adhesion, aggregation and release of agents further promoting aggregation and causing vasoconstriction and (iii) activation of the clotting mechanism plays important role in the formation of fibrin takes place. 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 atherosclerosis (Roberts 1995). 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 agents (Joseph, 2003). Platelet

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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. Hence, an attempt has been made to evaluate antithrombotic effect of ethanolic extract of bacopa monniera (Brahmi) on thrombosis in mice, using bleeding time method.

The vitamin-K reduces the bleeding time and mean λmax 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 enhance the coagubility of blood (Dowd et al., 1995). Heparin sodium enhanced the bleeding time and mean λmax in mice by reducing the coagubility of blood. The present data supported by earlier reports which states that the heparin binds to enzyme inhibitor antithrombin-III and inactivates thrombin & other proteases involved in blood clotting. It is also reported by many scientists that heparin and its low molecular weight derivatives prevent thrombosis (Ahuja et al., 1946; Nahas et al., 1975; Bjork and Lindahl, 1982; Dvorak et al., 2010).

The ethanolic extract of Bacopa monniera significantly prevented thrombosis as these extracts enhanced the bleeding time and mean λmax. The extract of brahmi potentiated the anti-coagulating action of heparin sodium and suppressed the coagulating effect of vitamin-K as and when given in combination with these agents. This is possible only either by suppressing the various proteases enzymes involved in blood clotting or by reducing the level of prothrombin & other coagulating factors. Present results are in favour of the earlier studies with extracts or products of numerous plants possessing antioxidant properties which prevents clotting & reducing the risks of thrombosis (Sinha et al., 2002; Voko et al., 2003; Di-santo et al., 2003; Dar and Tabassum, 2012). The ethanolic extract of Bacopa monniera (Brahmi) when administered intraperitoneally, produced dose dependent antithrombotic activity in mice. Ethanolic extract of Bacopa monniera in the dose 30 mg/kg showed increased bleeding time & mean λmax as compared to vehicles (normal saline and distilled water) treated control groups of mice. Bacopa monniera, a natural antioxidant, possibly attenuated the platelet aggregation or formation of thrombi by suppressing lipid peroxidation activating acetyl hydroxylase, an enzyme present within plasma lipoproteins that rapidly destroys platelet activating factor (Kishore and Singh, 2005). The present hypothesis is supported by the previous research that reveals oxidants or free radicals and certain biochemical agents like adenosine diphosphate, adrenaline and collagen induced platelet aggregation or formation of thrombi by promoting PAF activity and lipid peroxidation (Halliwell and Gutteridge, 1989). Shilajit a herbo-mineral antioxidant significantly attenuated the oxidants induced platelet aggregation or thrombus formation in human (Ghosal, 2001). The present results are also supported by various earlier studies which concluded that numerous flavonoids compounds have antioxidant properties, reducing thrombotic tendencies by inhibiting platelet aggregation (Knekt et al., 1996). It is also reported that the phytochemical analyses of Bacopa monniera were found to possess antioxidant potential. The chloroform extract of Bacopa monniera also have clot lytic properties in different blood samples (Das and Rahman, 2014).

Conclusion

In in-vivo antithrombotic screening studies done in mice, showed that ethanolic extract of Bacopa monniera possessed good antithrombotic activity in per-se as well as in presence of heparin sodium (an anticoagulating agent). The ethanolic extract of brahmi also reversed vitamin-K (a coagulating agent) induced blood viscosity. Therefore, the extract of Bacopa monniera 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 Bacopa monniera should be thoroughly investigated phytochemically and pharmacologically to exploit its medicinal and pharmaceutical potentialities.

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