

ANTITHROMBIC ACTIVITY OF *CRATAEGUS OXYCANTHA* LINN. MOTHER TINCTURE IN ALBINO MICE

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ABSTRACT

Antithrombic activity of *Crataegus oxycantha* Linn. mother tincture was studied in adult albino mice. Paralysis and death of an animal observed after intravenous injection of collagen + epinephrine mixture was considered as an index of thrombosis which was later confirmed microscopically by observing the aggregation of disintegrated platelets along with white blood corpuscles and red blood corpuscles in the blood capillaries of heart and lungs tissues. On the contrary, the ability of *Crataegus oxycantha* Linn. mother tincture to protect the mice from the paralytic or lethal effect of platelet aggregating agent was considered as an index of antithrombic activity of the drug. The present result showed that *Crataegus oxycantha* Linn. mother tincture when administered in doses of 0.05ml/mouse twice daily for three days before the intravenous injection of collagen + epinephrine mixture (0.1 ml/mouse) had considerable protective effect (55.6%) as compared to that observed in both the control groups.

INTRODUCTION

Crataegus oxycantha Linn. commonly known as "Hawthorn or hedge thorn" is considered as one of the most beautiful spring flowering shrub and has been a well loved plant throughout the ages. To the Greeks, it symbolized hope and the flowering branches decorated weddings. "A well-known physician, the late Dr. Green, of Ennis, County Clare, Ireland, attained an extended reputation in the treatment of heart disease, but kept the remedy a secret. Upon his death in 1894, his daughter revealed the famous cure to be a tincture of the ripe berries of *Crataegus oxycantha*". It is found in most herbal preparations for heart weakness, irregular heart beats, hardening of the arteries, artery spasms, and angina. The increase in coronary blood flow is attributed to the flavonoid components while procyclins apparently slow the heart¹.

The aim of this study was to investigate antithrombic activity of *Crataegus oxycantha* Linn. mother tincture in

experimentally induced thrombosis in albino mice in order to support its homoeopathic clinical use in aneurysm and thromboembolism².

MATERIALS AND METHODS

Animals

Albino mice of either sex, weighing between 25 -35 g. were purchased from Central Animal House, Banaras Hindu University, Varanasi and were kept in the departmental animal house for about 2 weeks in order to acclimatize to the environmental conditions (10h darkness, 14 h light). They were fed Gold Mohur Pallet diet (Lipton India Ltd.) and water *ad-libitum*.

Platelets aggregating mixture

Platelets aggregating mixture containing collagen (150g) and epinephrine (1.80g) (Sigma) in 0.1 ml of 0.154 M sodium chloride solution. The mixture was always prepared fresh before use on the day of experiment, mixed well and kept at 21°C before injecting into the mice.

Drug

Crataegus oxycantha Linn. mother tincture containing 60% alcohol v/v (M. Bhattacharya & Co, Varanasi).

Experimental

Antithrombic activity of *Crataegus oxycantha* Linn. mother tincture was evaluated in adult albino mice according to the method of DiMinno and Silver³. Sixty two adult albino mice were divided into three groups. The mice of first two groups served as controls and given distilled water and alcohol respectively. The mice of third group were administered *Crataegus oxycantha* Linn. mother tincture, in doses of 0.05 ml/ mouse orally twice daily for 3 days and once in the morning on fourth day. Two groups of Mice kept under control received equivalent volume of distilled water and/or alcohol (60% v/v) respectively. One hour later, mice of all the groups were injected freshly

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prepared aggregating mixture in doses of 0.1 ml/mouse intravenously through the tail vein via 27 gauge needle. Thereafter, animals were observed for the gross effects of aggregating mixture on the central nervous system and on the behaviour, until, paralysis or death of an animal supervened within 15 min of the platelet aggregating challenge which was considered as an end point of thromboembolic activity. The ability of *Crataegus oxycantha* Linn. mother tincture to protect the mice from the paralytic or lethal effect of the platelet aggregating agent was considered as an antithrombic activity of the drug. Later on, all the animals (both dead and alive) were sacrificed, their heart and lungs were removed, examined for gross morphological changes and preserved in 10% formalin for histological study.

Histological examination of the lungs and heart were performed to determine whether vessels of the microcirculation of these organs were occluded by thrombi consisting of platelet aggregates formed in response to the collagen-epinephrine injection. The preserved tissue was dehydrated gradually by passing through different grades of alcohol and subsequently hardened first in benzene and then in xylene. Later, paraffin blocks of processed tissues were prepared. The tissue sections of desired thickness

(5mm) were cut on a rotary microtome, mounted on a microscopic slides, and stained with haemotoxylin and eosin for histological examination.

Statistical analysis

Total number of mice in each group responded to platelet aggregating agent were noted and the results were analysed by "chi-square" test. The level of significance of difference between control and drug treated groups were calculated at P value < 0.05.

RESULTS

The data for antithrombic activity of *Crataegus oxycantha* Linn. mother tincture in experimentally induced thrombosis in mice by platelet aggregating agent (collagen + epinephrine mixture) are presented in Table I

Most, if not all, mice of control distilled water group, soon after intravenous injection of platelet aggregating agent, jumped on the table, developed large protruding eyes, became immobile and began to gasp for breath. This was followed by shallow but rapid respiration. About 40% of mice died within 2-5 min of injection. Alive mice remained

Table 1

Antithrombic activity of *Crataegus oxycantha* Linn. Mother Tincture in Albino Mice

Groups	Treatment	Volume of each dose admin. (ml/mouse)	Total number of doses admin. @	Number of mice challenged with aggregating	Number of mice not responded to aggregating agent	Number of mice responded to aggregating agent challenge	Number of mice showed sluggish movement	Number of mice showed paralysis or death	Percent protection Total mice responded
I	Distilled Water	0.05	7	17	2	4	11	15	11.8
II	Alcohol (60% V/V)	0.05	7	17	3	6	8	14	17.6*
III	<i>Crataegus Oxycantha</i> mother tincture	0.05	7	18	10	5	3	8	55.6**

* Not significant from control I,

** Significant from both controls (I and II) (P value < 0.05).

@ The doses were administered twice daily for 3 days and once in the morning of the 4th day.

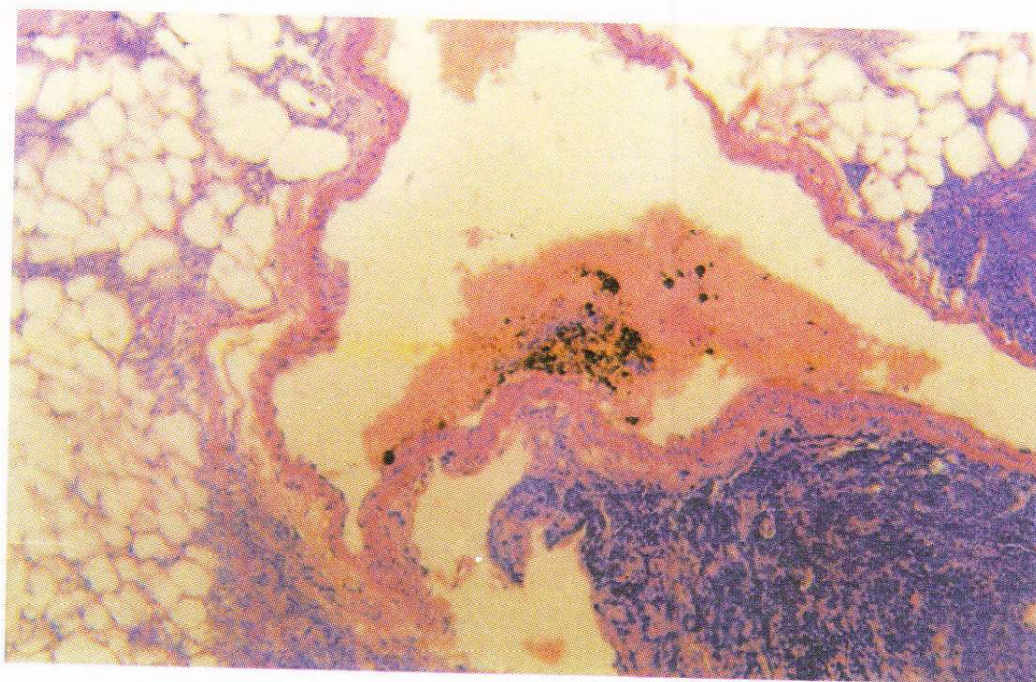


Fig. 1. Photograph (x 100) showing aggregation of disintegrated platelets along with white and red blood corpuscles in the lumen of the blood capillaries of lung parenchyma after intravenous injection of collagen+epinephrine mixture in control adult mice

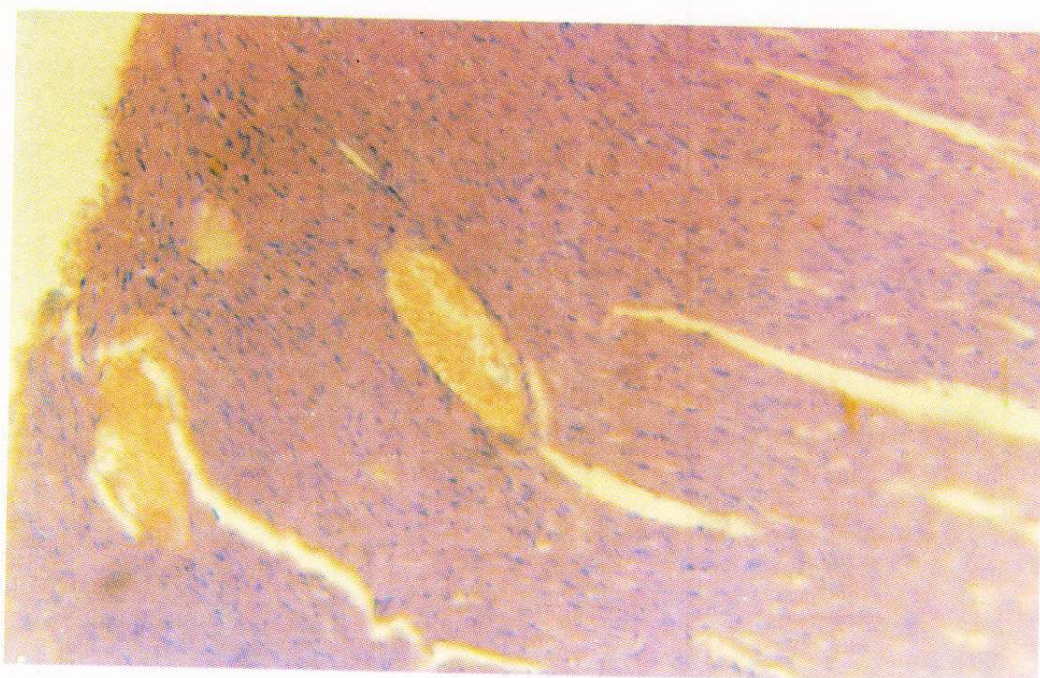


Fig. 2. Photograph (x 100) showing aggregation of disintegrated platelets along with white and red blood corpuscles in the lumen of the blood capillaries of heart tissue after intravenous injection of collagen+epinephrine mixture in control adult mice

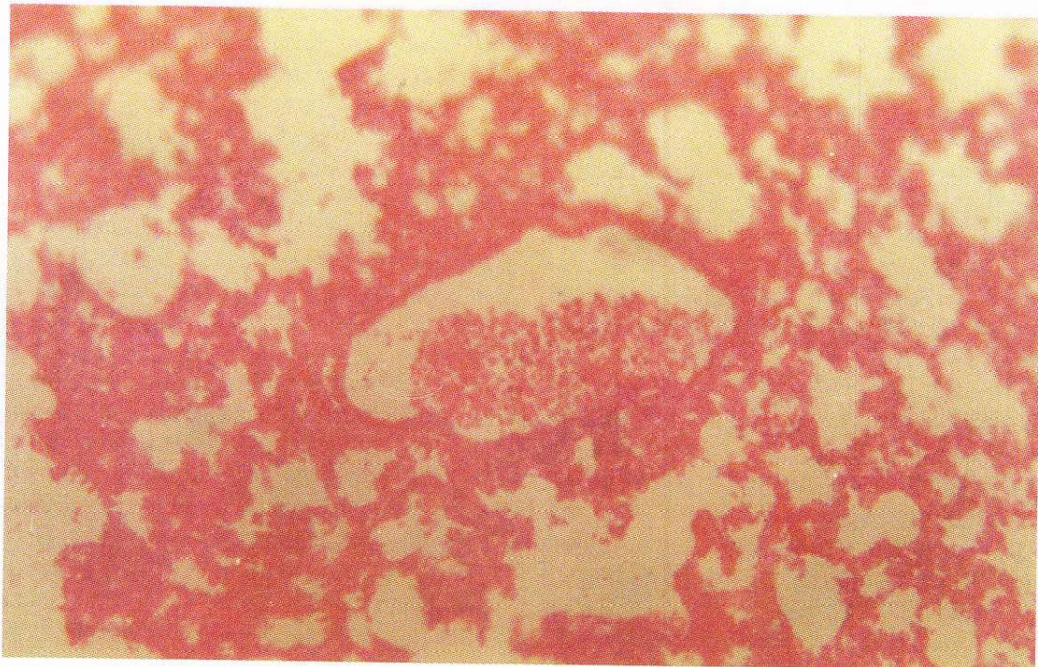


Fig. 3. Photograph (x 100) showing intact platelets, and white and red blood corpuscles in the lumen of the blood capillaries of lung parenchyma in *Crataegus oxycantha* Linn. mother tincture pretreated adult mice after intravenous injection of collagen+epinephrine mixture

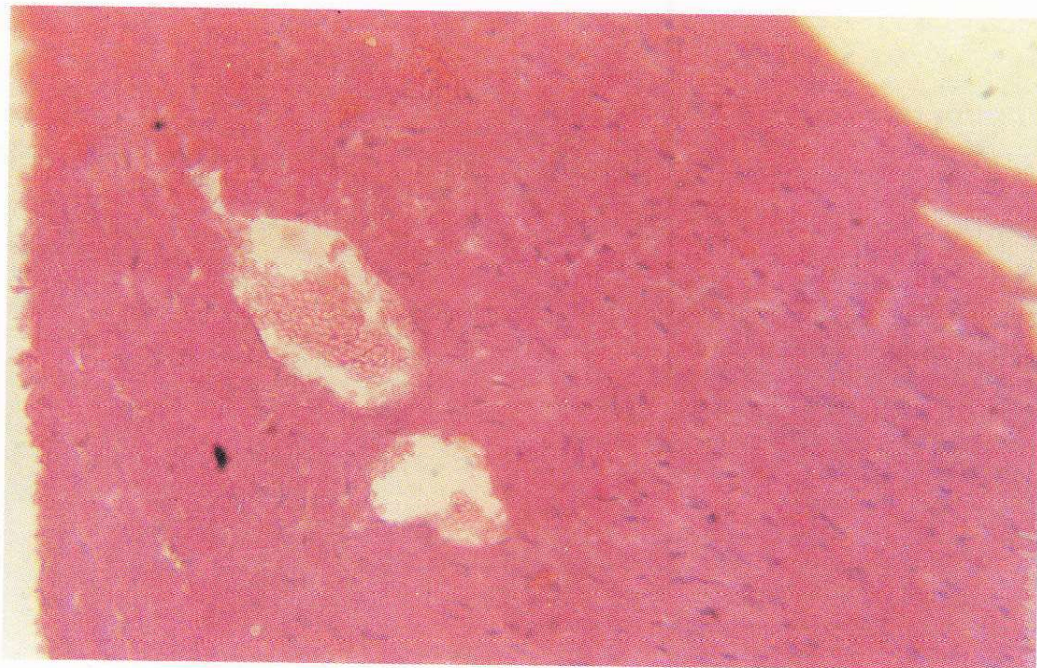


Fig. 4. Photograph (x 100) showing intact platelets, and white and red blood corpuscles in the lumen of the blood capillaries of heart tissue in *Crataegus oxycantha* Linn. mother tincture pretreated adult mice after intravenous injection of collagen+epinephrine mixture

immobile with rapid shallow respiration for at least 20 min after the injection. Likewise, mice of alcoholic group (second control) behaved very similar to that of distilled water group except that the mortality was reduced to 18%. On the contrary, among 18 mice pretreated with *Crataegus oxycantha* Linn. mother tincture for 3 days, only two mice died, one paralysed and 5 mice showed sluggish movements after intravenous injection of platelet aggregating agent. Remaining 10 mice behaved normal indicating that *Crataegus oxycantha* Linn. mother tincture had considerable protective effect (55.6%) against the observed response to the platelet aggregating agent in both the control groups.

Morphological studies of lungs and heart revealed marked congestion and infiltration of both the organs in control groups challenged with platelet aggregating agent, while, such changes were not observed in *Crataegus oxycantha* Linn. mother tincture pretreated animals. Histological studies of the lungs (Fig. 1) showed marked aggregation of disintegrated platelets along with white blood corpuscles and red blood corpuscles in the blood capillaries of lung parenchyma in both control groups challenged with platelet aggregating agent. Similar effect was also observed in blood capillaries of the heart tissue, but to a lesser degree in these animals (Fig. 2). On the other hand, animals protected from the paralytic effect of platelet aggregating agent in *Crataegus oxycantha* Linn. mother tincture pretreated group, did not reveal any aggregation of platelets (Figs. 3 and 4), and only an insignificant platelets aggregation in those animals who responded to aggregating challenge in this group, in the blood capillaries of lung parenchyma and of heart tissue.

DISCUSSION

Crataegus oxycantha Linn. is a folk remedy for arteriosclerosis, dropsy, dyspnoea, hypertension, nephrosis and sore throat⁴. The Wealth of India describes the fruit extract as a cardiac tonic, used for organic and functional heart diseases such as dyspnoea, hypertrophy, valvular insufficiency and heart oppression⁵. In animal studies, hawthorn extract has been documented to increase coronary blood flow and reduce peripheral resistance both *in vitro* and *in vivo* studies⁶. The hypotensive activity of hawthorn has been attributed to a vasodilatation action caused by its flavonoid components⁷⁻⁸. In Homoeopathy, the drug has been recommended for chronic heart diseases with extreme weakness, thromboembolic conditions, and in arteriosclerosis and is said to have a solvent power upon crustaceous and calcareous deposits in arteries in the latter disease².

The results of the present study showed that of all the mice of both control groups given intravenous injection of collagen + epinephrine mixture, more than 80% of animals responded immediately. They jumped on the table, developed large protruding eyes, became immobile and began to gasp for breath. Paralysis and death occurred in 47% (in alcoholic group) to 64.7% (in distilled group) of animals within 2-5 min of the injection. On the other hand, of the 18 mice pretreated with *Crataegus oxycantha* Linn. mother tincture, 10 mice showed complete protection as they did not respond and out of the 8 mice responded only two mice died and one paralysed after the intravenous injection of collagen + epinephrine mixture (Table I). Histological studies revealed aggregation of disintegrated platelets along with white blood corpuscles and red blood corpuscles in the blood capillaries of the lung and heart tissues after the intravenous injection of platelet aggregating agent in control animals while animals pretreated with *Crataegus oxycantha* Linn. mother tincture either did not reveal or had very mild aggregation of platelets in the blood capillaries of lungs and heart tissues after such injection, indicating thereby that *Crataegus oxycantha* Linn. mother tincture had considerable protective effect (55.6%) as compared to that observed in control mice.

CONCLUSION

It is concluded that administration of *Crataegus oxycantha* Linn. mother tincture three days before collagen + epinephrine mixture injection, afforded significant protection against the paralytic and lethal effect induced by this mixture in control animals.

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