

Evaluation of Antihypertensive Effect of Fruit Beverage of *Crataegus crenulata* Roxb. : A wild Shrub of Himalayan Hills

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ABSTRACT

Morphological and biochemical studies of *Crataegus crenulata* Syn. *Pyracantha crenulata* fruits have been carried out and an herbal formulation was prepared from its fruit juice. The small sized berries weighing 250 mg each and 4000±32 fruits in 1 kg weight contain 25 per cent fruit juice. Biochemical analysis of fruit juice quantified 0.11±0.015 percent protein, 0.08±0.001 percent calcium, 0.022±0.001 percent magnesium, 0.43±0.003 percent potassium, 73±2 percent moisture, 1.006± 0.04 percent flavonoides, 0.38±0.02 mg/100g Vitamin 'A', 101±9 µg/100g vitamin B₁₂, 1.2±0.03 mg/100g Vitamin 'C', 17.5±1.02 mg/100g Vitamin 'E'. The present study was designed to investigate the efficacy of the herbal formulation from *C. crenulata* fruits in two forms of experimental hypertension: cadmium chloride induced and in normotensive control in animal module. Since the blood pressure falls down independently in the hypertensive and normotensive rats. The study strongly suggested *C. crenulata* possesses anti-hypertensive or hypotensive effects.

Keywords: *Crataegus crenulata*; Hypertension; Anti-hypertensive effect; Flavonoides; Uttarakhand; Ghingaroo

1. INTRODUCTION

Crataegus crenulata Roxb. Syn. *Pyracantha crenulata* (D. Don) M. Roemer, Fam. Rosaceae) is endemic to Himalayan hills ranging from 900 m to 2400 m amsl¹. It is also known as Hawthorn or Himalayan Firethorn which is locally known as *Ghingharoo* in Uttarakhand². This dense bushy shrub (Fig.1) grows widely in abundance in barren, rocky and dry grasslands. These spiny bushes are 5 ft to 15 ft high profusely branched with dark green leaves and are laden with dark red coloured, pulpy berries (Figs. 2 (a) & 2(b)) a pomlet fruit type during the month of July-September. Each berry contains 5 triangular brown coloured seeds covered with hard seed coat. Stem bark is dark brown and turns to glabrous when old. Flowering occurs during the month of April-May. The white coloured inflorescence is a compound corymb having many flowers in it. Flower is hermaphrodite (bisexual) having 20 stamens and 01 ovary. This perennial, deciduous and thorny shrub is commonly known as Himalayan Firethorn.

Hawthorn species (*Crataegus* spp.) are traditionally used for cardioprotective benefits³. Countries like China and few European countries have included *Crataegus* in their Pharmacopia's as herbal drug⁴. Cardiovascular diseases account for the death of about 17 million people per year globally². Changed food habits and modern life styles have further aggravated this problem considerably. Prescribed antihypertensive agents being marketed by the pharma industry have to be taken infinitely and the side effects emerging due to long term use are less documented. The plant based drugs



Figure 1. *Crataegus crenulata* plant with fruits.

on the other hand have the advantage of lesser side effects and low cost. There continues to be a high demand for plant based antihypertensive agents. Flavonoides and oligomeric proanthocyaninidins have been reported as the bioactive constituents of some of the standardised preparations from the different species of *crataegus* for the treatment of mild forms of heart problems^{4,5}.

Conventionally this plant is exploited by the local inhabitants for fencing of agricultural fields and making tool handles. However, no conventional knowledge on medicinal uses of *C. crenulata* is available. Nevertheless, several reports



Figure 2. (a) Fruit bearing branch of *Crataegus crenulata*, (b) Harvested Fruits

on medicinal properties of other species of *Crataegus* are available in the literature^{6,7}. Presence of bio-flavonoides in many species of *Crataegus* is useful in the treatment of disorders of the heart and circulation system especially in case of angina⁸. The fruits of *Crataegus* are also having antispasmodic, diuretic, sedative, vasodilatation properties. The fruits and flowers of *Crataegus* are having hypotensive properties hence are useful in case of high blood pressure⁹.

Owing to its nutraceutical and pharmaceutical attributes, Defence Institute of Bio-Energy Research (DIBER), Haldwani has made a successful attempt in exploitation of this plant species. Himalayan Firethorn berries are very nutritious, having flavonoides, vitamin A, vitamin B₁₂, vitamin C, vitamin E, protein, calcium, magnesium, potassium and oligomeric proantho-cyaninidins¹⁰. Scientific research has shown that this shrub has potential application for treatment of hypertensive patients. Clinical trials on hypertensive heart patients have revealed that the total flavonoides of fruits lowers the blood cholesterol level and showed significant improvement in heart patients¹¹. Similarly *Crataegus* leaves are also found useful for antioxidant, immune-modulatory and anti-inflammatory activities. Thus fruits of this plant seems to be a really good natural heart tonic.

Crataegus has also been identified for environmental point of view in land reclamation in fragile mountain ecosystem. This plant has an extensively developed root, which holds the soil and help in reducing soil erosion and landslides¹². This thorny bush has proven to be beneficial for the farmers as well because it acts as a bio-fence around their agricultural fields and prevents stray and wild animals from damaging it.

Antioxidants present in berries of hawthorn are helpful to reduce the damage from free radicals. During eighteenth century medical system in America recognised this valuable shrub for its benefits in cardiac and respiratory ailments¹³. Clinical studies have also shown that decoction of *Crataegus* fruits have healing effects on the heart muscles and in keeping the blood pressure normal. Besides its hypertensive effects the fruits of hawthorn are used in preparations of direct and mild heart tonic. It is especially indicated in the treatment of weak heart combined with high blood pressure, heart muscles weakened in old age. *Crataegus* leaves were also found beneficial in enhancing memory of the brain cells by increasing their blood supply. Present study was therefore undertaken to validate the antihypertensive potential of fruit beverage of *Crataegus* developed by DIBER.

2. MATERIALS AND METHODS

2.1. Study Material

Mature fruits of *C. crenulata* were harvested from their natural habitat in the Himalayan hills of Uttarakhand. The plant material was identified and authenticated by the Research Unit of DIBER, Pithoragarh. Natural specimens of *C. crenulata* were also submitted in the DIBER herbarium for reference purposes.

2.2. Formulation of the Herbal Beverage from Fruits

For juice (herbal beverage) (Fig. 3) preparation the fully mature harvested fruits used to wash thoroughly with tap water to remove the adhering soil particles. The washed fruits were dipped in sterile distilled water for 30 minutes before extraction of the juice. Equal amount of fruit juice and water (v/v) was taken in a separate container and 70 per cent sugar (70 g sugar +30 ml water) was added to it and heated to just boiling point. After cooling down the beverage Sodium benzoate solution (700 ppm) was mixed to it as a preservative. The beverage so prepared is filled in air tight clear pet bottles and stored in cool place.



Figure 3. *Crataegus* fruit beverage.

2.3. Bio-Chemical Composition

Biochemical composition of *Crataegus* berries was evaluated by estimation of moisture per cent, flavonoids, vitamin A, vitamin B₁₂, vitamin C, vitamin E, protein content, calcium, magnesium, potassium and flavonoides (Table 1). Heavy metals like lead, arsenic, mercury and cadmium were also estimated by standard methods^{14,15}.

2.4 Animals Used for Anti-Hypertensive Study

Wister male rats weighing 200 g - 250 g were used for the antihypertensive study. The rats were housed in standard environmental conditions (Temp 25±1 °C, humidity 60-70 per cent RH, 12 hr light/dark cycle) and fed with rodent diet per oral, intra venous and water ad libitum. The experimental protocol was approved by Institutional animal ethics committee for the purpose of control and supervision of experiments on animals (CPCSEA) Ministry of environment and forest Govt. of India.

Table 1. Biochemical composition of *Crataegus crenulata* fruits

Biochemical constituent	Quantity
Protein	1.8 ± 0.015%
Total Sugar	59.18 ± 1.03%
Calcium	3.08 ± 0.001%
Magnesium	1.4 ± 0.001%
Sodium	1.00 ± 0.2%
Potassium	1.43 ± 0.03%
Vitamin A	0.38 ± 0.02 mg/100g
Vitamin B ₁	0.50 ± 0.04 mg/100g
Vitamin B ₂	17.7 ± 1.10 mg/100g
Vitamin C	55 ± 0.03mg/100g
Vitamin E	272 ± 1.02 mg/100g
Flavonoides	2.9 ± 0.04 %
Total Soluble Solids	84.34 ± 2.1 %

2.4.1. Anti-Hypertensive Activity in Normotensive Rats (Non-Invasive Model)

All the animals were trained for one week to remain in the restrainer for a period of 4-6 hr. per day. The mean blood pressure was measured using LE-5002 Storage Pressure Meter, the indirect blood pressure measuring system (UGO, Basile, Italy) from the tail of pre-warmed un-anaesthetised rats by tail-cuff technique for this purpose all experimental animals were pre-trained. From every animal three readings was recorded. After initial 07 days period, trained groups received different doses of test materials p.o. (0.25,5.0,1.0 and 2.0 ml/ 25 g mouse) for two weeks. The control group received only the vehicle, in a volume corresponding to the test material.

2.4.2. Anti-Hypertensive Activity in Normotensive Rats (Invasive Model)

The rat was anaesthetised by i.p injection of urethane (25 per cent, 0.6 ml/100g body weight). Tracheostomy was performed and polyethylene cannula inserted to allow the rat to have spontaneous breathing. Carotid artery was dissected, vagus nerve separated and carotid artery cannulated and connected to the pressure transducer of multi-channel polygraph (Expermeteria, Hungary) for recording the blood pressure and heart rate. Jugular vein was also cannulated for drug administration. After surgery, 30-40 minutes time was allowed for cardiovascular indices to stabilize. The test doses (25,50 and 100 µl/rat) of formulation were administered i.v and effect on blood pressure and heart was recorded.

2.4.3. Anti-Hypertensive Activity in Cadmium Chloride Induced Hypertension

Hypertension was produced by the chronic administration of CdCl₂ (1 mg/Kg, i.p. for 2 weeks). The rat was anaesthetised by i.p. injection of urethane (25 per cent, 0.6 ml/100g body weight). The surgical procedure as described in 2.5 was followed. The formulation at test dose 25 µl/rat, 50 µl/rat, and 100 µl/rat was administered i.v. The effect on blood pressure and heart was monitored.

3. RESULTS

3.1 Biochemical Composition

Biochemical analysis of *Crataegus* berries is shown in

Table 1. Flavonoids and oligomeric proantho-cyaninidins are the important biologically active compounds occurring in *Crataegus* fruits. Heavy metals like lead, arsenic, mercury and cadmium are present below the permissible limit.

3.2 Anti-Hypertensive Activity

In anti-hypertensive activity studies (normotensive rats, noninvasive model), formulation administered at the doses of 1 ml/rat, 1.5 ml/rat, and 2.0 ml/rat, to conscious rats produced lowering in the blood pressure in a biphasic manner. However, no significant effect on heart rate was observed. (Figs. 4-5)

In case of normotensive rats, (noninvasive model), at all the test doses, the formulation lowered the mean blood pressure up to 5 h. No significant effect on heart rate was observed. Blood pressure was lowered up to 4 hours at the dose of 100 µl/rat in cadmium chloride induced hypertensive rats (Figs. 6-7).

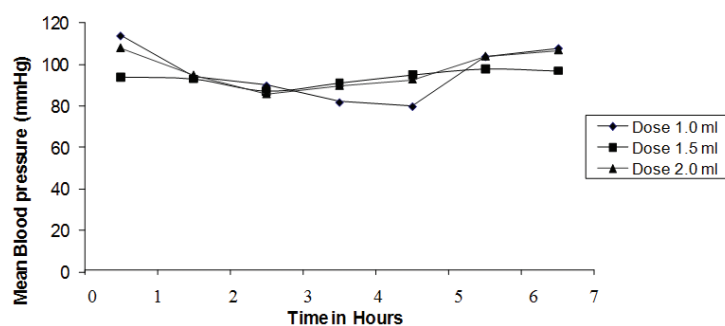


Figure 4. Effect of oral dose of *Crataegus* on mean blood pressure (mmHg) in normotensive conscious rats.

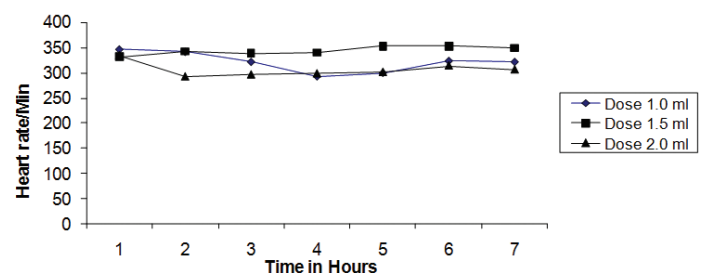


Figure 5. Effect of oral dose of *Crataegus* on heart rate per min of normotensive conscious rats.

4. DISCUSSION

Crataegus is a multipurpose thorny shrub of hills having potent medicinal attributes in every part of the plant found useful in one or the other ailments. By the early 1800s, American doctors recognised the medicinal properties of *Crataegus* and began using it for the treatment of circulatory disorders and respiratory illnesses. Antioxidants present in berries of hawthorn are helpful in reducing the damage from free radicals. Western herbalists consider that the regular use of its fruit juice restores the heart health due to the presence of bio-flavonoides and antioxidants. Present investigation validated the hypotensive properties of *C. crenulata* fruit beverage in animal model. Significant improvement in hypertensive animals was observed in experimental animals which validated the antihypertensive properties present in the fruit beverage of *Crataegus crenulata*.

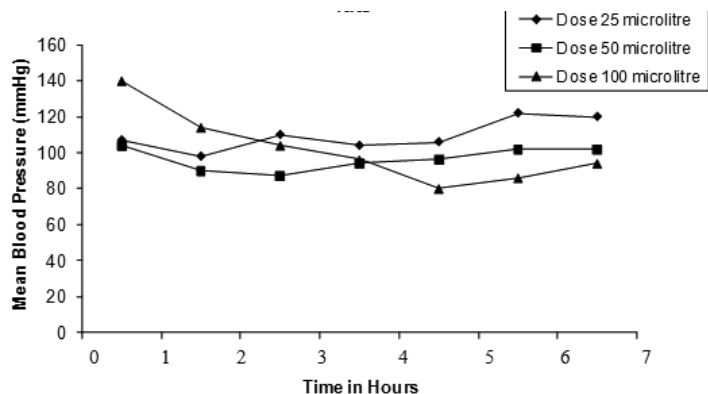


Figure 6. Effect of intravenous dose of *Crataegus* on mean blood pressure (mmHg) in CdCl₂ induced hypertensive rats

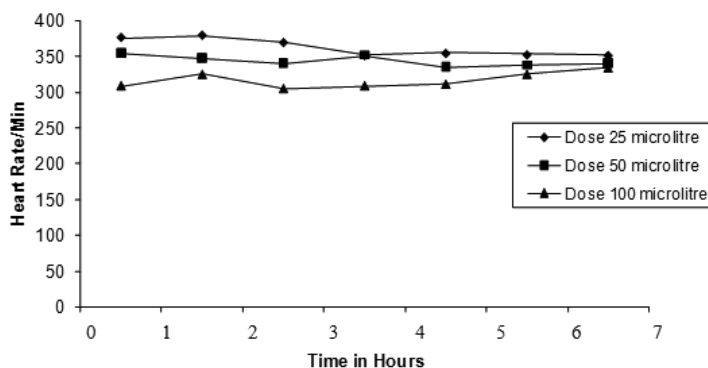


Figure 7. Effect of intravenous dose of *Crataegus* on Heart rate per min in CdCl₂ induced hypertensive rats

Presence of bioflavonoid in *Crataegus* is useful in the treatment of disorders of the heart and circulatory system especially in case of angina¹¹. The fruits are also having antispasmodic, diuretic, sedative and vasodilatation properties¹¹. The fruits and flowers of *Crataegus* are having hypotensive properties hence are useful in case of high blood pressure¹¹.

Crataegus has potential for treatment of hypertension. Clinical trials on heart patients with hypertension have shown that total flavonoides of *Crataegus* reduce cholesterol level and improve cardiac functions¹³. Similarly *Crataegus* leaves are antioxidant, immunomodulatory and anti inflammatory¹³. No doubt this plant is a storehouse of bioactive compounds with a variety of medicinal attributes.

Crataegus is commonly useful to strengthen the heart and its berries are especially indicated in the treatment of weak heart combined with high blood pressure, old age, inflammation, arteriosclerosis and nervous heart problems. Decoction of shoots and bark of *Crataegus* stops excess menstrual flow¹³. Prolonged use is necessary for the efficacious treatment, which is normally used either as a tea or a tincture.

5. CONCLUSION

The study conclude that *Crataegus crenulata* is an important multi-utility plant of hills. Fruits of the plant were found rich in flavanoids, vitamins, proteins and minerals. Fruit juice is having cardio-tonic properties. Herbal formulation prepared from the fruit of *C. cranulata* possessed significant

anti-hypertensive properties. Antioxidants present in berries of this shrub are helpful to reduce the damage from free radicals. Now a days *Crataegus* fruit juice is being considered as a heart tonic due to the presence of bio-flavonoides. It also strengthens the heart muscles and normalizes blood flow. The fruits of this plant are also having antioxidant properties and hence regular use of its juice have a positive effect on heart health. Thus it can be concluded from the study that *Crataegus* fruit juice has properties of a heart tonic and it may prove highly useful in the treatment of irregular heartbeat, high blood pressure, chest pain, hardening of the arteries, and heart failure. Since this plant grows in abundance in the Himalayan hills, it could be exploited scientifically for commercial use.

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He is one of the main contributors in study of antihypertensive properties of fruit beverage of *Crataegus*.

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He has contributed actively in formulation of herbal health beverage from *Crataegus* and in the study of antihypertensive effect of *Crataegus* fruit juice and its biochemical analysis.

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