# HAWTHORN (CRATAEGUS OXYCANTHA) BERRIES LOWERS ELEVATED SERUM LIPIDS IN LAB ANIMALS

**MOHAMMAD SUALEH[1], MIRZA TASAWER BAIG[1], ZAFAR ALAM MAHMOOD[2], SAAD BIN ZAFAR MAHMOOD[3] AND KHALID KHAN[4]**

*[1]Department of Pharmacognosy, Federal Urdu University, Karachi-Pakistan*
*[2]Colorcon Limited, Dartford, Kent-England*
*[3]Ziauddin Medical College, Ziauddin University, Karachi-Pakistan*
*[4]Drug Testing Laboratory, Peshawar-Pakistan*
*Corresponding author e-mail: baigutdu@hotmail.com*

**Abstract**

The purpose of the present study was investigate the antihyperlipidemic effect of Hawthorn berries (*Crataegus oxycantha*) after oral administration of crude extract in lab animals (albino rabbits), as well as to report the chemical analysis findings relating to major bioactive compounds (flavonoids and procyanidins) present in the species of *C. oxycantha* available in Pakistan. Albino rabbits were made hyperlipidemic by feeding an atherogenic diet formulation. The antihyperlipidemic test was carried out at one dose level, 100 mg / kg body weight. Blood samples were obtained from the animals of both groups *(pl define groups briefly here also*) before starting the experiment (for initial value), after feeding atherogenic diet for 30 days and then at 20, 40 and 60 days of the treatment. Both biochemical - alkaline phosphatase, SGPT (ALT) and SGOT (AST) and lipid profile - total cholesterol, HDL, LDL and Triglycerides were studied. The quantitative analysis of the bioactive compounds (Flavonoids and Procyanidins) of *C. oxycantha* (berries) was also performed (by using ultrasonic and classical maceration method). Promising antihyperlipidemic effect of Hawthorn berries was observed in lab animals. Significant reduction was noted in triglyceride, (upto 57.75%), total cholesterol, (71.33%) and LDL (84.03%), as compared to ……., while no difference recorded in the HDL profile between test and control group. All twelve subjects completed the study. No mortality was observed.

## Introduction

*Crataegus oxycantha* (local name - Ban-Sangli or Narghuncha or Ghunza) is a member of the Rosaceae family. The plant is cultivated in Murree hills 1300-3000 M, in NWFP province and Qalat, Quetta and Hanna in Balochistan province of Pakistan. Flowering time is reported between April to July. Later in the year it bears numerous small, oval dark red fruit about 1 cm long, berry-like, but structurally a pome containing two seeds (Baquar 1989, Kirtikar and Basu 1984).

Traditionally, *C. oxycantha* is reported to posses cardiac tonic properties, and are widely used for remedial the conditions including angina, hypertension, arrhythmias, and congestive heart failure. The main constituents of Crataegus are amines, triterpene saponins, flavonoids and their glycosides, catechin and epicatechin, and oligomeric proanthocyanidins. The primary cardio protective activity of this plant is generally attributed to its flavonoid and oligomeric proanthocyanidin (OPC) contents. Although numerous flavonoid molecules have been found to have positive effects on the cardiovascular system, it appears the combination of flavonoid-based constituents in Crataegus provides the beneficial cardiovascular activity attributed to this botanical drug (Anonymous, 1998, Wichtl, 1994)

Flavonoids are found in many foods and botanical medicines and have been shown to increase collagen cross-linking in the vascular endothelium, strengthening blood vessels, as well as having potent antioxidant activity. Recent epidemiological studies have found an association between dietary flavonoid intake and reduced risk of heart disease, myocardial infarction, and stroke (Hertog et al., 1993 and 1995)

**Subjects:** Adult albino rabbits (giant white strain) weighing 1.6 to 2.7 kg were procured from the animal house of Pakistan Council for Scientific and Industrial Research (PCSIR), Government of Pakistan, Karachi – Pakistan. The animals were kept in cages with proper aeration and lighting. Initially, they were given a controlled diet consisting of wheat and lusern (*Medicago sativa*, family Fabaceae) and water for two weeks. This was a period of acclimatization.

## Materials and Methods

***Hawthorn Extract:*** The dried fruit (berries), 10 kg was soaked in 30 L ethanol (95 %) for thirty days at room temperature. After this period the solvent was filtered out and the material was again soaked in the same quantity of fresh ethanol for thirty days. The process of soaking was repeated thrice using same parameters.

Finally the combined ethanolic extract was subjected to rotary evaporator to evaporate ethanol. A light brown dried material (600 g) was obtained which was further subjected to lyophilization and then used in the study.

***Food Records and administration:*** Twelve rabbits were made hyperlipidemic by feeding an atherogenic diet (containing 60g lucern, 50g wheat flour, hydrogenated groundnut oil 5g, sodium chloride 4g and cholesterol powder 400mg per kg body weight) for 30 days. Rabbits were divided into two groups (group 1 – control and group 2 – test), each group comprising of six animals, three males and three females. The test was carried out at one dose level, 100 mg / kg body weight. Group 1 (Control group) was given control diet consisting of wheat and lusern and water during the study. The Test group, was also given controlled diet consisting of wheat and lusern and water during the study. While the extract (100 mg / kg body weight) was administered by oral route as a watery suspension using 25ml disposable plastic syringes without needle (Ram et al., 1997).

***Blood Sample and analysis:*** Blood samples were obtained from the animals of both groups before starting the experiment (for initial value), after feeding atherogenic diet for 30 days and then at 20, 40 and 60 days of the treatment. Both Lipid and biochemical profiles were studied to ascertain the antihyperlipidemic effect and safety profile. Blood samples were collected from the marginal ear vein of each rabbit and the serum separated for estimation of Biochemical (AST, ALT, ALP ……) and Lipid profile components (Cholesterol, HDL, LDH, triglycerides) using "Merck Microlab 200", a fully computerized semi auto-analyzer (Anonymous 1994). LDL cholesterol was calculated by using the method described by Marshall (1992).

***Determination of flavonoids and procyanidins:*** An ultrasonic and classical maceration method (Velickovic et al., 2007) was used to quantify the flavonoids and procyanidins.

**Results**

The over all summary of all the parameters (biochemical and lipid profile) studied to establish antihyperlipidemic effects are presented in Table – 1 and 2 respectively. The values of various lipid profile components (total cholesterol, triglyceride, HDL and LDL) and biochemical parameters are given in mean ± S.E.M. Analysis to determine differences in test and control groups was carried out by the t-test of significance and $p > 0.005$ was considered significant (Walpole, 1982). The values obtained in the study were also compared with standard normal values of the biochemical and lipid profile given for rabbits (Table 1 & 2). All twelve subjects completed the study. No mortality was observed.

***Effect on Lipid Profile:*** All parameters (triglyceride, total cholesterol, HDL and LDL) were increased after atherogenic diet. The antihyperlipidemic effect of the test group was calculated and compared with the control group on the basis of reduction in average values. The values obtained after atherogenic diet was the base line, while values recorded after 60 days of treatment was the final results for the three parameters, triglyceride, cholesterol and LDL. However, for HDL, the initial value (before the atherogenic diet) was considered as base line.

**Table 1.** Results of the biochemical parameters obtained from test (hawthorn group) and control group.

| Parameters | Normal * Value | Initial value | After Atherogenic Diet | After 20 Days | After 40 Days | After 60 Days | % | *t-value* |
|---|---|---|---|---|---|---|---|---|
| **Control group** | | | | | | | | |
| Alkaline Phosphatase (U/L) | 06.31-115.31 | 22.724±1.9 | 28.694±2.4 | 29.117±2.1 | 28.016±2.2 | 27.622±1.8 | 3.73 | 0.348 |
| SGPT (U/L) | 00.00– 07.18 | 43.011±1.8 | 130.311±8.5 | 119.732±9.4 | 107.705±9.5 | 96.520±9.4 | 25.93 | 2.644 |
| SGOT (U/L) | 14.77-165.41 | 65.446±4.5 | 157.662 ±10.8 | 147.356±9.6 | 134.275±7.2 | 119.400±6.0 | 24.26 | 3.074 |
| **Test group** | | | | | | | | |
| Alkaline Phosphatase (U/L) | 06.31-115.31 | 21.745±1.9 | 30.807±2.4 | 31.296±2.5 | 32.812±2.4 | 32.991±2.3 | 7.08 | 0.02 |
| SGPT (U/L) | 00.00– 07.18 | 43.584±1.8 | 129.323±8.5 | 120.924±8.7 | 110.491±10.1 | 84.188±9.8 | 34.90 | |
| SGOT (U/L) | 14.77-165.41 | 64.972±4.5 | 156.622±10.8 | 151.867±9.7 | 131.34±8.6 | 91.515±7.5 | 41.56 | 4.926 |

* Howard HT H, and Nathan CC. Accumulation of low density lipoprotein associated cholesterol in calcifying vesicle fraction correlates with intimal thickening in thoracic aortas of juvenile rabbits fed a supplemental cholesterol diet. *Lipids in Health and Disease*. 2006; 5:25-31. (2006).

*Ignasi M, Rafaela C, Josep P, Roser V, and Santiago L. Hematology and Serum Chemistry Values of the European Brown Hare. *Veterinary Clinical Pathology.* 2003; 32:195-198.

Significant reduction was noted in triglyceride, total cholesterol and LDL, while no difference recorded in the HDL profile between test and control group (Table 1 & 2). Triglyceride decreased up to 57.75%, cholesterol 71.33% and LDL 84.03% in the test group. In comparison, the control group responded as 22.21%, 19.44% and 16.66% respectively. No significant difference was noted in HDL value between test and control groups

***Effect on Biochemical Parameters:*** All biochemical parameters also behaved in a similar pattern. The values (alkaline phosphatase, SGPT and SGOT) increased after atherogenic diet. The effect in decreasing the elevated values in the test group was compared with the control group on the basis of the final values obtained after 60 days of treatment. The base line values were considered after atherogenic diet. A significant reduction was noted in SGPT and SGOT values between the test and control group. However, no difference was observed in case of alkaline phosphatase. The SGPT and SGOT values decreased up to 34.90% and 41.56% in the test group, compared to 25.93% and 24.26% .

**Table 2. Results of the lipid profile obtained from test (hawthorn group) and control groups.**

| Parameters | Normal * Value | Initial value | After Atherogenic Diet | After 20 Days | After 40 Days | After 60 Days | % | *t value* |
|---|---|---|---|---|---|---|---|---|
| **Control group** | | | | | | | | |
| Triglyceride (mg/dl) | 13.00-148.00 | 15.496±0.4 | 62.710±7.9 | 57.302±7.2 | 53.241±6.6 | 48.780±6.2 | 22.21 | 1.381 |
| Total Cholesterol (mg/dl) | 11.00-25.00 | 21.225±0.8 | 117.134±4.9 | 110.063±5.0 | 102.492±5.2 | 94.293±5.3 | 19.49 | 1.626 |
| HDL | <05.00 | 3.802±0.2 | 15.123±0.7 | 13.783±0.7 | 12.268±0.5 | 10.864±0.2 | 28.16 | 5.362 |
| LDL | <10.00 | 10.380±0.8 | 73.507±7.8 | 70.234±7.5 | 65.190±7.9 | 61.257±7.5 | 16.66 | 0.115 |
| **Test group** | | | | | | | | |
| Triglyceride | 13.00-148.00 | 16.089±0.4 | 64.428±7.9 | 50.453±6.5 | 36.338±3.9 | 25.928±2.8 | 59.75 | 4.578 |
| Total Cholesterol | 11.00-25.00 | 20.149±0.8 | 120.140±4.9 | 90.569±9.2 | 57.664±7.5 | 34.438±3.8 | 71.33 | 13.544 |
| HDL | <05.00 | 3.108±0.2 | 15.482±0.7 | 13.888±0.5 | 12.213±0.3 | 10.619±0.4 | 31.41 | 5.677 |
| LDL | <10.00 | 9.998±0.8 | 75.373±7.8 | 10.619±0.4 | 28.933±8.0 | 12.034±4.2 | 84.03 | 7.129 |

* Howard HT H, and Nathan CC. Accumulation of low density lipoprotein associated cholesterol in calcifying vesicle fraction correlates with intimal thickening in thoracic aortas of juvenile rabbits fed a supplemental cholesterol diet. *Lipids in Health and Disease*. 2006;5:25 – 31 (2006).

*Ignasi M, Rafaela C, Josep P, Roser V, and Santiago L. Hematology and Serum Chemistry Values of the European Brown Hare. *VeterinaryClinical Pathology.* 2003;32:195-198.

## Discussion

*Crataegus oxycantha* has been also been used traditionally as a cardiac tonic and current uses include treatment for angina, hypertension, arrhythmias, and congestive heart failure. Animal studies have also indicated that Crataegus extracts may also have potential use as anti-ischemic and lipid-lowering agents. The flavonoid content of the crude drug is approximately 1% for the leaves and flowers but only about 0.1% for the berries. The content of oligomeric procyanidins in the leaves and flowers is about 1-3% (Busse 1996). In the present study, the C. oxycantha species available in Pakistan, showed 0.075% flavonoids and 2.68% procyanidins.

Procyanidins from hawthorn extracts help reduce cholesterol levels and decrease the size of atherosclerotic plaques. This action could be due to hawthorn's ability to increase the integrity of the collagen matrix of the vessel walls, making them stronger, which may help prevent the development and further progression of atherosclerotic plaque (Wegrowski *et al.,* 1984). The beneficial effects of hawthorn in the treatment of angina are due to improvement in the blood and oxygen supply of the heart, resulting from dilation of the coronary vessels, as well as improvement of the metabolic processes in the heart (Murray and Pizzorno 1991). Most clinical studies use 100-250 mg hawthorn flower extract. Long term oral studies and *in vitro* tests have shown no evidence of risks or any other contraindications with the use of hawthorn extract (Schlegelmilch and Heywood 1994 and Schulz *et al.,* 1998).

Results of the lipid profile obtained in the present study are quite encouraging. Triglyceride decreased up to 57.75%, total cholesterol 71.33% and LDL 84.03% in lab animals. In comparison, the control group responded as 22.21%, 19.44% and 16.66% respectively. No significant difference was noted in HDL value between test and control groups, confirming that hawthorn berries have no influence in increasing the DHL level. The atherogenic diet was responsible for the over all increase in HDL level in both test and control groups at the end of 60 days of treatment.

The lipid lowering effect of *Crataegus oxycantha* may be ascribed to it's high content of flavonoid compounds, particularly the Oligomeric proanthocyanidins (OPCs), Crataegus has significant antioxidant activity (Rakotoarison *et al.,* 1997). In addition, it increases coronary blood flow enhancing oxygen flow and utilization by the heart (Loew, 1994, Schussler *et al*., 1995). Crataegus extracts also have a positive inotropic effect on the contraction amplitude of myocytes (Popping *et al.,* 1995). Due to the flavonoid content, extracts of

this herb exert considerable collagen stabilizing effects, enhancing integrity of the blood vessels (Gabor 1972). Extracts of Crataegus prevent elevation of plasma lipids, such as total cholesterol, triglycerides, and LDL- and VLDL-fractions, in rats fed a hyperlipidemic diet (Shanthi *et al.,* 1994). Crataegus up regulates hepatic LDL-receptors, resulting in greater influx of plasma LDL-cholesterol into the liver. It also prevents the accumulation of cholesterol in the liver by enhancing cholesterol degradation to bile acids, promoting bile flow, and suppressing cholesterol biosynthesis (Rajendran *et al.,* 1996).

To assess the effect of all the extracts on liver function as well as to establish the safety profile, various biochemical parameters, Alkaline phosphatase and Transaminase Enzymes (SGPT and SGOT) were also studied. Both SGPT and SGOT reduced significantly but no significant effect was noted in the reduction of alkaline phosphatase in the test group. However, the values of alkaline phosphatase in both the group noted to be well within the limit. The transaminase enzymes show a low level in the plasma under normal circumstances. With the accelerated death of cells - virus infection, necrosis - soluble enzymes enter the bloodstream. Parallel determination of both transaminase activities is of great importance in the diagnosis and evaluation of liver and heart diseases. Myocardial infarction significantly enhances the activity of AST (SGOT) while the activity of ALT (SGPT) is elevated moderately. On the other hand, hepatocellular tissue destruction enhances both activity, but the level of ALT (SGPT) is higher, than that of AST (SGOT). Acute hepatitis is likely when the quotient of activities of AST/ALT is under the value of 1.3; on the other hand, acute myocardial infarction is followed by a higher value of AST/ALT quotient (Dufour *et al.,* 2001). Earlier studies on antihyperlipidemic effect of *C. oxycantha* reported by various authors mainly based on the determination of lipid profile. However, in the present study, biochemical parameters were also studied and reported to ascertain the safety profile of the plant as well.

## Conclusion

The present study provides satisfactory data to conclude that the crude extract of *Crataegus oxycantha (Hawthorn berries)* available in Pakistan contains considerable quantity of bioactive compounds (Flavonoids and Procyanidins) and are capable of lowering triglyceride, cholesterol and LDL in lab animals. The decrease in SGPT (ALT) and SGOT (AST) values as compared to ……..further supports its safety profile. Based on results, it could be suggested that the extract have strong potential to be used as lipid lowering component, further clarified scientifically in pharma-industry, possibly in humans as well in future. However, a short and long term toxicity study is also suggested, which is under way and will be reported along with the haematological data and pathological changes observed in various organs after oral administration.

## References

Anonymous. (1994). Diagnostica Merck. *Merck Microlab 200 Manual*. Merck KGaA, Darmstadt, Germany.

Anonymous. (1998). Physician Desk Reference for Herbal Medicine: 1st edition, published by Medical Economics Company, N.J.

Baquar, S.R. (1989). *Medicinal and Poisonous Plants of Pakistan*. 1st edition, published by Printas, Karachi-Pakistan.

Blesken, R. (1992). Crataegus in cardiology. *Fortschr Med.*110: 290-292.

Busse W. (1996). Standardized *Crataegus* extract clinical monograph. *Quarterly Review of Natural Medicine* (fall):189-197.

Dufour D.R., Lott, J.A. and Henry, J.B. (2001). Clinical Enzymology – In *Clinical Diagnosis and Management by Laboratory Methods* (ed., Henry, J.B), 29th edition 1: 281-303.

Gabor, M. (1972). Pharmacological effects of flavonoids on blood vessels. *Angiologica* 9: 355-374.

Hertog, M.G.L., Feskens, E.J.M. and Hollman, P.C.H. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease, the Zutphen Elderly Study. *Lancet*. 342: 1007-1011.

Hertog, M.G.L., Kromhout, D. and Aravanis, C. (1995). Flavonoid intake and long-term risk of coronary heart disease and cancer in the Seven Countries Study. *Arch. Intern. Med.* 155: 381-386.

Kirtikar, K.R. and Basu, B.D. (1984). *Indian Medicinal Plants.* 2nd edition (in four volumes), Published by, Lait Mohan Basu, Allahabad, India.

Loew, D. (1994). *Crataegus*-special-extracts in heart failure. Established pharmacological and clinical results. *Der. Kassenarzt.*15: 43-52.

Marshall, WJ., Lipids, and Lipoproteins. (1992). In *Illustrated Text Book of Clinical Chemistry*, 2nd edition, Gower Medical Publishing, London 222-237.

Murray, M.T. and Pizzorno, J.E. (1991). *Atherosclerosis* – In Encyclopedia of Natural Medicine, published by Prima Publishing, CA. 1991:156-170.

Popping, S., Rose, H., Ionescu, I. (1995). Effect of a hawthorn extract on contraction and energy turnover of isolated rat cardiomyocytes.*Arzneimittelforschun* 45: 1157-1161.

Rajendran, S., Deepalakshmi, P.D. and Parasakthy, K. (1996). Effect of tincture of Crataegus on the LDL-receptor activity of hepatic plasma membrane of rats fed an atherogenic diet. *Atherosclerosis* 123: 235-241.

Rakotoarison, D.A., Gressier, B. and Trotin, F. (1997). Antioxidant activities of polyphenolic extracts from flowers, in vitro callus and cell suspension cultures of Crataegus monogyna. *Pharmazie.* 52: 60-64.

Ram, A., Lauria, P., Gupta, R. (1997). Hypocholesterolemic effects of Terminalia arjuna tree bark. *J. Ethnopharmacol.* 55: 165-169.

Schlegelmilch, R. and Heywood, R. (1994). Toxicity of *Crataegus* (hawthorn) extract (WS1442). *J. Amer. Coll. Toxicol***.** 13(2): 103-113.

Schulz, V., Hansel, R. and Tyler, V.E. (1998). *Rational Phytotherapy***.** Berlin: Springer-Verlag.

Schussler, M., Holzl, J. and Fricke, U. (1995). Myocardial effects of flavonoids from Crataegus species. *Arzneimittelforschung* 45: 842-845.

Shanthi, S., Parasakthy, K., Deepalakshmi, P.D. and Devaraj, S.N. (1994). Hypolipidemic activity of tincture of Crataegus in rats. *Indian J. Biochem. Biophys.* 31: 143-146.

Velickovic, D.T., Nikolova, M.T., Ivancheva, S.V., Stojanovic, J.B. and Velikovic, V.B. (2007). Extraction of flavonoids from garden (Salvia officinalis L.) and glutinous (Salvia glutinosa L) sage by ultrasonic and classical maceration. *J. Serb. Chem. Soc.* 1: 73-80.

Walpole, R.E. (1982). Introduction to Statistics, 3rd edition, Macmillan Publishing Co., Inc, New York.

Wegrowski, J., Robert, A.M. and Moczar, M. (1984). The effect of procyanidolic oligomers on the composition of normal and hypercholesterolemic rabbit aortas. *Biochem. Pharm.* 33: 3491-3497.

Wichtl, M. (1994). *Herbal Drugs and Phytopharmaceuticals.* A handbook for practice on a scientific basis – English Edition, (Editor: Norman Grainger Bisset) published by Medpharm, Stuttgart, Germany.