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Hawthorn Extract - Viable Treatment for Cardiovascular Disease or Unscrupulous Herbal Supplement?

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Abstract:

In the past Hawthorn leaves, berries and flowers have been used in Asia to treat hypertension (high blood pressure) and other cardiovascular diseases. It is hypothesized that the Hawthorn extract acts as a vasodilator increasing the size of the lumen of blood vessels by relaxing the smooth muscle. This occurs by decreasing the amount of calcium that is present in the cytosol by either increasing the effectiveness of the Ca^{2+}-ATPase pump or by altering the Na^{+}-Ca^{2+} antiport exchanger. With this decrease in cytosolic calcium the calcium unbinds from CaM causing the enzyme myosin phosphatase to remove phosphate from the myosin thus causing the smooth muscle to relax. The commercially available Hawthorn extract used in these studies did not have a significant effect on coronary or pulmonary arteries. The denuded and intact LADs did have a significant relaxation at higher concentrations which was likely due to the ethanol that was used in the formulation of the extract.

Introduction:

Crataegus laevigata, more commonly known as the English Hawthorn, is a tree native to western and central Europe and parts of Asia but can also be found in the United States including the state of Michigan. It can grow to be approximately 20 to 25 feet high and have a spread of 15 to 25 feet. It grows in most soil types but prefers fertile soil consisting of clay, sand and silt. The tree blossoms red and white flowers in the spring and produces bright red berries during the late summer and early fall (Gilman and Watson, 1993). In the past the Hawthorn berries, leaves and flowers were used in parts of Asia to treat hypertension and other cardiovascular diseases.

Cardiovascular disease is a group of closely related diseases that involve many of the body's organs, and according to the Morbidity and Mortality Weekly Report (MMWR) of 2006 is the leading cause of morbidity and mortality in the United States responsible for 26% of

deaths. Cardiovascular disease frequently targets the arteries of the coronary and pulmonary circulations. The arteries involved in the coronary circulation are responsible for providing oxygenated blood throughout the myocardium, the middle layer of the heart wall, that is directly responsible for pumping blood. The coronary circulation originates in the ascending aorta with branches to the left and right coronary arteries. The left coronary artery supplies the circumflex artery and the left anterior descending coronary artery (LAD), one of the arteries studied in this experiment. The coronary veins ultimately return the blood that is now low in oxygen back to the right side of the heart. Once the blood has returned to the heart it can be re-oxygenated via the pulmonary circulation.

Pulmonary circulation begins when the deoxygenated blood in the right ventricle gets pumped to the lungs via the pulmonary arteries. Inside the lungs these arteries parallel the airways as they branch frequently and become narrower and smaller. The branching ends at the pulmonary capillaries which surround interconnected sacs called alveoli. At this location, oxygen moves from the inhaled air to the blood and carbon dioxide moves out of the blood. Once the blood has been oxygenated in the capillaries, it returns to the left atrium of the heart via the pulmonary veins completing the pulmonary circuit. Once the oxygenated blood is back in the heart, it can be re-circulated throughout the body including the coronary circulation.

The coronary and pulmonary arteries contain three primary layers known as tunics. The innermost layer is the tunica intima and contains the endothelium. The endothelium is known to play an important role in the relaxation of the arteries (Furchgott and Zawadzki, 1980) and in this experiment it will be studied by denuding some of the arteries. The outermost layer known as the tunica externa is composed of connective tissue that helps stabilize the vessel. The smooth muscle of the artery is located in the tunica media (i.e. middle layer of the blood vessel wall) and

has the ability to contract or relax depending on the chemical, physical, and/or electrical stimuli present. It's speculated that Hawthorn extract might be capable of dilating the lumen of these arteries, by relaxing the smooth muscle, which would cause a concomitant increase in blood flow in the heart and lungs. This increase in blood flow may cause the heart to work more efficiently, thus potentially changing how many people are treated for cardiovascular disease as well as decreasing the morbidity and mortality associated with cardiovascular disease.

In the smooth muscle that surrounds the arteries, the amount of Ca^{2+} present in the intracellular fluid is directly related to the strength of the contraction of the muscle. When a vasoconstrictor stimulus is present, Ca^{2+} enters the smooth muscle cell via voltage gated Ca^{2+} channels in the cell membrane and from intracellular Ca^{2+} stores. The cytosolic Ca^{2+} then binds to calmodulin (CaM), a protein found in the cytosol. The Ca^{2+} -calmodulin complex then activates myosin light chain kinase (MLCK), an enzyme which phosphorylates the myosin allowing for the contraction to occur. Consequently in order for the smooth muscle to relax the intracellular Ca^{2+} concentration must decrease. This may be accomplished by removing the free cytosolic Ca^{2+} by means of pumping the Ca^{2+} back into the sarcoplasmic reticulum through Ca^{2+} -ATPase or out of the cell using the Ca^{2+} -Na^{+} antiport exchanger. With the decrease in concentration of cytosolic Ca^{2+}, the Ca^{2+} unbinds from CaM causing the enzyme myosin phosphatase to remove phosphate from the myosin thus causing the relaxation of the muscle.

Although both the pulmonary and coronary smooth muscles rely directly upon the concentration of the cytosolic Ca^{2+} to contract or relax they both do not always respond the same way when a pathological condition arises. For example when the human body becomes deprived of adequate oxygen supply causing hypoxia the coronary smooth muscle will dilate in order to increase the blood flow to the heart causing more blood to be pumped to the body. However the

pulmonary smooth muscle will contract in order to decrease the amount of blood flow to the hypoxic regions of the lung in order to enhance ventilation-perfusion matching.

Since the Hawthorn extract is hypothesized to act as a vasodilator (Chen et al, 1998; for review see Furey and Tassell, 2008) by relaxing the smooth muscle, it may effectively decrease the amount of Ca^{2+} that is present in the intracellular fluid. This may be accomplished by increasing the effectiveness of the Ca^{2+} -ATPase pump or by altering the Na^{+} -Ca^{2+} antiport exchanger thus decreasing the amount of Ca^{2+} present in the cytosol. This current study evaluates the effects of the Hawthorn berry, flower and leaf on the coronary and pulmonary arteries to determine if the proposed relaxation of the smooth muscle actually occurs and to gain insight into the mechanism of action. By studying both the coronary and pulmonary arteries it will allow us to understand how Hawthorn extract impacts more than one organ and to study any similarities or differences between them.

Procedure:

Porcine hearts and lungs were obtained from the local abattoir and the left anterior descending coronary arteries (LAD) were carefully dissected from the hearts and intra-pulmonary arteries were dissected from the lungs. If the arteries were stored overnight they were put in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer solution (made of 140 mM NaCl, 4.7 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, 10 mM HEPES, and 11.1 mM glucose) with the pH adjusted to 7.4 and stored at 4 degrees Celsius. On the day of the experiment, the previously dissected arteries were cut into 5mm rings, connected to a force transducer, mounted in an organ bath containing 25 mL of Krebs-Henseleit solution (made of 118 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$ and 11 mM glucose), heated to 37 degrees Celsius, bubbled with a gas mixture containing

95% O_2 and 5% CO_2, and loaded with 7 grams of passive tension (Fig. 1). Some of the arterial rings were denuded by gently rolling the compressed blood vessel between the thumb and forefinger. After a 1 hour equilibration period, the arteries were exposed to a cumulative dose response of potassium chloride (KCl; 15-60 mM). Changes in vascular reactivity were recorded for 5 minutes at each dose. Subsequent dose responses were also recorded at 5 minute intervals. The arteries were then treated with sodium nitroferricyanide (SNP; $1x10^{-6}$ M - $3x10^{-3}$M). The data were recorded as change in tension from 7 grams using LabScribe version 2.0 software from iWorx.

The organ baths were then drained and rinsed with the Krebs-Henseleit buffer solution three times and allowed to equilibrate for approximately 30 minutes. 60 mM KCl was then added once again. Following the addition of KCl, methyl acetylcholine (mACh; $1x10^{-7}$ M – $3x10^{-4}$ M) was added using a cumulative dose response protocol as described for SNP. The data were once again recorded as change in tension from 7 grams. The arteries were once again drained and rinsed three times with Krebs-Henseleit buffer solution and allowed to equilibrate for approximately 30 minutes. 60 mM KCl was again added. The Hawthorn extract was obtained from Herb Pharm located in Oregon and composed of the *Crataegus* flower, leaf, and berry. The producers dissolved the *Crataegus* species in organic grain alcohol and water. The Hawthorn extract was then added to denuded and intact arteries at increasing logarithmic ratios. Ethanol was added using the same logarithmic ratios as the Hawthorn and served as a control. The data were once again recorded as change in tension from 7 grams. The order was the randomized throughout the experiment.

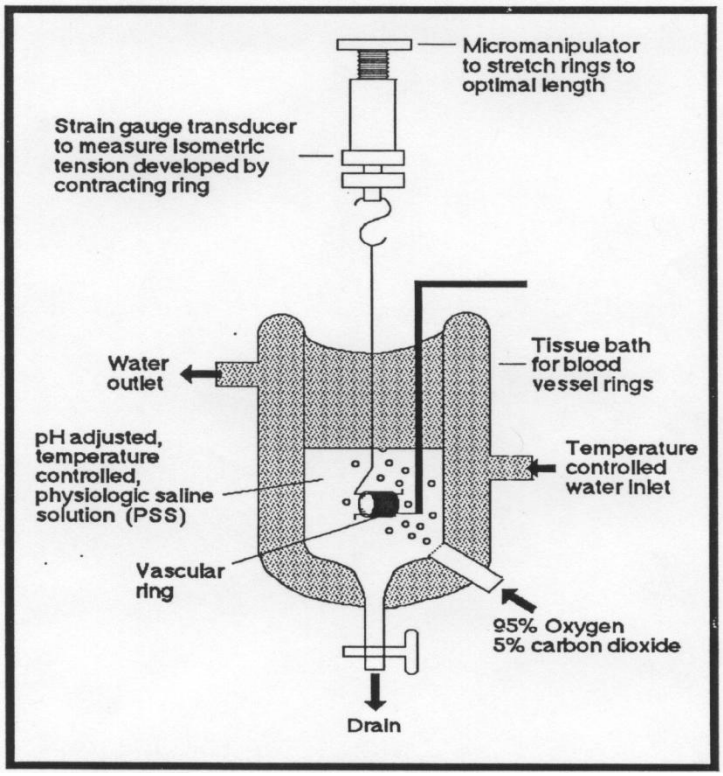

Figure 1. Schematic diagram of laboratory set-up used to perform experiments with arteries. Created by Donald B. Stratton, Ph.D. (Drake University, Des Moines, Iowa).

Results:

Potassium chloride (KCl) was added as a cumulative dose response (15, 30, 45, 60 mM) during the experimental process to constrict the arteries. Results showed a significant constriction at the higher concentration levels for both the denuded and intact pulmonary and coronary arteries. However there was no significant difference in constriction among the four groups (Fig. 2). After the cumulative doses of KCl were added, increasing concentrations of sodium nitroferricyanide (SNP; $1x10^{-6}$ M - $3x10^{-3}$M) were used to relax the arteries. A significant relaxation was recorded in the intact coronary arteries at doses at or greater than 10^{-4} M and in the denuded coronary arteries at doses greater than $3x10^{-5}$ M (Fig. 3). No significant relaxation occurred in either of the pulmonary arteries.

Once the baths had been rinsed and the arteries constricted again using 60 mM KCl, the arteries were then relaxed using increasing concentrations of methyl acetylcholine (mACh; $1x10^{-7}$ M – $3x10^{-4}$ M). However upon the addition of mACh, no relaxation occurred in any of the arteries but a significant constriction was found in doses at or greater than 10^{-6} M in the coronary arteries and at or greater than 10^{-5} M in the pulmonary arteries (Fig. 4).

When the proposed vasodilator, the Hawthorn extract, was added to the baths no significant changes were observed for either the intact or denuded pulmonary arteries but a significant dilation did occur at the highest doses for both the denuded and intact coronary arteries (Fig. 6). However, when these results were compared with the data collected from the ethanol testing (the control group) the exact same results were found (Fig. 5). The ethanol had no significant effect on the pulmonary arteries but had a significant dilation for both the denuded and intact coronary arteries at the highest dose.

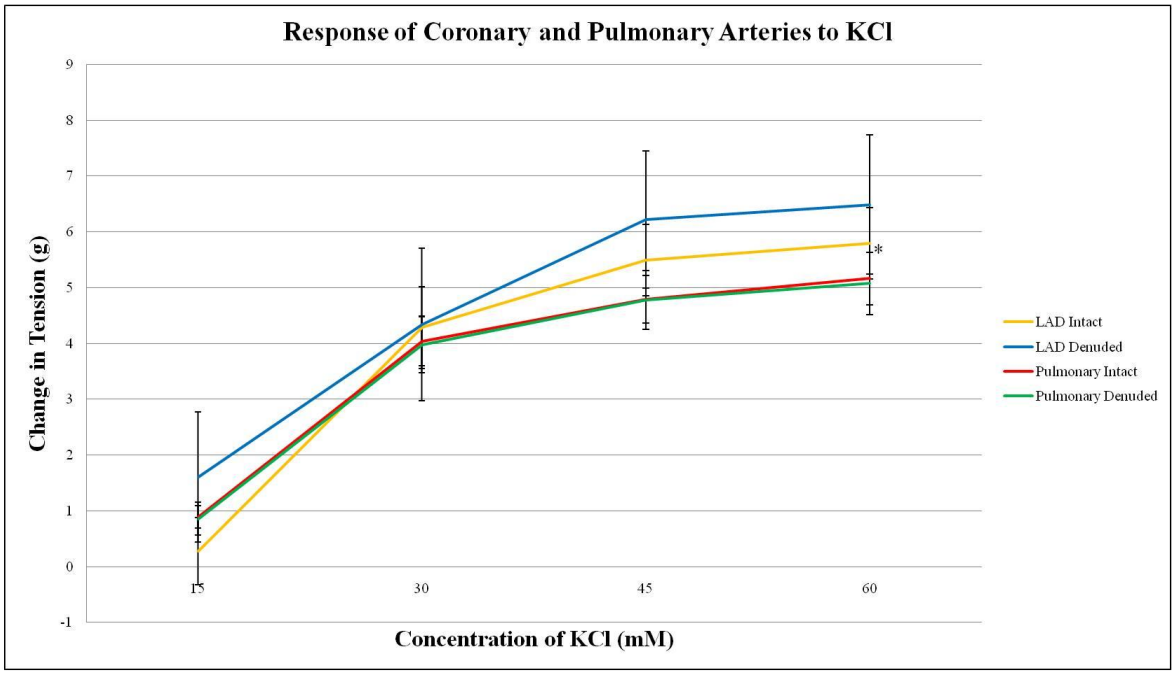

Figure 2: The above data shows the relationship between coronary and pulmonary arteries and their individual responses to increasing doses of KCl, a known vasoconstrictor.
*Significant constriction was found in the intact LAD at all concentration levels.

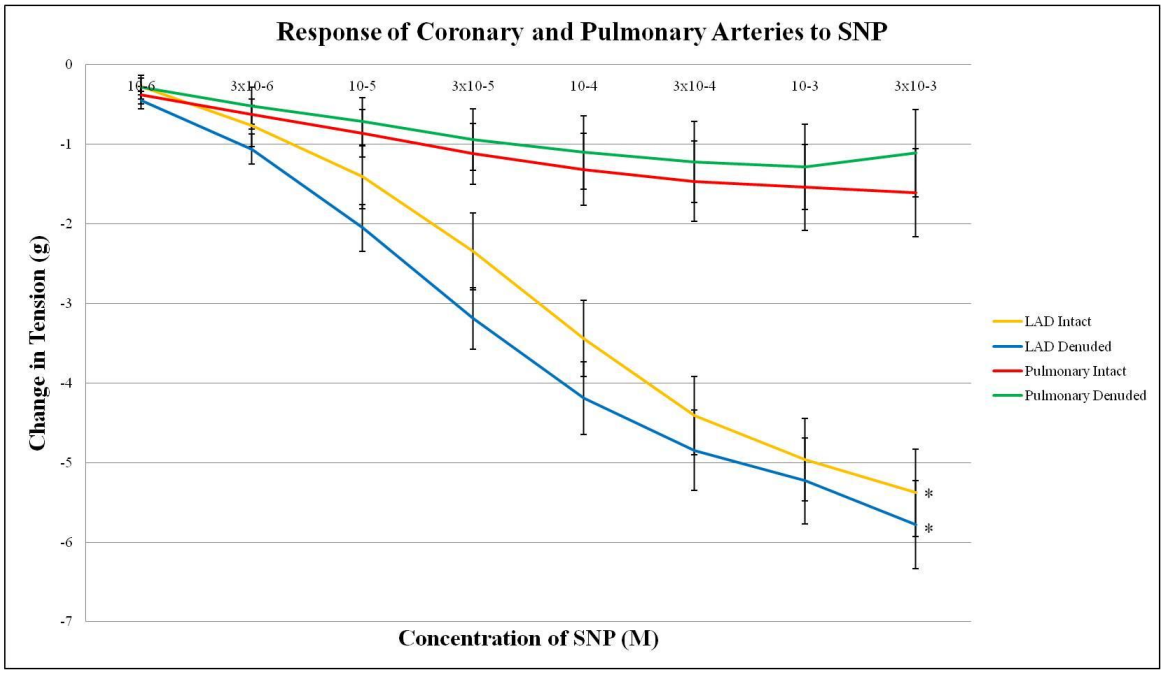

Figure 3: The above data shows the relationship among denuded and intact coronary and pulmonary arteries and how each artery was affected by an increasing doseof SNP. *Significant relaxation occurred at doses at or greater than 10^{-4} M in the intact LAD and at $3x10^{-5}$ M or greater in the denuded LAD.

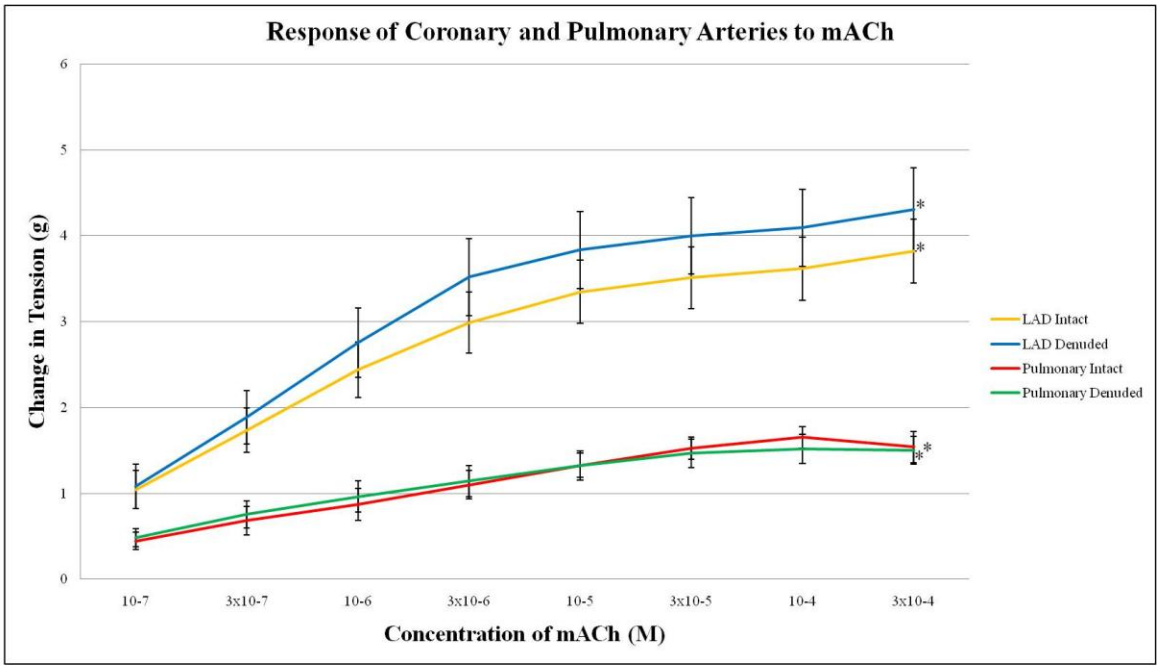

Figure 4: The above data shows the relationship between coronary and pulmonary arteries in response to mACh and whether denuding has any effect on those responses. *Significant contraction was found in doses at or greater than 10^{-6} M in the LADs and at or greater then 10^{-5} M in the pulmonary arteries.

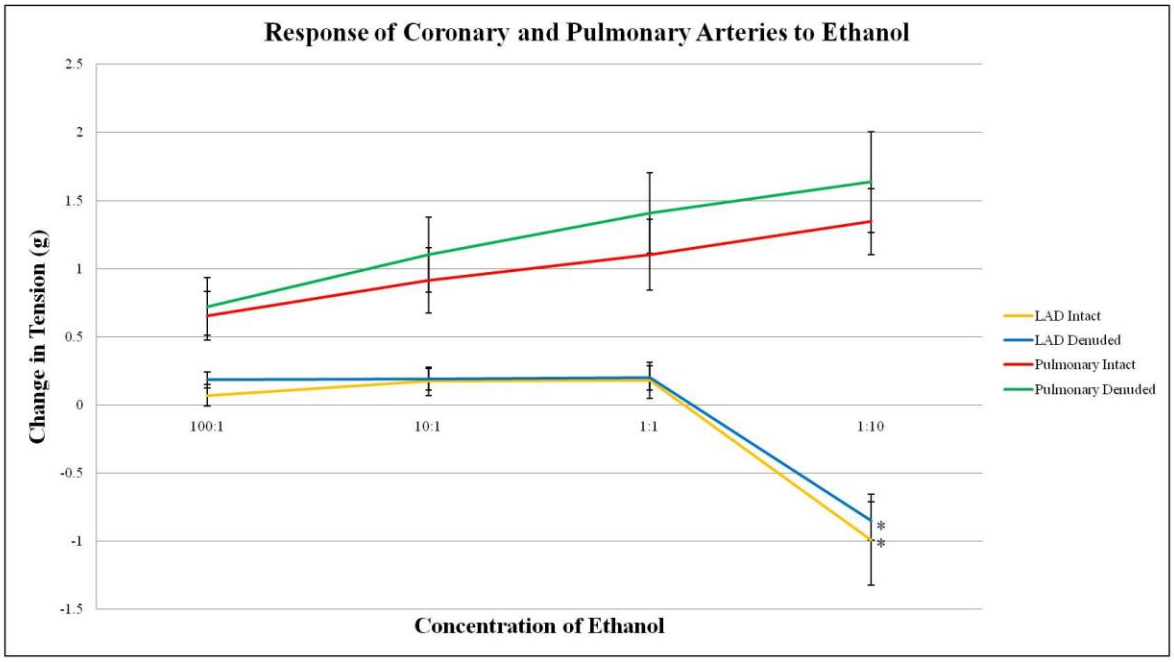

Figure 5: The above data shows the relationship between denuded and intact LADs and denuded and intact pulmonary arteries and their response to increasing amounts of ethanol.
*Significant dilation was found at the highest doses for both the LAD intact and denuded. Ethanol had no effect on the pulmonary arteries.

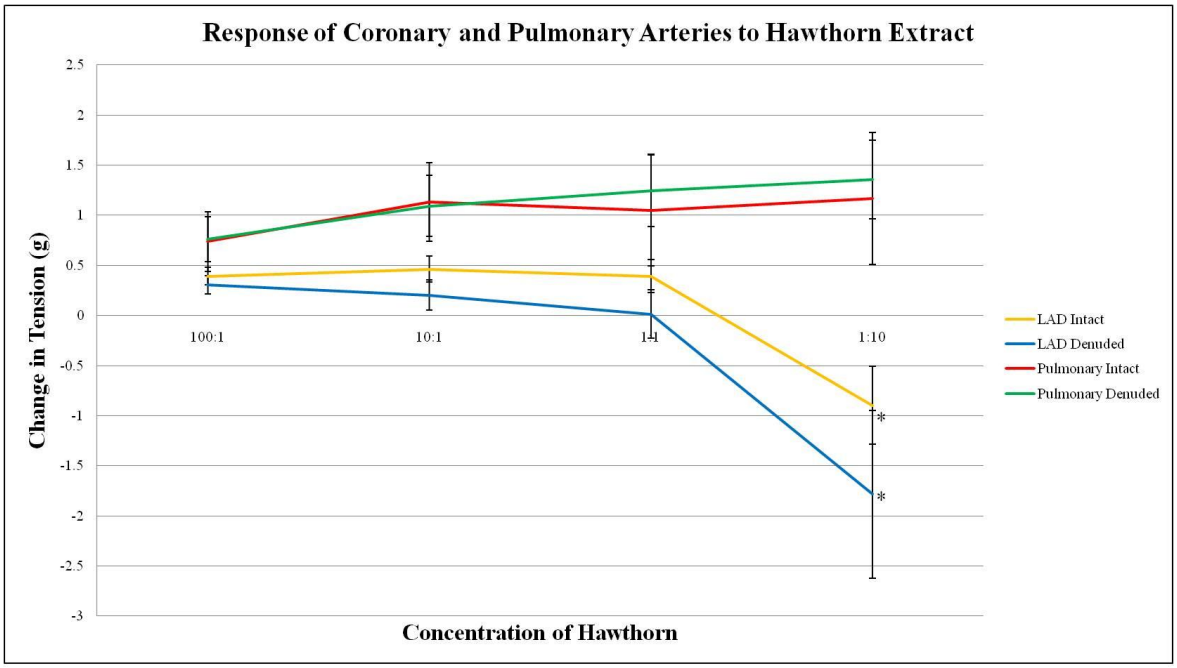

Figure 6: The above data shows the relationship between intact and denuded LADs and intact and denuded pulmonary arteries and their response to increasing amounts of the Hawthorn extract.
*Significant dilation was found at the highest doses for both denuded and intact LADs. There was no significant effect on either of the pulmonary arteries.

Discussion:

The results that were obtained did not support the proposed hypothesis that the Hawthorn extract would act as a vasodilator in the coronary and pulmonary arteries. The findings obtained showed no significant dilation that could not be explained by the results from the control (ethanol). The results that were obtained were also not consistent with the results from Kim et al (2000) which showed the Hawthorn acting as a vasodilator. However the cause of this discrepancy could be caused by a number of things including the source of the specific Hawthorn extract that was used in this protocol. The extract used was not Food and Drug Administration (FDA) approved making the contents of the extract questionable. Since this is a nutritional supplement FDA approval is unnecessary. Only drugs require FDA approval. The discrepancy could have also been caused by the species of Hawthorn that was used by Kim et al (2000) while the extract that was used in this experiment was from the flowers, leaves and berries of the *Crataegus* species dissolved in water and organic grain alcohol. Both of these could have caused the noted differences in results.

In this specific study removing the endothelium of the arteries making it denuded or leaving it intact was also taken into consideration. Since smooth muscle responds differently to various chemical stimuli depending on whether the endothelium is intact or not, this factor was taken into consideration. The denuding process was performed by rubbing the artery between the thumb and forefinger and then using methyl acetylcholine (mACh) to test whether the artery was entirely denuded. If the artery had been denuded successfully the mACh would bind to receptors in the smooth muscle causing the artery to constrict. If the endothelium was left intact the artery would show a relaxation based on the mACh binding to receptors in the endothelium. Based on the results obtained a significant constriction was observed in both the denuded and

intact coronary arteries at or above 10^{-6} M and in both the pulmonary arteries at or above 10^{-5} M. This verifies that the denuded arteries were indeed denuded and that the intact arteries were probably partially denuded to some extent as well since no relaxation occurred.

The control that was used, ethanol, is a known vasodilator which was confirmed in the coronary arteries but showed a constriction in the pulmonary arteries. Further research needs to be done to explain why this constriction occurred when ethanol is a known vasodilator. It's important to note that pulmonary arteries constrict in response to ethanol as observed by Jakupi et al (1991).

Further research needs to be conducted in order to determine whether the Hawthorn extract truly acts as a vasodilator or not. It may be advantageous to find an actual source of Hawthorn and make the extract using the protocol from Kim et al (2000). From there further testing can be done to broaden the information known about Hawthorn and to better determine that it acts as a vasodilator and could therefore help people that are suffering from cardiovascular disease.

References:

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