The effect of *Crataegus Oxyacantha* θ on homocysteine levels in males

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DECLARATION

I declare that this dissertation is my own and unaided work. It is being submitted for the degree of Masters of Technology, Homoeopathy, at the University of Johannesburg. It has not been submitted before to any other institution to obtain a research diploma or degree. The study met all ethical research standards and was approved by the Committee for Academics Ethics, of the Faculty of Health Sciences of the University of Johannesburg on the 26 February 2010 with ethical clearance number AEC 11/02-2010, and higher degrees clearance number HDC 11/02-2010.

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Signature of candidate

The ____ day of ____ 2011

UNIVERSITY OF JOHANNESBURG
ABSTRACT

Cardiovascular disease and its complications accounts for about half of all deaths worldwide. As conventional risk factors do not successfully explain all of these cases, homocysteine (Hcy) appears to be a new and promising field to investigate as an accompanying risk factor for the development of cardiovascular disease (Stanger et al., 2004). Hyperhomocysteinemia, or elevated Hcy levels, have been shown to be directly linked to the development of cardiovascular disease (Wald and Morris, 2002).

Crataegus oxyacantha Mother Tincture (0) has been used over centuries for various cardiovascular disease conditions and is considered to have cardio-protective properties (Rose and Treadway, 1999), however its effect on homocysteine levels has not been researched.

The aim of this double-blind placebo-controlled study was to determine the effect of homoeopathically prepared Crataegus oxyacantha 0 on Hcy levels in males aged 25-35 years of age by measuring Hcy levels in the blood over a three week period. Participants attended an initial consultation where the procedure of the research was discussed, a short medical history was taken, and a full cardiovascular examination together with vital signs was assessed. Thereafter a pathology laboratory (Lancet laboratories) measured Hcy levels of the participants. Those participants that qualified for the study were divided into two groups of fifteen. The experimental group received a 25mL bottle of Crataegus oxyacantha 0 and the placebo group received a 25mL bottle of alcohol identical in appearance and taste. Participants were informed not to make any substantial changes to their diet and lifestyle. After three weeks a second Hcy test was completed and a follow up consultation was scheduled.

Collected data was statistically analyzed and a Chi Square goodness of fit test was utilized to determine if there was any significant decrease in Hcy levels in the participating individuals.

Preliminary findings suggest that Crataegus oxyacantha 0 was not effective in reducing plasma Hcy levels in adult males with Hcy levels of 6.3 mmol/L and higher, however more research over an extended period of time is needed to confirm these findings.
I dedicate this to all who supported me throughout the years.
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Supervisor: Dr J. Peilow
Co-supervisor: Dr U. Hohl
To all the participants in this study
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CHAPTER ONE

INTRODUCTION

1.1 Problem statement

Cardiovascular disease and its complications account for about half of all deaths world-wide and has a huge economic impact on society and our already over-burdened healthcare system (Stanger et al., 2004). There is therefore a need to focus on the prevention of cardiovascular disease. As conventional risk factors do not account for all cardiovascular deaths, additional factors should be recognized in order to minimize or lower the risk associated with cardiovascular disease. One such factor is high homocysteine levels, which are directly linked to cardiovascular disease (Wald and Morris, 2002).

Homocysteine is an amino acid produced by the body from the amino acid methionine, which is found in normal dietary protein (Holford, 2004). Ideally, homocysteine should be present in the blood in quantities less than 6.3 mmol/L (Robinson et al., 1995). If an individual is deficient in certain micro-nutrients, exercises excessively, smokes, abuses alcohol, uses certain medications, or has a specific genetic enzyme deficiency, homocysteine may elevate even further and this then increases the overall risk for developing cardiovascular pathology (Holford, 2004).

Numerous studies have indicated an independent relationship between mild hyperhomocysteinemia and cardiovascular disease (Stanger et al., 2004).

Crataegus oxyacantha Mother Tincture (Ø) has been used over centuries for various cardiovascular disease conditions and is considered to have cardio-protective properties (Rose and Treadway, 1999), due to the fact that it contains specific alkaloids which are responsible for its effect on the cardiovascular system (Chang et al., 2005).

Even though a volume of research has been conducted on the use of Crataegus oxyacantha Ø in the treatment of cardiovascular disease, no research has been undertaken on the effect thereof on homocysteine levels.
1.2 Aim of study

The aim of this study was to determine the effect of *Crataegus oxyacantha* Ø on homocysteine levels in healthy males by measuring homocysteine levels in the blood.

1.3 Importance of the study

It was projected that *Crataegus oxyacantha* Ø would have a statistically significant effect on the reduction of Hcy levels with the individuals that took part in this study. This would allow for further research into the field of Hcy, *Crataegus oxyacantha* Ø and its effects on the cardiovascular system.

1.4 Hypothesis

It was hypothesised that a regimen of 20 drops of *Crataegus oxyacantha* Ø taken orally three times daily, would be more effective than placebo in reducing Hcy levels in males.

1.5 Null hypothesis

The null hypothesis is that the homoeopathically prepared *Crataegus oxyacantha* Ø taken orally 3 times daily is not more effective than placebo in reducing Hcy levels in male individuals.
CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Cardiovascular disease and its complications account for about half of all deaths world-wide and has a huge economic impact on society and our already over-burdened healthcare system (Stanger et al., 2004). As conventional risk factors do not account for all cardiovascular deaths, additional factors should be recognized in order to minimize or lower the risk associated with cardiovascular disease.

One such factor is high $\textit{Hcy}$ levels, which are directly linked to cardiovascular disease (Wald and Morris, 2002) and numerous studies have indicated an independent relationship between mild hyperhomocysteinemia and cardiovascular disease (Stanger et al., 2004). There is therefore a need to focus on the prevention of cardiovascular disease. Homocysteine ($\textit{Hcy}$) is an amino acid produced by the body from the amino acid methionine, which is found in normal dietary protein (Holford, 2004). Ideally, $\textit{Hcy}$ should be present in the blood in quantities less than 6.3mmol/L (Robinson et al., 1995). If an individual is deficient in certain micro-nutrients, exercises excessively, smokes, abuses alcohol, uses certain medications, or has a specific genetic enzyme deficiency, $\textit{Hcy}$ may elevate even further and increase the overall risk for developing cardiovascular pathology (Holford, 2004).

$\textit{Crataegus oxyacantha}$ Mother Tincture ($\varnothing$) has been used over centuries for various cardiovascular disease conditions and is considered to have cardio-protective properties (Rose and Treadway, 1999), due to the fact that it contains specific alkaloids which are responsible for its effect on the cardiovascular system (Chang et al., 2005). Even though a volume of research has been conducted on the use of $\textit{Crataegus oxyacantha}$ $\varnothing$ in the treatment of cardiovascular disease, no research has been undertaken on the effect thereof on $\textit{Hcy}$ levels.
2.2 Homocysteine (Hcy)

Hcy is a non-protein forming, sulphur-containing amino acid manufactured by the body from normal dietary protein. The human body naturally turns Hcy into one of two beneficial substances, namely glutathione and S-Adenosyl Methionine (SAMe) (Solomon and Duda, 1998). Glutathione is one of the body’s most important anti-oxidants which neutralize free radicals in the body and SAMe is a methyl donor which helps to maintain chemical balance based upon its ability to add or subtract molecules called methyl groups (Holford and Braly, 2003).

2.2.1 History of Hcy

In 1932 Vincent DuVigneaud, an American biochemist, discovered a new amino acid from the removal of the methyl group of methionine. Since the amino acid methionine has the same functional group, amino group and carboxyl group as cysteine, DuVigneaud named it ‘homocysteine’ (Hofmann, 1987).

After the discovery of Hcy, this molecule was only regarded as an intermediate in methionine metabolism. Very little was known about the biomedical significance of Hcy up until further studies were done in 1962 (Hoffman, 1987).

In 1962 scientists in Belfast, Northern Ireland, began to study the urine of children with mental retardation for the presence of amino acids, using newly developed techniques of paper and column chromatography (McCully, 1969). Numerous children in the study were found to have elevated levels of Hcy in their urine, and hence the disease was called homocysteinuria. These children suffered from various other abnormalities in addition to mental retardation, including accelerated growth, dislocated ocular lenses, osteoporosis and other skeletal problems, and a tendency to develop cardiovascular pathology (Hoffman, 1987).

In 1969 Kilmer S. McCully was the first to hypothesize the relationship of severely elevated Hcy concentrations with premature atherosclerosis and cardiac pathology. These levels were documented at levels of 100mmol/L and above (McCully, 1969).
2.2.2 *Hcy* synthesis

*Hcy*, with the formula HSCH₂CH₂CH(NH₂)CO₂H, formed during the demethylation of methionine, is at the crossroads of two metabolic pathways: remethylation, which is methylcobalamin- and methyltetrahydrofolate-dependent; and transsulphuration, which is pyridoxal-5'-phosphate-dependent (Hoffman, 1987).

*Hcy* is the endogenous product of all transmethylation reactions that use SAMe as a methyl donor. Methionine intake and transmethylation activity determine the input of *Hcy* into the system. A certain amount of that *Hcy* is catabolically eliminated by trans-sulphuration to cysteine, however 30% in humans is conserved by remethylation to methionine, using two independent remethylation pathways. *Hcy* can be remethylated to methionine by the cobalamin dependent enzyme MS, using 5-methylfolate as co-substrate, which is supplied by mutated methylenetetrahydrofolate reductase (MTHFR) (Hoffman, 1987).

2.2.3 Determining *Hcy* Levels

A fasting blood sample obtained by a trained phlebotomist from a pathology laboratory is used to determine blood *Hcy* levels. One vial of an EDTA tube is used to collect 10mL of blood. The plasma is separated from the blood cells by centrifugation. This is done as soon as possible after collection as erythrocytes continue to export *Hcy* in collected whole blood. Aliquots of plasma are then used to determine *Hcy* by the use of a chromatograph (Solomon and Duda, 1998).

Fasting levels of *Hcy* are observed to stay constant over time for individuals in stable health without dietary changes (Refsum *et al.*, 2004). Fasting levels are recommended because a protein-rich meal can induce increases in *Hcy* levels for the next several hours (Verhoef *et al.*, 2005). This is explained by the digestion of dietary protein yielding the amino acid methionine which serves as a precursor for *Hcy*. Methionine loading is a research tool for investigating *Hcy* metabolism but is not recommended for routine diagnosis (Refsum *et al.*, 2004).

2.2.4 Normal *Hcy* levels

The normal *Hcy* level according to Lancet laboratories is a value less than 10mmol/L (Hepton, 2009). However, a large scale survey published in the American Heart Association Journal
Circulation stated that any Hcy score above 6.3mmol/L is associated with a greater risk of cardiovascular disease (Robinson et al., 1995).

Fig 2.1 shows the graphical presentation of Hcy and the ratio of risk to develop coronary artery disease.

(Sourced from Life Extension, 2003)

**Figure 2.1** Hcy and the ratio risk to develop coronary artery disease

Figure 2.1 above illustrates the results of the American Heart Association study: incremental increases in homocysteine levels correlate with increased risk for coronary artery disease. Levels of risk: 15.0=high risk; 9.0=moderate risk; 7.0=low risk (Life extension, 2003).

### 2.2.5 Homocystinuria

Homocystinuria or cystathionine beta synthase deficiency is the term referring to a genetic condition producing elevated levels of Hcy in the urine. The markedly elevated Hcy concentrations in classical homocystinuria are due to cystathionine beta synthase deficiency resulting in a reduced trans-sulphuration pathway activity (Fowler et al., 1971). This condition may cause different disorders in different systems of the body.
The most prevalent symptoms are associated with the ocular, musculoskeletal, central nervous and the cardiovascular systems (Mudd et al., 1985).

Homocystinuria is listed as a rare disease by the Office of Rare Diseases (ORD) of the National Institute of Health (NIH). This means that homocystinuria affects less than 200 000 people in the United States. The reported worldwide incidence is around 1 in 344 000 people (Yap, 2003).

2.2.5.1 Ocular symptoms

Ocular symptoms may include ectopia lentis, which is the dislocation of the lens from its normal position. This usually occurs after one year of age (Mudd et al., 1985) and according to Mulvihill et al., (2001) it rarely occurs during infancy.

2.2.5.2 Musculoskeletal symptoms

Individuals with homocystinuria are at risk for osteoporosis. The vertebrae and long bones of extremities are usually affected. For diagnostic purposes it is radio-graphically detected by imaging the lateral view of the lumbar spine of individuals. Fifty percent of individuals with homocystinuria will suffer from osteoporosis by the age of 13 (Yap, 2003).

According to Yap (2003), scoliosis is common in individuals with homocystinuria. Scoliosis is an abnormal curvature of the spine. Scoliosis is a common disorder diagnosed in girls aged between 10 and 16 years of age.

2.2.5.3 Cardiovascular symptoms

Vascular system complications such as thrombo-embolisms are usually the main cause of death in individuals with homocystinuria. Homocystinuria may affect any blood vessels in the body (Yap, 2003).

2.2.5.4 Central nervous system symptoms

Central nervous system developmental delays are the first abnormal signs in individuals with homocystinuria. Many individuals have psychiatric problems including personality disorders,
anxiety, depression, obsessive-compulsive behaviour, and psychotic episodes (Abbott et al., 1987).

2.2.6 Prevalence of elevated Hcy levels

Around 30% of elderly people, male and female, have elevated levels of Hcy. Vegetarians have a 29% elevated level of Hcy. These increased levels of Hcy in vegetarians are from the relative lack of methionine and vitamin B12 in the diets of vegetarians. Approximately 5% of omnivores are found to have elevated Hcy levels and 13% to 47% of symptomatic atherosclerosis patients have elevated levels of Hcy (Krajcovicova-Kudlackova et al., 2000).

Hcy levels are found to be higher in males compared to pre-menopausal women (Giltay et al., 1998). Slightly elevated levels of Hcy are noted in menopausal females. It is hypothesized that the lower levels of Hcy in menopausal women and males are largely due to the fact that progesterone, which is a hormone predominately excreted by the female, may have a Hcy lowering effect (Holford, 2004).

2.3 Conditions associated with elevated Hcy levels

Increased levels of Hcy have been linked to several conditions including cardiovascular, central nervous system and metabolic disorders (Holford, 2004).

2.3.1 Hcy and heart disease

Excessive Hcy levels are not the only risk factor for many forms of cardio-vascular diseases, however excess Hcy correlates with the occurrence of cardiovascular disease more closely compared to elevated levels of cholesterol (Graham, 1997).

2.3.1.1 Hcy and atherosclerosis

Elevated levels of Hcy stimulate atherosclerosis. The three main processes in which elevated levels of Hcy stimulates the formation of atherosclerosis are by increasing oxidative stress, impairing endothelial function and the induction of thrombosis (Guthikonda and Haynes, 2006).
2.3.1.1 \textit{Hcy} and oxidative stress

A study performed to determine how \textit{Hcy} plays a role in oxidative stress notes that \textit{Hcy} induces oxidative stress by up-regulating protease activated receptors (PAR) and in turn promotes production of reactive oxygen species (ROS). PAR’s sense various molecules outside cells and activate inside signal transduction pathways while ROS is the main chemical molecule in oxidative stress situations (Tyagi \textit{et al.}, 2005).

2.3.1.2 \textit{Hcy} and impaired endothelial function

\textit{Hcy} impairs endothelial functioning through compromised vascular endothelial growth factor (VEGF). VEGF is a signalling protein which is produced by the cells that is responsible for vasculo-genesis and angiogenesis. These two processes are part of the system that restores the oxygen supply to tissues when blood circulation is inadequate (Yan, \textit{et al.}, 2010).

2.3.1.3 \textit{Hcy} and induction of thrombosis

Induction of thrombosis occurs when \textit{Hcy}, at specific relevant concentrations, induces the expressions of tissue factor by monocytes. Tissue factor is also known as platelet tissue factor, factor III, thrombokinase, or CD142 and is responsible for blood coagulation (Khajuria and Houston, 2000).

2.3.1.2 \textit{Hcy} and blood clotting

It has been proposed that excessive \textit{Hcy} may cause abnormal blood clotting by inhibiting the production of tissue plasminogen activator. This in turn inhibits the breakdown of fibrinogen and leads to increased risk of abnormal blood clotting (Faloon, 2001).

Fibrinogen is a soluble glycoprotein produced by the liver. Fibrinogen is converted by thrombin to fibrin during the normal physiological process of blood coagulation (Kessler, 2007).

2.3.2 \textit{Hcy} and ageing

During a study to determine if \textit{Hcy} may accelerate endothelial cell senescence, Xu \textit{et al} (2000) discovered that \textit{Hcy} accelerates the shortening of telomeres. Telomeres are a region on DNA at
the end of a chromosome, which protects the chromosome from deterioration and which play an important role in cell providence and ageing by altering cellular response to stress and growth stimulation on the basis of previous cell division and DNA damage (Aubert and Lansdorp, 2008).

An endothelial-cell-culture test system was set up in order to examine the effects of \( \text{Hcy} \) on cell senescence. \( \text{Hcy} \) increased the amount of telomere length lost per population of almost a three-fold increase in telomere shortening indicating that increased \( \text{Hcy} \) levels increases the process of aging (Xu et al., 2000).

### 2.3.3 \( \text{Hcy} \), Crohn’s disease and ulcerative colitis

Crohn’s disease is an auto-immune inflammatory bowel disease which is also known as regional enteritis. Crohn’s disease may affect any part of the gastro-intestinal tract from the mouth to the anus. Crohn’s disease may present with a wide variety of symptoms, which includes abdominal pain, diarrhoea, vomiting and weight loss (Baumgart and Sandborn, 2007).

Ulcerative colitis is a form of an inflammatory bowel disease which is usually limited to the colon. Ulcerative colitis is characterised by inflammation, ulcers or open sores and symptoms may include diarrhoea and / or haematochezia. It differs from Crohn’s disease in terms of the area of the gastro-intestinal tract effected (Lennard-Jones, 1989).

\( \text{Hcy} \) may exacerbate and even be an underlying cause of Crohn’s disease and ulcerative colitis according to Danese et al (2005).

Increased \( \text{Hcy} \) contributes to the pathophysiology of several chronic inflammatory bowel diseases. \( \text{Hcy} \) is increased in both the mucosa and plasma of patients with Crohn's disease and ulcerative colitis and contributes to the inflammatory state of the mucosal endothelium (Danese et al., 2005).

### 2.3.4 \( \text{Hcy} \) and age-related hearing loss

Hearing impairment is one of the 4 most common conditions in geriatric patients. It is also noted that the biological basis of age related hearing loss in unknown (Houston et al., 1999).
According to the authors of a study published in the American Journal of Clinical Nutrition, elevated $Hcy$ levels are considered to be an underlying risk factor for age-related hearing loss. This may be attributed to high levels of $Hcy$ adversely affecting blood circulation to the cochlea (Houston et al., 1999).

Contradictory to the above study of Houston et al., (1999), a study performed in 2000 showed that there was no association between hearing level and vitamin B12 or folic acid deficiencies in elderly patients (Berner, 2000). Further studies need to be conducted in order to fully understand the relationship between $Hcy$ and hearing loss.

### 2.3.5 $Hcy$ levels and age-related macular degeneration

Age-related macular degeneration (ARMD) is a debilitating ocular condition, which may result in permanent blindness. Snow and Seddon (1999) note that ARMD is the leading cause of irreversible vision loss and blindness in the United States.

Kamburo et al (2005) and Nowak et al (2005) substantiate that $Hcy$ is a risk factor for the development of ARMD.

A study published in the Ophthalmic Journal of Epidemiology states that the aetiology of ARMD remains unknown, although some epidemiological findings point to a cardiovascular risk profile among individuals being affected. This may point out that the causal pathways for cardiovascular disease and ARMD may be the same (Snow and Seddon, 1999).

Christen et al., (2009) studied the effect of a daily supplement containing vitamin B6, B9 and B12, which are known to reduce $Hcy$, in females with a high risk of cardiovascular disease. They found that the treatment may reduce the risk of age-related macular degeneration.

### 2.3.6 $Hcy$ and cancer

Wu and Wu (2002) performed a study to determine if hyperhomocysteinemia is a risk factor for cancer. They proposed not only is $Hcy$ a well known risk factor for all types of cancer, but that $Hcy$ could be used as a tumour marker for various types of cancer.
In another study plasma $Hcy$ levels were measured in breast cancer patients and in healthy controls. Women with the highest plasma $Hcy$ levels had a 2.89-fold increased risk of breast cancer compared to women with the lowest level of plasma $Hcy$. Moreover, a similar pattern of enhanced breast cancer risk at higher plasma $Hcy$ levels was observed in both pre-menopausal and post-menopausal women (Chou et al., 2006).

It is important to note the fact that the study performed by Wu and Wu (2002) was done on individuals that were not treated with anti-folate drugs, which may have shown elevated $Hcy$ levels.

Weinstein et al (2001) explored the relationship between serum $Hcy$ and invasive cervical cancer. A large case-control study was conducted in five areas in the United States. In addition to the $Hcy$ screening, exposure to human papillomavirus (HPV) type 16, the most prevalent oncogenic type, was assessed using an enzyme-linked immunosorbent assay. Invasive cervical cancer risk was substantially elevated for women who fell into the higher level $Hcy$ group ($>6.31$ mmol/L). Serum $Hcy$ was strongly and significantly predictive of invasive cervical cancer risk.

### 2.3.7 $Hcy$, Alzheimer’s and dementia

According to Brookmeyer et al (1998) Alzheimer’s disease accounts for more than seventy percent of all cases of dementia. Vascular factors are now considered to be an underlying cause of Alzheimer’s disease. Hofman et al (1997) states that it is now recognised that patients with cardiovascular risk factors and a history of stroke have an increased risk of both vascular dementia and Alzheimer’s disease.

Elevated $Hcy$ has been associated with poor levels of cognition and dementia in cross sectional studies. Seshadri et al., (2002) states that with plasma $Hcy$ levels greater than 14 mmol/L, the risk of Alzheimer’s disease nearly doubles.
2.4 Substances affecting levels of $Hcy$

2.4.1 Substances that may lower $Hcy$ levels

Current nutraceutical formulations used in the treatment of high $Hcy$ levels consist of various micro-nutrients namely riboflavin, pyridoxine, cobalamin, inositol, zinc, folic acid and tri-methyl glycine. These micro-nutrients are directly involved in the enzymatic processes which are responsible for the conversion of $Hcy$ to SAMe and glutathione. If the diet does not provide optimal amounts of these micro-nutrients, the enzymes that convert $Hcy$ are impaired, thus leading to raised $Hcy$ levels (Holford, 2004).

2.4.1.1 Vitamin B2

Vitamin B2, also known as riboflavin, is a micronutrient that plays an important role in health in humans. Vitamin B2 is commonly found in dietary products such as milk, cheese, leafy green vegetables, liver, kidneys, legumes, tomatoes, mushrooms and almonds (Jane and Drake, 2007).

In one study a group of individuals received 1.6mg/d of vitamin B2, in which $Hcy$ levels were measured and compared to a placebo group over a period of 12 weeks. It was concluded that supplementation of vitamin B2 over a period of 12 weeks was effective in reducing total $Hcy$ levels in the participating individuals (McNulty et al., 2006).

2.4.1.2 Vitamin B6

Vitamin B6 or pyridoxine as its other commonly used name, is a water soluble vitamin in the B complex family. It mainly plays a role in macro-nutrient metabolism of protein and carbohydrates. Vitamin B6 may be found in meats, vegetables, nuts and bananas (McCormick, 2006).

Results published from a study in the Journal of the American Medical Association states that the intake of vitamin B6 above the recommended dosages may lower $Hcy$ and may be important in the primary prevention of coronary heart disease (Rimm et al., 1998).
2.4.1.3 Vitamin B9

Vitamin B9 is more commonly known as folic acid or folate. Folic acid also belongs to the water soluble B vitamin family. Folic acid is responsible for the repair and maintenance of DNA and is also very important for cell division and cell growth (Litwack, 2008).

In 1996 the American Food and Drug Administration issued a regulation that all enriched grain food products are required to be fortified with folic acid to reduce neural tube defects. A study was performed to assess the effect of folic acid fortification on folate status. In the study they measured plasma folate and total $Hcy$ levels. In the outcome of the study it was noted that the prevalence of $Hcy$ concentrations decreased from 18.9% to 9.8% (Jacques et al., 1999).

2.4.1.4 Vitamin B12

Vitamin B12, also known as cobalamin, is also one of the water soluble vitamins of the B complex family. Although it has many functions it mainly plays a role in the functioning of the central nervous system, nerve functioning and various enzymatic processes (Sareen, 2009).

In a review for a Guide for the Primary Care Physician laboratory tests state that with supplementation of vitamin B12 a lowering effect on $Hcy$ may be seen, and a total $Hcy$ serum test may be used to identify or diagnose atypical and subclinical vitamin B12 deficiencies (Snow, 1999).

Figure 2.2 illustrates the role where the B complex vitamins and co-factors influence $Hcy$ metabolism.
2.4.1.5 Betaine

Betaine, which is also known as trimethyl glycine (TMG), is an amino acid commonly found in small amounts in quinoa, spinach, wheat bran and lamb (Holford and Braly, 2003).

The effect of betaine was studied on plasma Hcy levels. In this study 11 males and 24 females were given 6000mg of betaine over a period of time. The mean Hcy concentration decreased and further decreased with the addition of 1000mg of folic acid (Alfthan et al., 2004).

TMG provides a methyl group in the conversion of Hcy to methionine by the enzyme betaine-homocysteine methyltransferase. Therapeutic doses of betaine (TMG) are at least partially effective in lowering high homocysteine levels. One molecule of TMG can maximally remethylate four molecules of homocysteine (Kalkiri, 2003).
2.4.1.6 Choline

Choline is a water soluble nutrient chemically related to the B complex vitamins although it is not considered as a B complex vitamin. It plays an important role in the structure of cell membranes. Choline containing foods include almonds, beef and beef liver, cauliflower, eggs and tofu (Nix, 2005).

Canty (1998) states that choline may help to lower \( \text{Hcy} \) via its metabolism within the body to form betaine. Betaine, which may be converted to choline, is about as effective as pyridoxine or folate in normalizing hyperhomocysteinaemia (Dudman, 1993).

2.4.1.7 Arginine

Arginine is a non essential amino acid which can be manufactured by the body. The best sources include dairy products such as cottage cheese, ricotta, milk, yogurt and whey protein. Plant sources include wheat germ, buckwheat, granola and nuts. Arginine serves as a precursor to nitric oxide, reduces healing times after injury and assists in lowering elevated blood pressure (Stargrove, 2008).

West et al (2005) studied the effect of l-arginine and the hemodynamic responses to stress, and whether it may reduce \( \text{Hcy} \) levels in hypercholesterolemic men. He noted that when administered intravenously, l-arginine substantially reduces blood pressure and peripheral vascular resistance in healthy adults and in patients with vascular disease. Plasma \( \text{Hcy} \) decreased on average by 2mmol/L in both healthy and unhealthy individuals during the study (West et al., 2005).

2.4.1.8 Creatine

Creatine is an amino acid manufactured by the liver, pancreas and kidneys that naturally occurs in the human body. Its primary function is to supply energy to the cells of muscles. It is a very common dietary supplement among athletes to enhance endurance and performance (France, 2004).

Supplemental creatine monohydrate is hypothesized to lower elevated \( \text{Hcy} \) levels by “sparing” methyl groups normally used for the production of endogenous creatine, permitting these methyl groups to be utilized for the proper metabolism of \( \text{Hcy} \) (Korzun, 2004).
2.4.1.9 N-acetyl-cysteine (NAC)

N-acetyl-cysteine is an amino acid and is used for various disease conditions which includes: acetaminophen overdosing, strengthening the immune system, lowering cholesterol, counteracting liver toxicity and fighting infections (Murray, 2000).

N-acetyl-cysteine may lower $Hcy$ levels in females. A study done on healthy non-pregnant females presented quick and highly significant results. Not only did plasma $Hcy$ levels decrease, plasma concentrations of the antioxidant glutathione increased (Roes and Rainmaker, 2002).

2.4.1.10 Dietary Fibre

Dietary fibre is the indigestible portion of an edible plant. It is divided into two main categories, namely soluble and insoluble. Examples of foods containing soluble fibre include legumes, oats, rye, prunes, plums, broccoli and psyllium seed husks. Examples of insoluble fibre foods include wheat, corn bran, nuts seeds, potato skins, flax seeds and tomato skins (Cherbut, 1995).

Increased consumption of dietary fibre is widely recommended to maintain or improve health, but knowledge of the relation between dietary fibre sources and cardiovascular disease risk factors is limited. Lairon et al (2005) examined the relationship between the source or type of dietary fibre intake and cardiovascular disease risk. The highest total dietary fibre and non-soluble dietary fibre intakes were associated with a significantly ($p < 0.05$) lower risk of overweight and elevated waist-to-hip ratio, blood pressure, plasma apolipoprotein (apo) B, the apo B:apo A-I ratio, cholesterol, triacylglycerol, and $Hcy$.

Soluble dietary fibre was shown to be less effective. Fibre from cereals was associated with a lower body mass index, blood pressure, and $Hcy$ concentration; fibre from vegetables with a lower blood pressure and homocysteine concentration; and fibre from fruit with a lower waist-to-hip ratio and blood pressure (Lairon et al., 2005).

Dietary fibre intake is inversely correlated with several cardiovascular disease risk factors in both sexes including $Hcy$, which supports its protective role against cardiovascular disease and recommendations for its increased consumption (Lairon et al., 2005).
2.4.1.11 Thyroxine

Levothyroxine is also known as l-thyroxine, or commonly abbreviated as T4, and is a common synthetic form of thyroxine used as a hormone replacement in patients with thyroid conditions (Vaidya and Pearce, 2008).

In a small study published by the Annuals of Internal Medicine found that the use of l-thyroxine in patients presenting with hypothyroidism lowered Hcy levels. The study took place over a 3 month period and the plasma Hcy levels increased again after termination of treatment with thyroxine (Hussein et al., 1999).

2.4.1.12 Omega-3 Fatty Acids

A study published by Li et al (2006) investigated the possibility of a relationship between plasma Hcy and poly-unsaturated fatty acids in healthy Australian males. Evidence from this study indicated that an increased concentration of poly-unsaturated fatty acids (n-3 PUFA) in tissues has a beneficial effect on cardiovascular health. These findings provide further evidence that increased consumption of dietary n-3 PUFA increases the concentration of n-3 PUFA in plasma phospholipids, which is associated with a protective effect against cardiovascular diseases and lower plasma Hcy levels. The mechanism that might explain the association between plasma n-3 PUFA and Hcy levels however is not clear (Li et al., 2006).

2.4.1.13 Aspirin

Asprin is a salicylate drug, which is commonly known as acetylsalicylic acid, and is used for its analgesic, antipyretic, anti-inflammatory and anti-platelet effects (Millwood, 2007).

According to Schroeksnadel et al., (2005) aspirin not only regulates the formation of Hcy in humans but is also very effective in reducing inflammation, which is another known risk factor in cardiovascular disease and neurodegenerative diseases.

2.4.2 Substances that may increase Hcy levels

2.4.2.1 Fructose
Fructose is a simple sugar naturally found in fruits, although it is a common sweetener added to various processed foods. It is sweeter than sucrose or glucose when compared with equal amounts. For this reason, and its inexpensive cost, it is used as a common bulk sweetener in many foods (Rizkalla, 2010).

Syndrome X is a group of several metabolic disorders that includes hyperinsulinemia, hypertriglyceridemia, and hypertension and is associated with severe vascular morbidity (Garcia-Larajhu et al., 2010).

Hyperhomocysteinemia is a risk factor for cardiovascular and cerebrovascular diseases, often exhibited by insulin-resistant patients (Fanapour et al., 1999). Oron-Herman (2003) investigated the relationship between syndrome X and hyperhomocysteinemia in a rat model. Two groups of rats were fed either a fructose-enriched diet or standard rat chow for 5 weeks. Systolic blood pressure (SBP), as well as fasting plasma insulin, triglycerides, total cholesterol, and total Hcy levels were determined at the beginning and at the end of the study. Hcy concentration was 72% higher after 5 weeks on the fructose diet (Oron-Herman et al., 2003).

2.4.2.2 Chlorogenic Acid

Chlorogenic acid is a major polyphenol in coffee and black tea and has been known to be a very potent anti-oxidant (Johnston, 2003).

The effect of chlorogenic acid on Hcy levels was studied in 20 males and 20 females who regularly consumed coffee on a daily basis. In the study chlorogenic acid was consumed by the individuals in amounts of up to 1000 mg per day. It was noted that consumption of chlorogenic acid ingested from coffee raised total Hcy levels in plasma (Olthof et al., 2001).

The study on the effect of chlorogenic acid and Hcy was also simultaneously compared with the effect of quercetin-3-rutinoside. Quercetin-3-rutinoside is one of the major flavonols in tea and apples. It was noted that quercetin-3-rutinoside had no significant effect on Hcy concentration (Olthof et al., 2001).
2.4.2.3 Metformin

Metformin is a common oral drug in the biguanide classification of anti-diabetic drugs. It is commonly prescribed as a first line of treatment to control elevated blood glucose levels in patients presenting with type 2 diabetes (Stargrove, 2008).

A study was performed on 40 individuals with non insulin dependent diabetes mellitus (NIDDM) who received a dosage of metformin of 500 -2550mg per day for 6 months and longer, and 71 individuals with NIDDM not treated with metformin. The total Hcy was compared between the 2 groups. The results of the study confirmed the elevating effect of metformin on total serum Hcy in NIDDM patients (Hoogeven, 1997).

Metformin-induced increases in Hcy occur as a result of metformin-induced malabsorption of vitamin B12. This negative effect can be counteracted by using supplemental vitamin B12 in conjunction with metformin (Carlsen, 1997).

2.4.2.4 Methotrexate

Methotrexate is classified as an anti-metabolic drug which blocks the metabolism of cells in the body. It is commonly prescribed in certain diseases which are associated with rapid cell division and growth such as cancer and psoriasis (Cronstein and Bertino, 2000).

Mindell (1997) notes that elevated Hcy levels can occur as a result of methotrexate therapy due to methotrexate causing the depletion of folic acid.

2.4.2.5 Alcohol (ethanol)

Barak et al (2001) studied the effect of alcohol on Hcy. Results of the study showed that chronic ethanol administration impairs methionine synthetase activity and decreases SAMe levels in the liver, indicating interference with Hcy remethylation.

Cravo et al (1996) notes that beer consumers had significantly lower concentrations of Hcy compared with drinkers of wine or spirits. The results of the study suggest that interference with folate or vitamin B6 metabolism through chronic alcohol intake may impair the disposal of Hcy through the transmethylation or trans-sulfuration pathways.
The reason for total lower $Hcy$ levels with individuals drinking beer compared to wine and spirit drinkers is due to the vitamins contained in beer. More specifically it is the beneficial vitamin B6 contained in beer which is regarded as the origin of the $Hcy$ lowering effect of beer (Schlienger, 2003).

Carmel and James (2002) states that alcohol abuse is an important risk factor for severe hyperhomocysteinemia. Alcohol has a complex direct effect on $Hcy$ metabolism, and indirect effects mediated by interactions with vitamin metabolism and other factors. Both transmethylation and trans-sulfuration pathways are affected. Alcohol abuse is a common cause of hyperhomocysteinemia that often fluctuates and is sometimes severe.

Robinson et al (2005) states that raised plasma $Hcy$ levels in alcoholism are one of the primary causes of dementia and heart disease.

2.4.2.6 Tobacco

According to Obczack (2003), there is evidence that certain lifestyle factors such as cigarette smoking may affect $Hcy$ levels.

The effects of smoking on the cardiovascular system were studied on 1432 individuals that underwent percutaneous transluminal coronary angioplasty (PTCA). PTCA is primarily performed to improve health related quality of life in patients with symptomatic coronary artery disease. Beside the fact that $Hcy$ increases are noted, he further explains that smoking causes endothelial dysfunction, dyslipidemia, increases fibrinogen, C-reactive protein and insulin resistance. These are the most important risk factors to consider in preventing cardiovascular disease. The beneficial effects of statins and anti-oxidants for cardiovascular disease are also counteracted by smoking (Tsiara et al., 2003)

2.4.2.7 Niacin

The term niacin refers to nicotinamide (nicotinic acid amide), nicotinic acid (pyridine-3-carboxylic acid), and derivatives that exhibit the biological activity of nicotinamide (Institute of Medicine Report U.S., 1998).
Niacin (vitamin B3) is a member of the water soluble B complex group and can be found in many different food sources including liver, kidneys, chicken, beef, tuna, salmon, milk, eggs, avocados, dates, carrots, nuts, legumes and mushrooms (Insel et al., 2010).

Both Basu et al (2002) and Garg et al, (1999) studied the $Hcy$ elevated effect that may be experienced with the consumption of high levels of niacin. The underlying physiological effect may be explained through that niacin is excreted as methylated pyridines requiring methionine as a methyl donor, which thus in turn increases $Hcy$.

The study performed by Basu et al (2002) used dosages of niacin at 400 – 1000mg/kg on Sprague-Dawley rats and the study of Garg et al (1999) was performed on humans with dosages of 100-1000mg of niacin per day.

2.4.3 Exercise and its affect on $Hcy$ levels

The relationship between physical exercise and total levels of $Hcy$, vitamin B12 and folic acid has not been fully examined. König et al (2003) investigated the influence of extensive endurance training and acute intense exercise on 42 male triathlon athletes. It was concluded that although intense exercise acutely increased $Hcy$ levels, chronic endurance exercise was not associated with higher $Hcy$ levels.

Interestingly it was noted in the same study that athletes with the highest training volume also had the highest plasma folate levels, and showed a decrease in $Hcy$ levels following the training period as well as much lower increase of the $Hcy$ after acute intense exercise (König et al., 2003).

2.5 Crataegus oxyacantha Ø

2.5.1 Introduction

Different species of Crataegus have been used all over the world as medicinal plants. In European herbal medicine hawthorn is commonly used for various heart diseases. In some European countries hawthorn preparations are approved drugs for the treatment of mild forms of heart insufficiencies including Germany, Austria and Switzerland. In other countries such as the USA, it is regulated as a dietary supplement (Preedy and Watson, 2008).
2.5.2 General descriptions of *Crataegus oxyacantha* Ø

*Crataegus* is part of the rose family and is a spiny tree or shrub, which may reach a height of 8 feet. It bears small white five-petaled blossoms in late spring, which then changes to red berries in the summer (Balch, 2002).

2.5.3 Habitat and Cultivation

The small spiny tree is widespread throughout northern and eastern Europe and north eastern America in temperate zones (Stargrove, 2008).

2.5.4 History of *Crataegus oxyacantha* Ø

Historically, *Crataegus* fruits have been used for many years before the healing properties of the flowers and the leaves became part of medicinal use (Stargrove, 2008).

A physician, Dr Green, attained an extended reputation in the treatment of heart disease keeping his remedy a secret. At the time of his death in 1894, his daughter revealed the fact that this ‘famous cure’ was a tincture of the ripe berries of *Crataegus oxyacantha* (Anon, 2011).

2.5.5 Nomenclature

*Crataegus* is also known as Hawthorn, Haw, Hedgethorn, May bush, May blossom, May Day Flower, Ban-sangli and White Thorn. All of these names correspond to the genus *Crataegus*. It originates from the Greek word ‘kratos’ which directly translates as ‘hardness of wood’ and is a member of Rosaceae family (Amy *et al.*, 2006).

Hawthorn includes the species *Crataegus monogyna*, *Crataegus laevigata*, *Crataegus rhipidophylla* *Crataegus douglas*, *Crataegus colombian*, *Crataegus cuneata*, *Crataegus pinnatifida*, and other *Crataegus* species, which are all used interchangeably with *Crataegus oxyacantha* (Deutscher Apotheker Verlag, 2000).
2.5.6 The chemical composition of *Crataegus oxyacantha* Ø

Flavanoids are a class of secondary metabolites originating from plants which play no role in the growth and development of plants (Grotewold, 2008).

The flavanoids spectrum of *Crataegus* consist of quercetin, hyperoside, rutin, flavonoglycosyls, and vitexin-4'-rhamnoside. It also consists of glycosides, oligomeric proanthocyanidin, epicatechol, anthocyanidins, saponins and tannins (Verma et al., 2007).

Other chemical constituents include cratetegen; cardiotonic amines (phenylethylamine, tyramine, isobutylamine, omethoxy phenylethylamine); choline and acetylcholine; purine derivatives (adenosine, adenine, guanine, and caffeic acid); amygdalin; pectins and triterpene acids (ursolic acid, oleonic acid and crategolic acid) (Verma et al., 2007).

*Crataegus oxyacantha* Ø contains two very specific alkaloids: hyperoside and procyanidin (Bahorun, 1994), which are responsible for its protective effect on the cardiovascular system (Chang et al., 2005).

Hyperoside is a chemical compound that is classified as the 3-0-galactoside of quercetin. The medicinally active compound may also be isolated from plants such as *Drosera rotundifolia, Stachys, Prunella vulgaris, Rumex acetosella*, and St. Johns Wort (Lui et al., 2005). Hyperoside also demonstrates an inhibitory effect on thromboxane A2 (Vibes et al., 1994).

The plant also consists of high doses of vitamin C (Verma et al., 2007).

2.5.7 Parts used of *Crataegus oxyacantha*

Although the leaves, flowers and berries are most commonly used, the fruits and roots are also used traditionally, but lack of comprehensive pharmacological and clinical data makes the use unclear (Stargrove, 2008).

2.5.8 Clinical application of *Crataegus oxyacantha* Ø

2.5.8.1 *Crataegus* and cardiac failure
The main indication for the use of *Crataegus* is cardiac failure and modulation of blood pressure (Schmidt *et al.*, 1994). Stansbury (1990) states that *Crataegus oxyacantha* may function similarly to calcium channel blockers due to the active flavanoids present in the plant, resulting in its effect on blood pressure.

Heart failure, also known as congestive cardiac failure, is defined as the inability of the heart to supply sufficient blood flow to meet the rest of the body’s needs (McMurray and Pfeiffer, 2005). The functional classification relies on the New York Heart Association Functional Classification Criteria Committee, New York Association (1964). Table 2.1 shows the different classification of heart failure on the New York Heart Association Functional Classification system.

<table>
<thead>
<tr>
<th>Class I</th>
<th>No limitation is experienced in any activities; there are no symptoms from ordinary activities.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class II</td>
<td>Slight, mild limitation of activity; the patient is comfortable at rest or with mild exertion</td>
</tr>
<tr>
<td>Class III</td>
<td>Marked limitation of any activity; the patient is comfortable only at rest.</td>
</tr>
<tr>
<td>Class IV</td>
<td>Any physical activity brings on discomfort and symptoms occur at rest</td>
</tr>
</tbody>
</table>

Table 2.1 Classification of heart failure (Criteria Committee, New York Association, 1964).

Congestive heart failure typically presents clinically with shortness of breath, hypoxemia, hypotension and renal insufficiency (Poppas and Rounds, 2002).

*Crataegus* is clinically indicated for strengthening the heart muscle thereby reducing heart congestion (Fong and Bauman, 2002) and dilating coronary blood vessels (Zapfe, 2001). *Crataegus oxyacantha* Ø moderates blood pressure and also improves oxygen supply to the heart (Schmidt *et al.*, 1994). It improves the health of the blood vessels by decreasing the size of existing atherosclerotic plaques, promotes the integrity of the collagen content of blood vessels and aids in lowering cholesterol levels (Weimayr and Ernst, 1996).
**2.5.8.2 *Crataegus oxyacantha* Ø in the treatment of angina**

In 1979, Petkov first noticed the beneficial properties of *Crataegus oxyacantha* in patients presenting with angina in a study researching plants with hypotensive, anti-atheromatous and coronary dilating properties (Petkov, 1979).

A study conducted by Hanack and Briickel (1983) found that 180mg of *Crataegus oxyacantha* standardised to contain 18.75% oligomeric proanthocyanidin, one of the most important constituents in *Crataegus oxyacantha*, improved heart function and further improved the ability to exercise in angina patients.

In the late 90’s Miller (1998) and Stansbury (1990) further validated the beneficial properties of *Crataegus oxyacantha* as a cardiac tonic for the treatment of angina.

**2.5.8.2 *Crataegus oxyacantha* Ø and arrhythmias**

Miller (1998) describes that *Crataegus oxyacantha* has been used traditionally as a cardiac tonic and current uses include the treatment of arrhythmias.

Newer studies are still being performed that validate the beneficial effect of *Crataegus* on arrhythmias. Chang (2004) states that with the rise of use of vitamins and herbal supplements in the general population, it is very important to assess the potential impact of these supplements on cardiac arrhythmias. Some may have a proarrhythmic effect either alone or with other supplements or prescribed medication. According to Chang (2004), *Crataegus* is one of the more effective and safer anti-arrhythmic agents affecting the cardiovascular system.

**2.5.8.3 *Crataegus oxyacantha* and the effect on collagen of the arterial system**

*Crataegus oxyacantha* may improve the structural integrity of the collagen content of arteries due to the high content of oligomeric proanthocyanidin, which has protective properties on collagen of the cardiovascular system (Murray, 1993). The collagen content is responsible for the structural integrity of the arterial system of the body (Pope and Nicholas, 1975).

*Crataegus oxyacantha* may also improve the integrity of the collagen content of blood vessels, by lowering total cholesterol and the size of existing atherosclerotic plaques, which has an undeviating harmful effect on the collagen of blood vessels (Kovach, 1959; Schussler, 1995)
2.5.8.4 Crataegus oxyacantha and coronary blood flow

One of the key actions of Crataegus oxyacantha is to improve circulation in the cardiovascular system and more specifically to the heart. This may occur as a result of Crataegus oxyacantha relaxing the coronary arteries or via an increase in contraction and relaxation velocities, which increases the diastolic interval, allowing more time for blood to pass through the coronary arteries (Hawthorn, 2000).

2.5.8.5 Crataegus oxyacantha and oxygen utilisation

In a study performed by Echte (1960), on the effects of Crataegus oxyacantha on the cardiovascular system, Echte noted that Crataegus oxyacantha improves the utilization of oxygen by the muscles of the heart and further improves blood circulation to the heart.

Kandziora (1969) measured the effect of Crataegus oxyacantha berries on oxygen utilization by the heart during exercise in coronary perfusion patients. Crataegus oxyacantha treatment resulted in a 77% reduction in oxygen utilization compared to a 25% reduction in patients receiving orthodox treatments. Kandziora (1969) concluded that Crataegus oxyacantha exerts an oxygen-sparing effect on heart muscle that is under stress.

A Crataegus extract prepared from the leaves and flowers standardised to 2.2% flavonoids was investigated on its effect on refractory period, contraction behaviour and oxygen consumption of the heart. The result of the study stated that the isolated cardiac cells were more efficient in oxygen consumption with treatment of Crataegus extract compared to beta-antagonists and cardiac glycosides (Poeping, 1994).

2.6 Therapeutic Dosages of Crataegus oxyacantha Ø

The recommended safe therapeutic dosage for Crataegus oxyacantha herbal tincture is twenty drops three times daily 15 minutes before or after a meal (Murray and Pizzorno, 2000).

Bone (2005) describes the typical adult dosages as follows

- 0.75 to 6g/day of dried flower, leaf or by infusion
- 3-6mL/day of 1:2 liquid extract of hawthorn leaf
- 3.5 to 17.5mL/day of 1:10 tincture of hawthorn leaf

Herbal extracts and herbal tinctures are made from a 25% alcohol and water mixture as the solvent. Usually extracts are more concentrated than herbal tinctures, because they distill off some of the alcohol. A tincture is typically 1:10 or 1:5 concentrations while an extract is 1:1 (Rector-Page, 1998).

Cardioactivity has been observed in vitro and in animal and human clinical trials. In general, preparations administered orally are reported to have a more prolonged effect than those administered parenterally (Ammon & Kaul, 1994).

Homeopathic Mother Tinctures are prepared from the fresh embryonic parts of the plant in a 1:10 ratio. These mother tinctures are typically prepared in glycerine. Recommended dosages for homeopathic Mother Tinctures is usually 1-5 drops although the dose may be increased to 10, 20 and even 30 drops per day (Chatterjee, 2003).

2.7 Contra-indications

Safety during breast-feeding has not been established (Medicine net, 2005). *Crataegus oxyacantha* Ø should not be used in pregnancy because of its demonstrable action on the uterus through reduction of tone and motility as shown in vivo and in vitro studies (Ammon & Handel, 1981).

2.8 Drug Interactions

2.8.1 Digitalis

In 1994, Djumlija discouraged the use of *Crataegus oxyacantha* Ø, as it was thought to potentiate the therapeutic effects of digitalis.

*Crataegus oxyacantha* Ø interactions are likely with agents that have an effect on the cardiovascular system. It has shown synergy with digitalis by enhancing the effect of cardiac glycosides. This effect is thought to be due to an inhibitory effect on cAMP-PDE and thus effects calcium channels (McGuffin, et al., 1997).
In 1998 Mashour et al. states that more studies are undoubtedly needed to effectively and safely use Digitalis with Crataegus.

### 2.8.2 Beta blockers

Beta blockers, which are also known as beta adrenergic blocking agents, are pharmaceutical drugs commonly used for arrhythmias, migraines, hypertension and cases of angina (Khan, 2007).

Combining beta blockers (which are commonly prescribed to lower blood pressure) with Crataegus oxyacantha may increase blood pressure in hypertensive patients (Barnes et al., 1996).

### 2.9 The effect of Crataegus oxyacantha Ø on cardiac enzymes

#### 2.9.1 Thromboxane A2

In one study thromboxane A2, a vasoconstrictor enzyme found in the body which plays a major role in promoting blood clotting, was exposed to Crataegus oxyacantha flower extract in vitro. Crataegus oxyacantha inhibited the activity of thromboxane A2, thus reducing blood clotting activity. The principal active constituents responsible for the inhibition of the enzyme were catechin, epicatechin and hyperoside (Vibes et al., 1994).

#### 2.9.2 Angiotensin Converting Enzyme (ACE)

Angiotensin Converting Enzyme (ACE) plays an important role in the body’s rennin angiotensin system (RAS). RAS regulates extracellular volume of blood plasma and lymph fluid. RAS also plays an important role in arterial vasoconstriction (Kierszenbaum, 2007).

Uchida et al., (1987) suggests that Crataegus oxyacantha may inhibit ACE. Effects of condensed tannins isolated from Crataegus oxyacantha on the activities of ACE and various proteases were examined in vitro. Among the various condensed tannins tested, procyanidin B-5 3,3'-di-O-gallate and procyanidin C-1 3,3',3''-tri-O-gallate strongly inhibited the activity of ACE. Uchida et al (1987) further suggests that the inhibitory effects of condensed tannins on the activities of ACE are specific.
2.10 Safety of *Crataegus oxyacantha* Ø

*Crataegus oxyacantha* Ø can safely be used for long periods of time without the risk of toxicity or side effects. There are no reports of adverse effects with low doses however higher doses increase the risk of drug induced hypotension and sedation. It should not be used in children under 2 years of age (Hawthorn, 2000).

Human subjects taking 180 - 900 mg daily of hawthorn preparations consisting of extracts of leaf with flower, leaf with flower and fruit, and fruit only (standardized to 5% OPCs, 19% OPCs, and 2.2% flavanoids, respectively) reported no side effects over that specific time frame (Reutxer, 1994).

With the use of *Crataegus oxyacantha* Ø side effects are rare if consumed in recommended dosages. In a human clinical study of 136 patients treated with *Crataegus oxyacantha* extract, no changes in blood status, liver enzymes, electrolytes, glucose, or erythrocyte sedimentation rate were observed (Weikl, 1996).

Some of the most common adverse effects that may be experienced are vertigo and dizziness. *Crataegus oxyacantha* Ø can rarely cause nausea, gastrointestinal complaints, fatigue, sweating and a rash on the hands. Even less commonly reported side effects are palpitations, headache, dyspnoea, nosebleeds, sleeplessness, agitation and circulatory disturbances (Daniele *et al*., 2006).

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**CHAPTER THREE**

**METHODOLOGY**
3.1 Sample group

The sample group consisted of 30 male participants, recruited from the University of Johannesburg Doornfontein Health centre Campus. Once the participants were recruited the following criteria were evaluated in order to be included in the study.

Participants had to be

- Male between the ages of 25 and 35
- Available for the full duration of the study
- With a fasting Hcy level of 6.3mmol/L or higher.

Participants in the following categories were not regarded for enrolment

- On any chronic medication
- On any nutraceutical or Hcy lowering formulations
- With uncontrolled hypertension
- Participants with controlled hypertension for 6 months and on anti-hypertensive medications will not be excluded

3.2 Research procedure

The research was a double-blind placebo-controlled study that took place over 3 weeks, evaluating the effect of *Crataegus oxyacantha* Ø on Hcy levels in 30 healthy male individuals aged between 25 and 35.

The initial consultation included the following:

- The interested participant was informed of the research and the specific procedures pertaining to it
- Signing the Participant Information and Consent form (Annexure C)
- Biographical information and the medical history of each participant was recorded (Annexure D)
- A full cardiovascular examination was performed and the vital signs of each participant was assessed (Annexure E).
The potential participants reported to a Lancet laboratory. A fasting blood sample was taken and participants were advised not to consume any food or drink 8-12 hours before the collection of blood. A trained phlebotomist collected 1 EDTA tube of blood from the participant which was then used to determine the homocysteine level of the individual. The results were sent to the researcher when completed.

Based on the initial consultation and the results of the homocysteine test, those participants that qualified were then included in the study and randomly assigned to either the experimental or control group. Fifteen participants received the homeopathically prepared *Crataegus oxyacantha* Ø and fifteen participants received the placebo. The participants were not matched according to the severity of *Hcy* levels or additional risk factors. Participants were advised not to make any drastic changes to their diet and lifestyle.

The participants were notified 3 weeks later by the researcher to report to a Lancet laboratory for the final measurement of homocysteine. The final results were then sent to the researcher.

The final consultation included:

- Performing a full cardiovascular examination and assessing the vitals of each participant (Appendix D)
- Discussing the individual results of the homocysteine tests with the participant.

### 3.3 Administration of medication

Participants took 20 drops, which is equal to 1.0ml, 3 times daily. Each participant received a supply for 3 weeks of either the *Crataegus oxyacantha* Ø or the placebo. They were instructed to take the medication orally 15 minutes before a meal, 3 times daily.

The homeopathically prepared remedy *Crataegus oxyacantha* Ø and a 20% ethanol placebo was prepared according to HAB method 2A of the German Homeopathic Pharmacopoeia (Deutscher Apothekeker Verlag, 2000), randomised and numbered by Comed Health. It was made available to the researcher in numbered 50ml bottles and dispensed in numerical order. Each participant was randomly allocated a number to receive the medication. Neither the researcher nor the participants were aware which remedy they had received. A record of all medication which was dispensed will be kept by the researcher. The study was unblinded and all the information was made available once the study was completed.
3.4 Reliability and validity

Quality assurance was guaranteed by the suppliers of the medication as well as by the distributors of the product, Comed Health. Comed Health performed all of the quality control and quality assurance on the product. All of the *Crataegus oxyacantha* Ø that was used in this study was from the same manufacturing batch to ensure standardization of its contents. Comed Health manufactured the placebo and was responsible for matching the characteristics of taste and appearance exactly to that of *Crataegus oxyacantha* Ø used in the study.

Validity of the *Hcy* tests was assured as the *Hcy* tests were carried out at Lancet laboratories. All additional tests (blood pressure and vitals) were performed by the same researcher with the same diagnostic equipment.

3.5 Collection and analysis of data

The data collected from the *Hcy* levels done by Lancet laboratories was statistically analyzed utilizing a Chi Square goodness of fit test was utilized to determine if there was a significant decrease in *Hcy* levels in the participating individuals (Steffens, 2009).

3.6 Ethical consideration

The participation in this study was completely voluntary and participants were free to refuse treatment or to withdraw from the study at any given time. All the participants signed a consent form. Anonymity was maintained and all information submitted by participants was treated as confidential. *Crataegus oxyacantha* Ø is generally well tolerated and side effects are rare and no side effects were noted during the span of the study.

The study was passed by the Higher Degree Committee of the University of Johannesburg, met all ethical research standards and was approved by the Committee for Academic Ethics of the Faculty of Health Sciences of the University of Johannesburg with ethical clearance number AEC 11/02-2010, and higher degrees clearance number HDC 11/02-2010.
All participants’ details were kept confidential throughout the study, under lock and key at the homoeopathic clinic at UJ. Privacy was protected throughout the study and all consultations took place in a private setting.
CHAPTER FOUR

RESULTS

4.1 Introduction

This was a double blind placebo controlled study involving 30 participants. The study assessed the effect of *Crataegus oxyacantha* on Hcy levels of these individuals over a period of 3 weeks.

The study timeline was from April 2010 to June 2010. Thirty male participants aged from 25 – 35 years with a fasting Hcy blood level of 6.3mmol/L or higher took part in the study. Thirty participants took part in the study, of which all thirty participants successfully completed their treatment. Fifteen participants were in the experimental group and 15 participants were in the control group.

4.2 Study compliance

All 15 participants in the experimental group and all 15 participants in the control group were included in the statistical analysis of the study. All of the 30 starting participants followed through and no participant discontinued the study. Table 4.1 shows the figures in table form.

Study compliance regarding taking of the medication was not measured although where medication was received it was signed off by the researcher and the participant.

Table 4.1 Percentage of participants that completed the study

<table>
<thead>
<tr>
<th>Faction</th>
<th>Participants</th>
<th>Total completed study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>n=15</td>
<td>100 %</td>
</tr>
<tr>
<td>Experimental</td>
<td>n=15</td>
<td>100 %</td>
</tr>
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</table>
4.3 Consultation

The initial consultation included the following

- The interested participant was informed about the research and the specific procedures that was included in the study
- The interested participant was required to sign the Participant Information and Consent form (Annexure C)
- All of the biographical information was recorded and a short medical history was taken (Annexure D)
- A complete cardiovascular examination was performed and the vital signs (blood pressure, pulse, respiratory rate and vitals) were examined (Annexure E and F)

4.4 First Hcy reading

An average was used to compare the total $Hcy$ readings between the two groups to determine whether the two groups were similar. The test revealed that the mean $Hcy$ on day one for the experimental group was 9.0mmol/L and for the placebo group was 8.8mmol/L. This indicated that the two groups were similar. The exact $Hcy$ readings of each participant are listed in Annexure A.

4.5 Analysis of Participant Questionnaire

4.5.1 Age

The mean age of the control group was calculated at 28.2 years of age and the mean age of the experimental group were calculated at 30.1 years of age. This indicates that the factions were similar and can be used to evaluate the outcome based in the study. Table 4.2 presents the mean age in the control group and the mean age in the experimental group.

Graph 4.1 presents the mean ages of the control group compared to the experimental group. Graph 4.2 doubles to show the minimum and maximum ages of the two groups in this study. This indicates and substantiates that the two groups in this study were similar in age.
Table 4.2  The mean age in the control group compared to the experimental group

<table>
<thead>
<tr>
<th>Faction</th>
<th>Mean Age in years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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</tr>
<tr>
<td>Experimental</td>
<td>30.1</td>
</tr>
</tbody>
</table>

Graph 4.1  Graphical presentation of the mean age of the control group compared to the experimental group
Graph 4.2 presents all the individual ages of the control group compared to the experimental group in ascending order in a linear presentation.

4.5.2 Past and current medical history

Although no specific past or current medical history information was noted during the analysis of the participant questionnaire that pertained to the study, it was still significant to exclude certain individuals from the study.

4.5.3 Use of prescription medication

All individuals in the control group and the experimental group reported that they did not use any other prescription medication during the time span of the study. Neither did they use any medication before the start of the study. Table 4.3 presents the percentage use of medication of the control group and experimental groups.

Table 4.3 Percentage of participants that used prescription medication during the study

<table>
<thead>
<tr>
<th>Reported use of medication during the study</th>
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<th>Experimental Group</th>
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<td>No</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Yes</td>
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</table>
4.5.4 Use of nutraceutical supplementation

Table 4.4 presents the use of any supplementation during the study in the control group and the experimental group. All the participants were strongly advised not to use any nutraceutical supplementation.

Table 4.4 Percentage of participants that used nutraceutical supplementation during the study

<table>
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<tr>
<th>Reported use of nutraceutical supplementation during the study</th>
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<th>Experimental Group</th>
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<tr>
<td>Yes</td>
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<td>0</td>
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4.6 Blood pressure

4.6.1 Systolic blood pressure

For statistical purposes the blood pressure readings were divided into systolic and diastolic readings. One blood pressure reading was taken on day 1 before the start of the study and another was taken at the end of the study. Although the primary outcome of this study was based on the Hcy levels, blood pressure was taken to further evaluate participant safety and the effect of the medication.

The initial mean systolic blood pressure in the control group was calculated at 123.5 mm/Hg compared to the initial mean systolic blood pressure in the experimental group of 123.7 mm/Hg.

The concluding mean systolic blood pressure in the control group was calculated at 122.4 mm/Hg and the concluding mean systolic blood pressure in the experimental group was calculated at 123.9 mm/Hg.

The mean systolic blood pressure of the control group compared to the experimental group is shown in Table 4.5 and Graph 4.3.
Table 4.5  Mean systolic blood pressure readings in mm/Hg

<table>
<thead>
<tr>
<th>Mean systolic blood pressure readings</th>
<th>Systolic blood pressure reading 1</th>
<th>Systolic blood pressure reading 2</th>
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</thead>
<tbody>
<tr>
<td>Control group</td>
<td>123.5</td>
<td>122.4</td>
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<tr>
<td>Experimental group</td>
<td>123.7</td>
<td>123.9</td>
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</table>

Graph 4.3 presents the change in mean systolic blood pressure in the control and the experimental group after 3 weeks

4.6.2 Diastolic blood pressure

The initial mean diastolic blood pressure in the control group was calculated at 79.5 mm/Hg compared to the initial mean diastolic blood pressure in the experimental group of 79.6 mm/Hg. The concluding diastolic blood pressure in the control group was calculated at 79.3 mm/Hg and the concluding diastolic blood pressure in the experimental group was calculated at 80.3 mm/Hg.

The mean diastolic blood pressure of the control group compared to the experimental group is shown in Table 4.6 and Graph 4.4.
Table 4.6 Mean diastolic blood pressure readings in mm/Hg

<table>
<thead>
<tr>
<th>Mean diastolic blood pressure readings</th>
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<td>Control group</td>
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<tr>
<td>Experimental group</td>
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<td>80.3</td>
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</table>

Graph 4.4 presents the change in mean diastolic blood pressure in the control and the experimental group after 3 weeks

4.6.3 Side effects

According to literature vertigo and dizziness are the most common symptoms to be experienced by individuals using *Crataegus oxyacantha*. No individual in the control or the experimental group reported any side effects during the time span of the study.

4.6.4 Hcy readings
In graph 4.5 it shows all the initial documented $Hcy$ levels of the individuals, where it compares the control group with the experimental group. This indicates that the groups were fairly similar and that it may be compared. All of the $Hcy$ levels that were measured were above 6.3mmol/L.

**Graph 4.5 Initial $Hcy$ levels of the control group compared to the experimental group in ascending order**

Graph 4.6 represents the mean initial $Hcy$ levels of the control group compared to the experimental group.
Graph 4.6 Graphical presentation of mean initial $Hcy$ levels of control and experimental group

Graph 4.7 represents the mean resulting $Hcy$ levels of the control group compared to the experimental group at the end of week 3 of the study.

Graph 4.7 Graphical presentation of mean resulting $Hcy$ levels of control and experimental group
In graph 4.8 is shows the difference of $Hcy$ levels in the control group and the experimental group. The mean $Hcy$ level of the control group show a slight decrease and the mean $Hcy$ level of the experimental group shows a slight increase.

**Graph 4.8  Mean $Hcy$ levels changes during the study. Experimental compared to control**

In graph 4.9 it shows the concluding $Hcy$ levels of the control group compared to the experimental group.
4.7 Statistics

Due to the small group of participants used in the study a 0.05 % level of significance was used to validate the statistical data.

Table 4.7 represents the statistical analysis of the Hcy levels for the 3 week period of the control group.

<table>
<thead>
<tr>
<th>Chi Square Value</th>
<th>Degree of freedom</th>
<th>p-value</th>
<th>N</th>
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</thead>
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Table 4.8 represents the statistical analysis of the Hcy levels for the 3 week period of the experimental group.

<table>
<thead>
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<th>Chi Square Value</th>
<th>Degree of freedom</th>
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<tbody>
<tr>
<td>6.619</td>
<td>2</td>
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CHAPTER FIVE

DISSCUSION OF RESULTS

5.1 Introduction

Thirty male individuals participated in the study and all of these participants completed the study. All the inclusion criteria were met and diagnostic testing was done to determine all of the participants were healthy. Elevated blood pressure was used on exclusion criteria. The participants were randomly divided into the experimental and control groups. The medication was randomised by an independent person at Comed Health. Fifteen participants were selected for the experimental group and fifteen participants were selected for the control group. Hcy levels were measured by a diagnostic laboratory, Lancet and the results were evaluated.

The data collected from Lancet laboratories and data derived from the patient questionnaire was collected, tabulated and is discussed below. The data was statistically analysed using the Chi Square goodness of fit test to determine if there was a statistically significant lowering of total Hcy with the use of Crataegus oxyacantha O over the 3 week study.

5.2 Summary of results

No irregularities were noted during the time span of the study and adherence to the study was sufficed. All 30 participants that enrolled for the study completed the study with a 100% compliance rate.

The mean age in the control group was 28.2 years and 30.1 in the experimental group.

5.2.1 Hcy readings

The Chi Square goodness of fit test showed that there was no significant decrease in the total Hcy levels during the 3 week period of the study (p=0.03654) in the experimental group. There was a slight increase in Hcy in the experimental group. No statistically significant lowering effects were seen in the control group (p=0.19829).
In graph 4.9 it shows a slight increase in mean $Hcy$ levels in the experimental group and it shows a slight decrease in $Hcy$ levels in the control group.

5.2.2 Systolic blood pressure readings

BP readings were not statistically analysed. The mean systolic blood pressure of the control group decreased from 123.5 mm/Hg to 122.4 mm/Hg.

The mean systolic blood pressure of the experimental group slightly increased from 123.7 mm/Hg to 123.9 mm/Hg.

It should also be noted that *Crataegus oxyacantha* Ø should be used for longer than 3 weeks to experience a therapeutic normo-static effect on blood pressure.

5.2.3 Diastolic blood pressure readings

The mean diastolic blood pressure in the control group decreased slightly from 79.5 mm/Hg to 79.3 mm/Hg.

The mean diastolic blood pressure in the experimental group increased from 79.6 mm/Hg to 80.3 mm/Hg.

5.2.4 Final finding

The results thus indicate that that the homeopathic remedy, *Crataegus oxyacantha* Ø taken 3 times daily, is not more effective in lowering $Hcy$ levels in healthy males with a $Hcy$ level higher than 6.3mmol/L.

5.3 Null hypothesis

The null hypothesis was maintained. The null hypothesis is that the homoeopathically prepared *Crataegus oxyacantha* Ø taken orally 3 times daily is not more effective than the placebo in reducing elevated $Hcy$ levels in male individuals.
5.4 Placebo effect

The results of the present study show that the $Hcy$ levels of the placebo group stayed the same and were not significantly changed during the span of the study.

The placebo effect is the difference in outcome between the placebo group and an untreated group in an unbiased experiment.
CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

It may be concluded that *Crataegus oxyacantha* Ø prepared homeopathically is not effective in lowering total *Hcy* levels in males aged 25 to 35. There was a slight increase in total *Hcy* levels in the treatment group although there are various factors that may have influenced the final outcome of this study.

The factors that may have influenced the final outcome of the study are listed in the recommendations list.

The study also provides further evidence of safety of *Crataegus oxyacantha* prepared homeopathically as no participant reported any adverse affects during the study.

6.2 Recommendations

The following recommendations for future studies should be made:

- The study period should be extended to 12 weeks or even 6 months to determine the long term effect of *Crataegus oxyacantha* on *Hcy* levels.
- The sample size should be enlarged to increase statistical analysis and interpretation
- Female participants should be included in the study separately to evaluate the effect of *Crataegus oxyacantha* on female individuals.
- The dose and frequency of the medications given out should be investigated
- Standard Operating Procedures for the determination of *Hcy* should be investigated to ensure the validity of the laboratory findings
- A standardised diet should be followed during the course of the study.
CHAPTER SEVEN

REFERENCES:


Annexure A

Blood pressure representation of participants

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Annexure B

*Hcy* levels of participants

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Annexure C
Participant Information and Consent Form

My name is Petrie Joubert and I am a registered final year student for the M-Tech Homoeopathy degree through the University of Johannesburg. One of the requirements for the completion of the above degree is a research study on a related subject. The study that I am undertaking is to determine the effect of homoeopathically prepared *Crataegus oxyacantha Ø* on homocysteine levels in males and hopefully discovering additional therapeutic effects of *Crataegus oxyacantha Ø*.

Homocysteine is an amino acid produced by the body from the amino acid methionine, which is found in normal dietary protein. Ideally, it should be present in blood in quantities less than 6.3mmol/L. In certain individuals homocysteine levels become raised and increase the risk for developing cardiovascular disease.

This is a double blind, placebo-controlled study that will be taking place over a period of 3 weeks.

The initial consultation will include:

- Informing you of the research and the specific procedures of the study
- Signing of the Participant Information and Consent form
- Recording biographical information and a short medical history
- Performing a full cardiovascular examination and assessing vital signs

Inclusion criteria

- Male participants between the ages of 25 and 35
- Participants must be available for the full duration of the study
- Participants with a fasting homocysteine level of 6.3mmol/L and higher.

Exclusion criteria

- Participants on any medication
- Participants on any nutraceutical or homocysteine lowering formulations
- Participants with uncontrolled hypertension will be excluded from the study.

Participants with controlled hypertension that are under medical treatment with the same anti-hypertensive medication for the past six months will not be excluded from the study.
Participants of this study will be asked to report to a Lancet laboratory where a fasting blood sample will be taken. As this is a fasting test you are advised not to eat or drink for 8-12 hours before the collection of blood. A trained phlebotomist will collect 1 EDTA tube of blood which will then be used to determine the homocysteine levels in your blood. The results will be sent to the researcher when completed.

If however you don’t meet the inclusion criteria of the study, you will be fully informed of the situation and if relevant, be referred to your medical practitioner for further evaluation.

This is a double blind, placebo-controlled study. As a participant of this study you will either receive the medication or a placebo manufactured to be similar in taste and appearance to the medication. Neither you nor the researcher will have knowledge on which medication was given to whom.

The researcher will supply you with a 50mL bottle of *Crataegus oxyacantha* Ø or a placebo. The prescribed dosage will be 20 drops 3 times daily taken 15 minutes before or after a meal.

You, as a participant will be notified 3 weeks later by the researcher to report to a Lancet laboratory for the final measurement of homocysteine.

A final consultation will include:
- Performing a full cardiovascular examination and assessing the vital signs of each participant (Annexure E and F)
- Discussing the individual results of the homocysteine tests with each participant

The timeline of the study is as follows:

<table>
<thead>
<tr>
<th>Week 1</th>
<th>Week 2-4</th>
<th>Week 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial consultation</td>
<td>Clinical study</td>
<td>Follow up consultation</td>
</tr>
<tr>
<td>Checking for initial qualifying criteria</td>
<td>First and final homocysteine test</td>
<td>Assessment of results</td>
</tr>
</tbody>
</table>

As a participant of the study you are requested not to make any drastic changes to your diet and lifestyle and to use only the prescribed medication.
A preparation of *Crataegus oxyacantha* Ø will be used in this study. Clinically it is indicated for the improvement and strengthening of cardiovascular health.

*Crataegus oxyacantha* Ø is generally well tolerated and side effects are rare. Vertigo and dizziness are the most common adverse reactions. Should you experience new or any other symptoms or problems, please notify the researcher, and if necessary you will be referred to your health care provider. Your participation in this study is voluntary and you will not be paid. You may withdraw from the study at any time and for any reason. All information submitted by you would be treated as strictly confidential, with only the researcher and the supervisor having access to the collected data.

I, the participant, have been fully informed of the procedure of the study including the potential risks and benefits. In signing this consent form I agree with the method of treatment and understand that I am free to withdraw my consent and discontinue from the study at any time.

Signature______________________  Date_______________________

I, the researcher, have fully explained the techniques and purpose of treatment used in this research. Any questions or concerns from the participants during the course of this study will be answered to the best of my ability or feel free to contact any of the research supervisors.

Signature______________________  Date_______________________

Thank you for participating.

Research Supervisor:    Dr J. Pellow  011 599 6828
Research Co-supervisor: Dr U. Hohl  012 346 1230
Researcher:            Petrie Joubert  083 381 6597
Annexure D
Biographical Information and Patient History Sheet

<table>
<thead>
<tr>
<th>Date</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Surname</td>
<td></td>
</tr>
<tr>
<td>First names</td>
<td></td>
</tr>
<tr>
<td>Address</td>
<td></td>
</tr>
<tr>
<td>Contact number</td>
<td></td>
</tr>
<tr>
<td>E-mail</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>Date of birth</td>
<td></td>
</tr>
<tr>
<td>M-S-W-D</td>
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</table>

Past and current medical and history and treatment

____________________________________________________________________________
____________________________________________________________________________
____________________________________________________________________________

Current medication and supplementation

____________________________________________________________________________
____________________________________________________________________________
____________________________________________________________________________

Family medical history

____________________________________________________________________________
____________________________________________________________________________
____________________________________________________________________________
First Consultation

<table>
<thead>
<tr>
<th>Blood pressure</th>
<th>Date and Time</th>
<th>Reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clubbing</td>
<td></td>
<td></td>
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<tr>
<td>Anaemia</td>
<td></td>
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</tr>
<tr>
<td>Jaundice</td>
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<tr>
<td>Circulation</td>
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<td></td>
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<tr>
<td>Oedema</td>
<td></td>
<td></td>
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<tr>
<td>Lymphadenopathy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dehydration</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Second Consultation
<table>
<thead>
<tr>
<th>Blood pressure</th>
<th>Date and Time</th>
<th>Reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulse</td>
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<td></td>
</tr>
<tr>
<td>Dehydration</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Annexure E
Procedure for Cardiovascular Examination

(Bickley and Szilagyi, 2008).

- Position patient comfortably on an examination bed
- Position bed at 30 degrees angle
- Assess peripheral arterial pulses
- Assess heart rate, rhythm, amplitude, and contour
- Examine digits of both hands for the presence of clubbing
- Check for oedema
- Assess jugular venous pressure and pulse
- Inspect thorax for any irregularities or imperfections
- Palpate apex beat, left sternal border, left second intercostals space, the right second intercostals space, and the epigastric area
- Auscultate with a stethoscope to exclude bruits, thrills or murmurs over carotid arteries, apex beat, left sternal border, left and right second intercostals
Annexure F

Measuring Blood Pressure

(Bickley and Szilagyi, 2008).

- Locate radial pulse on the arm of the patient
- Locate brachial artery on the same arm
- Attach the blood pressure cuff firmly 2cm above the crease of the same arm
- Inflate blood pressure cuff while assessing the radial pulse
- Record the reading on the blood pressure dial at the termination of the radial pulse on inflation of the cuff
- Deflate the cuff slowly
- Then place stethoscope over brachial artery and repeat the inflation process to 30mmHg above last recorded reading
- Then start to deflate the blood pressure cuff
- The first audible sound should be recorded as the systolic blood pressure reading
- At the cessation of the sound through the stethoscope, record the reading as the diastolic blood pressure
- Further deflate the blood pressure cuff