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EFFECTS OF ALPHA-METHYLDOPA ON THE SYMPATHETIC NERVOUS SYSTEM ACTIVITY IN HEALTHY PARTICIPANTS

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SUMMARY

Methyldopa (L-alpha-Methyl-3,4-dihydroxyphenylalanine) is a catecholamine used as an antihypertensive agent.[1] Alpha-Methyldopa is not used as frequently anymore due to side effects, but it is still used especially in developing countries due to its low cost. Indications are mostly for the management of pregnancy-induced hypertension (PIH), as it is relatively safe in pregnancy compared to other antihypertensive drugs. This project is intended to increase the already-existing knowledge base of the mechanism of pharmacological action and to stimulate further investigation through research.

The sympathetic nervous system is a division of the autonomic nervous system and it is responsible for the “flight-or-fight” response. It is involuntary and constantly active to maintain homeostasis in the human body. Sympathetic responses include an increase in heart rate, blood pressure and cardiac output, dilation of pupils and bronchioles, constriction of blood vessels, contraction of sphincters and inhibition of gut motility and secretions.

The purpose of this study is to evaluate the activity of the sympathetic nervous system of volunteers by three different techniques (QT interval and Heart rate variability and Skin conductance) after a week of a bi-daily dosage of alpha-methyldopa.

All volunteers received either 250mg alpha-methyldopa orally or a placebo tablet in a randomized, double blind, placebo controlled study design. The correlation between the following techniques was also evaluated: Skin conductance as measured by the ProComp Infiniti Biofeedback apparatus, QT interval on ECG and HRV measured with Viport apparatus. A salivary sample was collected to evaluate the effect of alpha-methyldopa on salivary cortisol using an ELISA kit for analysis.

OPSOMMING

Metieldopa (L-alfaMetiel-3,4-dihydroksiefenielalanien) is ‘n katesjolamien wat gebruik word as ‘n antihipertensiewe middel.[1] Alfa-Metieldopa word nie meer alledaags gebruik nie as gevolg van sy newe effekte. Omdat dit so goedkoop is word dit nog steeds gebruik in ontwikkelende lande. Indikasies is meestal vir die beheer van swangerskap-verwante hipertensie, omdat dit relatief veilig is in vergelyking met ander antihipertensiewe medikasie. Hierdie projek het ten doel om die alreeds bestaande data basis van die meganisme van farmakologiese aksie aan te vul en verdere navorsing te stimuleer.

Die simpatiese senuweestelsel is ‘n vertakking van die autonome senuwee stelsel wat verantwoordelik is vir die “vlug-of-veg” reaksie. Dit is onwillekeurig en konstant aktief besig om homeostasis te handhaaf in die menslike liggaam. Simpatiese reaksies sluit in ‘n vehoging in pols, bloeddruk, kardiale uitset, vergroting van pupille en brongiole, vernouing van bloedvate, sametrekking van sfinkters en inhibisie van gastroïntestinale beweeglikheid en uitskeiding.

Die doel van die studie is om die simpatiese senuweestelsel te evalueer in vrywilligers deur middel van 3 verskillende tegnieke (QT interval, harttempo veranderlikheid en velgeleiding) na ‘n week van tweemaal daaglikse dosering van alfa-metieldopa.

Alle vrywilligers het of 250mg alfa-metieldopa or a plasebo tablet oral ontvang in ‘n ewekansige, dubbel blinde, plasebo gekontrolleerde studie ontwerp. Die korrelasie tussen die volgende tegnieke is ook geevalueer: velgeleiding gemeet d.m.v. die ProComp Infiniti Biofeedback apparaat, QT interval op EKG en hartklop veranderlikheid gemeet met ‘n Viport apparaat. ‘n Speeksel monster is versamel om die effek van alfa-metieldopa op speeksel kortisol te evalueer deur gebruik te maak van ‘n ELISA metode vir analise.

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LIST OF ABBREVIATIONS

A

ANS	Autonomic nervous system
ADHD	Attention deficit hyperactivity disorder

B

BP	Blood pressure
BPV	Blood pressure variability
BRS	Baroreceptor sensitivity

C

CBG	Corticoid binding globulin
CSI	Cardio stress index

D

DM	Diabetes Mellitus

E

EDR	Exosomatic Electrodermal Response
ELISA	Enzyme-linked immunosorbent assay

G

GSR	Galvanic Skin Response

GIT	Gastrointestinal tract

H

HPA	Hypothalamic–pituitary–adrenal
HR	Heart Rate
HRV	Heart rate variability

N

NE	Norephinephrine

P

PIH	Pregnancy-induced hypertension

Q

QTvi	QT variability index

R

RRSD	Standard Deviation of RR interval

T

TMB	Tetramethylbenzidine

CHAPTER 1

LITERATURE REVIEW

1.1 Methyldopa

Methyldopa (L-alpha-Methyl-3,4-dihydroxyphenylalanine) is a catecholamine used as an antihypertensive agent. [1] Methyldopa is not used so frequently anymore due to side effects, but it is still used, especially in developing countries because of its low cost. It is mostly indicated for hypertension in particularly the management of pregnancy-induced hypertension (PIH), as it is relatively safe in pregnancy compared to other antihypertensive drugs. Methyldopa as treatment for severe dyskinesias is also recognised. [2] This project is intendeds to add to the already-existing knowledge base of the mechanism of pharmacological action and to stimulate further investigation through research.

According to the package insert of methyldopa, the exact mechanism of pharmacological action is not known. [3] Various mechanisms of action have been hypothesized in previous studies. [4] The antihypertensive effect most commonly accepted is the metabolism of methyldopa to alpha-methylnorephinephrine. Methyldopa is metabolized by L-aromatic amino acid carboxylase in adrenergic neurons to alpha-methyldopamine which is then converted to alpha-methylnorephinephrine. Thus when adrenergic neurons discharge their neurotransmitters, alpha-methylnorephinephrine is released instead of norephinephrine. [5] alpha-methylnorephinephrine is as potent a vasoconstrictor as norephinephrine (NE), thus its substitution for norephinephrine in peripheral adrenergic neurosecretory vesicles does not alter the vasoconstrictor response to peripheral adrenergic neurotransmission. Adrenergic neuronal outflow is inhibited due to the effect of alpha-methylnorephinephrine in the central nervous system. According to the literature methylnorephinephrine probably acts as an agonist at

presynaptic α_2 adrenergic receptors in the brainstem, attenuating NE release and thereby reducing the output of vasoconstrictor adrenergic signals to the peripheral sympathetic nervous system. [5]

The most common side effect according to the package insert of Methyldopa is drowsiness. This will decline naturally after the first 2-3 days of usage. Some other frequent adverse events include depression, psychic effects, impaired mental acuity, nightmares, nausea, dryness of the mouth, nasal stuffiness, weakness, dizziness, light-headedness, headache, oedema and disorders of sexual function. [3]

Caution should be taken when methyldopa is administered to people with impaired kidney or liver function or with a history of liver disease or mental depression. As expected, methyldopa is contra-indicated in anyone sensitive to the compound. It is also reported that porphyria is aggravated by methyldopa.

The peak plasma concentration is achieved after 2 to 3 hours, whereas the maximum fall in arterial pressure is only observed after 6 to 8 hours.

The initial treatment dosage is 250mg orally, 2 to 3 times daily. Thereafter the maintenance dosage is between 500 mg and 2g orally divided in 2 to 4 doses.

1.2 Sympathetic activity

The autonomic nervous system (ANS) is divided into subsystems. The two subtypes are the sympathetic- and parasympathetic nervous system, which leave the central nervous system at different sites and have opposing effects. Some textbooks categorize the enteric nervous system as a subdivision of the ANS.

The enteric nervous system controls the gastrointestinal system and can function autonomously. Extensive communications between the enteric and the

sympathetic and parasympathetic systems exist to control the digestive system and its physiological demands in the human body.

The sympathetic nervous system (SNS) is also known as the “flight-or-fight” response whereas the parasympathetic is much smaller and controls the vegetative function or “rest-and-digest” response. [6] The parasympathetic nervous system (PSNS) also regulates functions such as sexual arousal, salivation, lacrimation (tears), urination, digestion, and defecation. Due to the neuronal pathways being much longer, the PSNS is a much slower system. The PSNS has only two types of receptors: muscarinic and nicotinic receptors.

The SNS is involuntary and constantly active to maintain homeostasis in the human body. It has two types of receptors, alpha- and beta-adrenoceptors and can be subdivided into 5 subtypes: alpha-1, alpha-2, beta-1, beta-2 and beta-3 receptors.

Alpha-1 receptors are responsible for smooth muscle contraction and are found in most arteries and in sphincter muscles of the gastrointestinal tract (GIT) and bladder. Alpha-2 receptors are found in presynaptic nerves and also in parts of the GIT. Epinephrine and norepinephrine stimulate the alpha-adrenoceptors, causing the arteries to constrict and blood pressure to increase. An alpha-blocker will thus cause vasoconstriction and can be used to treat hypertension.

Beta-adrenoceptors help with the regulation of both cardiovascular and metabolic functions. [7] Beta1-adrenoceptors are predominantly found in the heart and when they are stimulated, the heart rate and contractility will increase. Beta-2 receptors are responsible for smooth muscle relaxation and are found, among others, in the bronchioles of the lung and in the GIT. Beta-3 receptors are found mainly in adipose tissue and regulate lipolysis and thermogenesis.

Sympathetic responses include an increase in heart rate, blood pressure and cardiac output, dilation of pupils and bronchioles, constriction of blood vessels,

contraction of sphincters and inhibition of gut motility and secretions. These responses require energy and make it possible for us to react in a stressful or emergency situation. Heart rate variability (HRV), blood pressure variability (BPV) and baroreceptor sensitivity (BRS) are often used as a measure of autonomic activity. [8]

1.3 Heart rate variability

A healthy heart does not beat regularly to the millisecond, rather slightly irregularly so it can react quickly to stimuli. This discreet fluctuation of the heart‘s rhythm is known as heart rate variability. [11] If there is a disruption to the fine adjustment of the heart rhythm regulation, this can be shown on the basis of a mathematical determination of the HRV from the ECG signal and can provide information not only about the heart function, but also about the entire state of health. [10] HRV is a non-invasive method to evaluate the autonomic influence because it is regulated by the autonomic nervous system.

Parasympathetic activity decreases heart rate and increases HRV whereas sympathetic activity increases heart rate and decreases HRV. [9]
HRV is known to decrease with age. [10] Cross sectional studies have shown that fit and healthy individuals have a higher HRV. [11] HRV can now easily be analysed through the availability of mobile heart rate monitors to sport, scientific and medical personnel. [12] In patients with coronary heart disease, hypertension and heart failure, HRV can be used as a prognostic tool. [13]

The Cardio Stress Index (CSI) is calculated using the parameters of a typical ECG; the RS length, HR, rhythm and RRSD (Standard Deviation of RR interval/individual heart beats). [14] These parameters are transformed via algorithms to give the CSI which then can provide information about HRV. The ECG signal is transferred following Einthoven’s method via the three electrodes of the device.

The range of measurement is between 0 and 100. A normal CSI is considered between 0 and 20 and corresponds to a normal variability. Above 20 indicates reduced variability, thus a low HRV. [15] A decrease in RRSD-values indicates a reduced HRV which means an increased Cardio Stress Index (CSI). Very high RRSD-values may be an indicator of arrhythmia.

1.4 QT interval on ECG

A typical electrocardiograph consists of a complex cycle of de-polarization and re-polarization generating waveforms as in Figure 1. [16] The QT interval is the duration from ventricular de-polarization to re-polarization, in a resting ECG. It is an easy biomarker for autonomic balance between sympathetic and parasympathetic activities.

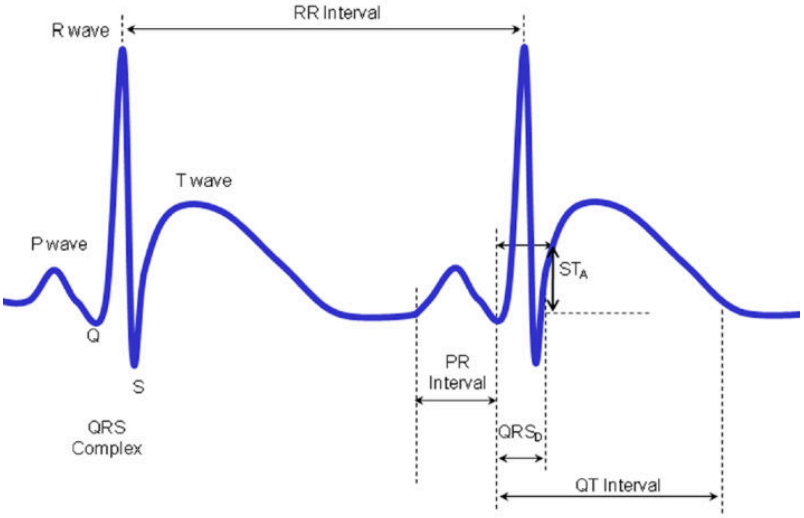

Figure 1: Electrocardiographic waveforms and intervals [16]

The QT variability index (QTvi) reflects sympathetic activity to some extent. [17] According to Boettger *et al.* changes in HRV mainly reflect the efferent parasympathetic modulation on the heart at the sinus node level, beat-to-beat QTV depicts the temporal fluctuation in ventricular repolarization and provides information on the phase in which the heart is most susceptible to arrhythmias. [18]

A longer heart rate-corrected QT interval (QTc) represents predominance of sympathetic activity within autonomic balance. [25]

There are a few inherent methodological limitations to QT interval, because the QT interval varies with heart rate. An inverse relationship exists between HR and the QT interval. Exercise will shorten the QT interval as a result of the increase in heart rate with the activation of the sympathetic nervous system and increasing of catecholamine levels. Increasing the heart rate from 60 to 160 bpm will typically shorten the QT interval by 25–40%. [26] It has been found that atropine shortens the QT interval similar to exercise. [27]

A corrected QT interval (QTc) can be calculated by using several QTc prediction formulae. The most popular and also most criticized formula is the Bazett's formula, which is commonly used for clinical and research purposes. [19]
The participant's QT interval (measured in seconds) is divided by the square root of their RR interval (also measured in seconds). This is Bazett's formula (QTc = QT/√RR).

A normal QTc interval range is considered between 0.35–0.44 seconds.
According to Graff *et al.* there is a limitation to the ability to reliably detect small changes of <5% of the interval in the QT interval. [20]

A longer QT interval is the results of unfavorable predominance of sympathetic activity as well as dispersion of depolarization within the autonomic balance. [21-23] In a healthy population a longer QTc has been found to be predictive of coronary heart disease mortality. The prolongation causes ventricular electrical instability which increases the risk of fatal myocardial infarctions. [24] Prolonged QT interval has also been associated with an increase in age and diabetes mellitus (DM). According to Nagaya *et al.*glucose tolerance may be affected in healthy individuals with prolonged QT interval, leading to the development of DM. [25] It has been a tendency the last couple of years to focus more on drugs that prolong

the QT interval, leading to several drugs being withdrawn from the market (e.g., terfenadine, astemizole, cisapride, and grepafloxacin). [16]

1.5 Skin conductance

Skin conductance, also known as Galvanic Skin Response (GSR), measures perspiration produced on the palmar surface indicating changes in sympathetic arousal. Skin conductance is only affected by sympathetic activity whereas heart rate and blood pressure are influenced by both sympathetic and parasympathetic nervous system. [28]

The eccrine glands found in the palmar and plantar region are filled with sweat, with the activation of the sympathetic nervous system causing skin resistance to decrease and the skin conductance to increase before the sweat is reabsorbed. [29-30] The correlation between the sympathetic nervous system and skin conductance indicates that an increase in autonomic arousal results in an increase in skin conductivity.

Skin conductance has a direct correlation with the activity of filling of sweat ducts and the reabsorption process and not with the production and evaporation of sweat from skin. [31] A decrease in sweat activity can be an indicator of various diseases such as diabetic neuropathy or Ross syndrome.

There are 2 methods in which the skin conductance can be measured: endosomatic or exosomatic:

Endosomatic is a method that involves microneurography i.e. electrodes that are directly inserted into the skin neurons. No external current is applied. This is the more invasive procedure and gives a direct measurement of the electrical activity of the skin's neurons. [51]

The exosomatic procedure only requires 2 small electrodes to be placed on to the skin's surface. A small electrical current passes over the surface between the two electrodes and records the skin potential response. [51]

Exosomatic skin conductance activity is a non-invasive method that can easily be used. Pain and stress have reportedly been measured with skin conductance in both preterm and term infants which is unaffected by environmental temperature or by cardiorespiratory status. [32] According to Bini et al, environmental temperatures within normal range will not affect sweating activated through skin sympathetic nerves. [33]

1.6 Salivary cortisol

Salivary analysis is becoming more significant in hormonal analyses. [34]
Secretion of each gland contributes to the total unstimulated saliva; 65% by submandibular, 23% by parotid, 8% by Von Ebner and 4% by sublingual glands. Cortisol or hydrocortisone is a glucocorticoid produced in the cortex of the adrenal gland.

Cortisol's primary functions in the body are increasing blood glucose through gluconeogenesis, suppressing the immune system and assisting in fat, protein, and carbohydrate metabolism. [35]

90% of the circulating cortisol is bound to corticoid binding globulin (CBG), 7% is bound to albumin and only 1–3% is unbound and represents the active form of cortisol responsible for biological functions. Total cortisol is therefore not an accurate measurement of the active cortisol levels. To measure the free plasma cortisol directly is time-consuming and labour intensive. Salivary cortisol only represents the bioactive fraction, which is the unbound and biologically active form of circulating cortisol, not bound to CBG or other proteins. [36]

Compared to the serum-free cortisol, the cortisol concentration in saliva is only 50-60% of its equivalence. The salivary cortisol/cortisone ratio is approximately 1:4 where in the serum the ratio is 8:1. [34] Cortisol can be detected in the serum, saliva, urine and faeces. [36] Urine and faecal sampling has a huge disadvantage because minor and rapid changes in concentrations cannot be detected. [37] Serum sampling is widely used in clinical settings but the venipuncture itself can also cause an increase in cortisol levels. Salivary and serum cortisol has a high correlation, ranging from r = 0.6-0.99. [38]

Therefore salivary cortisol is an acceptable method of sampling which presents many advantages;

1) The most important advantage of salivary sampling is the easy and non-invasive nature of the measurement.
2) The sampling is independent of a laboratory thus samples can be taken at unlimited frequencies in a variety of clinical settings or at home. [39]
3) Salivary cortisol concentration is independent of flow rate of saliva. [37]
4) Only free cortisol is in saliva thus no separation manipulation is necessary. [37]

The diurnal rhythm of cortisol is at the highest concentration just after awakening in the morning and decrease in concentration throughout the day until reaching the lowest point around midnight. [40] Late-night salivary cortisol measurements are an accepted method to screen patients for Cushing's syndrome. It is also a way to assess secretory activity and rhythm of the hypothalamic–pituitary–adrenal (HPA) axis. [41]

Various studies have been done to evaluate cortisol levels. Smoking and stress have both been found to stimulate the release of cortisol. [42] Exercise has a linear relationship with cortisol, increasing with intensity and duration of the exercise. [38] On the other hand, high cortisol levels have been associated with Cushing's

syndrome, muscle weakness, osteoporosis, hypertension, diabetes mellitus (DM) and a susceptibility to infections. [43]

CHAPTER 2

AIM AND OBJECTIVES

2.1 Aim

The aim of this study is to evaluate the activity of the sympathetic nervous system of volunteers by three different techniques (QT interval and Heart Rate Variability and Skin conductance) after a week of a bi-daily dosage of alpha-methyldopa.

2.2 Objectives

i) To evaluate the correlation between the following techniques in measuring sympathetic activity;

- Skin conductance as measured by the ProComp Infiniti Biofeedback apparatus,
- QT interval measured by 12 lead ECG,
- HRV measured with Viport apparatus.

ii) To evaluate the effect of alpha-methyldopa on salivary cortisol using an ELISA kit.

CHAPTER 3

MATERIALS AND METHODS

3.1 Clinical Trial

3.1.1 Study design

In this parallel double-blind placebo controlled study 250mg alpha-methyldopa was randomly assigned to healthy volunteers. Participants consisted of healthy male and female volunteers between 18 and 30 years old. Only volunteers who were willing to sign the informed consent form were enrolled.

Twenty-two participants were screened and enrolled in the study. Two participants withdrew from the study and did not complete the final visit of the study.

Depending on results of this pilot study further investigation with a larger sample size can be evaluated in further research.

There were 2 site visits:

At Visit 1: Baseline, the following was done:

- Signing of informed consent was done before any study procedures were performed,
- Assessing inclusion and exclusion criteria to determine subject's eligibility,
- Biographical data was collected, such as age and sex,
- Blood pressure was measured,
- Heart rate variability (HRV) was measured,
- ECG was measured,
- Skin conductance was measured,
- Salivary cortisol sample was collected,

- A patient diary was handed out to note any adverse events.

At Visit 2: volunteers returned after 1 week of treatment to complete the End of study visit.

- Adverse events were assessed if applicable,
- Blood pressure was measured,
- Heart rate variability (HRV) was measured,
- ECG was measured,
- Skin conductance was measured,
- Salivary cortisol sample was collected,
- Compliance with study drug was assessed,
- Patient diaries were collected.

Volunteers were monitored in the sitting position.

For the salivary cortisol test, no food, drinks, gum or brushing of teeth were allowed 30min before sampling.

3.1.2 Ethical considerations

This clinical study protocol has been approved by the University of the Pretoria Research Ethics Committee (Protocol S198/2010). The study and informed consent have been conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

3.1.3 Patient selection

After permission had been given by Prof G Lindeque the Deputy Dean of Medicine, students and personnel of the University of Pretoria were invited to volunteer for the study.

3.1.4 Study drug regime

Volunteers were randomly divided into 2 groups. All volunteers received either 250mg alpha-methyldopa (HY-PO-TONE® 250) or a placebo tablet. The placebo tablet was supplied and manufactured by Aspen Pharmacare. The study drug or placebo was taken oral, bi-daily for one week, in a parallel, randomized, double blind, placebo controlled study design.

3.1.5 Inclusion and exclusion criteria

3.1.5.1 Inclusion criteria

- Healthy males or females aged 18-30 years
- Female patients using reliable contraception if of childbearing potential.

3.1.5.2 Exclusion criteria

- All participants that do not comply with the above inclusion criteria
- Any medication, vitamins and/or supplements during the study period
- Refusal to freely give written informed consent
- Extreme low blood pressure (systolic BP < than 90 mm Hg or diastolic BP < than 60 mm Hg)
- A history of cardiovascular, hepatic, respiratory, renal impairment, pulmonary metabolic, and/or orthopaedic disease requiring medical attention were excluded
- Psychological disorders
- Pregnant or lactating females
- A subject that has taken part in any other clinical trial within the last month

3.2 Vitals

Blood pressure and pulse were measured using an Microlife blood pressure monitor (Model: BP3BT0-A). Arm measurements were done. Subjects were measured in sitting position after 5 minutes of rest.

3.3 ProComp Infiniti Biofeedback apparatus

Biofeedback is a painless and non-invasive procedure to measure the skin conductance.

Sensors/ electrodes were attached to the index and middle finger after the area had been wiped with an alcohol swab. The electrodes were connected to the ProComp Infiniti Biofeedback apparatus which was also connected to a laptop computer.

Using the BioGraph Infiniti Software 3.0 program the skin conductance could be measured while subjects were in a sitting position. A reading of 5 minutes was taken for each subject at the baseline and at the final visit.

3.4 ECG

Electrocardiogram (ECG) measures the electrical activity of the heart.

12 self –adhesive electrodes were placed on a subject's arms, legs and chest to record the ECG of a subject. The site where the electrodes were placed was first cleaned with an alcohol swab; if necessary the hair was shaved off to ensure the electrode came in contact with the skin.

Any mobile phones or electrical devices were removed in the near vicinity of the subject. After 5min in the supine position the ECG was taken with the Edan SE-1200 ECG machine.

The QT/QTc was calculated automatically by the ECG machine with a paper speed of 25mm/s.

3.5 Viport

The Viport is a small electronic handheld device that was used to measure HRV via CSI. The data was collected on the basis of a digital multi-channel ECG system while subjects were in the sitting position. The ECG signal is transferred following Einthoven's method via three electrodes at the back of the device.

Before the device was used, the electrodes were prepared by adding electrode gel to each electrode to make them moist. The Viport was positioned on the subjects bare chest three fingers width below the left clavicle. All the electrodes were in direct contact with the skin during the entire measurement. After approximately 2 minutes of data recording the Viport computed an individual electrocardioportrait (ECP) on the integrated colour display from the recorded signal, as well as the following values:

- Cardio Stress Index (range 0-100)
- Heart rate (beats/min)
- Rhythm (rhythmic yes/no)
- QRS duration (the length of ventricle activity in your heart)
- RRSD (ms) (the standard deviation of the interval between the individual heart beats)

The RS length, HR, rhythm and RRSD of the ECG is used to calculate the CSI.

Via algorithms these parameters are transformed to give CSI which then can provide information about HRV.

The range of measurement is between 0 and 100. A normal CSI is considered between 0 and 20 and corresponds to a high variability. Above 20 indicate reduced variability.

3.6 Salivary Cortisol ELISA

Saliva is a convenient, non-invasive method of determining hormonal concentrations. [44] Studies have proven the validity of salivary assays in the clinical and non-clinical field. [45] An Enzyme-linked immunosorbent assay (ELISA) for the in-vitro diagnostic quantitative determination of free cortisol in human saliva will be done. The Cortisol ELISA kit (RE52611) from IBL International that was used has an analytical sensitivity (limit of detection) of up to 0.05 ng/mL and a functional sensitivity of up to 0.30 ng/mL.

Salivary cortisol levels peak in the first 90 minutes after awakening. To control for diurnal variation in cortisol concentration within participants each visit was conducted at approximately the same time of day.

No food, drinks, gum or brushing of teeth was allowed 30min before sampling. If the participant did take any food, drinks, gum or brushed of teeth they had to rinse their mouth thoroughly with cold water 5 minutes prior to sample collection.

Saliva was collected in an eppendorf tube. A minimum of 0.5ml liquid was collected. Samples were frozen at –20°C prior to laboratory testing. The protocol of the ELISA kit (RE52611) from IBL International was followed for samples collected. According to a study done by Westermann *et al.*, the saliva cortisol range measured in ng/mL in healthy subjects is between 2.0 – 14.1 before midday and between 0.4 – 4.1 after midday. [46]

3.6.1 Materials supplied by kit:

Microtiter wells, 12x8 (break apart) strips, 96 wells;
Wells coated with an anti-cortisol antibody (polyclonal).

Standard (Standard 0-6), 7 vials, 1 mL each, ready to use;
Concentrations: 0, 0.1, 0.5, 1.5, 4, 10 and 30 ng/mL, contains 0.003% Proclin as a preservative.

Control low / Control high, 2 vials, 1.0 mL each, ready to use; Contains 0.003% Proclin as a preservative.

Enzyme Conjugate, 1 vial, 26 mL, ready to use; Cortisol conjugated to horseradish peroxidase; contains < 0,019% BND and < 0,017% MIT as preservative.

Substrate Solution, 1 vial, 25 mL, ready to use; Tetramethylbenzidine (TMB).

Stop Solution, 1 vial, 14 mL, ready to use; contains 0.5M H_2SO_4.

Wash Solution, 1 vial, 30 mL (40X concentrated).

3.6.2 Materials not supplied by kit:

A microtiter plate calibrated reader (450±10 nm)

Calibrated variable precision micropipettes (100 µL, 200 µL).

Absorbent paper.

Distilled or deionized water.

3.6.3 Principle of test

The DRG Salivary Cortisol HS ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with a polyclonal rabbit antibody directed towards an antigenic site on the cortisol molecule. Endogenous cortisol of a donor sample competes with a cortisol-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of cortisol in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of cortisol in the donor sample.

3.6.4 Method

3.6.4.1 Preparations of reagents

All the reagents were brought to room temperature (18-25ºC) prior to doing the assay.

The wash solution was diluted by adding 390ml deionised water to 10ml of the 40x concentrate wash solution to get a total volume of 400ml.

3.6.4.2 Specimen collection, storage and preparation

Duplicate saliva samples were collected using DRG® Sali-tubes (SLV-4158). Saliva samples were collected at fasting or before a meal, if not, the subject was instructed to rinse his/her mouth thoroughly with cold water for 5 minutes. Samples

were collected approximately the same time of day during each visit to eliminate diurnal fluctuation.

The samples were stored at -20ºC until all the samples were collected and the assay could be performed. Samples remain stable for ≥ 6 months at -20ºC.

The frozen samples were brought to room temperature on the day of the assay procedure. They were carefully mixed and centrifuged for 10min at 2000xg. Only the clear colourless supernatant was used.

3.6.4.2 Assay procedure

Before the assay was started a pre-test was done. One randomly selected sample was used to make a 1:10 and 1:100 dilutions. These dilutions plus the undiluted sample was used to do the complete assay procedure. Because the undiluted specimen was found to contain more than the highest standard, all the saliva samples were diluted to 1:10 by adding 10µl saliva to 90µl Standard 0.

All the samples were used to follow the following assay procedure:

100µl of each standard, control and samples were dispensed into appropriate wells. Only 9 out of the 10 subjects' samples were used for the analysis with the ELISA kit due to the limited wells available on the plate. The 9 samples were randomly selected from the available 10 samples.

200µl of the Enzyme conjugate was pipetted into each well and mixed thoroughly for 10 seconds.

The microtiter plate was incubated for 60min at room temperature on a shaker at 300rpm.

The contents were briskly removed from the plate. The wells were rinsed 5 times with the diluted wash solution using 400µl per well each time around.

200µl of the substrate solution was added to each well.

The plate was incubated for 30min at room temperature.

The enzymatic reaction was stopped by adding 100µl of the stop solution to each well.

The absorbance was measured at 450nm within 10 minutes after adding the stop solution.

A standard curve was generated using the controls.

3.7 Statistical analysis

Statistical analysis was done with the support of Dr Lizelle Fletcher of the Statistics Department, University of Pretoria. The data analysis consisted of basic descriptive statistics, hypotheses testing and a correlation analysis. A one-sided T-test for paired observations was used to assess whether alpha methyldopa causes a decrease in sympathetic activity, while independent T-tests was used to evaluate the difference between the control and the treatment groups. Because the assumptions of the T-tests was violated and the sample was too small to rely on the central limit theorem, nonparametric tests (Wilcoxon signed-rank and Mann-Whitney U) was performed instead.

CHAPTER 4

RESULTS

Frequencies

Table 1: Patient demographics and baseline characteristics (n=20)

Baseline characteristics	Placebo Group	Treatment group
Age		
Mean	24.8	22.3
Range	21-29	18-29
Sex		
Male	8	6
Female	2	4

In total 20 subjects participated and completed the clinical trial. Twenty-two subjects were enrolled onto the study but two participants withdrew consent and did not return for the final visit.

The 20 subjects were randomly divided into the placebo or the treatment group.

Table 2: Descriptive statistics of variables at baseline and final visit for placebo group

Placebo Group										
	Baseline measurements (n=10)					**Final measurements (n=10)**				
	Minimum	**Maximum**	**Median**	**Mean**	**Std. Deviation**	**Minimum**	**Maximum**	**Median**	**Mean**	**Std. Deviation**
Vitals										
BP: systolic (mmHg)	108.00	149.00	119.00	124.90	13.51	105.00	140.00	124.50	123.20	11.30
BP: diastolic (mmHg)	61.00	109.00	78.50	79.30	12.83	70.00	91.00	80.50	80.10	5.74
Pulse (bpm)	52.00	97.00	75.50	74.90	14.45	55.00	100.00	79.50	80.10	12.29
Viport										
Pulse: Viport (bpm)	50.00	94.00	74.50	71.80	12.33	57.00	101.00	81.00	81.40	12.68
CSI	9.00	66.00	17.00	25.30	19.79	9.00	48.00	16.50	21.70	13.47
QRS (ms)	51.00	98.00	85.00	81.80	15.10	60.00	97.00	74.00	78.70	13.21
RRSD (ms)	33.00	72.00	62.50	58.20	12.58	32.00	64.00	40.50	41.70	10.70
ECG										
QT interval (ms)	323.00	412.00	373.50	369.50	28.52	320.00	398.00	361.50	358.20	20.89
Pulse: ECG (bpm)	45.00	99.00	69.00	72.10	17.16	53.00	95.00	71.50	72.90	10.95
QTc (ms)	358.00	449.00	391.50	400.40	25.13	343.00	431.00	396.50	393.60	25.37
Biofeedback apparatus										
Skin conductance	1.97	4.92	2.84	3.04	0.92	0.81	6.92	3.86	3.21	1.95

Table 3: Descriptive statistics of variables at baseline and final visit for treatment group

Treatment Group										
	Baseline Measurements (n=10)					**Final Measurements (n=10)**				
	Minimum	**Maximum**	**Median**	**Mean**	**Std. Deviation**	**Minimum**	**Maximum**	**Median**	**Mean**	**Std. Deviation**
Vitals										
BP: systolic (mmHg)	95.00	150.00	128.50	125.40	14.80	99.00	125.00	120.00	117.00	9.02
BP: diastolic (mmHg)	65.00	92.00	83.50	80.10	8.60	72.00	86.00	78.00	78.80	4.69
Pulse (bpm)	58.00	114.00	74.00	78.00	16.59	51.00	89.00	72.00	71.20	11.36
Viport										
Pulse: Viport (bpm)	58.00	98.00	74.50	77.00	11.03	59.00	100.00	71.50	74.40	13.20
CSI	9.00	89.00	21.50	30.40	24.53	9.00	48.00	16.50	19.40	12.32
QRS (ms)	49.00	91.00	74.50	72.50	13.08	49.00	95.00	67.00	72.70	15.12
RRSD (ms)	23.00	155.00	54.00	70.00	42.10	29.00	106.00	48.50	54.00	24.56
ECG										
QT interval (ms)	336.00	445.00	371.00	376.70	29.50	335.00	475.00	383.00	385.60	37.43
Pulse: ECG (bpm)	59.00	92.00	68.50	72.40	12.66	47.00	87.00	63.50	64.90	11.61
QTc (ms)	367.00	444.00	411.50	412.70	22.59	346.00	421.00	402.00	398.00	21.18
Biofeedback apparatus										
Skin conductance	2.11	5.75	2.64	3.03	1.14	0.78	8.71	3.91	3.30	2.11

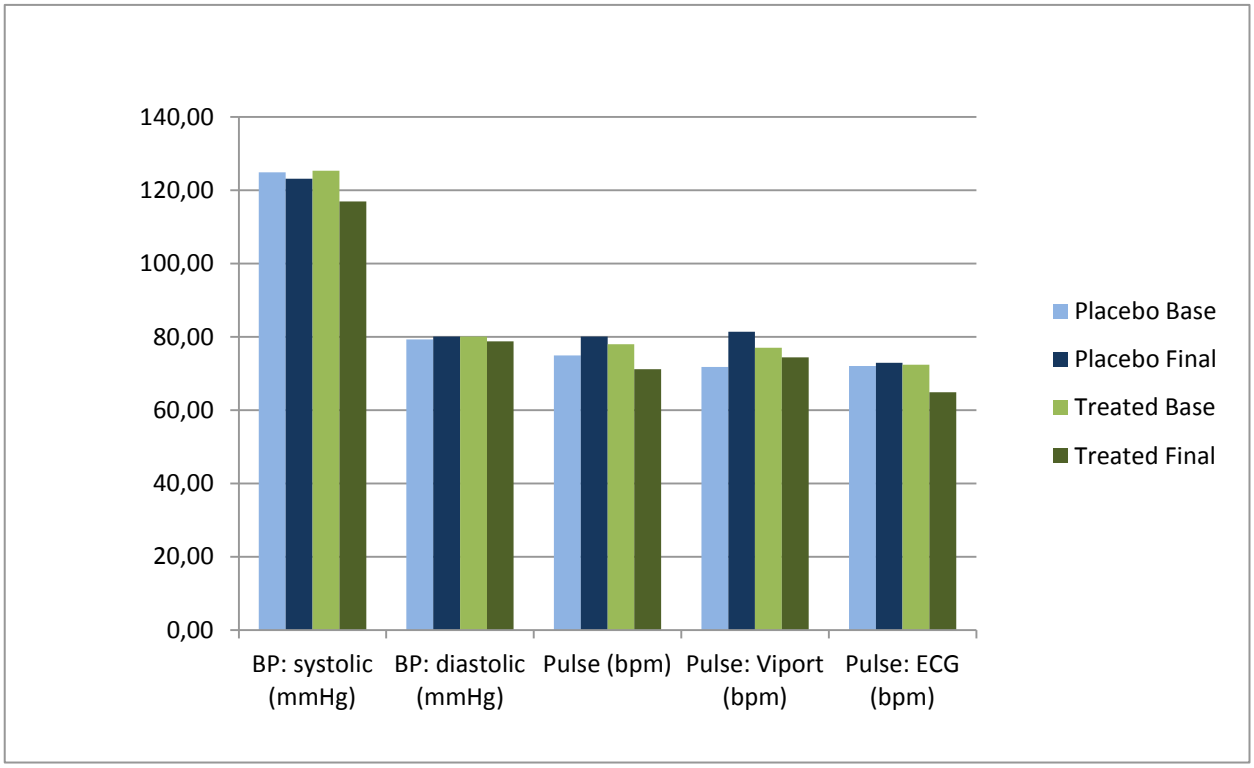

Figure 2: Comparison of mean vital values between baseline and final visit.

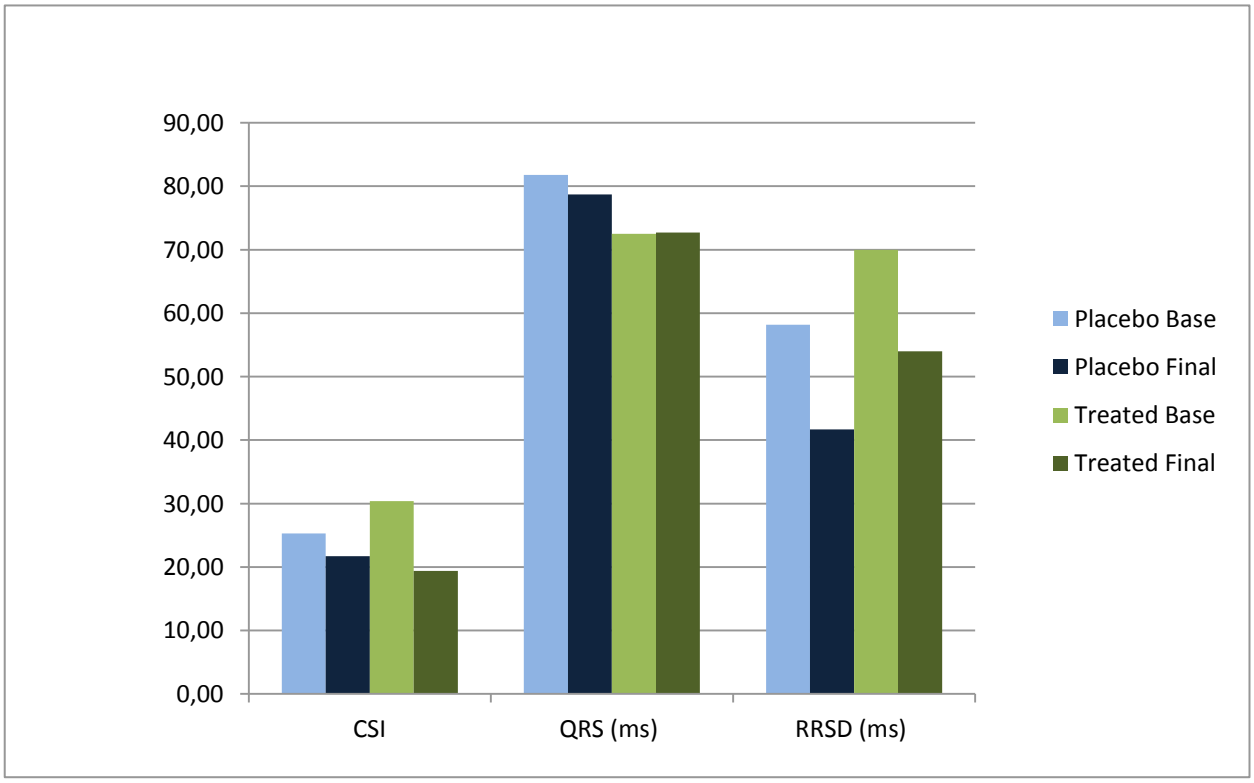

Figure 3: Comparison of mean Viport values between baseline and final visit.

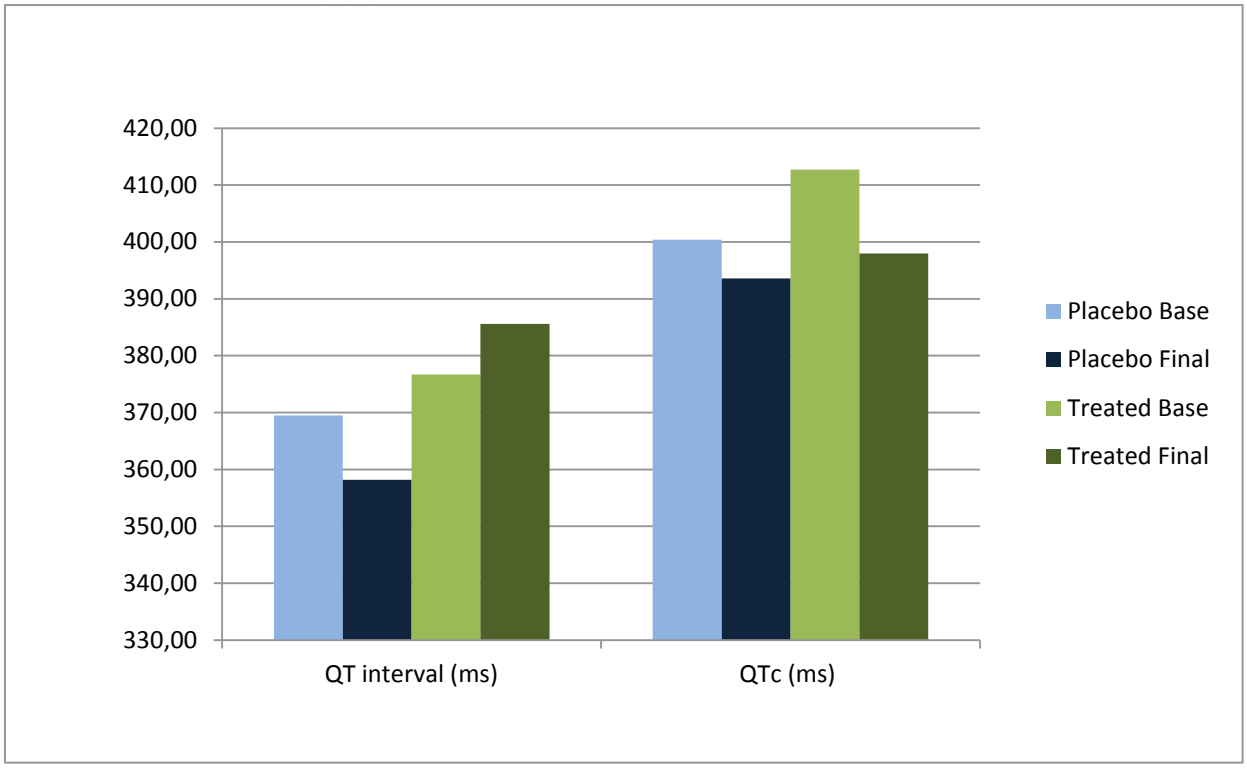

Figure 4: Comparison of mean QT values between baseline and final visit.

Figure 5: Comparison of mean skin conductance and salivary cortisol concentrations between baseline and final visit.

Table 4: Mann-Whitney U test to assess initial bias at baseline visit

	p-value
Vitals	
BP - systolic (mmHg)	0.791
BP - diastolic (mmHg)	0.519
Pulse (bpm)	0.970
Viport	
Pulse (bpm)	0.519
CSI	0.493
QRS (ms)	0.140
RRSD (ms)	0.820
ECG	
QT interval (ms)	0.940
QTc (ms)	0.190
Pulse (bpm)	1.000
Biofeedback apparatus	
Skin conductance	0.597

A Mann-Whitney test was done to determine if there was any initial bias at the baseline visit (Table 4). All the p-values were greater than 0.05 thus there was no significant difference prior to the evaluations for all the parameters.

Table 5: Wilcoxon signed ranks test: Within subject comparison

	p-value	
	Placebo Group	Treatment Group
Vitals		
BP - systolic (mmHg)	0.400	0.033*
BP - diastolic (mmHg)	0.297	0.318
Pulse (bpm)	0.023*	0.052
Viport		
Pulse (Viport)	0.006*	0.087*
CSI	0.271	0.004*
QRS (ms)	0.238	0.297
RRSD (ms)	0.003*	0.318
ECG		
QT interval (ms)	0.101	0.043*
QTc (ms)	0.110	0.012*
Pulse (ECG)	0.361	0.011*
Biofeedback apparatus		
Skin conductance	0.480	0.439

* Indicating statistical significance ($p < 0.05$)

Wilcoxon signed ranks test is a non-parametric test and was done to determine significant difference within the groups. Because the alternative hypothesis is one sided, the two-tailed p-value was divided by two. The smaller the p-value, the more evidence there is in favour of the alternative hypothesis. [47] With a p-value that is less than 0.01 the evidence that the alternative hypothesis is true is classified as “convincing”. Between 0.01 and 0.05 the evidence in favour of the alternative hypothesis is “strong”. There is a grey area between 0.05 and 0.10 that gives moderate evidence that the alternative hypothesis is true.

In Table 5 the Baseline and Final measurements were compared within each of the groups. In the placebo group the CSI, RRSD and the pulse values measured with the electronic device and Viport had significant differences. Within the treatment group there were statistical significant differences for systolic blood pressure, CSI, QT interval, QTc and all pulse measurements.

Table 6: Mann-Whitney U Test: Between group differences (i.e. final visit – base visit)

	p-value
Vitals	
BP - systolic (mmHg)	0.124
BP - diastolic (mmHg)	0.177
Pulse (bpm)	0.010*
Viport	
Pulse (Viport)	0.003*
CSI	0.053
QRS (ms)	0.218
RRSD (ms)	0.038*
ECG	
QT interval (ms)	0.026*
QTc (ms)	0.197
Pulse (ECG)	0.038*
Biofeedback apparatus	
Skin conductance	0.398

* Indicating statistical significance ($p < 0.05$)

The Mann-Whitney U test was used to compare the differences between the two independent groups (placebo and treatment group).Between the two group's statistically significant differences were found for RRSD, QT interval and all pulse readings.

CORTISOL ANALYSIS

Table 7: Salivary cortisol concentration and absorbance values of subjects in the placebo group

Placebo Group								
Subject nr	**Baseline Visit (n=10)**				**Final Visit (n=10)**			
	Absorbance 1 (at 450nm)	Absorbance 2 (at 450nm)	Average Absorbance (at 450nm)	Salivary Cortisol [ng/ml]	Absorbance 1 (at 450nm)	Absorbance 2 (at 450nm)	Average Absorbance (at 450nm)	Salivary Cortisol [ng/ml]
A01	0.65	0.56	0.60	7.30	0.56	0.57	0.57	9.92
A02	1.06	1.05	1.06	0.16	1.21	1.07	1.14	0.08
A03	1.15	1.14	1.14	0.08	1.38	1.21	1.29	0.02
A04	0.78	0.74	0.76	1.92	0.96	1.26	1.11	0.10
A06	0.88	1.28	1.08	0.13	0.97	0.97	0.97	0.34
A07	0.85	0.79	0.82	1.19	0.69	0.78	0.73	2.48
A09	0.88	1.27	1.08	0.14	0.75	8.79	4.77	0.00
A10	0.76	0.71	0.74	2.34	1.53	1.78	1.66	0.00
A11	0.52	0.50	0.51	15.95	1.54	1.69	1.62	0.00
Average	0.84	0.89	0.87	3.24	1.07	2.01	1.54	1.44
Min	0.52	0.50	0.51	0.08	0.56	0.57	0.57	0.00
Max	1.15	1.28	1.14	15.95	1.54	8.79	4.77	9.92
Median	0.85	0.79	0.82	1.19	0.97	1.21	1.14	0.08

Table 8: Salivary cortisol concentration and absorbance values of subjects in the treatment group.

Treatment Group								
Subject nr	**Baseline Visit (n=10)**				**Final Visit (n=10)**			
	Absorbance 1 (at 450nm)	Absorbance 2 (at 450nm)	Average Absorbance (at 450nm)	Salivary Cortisol [ng/ml]	Absorbance 1 (at 450nm)	Absorbance 2 (at 450nm)	Average Absorbance (at 450nm)	Salivary Cortisol [ng/ml]
B01	0.43	0.55	0.49	18.55	1.00	1.03	1.01	0.23
B02	1.12	1.04	1.08	0.13	1.13	0.92	1.02	0.22
B03	1.28	0.97	1.13	0.09	1.56	1.72	1.64	0.00
B04	1.10	1.19	1.14	0.08	1.06	1.08	1.07	0.15
B06	0.75	0.66	0.70	3.11	1.10	1.03	1.07	0.15
B07	1.46	1.39	1.43	0.01	1.30	1.43	1.37	0.01
B08	0.84	0.74	0.79	1.55	1.57	1.59	1.58	0.00
B09	0.92	1.55	1.23	0.04	1.80	1.84	1.82	0.00
B11	0.71	0.67	0.69	3.45	1.00	0.93	0.97	0.34
Average	0.96	0.97	0.97	3.00	1.28	1.29	1.28	0.12
Min	0.43	0.55	0.49	0.01	1.00	0.92	0.97	0.00
Max	1.46	1.55	1.43	18.55	1.80	1.84	1.82	0.34
Median	0.92	0.97	1.08	0.13	1.13	1.08	1.07	0.15

Table 9: Absorbance values of standard control cortisol samples

Standard [ng/ml]	Standard [ng/ml]*100	LOG[ng/ml]	Absorbance 1 (at 450nm)	Absorbance 2 (at 450nm)	Average Absorbance (at 450nm)
0.1	10	1.000	1.150	0.976	1.063
0.5	50	1.699	0.956	0.815	0.886
1.5	150	2.176	0.909	0.844	0.877
4	400	2.602	0.776	0.770	0.773
10	1000	3.000	0.540	0.521	0.531
40	4000	3.602	0.402	0.273	0.338

The absorbance values of the standard controls were obtained with the ELISA kit to generate a standard curve (Figure 2) of absorbance value versus concentration. Using the standard curve the concentrations of cortisol was calculated for each saliva sample.

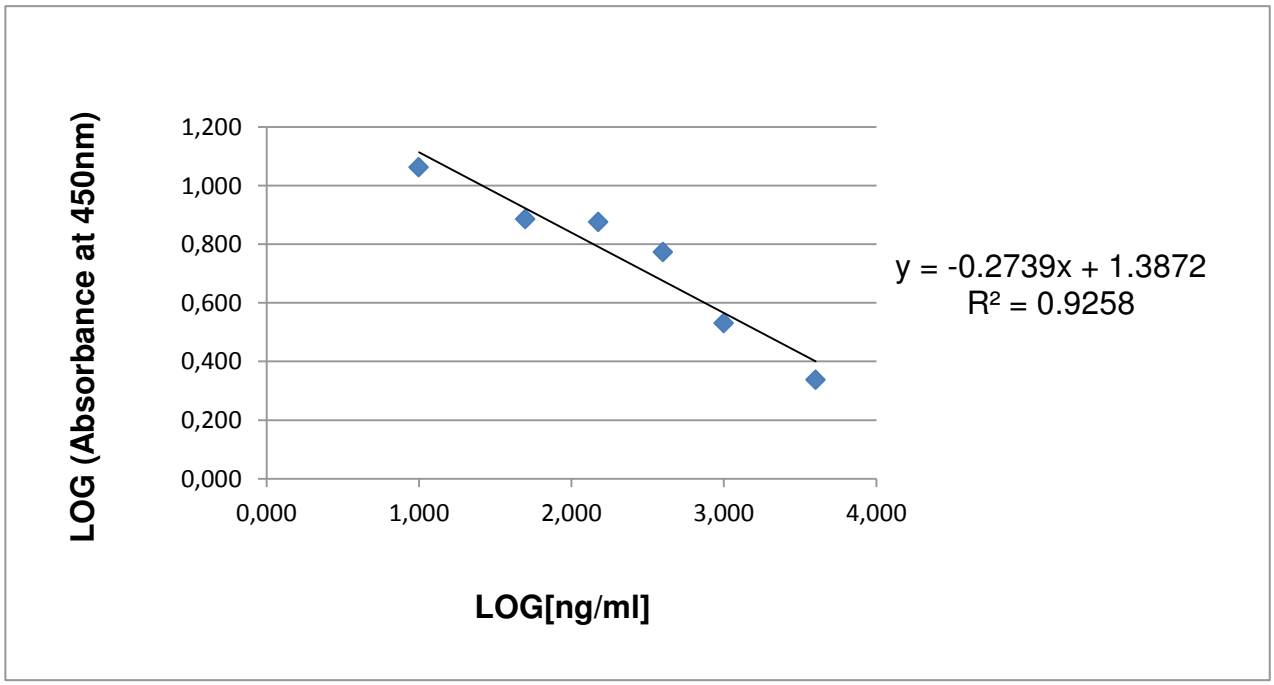

Figure 6: Standard curve of salivary cortisol

Table 10: Mann-Whitney Test to assess initial bias at base visit: Cortisol

	p-value
Salivary Cortisol	0.387

A Mann-Whitney test was done to determine if there was any initial bias at the baseline visit (Table 10). The p-value was greater than 0.05 thus there was no significant difference prior to the evaluations for all the parameters.

Table 11: Wilcoxon signed ranks test: Within subject comparison for salivary cortisol

	p-value	
	Placebo Group	Treatment Group
Salivary Cortisol	0.258	0.055

* Indicating statistical significance ($p < 0.05$)

Within the treatment group the p-value of salivary cortisol is 0.055, thus there is moderate evidence that the alternative hypothesis is true.

Table 12: Mann-Whitney U test: Between group differences (i.e. final visit – base visit) for salivary cortisol

	p-value
Salivary Cortisol	0.273

Salivary cortisol showed no significant difference between the two groups.

Table 13: Descriptive statistics: difference = final visit – base visit

	Placebo Group (n=10)				Treatment Group (n=10)			
	Min	Max	Mean	Std. Deviation	Min	Max	Mean	Std. Deviation
Vitals								
BP - systolic (mmHg)	-25.00	16.00	-1.70	12.95	-32.00	7.00	-8.40	11.81
BP - diastolic (mmHg)	-30.00	14.00	0.80	13.75	-13.00	12.00	-1.30	8.84
Pulse (bpm)	-7.00	17.00	5.20	7.44	-25.00	13.00	-6.80	12.36
Viport								
Pulse (Viport)	-4.00	21.00	9.60	8.59	-12.00	30.00	-2.60	12.56
CSI	-29.00	27.00	-3.60	16.47	-41.00	0.00	-11.00	13.27
QRS (ms)	-26.00	19.00	-3.10	14.01	-24.00	11.00	0.20	10.09
RRSD (ms)	-40.00	-1.00	-16.50	13.82	-123.00	11.00	-16.00	41.12
ECG								
QT interval (ms)	-47.00	28.00	-11.30	24.24	-8.00	30.00	8.90	13.10
QTc (ms)	-27.00	11.00	-6.80	14.36	-42.00	13.00	-14.70	16.06
Pulse (ECG)	-18.00	18.00	0.80	10.23	-23.00	4.00	-7.50	9.30
Biofeedback apparatus								
Skin conductance	-1.68	4.19	0.17	1.82	-3.31	6.26	0.27	2.44
ELISA								
Salivary Cortisol	-2.62	15.94	1.81	5.50	-0.09	18.32	2.88	5.94

Figure 7 and Figure 8

Adverse events for both groups were collected at the final visit. Both groups experienced adverse event, but the treated group had a higher incidence and variety of events.

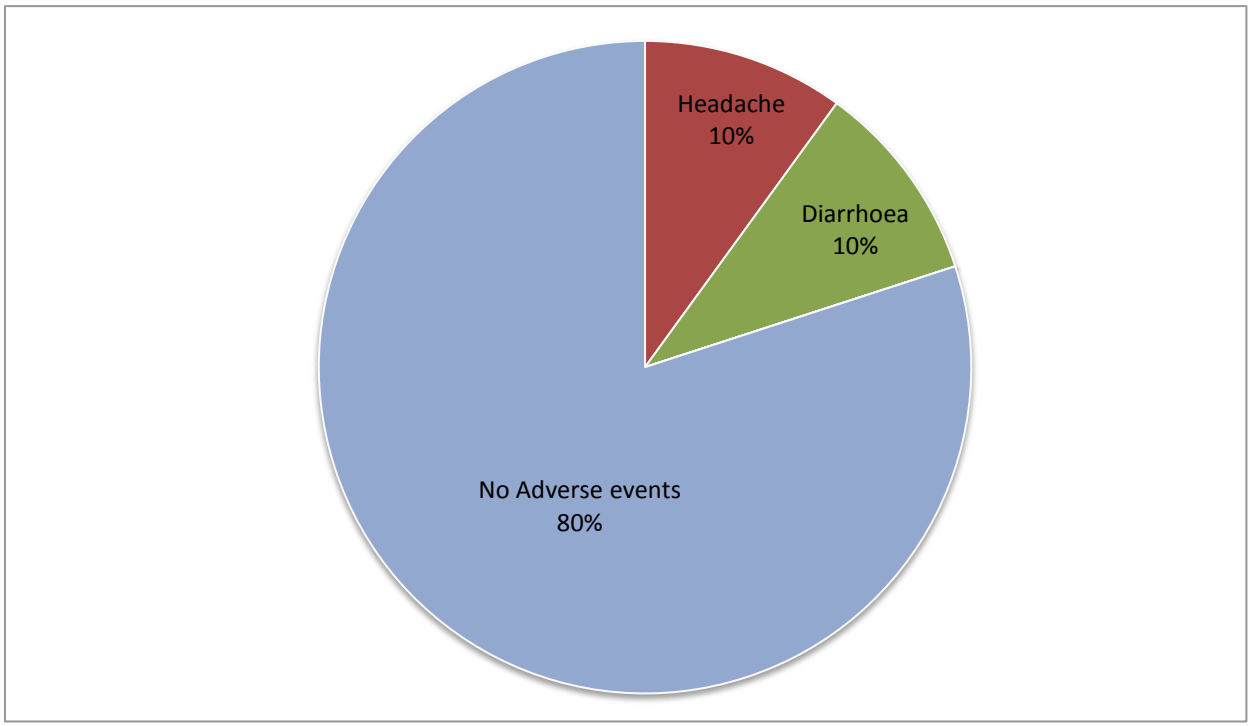

Figure 7: Frequency of adverse events in placebo group

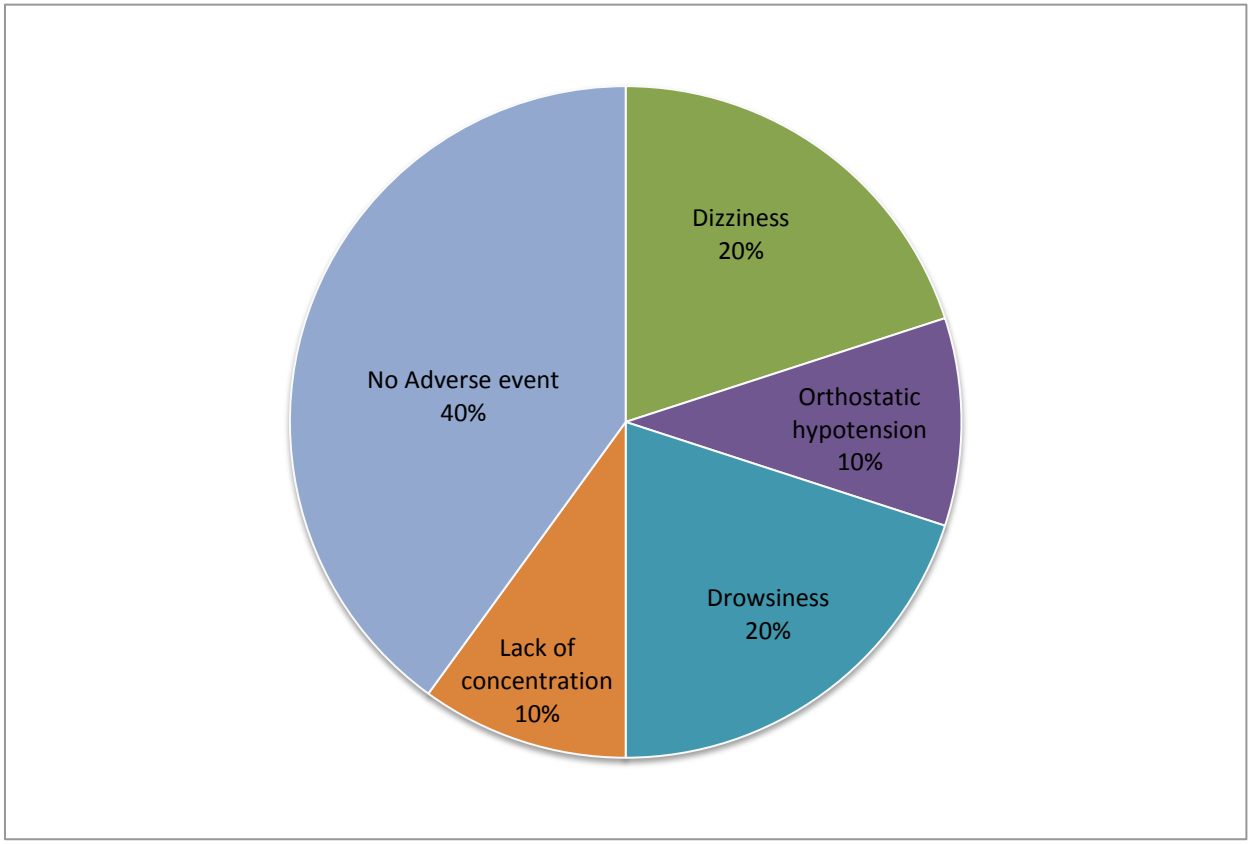

Figure 8: Frequency of adverse events in treatment group

CHAPTER 5

DISCUSSION

Demographics

The average age of the volunteers was 23.55 years, with a range of 18 to 29 years (Table 1).

Two subjects, one from each group, withdrew participation from the study and thus did not return for the final visit. 20 subjects did complete the study, 10 in the placebo group and 10 in the treatment group.

Only 30% of the volunteers were female and the remaining 70% male. Although this is not a representation of the South African population, the volunteers were randomly selected.

The difference between genders was not compared in this study although previous studies have indicated a variance between gender groups. Chauhan *et al* indicated that at rest woman have longer QTc intervals, less QT dispersion and lower amplitude than men. [55] And according to Larsson *et al* *w*omen in general were found to have significantly higher levels of morning cortisol than men. [56] This oversight can be noted as a limitation in the study.

Adverse events

Adverse events were observed in both the treatment and the placebo group. In the placebo group 2 subjects each experienced headache and diarrhoea respectively (Figure 7).

Adverse events were more frequent in the treatment group. These adverse events were anticipated since it is listed as common side effects of Methyldopa. Participants were informed of these possible side effects in the patient informed consent. Dizziness and drowsiness were both observed in 20% of the group while lack of concentration and orthostatic hypotension were each observed in 10% of the group (Figure 8).

Blood pressure and Pulse rate

According to the American Heart Association the normal adult blood pressure is less than 120mmHg for systolic and less than 80mmHg for diastolic pressure. The average resting pulse rate is between 60 and 100bpm for a healthy adult.

The placebo group showed little statistical significance within the group comparing the Baseline visit to the Final visit (Table 5). No significant change was expected the placebo group, but an increase in all the pulse rates was observed. Both the pulse rates measured with the electric device and the Viport apparatus have strong evidence supporting the alternative hypothesis. The pulse rate measured with the ECG apparatus did increase but not statistical significantly.

The treatment group had more significant results within the group than the placebo group (Table 5). After a week of Methyldopa treatment, blood pressure and pulse decreased in the treatment group. The systolic blood pressure and all three pulse rates (measured with sphygmomanometer, Viport and ECG apparatus) decreased significantly. The diastolic blood pressure did also decrease however it was not significant.

Comparing the placebo group with the treatment group (Table 6 and Figure 2) there is statistical significance for all the pulse rates measured with the

different apparatus. Methyldopa thus has a convincing decreasing effect on the pulse rate. There was however no significant difference between the two groups for the blood pressure. Although there was a decrease in the treatment group for both systolic and diastolic blood pressure, if you compare the treatment group with the placebo group the difference is not significant. Since methyldopa is an anti-hypertensive agent, one would have expected a decrease in blood pressure. A reason for this occurrence may be the type of population that was used. All the subjects are young (18-30 years), healthy and normotensive so although an anti-hypertensive agent was administered, the body has homeostatic regulation that will counteract to regulate the blood pressure.

Cardio Stress Index (CSI) and Heart rate variability (HRV)

The Cardio Stress Index (CSI) is a converted measure of HRV. Various parameters of HRV were measured through frequency domains with the Viport apparatus and are represented as a percentage namely the CSI.
A normal CSI is considered between 0 and 20 and corresponds to normal heart rate variability (HRV).

Methyldopa suppresses the sympathetic nervous system causing Heart rate (HR) to decrease but inversely increasing HRV.

As seen in Table 5, in both the placebo and the treatment groups the CSI decreased. Within the groups only the treatment group had a statistically significant result. The decrease in CSI correlates with higher HRV.

Between the two groups there is moderate evidence that the alternative hypothesis is true (Table 6). Thus Methyldopa has a moderate increasing effect on HRV (Figure 3).

QRS complex and RRSD

A study done by Folino [48] indicated that an increased sympathetic stimulation causes a decrease in QRS complex duration. With methyldopa which decreases sympathetic output, the QRS complex will possibly increase. A normal QRS complex is considered between 60 and 100 milliseconds.

Within each of the two groups and between the two groups the QRS complex did not significantly change. Although the placebo group showed a slight decrease, the treatment group had a slight increase (Table 13). The average Baseline and Final visit QRS complex values for both the placebo group and the treatment group are within the normal range of 60 and 100 milliseconds.

The RRSD is the standard deviation of the interval between each heartbeat. A reduction in RRSD reflects a shift toward more sympathetic dominance. [50] A higher RRSD represents a higher discrepancy between the R-R intervals on an ECG. A low RRSD is accompanied by a low HRV which will lead to a high CSI. Very high RRSD's may be an indication of an arrhythmia.

Both RRSD variables decreased within their specific group, although only the placebo group showed a significant decrease, see Table 5. In the end when comparing the difference between the two groups, although there is an increase in HRV, there was an unexpected significant decrease (Table 6 and Figure 3).

QTc

A corrected QT interval (QTc) is used instead of the normal QT interval since QT varies with heart rate, therefore QTc is adjusted for effect of the heart rate on the QT interval. A normal QTc interval range is considered to be between 0.35–0.44 seconds.

Within the groups, (Table 5) both showed a decrease, nevertheless only the treatment group had a significant decrease. A longer heart rate-corrected QT interval (QTc) represents predominance of sympathetic activity within autonomic balance. [25] Thus we can assume the sympathetic activity was suppressed with Methyldopa.

However when comparing the groups with one another, there is no significant difference between the two groups, see Table 6 and Figure 4.

Skin Conductance

With the suppression of the sympathetic nervous system the skin conductivity is lowered in the skin due to the lack of sweat in the eccrine glands.

A slight increase in skin conductance was observed in both the placebo and treatment group comparing the baseline with the final measurement, which is the opposite effect that was expected (Table 5).

Although the skin conductivity increased, no significant result was obtained when comparing the difference between the placebo and the treatment group (Table 6 and Figure 5).

Salivary Cortisol

Only 9 out of the 10 subjects' samples were used for the analysis with the ELISA kit due to the limited wells available on the plate. The 9 samples were randomly selected from the available 10 samples.

To calculate the cortisol concentrations of the salivary samples a standard curve was generated using the absorbance values of the standard controls that were supplied.

The average absorbance value of each subject's salivary sample was used to calculate the cortisol concentration (Table 7 and 8). These average absorbance values were substituted in the equation (y = -0.2739x + 1.3872) of the standard curve (Figure 6). Where 'y' represents the absorbance value of the saliva, and 'x' is the cortisol concentration that was calculated.

A study done by Campisi [49] indicated that salivary cortisol levels elevate after sympathetic arousal and even stays elevated after 30 minutes when blood pressure and heart rate returned to their resting values.

Although a decrease was expected in the treatment group, both the groups had an increase in salivary cortisol, as seen in Table. With a p-value of 0.055 the treatment group showed moderate evidence that the alternative hypothesis is true.

No significant result was obtained when comparing the difference between the placebo and the treatment group. This correlates with a study done by Syviilahti, where cortisol levels fell slightly after treatment with Methyldopa, however this was still within normal range. [52] This study together with the study done by Syviilahti concludes that methyldopa does not cause any clinically important alterations in the anterior pituitary function. [52]

Attention deficit hyperactivity disorder (ADHD).

The knowledge of the effect of alpha-methyldopa on the SNS can be valuable in the treatment of attention deficit hyperactivity disorder (ADHD).
Accumulated evidence from a variety of studies has shown that alpha-2 agonists are beneficial for ADHD patients. In 2010 Clonidine, alpha-2 agonist, has been approved by the Food and Drug Administration (FDA) as an add-on to stimulant therapy or monotherapy in ADHD treatment.

ADHA possibly interfere with the functioning of the prefrontal cortex (PFC) and/or its connections with the basal ganglia and cerebellum. According to Arnsten et al. Alpha-2 agonists enhance the functional connectivity of prefrontal cortical networks through stimulation of post-synaptic alpha-2A receptors on the dendritic spines of prefrontal cortical pyramidal cells. [53] The research of Goldman-Rakic et al. was the first to reveal that catecholamines dopamine (DA) and norepinephrine (NE) are vital for PFC functioning. [54]

Although the sedative side-effect of Methyldopa remains a concern, this effect usually decrease spontaneously after 2-3 days.[3] Therefor Methyldopa can be helpful in the treatment of ADHD.

CONCLUSION

After a week of bi-daily dosing the effect of alpha-methyldopa on sympathetic activity was evaluated. Significant result was observed in a number of parameters. Comparing the placebo group with the treatment group there was statistical significance observed in CSI, HRV, RRSD, QT interval and in all the pulse rates measured with the different apparatus. RRSD did have a significant result however the decrease in value is an indicator of sympathetic predominance which is contradictory to the mechanism of action of methyldopa. If a larger sample size was used the results may have been different.

Parameters that did not have significant results include, BP, QRS complex, QTc, skin conductance and salivary cortisol. Since methyldopa is an anti-hypertensive agent a decrease in blood pressure was observed but it was not significant, possibly because the subjects that participated in the trial were young, healthy individuals. Skin conductance and salivary cortisol concentration did not decrease as expected but no significant increase was observed.

Adverse events were more frequent in the treatment group comparing to the placebo group. Dizziness and drowsiness were both observed in 20% of the group while lack of concentration and orthostatic hypotension were each observed in 10% of the group.

Alpha-methyldopa is an alpha-adrenergic agonist that inhibits sympathetic outflow. Alpha-methyldopa had a decreasing effect on BP, Pulse, CSI, RRSD and QTc whereas it had an increasing effect on QRS complex, HRV, QT interval, skin conductance and salivary cortisol. Most of the parameters indicated suppression in sympathetic activity with the exception of RRSD, skin conductance and salivary cortisol concentration.

The knowledge of the effect of alpha-methyldopa on the SNS is valuable in the treatment of attention deficit hyperactivity disorder (ADHD). Methyldopa can be considered in the treatment of ADHD.

Some limitations to the study include the small sample size and the parallel study design. A crossover study design would have been more appropriate.

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