



MAURITIUS RESEARCH COUNCIL

EFFECTS OF BLACK TEA CONSUMPTION ON ISCHAEMIC HEART DISEASES AMONG THE MAURITIAN POPULATION

Final Report

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MAURITIUS RESEARCH COUNCIL

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EFFECTS OF BLACK TEA CONSUMPTION ON ISCHAEMIC HEART DISEASES AMONG THE MAURITIAN POPULATION

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PROJECT SUMMARY

Data from the Central Statistics Office indicated that diseases of the circulatory system accounted for 40.2 % of the deaths in 2002. Epidemiologic studies report a reduced risk of ischaemic heart disease in subjects with a high phenolic intake through tea and other dietary sources, but findings are still conflicting. The potential protective effects of tea polyphenols have been attributed to its antioxidant, free radical scavenging, antithrombogenic, antimutagenic, anticarcinogenic and anti-inflammatory properties, lowering of plasma cholesterol and triglyceride levels, and reduction of blood pressure and platelet aggregation capacities. Recent animal experiments suggest that polyphenolics may also improve vascular function. Polyphenols remain the most abundant group of compounds in fresh tea leaves and are found in green and black tea beverages at 30-42% and 3-10% of the total dry matter respectively. In a previous systematic analysis of 9 commercially available black tea, we observed that Mauritian black tea infusates represent excellent sources of polyphenolic compounds including (+)-Catechin, (-)-Epicatechin, (-)-Epicatechin-3-gallate, Epigallocatechin, (-)-Epigallocatechin-3-gallate, gallic acid, procyanidin dimers B1 and B2, quercetin, myricetin and kaempferol derivatives. The infusates exhibited remarkably high *in vitro* antioxidant activities as evaluated by four independent assays. The antioxidant activities correlated strongly with levels of total phenols and proanthocyanidins. In line with certain studies on the phytochemistry and antioxidant capacities of the Mauritian diet and parallel to some previous non-communicable diseases intervention programme conducted by the Ministry of Health and Quality of Life, we propose to investigate the association between consumption of black tea and various selective fasting blood serum and urine biomarkers in a Mauritian population with ischaemic heart diseases. The clinical examination and analysis will be performed at the Cardiac Centre, Pamplemousses, in collaboration with Dr TK Guness and Dr OI Aruoma from The London South Bank University, UK.

This study, the first of its kind, will provide clinical data on the potential prophylactic propensities of Mauritian black tea against cardiovascular disease, which remains one of the major health threats to the Mauritian population.

1. PROJECT JUSTIFICATION

There is convincing biochemical, physiological, pharmacological, epidemiological and clinical evidence that excessive free radical production and lipid peroxidation are involved in diverse pathological conditions including atherosclerosis and cardiovascular diseases among others (Briviba *et al.*, 1997, Beckman and Ames, 1998, Aruoma and Halliwell, 1998, Mimic-Oka *et al.*, 1999, Garcia-Estan *et al.*, 2002). The cardiovascular disease is a heterogeneous group of disorders that affects the heart and blood vessels and are characterised by hypertension, congestive heart failure, reperfusion injury, acute myocardial infarction (heart attack), stroke, angina pectoris and arrhythmia. For instance, evidence suggests that damage to myocardial cell during reperfusion injury may be due to the generation of toxic, reactive oxygen species such as superoxide radical, hydrogen peroxide, and the hydroxyl radical (Garlick *et al.*, 1987, Bolli 1991, Valen and Vaage 1993) while the importance of oxidative stress in the development of atherosclerosis seems to be widely accepted.

The prevention of oxidative damage has been the subject of considerable investigation. In this respect plant polyphenolics and vitamins have been studied for their free radical scavenging and antioxidant properties (Bahorun *et al.*, 1995, Luximon-Ramma *et al.*, 2003, Bahorun *et al.*, 2004). Epidemiological studies have shown strong correlation between increased consumption of food phenolic antioxidants and a reduced risk of cardiovascular diseases (Yochum *et al.*, 1999, Singh *et al.*, 2002, Geleijnse *et al.*, 2002, Bagchi *et al.*, 2003).

Tea, one of the most consumed beverages in the world, has received considerable attention as a protective agent against cardiovascular disease and cancer, two important targets of preventive medicine for adults. Tea leaves are considered to be important sources of polyphenolics with about more than 35 % of their dry weight (Graham, 1982). Moreover, several laboratory studies on crude extract or constituents of black and green teas have shown inhibitory effects on oxidation, mutagenicity, inflammation and protection effects against certain neurodegenerative disorders (Choi *et al.*, 2001, Gupta *et al.*, 2002, Hong *et al.*, 2001, Katiyar *et al.*, 1999). Few cohort cross sectional studies reported an inverse association between consumption of green tea and various serum markers from cardiovascular patients (Imai and Nakachi 1995, Duffy *et al.*, 2001, Geleijnse *et al.*, 2002). No clear clinical evidence for protective effects of tea in humans, however, has been reported.

We previously screened, analysed and characterised the polyphenolic contents of 9 brands of Mauritian black tea infusates followed by an evaluation of the *in vitro* antioxidant capacities of the total extracts by four independent *in vitro* assays. Important levels of catechin derivatives, flavonols, procyanidin dimers and gallic acid from the infusates with remarkably high *in vitro* antioxidant efficacies were observed. The antioxidant activities correlated strongly with contents in total phenols and proanthocyanidins. Two research papers based on the results of this work are being prepared for submission to the peer reviewed journals "*Mutation Research*" and "*Journal of Pharmacy and Pharmacology*".

Since diseases of the circulatory system accounted for 40.2 % of the deaths in 2002 in Mauritius (Digest of Vital Health Statistics, 2002) and in line with several studies on the phytochemical and antioxidant capacities of the Mauritian diet, we are proposing the investigation, through a randomised double-blind, placebo controlled study, of the effects of black tea consumption on cardiovascular diseases among the Mauritian population. The study will be carried out on patients with diagnosed ischaemic heart diseases, selected from the waiting list for Angiography, at the Cardiac Centre, Pamplemousses in collaboration with Dr TK Gunness and Dr OI Aruoma from The London South Bank University, UK. It is interesting to note that a similar approach was used in the cross sectional survey of Uusitalo *et al.*, 1996 where the Government of Mauritius launched a non-communicable disease

intervention programme to determine the effect of reducing saturated fatty acid composition of ubiquitously used cooking palm oil on cholesterol contents in the population. The study was conducted during a five year intervention period. The positive findings of the study are now taken as a reference in the agenda of the World Health Organisation where Mauritius is stated as one of the developing countries having taken initiatives towards comprehensive intervention programme for the prevention of non-communicable diseases (WHO, 2001).

The proposed study, the first of its kind, will provide clinical and biochemical data on the potential antioxidant propensities and prophylactic capacity of the Mauritian black tea against cardiovascular diseases. It is interesting to note that the proposed study will be in line with several management strategies and therapeutic approaches towards the primary prevention of and protection against cardiovascular diseases among the Mauritian population at both the Governmental and institutional levels.

2. PROJECT DESCRIPTION

2.1 OBJECTIVES

Our broad objectives will be to

- survey and recruit patients with ischaemic heart diseases in a randomized sample representative of the Mauritian population (ethnicity, gender, age, social and economical status) and record their medical history,
- collect fasting blood serum and urine at baseline and after supplement of control volume of tea infusion and water from study group and control group respectively,
- optimize techniques to determine levels of specific biomarkers from above body fluid samples,
- investigate existing correlation between tea consumption and risk of cardiovascular diseases in Mauritian population

Our specific objectives will be to:

- select a randomised group of Mauritian population, with ischaemic heart disease, who has been referred to the Cardiac Centre, Pamplemousses for Angiography and a group of people showing no risk of cardiovascular diseases
- inform the people about the motives of the study and seek consent form for those willing to participate in the study,
- evaluate the levels of biomarkers of oxidative stress (total cholesterol, LDL, HDL, triglycerides, homocysteine, atrial natriuretic peptides, brain natriuretic peptides, 8-hydroxydeoxyguanosine, isoprostanes, hydroxyecosatetraenoic acid, products of protein damage, uric acid and glycosylated haemoglobin) from 8-10 hr fasting blood serum and urine at baseline from all participants
- supply the study group with control volume of tea infusion (Ouvagalia brand from Bois Chéri tea) and the control group with same amount of water for a defined period of time followed by a two week wash out period with water.
- study biomarkers as above from 8-10 hr fasting blood serum and urine on two weeks intervals during the supplement regime
- investigate the existing correlation between tea consumption and levels of biomarkers of cardiovascular diseases in Mauritian sample population.

2.2 BACKGROUND INFORMATION

With a population density of 1.20 million and a growth rate of 1.0 %, Mauritius is a cosmopolitan country with an extraordinary diversity and mixture of races comprising Hindu (52 %), Christians (28.3 %), Muslim (16.6 %) and Chinese (0.7 %) among others (Annual Digest of Statistics, 2002). Epidemiological data revealed a high prevalence of chronic diseases among the Mauritian populations. The distribution of deaths by Chapter of the International Classification of Diseases showed that diseases of the circulatory system accounted for 40.2 % of the deaths in 2002 in the country. Out of this, acute myocardial infarction represented 26.9 % with a further 13.3 % caused by other forms of ischaemic heart diseases (Digest of Vital and Health Statistics, 2002). The most worrying fact remains the 23.6 % deaths registered among the population under the age of sixty. Furthermore, deaths due to *Diabetes mellitus* represented 4.6 % of the total deaths in Mauritius in 2000. These values are becoming increasingly alarming.

In 1987 the Government of Mauritius launched a non-communicable diseases intervention programme to prevent and control diseases caused by unhealthy lifestyles because such diseases were occurring at increasing rates in Mauritius (La Vecchia et al., 1993, Gareeboo et al., 1988). Government limited the content of palm oil in cooking oil, which was used almost universally in Mauritius. Palm oil is high in saturated fatty acids and was then the main component of ration oil. The oil was gradually replaced by wholly soya bean oil during a five year cross sectional study from 1987 to 1992. Using cluster surveys on adults aged 25 – 75 years, they reported significant decreases in the prevalence of hypertension (15.0% to 12.1% in men and 12.4% to 10.9% in women); cigarette smoking (58.2% to 47.2% and 6.9% to 3.7% respectively); and heavy alcohol consumption (38.2% to 14.4% and 2.6% to 0.6% respectively). Mean population serum total cholesterol concentration fell appreciably from 5.5 mmol /l to 4.7 mmol/l ($P < 0.001$). However, the prevalence of overweight or obesity increased, and the rates of glucose intolerance changed little. The population frequency distributions of blood pressure, serum lipid concentration and the composite risk factor score shifted advantageously.

Similarly, epidemiological studies provide convincing evidence of the beneficial role of teas for the maintenance of health and prevention of chronic diseases. Teas are classified into three major categories: the non-fermented or green tea which represents about 20% of world-wide tea consumption, the partially fermented oolong or paochong tea representing only 2 %, and the fully fermented black or pu-erh tea with an 80% consumption (Balentine, 1992; Gupta *et al.*, 2002). The composition of tea varies with species, season, age of leaf, climate, and horticultural practices (Lin *et al.*, 1996). The constituents of green and black teas have been the subjects of intensive investigations for a long time (Hoefler and Coggon, 1976; Treutter, 1989; Lin *et al.*, 1998; Khokhar & Magnusdottir, 2002). Recent trends partially in response to claims of health benefits associated with the beverage, show an increased preference for fruit and herbal teas in continental Europe and to a lesser degree in the United Kingdom; however, black tea with added milk (white tea) remains by far the most common form consumed in the United Kingdom (The Tea Council, 2001) and in Mauritius.

Polyphenols are the most abundant group of compounds in fresh tea leaves and are found in green and black tea beverages at 30-42% and 3-10% of the total dry matter respectively (Graham, 1992). The major tea catechins are (+)-catechin, (-)-epicatechin, (-)-epicatechin-3-gallate, (-)-gallocatechin, (-)-epigallocatechin, (-)-epigallocatechin-3-gallate. In the manufacturing of black tea, the monomeric flavan-3-ols undergo polyphenol oxidase-dependent oxidative polymerization leading to the formation of bisflavanols, theaflavins,

thearubigins and other oligomers in the process commonly known as fermentation (Lin *et al.*, 1998). During the manufacture of oolong and black tea, the catechins (flavanols) are easily oxidized by polyphenol oxidase, and further polymerizations lead to theaflavins, thearubigins and compounds of higher molecular mass (Graham, 1992; Harbowy and Balentine, 1997; Wright *et al.*, 2002). These polyphenols are responsible for the characteristic reddish color and the astringency of the black tea (Rider *et al.*, 1992).

Tea catechins cause increased activities of phase II detoxifying enzymes, suppress extracellular signals and cell proliferation (Liang *et al.*, 1997), inhibit the induction of nitric oxide synthase (Lin and Lin, 1997), inhibit cyclooxygenase and lipoxygenase-dependent metabolism of arachidonic acid in human colon mucosa and colon tumor tissues (Hong *et al.*, 2001) and inhibit the growth of cancerous human colon and hepatic epithelial cells (Uesato *et al.*, 2001). (-)-Epigallocatechin-3-gallate (-)-EGCG has been shown to attenuate β -amyloid-induced neurotoxicity in cultured hippocampal neurons as well as inhibiting cloned rat brain Kv1.5 potassium channels (Choi *et al.*, 2001). Datla *et al.*, (2001) have shown that pre-treatment of rats with the citrus flavonoid tangeretin was neuroprotective in the 6-hydroxydopamine animal model of Parkinson's disease. This is in line with the report of Levites *et al.*, (2002) suggesting that tea extracts attenuated the neurotoxicity of 6-OHDA in rat pheochromocytoma (PC12) and human neuroblastoma (NB) SH-SY5Y cells *in vitro*.

Cardiovascular diseases are a heterogeneous group of disorders that affects the heart and blood vessels. These are characterised by angina pectoris, hypertension, congestive heart failure, reperfusion injury, acute myocardial infarction (heart attack), stroke and arrhythmia. There is now considerable biochemical, physiological and pharmacological data to support a connection between free radical reactions and cardiovascular tissue injury (Briviba *et al.*, 1997, Beckman and Ames, 1998, Aruoma and Halliwell, 1998, Mimic-Oka *et al.*, 1999, Garcia-Estan *et al.*, 2002). Evidence is accumulating that these disease conditions are directly or indirectly related to oxidative damage and share common mechanisms of molecular and cellular damage. Indirect evidences of free radical involvements in cardiovascular diseases can be demonstrated by the measurement of the products of free radical attack on biological substrates and intracellular and extracellular antioxidant capacity. Biomarkers of oxidative stress of interest are:

- Cholesterol and lipoprotein levels (total cholesterol, LDL, VLDL, triglycerides, HDL)
- Homocysteine - an independent risk factor of coronary heart diseases. It is derived from the demethylation of the amino acid methionine and it is believed that oxidation of homocysteine results in the formation of hydrogen peroxide, the triggering factor of lipid peroxidation.
- Atrial and brain natriuretic peptides – potent vasorelaxant substances of respectively the heart atria and ventricles and their release seem to be volume dependant in response to increase heart pressure
- Isoprostanes and hydroxyeicosatetraenoic acid (HETE) – products of oxidative lipid damage
- 8-hydroxydeoxyguanosine (8-OHdG) - a product of DNA base damage that can be assessed by HPLC with electrochemical detector
- Ortho-tyrosine and dityrosine – products of protein damage
- Uric acid – stabilises platelet aggregation and enhances thrombotic tendency, a risk factor of coronary mortality

There is accumulating evidence that consumption of both green and black tea is strongly associated with reduced risk of cancers and cardiovascular diseases (Duffy *et al.*, 2001, Arts *et al.*, 2001, Riemersma *et al.*, 2001 Miura *et al.*, 2001, Hodgson *et al.*, 2000, Katiyar *et al.*,

1999, Geleijnse *et al.*, 1999). A cross sectional study on the effects of drinking green tea on cardiovascular and liver diseases in a Japanese population revealed that an increased consumption of green tea is associated with decreased serum concentrations of total cholesterol and triglycerides together with a decreased proportion of low and very low lipoprotein cholesterol which resulted in a decreased atherogenic index (Imai K and Nakachi, 1995). In addition, antimutagenic effects (Shiraki *et al.*, 1994, Kuroda *et al.*, 1999; Gupta *et al.*, 2002), inflammation (Katiyar *et al.*, 1999) and protection against neurodegenerative diseases (Choi *et al.*, 2001) are widely discussed.

A previous systematic screening of the black tea infusates, obtained by infusion of tea leaves with boiled water for 5-10 mins, revealed important levels of phenolic comprising (+)-catechin, (-)-epicatechin, (-)-epicatechin 3-gallate, epigallocatechin, (-)-epigallocatechin 3-gallate, gallic acid, procyanidin dimers B1 and B2, quercetin, myricetin and kaempferol. These compounds are reputed for their potent free radical scavenging and reducing capacities (Ariga *et al.*, 1988, Arteel and Sies, 1999, Bors and Michel, 1999, Scott *et al.*, 1993, Rice-Evans *et al.*, 1995). In addition, the tea infusates exhibited remarkably high *in vitro* antioxidant capacities as assessed by four independent assays namely the Trolox equivalent antioxidant capacity (TEAC) [which is based on the ability of an antioxidant to scavenge the preformed radical cation ABTS⁺ (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) relative to that of the standard antioxidant Trolox], the ferric reducing antioxidant potential (FRAP) assay [which measures the antioxidant potentials of the plant extracts by their ability to reduce the Fe (III)-TPTZ complex to Fe (II)-TPTZ complex present in stoichiometric excess to the blue coloured ferrous form], the HOCl assay [which measures the protective effects against protein damage] and the deoxyribose assay [which measures protection against carbohydrate damage]. Because some of the above-mentioned assays were done in non-physiological pH values, it is difficult to extrapolate the results to physiological environment. It is highly stressed that proof of bioefficacy must emanate from application of reliable *in vivo* models where markers of baseline oxidative damage are examined from the standpoint of how they are affected by changes in diet or by antioxidant supplements.

2.3 SCOPE OF WORK

Mauritius has a relatively high prevalence of cardiovascular diseases, cancers and diabetes. Evidence from several studies have indicated that the phytochemical composition of Mauritian diet can be a rich source of protective antioxidant capacity. Our principal aim therefore is to investigate the *in vivo* cardioprotective effects of the polyphenolic rich Mauritian black tea (Bois Chéri tea) on patients with ischaemic heart disease at the Cardiac Centre, Pamplemousses, in collaboration with Dr T K Gunness and Dr OI Aruoma from The London South Bank University, UK. All clinical examination will be performed at the local Cardiac Centre with the expertise of Dr (Mrs) Roy and two technicians while biomarker analyses will be conducted at the University of Mauritius and The London South Bank University, UK.

2.4 METHODOLOGY

Preliminary contact and presentation:

A presentation on the proposed study was made on the 4th February, 2004 at the Cardiac Centre Conference Room, Pamplemousses, in the presence of medical and paramedical staffs from the Centre, members from the University of Mauritius and Dr OI Aruoma. Background scientific information, previous results obtained, aims and objectives, methodology, expected output and relevance of the proposed study were explicitly covered. The positive feedback and queries of the attentive audience clearly reflected their clear interest in the project. There were also an encouraging number of immediate responses for voluntary collaboration and participation in the setting up and the smooth running of the project at the Cardiac Centre. The project was greatly welcomed by the audience, as they seemed quite convinced about the beneficial effects of the study on the Mauritian population and the country as a whole.

Subjects:

- Randomised, double-blind sample of Mauritian population
- About 200 patients will be randomly selected from the waiting list for Angiography at the Cardiac Centre of Pamplemousses
- An open letter of invitation will be forwarded to each candidate, briefly explaining the purpose and benefits of the study. The patients will be requested to call at the Centre if willing to collaborate in the project
- The letter will highlight the points that the study is opened to all Mauritian ethnic groups from all regions of the island and from all socio-economic status. However, **patients will be excluded if**
 - 1) They do not belong to the age group of 45 - 70 yrs old
 - 2) They are current smokers or former smokers who had stopped for < 6 months
 - 3) Their alcohol intake is > 4 standard drinks/day
 - 4) They are postmenopausal women receiving hormone replacement therapy
- Patients retained for the study are those with a left ventricular ejection fraction > 40 %
- On their arrival at the Cardiac Centre, the patients will be asked to fill in the information sheet (Annex 1) by the help of the medical technicians. Additional information will be obtained during the study.
- From the total number of patients with ischaemic heart diseases obtained, half will be randomly selected for the study (medication + tea infusion) and the other half will act as placebo (medication + hot water) – provided all have nearly the same therapeutic profile
- Twenty (20) normal person will be recruited around the Cardiac Centre
- The Protocol will be submitted to the Ministry of Health and Quality of Life and needs to be approved by the Ethics Committee
- The study protocol will be fully explained to the subjects and they should give written, informed consent (Annex 2).

Study Design:

- Randomised, double-blind, placebo-controlled study with a 14-wk intervention period
- Subjects will be advised to maintain same diet as before and continue with their medication if any (lowering consumption of phenolic rich foods during the intervention might not reflect true picture of Mauritian diet)
- Study group will be asked to consume 3 x 200 ml of black tea/d (3 standard cups of 200 ml hot water each containing 2 g of tea leaves = a tea bag, infused for 5-7 minutes) for 12 wks without additives including milk and sugar. Study group then follow a 2 wk wash-out period by consuming same volume of hot water/d
- The 600 ml of black tea/d equate to 6 g tea leaves and this contains about 650 mg total phenols/d as supplement (assuming the infusion is from Bois Chéri tea brand– the richest Mauritian tea analysed)
- The equivalent of hot water consumed by the placebo group with their medication for the same period of time.

Blood samples and analysis

- All measurements of biomarkers will be performed at baseline and every 2 wk intervals during the intervention period
- Blood samples will be collected in the morning after the subjects had fasted overnight
- Blood samples for cholesterol analysis collected in test-tubes containing heparin powder. Plasma isolated by centrifugation (2000 x g, 10 mins at 0°C) and stored at -80°C to be used for biochemical analysis of total cholesterol, LDL, HDL and triglycerides
- Blood samples for analysis of homocysteine and neurohormones (ANP and BNP) and other markers of oxidative stress collected in plastic tubes containing potassium-EDTA as coagulant, immediately placed on ice. Plasma isolate by centrifugation (2500 x g, 10 mins at 4°C within 1 hr) and store at -80°C for analysis
- Total cholesterol, LDL, HDL, uric acid, glycosylated haemoglobin and triglycerides will be measured by standard Kits from RANDOX (**Techniques already validated: to be carried out at Cardiac Centre and University of Mauritius**)
- Homocysteine, 8-hydroxydeoxyguanosine, isoprostanes, hydroxyeicosatetraenoic acid measured by HPLC with electrochemical detector (**Techniques to be optimized: (Techniques already validated: to be carried out at University of Mauritius)**)
- Natriuretic peptides and products of oxidative protein damage by radioimmunoassay (**Techniques already validated: to be carried out at London South Bank University**)

Data Evaluation:

Data will be recorded and analyzed using Mixed Models procedures of the Statistical Analysis System (SAS; *PC SAS*, 2000). Initially, the model specified for this analysis will be unstructured specification for multivariate repeated measures analysis. Dietary supplemental treatment and sex and their interaction will be included in the model as fixed effects and individual patients will be the random variable or subject. The model will be modified to include time (months) as a repeated variable. The specified degrees of freedom estimation will be that of Kenward-Rogers (*PC SAS*, 2000). When F-test is significant, means will be separated by least squares means procedures of SAS. Relationships among variables will be examined by regression analysis (*PC SAS*, 2000). Also, limited nonparametric statistics (Chi-square test) appropriate for some of the data will be performed (*PC SAS*, 2000).

2.5 EXPECTED OUTPUT AND PLAN FOR INFORMATION DISSEMINATION

This study is the first of its kind on locally consumed black tea. It will give a clear indication of the effects of tea consumption on serum biomarkers in patients with cardiovascular diseases. Besides providing a base for an epidemiological evaluation of the potential prophylactic effects of tea polyphenols, the collective data can be used to implement management nutrition programs for the primary prevention of and protection against cardiovascular diseases among the Mauritian population.

Results obtained will be made available to local and international press (specialised journals, magazines, newspapers, radio, television etc...) to inform all those collaborating in the study and the population at large about the outcomes of the research study. In addition the outcome of the work will be taken up through oral communications in public forums as well.

2.6 RELEVANCE OF THE PROJECT

The proposed project is in line with certain management programs and therapeutic approaches towards the prevention of cardiovascular diseases among the Mauritian population at Governmental and institutional levels. Moreover, the most common approach to dealing with non-communicable diseases in many countries has been largely through the provision of health care, which has relied predominantly upon the development, and maintenance of specialised services that, due to their generally high cost, are not universally available and are unlikely to be so for the foreseeable future in many countries. This study will provide practices for a more economical means of protecting the body against various oxidative stresses. In addition the project will help to develop further the existing framework oriented towards polyphenolic and antioxidant research in Mauritius. It is important to note that this research area is rapidly developing and has proved to be of increasing importance to the pharmaceutical and medical sectors.

3. PRIOR RESEARCH WORK UNDERTAKEN BY PI

Experience relating to the proposed research project

Dr T. Bahorun

(Please see the CV and list of publications of Dr Bahorun attached)

Recent research work carried out by Dr Bahorun has allowed him to acquire a substantial knowledge of the characterization and evaluation of antioxidant capacities of biologically active extracts from both *in vivo* and *in vitro* cultures.

These studies mainly concerned the extraction, separation, purification, isolation and qualitative analyses of polyphenolic classes (phenolic acids, flavonoids, catechins, proanthocyanidins, anthocyanins) from a wide range of plant species. He has set up the scientific framework for the assessment of antioxidant capacities using 5 independent methods. He has successfully completed University and MRC funded projects related to the phytochemistry and antioxidant capacities of the Mauritian diets, traditionally used medicinal plants and *in vivo* and *in vitro*. He is currently working on a MRC funded project where the main objective is to investigate the molecular mechanisms of antioxidant protection in health and disease with particular reference to endemic Mauritian

plant extracts. His experience coupled with that of Dr Aruoma will be no doubt of utmost importance for the implementation of the proposed project.

Dr O.I.Aruoma

(Please see the CV and list of publications of Dr Aruoma attached)

Dr Aruoma has been involved in free radical research for the past 15 years and is one of very high international standing. Current research interest includes the role of oxidative stress, antioxidants, nutrition, gene expression, brain aging, drug metabolism and neuroinflammation in neurodegenerative diseases, neurochemistry and molecular mechanisms of the neuroprotective potentials of phytochemicals from medicinal and food plants.

Related work already in progress at the institutions

UNIVERSITY OF MAURITIUS, Department of Biosciences, Faculty of Science

In our line of study concerning the production of biologically active secondary metabolites, we have initiated and oriented our research activities towards both the *in vitro* and *in vivo* production and analyses of polyphenols in traditionally used exotic and endemic plants. Interesting results have been obtained from buckwheat (*Fagopyrum esculentum* Moench), hawthorn (*Crataegus monogyna* Jacquin), grape (*Vitis vinifera*), exotic (*Psidium cattleianum* Sabine) and about 60 endemic (*Syzigium*, *Monimiastrum*, *Diospyros*, *Eugenia* and *Trochetia*) Mauritian species. Antioxidant assays have been conducted using 5 different methods (antiloperoxidant assays, scavenging of H₂O₂ using the methodology described by Pick et al, 1980, Scavenging of superoxide anions using the xanthine-xanthine oxidase assay (Aruoma et al, 1980), scavenging of HOCl adapted from Weiss et al., (1982) and assessment of pro-oxidant actions to confirm activities of *in vivo*, *in vitro* extracts. A majority of these results have already been published in International journals. Recent research has been geared towards the screening of the Mauritian diets for prophylactic agents. Results have been obtained and published for phenolic and vitamin C contents and antioxidant activities of some commonly consumed Mauritian exotic fruits, vegetables and black teas. Cell cultures have also been induced from *Cassia fistula*, *Tagetes erecta* and *Crataegus monogyna*. These cultures are being screened for their polyphenolic contents and optimization experiments using precursor feeding and magnetic fields are ongoing. Antibacterial and antioxidant assays of the above total and fractionated extracts have being conducted.

The LONDON SOUTH BANK UNIVERSITY Department of Applied Science

London South Bank University (LSBU) is one of the largest Universities in England and Wales with a total student population of over 17,000. The range of the University's academic portfolio is unusually wide with a substantial engineering, science and computing faculty, an important range of courses in the built environment, a Business. LSBU incorporates the Royal College of Nursing Development Centre, is one of the largest providers of National Health Service training in London. The proposed research will be conducted within the Department of Applied Science at LSBU. The Department of Applied Science was reformed in January 2004 from the three divisions that were originally part of the School of Applied Science (Food & Bioscience, Human & Exercise Science, Applied Chemical Sciences). Research Centres at LSBU including human performance, nutrition and food research centres have continued to direct activities within their remits through the various incarnation of the Department. Other research centres monitor the work environment, heavy metal toxicology and bacterial locomotion. The Department has two large processing halls for chemical engineering and microbiology/biotechnology. The food processing hall has new equipments

for food science research. The Department is therefore one of the premier analytical facilities for science in London.

4. PREVIOUS WORK UNDERTAKEN AT THE UNIVERSITY OF MAURITIUS BY PRINCIPAL INVESTIGATORS

A) Dr Bahorun was the research investigator of two MRC/Private sector Collaborative Research Scheme projects;

1. Production of vacuum packed pickled local fruits, vegetables and spices
2. Packaging and commercialization of peeled fresh pineapple ready for consumption

Both projects have been successfully completed and final report submitted to MRC

B) Under the unsolicited MRC grant scheme Dr Bahorun has been awarded a 3 year grant for the study of polyphenolics, vitamins and the antioxidant status of the Mauritian diet.

This project has been successfully completed and the final report submitted to the MRC

C) “Extraction, Identification, Purification and Biological activities of plant polyphenolics from traditionally used in vivo and in vitro plant cultures”

This project funded by university of Mauritius has been successfully completed and final report has been submitted to UOM

5. RESULTS OF PREVIOUS WORK UNDERTAKEN BY PRINCIPAL INVESTIGATOR

The results of previous work conducted by the principal investigators have been published. A list of these publications is given in the attached CVs

6. REFEREES

Professor Francis Trotin,
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7. HAS THIS PROJECT PROPOSAL BEEN SUBMITTED TO OTHER FUNDING ORGANISATION

This project has not been submitted to other funding organization. It should be noted that this project will provide scope for the preparation of a PhD degree. In this context a student has been registered at the University of Mauritius.

8. FACILITIES, EQUIPMENT, OTHER RESOURCES

UNIVERSITY OF MAURITIUS, MUARITIUS	CARDIAC CENTRE, PAMPLEMOUSSES, MAURITIUS	THE LONDON SOUTH BANK UNIVERSITY, UK
<ul style="list-style-type: none">Laboratory space, glasswares, consumables etc	<ul style="list-style-type: none">Nursing staffs to obtain blood samples	<ul style="list-style-type: none">Laboratory facilities
<ul style="list-style-type: none">HPLC equipment and softwares	<ul style="list-style-type: none">Consumables	<ul style="list-style-type: none">Radioimmunoassays
<ul style="list-style-type: none">Freeze drying facilities	<ul style="list-style-type: none">Checking of BP, Pulse rate and weight	<ul style="list-style-type: none">Consumables
<ul style="list-style-type: none">Freezers, dessicators		
<ul style="list-style-type: none">TLC facilities		
<ul style="list-style-type: none">Normal pressure chromatography facilities		

9. DURATION:

Duration of Project: 15 months

10. COST ESTIMATE

		May-December 2004	Jan-July 2005
Consumables: <i>1. For blood collection, kits etc...</i> <i>2. Tea bags, cups, others</i> <i>3. Analysis</i> Extraction, marker analyses HPLC columns, HPLC solvents <i>4. Biological assays</i> <i>5. Glasswares</i>	Rs	175,000	200,000
6. Equipment, softwares/ Bibliography and Contingencies Lap top, Electrochemical detector (for the analysis of oxidative stress biomarkers), Reflotron apparatus with test strips, photocopies. Salaries for technicians, documentation, publications		1,308,000	
7. Travelling, Visits and short term attachments 1 visit for each principal investigator to London South Bank University/to University of Mauritius (1 wk) with per diem allowance at approved rate Air Fare ticket for 1 student to London South Bank University and living allowance (1 month)		160,000	100,000
TOTAL		1,643,000	300,000

NB: Funds (to be finalised) will have to be transferred to London South Bank University to cover partial cost of biomarker analyses

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ANNEX 1

Code Number:

EFFECTS OF BLACK TEA CONSUMPTION ON ISCHAEMIC HEART DISEASES AMONG THE MAURITIAN POPULATION

INFORMATION SHEET

**UNIVERSITY OF MAURITIUS, REDUIT, MAURITIUS
THE CARDIAC CENTRE, PAMPLEMOUSSES, MAURITIUS
THE LONDON SOUTH BANK UNIVERSITY, UK**

Contact details in Mauritius: Cardiac Centre, Telephone: 2438220 Facsimile: 2438384

DATE EXAMINED:

UNIT NO:

SURNAME:

OTHER NAMES:

DOB:

AGE:

SEX:

ADDRESS:

OCCUPATION:

TEL: (Home),

(Mobile)

EMAIL:

DIAGNOSIS:

CURRENT MEDICATION:

RISK FACTORS

Diabetes Recent (< 3 yrs) Insulin-dependant Non-insulin-dependant

Hypertension Recent Duration

Obesity Recent Duration

Hyperlipidaemia Recent Duration

Hypercholesterolaemia Recent Duration

Previous MI When

Angina When

Smoking Stopped < 6 mo < 1 yr Duration

Alcohol Stopped < 6 mo < 1 yr Duration

Family History with IHD

EXAMINATION

CVS Pulse BP

Echo EF

ECG ST Change Q wave

Angiography Coronary 1V 2V 3V

Urgent Semi-urgent Routine

SUMMARY 1. Disease

2. NYHA

3. Medical Decision (No treatment, Med treatment & FU, Dilatation & Stenting)

4. Patient added to waiting list

ANNEX 2

CONSENT FORM

EFFECTS OF BLACK TEA CONSUMPTION ON ISCHAEMIC HEART DISEASES AMONG THE MAURITIAN POPULATION

The purpose of this study and procedure being carried out by the clinicians at the Cardiac Centre, Pamplémousses, Mauritius as they are described below, have been explained to me to my satisfaction.

I understand that the purpose of this pilot study is to determine if a tea nutritional supplement can improve immunity and cardiovascular health, but at this time it may not be directly beneficial to me. I understand that I am one of 200 participants. The investigators have answered all of the questions that I have at present.

The research was designed in very close collaboration with a clinical consultant (Dr T K Gunness, Director of Cardiac Centre) and will be carried out by at the Cardiac Centre of the SSR Hospital, Mauritius. Analyses will be conducted under the co-supervision of the Principal Investigators, Dr Theeshan Bahorun and Dr Okezie I Aruoma at the University of Mauritius and London South Bank University. The information from this study will be used to help investigators determine the potential effectiveness of a nutritional supplement in improving levels of certain markers of immune function in the blood and to monitor cognitive functions.

Risks and Benefits

There are no known inherent or anticipated risks or discomforts associated with the nutritional supplement. There is no guarantee that I will directly benefit from the nutritional supplement that is provided in this study. However, there is a possibility that the nutritional supplement may improve blood levels of some nutrition-related immune markers and, by doing so, improve health.

Compensation

I understand that no monetary compensation will be offered for participation in this study. Transportation to the study visits will be arranged if needed. Any other expenses related to this pilot study must be agreed upon in writing by the study coordinator and myself.

Confidentiality

My records will be with strict confidentiality. Information will be used as a part of the study group to look for changes in biomarkers of health. All information that may be used in publication or other transmission will be coded to assure anonymity.

Other Information

I understand that I may refuse or otherwise withdraw my participation from this study without affecting my present or future care from those involved in the study or others. The tea supplement is available at no cost to me for the duration of the study.

The Ethics Committees of the Ministry of Health, University of Mauritius and the Cardiac Centre have reviewed and approved all aspects of this study. I may contact the patient representative at the Cardiac Centre if I have any questions or complaints about this study. I may also contact the Principal Investigator or Research Associate to resolve any questions or medical issues.

Clinical Consultant: Dr T K Gunness

Principal Investigators: Dr T Bahorun, Dr O I Aruoma

Research Associate: Mrs A Luximon-Ramma

All questions regarding this procedure and study have been explained to my full satisfaction in a Mauritian language in which I am fluent and in English. I have read and initialled each of the two pages of the consent form. I have also received a full copy of this signed consent form.

Printed name of Participant

Signature of Participant

Date of Participant's signature

Printed name of witness

signature of witness

Date of witness signature