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Original Research Article

Changes in blood enzymatic antioxidants: Catalase and Superoxide dismutase level in ageing rats fed with guava leaves supplemented diets

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Abstract: In recent years, there have been increasing evidences that reactive oxygen species (ROS) are associated with pathological conditions such as atherosclerosis and carcinogenesis as well as with ageing processes. Ageing is characterized by an increased production of ROS in somatic tissues. Young guava leaves have been reported to contain antioxidants that remove free radicals generated by the ROS. This was further investigated by supplementing the diet of ageing wistar rats with dried, ground young guava leaves and estimating catalase (CAT) and superoxide dismutase (SOD) levels in both tests and controls. 49 adult wistar rats were used for this study and grouped into four. Group A (male control fed with normal diet), Group B (female control fed with normal diet), Group C (male test fed with young guava leaves supplemented diet) and Group D (female test fed with young guava leaves supplemented diet). All the groups were fed with normal diet for two weeks after which they were fed according to group which lasted for a period of 8 weeks. The experimental period lasted for 10 weeks in total. After the expiration of the experimental period, the blood samples of the animals were collected by caudal puncture. The sera were separated and used to determine catalase and superoxide dismutase levels. The data presented in this study show clearly that there is a significant increase in antioxidant levels that could curb the effects of oxidative damage resulting from free radicals generation through the supplementation of rat diet with dried, ground young guava leaves. This by implication means, supplementing diets in man or taking fresh vegetables or processed leaves in form of tea can ameliorate ageing processes as well as preventing age-related disorders caused by free radicals generation in the body and oxidative stress.

Keywords: Free radicals, Oxidative-stress, Ageing, Antioxidants, *Psidium guajava* (guava*)* leaves, Catalase, Superoxide dismutase, Supplemented diets.

INTRODUCTION

Ageing can be defined as the accumulation of physical, psychological or social changes that occur in a biological system over time as a result of free radicals generated during metabolic processes. Oxidation by reactive oxygen species (ROS) has been implicated in the ageing process seen in biological system. Ageing is characterized by an increased production of ROS in somatic tissues [1] and an increase in the production of ROS may promote the induction of apoptosis. In recent years, there has been increasing evidence that reactive oxygen species are associated with pathological conditions such as atherosclerosis and carcinogenesis [2] as well as with ageing [3]. Free radicals are by-products of cellular metabolism that have direct effects on cell growth and development, cell survival and likely increase the pathogenesis of atherosclerosis, cancer, and several other conditions, including inflammatory disease; all of which are implicated in ageing process. Hence, free radicals are generated continuously in the body due to metabolism and diseases [4]. Examples of free radicals are reactive oxygen species such as superoxide anion ($0_{2^{\cdot}}$), hydroxyl radical ($OH^{\cdot}$), alkoxyl radical ($RO^{\cdot}$) and reactive nitrogen species such as nitric oxide ($NO^{\cdot}$) and nitric dioxide ($N0_{2^{\cdot}}$). Thus, nature has a defense mechanism against these free radicals which is the antioxidant scavenging mechanism that delays or prevents the spontaneous generation of these free radicals in the body. Antioxidants refer to compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions and which can thus prevent or repair damage done to the body's cells by oxygen. They act by one or more of the following mechanisms: reducing activity, free radical-scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen. Antioxidants work to protect the cells of the body against free radicals damage which occur during metabolism. However, since reactive oxygen species do have useful functions in cells, such as redox signaling, the function of antioxidant system is not to remove oxidants entirely, but instead to keep them at an

optimum level [5]; the balance between the antioxidant scavenging mechanism and the reactive oxygen species must be maintained in the system. But there could be an imbalance between free radical reaction and the scavenging capacity of the antioxidative defense mechanism with a higher production of the former and this result in oxidative stress [6] which can lead to cell injury and death. Oxidative stress is a strong candidate mechanism of ageing [7]. Epidemiological studies have shown that many phytonutrients of fruits and vegetables might protect the human body against damage by ROS. The consumption of natural antioxidant phytochemicals was reported to have potential health benefits [8].

Guava has been reported to be rich in tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fibre and fatty acids. Guava fruit is higher in vitamin C than citrus fruits (80 mg of vitamin C in 100g of fruit) and contains appreciable amounts of Vitamin A as well. Guava fruits are also a good source of pectin [9]. Guava has antioxidant properties attributed to polyphenols like (+)-gallocatechin, guaijaverin, leucocyanidin and amritoside found in its leaves. Guava leaves contain copious amounts of phenolic phytochemicals which inhibit peroxidation reaction in the living body and can therefore prevent various chronic diseases like diabetes, cancer, heart diseases [10] and can be said to slow down ageing processes.

MATERIALS

- 50 healthy wistar rats (males and females)
- Cage
- Eating and drinking trough
- Prepared dried, ground young guava leaves
- Animal feed (grower's mash)
- Weighing balance

PREPARATION OF SUPPLEMENTED DIETS

The young leaves of *Psidium guajava* were plucked from a local garden, air-dried and ground.

This was added to the animal feed as supplement in the ratio 1:2 (1 kilogram of dried, ground guava leaves to 2 kilogram of the animal feed).

EXPERIMENTAL DESIGN

49 wistar rats (26.5kg averagely) were obtained for this study. They were kept in a well ventilated cage in the animal house department of the school, at normal room temperature and supplied with adequate daylight and darkness. The animals were allowed to acclimatize for a period of two weeks during which they were fed with normal grower's mash and drinking water. After the termination of two weeks, the test animals were then fed with young guava leaves supplemented diets and drinking water for a period of eight weeks and the controls with grower's mash and drinking water for same period. All animals received humane care in compliance with the institution's guideline and criteria for humane care as outlined in the National Institute of Health Guidelines for the Care and Use of Laboratory Animals [11].

Treatment of the animals was in accordance with the Principles of Laboratory Animal Care. They were divided into groups as follows:

GROUP 1: Males control fed with normal feed and drinking water

GROUP 2: Females control fed with normal feed and drinking water

GROUP 3: Males test fed with supplemented diet and drinking water

GROUP 4: Female test fed with supplemented diet and drinking water

SAMPLE COLLECTION

At the expiration of the experimental period, the blood samples of the animals (both the controls and tests) were collected by cardiac puncture into plain bottle. The non-hemolysed sera were collected after centrifugation at 2000rpm for 5minutes into plain bottle.

BIOCHEMICAL ANALYSIS

DETERMINATION OF CATALASE ACTIVITY IN SERUM

Determination of catalase activity was based on the method of Sinha [12].

DETERMINATION OF SUPEROXIDE DISMUTASE (SOD) ACTIVITY IN SERUM

Determination of superoxide dismutase was by Misra and Fridivich method [13]

STATISTICAL ANALYSIS

The results were expressed in Mean±SE. ANOVA (one-way analysis of variance) method was used for the analysis of data. P-values<0.05 was considered statistically significant.

RESULTS

Table 1 and 2 show a significantly (P-value<0.05) increase in Catalase in males and females test respectively and their controls

Table 3 and 4 show a significantly (P-value<0.05) increase in Superoxide dismutase in males and females test respectively and their controls

Generally, the mean serum level of SOD in tests was found to be significantly higher than in controls and the mean serum level of CAT was significantly higher in tests than in controls (Table 5 and 6, P-value<0.05).

Table-1: Shows the MEAN±SD of CATALASE between male control and male test

GROUP	MEAN±SD	P-VALUE
MALES CONTROL	11.16±0.63	-
MALES TEST	13.16±1.02	*0.000

*p-value <0.05

Table-2: Shows the MEAN±SD of CATALASE between female control and female test

GROUP	MEAN±SD	P-VALUE
FEMALES CONTROL	11.72±0.96	-
FEMALES TEST	13.41±0.99	*0.000

* p-value <0.05

Table-3: Shows the MEAN±SD of SUPEROXIDE DISMUTASE between male control and male test

GROUP	MEAN±SD	P-VALUE
MALES CONTROL	0.99±0.16	-
MALES TEST	1.59±0.17	*0.000

* p-value <0.05

Table-4: Shows the MEAN±SD of SUPEROXIDE DISMUTASE between female control and female test

GROUP	MEAN±SD	P-VALUE
FEMALES CONTROL	1.19±0.16	-
FEMALES TEST	1.57±0.24	*0.000

*p-value <0.05

Table-5: Shows the MEAN±SD of CATALASE BETWEEN TESTS AND CONTROLS

GROUP	N	MEAN±SD	P-VALUE
CONTROLS	30	11.45±0.71	-
TESTS	19	13.25±1.00	*0.000

*p-value<0.05

Table-6: Shows the MEAN±SD of SUPEROXIDE DISMUTASE BETWEEN TESTS AND CONTROLS

GROUP	N	MEAN±SD	P-VALUE
CONTROLS	30	1.09±0.19	-
TESTS	19	1.58±0.19	*0.000

*p-value<0.05

* considered statistically significant

DISCUSSION

Oxygen-derived free radicals are responsible for the age-related damage at the cellular and tissue levels; they cause extensive damage to DNA, protein and lipid. In a normal situation, a balanced-equilibrium exists among oxidants, antioxidants and bio-molecules but excess generation of free radicals may overwhelm natural cellular antioxidants defense system, thus, leading to oxidative stress and over time cellular degeneration ensues with further cellular functional impairment such as wrinkles of the skin which results from weakling of the collagen in the skin and age-related diseases like atherosclerosis caused by narrowing of the artery and build-up of cholesterol around the arterial wall and many other age-related diseases. The accumulation of endogenous oxygen radicals generated in cells and the consequent oxidative modification of biological molecules (lipids, proteins and nucleic acid) during metabolism have been indicated as responsible for ageing, age-related diseases and later cell death.

In this study, the effect of *Psidium guajava* (guava) leaves; a phytochemical supplemented diet on catalase (CAT) and superoxide dismutase (SOD) of ageing rats was investigated. Catalase and superoxide dismutase are parts of the primary defense systems that protect the body against the deleterious effects of free radicals by removing them or preventing their chain reactions. There was a general significant increase in CAT and SOD levels in test animals (both males and females) compared to the control animals (both males and females) with p-value<0.05. The increase in the analytes (CAT and SOD) in this study is able to halt cellular damage that could be brought about by free oxidants in the body, thus, offering protection to the body. Hence, due to the threat of oxidative damage, there is a need of supplementing our diets so as to boost or augment the endogenous source of enzymatic antioxidants which confer natural protection against auto-oxidation in the body.

CONCLUSION

The health status of an individual is related to his eating habit or dietary intake. Since metabolic processes which generate these free radicals in the body cannot be halted, it is therefore essential that one considers what is being taken in whether it is more injurious to the health. Thus, this project in essence is to show that dietary intake of substances rich in antioxidants especially phytochemicals or its dietary supplement is associated with better health by enhancing immunity and offering protection against the harmful effects of free radicals and oxidative stress.

Based on the result of this study, supplementing the diet of rats with young dried ground guava leaves have augmented endogenous source of antioxidants. In order to boost endogenous source of antioxidants and retard ageing process and age-related diseases, there is a need to supplement our diet with antioxidants rich food. Phytochemicals or green-leafy vegetables such as guava leaves used for this research could be processed as green tea which can be taken as part of our diets.

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