

ORIGINAL RESEARCH ARTICLE

COCOA BEANS: AN ANTI-AGEING FUNCTIONAL FOOD WITH AN ABILITY TO INHIBIT GLYCATION AND OXIDATIVE STRESS

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ABSTRACT

Objective: Accelerated ageing may leads to decline in the quality of life at early age, which can be attributed to deleterious processes like glycation and oxidative stress. In this regard, the functional foods are useful to reduce the pace of ageing via interfering in aforementioned processes.

Methodology: The present study aimed to investigate the anti-ageing action of five common food substances i.e. cocoa beans powder, baking powder, spinach juice, coconut and olive oils using in-vitro assays i.e. anti-glycation and anti-oxidant.

Results: Our data exhibited cocoa beans powder to be best inhibitor of glycation (79%) at common tested strength of 1 mg/ml. None of the other test substances produced any remarkable anti-glycation effect. However, with an exception of coconut oil, all other test substance showed significant anti-oxidant property with the highest inhibition of 79% by olive oil. At the threshold of 50% inhibition, only cocoa powder presents itself in the center of Venn diagram.

Conclusion: The cocoa beans powder appeared to be the most potential functional food for slowing down the harmful processes of glycation and oxidative stress attributed to ageing-linked ailments and diabetic complications.

Keywords: Glycation; Oxidative stress; Cocoa beans powder; Functional food

INTRODUCTION

Getting old is the natural process, which is observing an unseen pace these days. By 2050, the old age will outnumber the young (Suzman and Beard, 2011). This is an alarming scenario because the world needs healthy individuals to function. Several stressors were reported to reduce the quality of life well before time (Campisi et al., 2019). On the very top lies the harmful biochemical reactions such as glycation and oxidative stress. The former leads to the accumulation of biological garbage inside the body (Sergiev et al., 2015) commonly known as Advance Glycation End products (AGEs) (Ross, 2015). While the later occurs due to imbalance between oxidants and antioxidants leading to build up of free radicals in the body (Pole et al., 2016). The

Maillard reaction (also known as glycation) occurs due to enzyme free reaction of carbohydrates with other biomolecules especially protein. This ultimately (after weeks) leads to the formation of AGEs (Goldin et al., 2006). Their levels were seen high in ageing linked impaired learning (Southern et al., 2007, Igase and Igase, 2018), renal damage (Sun et al., 2016) and alcohol independent fatty liver diseases (Leung et al., 2016). It is of note that the diabetic complications mainly happens due to the phenomenon of glycation (Ramasamy et al., 2005). The stress induced by accumulation of AGEs is termed as glycative stress (Yonei et al., 2020). Despite, there is significant contribution of AGEs in the development of multiple pathologies, no drug in available currently in the market to combat piling of AGEs in the body. A handful of inhibitors were

reported (Reddy and Beyaz, 2006) including the popular aminoguanidine, but none could pave its way to bedside due to significant off target actions (Thornalley, 2003, Campbell, 1996). Under such circumstances, the emerging field of nutritional pharmacology can be extremely useful. This field has introduced the concept of functional food i.e. the dietary substances which own a medicinal value along with the nutrition purpose. This is in line with the sayings of Hippocrates i.e. let food be thy medicine and medicine be thy food (Hasler, 2002). The advantage of utilizing this approach is that it can lead to prompt and low-cost medicinal substances because of the lack of conventional safety evaluation in its development process. Additionally, dietary intervention has higher complaints for ailments which are chronic and in nature and progresses insidiously. Keeping this into account, this study was designed to assess the anti-ageing potential of some commonly used dietary substances.

MATERIALS & METHODS

Selection of Test Substance

Five commonly used dietary substances i.e. cocoa beans powder, baking powder, spinach juice, coconut and olive oils were selected for this study.

Glycation Inhibition Assay

The assay was conducted using bovine serum albumin (BSA) and fructose using the procedure described earlier (Khan et al., 2017). Briefly, the BSA (10 mg/ml) and fructose (50mM) were mixed and incubated (60 °C) for 1 day in the presence of

the aforementioned test substances at working strength of 1mg/ml. Afterwards, the intrinsic fluorescence of AGEs were measured at excitation and emission wavelengths of 340 and 435 nm respectively. The assay was performed in triplicate.

Antioxidant Assay

The ability of test substance to scavenge free radical was assessed using DPPH (1-1 diphenyl-2-picryl-hydrazyl) assay as described earlier (Shen et al., 2010). In brief, the DPPH (0.1 mM in methanol) was mixed with test substances (working strength = 1 mg/ml), shaken and left at ambient temperature for thirty minutes followed by measurement of absorbance (517 nm). The assay was performed in triplicate.

Venn Diagram

In order to identify the most effective test substance, the Venn diagram was used at threshold of fifty percent inhibition.

Data analysis

The data is presented as mean \pm SEM of percent change performed in triplicate.

RESULTS

Following are the results of the study:

Glycation inhibition assay

The cocoa powder, among all test substances, showed significant inhibition of glycation (79%) as shown in Table-1. None of the other test substances has shown any promising inhibition as compared to the control.

Table-1 Effect of Various test substances on glycation inhibition

S.No.	Functional Food	Scientific name	Free radical Scaveng (%)
1	Cocoa powder	<i>Theobroma cacao</i>	79
2	Baking soda	<i>Sodium bicarbonate</i>	18
3	Spinach juice	<i>Spinacia oleracea</i>	2
4	Coconut oil	<i>Cocos nucifera</i>	0
5	Olive oil	<i>Olea europaea</i>	0
Aminoguanidine (3 mM; positive control)			72

Antioxidant Assay

With an exception of coconut oil, all other test substances demonstrated remarkable free radical

scavenging ability with the highest inhibition of 79% by olive oil (Table-2). The baking soda and spinach juice caused 55% and 62% inhibition respectively.

Table-2 Effect of Various Test Substance on Free Radical Scavenging

S.No.	Functional Food	Scientific name	Free radical Scaveng (%)
1	Cocoa powder	<i>Theobroma cacao</i>	72
2	Baking soda	<i>Sodium bicarbonate</i>	55
3	Spinach juice	<i>Spinacia oleracea</i>	62
4	Coconut oil	<i>Cocos nucifera</i>	0
5	Olive oil	<i>Olea europaea</i>	79
Ascorbic acid (1 mM; Positive control)			82

Venn Diagram

Among various test substances, the cocoa beans powder was able to show inhibition of glycation

and oxidative stress at set threshold of 50% inhibition (Figure-1).

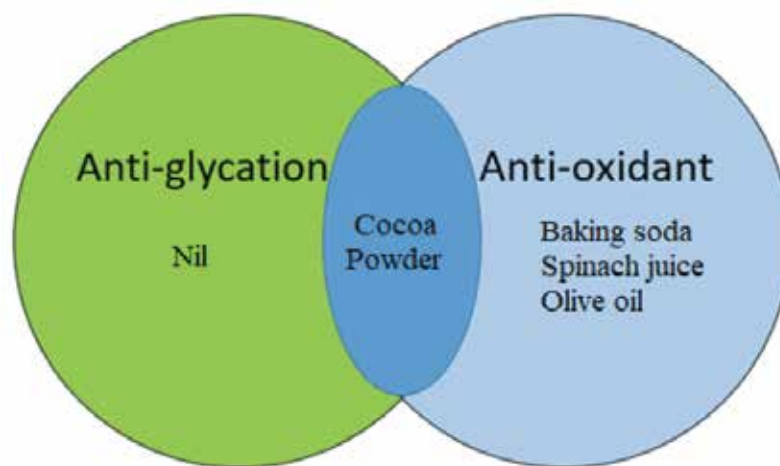


Figure-1 Venn diagram at Inhibition Threshold of 50 percent

DISCUSSION

The loss of functions of the body along with emergence of diseases is the hallmarks of ageing. Some ailments like diabetes mellitus also accelerate the process of ageing. Among the various underlying mechanisms, the theory of glycation holds a promising position and has been considered as a good target for pharmacological intervention. Keeping in view, the present study aims to assess the anti-ageing potential of several dietary substances through modulation of glycation.

Diet has long been considered as the determining factor for the development and cure of diseases. However, the lack of scientific evidences to support their use has rendered its widespread acceptance in the medical community. However, in modern times, the evidence based medicinal use of dietary substances is gaining attention over time leading to the popularity of the field of nutraceuticals especially for ailments which are chronic and insidious in

nature. On the top of it, there is strong patient compliance associated with the use of functional foods for the management of diseases. For the management of disease, one can often see these days that the diet experts (nutritionists) suggest the consumption of some constituent(s) (Solfrizzi et al., 1999), food (Berr et al., 2009) or recommend to follow some diet plan (Yannakoulia et al., 2015). Our data showed that among various dietary substances, only cocoa beans powder exhibit remarkable anti-glycation potential with an inhibition of 79% (Table-1). In similar lines, the cocoa beans rich diet has shown beneficial effect in galactose induce aging rat model, which was known to produce harmful effects through glycation (Yoo and Kim, 2021). Additionally, its polyphenolic extract was reported to prevent the aging induced cognitive impairment. (Bisson et al., 2008). Hence, taken together the inhibition of glycation, suggested in our study, most probably underlie the anti-ageing reports cited above. Ageing is a complex phenomenon and multiple mechanisms are reported to underlie its development. Along

with glycation, the oxidative stress is also considered as a major contributor (Srinivasan and nutrition, 2014). In this regard, with an exception of coconut oil, all other test substances (cocoa beans powder, baking soda, spinach juice and olive oil) showed remarkable radical scavenging action with a highest inhibition of 79% by olive oil (Table-2). It is of note that the cocoa beans powder also showed a good inhibition of 72%. Keeping into account the heterogeneous nature of ageing, the Venn diagram was plotted at 50% threshold inhibition. Our data showed that among all test substances, only cocoa bean powder has shown, in both assays, 50% inhibition, which presents it as the most promising anti-ageing dietary substance acting via interfering in multiple pathogenic pathways.

Hence, our data deduced that cocoa beans powder holds significant anti-glycation and anti-oxidant potential and its use is recommended for offering protection against accelerated aging and its associated morbidities.

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