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RESEARCH NEWS

PAGE - a simple method to detect the protective effects of medicinal plants against sugar induced protein damage

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This study reports a new application of polyacrylamide gel electrophoresis (PAGE), by which the medicinal plants that protect proteins from sugar induced damage can be recognised.

Diabetes mellitus affects hundreds of million people worldwide. Over five million deaths occurred in 2013 due to complications associated with diabetes. One of the key mechanisms causing long term complications of diabetes is initiated by the attachment of glucose to proteins in the body and this process is known as protein glycation (Goh & Cooper, 2008). Increase in the blood glucose level is a characteristic feature of poorly controlled diabetes. This creates an environment highly favourable to increasing protein damage caused by glycation. In the body, protein glycation continues to progress over a period of time (weeks to months) finally producing a complex group of compounds, which causes more damage to the proteins. Effects of the damage become more prominent in proteins such as collagen, which has longer life. These damaged proteins accumulate in various organs leading to long term diabetic complications. Protein damage caused by glycation affects the blood vessels and various organs such as the heart, kidney and eye leading to myocardial infarction (heart attack), stroke, kidney failure and blindness.

Slowing down of protein glycation is one way to delay or stop diabetic complications. Many studies have been conducted in the recent past to identify synthetic compounds that slow down protein glycation. However, such compounds did not reach the market due to safety issues. Plants with sugar lowering effects have been used in traditional medicine for thousands of years without side effects, and they may provide ideal sources for the discovery of compounds to protect proteins from sugar induced damage. Sri Lanka is blessed with a large number of plants, which are used to treat diabetes. However, the investigation for protective effects of these plants against glycation is not possible in a resource poor setting, due to the need of very expensive and specialised equipment such as high performance liquid chromatography systems and fluorescent spectrophotometers for these studies. A simple method, which can be conducted under a resource-poor setting was developed to detect the protective effects of medicinal plants against protein glycation.

The current study identified the usefulness of polyacrylamide gel electrophoresis (PAGE) in a new application, in which medicinal plants that can slow down the protein damage was identified. In this method the proteins are separated based on their net charge. The test protein (bovine serum albumin) was mixed with the sugar (glucose/ fructose/ ribose) and kept for four weeks at a temperature and pH that match the body conditions (37 °C and pH 7.4). In addition, either a plant extract or the standard inhibitor (aminoguanidine) was added into some of the mixtures. Sodium azide was used to stop microbial growth. Samples were collected from the reaction mixtures at intervals after allowing time for protein damage and analysed using the standard PAGE method (Laemmli, 1970). Changes in the speed of protein movement were compared after staining the protein bands (with Coomassie blue).

The protein bands move much faster towards the bottom of the gel (positive end), when they are damaged by glycation. The protein sample, which was left without sugar had the slowest movement. The increase

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in the movement was found to match with the degree of protein damage (Figure 1). This could be related to the decline in positive charges of the protein as they are the targets of glycation. As a result of the relative increase in the net negative charge, the glycated protein will become more attracted to the positive end of the gel. Protein movement was found to increase when the sugar concentration (Figure 2), incubation period (Figure 3) and the temperature was increased, and when a more reactive sugar (ribose) was used (Figure 3). Comparative movement of the protein was reduced when the standard inhibitor was used (Figure 4). Among the three sugars fructose was found to be better for the PAGE method, because the speed of protein damage occurred at an intermediate speed (speed of the damage with glucose was too slow and the speed was too fast with ribose).

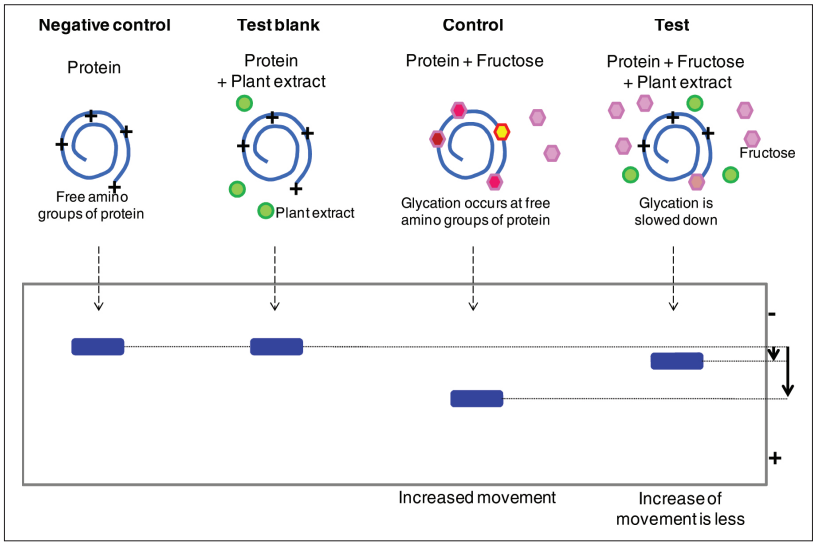

Figure 1: Principle behind the use of PAGE method to detect the protective effects of medicinal plants against protein glycation

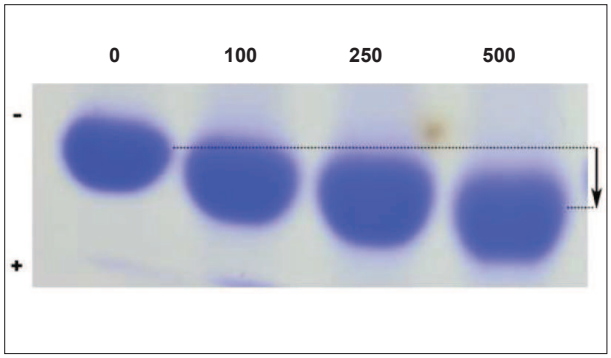

Figure 2: Effect of sugar concentration (0 – 500 mM) on protein movement
Source: Wijetunge & Perera, 2014

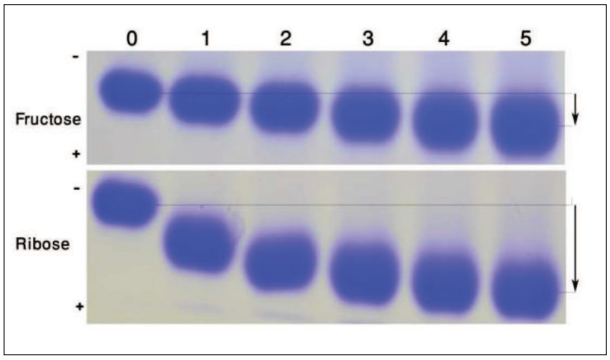

Figure 3: Effect of different sugars (fructose and ribose) and length of incubation (0 – 5 days) on protein movement
Source: Wijetunge & Perera, 2014

Protective effects of some plants such as *Phyllanthus emblica* (*Nelli*) fruit, *Syzygium aromaticum* (*Karabu*) flower buds and *Coriandrum sativum* (*Kottamalli*) seeds in preventing glycation have been previously reported, using the existing expensive methods. The PAGE method also has produced matching results with those three extracts validating the method. In addition, the protective effects of *Pterocarpus marsupium* (*Gammalu*) latex and *Phyllanthus debilis* (*Pitawakka*) plant (at a high sugar concentration) were also revealed for the first time (Table 1, Figure 4). The results suggest the value of these plants as treatment options to minimise chronic diabetic complications.

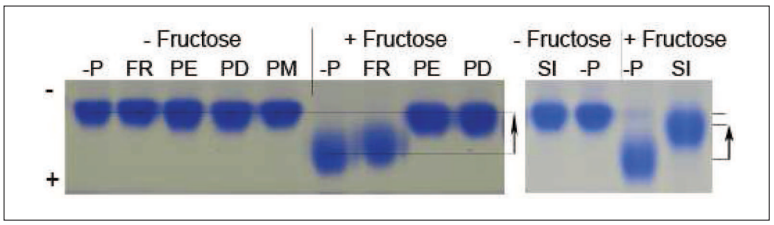

Figure 4: Effect of the presence of plant extracts and standard inhibitor on protein movement

-P: without plant extract; FR: *Ficus racemosa (Attikka);* PD: *Phyllanthus debilis (Pitawakka);* PE: *P. emblica (Nelli)* and PM: *Pterocarpus marsupium (Gammalu*) were used at 50 µg/mL, SI: standard inhibitor (1 mg/mL). Arrows show the comparative decrease in protein movement in the presence of extracts and standard inhibitor.
Source: Perera & Handuwalage, 2015

Table 1: Protective effect of medicinal plants against protein glycation

Botanical name	Common name	Plant part	Effect*
Coccinia grandis	*Kowakka*	Leaf	-
Ficus racemosa	*Attikka*	Stem Bark	++
Gymnema lactiferum	*Kurinnan*	Leaf	-
Gymnema sylvestre	*Masbedda*	Leaf	-
Musa X paradisiaca	*Alukesel*	Yam	-
Phyllanthus debilis	*Pitawakka*	Whole plant	+++++
Phyllanthus emblica	*Nelli*	Fruit	+++++
Pterocarpus marsupium	*Gammalu*	Latex	+++++
Strychnos potatorum	*Ingini*	Seeds	-
Tinospora cordifolia	*Rasakinda*	Leaf	-
Coriandrum sativum	*Kottamalli*	seeds	+++++
Cinnamomum zeylanicum	*Kurundu*	Bark	-
Syzygium aromaticum	*Karabu*	Flower bud	+++++

* Concentration of plant extracts: 50 µg/mL; +++++: Strong protective effect
Sources: Perera & Handuwalage, 2015; Perera & Wijetunge, 2015

This work has proved that the standard, simple PAGE method with albumin-fructose system is suitable to detect the protective role of medicinal plants against protein glycation.

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REFERENCES

1. Goh S.Y. & Cooper M.E. (2008). The role of advanced glycation end products in progression and complications of diabetes. *Journal of Clinical Endocrinology and Metabolism* **93**(4): 1143 – 1152.
DOI: http://dx.doi.org/10.1210/jc.2007-1817
2. Laemmli U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**(5259): 680 – 685.
DOI: http://dx.doi.org/10.1038/227680a0
3. Perera H.K.I. & Handuwalage C.S. (2015). Detection of protein glycation inhibitory potential of nine antidiabetic plants using a novel method. *Asian Journal of Medical Science* **6**(2): 1 – 6.
DOI: http://dx.doi.org/10.3126/ajms.v6i2.10584
4. Perera H.K.I. & Wijetunge D.C.R. (2015). Strong protein glycation inhibitory potential of clove and coriander. *British Journal of Pharmaceutical Research* **6**(5): 306 – 312.
DOI: http://dx.doi.org/10.9734/BJPR/2015/16190
5. Wijetunge D.C.R. & Perera H.K.I. (2014). A novel *in vitro* method to identify protein glycation inhibitors. *Asian Journal of Medical Science* **5**(3): 15 – 21.
DOI:http://dx.doi.org/10.3126/ajms.v5i3.8670