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Studies on antiglycation potential of some traditional antidiabetic plants

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ABSTRACT

The aim of the present study was to assess the in vitro antiglycation activity of aqueous extracts of some traditional antidiabetic plants by determining the degree of non-enzymatic hemoglobin glycosylation. In the present investigation, the best concentration and time to incubate glucose with hemoglobin was determined. Thereafter, the glycosylation degree of hemoglobin in the presence of plant extracts at different concentrations and in their absence was measured colorimetrically at 520nm.The inhibitory effect on glycosylation of hemoglobin at the four concentrations viz. 250 µg/ml, 500 µg/ml, 750µg/ml and 1000 µg/ml was estimated as follows: for M. fragrans 17.02%, 26.19%, 34.30% and 42.75%, T. indica; 31.77%, 41.14%, 51.63% and 60.20 %, C. bonducella; 44.01%, 60.13%, 69.70%, 76.33% respectively. The results of the study clearly highlighted that non-enzymatic nature of hemoglobin glycosylation could be effectively inhibited by the extracts of C. bonducella, T. indica and M. fragrans at a desirable concentration.

Keywords: Antiglycation, hemoglobin, Inhibition, Plant extracts

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INTRODUCTION

Diabetes mellitus is characterized by hyperglycaemia, lipidaemia and oxidative stress and predisposes affected individuals to long-term complications afflicting the eyes, skin, kidneys, nerves and blood vessels [1]. Protein glycation caused by sugars and reactive carbonyls is a contributing factor to diabetic complications, aging, and other chronic diseases [2]. Increased protein glycation and the subsequent build-up of tissue advanced glycation end products (AGEs) contribute towards the pathogenesis of diabetic complications. The accumulation of AGEs *in vivo* has been considered to play a major role in the pathogenic process of diabetes and its complications, including neuropathy, nephropathy, retinopathy, cataract and in other health disorder such as Alzheimer´s disease and aging [3, 4, 5]. Thus, the investigation of compounds with an AGEs inhibitor activity, would certainly offer a potential therapeutic approach for the prevention of diabetes or other pathogenic complications. Plants represent a vast source of potentially useful dietary supplements for improving blood glucose control and preventing long-term complications in type 2 diabetes mellitus [6]. Numerous plants have been documented as beneficial in the treatment of diabetes. The majority of traditional antidiabetic plants await proper scientific and medical evaluation for their ability to improve blood glucose control and or to prevent the diabetic complications. The potential of these traditional antidiabetic plants to inhibit glycosylation of hemoglobin and other plasma proteins, if explored will certainly prove to be beneficial for the prevention of diabetes and its other associated complications. *Myristica fragrans* (Family: Myristicaceae) commonly known as nutmeg, the seeds are widely used as a spice. The plant is known for its therapeutic properties like antimicrobial, antioxidant, anti-inflammatory, cytotoxic, antithrombotic, hypolipidaemic and antiatherosclerotic effects [7]. *Caesalpinia bonducella* (Family: Caesalpiniaceae) is prickly shrub claimed to have multiple therapeutic properties like, antidiuretic, anthelmintic, antibacterial, anti-anaphylactic, antiviral, antiasthmatic and antidiabetic [8-11]. *Tamarindus indica* Linn. (Family: *Leguminosae*) is also one of the popular drug in Ayurvedic system of medicine. Its fruit is regarded as a digestive, carminative, laxative, expectorant and blood tonic [12]. Other parts of the plant possess antioxidant, antihepatotoxic, anti-inflammatory, antimutagenic and antidiabetic activities [13, 14]. The aqueous extracts of the seeds of the aforesaid traditional antidiabetic plants were used for determination of their in vitro antiglycation activity.

MATERIALS AND METHODS

Plant material

The plant material was collected from local areas of Karad and was further identified and authenticated by the Department of Botany, Science College, Karad. The seeds of *M. fragrans*, *T. indica* and *C. bonducella* were cleaned, dried in a hot air oven (50 °C), powdered, passed through 60 mesh sieve (BS) and stored in an airtight container at 4 °C till further use.

Chemicals

Hemoglobin was purchased from Sigma Aldrich, USA. All the chemicals used in the study were of extra pure analytical grade.

Preparation of plant extracts

The aqueous extracts were prepared by extracting the powders of seeds of *M. fragrans*, *T.indica* and *C. bonducella* with hot water (70 °C) in a mechanical shaker (24 h), filtered and freeze dried.

Determining the best condition for hemoglobin glycosylation [15].

In order to find the best glucose concentration, hemoglobin 5 g/100 ml in 0.01 M phosphate buffer, pH 7.4 was incubated with different concentrations of glucose. The extent of glycosylation was measured by using colorimetric method. Thereafter, to assess the most useful time for glycosylation, hemoglobin 5g/ 100 ml with the best concentration of glucose was incubated at different times and the amount of glycosylation was measured.

Assay

1 ml of hemoglobin solution 5 g/100 ml and 1 ml of the solution containing glucose 2 g/100 ml and Gentamycin 20 mg/100 ml in 0.01 M phosphate buffer, pH 7.4 were incubated in the dark at room temperature. Then, the glycosylation degree of hemoglobin in the presence of different concentration of plant extracts and their absences were measured by the colorimetric method.

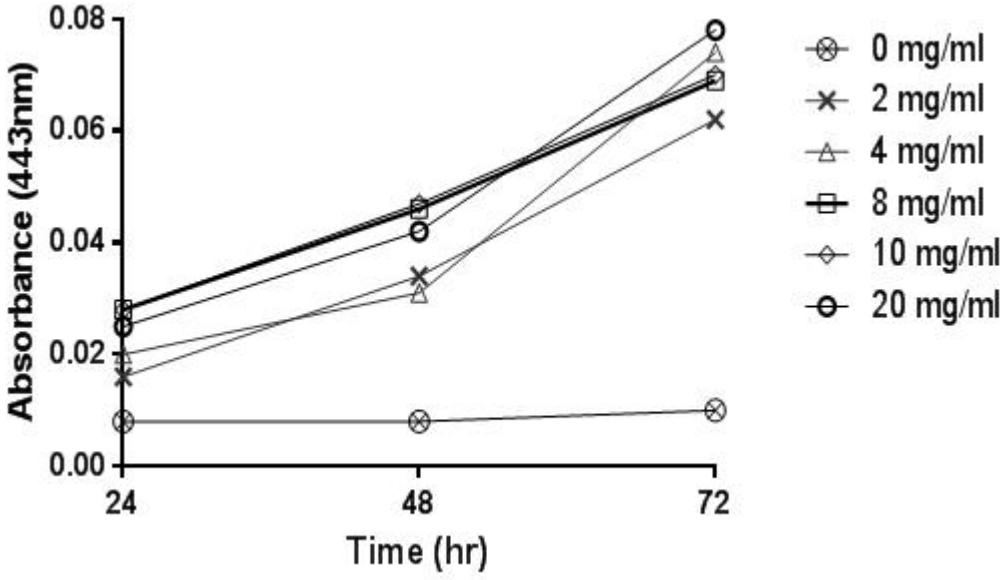

Figure 1. 1 ml of hemoglobin solution 5 g/100 ml and 1 ml of the solution containing different concentrations of glucose in 0.01 M phosphate buffer, pH 7.4 were incubated at room temperature at different time intervals

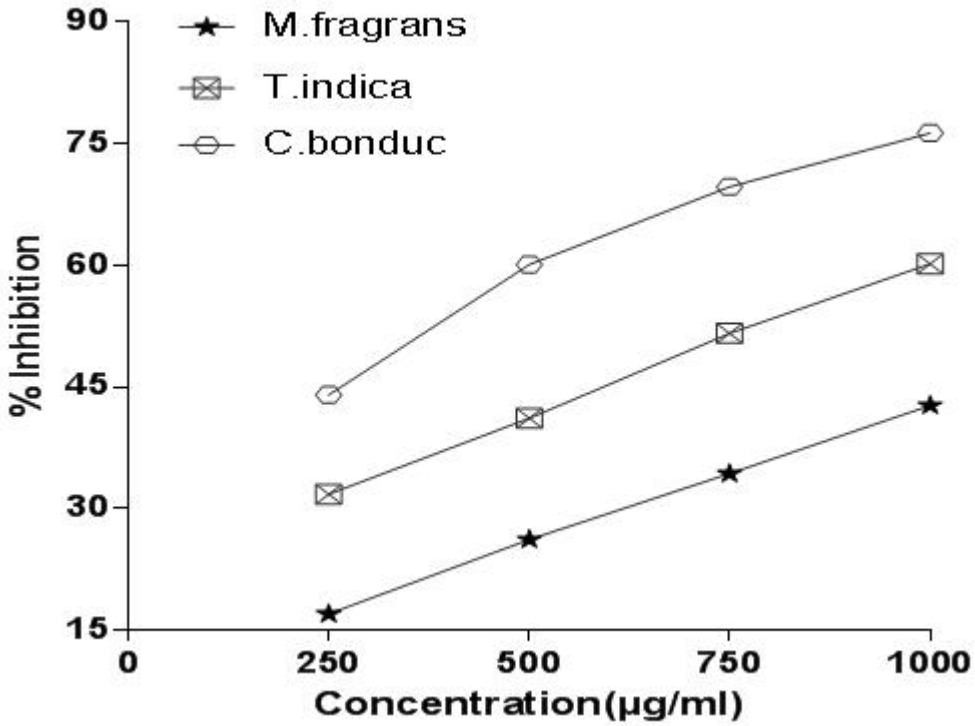

Figure 2. Inhibition of non-enzymatic glycosylation of hemoglobin by plant extracts

RESULTS

To study the antioxidant effects of selected plant extracts on glycosylation of hemoglobin, initially hemoglobin 5 g/100 ml was incubated in the presence of different concentrations of glucose and then the degree of glycosylation was measured by the colorimetric method. It was observed that up to the concentration of 2 g/100 ml stock solution of glucose, the amount of glycosylation increased linearly (Figure 1). So as to find the suitable time for incubation in this study, hemoglobin 5 g/100 ml was incubated in the presence of glucose 2 g/100 ml at different times and the degree of glycosylation was measured by the colorimetric method. The results exhibited that glycosylation increased up to the time of 72 h linearly and therefore, 72 h was chosen as the best time for this study (Figure 1). Different concentrations of the plant extracts were used in the study viz. 250 µg/ml, 500 µg/ml, 750µg/ml and 1000 µg/ml. The inhibitory effects on glycosylation of hemoglobin at these concentrations were estimated as 17.02%, 26.19%, 34.30% and 42.75%, for *M. fragrans* extracts. For *T. indica* it was observed as 31.77%, 41.14%, 51.63%, 60.20%, whereas for *C. bonducella* extract the inhibitory effects on hemoglobin glycosylation were observed as 44.01%, 60.13%, 69.70%, and 76.33% at a concentration of 250 µg/ml, 500 µg/ml, 750µg/ml and 1000 µg/ml respectively.

DISCUSSION

In diabetics, oxidative stress has been found to be mainly due to an increased production of free radicals due to persistent hyperglycemia and a sharp reduction of antioxidants defenses and the tissue antioxidant status which leads to the development of diabetic complications. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries [16]. Plant extracts may play an important role in the inhibition of the glycosylation end products. Our studies revealed that glycosylation of hemoglobin increases on its incubation with the increasing concentration of the glucose (2mg, 4mg,8mg 10mg and 20mg) over a period of 72hrs (Figure 1). However, the plant extracts promisingly inhibited the glycosylation of hemoglobin as shown in (Figure 2). The results of the studies clearly demonstrated that non-enzymatic nature of hemoglobin glycosylation could be effectively inhibited by the extracts of *C. bonducella, T. indica* and *M. fragrans* at a desirable concentration.

CONCLUSION

From the results it may be concluded that amongst the plant extracts studied, the aqueous extract of seeds of *C. bonducella* exhibited higher inhibition of glycosylation indicating that the extract decreases the formation of the

glucose- hemoglobin complex and thus amount of free hemoglobin increases. The vivo effect should be investigated so that it can be utilized to prevent or treat complication associated with diabetes.

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REFERENCES

[1] Elosta A, Ghous T, Ahmed N, *Curr Diabetes Rev,* **2012**,8(2), 92-108.
[2] Liu H, Liu H, Wang W, Khoo C, Taylor J, Gu L, *Food Funct,* **2011**, 2(8), 475-482.
[3] Majumdar M, Parihar P, *Asian Journal of Plant Science and Research,* **2012, 2(2),** 95-101.
[4] Rohilla A, Tiwari S, Rohilla S, Kushnoor A, *European Journal of Experimental Biology*,**2011**,1(4),72-80.
[5] Brownlee M, *Annu Rev Med,* **1995,** 46, 223-234.
[6] Gallagher AM, Flatt PR, Duffy G, Abdel Wahab, *Nutr Res,* **2003,** 23,413-424.
[7] Latha PG, Sindhu PG, Suja SR, Geetha BS, Pushpangadan P, Rajasekharan S, *Journal of Spices and Aromatic Crops,* **2005,** 14 (2), 94-101.
[8] Neogi NC, Nayak KP, *Indian J Pharmacol,* **1958,** 20, 95-100.
[9] Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Roy C, *Indian J Exp Biol,* **1968,** 6**,** 232-247.
[10] Aswar P, Kuchekar B, *Asian Journal of Plant Science and Research,* **2011**, 1(3), 91-102.
[11] Rao VV, Dwivedi SK, Swarup D, *Fitoterapia,* **1994,** 65,245-247.
[12] Komutarin T, Azadi S, Butterworth L, Keil D, Chitsomboon B, Suttajit M, *Food Chem Toxicol,* **2004,** 42, 649-658.
[13] Tsuda T, Watanable M, Ohshima K, Yamamoto A, Kawakishi S, Osawa T, *J Agri Food Chem,* **1994**, 42, 2671-2674.
[14] Rimbau V, Cerdan C, Vila R, Iglesia J, *Phytother Res,* **1999,** 13, 128-132.
[15] Fluckiger R, Winterhalter KH. *In vitro synthesis of hemoglobin,* North-Holland Publishing Company, Amsterdam, **1976**, pp.354-356.
[16] Rahimini R, *Biomed Pharmacother,* **2005**, 59,365-373.