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In vitro study on inhibition of glycosylation of ethanol leaf extract of *Gardenia latifolia* AIT

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Abstract
The inhibitory properties of ethanol extract of *Gardenia latifolia* on glycosylation formation, was investigated in haemoglobin using Gallic acid as Standard. The periodic glycosylation of haemoglobin at varying concentration of glucose shows a decrease in haemoglobin concentration indicating the glycosylation of haemoglobin. While the subsequent administration of *G. latifolia* ethanol leaf extract inhibit haemoglobin glycosylation, where a concentration of 20 mg/ml of the extract gave a significant inhibition by yielding haemoglobin concentration of 1.324 mg/ml for test extract as against 1.18 mg/ml for the standard. The present study suggests that the crude ethanol extract inhibits the binding of glucose to hemoglobin, since at higher concentration of glucose the concentration was found to be high.

Keywords: Haemoglobin, medicinal plants, gallic acid, ethanol, inhibition

Introduction
Diabetes is one of the endocrine disorders that severely affects people health and cause many complications in patients. According to the International Diabetes Federation (2015), over 400 million people are affected by diabetes worldwide, and 5 million deaths reported in 2015, every six seconds a person dies from diabetes, and also diabetes expenditure reached USD1.197 billion [1]. In diabetes, glucose forms covalent adducts with proteins through glycation. Glycation is non-enzymatic reaction between amino acid groups of protein and carbonyl group of reducing sugars that leads to formation of Advanced Glycation End Products (AEG) and thus clearly alters structure and function of proteins [2, 3]. Glycation of various structural and functional proteins including collagen and plasma proteins (as fibrinogen, albumin and globulins) due to high blood glucose levels is the major cause of pathogenesis of diabetic complications such as neuropathy, nephropathy and retinopathy. In addition to diabetes, AGEs is observed in several important diseases such as end-stage kidney and heart diseases, Alzheimer´s disease, arthritis and ageing. In addition to changing structure and function of proteins, Glycation have also deleterious effects on some other important molecules including nucleic acids and lipids that develop diabetic complications.
Control of plasma glucose could prevent the progression of most of the complications of diabetes and hemoglobinA1c is the most important criterion controlling these long-term complications [4]. Noticeably increase of the worldwide prevalence of type 2 diabetes is a true challenge for modern medicine. Thus, dietary supplements that can modulate glucose homeostasis would be desirable [5]. Use of medicinal plants for amelioration of various metabolic disorders is finding favor with researches owing to their lesser side-effects [6]. *Gardenia latifolia* (Rubiaceae) is commonly known as Indian boxwood or Ceylon boxwood, is a densely foliaceous small tree that occurs throughout the greater parts of Indian common in deciduous forests along the streams. The stem bark and fruits are reported to be used in the treatment of various ailments such as snake bite, skin diseases, stomach Pais, caries in humans and ephemeral fever in live stocks [7-9]. Fruits are used for making perfumes [10]. The present study was aimed at ascertaining the glycosylation inhibitory effects of the ethanol extract of *G. latifolia*.

Materials and Methods
Plant collection
The fresh aerial plant parts were collected from Kolli hills, Namakkal District, Tamil Nadu, India. The collected plant is identified by Botanical Survey of India (BSI/SRC/5/23/2013/Tech-795 & Serial No. 1), Coimbatore and the voucher specimens were

deposited at the herbarium of Department of Botany, National College (Autonomous), Tiruchirappalli-1.

Preparation of extracts

Plant material

Fresh and health leaves were collected from Kolli hills, Tamil Nadu, India. The leaves were washed thoroughly in distilled water and the surface water was removed by air drying under shade. The leaves were powdered with the help of mechanical blender and used for extraction.

Ethanol extract

Air dried powder of 10 g was placed in a conical flask containing 100 ml of ethanol plugged with cotton and then kept on a rotary shaker at 200 rpm for 24 hrs. Later, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 15 min. The supernatant was collected and the solvent was evaporated to make volume one fourth of its original volume.

Evaluation of haemoglobin glycosylation

Preparation of haemoglobin

The blood was collected from a healthy human volunteer and transferred into a blood bottle containing an anticoagulant. Hemolysate was prepared based on the principle of hypotonic lysis. The red blood collected were washed thrice with 0.14M NaCl solution and one volume of red blood cells suspension was lysed with two volumes of 0.01M phosphate buffer, pH 7.4 and 0.5 volume of CCl4. The haemolysate was then freed from the debris by centrifugation at 2300 rpm for 15 min at room temperature. The haemoglobin rich fraction ie the upper layer was separated and dispensed into sample bottle for storage and refrigerated until required for use [11].

Estimation of haemoglobin glycosylation

1 ml each of haemoglobin fraction was transferred into three test tubes, each containing 1 ml solution of different concentrations (2, 5, 10, and 20 mg/ml) of glucose in 0.01M phosphate buffer (pH 7.4). The contents were incubated at room temperature for 72 hrs. A blank solution in which the addition of glucose solution was omitted was used as the control. The amounts of hydroxymethylfurfural in nanomole released were estimated at different incubation periods of 0, 24 hrs, 48 hrs and 72 hrs which correspond to the degree of glycosylation [11].

Effect of extracts on haemoglobin glycosylation

To 1 ml of haemoglobin solution, 5µl of gentamycin and 25 µl of the plant extracts (30µg/ml) were added. The reaction was started by the addition of 1 ml of 2% glucose in 0.01M phosphate buffer (pH 7.4) and incubated in the dark at room temperature. The concentrations of glycated haemoglobin at the incubation period of 0, 24 and 72 hrs were estimated spectrophotometrically at 443nm [11].

Effect of extract at physiological glucose concentration

To 1 ml of haemoglobin solution, 1 ml of glucose solution (2mg, 10mg and 20mg in 20 ml each of 0.01M phosphate buffer, pH 7.4) and 5µl of gentamycin in 0.01M phosphate buffer (pH 7.4) were mixed and incubated in the dark at room temperature in the presence 30 µg/ml of Gallic acid and plant extracts respectively. Haemoglobin concentrations were estimated over an incubation period of 72 hrs spectrophotometrically at 443 nm, as an index for measuring the degree of haemoglobin glycosylation. The assay was carried out in triplicates [11].

Results and Discussion

Diabetes mellitus is an often life threatening chronic disorder with increasing incidence throughout the world. In recent years, there is a steady rise in the rate of incidence of Diabetes mellitus and estimated that 1 in 5 may be diabetic by 2025 [12]. Medicinal plants are ties of most effective plants were in part explained by the ability of the phytoconstituents to increase glucose transport and metabolism in muscle and/ or to stimulate insulin secretion [13]. The exposure of haemoglobin over a period of 72 hours to varying concentration of glucose (2mg, 5mg, 10mg and 20mg) showed decrease in the concentration of haemoglobin indicating an increase in the glycosylation of hemoglobin (Table 1). While Table 2 indicate the inhibition of glycosylation of hemoglobin as compared between the plant extract and a standard (Gallic acid). The Effect of *G. latifolia* ethanolic leaf extract at physiologic glucose concentration, compared with the data for standard (2mg/ml, 5mg/ml, 10mg/ml and 20mg/ml), showed an increase in the concentration of hemoglobin over all the periods of incubation, indicating inhibition of glycosylation. In comparison with standard, at 2mg/ml the increase in hemoglobin concentration was higher than that of the plant extract, but at 20mg/ml the increase in hemoglobin concentration was higher for the plant extract than for the standard (Tables 3, 4 & 5).

The glycosylated haemoglobin is an important clinical marker in diabetes which helps to determine the degree of protein glycation during diabetes. In persistent hyperglycemic state, formation of HbA1c occurred by nonenzymatic reaction between glucose and free amino groups of haemoglobin. HbA1c level in diabetes helps to evaluate long-termglycemic control, and it helps to assess the risk of the development or progression of diabetic complications. Published studies supported that reduction in HbA1c levels during the diabetes treatment considerably reduced microvascular complications [14]. The ethanol extract of *G. latifolia* showed reduction of HbA1c and improvement in Hb levels, and it might be due to blood glucose lowering effect of ethanol extract possibly through reversal of insulin resistance or increasing insulin secretion by regeneration of pancreatic β-cells. These results were in consistent with the results of *Leptadenia hastate* [15].

Increased concentration of glucose in the blood leads to its binding to haemoglobin which may result in the formation of the reactive oxygen species. The ethanol extract of *G. latifolia* exhibited a concentration dependent increase in % inhibition of glycosylation, suggesting that the plant extract decrease the formation of the glucose haemoglobin complex and thus amount of free haemoglobin increases. The ethanol and the last remaining aqueous extract displayed the highest inhibition of haemoglobin glycosylation at different physiological concentrations of the glucose over the period of 72 h which was comparable with the standard drug. The activity of crude ethanol extract from the leaves of *G. latifolia* was found to be better than standard drug.

It is concluded that the administration of *G. latifolia* ethanolic leaf extract inhibits glycosylation of hemoglobin as such the formation of advance glycated end-product may be inhibited by the plant extract. This observed effect might be attributed by the presence of bioactive compounds in the plant extract like flavonoids, alkaloids, phenols and sterols. This needs further investigation specific bio active compound responsible for such activities.

Table 1: Estimation of haemoglobin glycosylation over the Period of 72 hours

Glucose Concentration (mg/ml)	Incubation period		
	24 hrs	48hrs	72 hrs
2	1.02 ± 0.32	1.08 ± 0.82	1.09 ± 0.21
5	0.982 ± 0.28	0.991 ± 0.14	0.942 ± 0.39
10	0.972 ± 0.38	0.984 ± 0.48	0.971 ± 0.41
20	0.843 ± 0.19	0.851 ± 0.05	0.871 ± 0.20

Values are expressed as mean ± SD, n = 3

Table 2: Effect of plant extracts on haemoglobin glycosylation

Extracts	Incubation period		
	24 hrs	48hrs	72 hrs
Standard (Gallic acid)	0.865 ± 0.42	0.971 ± 0.21	1.18 ± 0.001
Ethanol	0.674 ± 0.34	0.712 ± 0.40	0.748 ± 0.37

Values are expressed as mean ± SD, n = 3

Table 3: The comparative effects of plant extracts on haemoglobin glycosylation at physiological glucose concentration after 24hrs of incubation

Glucose Concentration (mg/ml)	Standard (Gallic acid)	Plant extract (Ethanol)
2	1.012 ± 0.12	0.653 ± 0.02
5	1.078 ± 0.21	0.701 ± 0.76
10	1.143 ± 0.04	0.752 ± 0.05
20	1.25 ± 0.27	0.975 ± 1.04

Values are expressed as mean ± SD, n = 3

Table 4: The comparative effects of plant extracts on haemoglobin glycosylation at physiological glucose concentration after 48 hrs of incubation

Glucose Concentration (mg/ml)	Standard (Gallic acid)	Plant extract (Ethanol)
2	1.114 ±0.18	0.861 ± 1.43
5	1.187 ± 0.92	0.954 ± 0.81
10	1.25 ± 0.14	1.03 ± 0.02
20	1.29 ±0.21	1.21 ± 0.05

Values are expressed as mean ± SD, n = 3

Table 5: The comparative effects of plant extracts on haemoglobin glycosylation at physiological glucose concentration after 72hrs of incubation

Glucose Concentration (mg/ml)	Standard (Gallic acid)	Plant extract (Ethanol)
2	1.272 ±0.21	0.943 ± 0.31
5	1.296 ± 0.71	1.07 ± 0.91
10	1.31 ± 0.05	1.28 ± 0.25
20	1.324 ± 0.31	1.32 ± 0.92

Values are expressed as mean ± SD, n = 3

References

1. Atlas ID: International Diabetes Federation, Brussels, 2015.
2. Friguet B, Stadtman ER, Szweda LI. Modification of glucose-6- phosphate dehydrogenase by 4-hydroxy-2-nonenal. Formation of cross-linked protein that inhibits the multicatalytic protease. J Biol Chem. 1994; 269(34):21639-21643.
3. Bulteau AL, Lundberg KC, Humphries KM, Sadek HA, Szweda PA, Friguet B *et al*. Oxidative modification and inactivation of the proteasome during coronary occlusion/reperfusion. J Biol Chem. 2001; 276(32):30057-30063.
4. Haddadinezhad S, Ghazaleh N. Relation of fasting and postprandial and plasma glucose with hemoglobinA1c in diabetics. Int J Diabetes Dev Ctries. 2010; 30(1):8-10.
5. Winiarska H, Dworacka M, Borowska M, Iewicz-Kozowska TB, Gorecki P, Mœcisz A. The effects of plant extracts of *Medicago sativa* and *Trigonella foenum-graecum* on postprandial glucose levels in type 2 diabetic rats. Herba Polonica. 2007; 53(5):34-44.
6. Kavishankar GB, Lakshmidevi N, Mahadeva Murthy S, Prakash HS, Niranjana SR. Diabetes and medicinal plants-A review. Int J Pharm Biomed Sci. 2011; 2(3):65-80.
7. Reddy KN, Subbaraju GV, Reddy CS, Raju VS. Ethno veterinary medicine for trating live stck in Eastern Ghats of Andhra Pradesh. Ind. J Trad Knowl. 2006; 53:68-372.
8. Madava Chetty K, Sivaji K, Tulasi Ra K. Flowering Plants of Chittor District, First ed., Students Offset Printers, India, 2008, 57-59
9. Dr. Due's Phytchemical and Ethnbotanical Databases
10. Chandra Prakash K. Aboriginal uses and management of ethnobotanical spices in deciduous forests of Chhattisgarh state in India. J Ethnobiol Ethnomed, 2009, 5-20
11. Adisa RA, Oke J, Olomu SA, Olorunsogo O. Inhibition of human haemoglobin glycosylation by flavonoid containing leaf extract of *Cnestis ferruginea*. J Camer Acad Sci. 2004; 4:351-359.
12. Romila Y, Mazumder PB, Dutta Choudhury MA. Review on Antidiabetic Plants used by the People of Manipur Characterized by Hypoglycemic Activity. Assam University Journal of Science & Technology: Biological and Environmental Sciences. 2010; 6:167-175.
13. Edwards CA, Black burn NA, Craigne L, Daavidson P, Tomlin J, Sugden K *et al*. Viscosity of food gums determined *in vitro* related to their hypoglycemic actions. Am J Cli Nutr. 1987; 46:72-77.
14. Calisti L, Tognetti S. Measure of glycosylated hemoglobin. Acta Bio Medica de Ateneo Parmense. 2005; 76(3):59-62.
15. Ukwuani AN, Igbokwu MO. *In vitro* antidiabetic effect of *Leptadenia hastata* leaves fractions. Biosci Res Tod World. 2015; 1:40-46.