Inhibition of human haemoglobin glycosylation by flavonoid containing leaf extracts of *Cnestis ferruginea*

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ABSTRACT

The decrease in the amount of residual haemoglobin following the incubation of haemoglobin with varying concentrations of glucose over specified periods was used as an index of human haemoglobin glycosylation *in vitro*. In this study the inhibitory effect of flavonoid-rich methanolic extracts of a herbal remedy-*Cnestis ferruginea* (Cn.f) on glycosylation was compared to that of quercetin – a standard flavonoid and a known antioxidant. The effect of these extracts was also investigated at physiological concentrations of glucose (≤1mg/ml). Time-dependent haemoglobin glycosylation increased in a concentration-dependent manner up to 20mg glucose/ml. Glycosylation in the presence of 20mg glucose/ml was about 72% while varying concentrations of methanolic extracts (10, 20, 30µg/ml) of Cn.f reduced the glycosylation to 41, 31, and 29%, respectively. Similar effects were obtained for quercetin (10, 20, 30µg/ml). However, results obtained from experiments carried out on the possible effects of the extracts of Cn.f (10, 20, 30µg/ml) on haemoglobin glycosylation in the presence of physiological concentrations of glucose (≤ 1mg/ml) indicated that there was no significant glycosylation ($P \geq 0.05$) at these concentrations of glucose up to 1mg/ml. Furthermore, a 4% inhibition of glycosylation was observed after 24hrs of incubation with 10µg/ml extract of Cn.f in the presence of 1mg glucose/ml. The extent of inhibition at this concentration of glucose increased from 5.5 to 7% after 48 and 72hrs, respectively. Using these same concentrations (20 and 30µg/ml) of methanolic extracts of Cn.f or quercetin, the inhibition was total even after prolonged hours of incubation. From these results, the leaf extracts of *Cnestis ferruginea* inhibited haemoglobin glycosylation almost to the same extent as quercetin, thus indicating that the medicinal plant could prove useful in drug development and treatment of diabetes mellitus.

Keywords: Haemoglobin glycosylation, *Cnestis ferruginea*, Quercetin, Diabetes, Inhibition.

1. INTRODUCTION

In general, protein molecules bind non-enzymatically with glucose or other sugars to form initially unstable aldimine and ketamine adducts and finally, more stable structures called advanced glycated end-products (AGEs) (Brownlee, *et al.*, 1988). Allen *et al.*, (1958) first reported that about 5% of haemoglobin in a population of normal red cells is covalently linked to glucose, ensuing in the formation of a distinct minor component called HbA_{1c}. Recently, attention has been focused on the non-enzymic glycosylation of human haemoglobin following the discovery that untreated diabetic patients exhibited a two- fold increase in this minor fraction (Rahbar, 1968). In this regard, AGEs have been implicated as a major pathogenic process leading to diabetic complications (McCance, *et al.*, 1993). In fact, non-enzymic glycation of proteins is now known to be the source of free radicals in diabetes, thus aggravating the state of an increased oxidative stress in diabetes which is accompanied by a lowered antioxidant capacity and an elevated oxidation of cellular components and lipoproteins (Brownlee, 1994). By simple extrapolation therefore, an inhibition of the glycation process could prevent or somewhat delay the onset and /or the progression of these complications.

In view of the fact that the glycosylation reaction involves the formation of reactive oxygen species (Thornalley, *et al.*, 1984), it seems likely that the presence of naturally occurring antioxidants may block the formation of AGEs. Indeed, the use of such co-adjuvants therapy for the treatment of diabetes complications is now not uncommon.

Flavonoids are a family of naturally occurring benzo-gamma-pyrone derivatives of low molecular weight which are ubiquitous in photosynthesizing cells and thus occur both in the human diet and in herbs used as folk medicines (Kuhnau, 1970)). They have been isolated from fruits, vegetables, nuts, seeds, stems, flowers, barks of trees, tea, wine and marine organisms (Hermann, 1976). Interestingly, they modulate the activity of a large variety of enzymes, exhibit free radical scavenging activity, chelate certain metal cations, possess antioxidant properties, enhance resistance of low density lipoprotein to oxidation, protect against peroxidative membrane damage and affect cellular protein phosphorylation (Robak and Gryglewski, 1988; Liu, *et al.*, 1992; Harbon, 1994; Glttertog, *et al.*, 1997; Ishikawa, *et al.*, 1997; Furhman, *et al.*, 1997).

Cnestis ferruginea is a medicinal plant belonging to the family of Connaraceae. Its roots and fruits are being used by the West African straditional healers for therapeutic purposes such as an antigonorrhoea, antidiarhoeal, antibacterial, analgesic, and as a remedy against dental caries, pyorrhea, and snake-bite (Kerharo and Bourquet, 1950; Dalziel, 1937). Phytochemical screening of the petroleum ether fraction of the fruit of *Cnestis ferruginea* revealed the presence of flavonoids, combined anthraquinones, saponins, tannins, steroidal glycosides (Ogbechie *et al.*, 1987) and an isoflavone glycoside (Parvez and Rahman, 1992).

The aim of this study was to assess the potency of the leaf extracts of *Cnestis ferruginea* as an inhibitor of haemoglobin glycosylation. On the basis that this reaction is an oxidation reaction, the biologically active naturally occurring flavonoids such as anthraquinones contained in the leaves of *Cnestis ferruginea* which acts as antioxidants should be able to reduce it.

2. MATERIALS AND METHODS

2.1 Plant Material and extract preparation

The leaves of *Cnestis ferruginea* were obtained from a forest in Mamu, a village in Oyo State of Nigeria. The samples were authenticated and identified by Mr. T.K. Odewo of the Herbarium, Forest Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. A voucher specimen (No. 106524) of the plant was deposited at FRIN. The fresh leaves of the plant were thoroughly rinsed in distilled water, spread on trays and allowed to air-dry for one week at room temperature. The dry leaves were blended into powder and 200g of the powder was soaked in 2 liters of methanol (95% v/v) and kept in the dark for 4 days following which the solvent with extract was collected and evaporated at 40°C on a rotary evaporator until a dark greenish brown sticky substance was obtained. The residue was then processed to give 24.6g (12.3% yield) solid crude extract, which was stored in a freezer until use. The extract was tested for the presence of flavonoids following the method described by (Cuendet, *et al.*, 1997) in a reduction reaction with 2,2 Diphenyl-1-picrylhydrazyl (DPPH) radical. Thin layer chromatographic (TLC) plates were developed and dried after which the plates were sprayed with 0.01% DPPH solution in methanol. The plates were examined 30 min later. Active compounds appear as yellow spots against a purple background. The total phenolic compound was also de-

termined using the methods of Gow-Chin Yen and Pin-Der Duh, (1994).

2.2 *Collection of blood samples and preparation of haemoglobin*

Thirty milliliters (30ml) of blood was collected from 10 commercial donors of the University College Hospital, Ibadan, after obtaining their consent. The samples were collected in vernoject bottles containing Ethylene diamine tetraacetic acid (EDTA) as anticoagulant. Haemolysate was prepared following the procedure of Asgary, et al., (1999) based on the principle of hypotonic lysis. According to the procedure, the red blood cells were washed thrice with 0.14M NaCl solution and 1 volume of red blood cells suspension was lysed with 2 volumes of 0.01M phosphate buffer, pH 7.4 and 0.5 volume of carbon tetrachloride. The haemolysate was then freed from the debris by centrifugation at 2300rpm for 15 mins at room temperature. The haemoglobin rich fraction (upper layer) was separated and dispensed into sample bottles for storage at –10°C until required for use.

2.3 Estimation of Haemoglobin glycosylation

Haemoglobin concentrations were estimated spectrophotometrically at 540nm by the method of Drabkin and Austin (1932).

Non-enzymic glycosylation of haemoglobin was estimated by a modification of the method of Fluckiger and Winterhalter, (1976) according to Parker, *et al.*, (1981). To 1ml of haemoglobin fraction was added lml each of solution containing different concentrations of glucose in 0.01M phosphate buffer pH 7.4. The contents were incubated at room temperature for 72 hrs. The final concentrations of glucose used in this study were 2, 10, and 20mg/ml. Blank in which the addition of glucose solution was omitted was used as control. The amount of hydroxymethylfurfural (nanomole HMF) released were estimated at different incubation periods of 0, 24hr, 48hr, and 72hr and corresponds to the degree of glycosylation. All determinations were carried out in triplicates.

2.4 Assays

a) Effect of extract on haemoglobin glycosylation

The effects of quercetin - a known flavonoid (purchased from Sigma-Aldrich Chem. Co. St. Louis, USA) which was used as a standard and extracts of Cn.f were tested on haemoglobin glycosylation, in this experiment. To 1ml of haemoglobin solution was added 5µl of gentamycin and quercetin or extracts of Cn.f (10- 30µl). The reaction was started by the addition of 1ml of (2%) glucose solution in 0.01M phosphate buffer pH 7.4 and incubated in the dark at room temperature. The concentrations of glycated haemoglobin at the incubation periods (0, 24, and 72hrs) were estimated colorimetrically at 443nm by the method of Parker et al., (1981). All assays were conducted in triplicates.

b) Effect of extract at physiological glucose concentration

1ml of haemoglobin solution, 1ml of glucose solution and 5ìl of gentamycin in 0.01M phosphate buffer pH 7.4 were mixed and incubated in the dark at room temperature in the presence or absence of 10µg, 20µg or 30µg/ml of quercetin or the extracts of *Cnestis ferruginea* respectively. Different concentrations of glucose 1mg, 2mg, 4mg, 6mg, 8mg, 10mg, 15mg and 20mg in 20mls each of 0.01M phosphate buffer, pH 7.4 were used. Haemoglobin concentrations were estimated daily throughout the incubation period (72hrs) as an index for measuring the degree of haemoglobin glycosylation. Assay was carried out in triplicates. The absorbance read at 443nm, colorimetrically, according to the procedure of Parker *et al.*, (1981).

2.5 Statistical Analysis

Glycosylated haemoglobin is expressed in nmole HMF ± standard deviation. The level of significance of difference (5%) between glycosylated haemoglobin levels at zero and other time intervals, inhibitory activity of methanolic extracts of *Cnestis ferruginea* and quercetin on human haemoglobin glycosylation at the varied periods of incubation were analysed using a one-way analysis of variance (ANOVA). Graphs were plotted with microsoft excel on windows version.

3. RESULTS

3.1 Polyphenolic content of Extracts

Fresh extracts of Cn.f contain some compounds, which reduced the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical in a TLC autographic assay by forming a yellow colouration on a purple background when sprayed with Diphenyl-Picryl, Hydrazine (DPPH) on a chromatogram. Also these compounds exhibited strong yellow or blue fluorescence under UV light. The total phenolic compound obtained in the extract was 875mg/g as catechin equivalents .

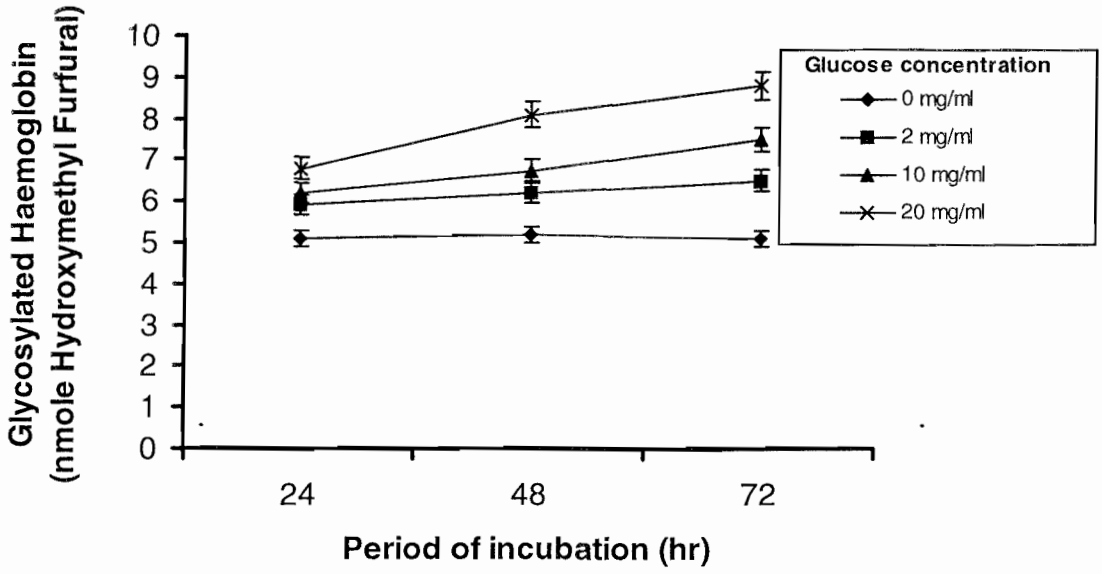

Fig 1: Time dependent glycosylation of human haemoglobin at varying concentration of glucose

3.2 Time-dependent haemoglobin glycosylation

Fig. 1 shows the level of haemoglobin glycosylation in the presence of varying concentrations of glucose at different periods of incubation. Exposure of haemoglobin to varying concentrations of glucose (2, 10, 20mg/ml) increased the concentration of hydroxymethylfurfural to different extents throughout the incubation periods up to 72hrs. For example, at 2mg glucose/ml, glycosylated haemoglobin concentration was increased from 5.10 ± 0.49nmoleHMF to 6.50 ± 0.750 nmoleHMF or by 27% at the end of incubation for 72hrs. Similarly, glycosylated haemoglobin concentration was increased to 6.20 ± 0.703 nmoleHMF or by 22% within the first 24hrs of incubation with 10mg glucose/ml. By the end of 48 and 72hrs incubation period, in the presence of 10mg glucose/ml, glycosylated haemoglobin had been elevated to 6.75 ± 0.702 and 7.510 ± 0.602 nmoleHMF or by 32% and 47%, respectively. The results show furthermore, that glycosylated haemoglobin was increased to 6.80 0.534 nmoleHMF or by 33% within the first 24hrs of incubation on increasing the glucose concentration two fold (20mg glucose/ml). Moreover, the concentration of glycosylated haemoglobin was raised to 8.10 ± 0.752 or by about 60% and 8.78 ± 0.881 nmoleHMF or by 72% following incubation for 48 and 72hrs respectively, in the presence of 20mg glucose/ml. In summary, the glycosylation of haemoglobin is both concentration and time-dependent.

3.3 Haemoglobin glycosylation in the presence of quercetin and extracts of Cn.f.

The results obtained from measurement of time- dependent haemoglobin glycosylation in the presence of varying concentrations of glucose revealed that 20mg glucose/ml optimally glycosylated haemoglobin. This concentration of glucose was therefore used in assessing the effects of quercetin and extracts of Cn.f on haemoglobin Fig. 2 shows the percentage inhibition of haemoglobin glycosylation. following incubation with 20mg glucose and quercetin or extracts of Cn.f. Increases in haemoglobin levels or reduction in haemoglobin glycosylation in comparison with controls were observed at all the concentrations of the quercetin and extracts of Cn.f (10, 20, 30) µg/ml used, irrespective of the incubation period. For example, incubation with 20mg/ml glucose for 72hrs resulted in 72% glycosylation of haemoglobin whereas increasing concentrations of quercetin and extracts of Cn.f resulted in an inhibition of glycosylation in a concentration -dependent manner. The degrees of inhibition using 10, 20, and 30µg/ml quercetin were 60, 67, and 75%, respectively. Similarly, 10, 20, and 30 µg/ml methanolic extracts of Cn.f prevented glycosylation by 59, 69, and 71% respectively. The effect of quercetin was highest after 72 hrs of incubation, however, there was no significant difference (P = 0.05) between their inhibitory effects on glycosylation. In this regard, the longer the incubation period, the greater the effects of the

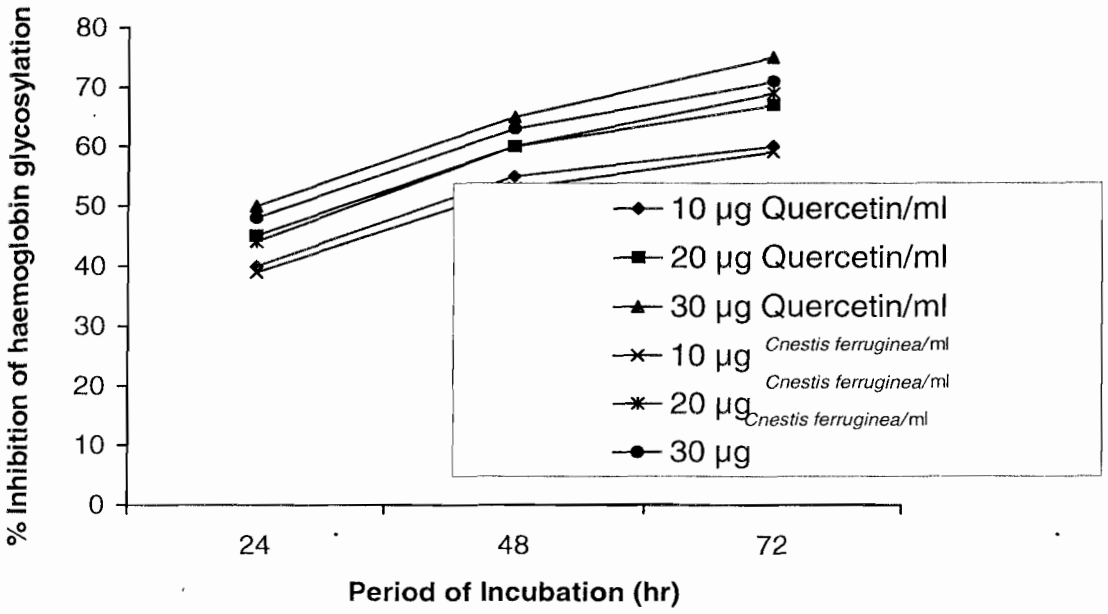

Fig 2: Comparative effects of varying concentrations of quercetin and extracts of Cnestis ferruginea on human haemoglobin glcosylation in the presence of 20 mg glucose/ml

antioxidants on the process of glycosylation. Also, the higher the concentration of the antioxidant, the greater the extent of prevention of glycosylation. The degree of prevention of glycosylation by quercetin and extracts of Cn.f were similar over the varying periods of incubation used in this study.

The effects of varying concentrations of quercetin and methanolic extracts of *Cnestis ferruginea* on haemoglobin glycosylation investigated in the presence of physiological concentrations of glucose (≤ 1mg/ml) show no significant glycosylation of haemoglobin at varying concentrations of glucose up to 1mg/ml. Infact, a 4% inhibition of glycosylation was observed after 24hr of incubation with 10µg/ml extract of Cn.f in the presence of 1mg/ml glucose, while the extent of inhibition at this concentration of glucose increased from 5.5 to 7% after 48 and 72hrs, respectively.

Similar results were observed with quercetin. Using 20µg/ml quercetin and 30µg/ml quercetin and *Cnestis ferruginea* respectively, prevention of glycosylation was total. Furthermore, these substances did not allow glycosylation to take place even after prolonged hours of glycosylation (Tables 1, 2 and 3).

Table 1: Effect of 10ìg/ml methanolic extracts of *Cnests ferruginea* and 10 ìg/ml quercetin on Haemoglobin glycosylation in the presence of physiological concentration of glucose (≤1mg/ml)

	Concentration of glycosylated haemoglobin (nanomol Hydroxymethylfurfural)								
	Control			+Quercetin (µg/ml)			+C.ferruginea (µg/ml)		
Gluc µg/ml	24hr	48hr	72hr	24hr	48hr	72hr	24hr	48hr	72hr
0	5.10±0.43	5.20 ±0.51	5.10±0.53	5.10±0.49	5.2±0.49	5.10±0.54	5.10±0.49	5.20±0.49	5.10±0.50
50	5.20±0.45	5.25 ±0.52	5.26±0.51	5.23±0.50	5.22±0.48	5.22±0.49	5.20±0.46	5.22±0.48	5.21±0.49
100	5.22±0.51	5.31 ±0.53	5.38±0.49	5.22±0.51	5.20±0.51	5.21±0.49	5.18±0.50	5.21±0.48	5.20±0.45
200	5.28±0.52	5.34±0.525	5.43±0.53	5.19±0.48	5.19±0.49	5.19±0.52	5.19±0.52	5.20±0.47	5.19±0.43
300	5.31±0.53	5.38 ±0.53	5.46±0.53	5.19±0.49	5.18±0.47	5.17±0.53	5.19±0.52	5.19±0.51	5.17±0.52
400	5.33±0.49	5.40 ±0.52	5.49±0.49	5.18±0.51	5.17±0.46	5.14±0.51	5.18±0.50	5.17±0.44	5.15±0.51
500	5.36±0.50	5.44 ±0.52	5.53±0.52	5.19±0.49	5.15±0.48	5.13±0.49	±0.48	5.16±0.45	5.14±0.50
750	5.38±0.53	5.48 ±0.55	5.57±0.54	5.18±0.49	5.15±0.49	5.12±0.49	5.18±0.49	5.14±0.47	5.12±0.49
1000	5.40±0.54	5.52 ±0.51	5.61±0.53	5.17±0.48	5.10±0.49	5.01±0.48	5.19±0.48	5.10±0.45	5.02±0.49

Each value is a mean of 10 different estimations ± standard deviation. Values are not significantly different at P≥0.05
Gluc= Glucose

Table 2: Effect of 20ìg/ml methanolic extracts of *Cnests ferruginea* and 20 ìg/ml quercetin on Haemoglobin glycosylation in the presence of physiological concentration of glucose (≤1mg/ml)

	Concentration of glycosylated haemoglobin (nanomol Hydroxymethylfurfural)								
	Control			+Quercetin (µg/ml)			+C.ferruginea (µg/ml)		
Gluc µg/ml	24hr	48hr	72hr	24hr	48hr	72hr	24hr	48hr	72hr
0	5.10±0.43	5.20 ±0.51	5.10±0.53	5.10±0.49	5.21±0.51	5.10±0.53	5.10±0.49	5.20 ±0.49	5.10±0.50
50	5.20±0.45	5.25 ±0.52	5.26±0.51	5.22±0.50	5.21±0.49	5.20±0.50	5.20±0.46	5.22±0.48	5.21±0.49
100	5.22±0.51	5.31 ±0.53	5.38±0.49	5.21±0.49	5.20±0.48	5.19±0.45	5.18±0.50	5.21±0.48	5.20±0.45
200	5.28±0.52	5.34±0.525	5.43±0.53	5.20±0.48	5.19±0.50	5.18±0.47	5.19±0.52	5.20±0.47	5.19±0.43
300	5.31±0.53	5.38 ±0.53	5.46±0.53	5.19±0.47	5.18±0.48	5.15±0.49	5.18±0.50	5.18±0.51	5.17±0.52
400	5.33±0.49	5.40 ±0.52	5.49±0.49	5.17±0.48	5.15±0.47	5.14±0.50	5.18±0.49	5.17±0.44	5.15±0.51
500	5.36±0.50	5.44 ±0.52	5.53±0.52	5.18±0.48	5.12±0.46	5.11±0.51	·±0.48	5.16±0.45	5.14±0.50
750	5.38±0.53	5.48 ±0.55	5.57±0.54	5.17±0.52	5.10±0.48	5.09±0.49	5.18±0.49	5.14±0.47	5.12±0.49
1000	5.40±0.54	5.52 ±0.51	5.61±0.53	5.16±0.47	5.08±0.49	5.08±0.48	5.18±0.49	5.10±0.45	5.02±0.49

Each value is a mean of 10 different estimations ± standard deviation. Values are not significantly different at P≥0.05
Gluc= Glucose

4) DISCUSSION

The speculation about the involvement of reactive oxygen species (ROS) in the pathophysiologic process of diabetes mellitus emanated from the observation that alloxan induces diabetes in rats via these oxidative species (Heikkila, *et al.*, 1976). The importance of erythrocytes in the pathophysiologic mechanisms in diabetes mellitus has become very evident from several abnormal features demonstrated for red cells of diabetic patients (Heikkila, *et al.*, 1976). In particular this include altered oxygen transport and metabolism (Jones and Peterson, 1981),

Table 3: Effect of 30ìg/ml methanolic extracts of *Cnests ferruginea* and 30 ìg/ml quercetin on Haemoglobin glycosylation in the presence of physiological concentration of glucose (≤1mg/ml)

	Concentration of glycosylated haemoglobin (nanomol Hydroxymethylfurfural)								
	Control			+Quercetin (µg/ml)			+C.ferruginea (µg/ml)		
Gluc µg/ml	24hr	48hr	72hr	24hr	48hr	72hr	24hr	48hr	72hr
0	5.10±0.43	5.20 ±0.51	5.10±0.53	5.10±0.43	5.20±0.51	5.10±0.53	5.10±0.43	5.20 ±0.51	5.10±0.53
50	5.20±0.45	5.25 ±0.52	5.26±0.51	5.22±0.49	5.20±0.49	5.20±0.48	5.21±0.49	5.20±0.48	5.19±0.49
100	5.22±0.51	5.31 ±0.53	5.38±0.49	5.20±0.48	5.19±0.48	5.18±0.50	5.20±0.48	5.19±0.49	5.17±0.48
200	5.28±0.52	5.34±0.525	5.43±0.53	5.19±0.50	5.18±0.47	5.16±0.51	5.18±0.50	5.17±0.48	5.16±0.48
300	5.31±0.53	5.38 ±0.53	5.46±0.53	5.18±0.48	5.16±0.48	5.15±0.50	5.16±0.51	5.15±0.49	5.15±0.51
400	5.33±0.49	5.40 ±0.52	5.49±0.49	5.19±0.49	5.15±0.46	5.13±0.51	5.15±0.48	5.13±0.47	5.13±0.50
500	5.36±0.50	5.44 ±0.52	5.53±0.52	5.17±0.50	5.13±0.49	5.12±0.49	±0.49	5.12±0.48	5.12±0.49
750	5.38±0.53	5.48 ±0.55	5.57±0.54	5.15±0.48	5.12±0.48	5.10±0.49	5.11±0.48	5.10±0.47	5.12±0.49
1000	5.40±0.54	5.52 ±0.51	5.61±0.53	5.11±0.47	5.09±0.48	5.08±0.48	5.10±0.49	5.08±0.48	5.08±0.48

Each value is a mean of 10 different estimations ± standard deviation.
Values are not significantly different at P≥0.05
Gluc= Glucose

glycation of membrane and cytosolic proteins (Gabbay, *et al.*, 1976), decreased life span (Pescarmona, *et al.*, 1982), and increased glycation of haemoglobin (Trivelli, *et al* 1971), a feature now generally used as an index of blood glucose control (Goldstein, 1995). It is well known that if untreated, diabetic patients are prone to developing several long term complications which could lead to increased morbidity and early mortality (Kannel, *et al* 1979; Lyons, 1991). Indeed, diabetic patients have an elevated oxidative stress, which is accompanied by a decreased antioxidant status and an increased oxidation of cellular lipoprotein components (Baynes, 1991). The fact that inhibition of glycosylation and oxidation processes by co-adjuvant therapy could prevent or at least delay the onset and/or the progression of these complications has stimulated current search for drugs with such properties.

The results obtained in this study revealed that the degree of haemoglobin glycosylation increases with the period of incubation with glucose in a concentration – dependent manner up to 20mg glucose/ml where glycosylation appeared to be saturated. This finding is in agreement with earlier reports of Asgary, *et al.*, (1999) in which the amount of glycosylation increased linearly up to 2% glucose. Furthermore, we have shown in this study that the process of glycosylation in red cell occurs slowly thus confirming that the process involves the non-enzymic condensation of two abundant reactants, glucose and haemoglobin. The extent of glycosylation increases with the period of exposure to glucose, glycosylation being maximum after 72hrs of incubation.

Experiments designed to determine the effects of the graded concentrations of flavonoid containing leaf extracts of Cn.f on haemoglobin glycosylation were carried out at varying periods of incubation (24-72hrs) using the level of nmole Hydroxymethylfurfural as an index of measurement. The finding that there were reductions in the haemoglobin glycosylation in comparison with controls at all the concentrations of quercetin and extracts of Cn.f used irrespective of the incubation period (Fig.2), is consistent with the findings of Odetti *et al.*, (1990) that haemoglobin glycosylation was significantly reduced in diabetic rats treated with rutin, a flavonoid. This data further revealed that maximum inhibition up to 75%and 71% occurred in the presence of quercetin and Cn.f respectively, when 30µg/ml of either compound was used. It seems likely from these results that the compounds probably flavonoids in the methanolic extracts of Cn.f and quercetin are acting as antioxidants by interfering with the oxidative process of glycosylation since the glycosylation reaction occurs via intermediates that are free radicals. This is further supported by the result of the evaluation of the radical scavenging properties of the extracts of Cn.f against the DPPH radical. By using the DPPH as a TLC spray reagent, compounds which are polyphenols in the extracts of Cn.f donated electrons or hydrogen to quench the electron mobility in DPPH thus forming complexes observed as yellow spots on the chromatogram (Takao *et al.*, 1994). The prevention of the non-enzymic haemoglobin glycosylation by Cn.f gives the impression that this extract might have an effect even on the process of forming the small proportion (5 – 7 %) of HbA_{1c} that exists in non-diabetic adults.

Experiments were therefore carried out to investigate the effect of the extracts on haemoglobin glycosylation in the presence of physiological concentration of glucose (=1mg/ml). The data obtained showed that quercetin and extracts of Cn.f inhibited human haemoglobin glycosylation insignificantly (7%) in the presence of physiological concentrations of glucose (=1mg/ml), (Tables 1, 2, & 3). Although, the long-term effect of HbA_{1c} component in non-diabetics is not yet clear, this result could be interpreted to mean that if Cn.f is ingested by non-diabetic individuals, the formation of the small proportion of HbA_{1c} that accumulates over a long period of years could be inhibited. However, since the leaf extract used in this study is crude, it seems possible that other components in the crude extract may interfere with the potency or otherwise of the constituent flavonoid. Thus, it has becomes pertinent to purify, characterize and re-evaluate the inhibitory action of the constituent bioactive flavonoids or polyphenolic compounds in the leaf of Cn.f using bioassay guided techniques with a view to using the active principles in the chemotherapy of diabetes mellitus.

In conclusion, it seems reasonable therefore to suggest that ingestion of leaves of *Cnestis ferruginea* may prevent the formation of AGEs in diabetes and thereby possibly reduce or delay the progression of long- term complications seen in the disease condition considering the high polyphenols present in it. The safe dose to be taken will however, depend on the outcome of results of the toxicity studies on the leaves of Cn.f.

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