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International Journal of Current Research
Vol. 7, Issue, 10, pp.21000-21006, October, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

MODULATORY EFFECTS OF *HIBISCUS ROSA-SINENSIS* L. DURING 10% D-GLUCOSE FEEDING IN RATS

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ARTICLE INFO

Article History:
Received 05th July, 2015
Received in revised form
15th August, 2015
Accepted 21st September, 2015
Published online 20th October, 2015

Key words:

Hibiscus rosa-sinensis,
10% D-glucose, Insulin,
Lipids, Glycoproteins.

ABSTRACT

In the present study, the modulatory effect of *Hibiscus rosa-sinensis* flower petals treatment on the levels of plasma glucose, insulin, lipids and glycoproteins in 10% D-glucose feeding for 4 weeks were examined. The *H. rosa-sinensis* flower petals (1000mg/kg of body weight) was mixed with normal chow diet and administrated for 4 weeks as preventive and curative effects. The levels of plasma glucose, insulin, lipids and glycoproteins were increased significantly, whereas the levels of plasma HDL was decreased in 10% D-glucose fed rats. Administration of *H. rosa-sinensis* flower petals reversed these hyperglycemic and hyperlipidemic statuses in 10% D-glucose fed rats. These observed effects were found to positively correlate with the proanthocyanidins and β-carotene content of the *H. rosa-sinensis* flower petals. The present study concludes that the *H. rosa-sinensis* flower petals possess a significant beneficial effect on glucose, insulin, lipid profiles and glycoprotein moiety in addition to its antidiabetic and antiatherosclerotic effects. Thus, the results of the present study indicate a positive role of *H. rosa-sinensis* as a therapeutic agent for diabetes and atherosclerosis in 10% D-glucose fed rats.

***Citation*:** Shanmugham Bhuvana and Vava Mohaideen Hazeena Begum, 2015. "Modulatory effects of *Hibiscus rosa-sinensis* L. during 10% d-glucose feeding in rats", *International Journal of Current Research*, 7, (10), 21000-21006.

INTRODUCTION

Glucose is the main fuel for energy requirement of the body. Therefore, a continuous supply of glucose is necessary to ensure proper function and survival of all organs (Saravanan *et al*., 2002). Acute oral glucose loading can cause an acute oxidant stress in healthy subjects (Ceriello *et al*., 1998). The elevated glucose, a hallmark in all forms of diabetes, may directly contribute to the altered cardiac contractile function in individual ventricular myocytes (Ren *et al*., 2003). Moreover, diabetes mellitus is a well-established risk factor for cardiovascular disease (Grundy *et al*., 1999). Also insulin resistance is a metabolic disorder that is increasing worldwide and plays a role in the pathophysiology of the most common human hypertension, obesity, dislipidemia and coronary heart disease (Aoeli *et al*., 2001). Impaired metabolism of glycoproteins has been proposed as a marker of an acute-phase response in cardiovascular diseases (Pickup and Crook, 1998). The increased glycoprotein levels were associated with an increase in vascular oxidative stress along with the development of metabolic diseases such as obesity, diabetes and atherosclerosis are now known to also play a role in vascular pathophysiology (Crook *et al*., 2003). In the high level of glucose state, glucose is used by the insulin-independent pathways, leading to the synthesis of oligosaccharide moieties of glycoprotein; hexose, hexosamine, fucose and sialic acid have an important role in protein stability, function, and turnover (Begum *et al*., 1978). Raised levels of glycoproteins may also be a predictor of angiopathic complications (Konukoglu *et al*., 1999).

Traditional medicines and herbs would probably open new therapeutic avenues for multi-factorial disease, such as diabetes mellitus, since their complex components often provide versatile bioactivity and varied mechanism of action (Kim *et al*., 2002). Diabetic patients and healthcare professions are increasingly considering complementary and alternative approaches, including the use of medicinal herbs with anti-hyperglycemic activities (Park *et al*., 2005). The study of such medicines might offer a natural key to unlock a diabetologist's pharmacy for the future (Anjali and Manoj, 1995).

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Hibiscus rosa-sinensis L. (Malvaceae) is an evergreen woody, glabrous plant well known for its beautiful flowers. It blossoms almost throughout the year and seldom sets seeds under cultivation (Akhtar *et al*., 1992). The flowers of *H. rosa-sinensis* have been reported in the ancient Indian medicinal literature with beneficial effects in heart diseases (Nadkarni, 1976). The flowers and leaves have been traditionally used to treat conditions such as cancer and gallbladder attacks, to lower blood pressure, to relieve dry coughs, and topically to treat skin afflictions (Duke, 1985). The flowers contain substantial quantities of flavonoids, proantocyanidins (Bruneton, 1999), anthocyanins (Nakamura *et al*., 1990), β-carotene (Chauhan *et al*., 1979) which are associated with antioxidant, fever-reducing (antipyretic), pain-relieving (analgesic), and spasm-inhibiting (spasmolytic) activities (Dafallah and Al-Mustafaz, 1996; Salah *et al*., 2002). Of the many polysaccharides the acidic polysaccharides show the most interesting properties presumably promote wound healing and also immune-modulating (Muller and Franz, 1992; Brunold *et al*., 2004). There is also a high concentration (15 to 30%) of simple organic acids such as citric and malic acids (Bruneton, 1999). In recent times, both experimental and clinical studies have shown that the dried flower powder of *H. rosa-sinensis* has significant protective effects in ischemic heart disease (IHD) (Yamasaki *et al*., 1996) and for diabetes (Sachdewa and Khemani, 2003). *H. rosa-sinensis*, which contains flavonoids and anthocyanosidic polymers as primary organic components, are currently used in the therapy of vascular diseases (Murray and Pizzorno, 1999).

The aim of this study was therefore to determine plasma glucose, lipids and glycoprotein concentrations in 10% D-glucose fed rats which could simulate insulin resistance in type II diabetes mellitus during the administration of *H. rosa-sinensis* flower petals.

MATERIALS AND METHODS

Plant material

The flowers of *H. rosa-sinensis* were collected from the Kuttalam located in Nagapattinum district, Tamilnadu, India, during spring and rainy seasons (Kholkute *et al*., 1977). The botanical identity was authenticated by Dr. M. Jegadeesan, Professor and Head, Department of Environmental and Herbal Sciences, Tamil University, Vakaiyur, Thanjavur, Tamilnadu, India. The specimen was deposited in Tamil University Herbarium (Acc No. 268).

Preparation of H. rosa-sinensis mixed rat chow diet

The flower petals were air dried under shade and pulverized to powder form. The powder was mixed in balanced commercial pellet diet (Hindustan Lever Ltd, Mumbai, India) with a composition of 5% fat, 21% protein, 55% nitrogen-free extract, and 4% fiber (wt/wt) with adequate mineral and vitamin levels for the rat.

Animals

Male albino wistar rats (body weight, 175-200gm body weight) divided into four groups of six each. The animals were maintained under a constant 12 hour light-dark cycle. Diet and water were provided *ad libitum*. All animal experiments were conducted as per the instructions of Institutional Animal Ethics Committee.

Glucose feeding

Experimental rats were treated with 10% D-glucose solution (Midaoui *et al*., 2003) to drink orally for the daytime. Without any treatment was taken as the normal rats. Neither death nor any other adverse effect was observed at the tested concentration throughout the study.

Experimental Design

Group I : Served as normal, given water and normal chow diet.

Group II : Experimental rats given 10% D-glucose to drink at the daytime in addition to a normal chow diet for 4 weeks.

Group III : Experimental rats given to drink 10% D-glucose and simultaneously received with *H. rosa-sinensis* flower petals powder (1000mg/kg body weight) mixed with normal chow diet for 4 weeks at the daytime as preventive.

Group IV : After 4 weeks of 10% D-glucose treated rats received with *H. rosa-sinensis* flower petals powder (1000mg/kg body weight) mixed with normal chow diet for 4 weeks at the daytime as curative.

At the end of experimental period, the rats were deprived of food overnight and sacrificed by cervical dislocation. Blood was collected in tubes containing potassium oxalate and sodium fluoride solution. The plasma was separated for the estimation of glucose, insulin, lipid profiles and glycoproteins.

Biochemical analysis

Determination of plasma glucose and plasma insulin

Plasma glucose was determined by the O-toluidine method by Sasaki *et al.* (1972). Plasma (0.1 ml) was precipitated with 1.9 ml of 10% TCA (tricholoro acetic acid) and centrifuged. Supernatant (1.0 ml) was mixed with 4.0 ml of O-toluidine reagent, kept in a boiling water bath for 15 minutes, and cooled. The absorbance was read at 620 nm. Glucose was expressed as milligrams per deciliter of plasma.

Plasma insulin was estimated using enzyme-linked immunosorbent assay kit (Boehringer, Mannheim, Germany) using human insulin as the standard (Anderson *et al*., 1993).

Determination of lipids

The plasma contents of total cholesterol (Allein *et al*., 1974), triglycerides (Werner *et al*., 1981) and HDL-cholesterol (Allein *et al*., 1974) were determined by using the Boehringer Mannheim Assay Kids and phospholipids were determined by using the method of Zilversmit and Davis (1950) with Fiske and Subbarrow (1925). The levels of LDL cholesterol and VLDL were calculated using Friedewald formula (Friedewald *et al*., 1972).

VLDL-cholesterol = Triglycerides / 5
LDL-cholesterol = Total cholesterol – (HDL+VLDL)
Atherogenic index = LDL/HDL.

Determination of hexose, hexosamine, sialic acid, and fucose

Hexose was estimated by the method of Niebes (1972). Briefly, to 0.5 ml of the plasma, 0.5 ml of 5% phenol and 2.5 ml of concentrated H_2SO_4 were added. For a blank, 0.5 ml of 0.1N NaOH was treated in the same way. The tubes were then heated in a boiling water bath for 20 minutes, and the absorbance was read at 490 nm.

Hexosamine was estimated by the method of Elson and Morgan (1933). To the plasma (0.5 ml/0.1 ml), 2.5 ml of 3N HCl was added and boiled for 6 hours in a boiling water bath and then neutralized with 6N NaOH. To 0.8 ml of the neutralized sample, 0.6 ml of acetyl acetone reagent was added and heated in boiling water for 30 minutes. After cooling, 2 ml of Ehrlich reagent was added and mixed well. The color developed was read at 540 nm.

Sialic acid was estimated by the method of Warren (1959). Briefly, to 0.5 ml of plasma, 0.5 ml of water and 0.25 ml of 0.025-mmol/l periodic acid were added and incubated at 37°C for 30 minutes. To this, 0.2 ml of 4% sodium meta-arsenate and 2 ml of thiobarbituric acid were added, heated in a boiling water bath for exactly 6 minutes, and cooled, and 5 ml of acidified butanol was added. The absorbance was read at 540 nm against the reagent blank.

Fucose was estimated by the method of Dische and Shettles (1948). To 0.5 ml of the plasma, 4.5 ml of H_2SO_4 (6:1 vol/vol in distilled water) was added and heated in a boiling water bath for 3 minutes. The samples were cooled, and 0.1 ml of cysteine hydrochloride reagent was added. For the blank, 0.5 ml of 0.1N NaOH was also treated in the same way. After 75 minutes in dark, the absorbance was read at 393 and 430 nm. The glycoprotein levels were expressed as milligrams per deciliter for plasma.

Statistical analysis

The values are expressed as mean ± standard deviation (SD). The results were computed statistically (Graphpad Instat) using one-way analysis of variance. Post hoc testing was performed for intergroup comparisons using the least significance test.

RESULTS

Table 1 shows the average weight gain by the rats during the experimental period of 4 weeks. The final body weight of 10% D-glucose fed rats (group II) was significantly higher than that of the normal rats (group I). Treatment with *H. rosa-sinensis* flower petals reduced the bodyweight gain significantly.

Fasting plasma glucose levels were determined every other week, and non-fasting plasma glucose and insulin levels were measured at the end of the treatment. A significant hyperglycemia was developed in the 10% D-glucose fed group compared to the normal group for 4 weeks (Table 2). As a result of increased plasma glucose and insulin levels, homeostatic model assessment values for insulin resistance (HOMA-IR), calculated by insulin (μU/ml) x glucose (mM)/22.5 (Pickavance *et al*., 1999), of the 10% D-glucose fed (group II) was higher than that of the normal (group I). The insulin resistance indices of *H. rosa-sinensis* flower petals preventive (group III) and curative (group IV) were significantly reduced, when compared to the 10% D-glucose fed group (group II).

Table 1. Effect of *H. rosa-sinensis* on average weight gain by the animals during the experimental period of 4 weeks

	Initial Weight (gms)	Final weight (gms)
Group I	167.5 ± 11.62	206.3 ± 14.28
Group II	186.3 ± 13.18	258.8 ± 18.69 ^{a***}
Group III	191.3 ± 13.62	213.7 ± 15.57 ^{b***}
Group IV	196.3 ± 13.45	220.3 ± 16.61 ^{b**}

Values are expressed as mean ± SD of six rats. Treatment of groups: Group I: control; group II: 10% D-glucose fed rats; group III: 10% D-glucose fed rats treated simultaneously with *H. rosa-sinensis*; group IV: 10% D-glucose fed rats post-treated with *H. rosa-sinensis*. Comparisons are made: ^{a} with group I; ^{b} with group III. Symbols represent statistical significance: * P< 0.05; ** P< 0.01; *** P< 0.001.

Table 2. Effect of *H. rosa-sinensis* on plasma glucose, insulin and HOMA-IR in normal and experimental rats

	Glucose	Insulin	HOMA-IR
Group I	3.32 ± 0.18	38.48 ± 4.23	5.68 ± 0.82
Group II	5.47 ± 0.22 ^{a***}	78.14 ± 5.81 ^{a***}	19.00 ± 1.49 ^{a***}
Group III	3.41 ± 0.20 ^{b***}	42.33 ± 3.45 ^{b***}	6.40 ± 1.08 ^{b***}
Group IV	4.08 ± 0.16 ^{b***}	51.84 ± 3.89 ^{b***}	9.40 ± 1.16 ^{b***}

Values are expressed as mean ± SD of six rats. Treatment of groups: Group I: control; group II: 10% D-glucose fed rats; group III: 10% D-glucose fed rats treated simultaneously with *H. rosa-sinensis*; group IV: 10% D-glucose fed rats post-treated with *H. rosa-sinensis*. Units: glucose: mmol/l and insulin: μU/ml. Homeostasis Model Assessment was used to calculate an index of insulin resistance as insulin (μU/ml) x glucose (mmol/l)/22.5. Comparisons are made: ^{a} with group I; ^{b} with group III. Symbols represent statistical significance: * P< 0.05; ** P< 0.01; *** P< 0.001.

Table 3. Effect of *H. rosa-sinensis* on plasma total cholesterol, free fatty acids, phospholipids, c/p ratio and atherogenic index in normal and experimental rats

	Group I	Group II	Group III	Group IV
Total cholesterol	117.69 ± 8.56	195.81 ± 14.26 ^{a***}	118.65 ± 8.66 ^{b***}	120.45 ± 8.82 ^{b***}
Free fatty acids	29.86 ± 2.19	55.22 ± 4.72 ^{a***}	30.31 ± 2.17 ^{b***}	29.98 ± 2.35 ^{b***}
Phospholipids	65.74 ± 4.86	92.82 ± 6.78 ^{a***}	71.41 ± 4.82 ^{b***}	67.50 ± 4.79 ^{b***}
C/p ratio	1.78 ± 0.09	2.07 ± 0.15 ^{a**}	1.70 ± 0.14 ^{b***}	1.80 ± 0.11 ^{b**}
Atherogenic index	1.74 ± 0.12	4.89 ± 0.42 ^{a***}	1.87 ± 0.12 ^{b***}	2.03 ± 0.13 ^{b***}

Values are expressed as mean ± SD of six rats. Treatment of groups: Group I: control; group II: 10% D-glucose fed rats; group III: 10% D-glucose fed rats treated simultaneously with *H. rosa-sinensis*; group IV: 10% D-glucose fed rats post-treated with *H. rosa-sinensis*. Units: total cholesterol; free fatty acids and phospholipids: mg/dl. Comparisons are made: ^{a} with group I; ^{b} with group III. Symbols represent statistical significance: * P< 0.05; ** P< 0.01; *** P< 0.001.

Table 3 shows the plasma concentration of total cholesterol, free fatty acids, phospholipids, cholesterol/phospholipid ratio and the atherogenic index. The concentrations of total cholesterol, phospholipids and free fatty acids were

significantly higher in 10% D-glucose fed rats (group II) as compared to normal rats (group I). Administration of *H. rosa-sinensis* flower petals to preventive (group III) and curative (group IV) rats were significantly lower the levels as compared to 10% D-glucose fed rats (group II).

Table 4. Effect of *H. rosa-sinensis* on plasma triglycerides and lipoprotein in normal and experimental rats

	Group I	Group II	Group III	Group IV
Triglycerides	51.93 ± 3.68	65.49 ± 4.87 ^{a***}	52.46 ± 3.76 ^{b***}	54.87 ± 3.81 ^{b**}
HDL	67.59 ± 4.67	39.70 ± 2.86 ^{a***}	61.55 ± 4.45 ^{b***}	59.60 ± 4.39 ^{b***}
LDL	39.70 ± 2.87	143.00 ± 10.29 ^{a***}	46.70 ± 3.36 ^{b***}	49.70 ± 3.62 ^{b***}
VLDL	10.38 ± 0.77	13.09 ± 1.03 ^{a***}	10.46 ± 0.84 ^{b***}	11.23 ± 0.78 ^{b**}

Values are expressed as mean ± SD of six rats. Treatment of groups: Group I: control; group II: 10% D-glucose fed rats; group III: 10% D-glucose fed rats treated simultaneously with *H. rosa-sinensis*; group IV: 10% D-glucose fed rats post-treated with *H. rosa-sinensis*. Units: triglycerides, HDL, LDL and VLDL: mg/dl. Comparisons are made: ^{a} with group I; ^{b} with group III. Symbols represent statistical significance: * P< 0.05; ** P< 0.01; *** P< 0.001.

Table 4 indicates the concentrations of plasma triglycerides and lipoproteins. There were significantly higher concentrations of plasma triglycerides, VLDL and LDL in 10% D-glucose fed rats (group II) as compared to normal rats (group I). The HDL concentration was significantly lower in 10% D-glucose fed rats as compared to the normal rats. It was significantly higher in rats treated with *H. rosa-sinensis* flower petals (group III and IV) as compared to those fed with 10% D-glucose (group II).

Table 5. Effect of *H. rosa-sinensis* on plasma total sialic acid, hexose, hexosamine and fucose in normal and experimental rats

	Group I	Group II	Group III	Group IV
Sialic acid	54.47 ± 3.79	71.82 ± 5.38 ^{a***}	56.88 ± 4.23 ^{b***}	60.43 ± 4.34 ^{b***}
Hexose	86.44 ± 6.31	126.82 ± 9.41 ^{a***}	94.11 ± 6.77 ^{b***}	100.86 ± 7.38 ^{b***}
Hexosamine	74.16 ± 5.42	100.74 ± 7.92 ^{a***}	80.63 ± 5.67 ^{b***}	86.93 ± 6.23 ^{b**}
Filose	28.41 ± 2.20	44.87 ± 3.98 ^{a***}	33.86 ± 2.36 ^{b***}	36.17 ± 2.56 ^{b***}

Values are expressed as mean ± SD of six rats. Treatment of groups: Group I: control; group II: 10% D-glucose fed rats; group III: 10% D-glucose fed rats treated simultaneously with *H. rosa-sinensis*; group IV: 10% D-glucose fed rats post-treated with *H. rosa-sinensis*. Units: total sialic acid, hexose, hexosamine and fucose: mg/dl. Comparisons are made: ^{a} with group I; ^{b} with group III. Symbols represent statistical significance: * P< 0.05; ** P< 0.01; *** P< 0.001

Table 5 shows the levels of plasma glycoproteins in normal and experimental rats. In 10% D-glucose fed rats (group II), plasma levels of sialic acid, hexose, hexosamine and fucose were significantly increased as compared to the normal rats (group 1). Administration of *H. rosa-sinensis* flower petals (group III and IV) significantly reversed the changes in plasma glycoproteins levels as compared to the 10% D-glucose fed rats.

DISCUSSION

In the present study, 10% D-glucose fed rats showed a significantly higher bodyweight gain than the normal rats, as expected but there were no preventive and curative supplementation with *H. rosa-sinensis* flower petals. The relationship between body weight and the risk of type II diabetes is successive and graded (Colditz *et al*., 1990). As a result of feeding 10% D-glucose for 4 weeks could develop a prediabetic state associated with overweight, hyperglycemia, hyperinsulinemia, insulin resistance and dyslipidemia. It is energy intake that matters in relation to the development of overweight (Astrup, 2001), and energy intake is often high when 10% D-glucose fed rats are consumed in large amounts.

In the present study, the levels of plasma glucose and insulin were significantly increased in 10% D-glucose induced diabetic rats. Continuous intake of 10% D-glucose was increased the hyperglycemia and insulin resistance. The resistance of insulin was expressed as HOMA-IR that also increased in the 10% D-glucose fed rats. It may be due to the reduced glucose oxidation that leads to hyperlipidemia that causing accumulation and increased synthesis of cholesterol, triglycerides, lipoproteins and free fatty acids. The capacity of an antidiabetic drug such as *H. rosa-sinensis* flower petals to decrease the elevated blood glucose to a normal level is an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes. However, *H. rosa-sinensis* flower petals treated rats inhibited the subsequent development of obesity and hyperglycemia in spite of continued access to the 10% D-glucose fed rats. The possible mechanism for the observed anti-hyerglycemic effect of *H. rosa-sinensis* flower petals may be due to the enhanced insulin sensitivity, increased the ability of insulin to mediate tissue glucose uptake, and was helpful to maintain glucose homeostasis and clear the postprandial glucose load. However, the effect of ameliorating insulin resistance of *H. rosa-sinensis* flower petals was reduces hyperinsulinemia and improves hepatic insulin resistance. Our findings coincide with those of earlier studies, which reported that the *H. rosa-sinensis* leaf extract showed hypoglycemic activity in albino rats (Sachdewa and Khemani, 1999) and also number of plants reduced the blood glucose levels in diabetic rats (Murakami *et al*., 1996; Fushiki *et al*., 1992).

The present study showed that consumption of 10% D-glucose fed rats led to significantly higher concentrations of plasma lipids than in the normal rats. This high cholesterol concentration in circulation may damage the endothelial cell membrane lining the large arteries and aorta, and may be an initial event in the aetiology of atherosclerotic rats (Mitchell *et al*., 1991). In the present study, treatment with *H. rosa-sinensis* flower petals to 10% D-glucose fed rats reduced the elevated concentrations of total cholesterol in the plasma as compared to normal rats. Previous studies of *H. rosa-sinensis* have shown a significant lowering cholesterol levels in animals (Saadany *et al*., 1991). An elevated total cholesterol-phospholipid (C/p) ratio is directly related to a high incidence of atherosclerosis (Brown and Goldstein, 1983). The reduced concentrations of phospholipids may also be due to enhanced activity of phospholipases. Increased circulation of free fatty acids might lead to insulin resistance, and ultimately to diabetes mellitus in genetically prone subjects by mechanism of lipotoxicity (Manco *et al*., 2004). Increased triglycerides store and lipolysis in adiopocytes were caused by hyperinsulinemia (Manco *et al*., 2004). Because the repressed plasma insulin levels via improvement of insulin resistance by *H. rosa-sinensis* flower petals treatment decreased the triglyceride store. Plasma triglyceride concentration was significantly reduced by *H. rosa-sinensis* flower petals administration, and these effects ameliorated insulin resistance.

VLDL and LDL, both these lipoproteins have a positive role in atherosclerosis (Ross, 1999). These lipoproteins are chemically modified by oxidation or glycation in the initial stages of atheroma formation. These oxidized or modified lipoproteins do not react with LDL receptors, leading to esterification of cholesterol and conversion of macrophages to foam cells, thereby contributing to the development of atherosclerosis (Parthasarathy *et al*., 1989). In the present study, the plasma VLDL and LDL concentrations were significantly higher in 10% D-glucose fed rats than in normal rats, but not in those preventive and curative treatments with *H. rosa-sinensis* flower petals. HDL is considered to be a beneficial lipoprotein (Miller *et al*., 1977), and has a negative effect in the development of atherosclerosis. It helps in the scavenging of cholesterol from the extrahepatic tissues in the presence of lecithin cholesterol acyl transferase (LCAT) and brings it to the liver. In the present study, the plasma HDL concentration was significantly lower in 10% D-glucose fed rats than in normal rats, whereas it was similar to that in the normal rats, on supplementation with *H. rosa-sinensis* flower petals. The observed hypocholesterolaemic effect of *H. rosa-sinensis* flower petals in the present study is probably due to the presence of the plant compounds like proanthocyanidins. In a different hypercholesterolamic model, proanthocyanidins were significantly lowered the amounts of cholesterol bound to aortic elastin compared to controls (Wegrowski *et al*., 1984).

Glycoproteins found in a variety of tissues including the arterial wall are very similar in structure and composition to those in plasma (Radhakrishnamoorthy and Berenson, 1973). Generalized abnormalities in glycoprotein metabolism are observed in both naturally occurring and experimental disease models (Pari and Ashokkumar, 2006). In the present study, the glycoproteins such as sialic acid, hexose, hexosamine and fucose concentrations were significantly higher in 10% D-glucose fed rats when compared with normal rats. Previous reports suggest that plasma concentrations of glycoproteins are significantly increased in various diseases such as diabetes mellitus, etc. (Latha and Pari, 2005; Prakasam *et al*., 2005; Pari and Ashok kumar, 2006). It may be due to the increased availability of glucose in the hyperglycemic state that accelerates the synthesis of basement-membrane components, that is glycoproteins (McMillan, 1972). This is because of depressed use of glucose by insulin-dependent pathways, thereby enhancing the formation of hexose, hexosamine and fucose for the accumulation of glycoproteins (Patti *et al*., 1999). This increase in plasma glycoproteins has been reported to be associated with the severity and duration of diseases. It is well established that vascular endothelium carries a high level of sialic acid (Born and Palinski, 1985), and the vascular damage leads to its release into the circulations. A relationship between serum sialic acid and microvascular complications has been observed (Yokoyama *et al*., 1995). The vascular permeability is regulated by sialic acid moieties, with increased vascular permeability resulting from the shedding of vascular endothelial sialic acid into the circulation (Born and Palinski, 1985). The finding of our observation is also in suggestive of the above statement, which showed increased plasma glycoprotein levels in 10% D-glucose fed rats.

Administration of *H. rosa-sinensis* flower petals to 10% D-glucose fed rats significantly reduced the glycoproteins levels to near normal levels. Therapeutic treatment with *H. rosa-sinensis* flower petals signifies its efficacy as antidiabetic and antiatherosclerotic activity. The antihyperglycemic action of *H. rosa-sinensis* flower petals, which is mediated via an enhancement of insulin action, as evidenced by the increased level of insulin in *H. rosa-sinensis* flower petals treated 10% D-glucose fed rats, may be responsible for the reversal of glycoprotein changes associated with high glucose levels. In recent times, many traditionally important medicinal plants have been tested for their efficacy against impaired glycoprotein levels in high glucose rats (Prakasam *et al*., 2005). *H. rosa-sinensis* flower petals was found to be rich in flavonoids, proanthocyanidins, anthocyanins (Nakamura *et al*., 1990), β-carotene (Chauhan *et al*., 1979). Flavonoids being polyphenolic in nature, it acts as antioxidants like vitamin E. So, this may also play a role in the modulation of glycoprotein components in 10% D-glucose fed rats.

From the results of the present study, it could be inferred that the phytoactive compounds such as flavonoids, proanthocyanidins, anthocyanins and β-carotene rich *H. rosa-sinensis* elicited definite beneficial effect by modulating the high glucose induced diabetes specific glycoconjucates in 10% D-glucose fed rats and thus possibly promotes to be an antidiabetic and antiatherosclerotic agents.

REFERENCES

Akhtar, H., Virmani, O.P., Popli, S.P., Misra, L.N., Gupta, M.H., Srivastava, G.N., Abraham Z., Singh, A.K., 1992. Dictionary of Indian Medicinal Plants, CIMAP, Luknow, India, p: 242.

Allein, C.C., Poon, L.S., Chan, C.S.G., Richmond W., Fu, P.C., 1974. Enzymatic determination of total serum cholesterol. *Clin. Chem*., 20, 470-475.

Anderson L., Dinesen, B., Jorgensen, P.N., Poulsen F., Roder, M.F., 1993. Enzyme immunoassay for intact human insulin in serum or plasma. *Clin. Chem*., 38, 578-582.

Anjali, P., Manoj, K.M., 1995. Some comments on diabetes and herbal therapy. Anc. Sci. Life 15, 27-29.

Aoeli, K., Taghibiglu, C.H., Van Iderstine, S.C., Lewis, G.F., 2001. Mechanism of hepatic very low-density lipoprotein over production in insulin resistance. *Trends Cardiovasc. Med.,* 11, 170-176.

Astrup, A., 2001. Healthy lifestyles in Europe : prevention of obesity and type II diabetes by diet and physical activity. *Pub. Healt. Nutr*., 4, 499-515.

Begum, N., Moses, G.P.S., Shanmugasundaram, K.R., 1978. Protein bound polysaccharides in human and experimental diabetes. *Arogya J. Health Sci*., 4, 140-144.

Born, G.V., Palinski, W., 1985. Unusually high concentration of sialic acid on the surface of vascular endothelium. *Br. J. Exp. Pathol*., 66, 543-549.

Brown, M.S., Goldstein, J. L., 1983. Lipoprotein receptors in the liver control signals for plasma chotesterol traffic. *J. Clin. Invest*., 72, 743-747.

Bruneton, J., 1999. Pharmacognosy, Phytochemistry, Medicinal Plants, second ed. Intercept, London, p. 24.

Brunold, C., Deters A., Knoepfel-Sidler, F., 2004. Polysaccharides from *Hibiscus sabdariffa* flowers stimulate proliferation and differentiation of human keratinocytes. *Planta Med*., 70, 370-373.

Ceriello, A., Bortolotti, N., Crescentini, A., 1998. Antioxidant defences are reduced during the oral glucose tolerance test in normal and noninsulin-dependent diabetic subjects. *Eur. J. Clin. Invest*., 28, 329-333.

Chauhan, J.S., Vidyapati, T.J., Gupta, A.K., 1979. A new flavonone glycoside from the stem of *Hibiscus mutabolis*. *Phytochemistry*, 18, 1766.

Colditz, G.A., Willett, W.C., Stampfer, M.J., Manson, J.E., Hennekens, C.H., Arky, R.A., Speizer, F.E., 1990. Weight as a risk factor for clinical diabetes in women. *Am. J. Epidemiol*., 132, 501-513.

Crook, M.A., Miell, J., Ameerally, P., Singh, N., Russell-Jones, D., 2003. Serum sialic acid, a reputed cardiovascular risk factor is related to serum leptin concentrations in Fijians. *Clin. Chim. Acta*., 331, 1-5.

Dafallah, A.A., Al-Mustafaz., 1996. Invertigation of the anti-inflammatory activity of *Acacia nilotica* and *Hibiscus sabdariffa*. *Am. J. Clin. Med*., 24, 263-269.

Dische, Z., Shettles, L.B., 1948. A specific color reaction of methyl pentoses and a spectrophotometric micro method for their determination. *J. Biol. Chem*., 175, 596-603.

Duke, J.A., 1985. Handbook of Medicinal Herbs. CRC Press, Bacaraton, Florida, pp. 228-289.

Elson, L.A., Morgan, W.T.J., 1933. A colorimetric method for the determination of glucosamine and chondrosamine. *Biochem. J*., 27, 1824-1828.

Fiske, C.H., Subbarrow, 1925. The colorimetric determination of phosphorus. *J. Biol. Chem*., 66, 375-400.

Friedewald, W.T., Levy, R.I., Fredrickson, D.S., 1972. Estimation of concentration of low-density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. *Clin. Chem*., 18, 439-502.

Fushiki, T., Kojima, A., Inoue, K., Sugimoto, E., 1992. An extract of from *Gymnema sylvestere* leaves and purified gymnemic acid inhibits glucose stimulated gastric inhibitory peptide secretion in rats. *J. Nutr*., 122, 2367-2373.

Grundy, S.M., Benjamin, R.J., Burke, G.L., 1999. Diabetes and cardiovascular disease: a statement for healthcare professional from the American Heart Association. Circulation 100, 1134-1146.

Kholkute, S.D., Mudgal, V., Udupa, K.N., 1977. Studies on the antifertility potentiality of *Hibiscus rosasinensis* (Parts of medicinal value, selection of species and seasonal variations). *Plants Med*., 31, 35.

Kim, Y.Y., Kang, H.J., Ko, S.K., Chung, S.H., 2002. Sopungsungiwon (sp) prevents the onset of hyperglycemia and hyperlipidemia in zucker diabetics fatty rats. *Arch. Pharmacol. Res*., 25, 923-931.

Konukoglu, D., Serin, O., Akcay, T., Hatemi, A., 1999. Relationship between diabetic angiopathic complications and serum total and lipid associated sialic acid concentrations. *Med. Sci. Res*., 27, 53-55.

Latha, M., Pari, L., 2005. Effect of aqueous extract of *Scoparia dulais* on plasma and issue glycoproteins in streptozotocin induced diabetic rats. Pharmazie 60, 151-154.

Manco, M., Calvani, M., Mingrone, G., 2004. Effects of dietary fatty acids on insulin sensitivity and secretion. *Diabet. Obes. Met*., 6, 402-413.

McMillan, D.E., 1972. Elevation of glycoprotein fucose in diabetes mellitus. Diabetes 21, 867.

Midaoui, A.E., Elimadi, A., Wu, L., Haddad, P.S., De Champlain, J., 2003. Lipoic acid prevents hypertension, hyperglycemia, and the increase in heart mitochondrial superoxide production. *Am. J. Hypertens*., 16, 173-179.

Miller, N.E., Thelle, D.S., Forde, O.H., Mjos, O.D., 1977. The Thromso Heart Study. HDL and coronary heart disease: a prospective case control study. *Lancet*, 965-967.

Mitchell, D.S.L., Porman, J.A., Smith, S.A., Overturf, M.L., Morrisett, J.D., Gotto Jr, A.M., 1991. Cholesterol metabolism in hypercholesterolemia resistant rabbits. Atherosclerosis 87, 169-181.

Muller, B.M., Franz, G., 1992. Chemical structure and biological activity of polysaccharides from *Hibiscus Sabdariffa*. *Planta Med*., 58, 60-67.

Murakami, N., Murakami, T., Kadoya, M., Matsuda, H., Yamahera, J., Yoshikawa, M., 1996. New hypoglycaemic constituents in "gymnemic acid" from a *Gymnema sylvestere Chem. Pharm. Bull*., 44, 469-471.

Murray, M., Pizzorno, J., 1999. Procyanidolic obligomers. In: Murray, M., Pizzorno, J., (Eds.), The Text book of Natural Medicine. Second ed. Churchill Livingston, London, pp. 899-902.

Nadkarni, A.K., 1976. Indian Materia Medica, Popular Prakashan Company, Bombay, India, 1, 1199.

Nakamura, Y., Hidaka, M., Masaki, H., Seto, H., Uozumi, T., 1990. Major anthocyanin of the flowers of Hibiscus (*Hibiscus rosa sinensis* L.). Agri. Biol. Chem. 54, 3345.

Niebes, P., 1972. Determination of enzymes and degradation products of glycosaminoglycans metabolism in the serum of healthy and varicose subjects. *Clin. Chim. Acta*., 42, 399-408.

Pari, L., Ashokkumar, N., 2006. Glycoprotein change in non-insulin dependent diabetic rats: effects of N-benzoyl-D-phenylalanine and metformin. *Therapie*, 61, 125-131.

Park, S.H., Ko, S.K., Chung, S.H., 2005. Euonymis alatus prevents the hyperglycemia and hyperlipidemia induced by high – fat diet in ICR mice. *J. Ethnopharmacol*., 102, 326-335.

Parthasarathy, S., Quinn, M.T., Schwenke, D.C., 1989. Oxidative modification of beta very low density lipoprotein potential role in monocyte recruitment and foam cell formation. Arteriosclerosis 9, 393-399.

Patti, M.E., Virkamai, A., Landaker, E.J., Kahn, C.R., Jarvinen, H., 1999. Activation of the hexosamine pathway by glucosamine in vivo induces insulin signaling events in skeletal muscles. Diabetes 48, 1562-1571.

Pickavance, L.C., Tadayyon, M., Widdowson, P.S., Buckingham, R.E., Wilding, J.P., 1999. Therapeutic index for rosiglitazone in dietary obese rats: separation of efficacy and haemodilution. *Br. J. Pharmacol*., 128, 1570-1576.

Pickup, J.C., Crook, M.A., 1998. Is type 11 diabetes mellitus a disease of the innate immune system? Diabelogia 41, 1241-1248.

Prakasam, A., Sethupathi, S., Pugalendi, K.V., 2005. Influence of *Casearta esculenta* root extract on glycoprotein

components in streptozotozin diabetic rats. Pharmazie 60, 229-232.

Radhakrishnamoorthy, B., Berenson, G.S., 1973. Structure of glycopeptides from a glycoprotein from bovine aorta. *J. Biol. Chem*., 248, 2000-2010.

Ren, J., Duan, J., Hintz, K.K., Ren, B.H., 2003. High glucose induces cardiac insulin-like growth factor I resistance in ventricular myocytes: role of AKT and ERK activation. *Cardiovas. Res*., 57, 738-748.

Ross, R., 1999. Mechanisms of disease. *N. Engl. J. Med*., 340, 115-126.

Saadany El, S.S., Sitohy, M.Z., Labib, S.M., El-Massry, R.A., 1991. Biochemical dynamics and hypocholesterolemic action of *Hibiscus sabbariffa* (Karkade). *Nahrung.,* 35, 567-576.

Sachdewa, A., Khemani, L.D., 1999. A preliminary investigation of the possible hypoglycemic activity of *Hibiscus rosa sinensis*. *Biomed. Environ. Sci*., 12, 222.

Sachdewa, A., Khemani, L.D., 2003. Effect of *Hibiscus rosa sinensis* Linn. ethanol flower extract on blood glucose and lipid profile in streptozotocin induced diabetes in rats. *J. Ethnopharmacol*., 89, 61-66.

Salah, A.M., Gothumbi, J., Viesling, W., 2002. Inhibition of intestinal motility by methanol extracts of *Hibiscus sabdariffa* flowers stimulate proliferation and differentiation of human keratinotes. *Planta Med*., 70, 370-373.

Saravanan, G., Pari, L., Venkateswaran, S., 2002. Effect of cogent db, a herbal drug, on plasma insulin and hepatic enzymes of glucose metabolism in experimental diabetes. *Diab. Obes. Metabol*., 4, 394-398.

Sasaki, T., Masty, S., Sonae, A., 1972. Effect of acetic acid concentration on the color reaction in the O-toluidine boric acid method for blood glucose estimation. Rinsho Kagaku. 1, 346-353.

Warren, L., 1959. The thiobarbituric acid assay of sialic acids. J. Biol. Chem. 234, 1971-1975.

Wegrowski, J., Robert, A.M., Moczar, M., 1984. The effect of procynidolic oligomers on the composition of normal and hypercholesterlemic rabbit aortas. *Biochem. Pharmacol*., 33, 3491-3497.

Werner, M., Gabrielson, D.G., Eastman, G., 1981. Ultramicro determination of serum triglycerides by bioluminescent assay. *Clin. Chem*., 27, 268-271.

Yamasaki, H., Usefuji, H., Sakihama, Y., 1996. Stress proteins and myocardial protection. *Arch. Biochem. Biophys*., 332, 183-186.

Yokoyama, H., Jensen, J.S., Jensen, T., Deckert, T., 1995. Serum sialic acid concentration is elevated in IDDM especially in early diabetic nephropathy. *J. Intern. Med*., 237, 519-523.

Zilversmit, D.B., Davis, A.K., 1950. Micro determination of plasma phospholipids by TCA precipitation. J. *Lab. Clin. Med.*, 35, 155-159.

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