Original Article

In Vivo Study Of Phospholipid Naringenin Complex In Diabetes Treatment

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DOI: 10.47750/pnr.2022.13.S10.439

Abstract

Diabetes mellitus is an endocrinological and metabolic disorder with an increasing global prevalence and incidence. Many medicinal plants have been provided a potential source of anti-diabetic principles and are widely used for the treatment of diabetes mellitus. Naringenin a flavonoid Several biological activities have been ascribed to this phytochemical, among them antioxidant, antitumor, antiviral, antibacterial, anti-inflammatory, antiadipogenic and cardioprotective effects. In addition, naringenin potentiates intracellular signalling responses to low insulin doses by sensitizing hepatocytes to ins Collulin. Thus this study evaluates the effect of Phospholipid complex of naringenin on various clinical parameters. Results showed that the Naringenin Phospholipid Complex at dose of (200 mg/kg) found to have body weight of 234.7±7.84 at 21 day. Also, the blood glucose level was observed to be 189.8±5.09 mg/dl for Naringenin PC complex. According to lipid profile outcomes the CHL, HDL, TG, DL were found to be 169.2±3.43mg/dl, 42.56±2.87 mg/dl, 166.7±3.60 mg/dl, 89.28±2.99mg/dl respectively for Polyherbal preparation (500 mg/kg). A significant fall in level of total cholesterol, triglycerides, LDL and VLDL in comparison to diabetic control was observed. However, HDL level was found to be increased as compared with untreated diabetic rats. In conclusion the Phospholipid naringenin complex have potential for treating diabetes.

Keywords: Diabetes, CHL, HDL, TG, DL, Naringenin, Herbal medicine, Phospholipid complex

INTRODUCTION

Diabetes mellitus is an endocrinological and metabolic disorder with an increasing global prevalence and incidence. High blood glucose levels are symptomatic of diabetes mellitus as a consequence of inadequate pancreatic insulin secretion or poor insulin-directed mobilization of glucose by target cells. conventional drugs treat diabetes by improving insulin sensitivity, increasing insulin production and/or decreasing the amount of glucose in blood. In addition to adverse effects, drug treatments are not always satisfactory in maintaining normal level of blood glucose and avoiding late stage diabetic consequences. (Blair, 2016; Ramachandran *et al*., 2010)

However, many medicinal plants have been provided a potential source of anti-diabetic principles and are widely used for the treatment of diabetes mellitus in various traditional systems of medicine worldwide and many of them are known to be effective against diabetes. Several scientists are reporting the hypoglycemic effects of pharmacologically active components of plants in diabetes patients (Rother, 2007; Kavishankar et al., 2011

Naringenin a flavonoid belonging to flavanones subclass, is widely distributed in several *Citrus* fruits, bergamot, tomatoes and other fruits, being also found in its glycosides form (mainly naringin). Several biological activities have been ascribed to this phytochemical, among them antioxidant, antitumor, antiviral, antibacterial, anti-inflammatory, antiadipogenic and cardioprotective effects. Nonetheless, most of the data reported have been obtained from *in vitro* or *in vivo* studies. (Kooti *et al*, 2016; Zeng *et al*., 2018).

It has also been reported to have a great ability to modulate signalling pathways related to fatty acids metabolism, which favours fatty acids oxidation, impairs lipid accumulation in liver and thereby prevents fatty liver, besides efficiently impairing plasma lipids and lipoproteins accumulation. In addition, naringenin potentiates intracellular signalling responses to low insulin doses by sensitizing hepatocytes to insulin, besides being able to traverse the blood–brain barrier and to exert diverse neuronal effects, through its ability to interact with protein kinase C signalling pathways (Felgines,2000; Kiran *et al*., 2017).

Although there is a huge amount of data on *in vitro* biological effects of naringenin, only few clinical studies have been carried out, mainly because of the reduced data on pharmacokinetic aspects, metabolic fate and chemical instability of this compound. Thus, this study analyses the phospholipid naringenin complex with respect to various pathological parameters playing essential role in diabetes (Gnananath,2017).

MATERIAL AND METHODS

Diabetes was induced in animals by a single intraperitoneal injection of a freshly prepared Streptozotocin (STZ). STZ solution of 10 mg/ ml was prepared in ice-cold citrate buffer 0.1 M, pH 4.5 kept in ice and was administered at a dose of 60mg/kg body weight on day 1st. Treatment was given after diabetes induction (day 3rd) for 21 days.

Table 1: Grouping and dosing: Animals were divided into five groups containing six animals in each.

Group	Dosing and treatment
I	Normal control (vehicle only, 1ml/100gm)
II	Diabetic control, Streptozotocin 60g/kg, i.p.
III	Standard, Diabetic rats were treated with Glibenclamide 0.25 mg/kg once daily for 21 days
IV	Diabetic rats were treated with Polyherbal preparation at 250 mg/kg once daily for 21 days.
V	Diabetic rats were treated with Polyherbal preparation at 500 mg/kg once daily for 21 days.

Physiological Parameters
Body weight of animals were measured using animal weighing balance.

Samples collection and storage
At the end of the experimental, animals were anaesthetized with intraperitoneal injection of Ketamine (50 mg/Kg i.p.) and blood was collected from retro-orbital puncture in blank (for serum) and EDTA containing apendorff tube (for plasma). The one drop of blood samples was immediately spread on the marked end of the gluco-strip. After few seconds the gluco-meter was display the blood glucose level. Serum and plasma were obtained by blood centrifugation at 3000rpm for 15 min. Animals were then sacrificed and pancreas were collected in 10% formalin for histopathology. All biological samples were store at −20 ◦C until analysis.

Serum analysis for Lipid profile:
Autospan Diagnostics kits were used for estimation of Total-Cholesterol, Cholesterol-HDL and Triglyceride.

Estimation of Total-Cholesterol
Cholesterol esters are hydrolysed by Cholesterol Esterase (CE) to give free cholesterol and fatty acids. In subsequent reaction, Cholestrol Oxidase (CHOD) oxidises the 3-oh group of free cholesterol to liberate Cholest-4-en-one and hydrogen peroxide. In presence of peroxidase (POD), Hydrogen Peroxide couples with 4-aminoantipyrine (4-AAP) and Phenol to produce Red Quinoneimine dye. Absorbance of coloured dye is measured at 505 nm and is proportional to amount of Total-Cholesterol concentration in the sample (Abell *et al*.,1952).

Estimation of HDL-Cholesterol
Low Density Lipoprotein (LDL) Cholestrol, Very Low-density Lipoprotein (VLDL) Cholesterol and Chylomicron fraction are precipitated by addition of Polyethylene Glycol 6000 (PEG). After centrifugation, the High-density Lipoproteins (HDL) fraction remains in the supernatant and is determined with CHOD-POD method. HDL cholesterol can be calculated by formula:
HDL-Cholesterol concentration (mg/dl) = Abs of test/Abs of standard ×50 × 2*

Estimation of Triglycerides
Triglycerides are estimated using accurate Triglycerides kit of Span Diagnostics Pvt. Ltd. Accurately triglycerides estimation kit is formulated using GPO and peroxide for quantitative estimation of serum triglycerides. This method is more specific due to use of lipase to liberate glycerol which is estimated (Ahmadi *et al*., 2008).

Statistical analysis
All the values are expressed as mean±standard error of mean (S.E.M.) and analyzed for ANOVA and posthoc Tukey-Kramer Multiple Comparisons Test by employing statistical software, GraphPad InStat 9. Differences between groups were considered significant at $P < 0.05$ levels.

Results & discussion
The Naringenin Phospholipid Complex at dose of (200 mg/kg) found to have body weight of 234.7±7.84 at 21 day. Also, the blood glucose level was observed to be 189.8±5.09 mg/dl for Naringenin PC complex. According to lipid profile outcomes the CHL, HDL, TG, DL were found to be 169.2±3.43mg/dl, 42.56±2.87 mg/dl, 166.7±3.60 mg/dl, 89.28±2.99mg/dl respectively for Polyherbal preparation (500 mg/kg). A significant fall in level of total cholesterol, triglycerides, LDL and VLDL in comparison to diabetic control was observed. However, HDL level was found to be increased as compared with untreated diabetic rats.

Table 2: Effect of Naringenin-PC on body weight in Streptozotocin induced diabetes in rats.

Groups	Treatment	Body weight (gm) (mean±SEM)			
		Initial	Day 7	Day 14	Day 21
I	Normal Control	231.5±5.36	233.7±5.66	234.2±5.77	235.5±5.38

II	Diabetic control	231.9±6.22	230.2±6.59	230.0±6.58	229.3±6.38
III	Standard (Glibenclamide 0.25 mg/kg)	233.6±4.28	235.8±4.58	234.9±4.63	235.4±4.76
IV	Phospholipid Complex (100 mg/kg)	232.0±3.24	230.8±3.52	234.7±3.44	232.8±3.89
V	Phospholipid Complex (200 mg/kg)	232.9±7.56	229.4±7.22	233.5±7.74	234.7±7.84

Table 3: Effect of Naringenin-PC on blood glucose level in Streptozotocin induced diabetes in rats.

Groups	**Treatment**	**Blood Glucose Level (mg/dl)**			
		Day 3	**Day 7**	**Day 14**	**Day 21**
I	Normal Control (NC)	112.4±5.37	109.3±8.76	113.5±8.69	109.5±7.35
II	Diabetic control	210.0±7.07^{a***}	275.7±10.74 ^{a***}	316.6±8.41 ^{a***}	300.7±6.50 ^{a***}
III	Standard (Glibenclamide 0.25 mg/kg)	218.6±8.75 ^{a***}	163.15±6.29 $^{a***, b***}$	148.1±5.79 $^{a***, b***}$	141.1±4.66 $^{a***, b***}$
IV	PC 100 mg/kg	210.0±8.84 ^{a***}	223.15±8.69 $^{a***, b***, c***}$	212.9±8.90 $^{a***, b***, c***}$	203.5±7.42 $^{a***, b***, c***}$
V	PC 200 mg/kg	217.0±8.54 ^{a***}	223.0±8.20 $^{a***, b***, c***}$	194.9±4.80 $^{a***, b***, c***}$	189.8±5.09 $^{a***, b***, c***}$

Values are mean ± SEM from a group of six animals. *p<0.05, **p<0.01 and***p<0.001

a- Significance difference as compare to normal control group

b- Significance difference as compare to Diabetic control group

c- Significance difference as compare to standard treated group

Table 4: Effect of Naringenin phospholipid complex on lipid profile in Streptozotocin induced diabetes in rats.

Groups	**Treatment**	**Lipid Profile**			
		CHL (mg/dl)	**HDL (mg/dl)**	**TG (mg/dl)**	**LDL (mg/dl)**
I	Normal Control (NC)	157.5±5.23	48.4±4.39	141.5±3.11	61.6±2.56
II	Diabetic control	183.4±4.38^{a***}	33.18±2.71 ^{a***}	183.5±2.96 ^{a***}	119.5±2.54 ^{a***}
III	Standard (Glibenclamide 0.25 mg/kg)	164.6±3.94$^{a**, b***}$	47.71±3.64 ^{b**}	164.1±3.74 $^{a***, b***}$	86.6±2.05 $^{a**, b***}$
IV	Polyherbal preparation (250 mg/kg)	169.0±3.52 $^{a***, b**}$	43.56±2.63 ^{b*}	169.9±3.32 $^{a***, b**}$	93.08±1.81 $^{a***, b***, c*}$
V	Polyherbal preparation (500 mg/kg)	169.2±3.43 $^{a***, b**}$	42.56±2.87	166.7±3.60 $^{a***, b***}$	89.28±2.99 $^{a***, b***, c**}$

Values are mean ± SEM from a group of six animals. *p<0.05, **p<0.01 and***p<0.001

a- Significance difference as compare to normal control group

b- Significance difference as compare to diabetic control group

c- Significance difference as compare to standard treated group

CONCLUSION

The common symptoms of diabetes, that is weight loss, blood glucose, levels of different lipids like LDL, HDL, TG & CHL were found to be lessened by the Phospolipid complex of naringenin in diabetic rats. It significantly reduced fasting glucose levels in diabetic rats and also reduced the lipid profile parameters in diabetic rats. conclusion, our histopathological investigation along with the biochemical evaluations suggests the strong antidiabetic potential of Phospholipid complex of naringenin.

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