

Study of hypoglycemic and anti-diabetic effect of grape fruit ethanolic extract on hyperglycemic glucose induced rats.

Izzeldin, O.M.¹; Elemam, A.²; Ahmed, M.A.²; Elhag, Yusra¹; Fath Elrhman, Sheraz ¹; Greeb Alla, Samah ¹; Elsadig. Tamadur ¹; and Abdulkarim, Hind ¹.

1 Faculty of science and technology, Omdurman Islamic University, Khartoum-Sudan

2 Faculty of pharmacy, Omdurman Islamic University, Khartoum-Sudan

Abstract

This experiment was conducted to study the hypoglycemic and anti-diabetic effect of grape fruit ethanolic extract (outer external layer) in hyperglycemic rats induced by injection of glucose at the rate of 2g/kg body weight, after fasting 18 hours.

According to our experiment rats weighing 80-120g were used for this study. Rats were divided into 5 groups (n=6). Group I was injected 10 mg/kg body weight of glibenclamide (Daonil), this is the standard group. Group II left as control and treated with 10 mg/kg distilled water, group III was treated with 200 mg/kg body weight and group IV was treated with 400 mg/kg of ethanolic grape fruit extract. The last group (group V) was treated with 800 mg/kg of the extract. The rats in the standard group have low concentration of blood glucose after 2 hrs, 4 hrs (86.3 – 76.8 mg/dl respectively). Group III (200 mg/kg extract) have blood glucose level of 121.8 and 124.2 mg/dl respectively. Group IV (400 mg/kg) have blood glucose 147.2 and 135.7 mg/dl respectively, while the rats in last group (800 mg/kg) have the lowest level of blood glucose 90.8 and 103 mg/dl respectively which was the best group, and the blood glucose was nearly similar to that of the standard.

Correspondence:

Dr. Omar Musa Izzeldin

Email: omermusaizzeldin@hotmail.com

Introduction:

Diabetes mellitus (D.M) is a major metabolic disorder, characterized by chronic glycaemia as relative or absolute lack of insulin or the action of insulin leading to structural changes in a range of cells specially those of the vascular system, nerves and the organs system ⁽¹⁾.

Type I diabetes mellitus (IDDM – insulin dependant D.M or juvenile diabetes) is

characterized by beta cells destruction caused by an autoimmuno-process, usually leading to

absolute insulin deficiency, over 95% of persons with type I develop the disease at the age of 25 with an equal incidence in both sexes. Type II diabetes mellitus (NIDDM, non-insulin dependent D.M or adult onset) is characterized by insulin resistance in peripheral tissue and insulin secretory defect of beta cell. NIDDM is usually

associated with family history of adults, elder age, obesity and lack of exercise, and it is more common in women⁽²⁾.

The outer layer of grape fruit contains emulin extract which lowers the carbohydrate, and reduces the glucose manufactured by the liver. It also lowers the high level of glucose in liver so it acts like insulin. It reduces the glucose level in the blood by about 27 %.

Naringin yellow flavonoid compound is found in the external layer of grape fruit. It has been identified as anti diabetic and hypoglycemic, in addition to hypolipidemic effect. Hesperedin is a flavonoid found in grapefruit, and is used as traditional remedy in some local tribes. The mechanism where by naringin and hesperdin have hypoglycemic and hypolipidemic action in type II diabetes have rarely been investigated⁽³⁾.

Ali and Elkader⁽⁴⁾ found that 80 mg/kg of naringin as citrus flavonoid have shown a great effect as hypoglycemic and anti-oxidant effect. Naringin inhibited glucose uptake in the in the intestine and also have inhibitory action towards glucose uptake in renal brush border membrane vesicle⁽⁵⁾. Effect of naringin (30mg/kg) with 50 mg/kg vitamin C was similar to the effect excreted by injection of 6 units/kg insulin⁽⁶⁾.

Materials and methods:

Method of ethanol extraction: 50 grams of dried sample (outer layer of the fruit) was extracted with 80% ethanol using shaker apparatus for about 48hours. Solvent was evaporated under reduced pressure using rotary evaporator apparatus. The extract was placed in

open Petri-dishes till complete dryness (yield about 15.6%).

Glucose-loaded model rats: Rats of either sex weighing 80-120 g were used. The animals were fasted for 18hours prior to the experiment and then allotted to five groups of 6 rats each. After administration of the extract, the five groups were injected intraperitonally with 50% glucose solution at a dose of 2g/kg body weigh. The glucose level was monitored at 0 hr, 2hrs and 4hrs intervals⁽⁷⁾.

Group I: Each rat was given 10 ml/kg body weight of glucose, then blood glucose was measured for each rat at 2hr and 4hr. This was considered as the control group.

Group II: Each rat was injected intraperitonally with 50% glucose solution at a dose of 2 g/kg body weight, then each rat was injected with 10mg/kg weight with glibenclamide. The glucose level was monitored at 0 hr, 2hr, and 4hr. This was considered as the standard group.

Group III: Each rat first injected with 50% glucose to induce diabetes, and then each rat was given the extract (0.2g/kg body weight) orally. The blood glucose was monitored at intervals of 2hr, and 4hrs.

Group IV: Each rat was first injected with 50% glucose to induce diabetes, then each rat was given an extract (0.4 g/kg body weight). The blood glucose was monitored at 2hrs, and 4hrs intervals.

Group V: Each rat injected with 50% glucose, and then each rat was given orally (0.8 g/kg body weight). The blood glucose was monitored at 2hrs, and 4hrs intervals.

The blood was taken by using capillary tube where the eye was pressed to outside by pressing its head down to allow the flow of blood to the capillary tube.

Glucose was estimated by glucose oxidase enzymatic method ⁽⁸⁾

Results:

In the standard and 800mg/kg treatment groups, there was no much difference between these two groups and others where the glucose level was 86.3 and 103.0 respectively (table 1).

Table 1: Effect of control, standard and different concentrations of grape fruit extract on blood glucose level at 2hr, 4hr intervals

Grape fruit Extract	Concentration of glucose after 2hr	Concentration of glucose after 4hr
control	132.7	127.8
standard	86.3	76.8
200 mg/kg	121.8	124.2
400mg/kg	147.2	135.7
800mg/kg	103	90.8
least significant difference (L.S.D)	20.3	40

If we compare concentration of glucose regarding time (2hrs & 4hrs) for all treatments (table 2), from the concentration of glucose after 4hrs is less significant (111.1 mg/dL) if we compared with 2hrs time (118.2 mg/dL). There was no significant difference of glucose at 2hrs, and 4hrs in groups

of (standard, 200 mg/kg, 400 mg/kg), but in group of (800 mg/kg) and especially after 4hrs this treatment record a significant differences (90.8 mg/dL) compared to (103.0 mg/dL) result obtained after 2hrs.

Table 2: Comparison of blood glucose level after 2hr, 4hr for all treatment, standard and (200 mg/kg, 400mg/kg, 800mg/kg)

Glucose level (mg/dL)	After 2hrs	After 4hrs	Significant difference
for all treatments	118.2	111.1	**
standard	86.3	76.8	ns
200 mg/kg	121.8	124.2	ns
400 mg/kg	147.2	135.7	ns
800 mg/kg	103.0	90.8	**

ns (no significant difference)

** There is significant difference at 1%

Discussion:

In the standard and 800mg/kg treatment groups, there was no much difference between these two groups and others where the glucose level was 86.3 and 103.0 respectively (table1). The other group recorded glucose concentration -after 2hrs-

132.7 mg/dL in control, 121.8 mg/dL in 200mg/kg, and 147.2 mg/dL in 400mg/kg. 200mg/kg record the least average (taken control and 400 mg/kg) we compare the concentration of these treatment. The decrease of level of glucose in group (800mg/kg treatment) we compare it to

the other treatments (control, 200 mg/kg, 400 mg/kg) it was 22.2%, 15.4% and 30% respectively. This proved that the treatment 800mg/kg after 4hr is the best treatment when compared to others.

If we compare concentration of glucose regarding time (2hrs & 4hrs) for all treatments (table 2), from the concentration of glucose after 4hrs is less significant (111.1 mg/dL) if we compared with 2hrs time (118.2 mg/dL).

As in table (2), there was no significant difference of glucose at 2hrs, and 4hrs in groups of (standard, 200 mg/kg, 400 mg/kg), but in group of (800 mg/kg) and especially after 4hrs this treatment record a significant differences (90.8 mg/dL) compared to (103.0 mg/dL) result obtained after 2hrs. The rate of decrease of glucose concentration was 11.9% which was similar to the rate in treatments 200 mg/kg, 400 mg/kg and 800 mg/kg for the results after 4hrs when compared to the results after 2hrs.

The ethanolic extracts of grape fruit include naringin and hesperedin. Naringin exert a variety of pharmacological effects such as anti oxidant activity, blood lowering, anti cancer activity, and inhibition of selected drug metabolizing cytochrome P450, including CYP 3A4 and CYP1A2 which may result in drug interaction in vivo. Ingestion of naringin may increase or decrease in circulating drug level.

Naringin is absent from juice ⁽⁹⁾, grape fruit juice did not produce interaction ⁽¹⁰⁾.

Naringin inhibited in vitro felodipine and nifedipine metabolism that was much less potent than its glycone naringenin ^(11, 12).

Now we are about to find the effect of grape fruit extract flavonoid in reducing the glucose level, but more research work must be carried not to find the effect of naringin, hesperedin, Emulin (separately or mixed) of pure forms of these components to produce anti diabetic and hypoglycemic, and hypolipimic effect. Also we need to know the different effect of naringin and it's a glycone naringenin.

Hesperidin and naringin both significantly increased the glucose kinase RNA level, while naringin lowered the RNA expression of phospho-enolpyruvate carboxkinase and glucose-6-phosphate in the liver ⁽³⁾. Hesperidin and naringin effectively lowered the plasma free fatty acids and plasma and hepatic triglyceride levels and simultaneously reduced the hepatic fatty acid oxidation. There was suppression of glucose-6-phosphate dehydrogenase and increasing in the plasma and hepatic cholesterol levels, this due to decrease in hydroxy-3-methylglutaryl. Coenzyme (HMG. GA) reductase and acyl CoA, cholesterol acyl transferase (ACAT) activities and increase fecal cholesterol ⁽³⁾.

Exogenous administration of individual gradual dose dependant of naringin to hyperglycemic rats causes a dose dependant decrease of the glucose level and an increase concentration of insulin, decrease of the H₂O₂ as well as an increase of the antioxidant enzymes (catalyse CAT, super-oxide

dismutase (SOD), glutathione peroxidase (GPX), and paraoxonase (PON)).

The greatest effect of naringin was observed at 80 mg/kg body weight ⁽⁴⁾.

Naringenin a flavonoid present in citrus fruits significantly inhibited glucose uptake in the intestine also has an inhibitory effect towards glucose uptake in renal brush-border membrane vesicles ⁽⁵⁾.

A dose of 30 mg/kg naringin and 50 mg/kg of vitamin C. was similar to the effect exerted by insulin 6units/kg ⁽⁶⁾.

Naringin have anti oxidant activity, blood lipid lowering, anti-cancer activity, inhibition of selected drug-metabolizing cytochrome P450 enzymes including CYP 3A4 and CYP 1A2 which may result in drug interaction in vivo, so this all effect the intestinal absorption of certain drugs leading to an increase or decrease in circulating drug level. Naringin inhibited in vitro felodipine and nifedipine metabolism, but this was much less in naringenin ⁽¹³⁾.

It seem that naringin have good effect in lowering the glucose and also reducing the level of lipids; but the issue in inhibition of absorption of certain drugs this will face the commercial pharmaceutical application of naringin as anti diabetic reagent; researcher may think about the effect of naringin (the aglycone of naringin), also the mixed effect of naringin and hesperidin, or the effect of emulin which reduce the level of blood glucose by 27%.

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