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Full Length Research Paper

Effect of *Citrus paradisi* and *Citrus sinensis* on glycemic control in rats

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This study was conducted to explore the effects of *Citrus sinensis* (orange juice) and *Citrus paradisi* (grapefruit juice) at three different doses alone and their two combinations on plasma insulin and blood glucose levels in healthy and diabetic rats. Diabetes was induced by alloxan after which rats were treated with *C. sinensis* and *C. paradisi* juices for six weeks, blood glucose and plasma insulin concentration was estimated. *C. sinensis* showed significant reduction in blood glucose and a significant rise in plasma insulin at all three doses. However *C. paradisi* revealed highly significant fall in blood glucose and highly significant rise in plasma insulin levels only at 0.5 ml/kg. Whereas combination dose group CSP-2 (5 + 0.3 ml/kg) showed highly significant reduction in blood glucose and highly significant rise in plasma insulin levels as compared to diabetic control. These results suggest that flavonoids and other essential nutrients present in citrus fruits juices might be responsible for these effects. Hence, it may be concluded that these juices may be used in combination to produce a synergistic effect in decreasing blood glucose and elevating plasma insulin levels.

Key words: Grape fruit, orange, glucose level, insulin.

INTRODUCTION

Diabetes mellitus is a metabolic disorder resulting in severe immediate and continuing consequences extending from brain damage to heart disease (American Diabetes Association, 2009). According to the report of International Diabetes Federation, the estimated number of people with diabetes increased universally in recent years and is expected to reach 380 million in 2025 (Samreen, 2009). Large number of agents is available for treatment but is associated with various serious adverse effects (Tahrani et al., 2010; Patel et al., 2012). Plants have been used over a long time for the treatment of different ailments. Fruits and vegetables have gained importance for the management of diabetes mellitus, for example pomegranate, grapes, guava, lemon, tomatoes and grape fruits. They have the capacity to re-establish function of pancreatic tissues by increasing insulin output, inhibiting intestinal absorption of glucose or helping metabolites in insulin dependent processes (Javascript, 2002; Free encyclopedia, 2002; Punitha et al., 2006; Malviya et al., 2010; Riaz et al., 2013).

This study was conducted on fresh juices of two fruits, *Citrus sinensis* (orange) and *Citrus paradisi* (grapefruit) belonging to Rutaceae family, since plants and plant derived phytochemical have great potential to treat and

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control diabetes (Behera and Yadev, 2013). Grapefruit contains phytochemicals including limonoids and lycopene. It is also an exceptional source of vitamin C, dietary fiber, vitamin A, potassium, folate and vitamin B5 (Mateljan, 2006). It also contains high levels of iron, calcium and other minerals. Pink and red varieties of grapefruits are rich in beta carotene, high in fiber and low in calories. They possess protective plant chemicals like phenolic acid, limonoids, terpenes, monoterpenes and bioflavonoids which protect against cancer and heart disease. The major bioflavonoid in grapefruit is naringin that gives grapefruit juice bitter taste (Giovannucci et al., 2002; Armando et al., 1998). Naringin exerts diverse pharmacological effects like antioxidant activity, blood lipid-lowering effect (Gorinstein et al., 2006), anti-carcinogenic activity (Armando et al., 1998) and inhibition of selected cytochrome P450 enzymes including CYP3A4 and CYP1A2 (Gao et al., 2006). Naringin being one of the components of these fruits have been reported for its antidiabetic effects (Pari and Suman, 2010). Grapefruit enhance appetite and is employed for its digestive, stomachic, antiseptic and diuretic properties (Herbal medicine, 2000).

Orange being a rich source of nutrients has also been known for a number of health and nutritional benefits. Orange contains nearly two hundred phytonutrients and flavonoids, which have shown activity against different types of cancers. They also have been reported to have strong anti-inflammatory and anti-oxidant properties and prevent bone loss (Liu et al., 2012; Peluso, 2006; Gao et al., 2006; Chiba et al., 2003). The essential oils in orange juice (*C. sinensis*) contain many constituents, including monoterpenes and sesquiterpenes with d-limonene as a major constituent (Graciela et al., 2003). Orange juice has been reported for cholesterol lowering effect in animal models (Kurowska et al., 2000) as well as humans (Roza et al., 2007). Its anti-inflammatory role in different disease is also well documented (Buscemi et al., 2012; Ghanim et al., 2010). Narirutin or Naringenin 7-O-rutinoside is another important flavonoid present abundantly in orange juice (Sawalha et al., 2009). It is absorbed well and shows good bioavailability (Manach et al., 2003). It is also shown to possess anti-inflammatory (Ha et al., 2012; Funaguchi et al., 2007), anti-allergic and anti-asthmatic effects (Rogerio et al., 2010; Funaguchi et al., 2007). Orange and grape fruit are both rich in phytochemical, flavonoids and vitamins which revealed to have strong anti-inflammatory and antioxidant properties (Liu et al., 2012; Peluso, 2006; Gao et al., 2006; Chiba et al., 2003).

MATERIALS AND METHODS

Animals

Present study was conducted after approval of the proposal by board of advance studies and research, University of Karachi on adult Wister rats with mean body weight of 220 ± 10 g. Animals were kept under controlled condition of temperature 23 ± 2°C and humidity 50 to 60%, with free access to food and water. Five rats were housed in each plastic cage measuring 32" × 18" × 16".

Citrus sinensis

C. sinensis was obtained from local market and recognized by Center of Plant Conservation, University of Karachi. The voucher specimen no.CS-10-10 was placed in the Department of Pharmacognosy, University of Karachi. *C. sinensis* was peeled and pressed by hand to yield fresh juice which was then used immediately after filtration. *C. sinensis* juice was administered in three different doses that is, 2, 5 and 8 ml/kg according to body weight through oral route.

Citrus paradisi

C. paradisi was also bought from local market, recognized by Center of Plant Conservation, University of Karachi. The voucher specimen no. CP-09-10 was kept in the Department of Pharmacognosy, University of Karachi. The fruits were peeled, pressed and fresh juice so obtained was filtered and used soon after yielding. *C. paradisi* was given in three doses that is, 0.1, 0.3 and 0.5 ml/kg according to body weight through oral route.

Combinations of *C. sinensis* and *C. paradisi*

C. sinensis and *C. paradisi* were also given orally in two combined doses that is, 2 + 0.1 and 5 + 0.3 ml/kg, and were abbreviated as CSP-1 and CSP-2.

Induction of diabetes

Diabetes was induced in overnight fasted rats by means of a single dose of alloxan monohydrate (Sigma chemicals, USA). Alloxan was administered in the dose of 180 mg/kg in normal saline by intraperitoneal injection. The glucose level in plasma was determined at 72 h after the administration of alloxan (Rohilla and Ali, 2012). Animals with blood glucose concentration more than 250 mg/kg were considered diabetic and used for the study.

Design of experiment

All rats were distributed in eleven groups each comprising of ten animals. One group received saline and served as normal control, while remaining ten groups induced diabetes received *C. sinensis* (three groups), *C. paradisi* (three groups), while two groups received *C. sinensis* and *C. paradisi* in combination, one group received standard drug glibenclamide and one group received saline served as diabetic control. All doses were given by gastric intubation on once daily basis for a period of six weeks. Entire study was performed under NACLAR guideline (Bernard, 2004). At the end of the investigational period, animals were deprived of food overnight, sacrificed by amputation and blood samples were collected in gel tubes and 3.2% sodium citrate containing tubes (9:1 v/v).

Estimation of blood glucose

Blood glucose was estimated by two methods, first through Accu-Chek glucometer by taking blood from tail vein of rats, after 72 h of intraperitoneal injection of alloxan to check the induction of diabetes. Second through blood collected in gel tubes. Serum was

Table 1. Effect of *C. sinensis* on glucose and plasma insulin levels.

Parameters	Groups				
	Normal control	Diabetic control	*C. sinensis* 2 ml/kg	*C. sinensis* 5 ml/kg	*C. sinensis* 8 ml/kg
Blood glucose (mg/dl)	93.7 ± 4.41	292.5 ± 19.53	227.81 ± 17.29*	218.65 ± 24.83*	223.9 ± 19.70*
Plasma insulin (µU/ml)	15.6 ± 0.35	7.9 ± 1.01	10.08 ± 0.35*	9.93 ± 0.47*	10.28 ± 9.1*

n=10, Values are means ± S.E.M. *P ≤ 0.05 significantly different as compared to control.

Table 2. Effect of C. paradisi on glucose level and plasma insulin levels.

Parameters	Groups				
	Normal control	Diabetic control	*C. paradisi* 0.1 ml/kg	*C. paradisi* 0.3 ml/kg	*C. paradisi* 0.5 ml/kg
Blood glucose (mg/dl)	93.7 ± 4.41	292.5 ± 19.53	186.39 ± 23.47**	180.37 ± 20.03**	169.93 ± 22.55**
Plasma insulin (µU/ml)	15.6 ± 0.35	7.9 ± 1.01	8.7 ± 0.68	10.29 ± 0.56*	11.01 ± 0.23**

n=10, Values are means ± S.E.M. *P ≤ 0.05 significantly different as compared to diabetic control. **p≤ 0.005 highly significant as compared to diabetic control

Table 3. Effect of combination doses of *C. sinensis* - *C. paradisi* and glibenclamide on glucose and plasma insulin levels.

Parameters	Groups				
	Normal Control	Diabetic Control	CSP -1 Treated	CSP -2 Treated	Glibenclamide
Blood glucose (mg/dl)	93.7 ± 4.41	292.5 ± 19.53	221.38 ± 14.5*	179.3 ± 27.06**	158.01 ± 18.37**
Plasma insulin (µU/ml)	15.6 ± 0.35	7.9 ± 1.09	8.76 ± 0.62	11.65 ± 0.54**	14.03 ± 0.62**

n=10, Values are means ± S.E.M. *P ≤ 0.05 significantly different as compared to control. **p≤ 0.005 highly significant as compared to diabetic control. CSP -1: 2+0.1 ml/kg/day *C. sinensis* and *C. paradise*, respectively. CSP -2: 5+0.3 ml/kg/day *C. sinensis* and *C. paradise*, respectively. Glibenclamide: 2.5 mg/kg.

separated by Humax 14K centrifuge at 2000 rpm for 10 min and then commercial kit of glucose (Human Diagnostic, Germany) was used to estimate blood glucose by Humalyzer 3000 Human Germany.

Estimation of plasma insulin

Blood was collected in 3.2% sodium citrate tubes and plasma was parted by Humax 14 K centrifuge at 3000 rpm for 15 min. Insulin was estimated using commercial ELISA insulin kit (Accu-Bind, Elisa Microwells, USA).

Statistical analysis

Entry of data and analysis was performed using 17th version of superior performance statistical software (SPSS). Quantities were presented as mean ± SD with 95% confidence interval. Analysis of variance (ANOVA) followed by post hoc was performed for comparisons of values with control. Values of $p < 0.05$ were considered statistically significant and $p < 0.005$ highly significant.

RESULTS

Table 1 reveals the effect of *C. sinensis* on blood glucose and plasma insulin levels in diabetic and control rats. There was significant decrease in blood glucose at 2, 5 and 8 ml/kg of *C. sinensis* in a dose dependent manner than diabetic control, while there was significant rise in insulin level at all three dose as compared to diabetic control. Table 2 displays the effect of *C. paradisi* on blood glucose and plasma insulin levels in diabetic and control animals. There was highly significant decrease in blood glucose at 0.1, 0.3 and 0.5 ml/kg of *C. paradisi* in a dose dependent manner than diabetic control. While there was highly significant increase in plasma insulin levels at 0.5 ml/kg and significant increase at 0.3 ml/kg. However, there was no change in plasma insulin level at 0.1 ml/kg. Table 3 shows the comparative effect of combination doses of *C. sinensis* and *C. paradisi* with standard drug glibenclamide on plasma glucose and insulin levels in diabetic control and normal control animals. There was highly significant decrease in blood glucose in animals treated by CSP-2 combination and glibenclamide. While significant decrease was observed in animals treated with CSP-1 combination as compared to diabetic control. There was also highly significant rise in plasma insulin at CSP-2 and glibenclamide treated animals as compared

to diabetic control. However, there was no change in plasma insulin level at CSP -1.

DISCUSSION

Type-I diabetes is categorized by a loss of insulin-producing beta cells islets of Langerhans in the pancreas leading to decrease in insulin. Diabetes was induced by the administration of alloxan to the experimental animals causing destruction of beta cells, a result consistent with several studies in rats (Mohammed et al., 2010; Prem et al., 2012). Findings of this study clearly indicates that diabetic animals treated with *C. sinensis* and *C. paradisi* had a reduced blood glucose concentration when compared to the diabetic control animals, a result almost similar to reference drug glibenclamide (Table 3).

Present study showed significant blood glucose lowering effect by *C. sinensis* at all three doses, while there was also significant increase in plasma insulin levels. On the basis of these results, it could be postulated that *C. sinensis* induced hypoglycemic effect may be due to the presence of high contents of flavonoids and monoterpenes in *C. sinensis*. The hypo-glycemic effect of monoterpenes had been previously demonstrated by Tavafi et al. (2011). Monoterpenes may have insulin mimetic properties or may be able to induce insulin production from the surviving beta cells enough to facilitate glucose uptake from the blood.

C. paradisi showed highly significant increase in plasma insulin levels at 0.5 ml/kg. This effect may be due to the presence of vitamin C in *C. paradisi* which acts as strong antioxidant. This effect might be also due to the presence of naringin. Since naringin is reported to produce hypoglycemic effect due to its strong anti-oxidation property (Pari et al., 2010). Several flavonoids and terpenoids are present in *C. paradisi*. It is therefore, logical to conclude that the hypoglycemic effect of grapefruit juice (*C. paradisi*) may be due to flavonoids and/or terpenoids content of *C. paradisi* (Cerda et al., 1988). While there was highly significantly decrease in glucose level by CSP-2. This might be due to synergic effect of naringin in *C. sinensis* and polyphenols in *C. paradisi*. Moreover naringin and vitamin C had been reported to produce hypoglycemic and hypo-cholesterolemic effects due to their strong anti-oxidant properties (Pari and Suman, 2010). While polyphenols and vitamin C in *C. paradisi* may be responsible for these beneficial effects due to their strong antioxidant property (Violi et al., 2010).

All antioxidants may produce a synergistic effect which may provide strengthening to the B-cells of pancreas to release more insulin. Combined management with naringin and vitamin C had been demonstrated to ameliorate streptozotocin-induced diabetes in rats (Punithavathi et al., 2008). It has also been reported that hesperidin and naringin are useful for improving hyperlipidemia and hyperglycemia in type - II diabetic animal models by partially regulating fatty acid and cholesterol metabolism and affecting gene expression of glucose-regulating enzymes (Jung et al., 2004; Jung et al., 2006). Though grapefruit juice has potent hypo-glycemic effects in experimental animals, however its use should be cautioned due to its enzyme inhibitory effects on the metabolism of many drugs (Bailey et al., 2000), which can lead to adverse effects from these medications (Gao et al., 2006).

Conclusion

From the results of the present study it may be concluded that combination of *C. sinensis* and *C. paradisi* is most effective in high doses, however further studies on more combination doses of *C. sinensis* and *C. paradisi* are required to reveal the role of these substances on reduction of blood glucose levels and combating diabetes mellitus. This study suggests that grapefruit juice and orange juice plays a good role in controlling the glucose level of experimental animals and can be applied clinically on patients with diabetes and lower cholesterol level.

Conflict of interest

There are no conflicts of interests

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