Hypoglycemic and Hypolipidemic Effects of Raisin Aqueous Extract “Karkni” in Alloxan-Induced Diabetic Rats

Fatma Ghrairi1, El Arem Amira2, Chahed Henda3, Lamia Lahouar2, Lotfi Achour2 and Salem Said1
1Laboratory of Biochemistry, Mohamed Karoui Avenue, Tunisia
2Higher Institutes of Biotechnology, Tahar Hadded Avenue, Tunisia
3Laboratory of Biochemistry, Hospital Farhat Hachad Sousse, Sousse, Tunisia

Abstract

The present study was carried out to investigate the possible antioxidant effects of raisin extract “Karkni” on biochemical parameters in alloxan-induced diabetic rats. Three doses of extract, 125, 250 and 375 mg/kg were orally administered daily to alloxan-diabetic rats for 4 weeks. Alloxan-induced diabetic rats showed significant increases in the levels of blood glucose, triglycerides, cholesterol, low density lipoprotein LDL-cholesterol, creatinine, uric acid , total protein, glycosylated hemoglobin (HbA1c), Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) while high density lipoprotein HDL cholesterol, total hemoglobin and lactate dehydrogenase (LDH) levels were significantly decreased compared to normal rats (P<0.05). The changes of the above parameters to their normal levels after 4 weeks of treatment were observed mainly with the second dose 250 mg/kg. The overall results suggest that the “Karkni” extract possesses potential hypoglycemic and hypolipidemic activity in alloxan-induced diabetic rats.

Keywords: Karkni; Rats; Alloxan; Hypoglycemic; Hypolipidemic; Biochemical parameters

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Diabetes is a major degenerative disease in the world today [1], affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders [2]. It is also associated with long-term complications, including retinopathy, nephropathy, neuropathy and angiospasm [3-5]. Controlling blood sugar levels and keeping these closer to normal values is able to reduce the risk of diabetes and death by these complications. Since dietary carbohydrates had the most direct impact on blood sugar levels, controlling the amount of carbohydrate consumed per meal is the focus of diabetes nutrition management [6]. Raisins, like all commonly consumed fruits, provide carbohydrates as the only caloric macronutrient but it is considered as a great source of many polyphenolic compounds (flavonoids, tannins, phenolic acid) playing important roles in nutrition and health care [7,8]. Spiller et al. [9] first studied the hypocholesterolemic effect of plant-based diets, which included more than one serving of sun-dried raisins daily. Keen and schram showed that a diet providing 2, 3.5 and 5.5 ounces of raisins per day for 4 weeks lowered ox-LDL levels [10]. Besides, research suggest that addition of raisins to the daily diet has been shown to lower total and LDL cholesterol levels, reduce markers of inflammation, increase plasma antioxidant capacity and lower circulating levels of oxidized LDL, a marker of coronary heart disease risk [11,12]. Also browning reaction products (BRPs) in raisin have been reported to prevent or retard oxidation reactions in lipid systems [13,14], because they have similar chemical properties as phenolic acids [15]. Experimental diabetes in animals has the advantage that it allows the analysis of the biochemical events that take place not only during the induction of a diabetic state but also after it has become established and during its evolution to a severe insulin deficiency or even death [16]. Alloxan is a specific toxin that destroys the pancreatic β cells, provoking a state of primary deficiency of insulin without affecting other islet types [17,18]. Hence, it was selected to induce diabetes in the present study and the principal objective was to determine the potential beneficial antidiabetic effects of three concentrations of raisin extract (125, 250 and 375 mg/kg) during 4 weeks on alloxan-induced diabetic rats.

Materials and Methods

Plant material

Dried fruit was extracted with distilled water by grinding with a mortar and pestle. It was incubated for 24 h and filtered using a Buckner funnel and Whatman No. 1 filter paper. It was the filtrate that was administered to the animals in the course of this study, fresh for a maximum of two days after which fresh extract was prepared.

Experimental animals

Male Wistar rats (Société des Industries Pharmaceutiques Tunis, Tunisia), weighing 200-250 g were housed under standard environmental conditions (23°C, 55 ± 5% humidity and a 12 h light/dark cycle) and maintained with free access to water and a standard diet ad libitum. Experimental diabetes was induced in rats by intraperitoneal injection of alloxan monohydrate at a dose of 120 mg/kg. After 2 weeks, animals with blood glucose levels of 200 mg/dl and above were considered diabetic. The rats were divided into 5 groups of 8 animals per group. Group 1: Non-diabetic untreated rats (Control), Group 2: Diabetic untreated rats, Group 3: Diabetic rats treated with 125 mg/kg “Karkni” extract (D+C1), Group 4: Diabetic rats treated with 250 mg/ kg of the “Karkni” extract (D+C2) and Group 5: Diabetic rats treated...
with 375 mg/kg of the "Karkni" extract (D+C3). After confirming diabetes, "Karkni" extract was administered orally, in the morning, once every 24 hours for 4 weeks. At the end of this treatment period, the animals were fasted overnight for 12 hours. Blood samples from rats were collected under mild ether anesthesia in heparinized tubes and centrifuged at 3000 g for 15 min at 4°C. Plasma samples were stored at -20°C in aliquots until analysis. All experimental protocols used were in accordance with the guidelines of the local Committee on Use of Laboratory Animals.

Experimental procedure

TC and HDL cholesterol, Creatinine, Urea, Uric acid, Total protein in plasma were determined by colorimetric method, whereas LDL cholesterol was calculated by the method of Friedewald et al. [19]. Coronary Risk Index (CRI) was calculated by dividing the Total Cholesterol (TC) by High density lipoprotein (HDL). Glycosylated haemoglobin (HbA1c) was estimated using whole blood. Hemoglobin, LDH, γ-GT, Alanine Aminotransferase, and Aspartate Aminotransferase activity levels in the plasma were estimated by using standard kits purchased from Biomaghreb [20-23].

Statistical analysis

Data were subjected to one-way analysis of variance for means of comparison, and significant differences were calculated according to Duncan multiple range test. Data are reported as means ± standard error of the means. Differences at P<0.05 were considered statistically significant. SPSS (version 11.0) was used to perform the statistical analysis.

Results

Effects of “Karkni” extract on blood glucose levels

The effects of “Karkni” extract on blood glucose levels in diabetic rats were shown in figure 1. The blood glucose levels of rats in the control group did not change during the experimental period. By contrast the blood glucose levels in diabetic group were significantly higher (P<0.05) than those of the treated rats in the other groups;
D+C1, D+C2 and D+C3. Of the 3 doses of raisin tested, the 250 mg/kg dose was found to be the most effective in reducing blood glucose levels during the 4 weeks.

Effects of “Karkni” extract on plasma lipid profile like TC, TG, LDL and HDL

In our study, induction of diabetes significantly altered the normal lipid profile levels compared to control rat (Table 1). Administration of three doses of “Karkni” extract significantly decreased (P<0.05) plasma TC, TG and LDL cholesterol levels compared to diabetic group. Moreover, the LDL cholesterol in the (D+C2) group was lower than that in the other groups (D+C1) and (D+C3). HDL cholesterol concentrations were increased in the (D+C2) and (D+C3) groups than in diabetic group. Consequently, the ratio of LDL/HDL ratio was decreased significantly in (D+C2) group than in the diabetic, (D+C1) and (D+C3) groups (p<0.05), whereas the coronary risk was significantly lower in the (D+C2) and (D+C3) groups than in the others groups.

Effects of “Karkni” extract on creatinine, Urea, Uric acid, Total protein, Hemoglobin and HbA1c levels

Plasma levels of blood uric acid were increased following diabetes induction (Table 2). There were significant difference between the diabetic and the control group, 187.42 ± 56.34 and 152.5 ± 61.51 µmol/l respectively. But the lowest levels were observed in (D+C2) group, (136.8 ± 21.01 µmol/l). Also the level of creatinine was increased in the diabetic group but the difference was not significant. Moreover, plasma protein levels were significantly increased in the diabetic rats (p<0.05). But after 4 weeks of treatment with “Karkni” extract, the levels of protein were decreased in (D+C1), (D+C2) and (D+C3) groups. Increased HbA1c levels were noticed in diabetic rats compared to control rats whereas hemoglobin and urea levels were significantly lowered in diabetic rats. But they were restored after four weeks of treatment by “Karkni” extract.

Effects of “Karkni” extract on enzymes activity

Induction of diabetes led to increased ALAT activity (Figure 2). After four weeks of treatment, activity of this enzyme was decreased in (D+C1) and (D+C2) groups but it still remained higher in the (D+C3) group. Concerning ASAT activity, it was also found that alloxan-induced diabetes increased, but it was significantly decreased in (D+C2) and (D+C3) group (Figure 3). Also a significant (P<0.001) reduction of LDH activity was observed in diabetic group compared to control rats. By contrast, γ-GT activity was increased significantly in diabetic rats (p<0.05). After 4 weeks, enzyme activity was restored in (D+C1), (D+C2) and (D+C3) groups.

Discussion

Raisin polyphenols have demonstrated significant antioxidant anticarcinogenic, anti-inflammatory, thermogenic, probiotic, and antimicrobial properties in numerous human, animal, and in vitro studies [6]. But the effect of raisin polyphenols in diabetes is still unknown. The present study was conducted to determine the effect of feeding 3 doses of raisin extract “Karkni” on the lipid parameters in alloxan rats. Alloxan, at a dose of 120 mg/kg body weight, caused sufficient damage to pancreatic β cells so that secreted insulin was not enough to regulate blood glucose and resulted in a significant increase in blood glucose levels [24-26]. In our experiment, we had shown that raisin extract decreased plasma glucose for 4 weeks compared with those of the diabetic’s controls. Both doses (125 and 250 mg/kg) of the extract showed a significant hypoglycemic effect but rats treated with 375 mg/kg of 'Karkni' extract showed significant increases in blood glucose after three weeks. These results can be related with the carbohydrates content in raisin. Previous studies had shown that hypoglycemic effect of many herbs was related to flavonoids content and these constituents can preserve the insulin-secreting capacity and viability of pancreatic β cells [27]. Therefore, the presence of these constituents in our variety of raisin may explain the hypoglycemic activity, and we can conclude that the antioxidative activities of raisin...
extract in alloxan-induced diabetes, at least in part, may be related to antihyperglycemic capability. The present study found that the intake of "Karkn" extract significantly lower plasma total cholesterol, triglycerides and LDL cholesterol concentrations but increased serum HDL cholesterol in (D+C1), (D+C2) and (D+C3) groups when compared with those of diabetic control. Also decreased level of coronary risk was observed in the same group but it was especially interesting in (D+C2) group. These results were similar to those found by Bruce et al. [11] and Gardner et al. [12]. High levels of circulating cardiac dysfunction/damage markers such as, LDL and uric acid, represent a sensitive predictor of increased cardiac complications [28]. In our study, "Karkni" extract showed a significant decrease in levels of uric acid in (D+C1) and (D+C2) groups when compared with those of diabetic control. But it was still remaining higher in (D+C3) group. In diabetic rats the elevation observed in uric acid level could be due to the increased abundance and activity of xanthine oxidase. It was responsible for the formation of uric acid and also serves as an important biological source of ROS (superoxide) that contribute to oxidative damage involved in many pathological processes such as diabetes, atherosclerosis, cancer and aging [29-31]. Moreover, decreasing levels of HbA1c observed in (D+C2) group can reduce the risk of lipid peroxidation [32]. These explain the important role of raisin to decrease cardiac damage markers in diabetic person with 250 mg/kg.

Also results presented in table 2 clearly revealed that the administration of the three concentrations of "Karkni" extract to the diabetic rats could restore the changes in the levels of hemoglobin, urea, creatinine and total protein after 4 weeks of treatment to their normal levels. Lal et al. proved that in diabetes, elevated levels of serum urea, uric acid and creatinine are observed which may be due to renal damage caused by abnormal glucose regulation or elevated glucose and glycosylated protein tissue levels [33]. So it could be concluded that the above action of raisin may be due to its potential to control hyperglycemia and renal damage in diabetic rats. Alloxan induces increase of AST, and ALT activity in rats (Figure 2). This results were similar than that observed with streptozotocin induced diabetic rats [34]. Restoration of the normal level of ALAT may indicate the normalizing effect of both 125 and 250 mg/kg of "Karkni" extract. As for ASAT activity, restoration of the normal level was observed only with 250 and 375 mg/kg of "Karkni" extract. A change in enzyme activity is presumed to be due to the decreased blood insulin concentration [35], but is also related to energy metabolism, as these enzymes play a role in gluconeogenesis [36]. As for LDH activity, results from our experiment show that diabetes decreases LDH activity (Figure 3). These result are similar than Tormo et al. [37], but not than Melinker et al. [38]. A highly significant elevation in the activity of γ-GT was observed in plasma of alloxan-induced diabetic rats. But after treatment of raisin, increased activity of this enzyme was lowered to near normal that indicates the possible prevention of necrosis in the liver by "Karkn" extract. The same result was observed by McLennan et al. [39]. Also, the increment of the activities of AST, ALT and LDH in plasma is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream [40], which gives an indication on the hepatoxic effect of alloxan, but raisin extract reduced the activity of these enzymes in plasma so the oral administration of "Karkni" extract had a beneficial effect on the diabetic state reducing the hyperglycemia as well as hyperlipidemia.

Conclusion

In conclusion, our study demonstrates that "Karkn" extract corrects hyperglycemia and dyslipidemia, thus improving the atherogenic index. The 250 mg/kg dose was found to be the most effective in restoring changes during the 4 weeks. This implies that raisin can prevent or be helpful in reducing the complications of diabetes. However, further investigations to fully identify the biologically active ingredients and to define the precise molecular mechanism(s) of these effects are required.

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