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The Effect of Hydro-alcoholic Extract of Grape Seed (*Vitis vinifera*) on Sugar and Lipids in Serum of Diabetic Rats

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Abstract: Now-a-days, no-drug treatments (medicinal plants) are novel therapeutic approaches in the treatment of diabetes. This study aimed at assessing the effect of grape seed extract on the blood glucose and lipid profile in diabetic rats. A total of 30 male adult rats were randomly selected and divided into 3 groups as; non-diabetic control, diabetic control and diabetic rats treated with hydroalcoholic extract of grape seed. In diabetic groups, alloxan monohydrate (120 mg kg^{-1}) was injected intraperitoneally to develop diabetes. Then the test group received intraperitoneal injection of hydro-alcoholic extract of grape seed (100 mg kg^{-1}). At last, glucose, cholesterol, triglyceride, HDL, VLDL, LDL and insulin contents of the rats' serum sample were determined. Diabetic rats treated with extract showed a significant decrease in blood glucose level ($p<0.05$). Furthermore, compared diabetic group, in the extract-treated rats, there was a significant decrease in serum contents of Total Cholesterol (TC), LDL, VLDL and TG but a significant increase in insulin level and HDL ($p<0.05$). These results show that the hydroalcoholic extract of grape seed may be effective in the treatment of diabetes. This effect can be due to the presence of flavonoides and their antioxidant features.

Key words: Grape seed, blood sugar, lipid profile, insulin, diabetic rats, Iran

INTRODUCTION

Diabetes is the most common endocrine disease being characterized with increased blood sugar (hyperglycemia) and disorders in metabolizing carbohydrates, lipids and proteins (Smith, 2003). In diabetes mellitus, chronic hyperglycaemia produces multiple biochemical sequelae and diabetes-induced oxidative stress could play a role in the symptoms and progression of the disease. Oxidative stress may result in overproduction of oxygen free-radical precursors and/or decreased efficiency of the antioxidant system (Soto *et al*., 2003). The oxygen free-radical generation is associated with auto-oxidation of glucose, impaired glutathione metabolism, alterations in the antioxidant enzymes and formation of lipid peroxides (Szudelski, 2001; Fukuda *et al*., 2004; Vaya and Aviram, 2001). There are various endogenous defence mechanisms against free radicals, such as the enzymes GSH, SOD, GPx and CAT whose activities eliminate superoxide, hydrogen peroxideand hydroxyl radicals (Soto *et al*., 2003).

Use of medicinal plants in medicine is increasing because of their widespread use and for their curing various diseases. In present study, the researchers have examined the role of alcoholic extract of grape seed on glucose serum, lipid profile and releasing insulin from pancreas of healthy animals and animals with diabetes caused by alloxan monohydrate.

MATERIALS AND METHODS

Plant material: From grapes, the seeds were removed and dried in shadow according to drying process . Then, the dried seeds were grinded to a uniform powder and weighed. The most important and essential part of extraction of plant material is the selection of a proper organic solvent which depends on the part and constituents of the plant. In this study, a mixture of ethanol and water in the ratio of 7:3 was prepared. Then, 500 g of the grape seed powder were extracted by maceration method for 3 days. The extracted material was filtered and the filtrated material was concentrated under vacuum evaporator until dryness.

Animals: In present study, the researchers used 30 male rats from Wistar race in weight range of 180 and 220 g. Animals were randomly divided to 3 groups of ten rats as control group: healthy rats which received physiologic serum equal to the injected extract volume. This was done to equalize the shock resulted from injection; diabetic

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control group: diabetic rats which were affected by single intraperitoneal injecting of 120 mg kg^{-1} monohydrate alloxan and were treated with physiologic serum; diabetic rats treated with grape seed extract: the rats which were affected as well as the second group. And 100 mg kg^{-1} hydro-alcoholic extract of grape seed injection was performed for 10 successive days after assuring that rates were affected by diabetes.

About 48 h after the last injection, the bleeding was performed from all groups and the resulted serum was used to determine blood glucose, cholesterol, triglyceride and lipoproteins (LDL and HDL) with enzyme kits (from Zist-Shimi, Iran).

During the study, storage, injection of various materials, bleeding and perishing animals were performed according to standard methods of working with laboratorial animals. In statistical survey of findings, the one-way ANOVA test was applied to compare average of each variable in test groups and then Tukey test was performed. Statistical analysis of findings was done with SPSS software and $p<0.05$ was treated meaningful.

RESULTS AND DISCUSSION

Results of biochemical tests of glucose, insulin, cholesterol, triglyceride, HDL, LDL and VLDL is shown in Table 1. In present study, the researchers examine the effect of hydro-alcoholic extract of grape seeds on biochemical parameters of blood. Results from this study show that in treated group, blood glucose, triglyceride, cholesterol, LDL, HDL and VLDL level had a meaningful decrease ($p<0.05$) compared to the diabetic control group and blood insulin and HDL level had a meaningful increase ($p<0.05$) compared to the diabetic control group. According to several studies, specific toxicity of alloxan for β-cells of pancreas is due to quick absorption of alloxan by pancreatic β-cells and free radicals production by alloxan. Free radicals can cause reversible or irreversible damages to cellular compound of creatures (such as proteins, lipids, carbohydrates, nucleic acids, etc.) and thereby affect cellular activities such as function of membrane, metabolism and gene expression. Therefore, some cells would lose their structure and activity. According researches, oxidative damage of free radicals is main cause of damage to cells and tissues in some diseases such as arthroscleroses, cancer, mellitus diabetes, etc. (Szkudelski, 2001). Antioxidants are compounds that protect cellular membrane and various components of creatures against oxidants. Mechanism of action of these compounds is gathering free radicals, transferring electron to these electrons and inactivation of them (Fukuda *et al*., 2004; Vaya and Aviram, 2001).

Table 1: Effect of Hydro-alcoholic extract of grape seed on level of serum glucose, Insulin, cholesterol, triglyceride and lipoproteins in studied group of rats (mean±SD)

Index	Control	Diabetic control	Treated by hydro-alcoholic extract of grape seed
Glucose (mg dL^{-1})	125±14.2	768±117.4	275±35.2
Triglyceride (mg dL^{-1})	107±14	227±15	131±29
Cholesterol (mg dL^{-1})	94.7±12.3	111.9±10.5	105.2±10.8
LDL (mg dL^{-1})	24±6	40±12	24±9
HDL (mg dL^{-1})	50±7	27±6	55±12
VLDL (mg dL^{-1})	22±2	46±3	27±5
Insulin (μu mL^{-1})	12.55±1.35	4.86±1.41	12.18±1.8

Mean glucose, triglyceride, cholesterol, LDL, HDL, VLDL and insulin difference of treated group with diabetic control group: meaningful ($p<0.05$)

Researches in recent years by Yassa *et al*. (2008), Puiggros *et al*. (2009) and Saad *et al*. (2009) also address to ability of grape seed extract to increase anti-oxidative defense and to control damages resulted from oxidative stresses. They point to presence of flavonoids as an essential factor in extract structure and suppose it probable that proanthocyanidin compounds existing in grape seed extract are among effective factors in incidence of anti-oxidative properties.

Irina *et al*. (2009) and Sano *et al*. (2007) pointed to decrease of Malondialdehyde and noted that 50-100 mg kg^{-1} of grape seed extract has an anti-oxidative characteristic and contribute to protect cells and control of their death.

According to results from this research, consumption of antioxidants existing in grape seed contributes to decrease damages to cells and specially accelerates restoration of pancreatic cells and subsequently increases insulin and decreases blood glucose.

In rats affected by diabetes with alloxan, increased blood glucose level can indirectly increase cholesterol, triglyceride, LDL, VLDL level of serum and decrease HDL level (Yanardag *et al*., 2002). Similarly, this accounts for to some extent undesirable changes serum lipids level in rats affected by diabetes in present study.

According to the results, disorders in lipids metabolism resulted from diabetes would be obviated by controlling blood glucose with grape seed extract. Therefore, this would also decrease HDL in addition to decreasing blood glucose, triglyceride, LDL and VLDL. This is in turn due to high level of anti-oxidative substances existing in extract of grape seed which can induce desirable metabolic changes associated to hepatic enzymes as to improve undesirable changes blood glucose and lipid levels.

CONCLUSION

According to this study, it can be concluded that one of mechanisms of hydro-alcoholic extract of grape seed effect on diabetes in rats is restoration of islets of Langerhans followed by increased insulin level.

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