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The Effect of Hydro – Alcoholic Seeds Extract of Ceratonia siliqua on the Blood Glucose and Lipids Concentration in Diabetic Male Rats

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Abstract—**In the present study, we attempted to investigate the effect of hydro-alcoholic seed extract of Ceratonia siliqua on the blood levels of glucose and lipids in diabetic male rats.**

Materials and Methods: In this experimental study, fifty-six adult male wistar rats each weighing 210-220g were randomly divided into seven groups of eight. These groups include: control group which left untreated; sham group which received distilled water; treated control group received only 600mg/kg seed extract; diabetic control group getting only streptozocine and three diabetic experimental groups 1, 2 and 3 receiving 150, 300 and 600 mg/kg extract respectively. Diabetes was induced by intraperitoneally injection (60mg/kg) of STZ. Following induction of diabetes, the extract dosages were administered to rats by gavageing method for sixteen days. Blood samples were taken from all groups and serum levels of glucose and lipids were measured by outoanalyzer. The data were analyzed by Microsoft Excel and Spss-18 software using ANOVA test. The results were expressed as mean±SEM and a P≤ 0/05 was set as significant value.

Finding: In three diabetic experimental groups 1, 2 and 3, concentration of glucose, total cholesterol and LDL-C decreased significantly in respect to diabetic control group, while triglyceride level was only declined in experimental group 2. In addition, the serum level of HDL-C in experimental group2 showed a considerable elevation.

Conclusion: According to our results consumption of hydro-alcoholic seed extract of Ceratonia siliqua can bring about a decline in blood glucose and lipids levels in diabetic male rats. The decrease in blood glucose level is probably due to the presents of fiber, phytosterols and tocopherol in the extract. Similarly these compounds could prevent the absorption of lipids and increase the concentration of bile acids, there by declining the blood lipids levels.

Key words: Certonia siliqua L. Seed, Glucose, Total cholesterol, LDL – C, HDL- C, Triglyceride, Rat.

I. INTRODUCTION

Diabetes Mellitus is an endocrine disease that causes disorders in carbohydrate, lipid and protein metabolism. This disease is defined by chronic hyperglycemia which is brought about by a deficiency in Insulin production and or by resistance to it (1). Hyperglycemia can give rise to other disorders in eyes, kidney, vessels and nervous system (2). At present, over 150 million persons suffer from hyperglycemia throughout the world; It is predicted that this number will be about 366 millions in 2030 (3). The most important and indeed the main method for treating diabetes is the use of insulin and hypoglycemic drugs; however, these substances have various unfavourite side effects (4). According to some reports, medicinal herbs can have applications in treatment of many diseases such as diabetes but their effectiveness has not been investigated properly and needs to be validated (5). One of these medicinal plants is Carob tree (Ceratonia siliqua l.) from fabaceae family which has a height of 7-12m. This evergreen tree is often monoecious and has pinnate leaves and raceme flowers (6). It has a kind of hanging pod fruit, with fleshy sheath and a length of 10 – 30 cm. Within the pod, there are 12-16 hard seeds. Ceratonia siliqua is native to Mediteranean area and is also found in Iran. It is widespread in Fars province, specially in Kazerun (around shapoor cave). In some area, Carob seeds is used like tea and coffee (7). In fact, it is a suitable replacement for cocoa, because it lacks caffeine and theobromine. Carob pods are used against cough; it is also used as styptic and its bark is applied against diarrhea (7, 8). Ceratonia pulp is prepared for treatment of hypercholesterolemia (9), as well as treating mouth inflammation(10). Similarly, Ceratonia siliqua seeds is useful to treat and improve diabetes symptoms because it has compounds such as fibers, phytosterols and tocopherol (11, 12). It has been determined that Carob seeds have no toxicity and limitation for consumption (13). Because of high prevalence of diabetes throughout the world and serious side effects of chemical drugs, it is necessary to develop and use drugs with least complications and high security degree for long term use. Since medicinal herbs are natural, and cause less complications and since they are used in traditional medicine to treat diabetes and there is no information about the effect of Ceratonia seed extract on diabetes, we decided to investigat the influence of hydro – alcoholic seeds extract of ceratonia siliqual on blood glucose and lipids levels.

II. MATERIALS AND METHOD

Animal groups

In this experimental study, 56 adult male wistar rats each weighing 210 -220g and 2/5-3 months old were divided in to 7groups of 8. Animals were kept under the same environmental conditions in 20-22c and 12 hours light and 12 hours dark cycle within standard cages. They were supplied with plenty water and food and the experiment was carried out only once. All ethical aspects were considered. Diabetes was induced intraperitoneally, by a single dose (60mg/kg) of STZ dissolved in normal saline (14). To make sure that rats were diabetic, after one week of STZ injection, blood samples were taken from their tails and glucose level

was measured by Easgluco set. Levels higher than 200mg/ dl are considered as diabetic basis and diabetic rats showed polydepssi and polyurea symptoms.

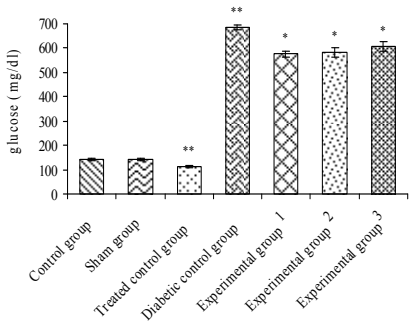
Method for preparing Hydro – Alcoholic seeds Extract of Ceratonia siliqua l.

At the beginning of may 2010, pods were collected around Kazerun city. After removing sheathes, seeds were grinded and a fine powder was obtained. One kilogram of this powder was soken in ethanol 96% and water in the ratio of 50 to 50 for 72 hours. This mixture was filtered, centrifuged for 8 min at 4500 RPM and the supernatant was dried in oven at $40c^{\circ}$. In order to produce the required dosages, various amounts of the brown extract were dissolved in distilled water. As mentioned, animals were divided in to7 groups Of 8 including: control group, shame group which received distilled water, diabetic control group which left untreated, treated control group receiving maximum dose of the extract (600 mg/kg) and 3 diabetic experimental groups 1,2 and 3 which got 150,300 and 600 mg/kg extract respectively. After 16 days, blood samples were taken from the heart under slight anesthesia by ether. Blood samples were kept for 20 minutes in lab conditions; after which, they were centrifuged for 15 minutes at 2000 RPM. The lipids and glucose levels were measured in the collected sera using auto analyzer and LDL-C was calculated by Freidewald formula as follow(15):LDL-C=(total-c)-(HDL+TG/5) .Data were analyzed by Excel, Spss-18 software and statistical test ANOVA. The results were plotted as Mean ± SEM and significant value was at $P \leq 0.05$.

III. RESULTS

According to the results the serum glucose level of the control and treated control groups were 142+6/87 and 113±4/07 mg/dl respectively whereas its level in diabetic control group and experimental groups 1, 2 and 3 were 685±8/42, 6.6±20/06, 582±14/84 and 576±11/67 mg/dl. As seen in chart1, the measured serum glucose concentration in diabetic control group increased significantly in respect to control group .

CHART 1:THE MEAN SERUM LEVELS OF GLUCOSE IN EXPERIMENTAL AND CONTROL GROUPS FOLLOWING ADMINISTERATION OF DIFFERENT CONCENTRATION OF HYDRO-ALCOHOLIC SEEDS EXTRACT OF CERATONIA SILIQUA L. SYMBOLS * AND ** INDICATE THE SIGNIFICANT DIFFERENCES.



The effect of hydro – alcoholic seeds extract of ceratonia siliqual on blood lipids concentration

The results showed no significant effect on serum lipids levels in control, sham and treated control groups. The total cholesterol levels in control and diabetic control groups were $60 \pm 2/97, 70 \pm 1/88$ mg/dl and in experimental groups1,2and3were $57 \pm 1/92, 55 \pm 2/30, 61 \pm 2/45$ mg/dl respectively. Chart 2 shows that mean total cholesterol of diabetic control group increases significantly in respect to control group; while, its concentrations in experimental groups 1, 2 and 3 declined significantly in comparison to diabetic control group . A significant increase was observed in LDL – C ($26 \pm 1/48$) in diabetic control group in respect to control group $(18 \pm 1/89)$. Similarly, LDL – C levels in experimental groups 1, 2 and 3 indicate a significant decrease in comparison to diabetic control group (Chart.3).

CHART 2: THE MEAN TOTALCHOLESTROL LEVELS AFTER USE OF DIFFERENT CONCENTRATION OF HYDRO-ALCOHOLIC SEEDS EXTRACT OF CERATONIA SILIQUA L. IN CONTROL AND EXPERIMENTAL GROUPS.

SYMBOLS * AND ** INDICATE SIGNIFICANT DIFFERENCES.

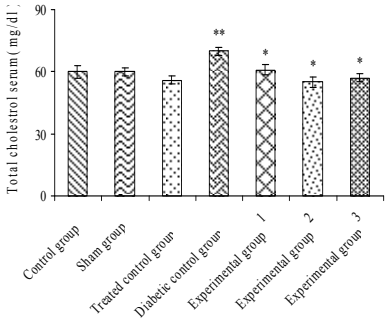
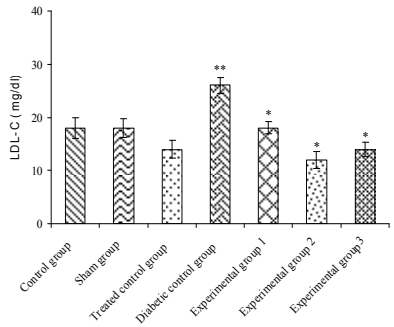


CHART 3: THE MEAN SERUM LEVELS OF LDL-C AFTER CONSUMPTION OF DIFFERENT CONCENTRATION OF HYDRO-ALCOHOLIC SEEDS EXTRACT OF CERATONIA SILIQUA L. IN CONTROL AND EXPERIMENTAL GROUPS.

SYMBOLS * AND ** INDICATE SIGNIFICANT DIFFERENCES.



Serum HDL-C level in diabetic control group was $22\pm$ 0/56 mg/dl which indicates a significant decrease in respect to control group $(25\pm0/95)$.

In addition, HDL-C level in experimental group 2 was $24\pm1/01$ mg/dl which indicates a significant increase in diabetic control group $(105\pm3/19)$ (Chart.4). Furthermore, the mean triglyceride concentration shows a significant decrease only in experimental group 2 with $92\pm3/17$ level mg/dl in comparison to diabetic control group(84$\pm$ 3/07) (Chart.5).

CHART 4: THE MEAN HDL-C SERUM LEVELS AFTER CONSUMPTION OF DIFFERENT CONCENTRATION OF HYDRO-ALCOHOLIC SEEDS EXTRACT OF CERATONIA SILIQUA L. IN CONTROL AND EXPERIMENTAL GROUPS.

SYMBOLS * AND ** INDICATE SIGNIFICANT DIFFERENCES.

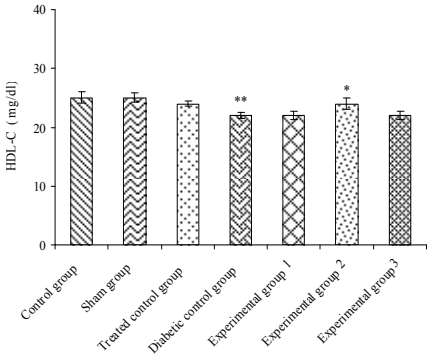
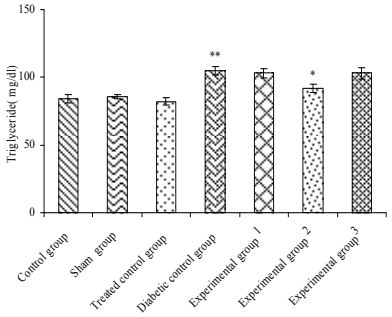


CHART5: THE MEAN TRIGLYCERIDE SERUM AFTER CONSUMPTION OF DIFFERENT CONCENTRATION OF HYDRO-ALCOHOLIC SEEDS EXTRACT OF CERATONIA SILIQUA L. IN CONTROL AND EXPERIMENTAL GROUPS.



Diabetes is considered one of the most prevalent diseases of endocrine system. As a result of this disease, the normal body metabolic functions become disordered. In spit of induced hyperglycemia, many body cells are not able to uptake glucose for nourishment (16). As expected, serum glucose concentration increased significantly following induction of diabetes in experimented rats. According to the results, hydro- alcoholic seed extract of ceratonia siliqua L can lower blood glucose concentration significantly. It has been shown that one of the main components found in seeds of ceratonia siliqua L are fibers (12). Studies indicate that consumption of high fiber foods can decrease plasma glucose concentration. Fibers are meanly carbohydrates produced by plants. However, human lack necessary enzyme to digest them. Chemical structure of polysaccharides fibers changes some features of digestive tract such as PH, folding and ionic charge. Adhesive composition of polysaccharides increases food particles adhesion which then, delays stomach offloading on one hand, and prevents sugar uptake by small intestine on the other (18-19). Lowering of serum glucose concentration can decrease insulin level . Studies indicate that usage of carbohydrates could modify blood glucose concentration through effecting the uptake of materials and influencing fermentation in large intestine (20). Similarly, food fibers can decrease blood glucose level by releasing insulin from liver and by elevating receptors sensitivity to insulin (21). Reports show that usage of seed powder of ceratonia siliqua l lowers blood sugar in rats, and it has been determined that this decrease is due to the presence of polysaccharide (particularly galactomannan) in seed endosperm. Blood glucose decrease after consuming ceratonia siliqua L seeds can also be due to the presence of polyphenols. Likewise, polyphenols can rise adhesiveness of digestive tract contents which is of importance in absorption of materials (22). In addition, it has been suggested that dietary carbohydrates play an important role in glucose homeostasis; there fore, they can control diabetes symptoms (23). Other studies indicate that tocopherol has positive effects on controlling metabolic processes in diabetic patients; this effect is due to its antioxidant effect on protein glycosylation and insulin sensitivity(24).

Furthermore, long term usage of tocopherol decreases blood sugar in diabetic and healthy people (25). There are some information about lowering plasma ALT by tocopherol and it is possible that simultaneous decline of glucose and insulin levels are the results of improvement in liver cells functions as well as decreasing liver resistance to insulin and glucose production (26). Tocopherol also in improves insulin function through its effects on plasma membrane (27).

IV. DISCUSSION

Presents of phytosterols, such as beta-sitosterol, campesterol and stigmasterol have been reported in the seeds of ceratonia siliqua l (12). Miyuki and his colleagoues (2006) gave Aloevera extract containing beta-sitosterol to diabetic and healthy rats; they observed a decrease in blood glucose level in diabetic rats (28). In another study, it was shown that plasma insulin level increases in normal and hyperglycemic rats receiving phytosterols orally. These compounds can reduce glucose concentration by stimulating pancreatic beta cells to secret more insulin in blood circulation; in this way, blood glucose level is controlled better (29).

Our results indicate that usage of hydro- alcoholic seed extract of ceratonia siliqua L. can significantly reduce blood total cholesterol, LDL-C and triglyceride in diabetic rats.

Similarly, the usage of this extract by diabetic rats shows a significant increase in HDL-C concentration. Plasma lipid concentration of diabetic patients is decreased by consumption of enough dietary fibers.

In their studies on diabetic patients, Manisha et al (2000) concluded that usage of fiber decreases total cholesterol, LDL-C and triglyceride levels, while HDL-C level showed no significant difference. They declared that the reason for this decline is an increase in bile acid due to fiber intake, which reduces cholesterol uptake (21, 30). In addition, fibers are able to bind to cholesterol and phospholipids thereby, interfere in their uptake (18). On the other hand polysaccharides bring about the usage of free cholesterol by blocking bile acids liver cycle (19). Other researches indicate that consumption of diet containing unsaturated fatty acid by rats with high cholesterol level reverses the correlation between unsaturated fatty acid and total cholesterol levels. It has been reported that materials rich in linoleic and oleic acid cause a significant decrease in total cholesterol and LDL-C concentrations; but have no significant effect on HDL-C level. It seems that saturated fatty acids elevate total cholesterol by mediating LDL-C receptor clearance and by controlling the expression of LDL-C receptors. Finally, it is possible that oleic acid normalizes the activity of LDL-C receptors and reduces cholesterol absorption(31).

As mentioned above, phytosterols such as beta-sitosterol, campesterol and stigmasterol are present in ceratonia siliqua l seed (12). These compounds can prevent the uptake of cholesterol from food and release of bily acids into the digestive tract by penetrating in to the micelles thereby lower blood fats concentrations (28). In their study on people with hypercholesterolemia, Ting et al (2004) observed a decrease in total cholesterol, LDL-C and triglyceride levels after usage of phytosterols (32).

Other researches also confirm this finding (33). Fat soluble tocopherol is among antioxidants found in ceratonia siliqua L seed (12). This compound prevents oxidation of unsaturated fatty acids; thereby changes cholesterol LDL-C, HDL-C and triglyceride plasma levels (34). According to some studies, alpha-tocopherol can reduce HDL – C and LDL- C concentrations in diabetic rats . In a study on rabbits, Singh et all observed that the use of tocopherol causes a significant decrease in serum LDL-C and cholesterol levels. Their results indicat that tocopherol can lower serum concentrations of lipoproteins and lipids by their per oxidation. Results of present study indicate that the oral administration of hydro- alcoholic seeds extract of ceratonia siliqua L to the experimental rat models suffering from diabetes mellitus can have hypoglycemic effects because it cause ideal and useful changes in serum lipids levels. By performing more research to confirm above results, one could recommend adding ceratonia siliqua seeds to diabetic patient diet.

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