Received 2017-05-03
Revised 2017-08-08
Accepted 2017-08-21

The Effect of Oral Administration of *Hypericum Perforatum* on Serum Glucose and Lipids, Hepatic Enzymes and Lipid Peroxidation in Streptozotocin-Induced Diabetic Rats

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Abstract

Background: In this research, the beneficial effects of oral administration of *Hypericum perforatum* (HP) on serum glucose and lipids, hepatic enzymes and the amount of malondialdehyde in Streptozotocin-induced diabetic rats are studied. **Materials and Methods:** In this experimental study, 32 male rats were randomly divided into 4 groups of control, treatment-control, diabetic and treatment-diabetic. HP was orally administered to treatment groups over a period of 6 weeks. Serum glucose levels, triglyceride, total cholesterol along with HDL and LDL were all evaluated prior to initiation of the treatment, and at 3rd and 6th (last) week of treatment initiation, and in the end of the treatment, malondialdehyde and aminotransferase enzymes of the liver were evaluated. **Results:** regarding serum glucose levels and body weight measured in the 3rd and 6th week, the treatment-diabetic group didn't show a significant change compared to the diabetic group, regarding serum total cholesterol and LDL levels, a significant decrease was observed and regarding serum HDL, a significant increase was documented. Furthermore, treating the treatment-diabetic group with HP did not result in any significant decrease in serum triglyceride, malondialdehyde or alanine aminotransferase but, in fact, did cause a significant decrease in aspartate aminotransferase. **Conclusions:** Oral administration of HP did in fact have a beneficial effect on lowering serum levels of total cholesterol, LDL and the hepatic enzyme aspartate aminotransferase and on raising the levels of HDL in diabetized rats with Streptozotocin. [GMJ.2017;6(4):319-29] DOI: 10.22086/gmj.v6i4.889

Keywords: Diabetes Mellitus; *Hypericum perforatum*; Blood Glucose; Lipids; Lipid Peroxidation

Introduction

Diabetes entangles multiple organs and cause an array of problems for each; among the most important long term complications which are caused by diabetes are retinopathy with impaired vision, nephropathy with renal insufficiency and failure, peripheral neuropathy and diabetic foot which leads to amputation, autonomic neuropathy and sexual dysfunctions, genitourinary complications, gastrointestinal problems, atherosclerosis and cardiovascular and cerebrovascular and peripheral arteriovenous structural damage [1]. A main cause in the development of atherosclerosis is the dyslipidemic condition in which diabetes patients are; raised triglyceride levels and lowered HDL levels are to be pointed out, and considering the liver's role in the metabolism of lipoproteins, tissue damage endured by the liver will exacerbate said conditions. In 2012, in the United States, 29.1 million people, an equivalent of 9.3% of the total population had become ailed with diabetes from which 8.1 million of them were undiagnosed [2] and this shows the silent malevolence of this illness. Cardiovascular diseases take up 65% of mortality of people suffering from diabetes, at the same time, mortality rate of those with diabetes are 2 to 4 times greater than those without said disease [3,4]. Even though the primary management of diabetes is through insulin and other hypoglycemic agents, the side effects they carry are numerous; including an increase in lipid storage, atrophy of the adipose tissue at the injection site and hypoglycemic shock, and in the long run, regardless of their effect, diabetes will still have its debilitating impact on the patient's body [5]. Even though diabetes is not fully mapped and figured out, a matter which had been subject of many researches were the effect of free radicals of oxygen and related oxidative stress which happens under certain circumstances in a patient's body afflicted by diabetes [6,7]. Therefore, a certain approach towards managing the disease can be through anti-oxidants, not as the main course of treatment, but one parallel with current treatment regimen of the disease, like said insulin and other hypoglycemic drugs.

During recent years, certain plants and their anti-oxidative properties have been evaluated and most have been proven to yield high efficacy and impact on managing one's diabetes [8]. In the modern world, herbal medicine's popularity is on the rise, due to low side effects and lower costs, one of which is *Hypericum perforatum* (HP) (a.k.a St. John's Wort plant) which has somewhat proven to have beneficial effects. HP is a flowering perennial plant species of the genus *Hypericum*, of the Hypericaceae family local to Western Europe, Asia and Northern Africa. Its most notable chemical constituents are hyperforin and hypericin, and several flavonoids like rutin, hyperoside, quercitrin, isoquercetin and quercetin which have high anti-oxidative properties along with anti-depressive ones [9-13]. In 2011, 366 million people were diabetic worldwide, and it is estimated that this number will rise to 552 million by the year 2030 [14]. Ergo, diabetes can be one of the main causes for morbidity and mortality in the future, and since it's considered a chronic ailment, pertaining cost of management on national and international scales will be catastrophic. Therefore, one possible alternative choice to drug regimens being HP and its effect on serum glucose level, serum lipid panel and hepatic enzymes and oxidative stress is the subject of investigation in this research. Our goal is to find out whether this plant yields positive outcome in regard to treating diabetes with herbal medicine which could in turn, result in reduced side effects and costs of treatment.

Materials and Methods

Animals

In this experimental study, 32 male rats of the Wistar strain (provided by Pasteur institute, located in city of Tehran), 3 months of age and in the body weight spectrum of 200-250 grams were used, all of which were kept in a 22-24 degrees Celsius cages with a diurnal/nocturnal cycle of 12hours/12hours with appropriate darkness and ventilation in groups of 4 in each cage. Subject animals had free access to tap water and specially-produced rat food (provided by "Khorak Daam Pars" co, Located in city of Karaj) for the 6 week du-

ration of the experiment. Handling and methodologies regarding this experimentation and animal care and use were followed thoroughly by the guidelines of national institutes of health of America (NIH). Also, the test subjects were chosen from those which were kept in normal environment and under a day-long fast while their post-fast serum glucose were ≤ 130 mg/dl. For that matter, blood sample was drawn from retro-orbital plexus using capillary vials and this sampling was done on all the animals. Next, the 32 chosen test subjects were randomly divided into 4 groups of control, treatment-control, diabetic and treatment-diabetic. For induction of diabetes, a single dosage of 60 mg/kg of body weight of Streptozotocin (STZ) (provided by Merck, located in Germany) diluted in cold normal saline solution was administered intra-peritoneally. A week after administration of STZ, the test subjects were assessed in order to make sure of their diabetes induction by checking urine glucose level with urine strip method, from which only those which had urine glucose level of 50mg/dl (which is the equivalent of 250mg/dl serum glucose) were chosen to be treated. In the following days, the classic manifestations of diabetes were documented among the diabetes-induced test subjects which included polydipsia, polyphagia and polyuria and weight loss. Serum glucose levels were assessed by enzymatic method of glucose oxidase (Ziest Chem, Iran) before commencement of the experiment and at weeks 2 and 4 using the spectrophotometer device. Also, total cholesterol, triglyceride and HDL cholesterol were evaluated and documented using exclusive kits (Ziest Chem, Iran) in accordance to provided guidelines. Moreover, LDL levels were assessed using the Friedewald equation by subtracting the amount of cholesterol associated with other particles, such as HDL and VLDL: $LDL \approx \text{Total Cholesterol} - \text{HDL} - (K * \text{Triglyceride})$ "K" is 0.20 if the quantities are measured in mg/dl and 0.45 if in mmol/l.

Preparation of Animal Food Containing Plant

At first, HP leaves were grinded using mechanical grinders and turned into a soft powder. Then, the powder was mixed with the test

subjects' food by a ratio of 1:6.25 Followed by adding water and creating a paste, which was then turned into appropriate sized pellets for the test subjects, then left to be dried and afterwards, was ready to be in the access of the test subjects along with their water [15].

Hepatic Enzyme Levels Evaluation

After 6 weeks at the end of the experimentation, the test subjects were ethically euthanized using diethyl ether (provided by Merck, Germany). Liver tissue was extracted and after irrigation with cold normal saline solution 0.9% NaCl and drying, a piece is dissected and weighed hastily, then again, along with cold normal saline %0.9 NaCl Solution, is put inside a tissue homogenizer (IKA, Germany) for 5 minutes and on 5000 rounds per minute (%10) and the homogenized solution, is then again, centrifuged in 4 degrees Celsius. Next, the top clear part is separated from the sediment and used for further measurements. For achieving levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), biochemical kits (provided by Ziest Chem Co, Iran) were used following specifications and guidelines provided by the company.

Malondialdehyde (MDA) Levels And Evaluation From Hepatic Tissue

In order to measure oxidative stress endured by the test subjects, a rather important biochemical marker, MDA, was assessed and measured in hepatic tissue. MDA measurements in supernatant tissue of the liver were performed using a method called TBARS (Thiobarbituric Acid Reactive Substances) in boiling point. In this method, MDA or similar compounds react with the Thiobarbituric Acid (TBA) and produce a pink-coloured gas with a maximum light-absorbance at 532 nanometer wavelength. This reaction occurs in an acidic environment with a pH around 2-3 and at about 90 degrees Celsius. After cooling down, light absorbance with spectrophotometer was evaluated and a standard slope measurement was created in accordance to different dilutions of tetraethoxypropane, then the absorbance values generated by the spectrophotometer were collated and compared with the standard slope [15,16].

Protein Evaluation of Hepatic Tissue

For this matter, Bradford protein assay method was chosen, in which bovine serum albumin is used as a standard and spectrophotometry is performed on the homogenized liver tissue, prepared as mentioned above [15].

Statistical Analysis

Statistically, all results were documented and published as Mean $\pm$ Standard Error of the Mean (SEM). After determining data distributions, in order to compare each parameter in any given group before and after the conducted experiment, Analysis of Variance (ANOVA) model method were used along with repetitive measuring, and for comparing any given group with another at any point along the experiment, one-way ANOVA test paired with Tukey's Test using SPSS software v16. Significant value for all analyses were appointed at $P < 0.05$.

Results

Body Weight

Body weight of the diabetic group, as expected, had lowered when measured at weeks 3 and 6, in comparison to their body weight a week prior to the beginning of the study and it was statistically significant at $P < 0.05$, and finally, the diabetic-treatment group, again, had lowered when measured at weeks 3 and 6, in comparison to their body weight a week prior to the beginning of the study and it was statistically significant at $P\text{-value} < 0.05$ (Figure-1).

Serum Glucose Levels

In the diabetic-treatment group, the increase in serum glucose levels at week 3 and 6 were also statistically significant ($P\text{-value} < 0.001$) and compared to the diabetic group, no statistically significant reduction is to be seen (Figure-2).

Serum Lipids

In the diabetic group, triglyceride levels in weeks 3 and 6 compared to a week prior to the experiment showed a significant difference ($P\text{-value} < 0.01$). In the diabetic-treatment group, a statistically significant rise in serum triglyceride levels were seen ($P\text{-value} < 0.01$) compared to levels evaluated at a

week prior to the study, statistically insignificant rise at week 6 compared to the week prior to initiation of the study (Figure-3). In regard to total cholesterol levels, in the diabetic group, a statistically significant rise in total cholesterol levels were documented when compared the values at 3rd week to the value documented at a week prior to the experiment ($P\text{-value} < 0.005$), and kept increasing to, yet again, a statistically significant level at 6th week of the study ($P\text{-value} < 0.005$). in the diabetic-treatment group, there was a significant rise to total cholesterol levels when compared the values from the 3rd week to the value acquired at week prior to the experiment ($P\text{-value} < 0.01$) and by week 6, there was an increase, but not statistically significant. Moreover, when comparing the two groups (diabetic and diabetic-treatment), a statistically significant difference was documented wherein the diabetic-treatment group had lower levels of total cholesterol both by 3rd and 6th week (respectively $P\text{-value} < 0.05$ and $P\text{-value} < 0.005$) (Figure-4). In accordance to HDL levels, in the diabetic group, acquired HDL values at weeks 3 and 6 shown a significant change when compared to its value acquired at a week prior to the study ($P\text{-value} < 0.01$). in the diabetic-treatment group, a significant rise was documented in HDL levels acquired at 3rd week when compared to baseline evaluated at a week prior to the study ($P < 0.05$) but the HDL evaluated at the 6th week in the same group, showed an increase, but not significantly different when compared to baseline evaluated at a week prior to the study. The HDL levels evaluated in these 2 groups, when compared to each other, showed a significant increase in the diabetic-treatment group when compared to the diabetic group at weeks 3 and 6 ($P\text{-value} < 0.005$) (Figure-5). When analyzing LDL levels, in the diabetic group there was a significant rise documented when comparing values acquired at 3rd week and a week prior to the study ($P\text{-value} < 0.005$) and this difference at 6th week was also significant ($P\text{-value} < 0.05$). In the diabetic-treatment group, there was a significant rise in LDL levels at 3rd week compared to the baseline at a week prior to the

study (P-value <0.01), however, the value of LDL acquired at week 6 showed no significant difference with the baseline. On the other hand, LDL levels in the diabetic-treatment group, when compared to the diabetic group, was significantly lower both at 3rd and 6th week of the experiment (respectively P-value <0.005 and P-value <0.001) (Figure-6).

Hepatic MDA Levels

The diabetic group had significantly higher levels of MDA compared to the control group. Also, MDA levels in the diabetic-treatment group showed a rise when compared to the control-treatment group, but not statistically significant. Moreover, When comparing the diabetic and diabetic-treatment groups, the latter had lower levels of MDA, but not statistically significant (Table 1).

Hepatic Aminotransferase Analyses

Levels of AST were significantly elevated in the diabetic group compared to the control group. Also, when comparing the control-treatment to diabetic-treatment regarding AST levels, there was a significant rise in the latter. When comparing the diabetic and diabetic-treatment groups, the latter also had significantly lower levels of AST (P-value <0.05) (Table-1). Levels of ALT were insignificantly elevated in the diabetic group compared to the control group. Also, when comparing the control-treatment to diabetic-treatment regarding AST levels, there was a rise in the latter, but statistically insignificant. When comparing the diabetic and diabetic-treatment groups, the latter also had lower levels of AST, but again, insignificantly (Table-1).

Discussion

The results of this study show that treating the diabetic test subjects would not lower levels of serum glucose or a significant improvement in their body weights, but in regard to total cholesterol and LDL levels, the decrease is significant along with a significant rise in HDL levels, which would in turn mean beneficial to the test subjects' overall health. And even though using HP didn't result in significant decrease in triglyceride levels or MDA levels or ALT, but

it did, in fact, significantly lower AST levels. Prior studies had shown that induction of diabetes by means of injection STZ intraperitoneally would result in certain degenerative changes in the Langerhans islet cells of the pancreas, which would eventually lead to a sustained loss of insulin-secreting cells to which the results are highly elevated levels of blood glucose and noticeable weight loss in the subjects, which are evident in this study as well. Moreover, after inducing diabetes, malevolent changes occur in the lipid and lipoprotein metabolism which will lead to certain array of disorders in the various functions that happen mainly in the liver regarding absorption of free fatty acids from the serum, oxidation of said FFA's and metabolization of them to certain metabolites, cholesterol and phospholipid synthesis and their endocinous secretion [17,18]. Moreover, in previous studies, increases in triglyceride and total cholesterol levels in induction of diabetes in mice with STZ had been documented [19,5], much like the present experimentation. On the other hand, diabetes induction in mice with alloxan or STZ have shown to indirectly increase levels of serum total cholesterol, triglyceride, LDL and VLDL and decrease in serum HDL levels by means of hyperglycemic events [20], which in terms can explain the array of malevolent disturbances in lipid panels of current test subjects in this study. Also, when diabetes sets in, free radicals of oxygen in various tissues lead to events of oxidative stress and lipid peroxidation, to which the best indicator is MDA which had shown a significant rise in the liver tissue in this study [15,21]. Needless to say, higher levels of MDA means a progression towards oxidative stressed endured by the tissue, liver in this case, and lipid peroxidation. Additionally, diabetes state effects the function of several hepatic enzymes, most importantly aminotransferases [22], which was also seen in the current study. In regards to beneficial outcomes of oral usage of HP, it's been previously proven to lower free radicals of oxygen such as superoxide and hydroxyl, protect cells against chemical damage due to environmental toxins, decrease lipid peroxidation in numer-

ous tissues and shield hepatocytes against chemical stress due to presence of certain antioxidants like flavonoids in this particular plant [23]. Therefore, consuming HP is still considered protective towards several tissue types, and effective on the oxidative stress events in diabetes mellitus state, and on biochemical changes in the blood [24].

Conclusion

Administration HP to diabetic rat resulted in lower levels of total cholesterol and LDL and AST while increasing HDL levels, and it

had no effect on serum glucose, Triglyceride, MDA and ALT levels.

Acknowledgments

This study was the result of MD thesis of Dr. Neda Mehdizade that financially supported by Shahed University (IR.shahed.REC.1394.47), Tehran, Iran.

Conflict of interest

The authors declared no conflict of interest.

Figure-1 – Body weight changes prior to and throughout the experiment in all four study groups, *P<0.05 ** P-value <0.01 compared to baseline of the same group

Figure-2 – Serum glucose changes prior to and throughout the experiment in all four study groups, * P-value <0.001 compared to a week prior to the experiment

Figure-3 – Serum triglyceride changes prior to and throughout the experiment in all four study groups, * P-value <0.05 ** P-value <0.01 compared to baseline of the same group

Figure-4 –Total cholesterol levels changes prior to and throughout the experiment in all four study groups, * P-value <0.05 ** P-value <0.01 compared to baseline of the same group. #P<0.05, ##P<0.005 compared to diabetic group of the same week

Figure-5 –HDL levels changes prior to and throughout the experiment in all four study groups, * P-value <0.05 ** P-value <0.01 compared to baseline of the same group. # P-value <0.005, ## P-value <0.001 compared to diabetic group of the same week

Figure-6–LDL levels changes prior to and throughout the experiment in all four study groups, * P-value <0.05 ** P-value <0.01 *** P-value <0.005 compared to baseline of the same group. # P-value <0.005, ## P-value <0.001 compared to diabetic group of the same week

Table-1: Hepatic MDA and Aminotransferase Levels After Experiment in All Four Study Groups

Study Groups	Control	Control+Treatment	Diabetic	Diabetic+Treatment
Hepatic Markers				
MDA (µg/mg of Protein)	24.87	27.16	46.73#	37.41
AST (IU/dl)	11.69	11.91	24.09*	15.06**
ALT (IU/dl)	22.11	25.78	29.32	27.32

P-value <0.005 compared to the control group, * P-value <0.05 ** P-value <0.05 compared to the control group

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