ORIGINAL ARTICLE

Comparison of Hypoglycemic Activity of Berberis Lycium Royle Stem Bark and Glimepiride in Type 2 Diabetes

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ABSTRACT

Objective: To compare the hypoglycemic activity of aqueous extract of stem bark of Berberis lycium Royle and glimepiride –a sulphonylurea in a type 2 diabetes mellitus induced male mice model.

Study Design: Randomized control trial.

Place and Duration of Study: This study was carried out in the animal house of National Institute of Health (NIH), Islamabad from 7th November 2013 till 21st January 2014.

Materials and Methods: Fifty albino Balb/C male mice were divided randomly into groups I-V (n=10). Group I served as normal control group. In rest of the forty mice from group II-V, type 2 diabetes mellitus was induced by administration of high fat diet (HFD) for two weeks followed by low dose (40 mg/kg) intra-peritoneal streptozotocin (STZ) injections for four consecutive days. Group II served as the disease control group, group III received the aqueous extract of stem bark of Berberis lycium Royle in dose of 50 mg/kg body wt. while group IV received the aqueous extract of stem bark of Berberis lycium Royle in dose of 100 mg/kg body wt. Group V was administered glimepiride in a dose of 2mg/kg body wt. herb extract and the drug was given orally once a day. Samples were taken at the end of five weeks for blood glucose and glycosylated hemoglobin (HbA1c %).

Results: The blood samples estimated for fasting blood glucose (FBG) and glycosylated hemoglobin (HbA1c %) levels showed that the aqueous extract of stem bark of Berberis lycium Royle in a high dose (100 mg/kg body wt.) showed the maximum lowering of FBG and HbA1c% levels followed by its low dose (50 mg/kg body wt.) Glimepiride also lowered the FBG and HbA1c% to normal limits but its extent was less than the aqueous extract of stem bark of Berberis lycium Royle.

Conclusion: The aqueous extract of stem bark of Berberis lycium Royle lowers the FBG and HbA1c levels in a type 2 diabetes induced male mice in a dose dependent manner.

Key words: *Berberis lycium Royle, Glimepiride, Streptozotocin, Type 2 Diabetes Mellitus Mellitus.*

Introduction

Diabetes mellitus once considered a single disease, is now known as a clinical syndrome[1] of multiple etiology, characterized by chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism resulting from defect in either insulin secretion, action or both.[2] Pharmaceutical companies have been working to discover the newer drugs to control it for quite long. Unfortunately; like the thorns are attached to roses, these drugs also bring with them some degree of adverse effects. Modern medicine has been famous for its efficient role in therapeutics but the side effects have always been an issue.[3] Currently the trends have started to shift more towards the natural products to combat the present increasing health issues.[4]

Berberis lycium Royle (family Berberidaceae) is a famous herb, known long for its medicinal value.[5] It is known as Barberry in English, Sumbloo in Urdu, Ziar largay in Pushto.[6] In Pakistan, it is abundantly found in Margalla Hills.[7] It is also distributed in northern areas such as Gilgit, Baltistan, Ghizer, Astor, Diamer and Swat, Khyber Pakhtunkhwa.[8] Berberis lycium Royle as an anti-diabetic agent has also been investigated. Studies have been conducted on its root, stem, leaves, fruit and root bark, in crude and extracted forms. Its stem bark has not been investigated for its glucose lowering property although the stem bark is readily available in local market and berberine is present in highest concentrations in roots followed by stem bark. The stem bark contains 4.2% alkaloids as compared to 5% in roots.[9] Berberine is known to possess a considerable anti-diabetic activity.[10] The anti-diabetic activity of Berberis lycium Royle has been compared with the current anti-diabetic agents like insulin, gliclazide, glibenclamide.[10,11] In the present study, aqueous extract of stem bark of the herb was selected and its blood glucose lowering properties were compared to another oral anti-diabetic drug; glimepiride.[10]

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Materials and Methods

A randomized controlled study was carried out in the animal house of National Institute of Health (NIH), Islamabad from 7th November 2013 till 21st January 2014. A total of fifty healthy male albino Balb/C mice, weighing 28-38g and aged between 6-8 weeks, having fasting blood glucose (FBG) levels not more than 110 mg/dl and HbA1c <6.0 were included in study. All mice were acclimatized for one week. Then they were randomly divided in five groups (group I-V), each group containing 10 mice (n=10). Group I (n=10) served as the normal control group. In rest of forty mice (group II-V), type 2 diabetes mellitus was induced by administration of high fat diet(HFD) for two weeks followed by low dose intra-peritoneal injection of streptozotocin (STZ), once daily for four consecutive days.[12,13] It was ensured to administer the freshly prepared STZ injections to mice. A persistent FBG level >250mg/dl was selected as the cut off point for the confirmation of diabetes.[14] Group II was the diabetes control group to which no drug or herb was given. Group III received 50 mg/kg body wt. (low dose) of aqueous extract of stem bark of Berberis lycium Royle while the group IV received 100 mg/kg body wt. (high dose) of aqueous extract of stem bark of Berberis lycium Royle. The group V received the drug; glimepiride 2 mg/kg body wt. The herb and the drugs were given orally once daily for five consecutive weeks. Mice were housed under the controlled conditions of room temperature 20+2o C, relative humidity 50%-70% and 12-h light-dark cycle. They were provided free access to water ad libitum. All mice received the care in accordance with the NIH guidelines. The stem bark of Berberis lycium Royle was collected from village Prang, Charsadda. It was identified by a botanist Ghulam Jillani at Herbarium section of Botany department, Peshawar University. It was then washed with water thoroughly and shade dried. It was grounded into a fine powder with the help of an electrical grinder and taken into a non-metallic jar. The bark powder was soaked in distilled water for 72 hours with periodic stirring. It was then filtered using Whatmann filter paper no 1.The filtrate was evaporated at 55 0C in a rotary evaporator at the research laboratory of Riphah Institute of Pharmaceutical Sciences (RIPS), Islamabad. The extract was obtained as a dark brown semi-solid sticky paste. It was stored in air tight glass bottles, protected from light and kept in refrigerator at 2-8 oC to be used throughout the experiment. The yield of aqueous extract of stem bark of Berberis lycium Royle with respect to the original dry plant material was about 25%.[15] Blood samples were taken at the mid-cycle i.e. week 5 for the confirmation of diabetes mellitus and the end of week 10 for final sampling. The 6-hr fasting blood samples were preferred as blood glucose levels vary widely together with food intake during a typical day.[16-18] Fasting blood glucose (FBG) levels were measured using glucose oxidase/ GOD POD method while glycosylated hemoglobin (HbA1C) of the mice were determined by cation exchange resin method.[19,20] Descriptive statistics were applied using one way ANOVA test on SPSS 20. The level of significance was pre-defined as <0.05 ($p<0.05$).

Results

The final blood sampling at the end of week 10 i.e. termination of study, showed the following results: Significant difference was observed between group II and III at the end of week 10 regarding the mean FBG levels determined by (457.3+19.6 vs. 87.2+1.8) $p<0.05$ and mean HbA1c% (9.8+0.5 vs. 4.7+0.1) $p<0.05$ as shown in figure 1 and 2. Thus it indicated that the low dose (50mg/kg body wt.) of aqueous extract of stem bark of Berberis lycium Royle significantly decreased the mean FBG and HbA1c levels in diabetic mice as compared to disease control group. Significant difference was observed between group II and IV at the end of week 10 in their mean FBG levels by Kit method (457.3+19.6 vs. 77.4+2.0) $p<0.05$ [table 8.7a] and the mean HbA1c% of group IV was statistically reduced (9.8+0.5 vs. 4.4+0.1) $p<0.05$ as shown in figure 1 and 2. Thus it indicated that the high dose (100mg/kg body wt.) of aqueous extract of stem bark of Berberis lycium Royle significantly decreased the mean FBG and HbA1c levels in diabetic mice as compared to disease control group. Significant reduction in the mean FBG (457.3+19.6vs.96.7+2.1) $p<0.05$ and HbA1c% levels (9.8+0.5vs.5.2+0.1) $p<0.05$ was observed in group V at the end of week 10 in comparison with group II (diabetes mellitus control group) as shown in figure 1 and 2.

Discussion

In this study, the hypoglycemic activity of stem bark of Berberis lycium Royle was observed and

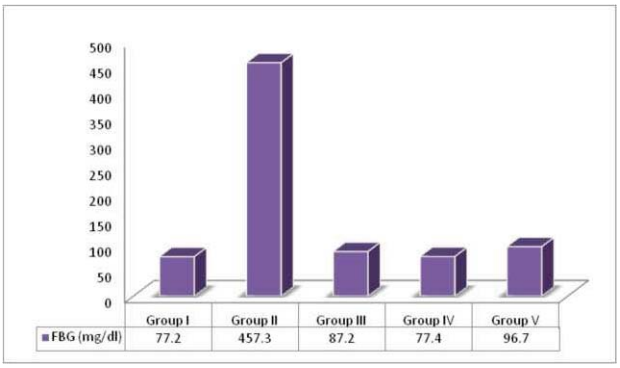

Fig 1: Effect of herb extract and drug on FBG levels of group I-V (N=50)

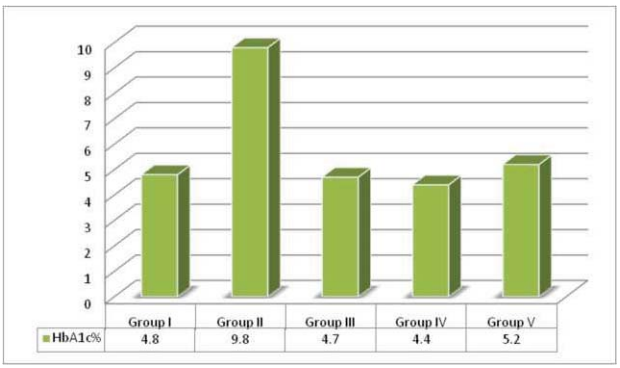

Fig 2: Effect of herb extract and herb on HbA1c levels of group I-V (N=50)

compared with glimepiride. The results indicated that the aqueous extract of stem bark of Berberis lycium Royle has a significant hypoglycemic effect ($p<0.05$), in a dose-dependent manner. FBG levels were lowest in the group receiving aqueous extract of Berberis lycium Royle stem bark at a dose of 100mg/kg body wt. ($p=0.00$) The levels were even lower than those of the normal control. However, statistically insignificant difference ($p>0.05$) was observed among the group III, IV, V in their FBG and HbA1c levels. These results correlate with the study carried by Gulfraz and Mahmood which showed hypoglycemic activity of methanolic extract of root of Berberis lycium Royle.[10] These results also correlate with the study done by Maqsood Ahmed which showed the glucose lowering ability of powdered root bark of Berberis lycium Royle and its extracts.[11] The other parameter of the study was the glycosylated hemoglobin (HbA1c) levels. The aqueous extract of Berberis lycium Royle stem bark also decreased the level of glycosylated hemoglobin (HbA1c%) in a dose dependent manner. High dose (100mg/kg body wt.) produced marked reduction in HbA1c level as followed by low dose (50mg/kg body wt.) ($p<0.05$) These results are in accordance with the work of Gulfraz and Mahmood on the extract of Berberis lycium Royle root.[10] Glimepiride also reduced the FBG and HbA1C% upto the normal levels but to a lesser extent then the herb stem extract. The glucose lowering effect of aqueous extract of stem bark of Berberis lycium Royle is probably due to presence of an alkaloid- berberine in stem.[21] A study by Yin J and co-workers in 2002 demonstrated the blood glucose lowering activity of berberine was similar to that of metformin.[22] Another study showed that berberine decreases blood glucose levels by increasing glucose transport by enhancement of GLUTs.[23] Berberine has also found to stimulate the activity of AMPK (AMP mediated protein kinase) by mitochondrial inhibition and thus enhancing the GLUT-4 and GLUT-1 translocations resulting in insulin independent mechanism of glucose consumption.[24] Further studies should be done to investigate the pharmacokinetic properties and drug interactions of the aqueous extract of stem bark of Berberis lycium Royle. So the desired effects produced by the herbal extract can be promptly achieved. Due to financial constraints, study could not be extended upto or beyond 12 weeks to further validate the HbA1c% levels.

Conclusion

The aqueous extract of stem bark of Berberis lycium Royle significantly lowered the fasting blood glucose and HbA1c levels in a diabetes mellitus type 2 induced male mice model in a dose dependent manner. The glucose lowering effects of the aqueous extract of stem bark of Berberis lycium Royle in type 2 diabetes mellitus induced male mice were comparable with the glucose lowering effects of glimepiride. Although the extent of the glucose lowering effects of extract was greater than glimepiride.

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