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ORIGINAL ARTICLE

Comparison of Anti-Diabetic Activity of Berberis lycium Royle Stem bark (Barberry) and Pioglitazone in Type 2 Diabetes Induced Mice Model

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ABSTRACT

Objective: To compare the anti-diabetic activity of aqueous extract of stem bark of *Berberis lycium Royle* and Pioglitazone –a thiazolidinedione in a type 2 diabetes mellitus induced male mice model.

Material and Methods: This Randomized control trial was carried out in the animal house of National Institute of Health (NIH), Islamabad for 10 weeks. Fifty albino Balb/C male mice were divided randomly into groups I-V (10 in each group). 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 Pioglitazone in a dose of 30mg/kg body wt. The herb extract and the drug was given orally once a day for six consecutive weeks. Samples were taken at the end of ten weeks.

Results: The blood samples estimated for fasting blood glucose (FBG) & 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.) maximally lowered the FBG and HbA1c% levels followed by its low dose (50 mg/kg body wt.) Pioglitazone also reduced 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, Diabetes Mellitus type 2, Pioglitazone, Streptozotocin,.

Author`s Contribution
[1]Conceived the topic of research and designed the study,[2]Literature review and manuscript writing,[3]Data Analysis and Discussion

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Introduction

Around the world, Diabetes affects 2-3% of the total population. So, Diabetes Mellitus is now a clinical syndrome.[1] It is characterized by long standing hyperglycemia along with the disturbances of carbohydrate, fat and protein metabolism due to a defect in either insulin secretion, action or both.[2] Everyday new

researches are taking place to discover more efficacious drugs. Thus modern medicine has been famous for its efficient role but the side effects have always been a grim.[3] So the trends have gradually started shifting towards the use of natural products.[4] *Berberis lycium Royle* (family Berberidaceae) is a famous medicinal herb.[5] It is known as Barberry in English.[6] *Berberis lycium Royle* is found in Pakistan, Indian Occupied Kashmir, Bhutan, Japan, China, Nepal, United Kingdom, Ireland, Turkey and parts of Asia, South America, Southern Argentina, Chile and Europe.[1,2] 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 been investigated. Studies have been conducted on its different parts like the root, stem, leaves, fruit and root bark, both in crude and extracted forms. Its stem bark has not been investigated yet, despite the fact that the stem bark is readily available in local market. *Berberine*-the active ingredient with the anti-diabetic potential, is present in highest concentrations in roots followed by stem bark.[9],[10] So far 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 anti-diabtic property was compared to another oral anti-diabetic drug; Pioglitazone.

Material and Methods

A randomized controlled study of ten weeks' duration was carried in the animal house of National Institute of Health (NIH), Islamabad. 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 and then they were randomly divided in five groups (group I-V), each group containing 10 mice in total. 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 freshly prepared Streptozotocin (STZ), once daily for four consecutive days.[12,13] 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; pioglitazone in a dose of 30mg/kg body wt. The herb and the drug were given orally once daily for six consecutive weeks. Mice were housed under the controlled conditions of room temperature 20 ± 2^{o} C, relative humidity 50%-70% and 12-h light-dark cycle. They were provided free access to water *ad libitum*. All mice were handled in accordance to the NIH guidelines.

The stem bark of *Berberis lycium Royle* was collected from village Prang, Charsadda. It was identified by a botanist at Botany department, Peshawar University. It was then washed thoroughly with water and shade dried. It was grounded into a fine powder with 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 ^{0}C 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 ^{o}C 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 end of week 4 for the confirmation of diabetes mellitus and at 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[1] 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 & III at the end of week 10, regarding the mean FBG levels (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 I & II. It was thus reported 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 & 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 and mean HbA1C% of group IV (9.8±0.5 vs 4.4±0.1) p<0.05 as shown in figure I & II. Thus, it is observed 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.96.1±2.4) p<0.05 and HbA1c% levels (9.8±0.5vs.5.1±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 I & II.

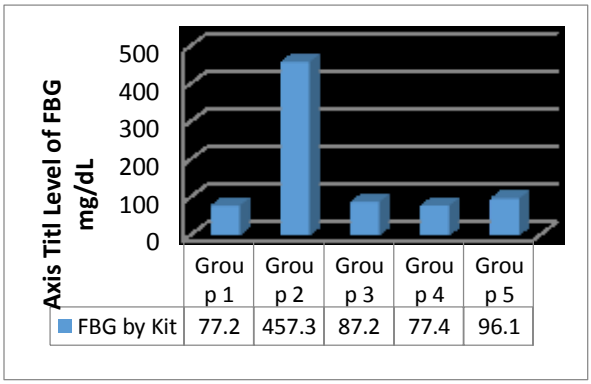

Figure I: Effect of herb extract and drug on FBG(mg/dl) levels of group I-V (n=50)

Maximum reduction was observed in high dose group, followed by low dose group and then pioglitazone group. Statistically difference was insignificant (p>0.05) among the group III, IV, V in their FBG and HbA1c levels.

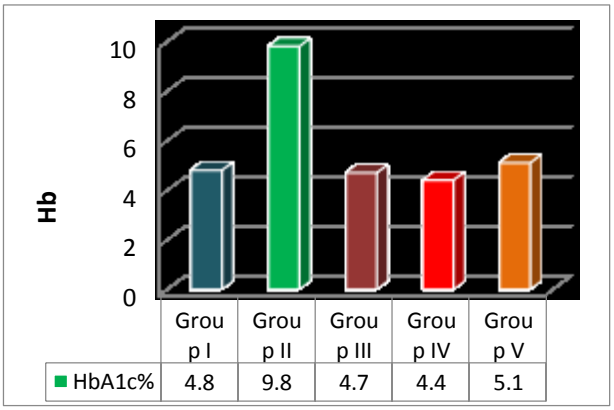

Figure 2: Effect of herb extract and herb on HbA1c levels (%) of group I-V (n=50)

Discussion

Naturopathy is the current trend now.[21] In this study, the hypoglycemic activity of stem bark of *Berberis lycium Royle* was observed and compared with pioglitazone. 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 reported 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 followed by the low dose (50mg/kg body wt.) (p<0.05) These results again are in accordance with the work of Gulfraz and Mahmood on the extract of *Berberis lycium Royle* root.[10] Pioglitazone 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.[22] A study by Yin J and co-workers in 2002 demonstrated the blood glucose lowering activity of *berberine* was similar to that of metformin.[23] Another study showed that *berberine* decreases blood glucose levels by increasing glucose transport by enhancement of GLUTs.[24] *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.[25]

Further studies are required to investigate the pharmacokinetic properties and drug interactions of the aqueous extract of stem bark of *Berberis lycium Royle* so that the desired effects produced by the herbal extract can be promptly achieved.

Conclusion

The aqueous extract of stem bark of *Berberis lycium Royle* significantly lowered the fasting blood glucose and HbA1c levels in 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 Pioglitazone. Although the extent of the glucose lowering effects of extract was greater than Pioglitazone.

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Study Limitations: Due to financial constraints, study could not be extended upto or beyond 12 weeks to further validate the HbA1c% levels

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