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Immunotherapy-induced hyperglycemia: Protective effects of *Berberis lycium* Royle and *Withania somnifera* extracts in rats

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Abstract
Levamisole (LEVA) and cyclophosphamide (CYP) are two of the most popular Immunomodulatory medications used to treat several serious diseases. However, these Immunomodulatory has been proven to increase vulnerability to type 1 diabetes mellitus. The purpose of the present study was to investigate the incidence of hyperglycemia during immunotherapy with cyclophosphamide and levamisole, and the possible benefit of providing plant extracts of *Berberis lycium* Royle and *Withania somnifera* as hypoglycemic agents. CYP and Levamisole were introduced in rats along with root extract of *Berberis lycium* Royle (BLR), and *Withania somnifera* (WS) to evaluate the average glucose level in rats and the HbA1c level was measured after 28 days. The result of the study showed a significant ($p<0.05$) increase in percent HbA1c level in the CYP group, LEVA group, and at its highest level in the CYP+LEVA group of rats compared to the groups treated with plant extracts. The HbA1c levels was found to be at the control level or non-diabetic range in the groups treated with plant extract. It was suggested that CYP and LEVA may have had an impact on glycemic control in the rats receiving immunotherapy therefore, patients with diabetes who are receiving cyclophosphamide immunotherapy may benefit from the combination of the two plant extracts.

Keywords: CYP-Cyclophosphamide, LEVA-levamisole, Diabetes, HbA1c, BLR-*Berberis lycium* Royle, WS-*Withania somnifera*

Introduction
According to WHO research, 65-80% of the poorer nations rely on medicinal plants for primary healthcare and medicines. The utilization of natural substances as antidiabetic medicines has attracted much interest in recent years. Around 800 plant species have been identified, and reports of their phytochemicals' hypoglycemic activity (Udayakumar*et al*., 2009) [19].

In the northern-western Himalayas between 850 and 3500 meters in Uttarakhand, *Berberis lycium Royle* (Indian lyceum, kilomda, Daruhaldi) is a commonly used traditional medicinal plant. It is a member of the *Berberidaceae* family. This plant's entire body has some therapeutic significance. Different ayurvedic treatments employed berries, root, bark, and stem (Bhattacharya *et al*., 1990) [5]. The locals of Uttarakhand used the berries and other components of this plant to treat diabetes, wounds, wound infections, diarrhea, and other conditions. The *Solanaceace* family member *Withania somnifera*, sometimes known as Indian ginseng or ashwagandha, has a highly distinct nomenclature in the Indian traditional medical system (Ayurveda). It is sometimes referred to as the "mother herb of Ayurveda" due to its widespread use in multi-herbal or mineral formulations for the treatment of various diseases. According to Sehgal *et al*. (2012), with anolides, which are regarded as the plant's trademark metabolite and are only generated in a select few genera of the *Solanaceae* family, are the primary pharmacological ingredients that contribute to biological activity Gupta & Singh, 2014) [11]. Withaferin A, Withanolides A, Withanolides D, and Withanone are the most common with anolides in plants. It is described as antidiabetic, antioxidant, anti-inflammatory, antimicrobial hepatoprotective, nephroprotective etc (Mishra *et al.*, 2000, Akhtar *et al.*, 2020, Melo *et al.*, 2021) [14, 13]. Cyclophosphamide and levamisole are the commonly immunomodulatory drugs used in the treatment of various major disease like cancer, organ transplantation, and hypersensitivity (Germoush & Mahmoud 2014, Cuce *et al*., 2015). Previous reports suggested that there is a possible relation between the cyclophosphamide-based treatment and type 1 diabetes (Ablamunits *et al.*, 1999, Reddy *et al*., 2006, Brode *et al.*,

2006, Kaur *et al*., 2010 García-Sáenz *et al*., 2018) [3, 15, 6, 12, 8].
The Immunomodulatory action of cyclophosphamide shown to promote susceptibility to type 1 diabetes mellitus in animal models. However the exact mechanism is not fully understood but there are reports that cyclophosphamide may temporarily depletes the suppressor cell and causes the destabilization of the local immune regulatory balance (Caquard *et al*., 2003, Gehi *et al*., 2003, Gaacia-saenze *et al.*, 2018) [7, 9, 8]. However contradictory role of cyclophosphamide in combinational therapy with glucocorticoids has been reported to be effective in treatment of type 1 diabetes (Yang *et al*., 2017) [21]. HbA1c is considered as a reliable measure for monitoring and managing chronic diabetes and is also recommended for the screening of pre-diabetes (Sherwani*et al.*, 2016) [16].
There have been reports of the hypoglycemic effects of berberine and *Withania somnifera*. According to Udayakumar *et al* (2009) [19], The HbA1c levels of alloxan-induced diabetic rats decrease after receiving *Withania somnifera* extract. However, Studies on *Berberis lycium* Royle, both alone and in combination with *Withania somnifera,* are lacking, though. Additionally, there are no known interactions between CYP and *Berberis lycium* Royle and any medications or herbal remedies. The purpose of this study was to investigate the incidence of hyperglycemia during immunotherapy with cyclophosphamide and levamisole, and the possible benefit of providing plant extracts of *Berberis lycium* Royle and *Withania somnifera* as hypoglycemic agents.

Materials and Methods

Chemicals and plant materials

Cyclophosphamide was bought From Sigma Eldrich Pvt. Ltd. HbA1c diagnosis kit was purchased from Erba Diagnostic Pvt.Ltd. The plant root of *Withania somnifera* were procured from the Medicinal Plant Research and Development Centre in Pantnagar, Uttarakhand and the root of *Berberis lycium* Royle were collected from village. Narendranagar, Uttarakhand and identified at Forest Research Institute, Dehradun, Uttarakhand.

Plant extract preparation

The roots of *Berberis lycium* Royle (BLR) and *Withania somnifera* (WS) were dried at room temperature in shady areas and ground into a fine powder. 50g of each powder sample was soaked in hydro-ethanol (1:1) at room temperature for 48 hours in a rotating shaker. With muslin cloth and then filter paper, the extract was filtered. 2-3 times were needed to complete this process in order to get a clear extract. The samples were held in a water bath at 50 °C for drying until the solvent evaporated, at which point semi-solid extracts were made.

Animals

Wistar rats of 2 to 2.5 months old were procured from ICAR-Indian Veterinary Research Institute, Bareilly, India. Experimental study was done accordance with institutional practice under the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals; (CPCSEA), India. The animals were fed with standard diet and water *ad libitium* throughout the experimental period and kept at 20-25 °C under a 12 hr/dark cycle.

Experimental design

Animals were divided into ten groups, five rats in each group and were treated as shown in Table1.
The aqueous suspension (1ml) of *Withania somnifera* and *Berberis lycium* Royle root extract were administered orally to the rats by gauzing every morning before meals for 28 days in doses of 200 mg/kg and 400 mg/kg, respectively. All groups, excluding the control and levamisole groups, received an oral dose of cyclophosphamide water suspension (1 ml) containing 200 mg/kg/body weight on days 9 and 16. The behavior and mortality of each animal was investigated. 28 days into the study, a blood sample was taken, and the HbA1C level was determined.

Statistical analysis

The experimental data were analyzed by statistical methods using a fully randomized design and one-way analysis of variance (ANOVA) using the programme SPSS version 16.0. All results were expressed as mean SEM. It was deemed statistically significant at a level of $p<0.05$.

Results and discussion

Withania somnifera and *Berberis lycium* Royle plant root extract supplementation did not result in any animal deaths or abnormal behavior during the course of the trial. Figure 1 shows the effects of various treatments on rats' HbA1c levels (%) after 28 days. Data analysis on the 28th day of treatment revealed that, when compared to the control group of rats, the HbA1c% level increased significantly in the cyclophosphamide (8.68%), levamisole (8.68%), and CYP+ LEVA (9.3%) groups of rats. However, the HbA1c level was discovered to be in the control or non-diabetic level, which lies between 6.06-7.26%, in the group of rats treated with plant extract. These results suggested that the cyclophosphamide and levamisole might have affected the glycemic control in rat during the study. Levamisole stimulated the immune system while also affecting glucose hemostasis. In rats, oral administration of a plant extract along with cyclophosphamide reduced the risk of diabetes development and maintained good glycemic control; however, the combined groups (CYP+WS+BLR200/400) significantly maintained glycemic control with HbA1C levels of 5.16% and 5.2% as compared to the other group of rats. Therefore, the two plant extracts together may be advantageous for diabetic patients receiving cyclophosphamide-based therapy. The research focused on the role of *Berberis lycium* Royle and *Withania somnifera* root extract in the immunotherapy-related prevention of type 1 diabetes.
Since earlier research has shown that there is evidence of drug-induced type 1 diabetes after treatments for cancer, organ transplantation, and hypersensitivity (Brode *et al*., 2006; Garca-Sáenz *et al*., 2018) [1, 8]. In the present study, it was found that cyclophosphamide and levamisole had an effect on HbA1c levels during an immunomodulation experiment on rats. According to Ablamunits *et al*. (1999) [3] and Gaacia-saenze *et al*. (2018) [8], type 1 diabetes and cyclophosphamide toxicity may indeed be related. The precise mechanism is not entirely understood, though. According to reports, there is an association between MHC Class-II genes and type-1 Diabetes induced during cyclophosphamide-associated immunosuppression therapy (Atlan-gepner & Bouabdallah 1998) [2]. CYP may momentarily reduce suppressor cell levels and disrupt the regional immunological regulatory balance (Gehi *et al*.**,** 2003**;** Yang *et al.*, 2017**)** [9, 21].

Levamisole was discovered to be the most dangerous medication for type 1 diabetes in the current study since it causes HbA1c levels to rise dramatically even when taken alone and has the greatest effect when combined with cyclophosphamide.

The presence of berberine in the *Berberis lycium* Royle root extract, which was previously found to have anti-diabetic activity but showed superior regulation when combined with *Withania somnifera*, may be responsible for the mechanism regulating glucose hemostasis. Such findings point to a synergistic mode of action against toxicity caused by CYP and impaired glycemic control. There have been claims that berberine possesses anti-diabetic properties (Zhou *et al*., 2008,) [22] Berberine was employed as a hypoglycemic drug in Chinese medicine as a result of its documented anti-hyperglycemic activity in treating diarrhoea in diabetic patients (Al-masri *et al*., 2009) [4]. According to Xia *et al*. (2011) [20], by inhibiting hepatic gluconeogenesis, berberine can lower fasting blood sugar levels in diabetic rats without requiring increased insulin levels.

Inhibiting protein tyrosine phosphatase 1B is how berberine exerts its anti-diabetic effects, according to Al-Masri *et al*. (2009) [4]. As insulin sensitivity increased, oral glucose tolerance improved and fasting blood glucose levels decreased, berberine demonstrated anti-diabetic efficacy. The suppression of intestinal glucose absorption, insulin mimics, stimulation of glycolysis, and increased GLP-I release are what give berberine its anti-diabetic properties (Suman *et al*., 2016) [18]. The anti-diabetic effects of *Berberis aristata* have been documented by Singh & Kakkar, P. (2009) [17] and found that it regulates glucose homeostasis via reduced gluconeogenesis and oxidative stress.

Table 1: Experimental group of rats

S. No	Groups	Treatments	No of rats
1.	Control	1ml water	5
2.	CYP	Cyclophosphamide 100mg/kg BW pop on 9th and 16th day	5
3.	LEVA	Levamisole 50mg/kg BW daily for 28 days (subcutaneously)	5
4.	CYP+LEVA	Cyclophosphamide 100mg/kg BW on 9th and 16th day and Levamisole (subcutaneously) 100mg/kg BW for28 days	5
5.	CYP+WS200	*Withania somnifera* extract 200 mg/kg BW, pop daily for 28 days Cyclophosphamide 100mg/kg BW pop on 9th and 16th day	5
6.	CYP+WS400	*Withania somnifera* extract 400 mg/kg BW, pop daily for 28 days Cyclophosphamide 100mg/kg BW pop on 9th and 16th day	5
7.	CYP+BLR200	*Berberis lycium* Royle extract 200 mg/kg BW, pop daily for 28 days Cyclophosphamide 100mg/kg BW pop on 9th and 16th day	5
8.	CYP+BLR400	*Berberis lycium* Royle extract 400 mg/kg BW, pop daily for 28 days Cyclophosphamide 100mg/kg BW pop on 9th and 16th day	5
9.	CYP+WS100+BLR100	*Withania somnifera 100mg/kg + Berberis lycium* Royle extract 100mg/kg BW, pop daily for 28 days Cyclophosphamide 100mg/kg BW pop on 9th and 16th day	5
10..	CYP+WS200+BLR200	*Withania somnifera*200mg/kg+ *Berberis lycium* Royle extract 200 mg/kg BW, pop daily for 28 days Cyclophosphamide 100mg/kg BW pop on 9th and 16th day	5

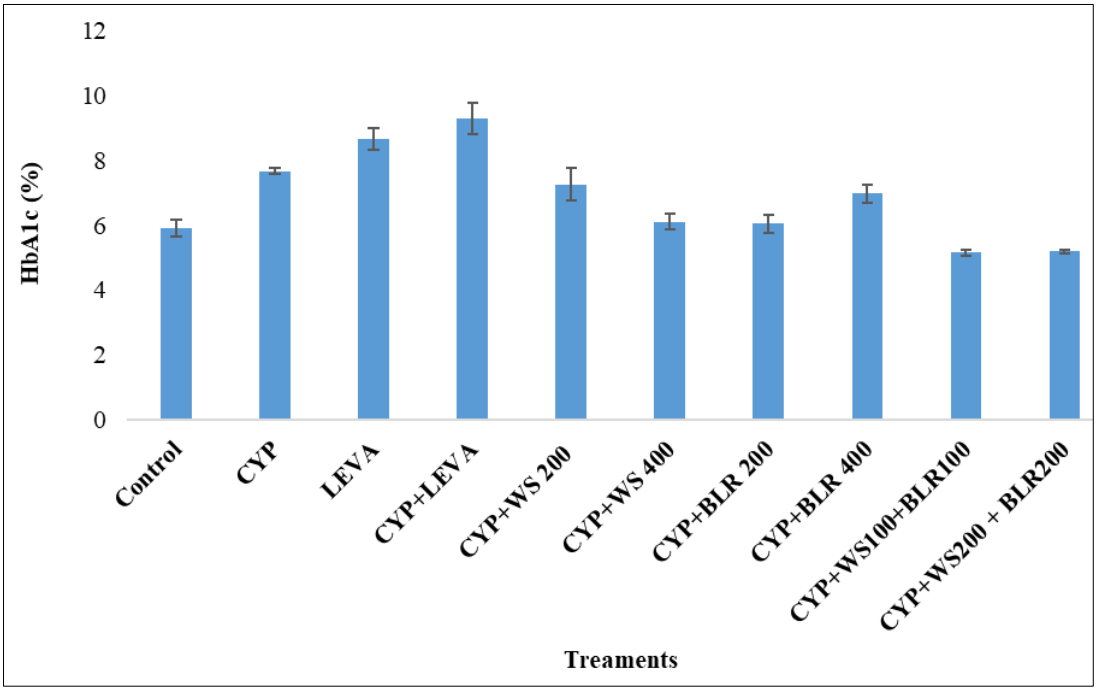

Fig 1: Effect on $HbA1_c$ (%) following administration of levamisole (subcutaneously) cyclophosphamide (orally on 9th and 16th day), hydroethanolic extract of *Withania somnifera* and *Berberis lyceum* Royle and their combination (orally) on 28th day in rats.

Conclusion

The findings of the current investigation revealed that CYP and LEVA may have repetitive an impact on glycemic control in the rats receiving immunotherapy. Although LEVA stimulates the immune system, it affects glucose hemostasis by raising HbA1c levels. Plant extracts given to mice together with cyclophosphamide may have reduced their likelihood of developing diabetes and kept their blood sugar levels under control (6.8-7.1%). However, the extracts CYP+WS100 +BLR100 and CYP+WS200+BLR200 maintain glycemic control in rats more successfully in co-administrations (5.6 and 5.2%, respectively). Therefore, patients with diabetes

receiving cyclophosphamide immunotherapy may benefit from the combination of *Berberis lycium* Royle and *Withania somnifera* extracts. However, as the current study was carried out to evaluate the Immunomodulatory effect of plant extract and was not based on a diabetic model, additional research is required to corroborate these findings.

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Disclosure Statement
The authors have no conflicts of interest to declare.

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